Curso 2010/11
CIENCIAS Y TECNOLOGÍAS/14
I.S.B.N.: 978-84-15287-35-3

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Bases filogeográficas y morfológicas de la diversificación del género *Dysdera* (Araneae, Dysderidae) en Canarias

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SOPORTES AUDIOVISUALES E INFORMÁTICOS
Serie Tesis Doctorales

A mi padre, por enseñarme todo lo que soy

A mi madre, por ser mi ejemplo a seguir en la vida

Soy de las que piensan que la ciencia tiene una gran belleza y que la investigación tiene un sentido casi poético.
Un sabio en su laboratorio no es solamente un teórico.
Es también un niño colocado ante los fenómenos naturales que le impresionan como un cuento de hadas.

Marie Curie

En dos palabras puedo resumir
cuanto he aprendido acerca de la vida:
sigue adelante.

Robert Lee Frost

Agradecimientos

AGRADECIMIENTOS

Llegados a este punto, miro hacia atrás, a todos estos años llenos de experiencias y momentos compartidos con tanta gente que me han ayudado a lo largo de este largo camino y han hecho posible que este proyecto haya llegado a buen puerto. No puedo más que emocionarme al recordar a todas esas personas que de alguna u otra forma han puesto su granito de arena en esta tesis, y ahora, al ver el fruto de tantos años de trabajo no puedo más que decir: ¡Gracias a todos!

Primero quiero agradecer a mi familia por estar apoyándome siempre en todos los aspectos de mi vida, por entender mis constantes ausencias, viajes, estancias, por los momentos en que no he estado presente y por ayudarme a conseguir esta meta. A mi padre y a mi madre a quien les debo todo, por su apoyo, por enseñarme que con ganas y esfuerzo se consigue todo lo que nos propongamos en la vida. Mi padre, ausente pero siempre presente en mis recuerdos y en cada momento de mi vida, estaría orgulloso de ver cumplido este sueño, por ser parte de el. A mi madre, mi ejemplo en la vida. Mujer luchadora donde las haya, siempre tirando hacia delante a pesar de los obstáculos. Por fortalecer la confianza en mi misma y darme ánimos en todo momento ¡Les quiero!

A Carmen, la mejor hermana y amiga que se puede tener. Hemos ido de la mano en muchos aspectos de la vida y también lo has estado durante la tesis. Hemos sido una piña en los malos momentos y me has ayudado a "tirar para adelante" siempre que lo he necesitado. Por tranquilizar a la "Pura"! Y ahora que eres tú la que está lejos, se te echa mucho de menos, pero ¡ánimo con tu sueño!

Al resto de mi familia (tíos, primos) por ser tan geniales, estar siempre unidos, por el gran apoyo recibido siempre, y por entender un poco mejor mi trabajo. Estos años han sido especialmente duros, han pasado muchas cosas que te truncan la vida y que te marcan para siempre. Gracias a todos los que nos han ayudado en los malos momentos.

A Salva, mi apoyo constante en cada momento. Por no dejarme caer y ayudar a levantarme cuando lo hacía. Por arrancarme siempre una sonrisa, incluso cuando pensaba que no había motivos para sonreír. Por entender las largas ausencias fuera y el tiempo dedicado a la tesis. Por los momentos compartidos y

los que nos quedan. Por tu gran implicación en todo este proceso, desde las largas jornadas de campo levantando piedras después del trabajo, durante los fines de semana y vacaciones, hasta tus sugerencias, comentarios, ideas en la escritura de la tesis ¡Mil gracias por todo!

A mis directores de tesis Pedro Oromí y Miquel Arnedo, les debo mucho para que esta tesis esté hoy finalizada. Por su implicación tanto profesional como personal en todo este proceso. Por el placer de haber podido trabajar con dos de los grandes en este campo ¡Moltes gràcies! A Pedro, le agradezco la confianza depositada en mí desde los inicios de mis pasos por el departamento, bajo la lupa, mirando arañas y despertando mi interés por este grupo. Por ayudarme en mi formación como investigadora, por tu paciencia y dedicación. Por tener siempre un hueco para todo lo que he necesitado, correcciones, sugerencias, opiniones, por defender y tirar siempre de la gente que trabaja a tu lado. Ha sido un gran placer compartir esta experiencia contigo. A Miquel, simplemente brillante en tu trabajo. Me has trasmitido tus conocimientos, tu pasión por la ciencia, la evolución, la curiosidad del por qué de las cosas. Tu capacidad crítica me ha enseñado a ver las cosas desde todas las perspectivas posibles y a no desistir ante los problemas que se nos iban planteando. Gracias por tener siempre una respuesta a casi todas mis dudas y por aportar todos tus conocimientos a mi formación como investigadora ¡Gracias por todo!

A mis niñas de Barcelona, que a pesar de la distancia han estado siempre tan presentes. A Leticia, mi gran amiga, confidente, gracias por todos los buenos y malos momentos compartidos, llenos de complicidad, risas durante las largas jornadas en el "fondo sur", en el laboratorio, en el campo y fuera del trabajo. Gracias por ser simplemente tan genial. Por estar siempre ahí para cualquier cosa que he necesitado. El trabajo siempre ha sido más fácil con tus aportaciones, sugerencias, comentarios, y el buen humor que desprendes. Gracias por escuchar mis constantes dudas sobre trabajo y cuestiones personales. Esta tesis es en parte también tuya y no habría sido igual sin ti ¡Mil gracias por TODO! A Isa, ¡mi niñaaa! La amiga que está siempre ahí, que me ha apoyado tanto en muchos momentos. Desprendes un entusiasmo contagioso por la vida, que nos arrastra a todos los que estamos a tu lado. Con esa fuerza y energía que te caracteriza, no hay nada que pueda contigo. Por tantas risas y experiencias inolvidables que hemos compartido y

por las que nos quedan. A Berta, por todos los buenos ratos en Barcelona, fuera y dentro de la facultad, por tus constantes ánimos y apoyo en todo momento. ¡Las quiero!

A todos los compañeros del Departamento de Biología Animal por todos estos años. A Heriberto, por todos los buenos ratos en el departamento y fuera de él, por tu apoyo incondicional en todos los aspectos de trabajo y a nivel personal. Por las jornadas en el campo, por todas la piedras que levantamos y las arañas que me traías desde cada lugar donde encontrabas. Por alimentar con tus "niñas" a mis arañas durante mis ausencias. A Helena, por saber hacerme ver las cosas desde otra perspectiva. Muchas gracias por los ánimos, especialmente en el periodo final de la tesis. A Toni, por toda su ayuda en los muestreos y su entusiasmo y buen humor en el trabajo. A David, por las horas de campo y cuidar de las arañas cuando no estaba. A Hermans, por todas las sugerencias y dudas que en multitud de ocasiones me resolvió.

A los profesores del Departamento de Biología Animal de la Universidad de La Laguna, a Rando, Marcos, Aurelio, Carolina, Ibáñez, Charo, Jacinto, Jorge Núñez, por echarme una mano cuando hizo falta.

Durante esta tesis he pasado largos periodos en Barcelona, que ha llegado a ser mi segundo hogar gracias a todas las personas con las que he compartido momentos inolvidables, horas de laboratorio, charlas, risas, lágrimas, comidas, cervezas, jornadas en la nieve… Gracias por hacer mi estancia en Barcelona inolvidable.

Gracias a Miquel y Mónica, desde los inicios cuando me ofrecieron su casa tanto a mí como a mi familia. A Mercè, mi profe del laboratorio, con ella aprendí los secretos de la PCR, los cleanings, la secuenciación. Gracias por las risas en los cafés y por todos los buenos momentos compartidos. A Nayra, la otra canaria, con ella reforzamos nuestra presencia isleña entre los catalanes, incluyendo nuestra jerga canaria y costumbres culinarias (mojo, papas, conejo al salmorejo…) ¡que a nadie dejó indiferente! Gracias por todo tu apoyo, personal, en el trabajo y durante las largas jornadas de campo al solajero. A Eva de Mas, siempre tirando para delante ¡gracias por los buenos ratos! A Cesc por la ayuda prestada en los análisis estadísticos de muchos de los artículos. A toda la gente que en un momento u otro

hizo mejor mi estancia allí: Marc, Lidia, Vera, Gemma, Facundo, Josep, Sara, Alberto, Enric, María Chatzaki, Sergi, Laura, Jenny, Mari Carmen, Marta, Núria, Dimitar, ¡y seguro que me olvido de alguien!

A los profesores de la Universidad de Barcelona también quiero agradecerles los buenos ratos compartidos, las charlas de Biodiversidad SEMBIO, las dudas resueltas. A Carles, el hombre pegado a un puro, ¡Macías! Gracias por los buenos ratos, comidas y cervezas compartidas, también por tus sugerencias y recomendaciones en algunos artículos. A Salvi, contigo nos poníamos al día en cotilleos y demás habladurías. Gracias por las risas, los buenos momentos y por las interesantes tertulias sobre evolución. A Eduardo, por prestarme la cámara de fotos en más de una ocasión. Al resto de profesores del Departamento de Biología Animal: Cruz Palacín, Xavi Turón, Marina Blas, Juli, a Nuria Bonada del Dpto. de Ecología, y a Julio Rozas y Marta Riurtot del Dpto. de Genética.

A mi compañera de piso Milena y a Trastu, y a Roser por tener siempre un hueco para mi y mi familia cuando me hizo falta.

El trabajo de campo ha sido una parte importante en esta tesis. La pregunta del millón: ¿Cuántas piedras se habrán levantado buscando arañas? Bastantes, nunca habría levantado tantas sin la ayuda de: Pedro, Heriberto, Salva, Helena, Toni, David, Sofía, Nayra, Rocío, Bárbara, Clare, Carmen y a muchos alumnos de la asignatura de artrópodos que me acompañaron en las salidas de campo ¡Los bocadillos y las chocolatinas Tirmas nunca supieron tan bien! También pude disfrutar de campañas de campo por la península, recorriendo cientos de kilómetros, con miles de anécdotas para contar en compañía de Miquel, Leticia, Vera, Isabel, Mariana y gente entusiasta interesada por nuestro trabajo. Gracias a la familia de Isabel Calderón por alojarnos en su casa en La Navillas. A la familia de Lina por prestarnos su casa en Lanzarote.

Mucha gente envió material desinteresadamente. Gracias a Manolo Arechavaleta, Laura García, Isamberto Silva y Dília Menenzes.

A Jordi Moya y Eva de Mas, por acogerme en su casa durante la corta estancia en Almería. Gracias Jordi por tus sugerencias y ayuda en la parte ecológica, que a pesar de que quedó pendiente, estaría bien retomar en un futuro.

Gracias a Brent Emerson, por su ayuda durante la estancia realizada en Norwich, por compartir tus conocimientos y las buenas ideas. Por todas las recomendaciones y sugerencias posteriores para mejorar los artículos. A Godfrey Hewitt, David Richarson y demás investigadores de la Universidad de East Anglia, por hacer tan enriquecedora esta estancia. Allí conocí gente maravillosa con la que compartí muy buenos momentos. Gracias a Eugenia, Álvaro, Allison, Lorenzo, Andrea Miraldo, Sandra, María, Gerardo, Cristiana, Claudia, Isabel, Yoichi. Gracias a Juan Carlos Illera por las recomendaciones previas a la estancia.

Gracias a Søren Toft por su buena acogida durante la estancia realizada en Aarhus, por la nueva experiencia de trabajar en ecología y por compartir sus conocimientos en este campo. Al resto de investigadores de la Universidad de Aarhus, especialmente a Trine Bilde, David Mayntz y Jens Olesen. A las chicas, Julieta gracias por los buenos ratos, a Marija, Cristina Tuni, Cristina Holm, María, Fabia, Cinzia Tesauro con quienes compartí muy gratos momentos. A Benigno por la ayuda prestada en cuestiones de la estancia.

A Pelucho, por brindarme su ayuda e ideas en muchas ocasiones. A todos los integrantes del Grupo de Ecología y Evolución en Islas del CSIC, especialmente a Patri, David, Bea, Aaron y Juan Carlos Illera, por ayudarme en diversas cuestiones siempre que los he necesitado. A Mariano, por las dudas resueltas en genética. A Oscar Moya, por sus aclaraciones con el NCA y sugerencias para mejorar los análisis genéticos.

A Patri, Rosi y Jairo por aclararme muchas dudas sobre la parte de papeleo, informes y burocracia del depósito de la tesis. A Marta Sansón, por las aclaraciones referentes a la Comisión Académica. A Fernando Vizcaíno por su inestimable ayuda en toda la parte engorrosa de papeleo durante todo el proceso que dura la tesis.

A los investigadores Stuart Longhorn y Bjarte Jordal por su ayuda desinteresada a la hora de redactar los informes europeos que me requerían para la tesis doctoral.

A los integrantes de la Asociación Canaria de Investigadores en Formación (ACIF) por luchar en contra de la precariedad en la investigación. Gracias a todos los que aportaron su granito de arena para alcanzar los importantes logros

conseguidos. A seguir luchando por la causa. ¡Investigar es trabajar! A David, Jairo y Kati, porque unidos conseguimos que se reconocieran nuestros derechos y se hiciera justicia, tras más de seis meses sin cobrar injustamente nuestras becas doctorales ¡Y doy gracias a que no consumamos nuestra huelga de hambre!

A los amigos de siempre, a los que aun están a mi lado y a los que hace tanto que no veo, pero también han sido importantes a lo largo de este periodo. A Sabrina, gracias por tu amistad, desde los inicios de la carrera hasta hoy, por estar siempre ahí. A Rosana, gracias por tu apoyo en los malos momentos y por las risas en los buenos. A Oliver, por estar en los inicios de este proyecto animándome siempre a seguir adelante. A Nica, Nisa, Ayoze, Ibra, Paloma, Tilman, Nayra.

Espero no haberme olvidado de nombrar a muchas personas que de una u otra manera me han acompañado en esta aventura y que han aportado su granito de arena para la realización de esta tesis, sabiendo que sólo con el apoyo de todos, este trabajo ha sido posible ¡Mil gracias!

Esta Tesis Doctoral ha sido realizada gracias a una beca de la Consejería de Educación, Universidades, Cultura y Deportes del Gobierno de Canarias.

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Capítulo 1

INTRODUCCIÓN

EVOLUCIÓN INSULAR: LAS ISLAS COMO LABORATORIOS EVOLUTIVOS Y ECOLÓGICOS

Desde los descubrimientos realizados por Darwin en su viaje a bordo del *Beagle* que darían lugar a su revolucionaria teoría de la evolución por selección natural (Darwin, 1859), las islas han despertado el interés de naturalistas y científicos, y han sido consideradas los tubos de ensayo para el estudio de determinados procesos evolutivos, ecológicos y biogeográficos (Mayr, 1967).

Las islas pueden clasificarse en tres tipos principales en función de su origen: (1) fragmentos insulares o islas continentales, que originariamente formaron parte de la masa continental, (2) islas darwinianas o islas oceánicas, que se han formado *de novo,* y (3) islas mixtas, fragmentos escindidos del continente que posteriormente han sido modeladas por eventos volcánicos (Gillespie & Roderick, 2002). Los procesos que rigen la dinámica de las poblaciones, la estructuración de las comunidades, la formación de nuevas especies y la extinción de taxones varían en función del tipo de isla. En los fragmentos continentales la mayoría de los nichos ya están ocupados e inicialmente el número de especies tiende a reducirse por procesos de competencia ecológica, reducción del área y relajación. A medida que aumenta el tiempo de aislamiento, se puede incrementar la diferenciación genética respecto a su origen de procedencia (Carson *et al.*, 1990; Gillespie & Roderick, 2002), y producirse procesos de relictualización de linajes y formación de paleoendemismos (Cronk, 1992).

Las islas oceánicas poseen cualidades adicionales que las hacen especialmente idóneas para testar hipótesis evolutivas (Mayr, 1967; Emerson, 2002), ya que son relativamente pequeñas, poseen límites conocidos y su fauna es generalmente menos rica y compleja que la de los continentes, facilitando el estudio de los procesos que generan la biodiversidad (Losos & Ricklefs, 2009). Cada una de las islas de los archipiélagos oceánicos se ha originado en diferentes periodos geológicos, constituyendo réplicas independientes de los procesos evolutivos de diferentes edades, lo que ha sido utilizado para establecer las

diferentes etapas del ensamblaje de las comunidades (Emerson & Gillespie, 2008). El aislamiento geográfico de las islas juega un papel fundamental durante los primeros estadios de colonización y diversificación de los organismos (White *et al.,* 2006; Fukami *et al.,* 2007). Así, en las islas donde el nivel de aislamiento es bajo, el número de especies que inicialmente colonizan el territorio es elevado y éstas pueden permanecer como metapoblaciones, por lo que la composición de la biota depende principalmente de las tasas de inmigración y extinción. Cuando el grado de aislamiento es intermedio, la inmigración suele ser suficientemente baja para permitir diferenciación genética de las poblaciones respecto a su fuente de colonización. Finalmente, en las islas con elevado aislamiento, los procesos de colonización son menos frecuentes, y se favorece la generación de nuevas especies por radiación adaptativa (Gillespie & Roderick, 2002).

Los archipiélagos oceánicos proporcionan múltiples oportunidades para el aislamiento poblacional y para la diversificación de los organismos, bien como resultado de la colonización entre islas y posterior evolución alopátrica, o bien por procesos de especiación intrainsular (Juan *et al.*, 2000; Gillespie & Roderick, 2002). Entre los factores responsables de la diversificación local destacan los procesos de vicarianza producidos por eventos geológicos, como por ejemplo el aislamiento poblacional debido a coladas volcánicas (p. ej. Beheregaray *et al.*, 2003), y los fenómenos de fragmentación ecológica (o creación de barreras ambientales) promovidas por la adaptación a determinados hábitats o nichos ecológicos (Roderick & Gillespie, 1998).

La Teoría del Equilibrio de la Biogeografía Insular (MacArthur & Wilson, 1963, 1967) y su posterior revisión (Lomolino, 2000), postulan que la biodiversidad insular es resultado del balance entre migración, especiación y extinción, los cuales son a su vez dependientes del grado de aislamiento y del área de la isla. A medida que aumenta el aislamiento (distancia) disminuye la tasa de inmigración, y la generación de nuevas especies se produce principalmente por procesos de especiación *in situ*, mientras que la tasa de extinción disminuye en función del incremento del área de la isla (Whittaker & Fernández-Palacios, 2007).

El tamaño (área) de las islas ha sido considerado un factor importante en la generación de nuevas especies (Paulay, 1994; Peck *et al.,* 1999; Losos & Schluter, 2000; Triantis *et al.,* 2008), ya que en las islas de mayor área existen más posibilidades de especiación alopátrica, gracias a la mayor variedad topográfica y disponibilidad de nichos ecológicos, comparado con las islas de menor tamaño (Losos & Ricklefs, 2009 y referencias incluidas). La disponibilidad de nichos ecológicos es un parámetro difícil de estimar, por ello la diversidad de hábitats y la altitud máxima han sido propuestas como variables alternativas (Cardoso *et al.,* 2010). En las islas de mayor tamaño, la diversidad de especies es superior, y se ha sugerido que una biodiversidad mayor podría a su vez incrementar la tasa de especiación (Emerson & Kolm, 2005). Las variables climáticas (temperatura y precipitación) fueron propuestas por Wright (1983), en su postulación de la teoría de *species-energy*, como factores determinantes del número de especies (ver Kalmar & Currie, 2006; Kreft *et al.,* 2008). Sin embargo, a pequeña escala, como es el caso de las islas oceánicas que poseen una mayor continuidad climática, la variación altitudinal parece jugar un papel más importante. La edad geológica de las islas (Baldwin & Sanderson, 1998; Borges & Brown, 1999) también es un factor importante a tener en cuenta. Así, las islas más antiguas han tenido más oportunidades de ser colonizadas y de generar nuevas especies, mientras que en las más jóvenes el corto espacio de tiempo transcurrido desde su formación se refleja en una menor diversidad y en valores inferiores de endemismos (Emerson & Oromí, 2005; Gillespie *et al*., 2008). Por otra parte, a mayor antigüedad geológica mayor erosión, que a largo plazo da lugar a la transformación y pérdida de hábitats. En este sentido, la extinción puede jugar además un papel fundamental en la estructuración de las comunidades de islas antiguas y en la disminución de su biodiversidad (Price & Clague, 2002; Whittaker *et al*., 2008, 2010). Así, la probabilidad de extinción aumenta con la edad geológica, que a su vez está inversamente correlacionada con el tamaño de las islas. La relación entre edad de las islas y diversidad de especies fue propuesta por Whittaker *et al*., (2008) en el G*eneral dynamic model of oceanic island biogeography* (*GDM*; Fig. 1.1), en el cual se exponen las etapas del denominado “ciclo vital” de los archipiélagos oceánicos en función de la edad geológica, la altitud y la complejidad topográfica de las islas.

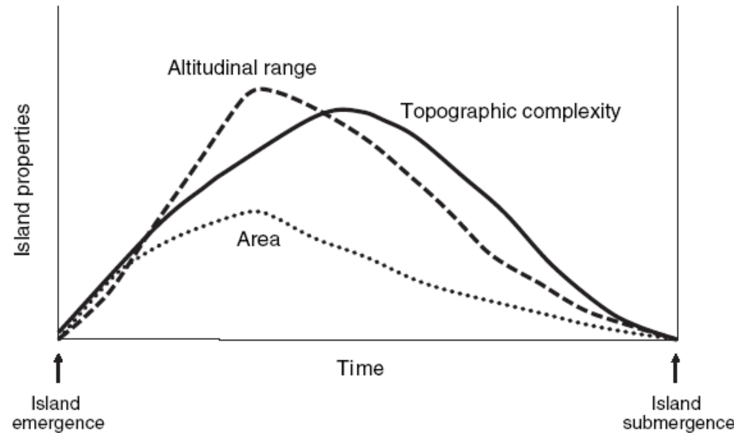

Fig. 1.1.- Relación existente entre la edad (eje abscisas, tiempo), y las características (área, altitud y complejidad topográfica) de una hipotética isla oceánica (eje ordenadas). Figura extraída de Whittaker *et al.*, (2008).

En las islas oceánicas, la radiación adaptativa es un proceso evolutivo especialmente importante en el origen y mantenimiento de la biodiversidad (Gillespie, 2002). Como consecuencia de este proceso, a partir de uno o pocos ancestros y en un corto periodo de tiempo, se origina un elevado número de nuevas especies que ocupan gran variedad de nichos ecológicos, facilitado por la ausencia de competición o de predadores (Schluter, 2000). La radiación adaptativa ha jugado un papel importante en la estructuración de las comunidades (Losos *et al.*, 1998), ya que la existencia de muchos linajes solapados geográficamente se ve facilitada por procesos de diversificación eco-morfológicos y etológicos en los organismos, debido a la presión ecológica y a la competencia por los recursos (Losos *et al.*, 2000; Schluter, 2000; Gillespie, 2004; Kozak *et al.*, 2005).

Se han descrito una serie de procesos evolutivos, como el desplazamiento morfológico (Brown, 1956; Grant, 1972) o ecológico de caracteres (Schluter, 2000), y la exclusión competitiva (Losos, 2000) entre especies simpátricas, los cuales facilitan la coexistencia y explotación de diferentes recursos por parte de las especies, evitando así la competencia entre ellas. De esta forma, se espera que las especies que habitan en simpatría posean más diferencias (morfológicas, ecológicas, etológicas o reproductivas) que las especies distribuidas

alopátricamente. No obstante, desde un punto de vista neutralista, si los recursos no son limitantes es posible la coexistencia de especies similares, ya que éstas responderán de forma parecida ante determinadas condiciones o características ambientales (Chesson, 2000; Leibold & McPeek, 2006), de forma que las especies pueden coexistir gracias a sus similitudes más que por sus diferencias (Hubbell, 2006).

La inclusión de información filogenética en estudios ecológicos nos brinda una herramienta indiscutible para entender el papel de la evolución en la composición y estructuración de las comunidades. En la Fig. 1.2 se observan diferentes ejemplos de estructuración de las comunidades, en los que están implicados varios factores: los filtros ambientales, las interacciones interespecíficas y los posibles cambios evolutivos que permiten la coexistencia ecológica entre especies (p. ej. competencia, desplazamiento de caracteres, exclusión competitiva, etc.). En la parte inferior del diagrama se puede apreciar cómo las especies pueden conservar las características biológicas propias del linaje (a y b) o, por el contrario, diversificarlas (c y d). A su vez, las especies que forman la comunidad pueden estar fuertemente influenciadas por los filtros ecológicos permitiendo su coexistencia (a y d), o bien la competencia por los recursos impide la coexistencia de especies que comparten el mismo nicho ecológico (b y c). Por lo tanto, la composición y estructuración de las comunidades debe entenderse desde un contexto filogenético, geográfico y ecológico, con una visión espacio-temporal (Emerson & Gillespie, 2008).

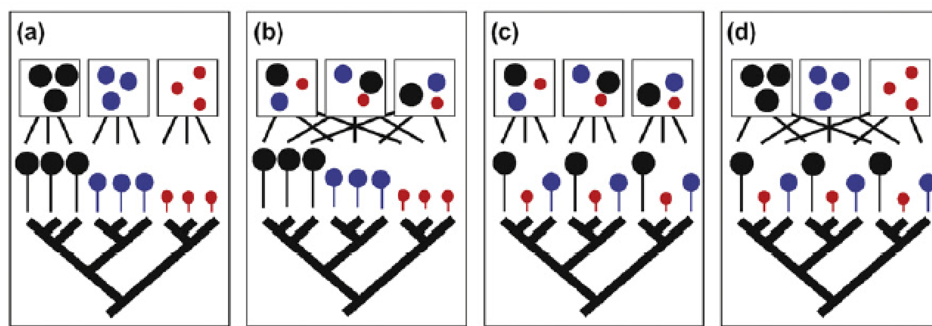

Fig. 1.2.- Esquema que representa las diferentes posibilidades evolutivas en la estructuración de las comunidades (ver texto para más detalle). Figura extraída de Emerson & Gillespie (2008).

SISTEMÁTICA Y FILOGEOGRAFÍA

La sistemática es el estudio de la biodiversidad y de sus orígenes. Su objetivo es entender las relaciones evolutivas entre los organismos, las especies y los taxones superiores, así como de otras entidades biológicas como los genes, y comprender la evolución de las características de los taxones, incluyendo rasgos intrínsecos (fenotipo/genotipo), interacciones ecológicas (ecología) y distribuciones geográficas (biogeografía y filogeografía). Una parte importante de la sistemática tiene que ver con el desarrollo de métodos relacionados con diferentes aspectos de la inferencia filogenética y la taxonomía (nomenclatura y clasificación). El primer paso para la reconstrucción filogenética consiste en la búsqueda de caracteres homólogos (origen en un ancestro común) en las unidades de estudio, para construir una matriz de caracteres y poder comparar los diferentes taxones. Los caracteres pueden ser morfológicos, moleculares (secuencias genéticas de ADN), etológicos o cualquier otro tipo de información susceptible de ser transmitida a la descendencia.

La sistemática filogenética o cladismo, término propuesto por Mayr (1969) y desarrollado por Hennig (1950, 1966), sentó las bases teóricas de la inferencia filogenética. Las relaciones filogenéticas de los organismos se expresan en forma de un árbol, donde los nodos terminales representan los taxones objeto de estudio y los nodos internos los ancestros hipotéticos. Las ramas que conectan estos nodos representan el proceso evolutivo acaecido entre un ancestro y su descendiente. Estas ramas a menudo se muestran de tamaño proporcional al número de cambios evolutivos que han tenido lugar en la misma. La rama más basal es la raíz, que representa el origen evolutivo del grupo.

El advenimiento de los ordenadores permitió la implementación computacional de diferentes métodos de inferencia filogenética. La parsimonia (Edwards & Cavalli-Sforza, 1963; Kluge & Farris, 1969; Farris, 1970; Fitch, 1971) es un método que no requiere la definición de un modelo evolutivo, pero permite incorporar información sobre la relevancia de los caracteres en forma de pesos o costes diferenciales. La parsimonia busca maximizar la congruencia entre los caracteres mediante la selección del cladograma que minimiza la homoplasia (evolución independiente) entre los caracteres, lo que desde un punto de vista

computacional se traduce en minimizar el número de cambios entre los diferentes estados de los caracteres. Otros métodos de inferencia filogenética requieren la definición de modelos probabilísticos de evolución de los caracteres (Lio & Goldman, 1998). Dentro de estos métodos destacan la máxima verosimilitud [*maximum likelihood* (ML)] (Cavalli-Sforza & Edwards, 1967; Felsenstein, 1981; ver Huelsenbeck & Crandall, 1997 para revisión del método) y la inferencia Bayesiana (Rannala & Yang, 1996; Huelsenbeck & Ronquist, 2001; Huelsenbeck *et al.*, 2001; Lewis & Swofford, 2001). Los métodos de máxima parsimonia (MP) y máxima verosimilitud (ML) se basan en criterios de optimización que permiten evaluar árboles alternativos y escoger el más óptimo (en MP el mejor árbol es el que minimiza la homoplasia, y en ML se selecciona el árbol más probable dados los datos y el modelo evolutivo). Por otro lado, la inferencia bayesiana utiliza algoritmos de *Markov Chain Monte Carlo* (MCMC) (Metropolis *et al*., 1953; Hastings, 1970) para aproximar la distribución posterior de los parámetros de interés, entre los que se cuentan la topología y la longitud de las ramas del árbol, y el resultado no es uno o varios árboles óptimos, sino una distribución de los mismos.

La máxima verosimilitud trata de construir el árbol estadísticamente más probable a partir de una matriz de datos y basándose en un modelo evolutivo determinado utilizando funciones de verosimilitud (Edwards, 1972). La inferencia Bayesiana (Rannala & Yang, 1996) utiliza la distribución de la probabilidad posterior (la probabilidad de que un árbol sea el correcto para los datos observados (Huelsenbeck *et al*., 2001)) para construir el árbol filogenético. El teorema de Bayes combina la probabilidad *a priori* de una filogenia con el valor de verosimilitud para producir una distribución de probabilidad posterior (PP). En este caso el resultado no es un árbol único, sino un conjunto de árboles que forman parte de la distribución posterior, de los cuales el de mayor valor de PP debe ser considerado como la mejor aproximación al árbol verdadero, y en este caso, el propio valor de PP constituye un soporte estadístico de las hipótesis evolutivas. Por el contrario, en los análisis de (MP) y (ML) el grado de soporte de los clados del árbol se estima *a posteriori* mediante diversos tipos de índices. Los valores de apoyo más frecuentemente utilizados pueden dividirse en cualitativos, como el apoyo de Bremer (*Bremer support*; Bremer, 1988) que se utiliza principalmente en MP, o

métodos basados en remuestreos al azar de la matriz de datos, ya sea con reemplazamiento como el *Bootstrap* (Felsenstein, 1985) o por eliminación como el *Jackknife* (Efron, 1979), que se utilizan indistintamente en MP o ML.

La filogeografía es una disciplina que trata de establecer los patrones y los procesos que rigen la distribución geográfica de los linajes y su diversidad genética (Avise *et al.,* 1987; Avise, 2000). La filogeografía sirve de puente entre la filogenia y la genética de poblaciones, y además incorpora información complementaria de otras disciplinas (paleontología, geología, etología, etc.), por lo que ha sido ampliamente utilizada en campos tales como la biología evolutiva, la ecología o la conservación (Avise, 2000). A través de la filogeografía se pueden establecer patrones de distribución de la diversidad genética desde un contexto geográfico y temporal, determinar procesos demográficos como flujo génico y tamaño efectivo poblacional, y distinguir entre procesos históricos (fragmentación, expansiones contiguas de rango o colonizaciones a larga distancia) y demográficos (restricción de flujo génico, interacción entre migración y deriva genética) (Avise, 2000; Templeton, 2004). Mediante la filogeografía comparada (Bermingham & Moritz, 1998; Moritz & Faith, 1998; Avise, 2000; Arbogast & Kenagy, 2001) podemos reconocer patrones evolutivos comunes en organismos procedentes de linajes independientes que están codistribuidos geográficamente, y así entender mejor los procesos históricos implicados en la estructuración de las poblaciones (p. ej. Zink, 1996; Schneider *et al.,* 1998; Sullivan *et al*., 2000; Victoriano *et al*., 2008). Si dos especies simpátricas muestran patrones filogeográficos similares, puede indicar que ambas especies estuvieron codistribuidas en el pasado, y que se han visto afectadas por los mismos cambios climáticos, condiciones ambientales, barreras geográficas o limitaciones de flujo génico (Irwin, 2002; Carstens & Richards, 2007; Garrick *et al.,* 2008). Por el contrario, si dos especies codistribuidas muestran patrones filogeográficos diferentes, puede indicar que características propias de las especies como especificidad de hábitat, comportamiento, ciclo de vida, capacidad de dispersión, etc., han influido en la estructuración genética de sus poblaciones (Taberlet *et al.,* 1998; Michaux *et al*., 2005; Bird, 2007; Hodges, 2007).

Los métodos estadísticos convencionales, como el *F* de Wright (1969) utilizado para detectar estructuración poblacional a partir de la distribución de los

alelos y genotipos, no son suficientes para la inferencia de procesos históricos, ya que no utilizan la información filogenética. El desarrollo de nuevas metodologías como la filogeografía estadística (Knowles & Maddison, 2002; Knowles, 2004; Nielsen & Beaumont, 2009) o el Análisis Filogeográfico de Clados Anidados (*Nested Clade Phylogeographic Analysis* (NCPA): Templeton *et al*., 1995; Templeton, 1998) permiten combinar la información filogenética y geográfica con las predicciones realizadas con frecuencias alélicas, considerando las premisas de la teoría de la coalescencia (Kingman, 1982; Hudson, 1990; Rosenberg & Nordborg, 2002). La teoría de la coalescencia, postulada por Kingman (1982), se basa en la reconstrucción de la historia evolutiva de los linajes genéticos hasta llegar (retrocediendo en el tiempo) a su ancestro común más reciente (*MRCA*), considerando la genealogía y la frecuencia alélica de los genes estudiados (Kingman, 1982; Donnelly & Tavaré, 1986; Kuhner, 2008; Fig. 1.3).

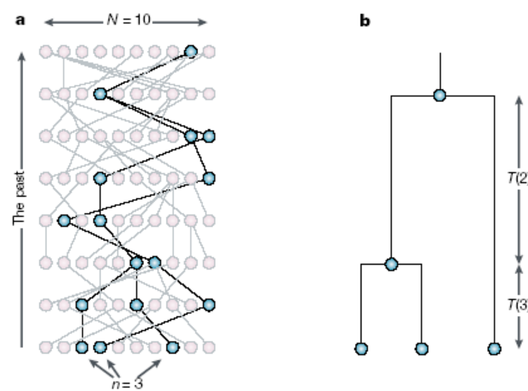

Fig. 1.3.- Esquema del principio básico en el que se basa la Teoría de la Coalescencia (a) Genealogía completa de una población de *N=10* individuos haploides, las líneas negras trazan, hacia el pasado, tres linajes diferentes muestreados *N=3*, hasta llegar a su *MRCA*. (b) La subgenealogía de estos tres linajes; *T(3)* y *T(2)* representan los tiempos entre cada evento de coalescencia. Figura extraída de Rosenberg & Nordborg (2002).

El tiempo que transcurre hasta que varios alelos coalescen en un único ancestro común depende principalmente del tamaño efectivo de la población (*Ne*): *Ne* pequeños se corresponden a tiempos de coalescencia menores. La probabilidad de que dos secuencias de una población (organismos diploides), bajo el modelo

neutralista de Wright-Fisher, coaleszan, retrocediendo en el tiempo, hasta su ancestro común en la generación anterior, es de 1/(2 *Ne*) para un locus nuclear y de 1/(*Ne*) para uno mitocondrial (Fu & Li, 1999). Actualmente muchos de los métodos existentes para el estudio de la filogeografía y la genética de poblaciones tienen su base en la teoría de la coalescencia.

Marcadores moleculares en filogenia y filogeografía

El ADN mitocondrial ha sido el marcador más utilizado en la reconstrucción filogenética y filogeográfica de muchas especies, ya que posee una serie de características óptimas para la inferencia filogenética (Wilson *et al.,* 1985; Avise *et al*., 1987; Moritz *et al*., 1987; Simon, 1991). La molécula de ADN mitocondrial animal posee una estructura simple (ausencia de regiones repetitivas, pseudogenes o intrones), circular, compuesta por 15000-17000 pb, que codifica para 37 genes (Brown, 1985; Ballard & Whitlock, 2004), y que además es fácil de amplificar y secuenciar gracias a las múltiples copias presentes en cada célula, y a la existencia de cebadores (*primers*) universales (p. ej. Kocher *et al.,* 1989; Folmer *et al.,* 1994 para invertebrados). La tasa de sustitución de los genes mitocondriales es elevada (Brown *et al.,* 1979), alrededor de 10 veces superior a la nuclear o cloroplástica, lo que permite detectar más rápidamente diferenciación de linajes a nivel intraespecífico y poblacional.

El tamaño poblacional efectivo (*Ne*) es menor que en los genes nucleares, ya que el genoma mitocondrial es haploide y principalmente de herencia materna; así, el número efectivo de alelos que se transmite a la descendencia suele ser ¼ para el ADN mitocondrial respecto al nuclear (Pamilo & Nei, 1988). Otra ventaja de los genes mitocondriales es la ausencia o escasez de procesos de recombinación (si bien se ha evidenciado en hongos, plantas y algunos animales; Birky, 2001). Algunas de las limitaciones que presenta esta molécula son la rápida saturación de las secuencias debida a múltiples sustituciones en la misma posición nucleotídica, una posible heteroplasmia (Avise *et al.,* 1987), la sensibilidad a procesos de selección (Hey, 1997), y la consecuente infraestimación de la diversidad genética (Zang & Hewitt, 2003), etc.

Muchos autores proponen el uso de marcadores nucleares en estudios filogeográficos y poblacionales para tener una visión más completa de los procesos evolutivos acaecidos en los organismos (Hare, 2001; Ballard & Whitlock, 2004), ya que la herencia uniparental de los genes mitocondriales nos permite entender sólo una mitad de la historia evolutiva de las especies (Ballard & Whitlock, 2004). El ADN nuclear presenta una serie de diferencias respecto al mitocondrial, como por ejemplo su herencia biparental (genoma diploide). Por este motivo posee un mayor tamaño poblacional efectivo (*Ne*), puede experimentar recombinación, selección, heterocigosis, posee tasas de sustitución inferior, etc. (Zang & Hewitt, 2003). Para establecer las relaciones filogenéticas a elevados rangos taxonómicos, los marcadores nucleares ribosomales (p. ej. *18S* y *28S* rRNA) son los más utilizados. Mientras que a niveles taxonómicos inferiores (de géneros a especies) o para establecer relaciones intraespecíficas y a nivel poblacional destacan los marcadores mitocondriales ribosomales (p. ej. *12S* y *16S* rRNA) y codificantes de proteínas (p. ej. *cox1*, *cytb*, *nad1*).

El alineamiento de las secuencias moleculares permite el establecimiento de la homología posicional entre dichas secuencias. La presencia de mutaciones de tipo inserción-deleción (indels) puede ocasionar problemas a la hora de identificar regiones homólogas, dificultando el alineamiento. El mantenimiento de la pauta de lectura aminoacídica de los genes proteicos o la estructura secundaria en los genes ribosomales, pueden ayudar a establecer la homología entre secuencias (Kjer, 1995). Pese a que los procesos de inserción-deleción muchas veces dificultan el alineamiento de las secuencias, son una importante fuente de información filogenética que no debe ser desechada (Lee, 2001), lo que ocurre cuando se consideran como *missing data* (Simmons *et al.,* 2001). La inclusión de la información aportada por los indels ha llevado a tratar los gaps bien como 5º estado, o bien como caracteres adicionales de ausencia/presencia (Barriel, 1994; Simmons & Ochoterena, 2000). Esta segunda opción evita el exceso de peso dado a indels de posiciones múltiples y que han sido probablemente resultado de un solo evento evolutivo.

Distinción entre árbol de especies y árbol de genes

La genealogía de los genes puede no coincidir con el árbol de las especies, debido a la estocasticidad del proceso genético (deriva genética y mutación) (Pamilo & Nei, 1988; Avise, 2004). Por ello, la inclusión de varios genes con diferentes características evolutivas, tanto en filogenia como en filogeografía, es imprescindible para determinar la filogenia real de una especie (Pamilo & Nei, 1988; Funk & Omland, 2003). Si la diferenciación entre las especies es relativamente reciente, se pueden dar procesos de retención de polimorfismos ancestrales (*incomplete lineage sorting*), las especies siguen compartiendo alelos, y no aparecen como monofiléticas (Tajima, 1983; Takahata & Nei, 1985; Nei, 1987; Takahata, 1989; Hudson, 1992; Lyons-Weiler & Milinkovitch, 1997). Los procesos de duplicación/extinción de genes, recombinación, transferencia horizontal, hibridación, introgresión entre especies emparentadas, o la existencia de flujo génico entre poblaciones pueden también interferir en la correcta identificación de los linajes (Maddison, 1997; Maddison & Knowles, 2006).

Desde un punto de vista neutralista, la probabilidad de coalescencia aumenta a medida que disminuye el tamaño efectivo de la población, y por ello los genes mitocondriales en principio podrían reflejar mejor el árbol de especies (Moore, 1995; Moore, 1997). Sin embargo algunos autores han cuestionado la utilidad del ADN mitocondrial en filogeografía y reconstrucciones filogenéticas aduciendo la evidente posibilidad de recombinación, la selección positiva y la tasa evolutiva errática, así como la posibilidad de barridos adaptativos que resulten en topologías distintas a las del árbol de especies (Galtier *et al*., 2009).

La magnitud de la discrepancia entre el árbol de especies y el árbol de genes radica en el tamaño efectivo de las poblaciones actuales (N_A, N_B, N_C y N_D) y ancestrales (N_{AB} y N_{ABC}), y en el tiempo de divergencia de las especies o poblaciones (T_{AB} y T_{ABC}) (Fig. 1.4; Nichols, 2001; Brito & Edwards, 2009; Degnan & Salter, 2005; Degnan & Rosenberg, 2006). La probabilidad de que el árbol de genes se corresponda con el árbol de especies es mayor para tiempos de coalescencia largos y tamaños efectivos de las poblaciones pequeños.

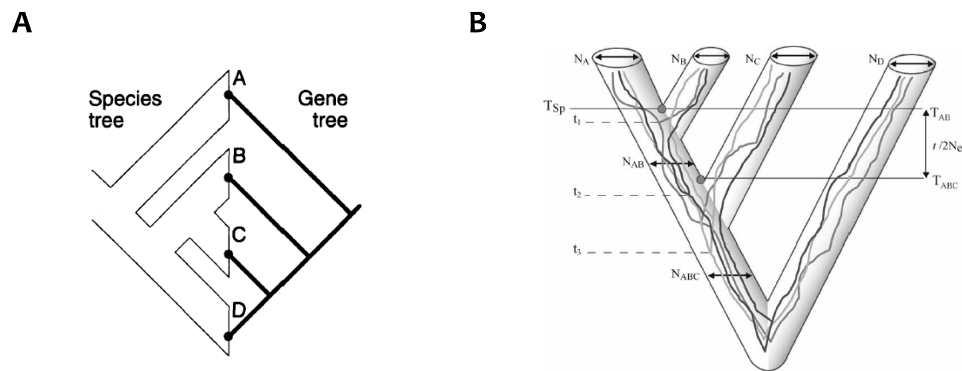

Fig. 1.4.- Diferencias entre árbol de especies (*A-D*) y árbol de genes (líneas interiores). En la Fig. A extraída de Maddison (1997) las especies *B* y *C* son hermanas, pero en el árbol de genes no aparecen emparentadas. En la Fig. B extraída de Brito & Edwards (2009) se observa cómo la ordenación estocástica de los alelos en función de la deriva genética depende del tamaño efectivo de las poblaciones actuales (*NA-ND*) y ancestrales (*NAB-NABC*), y del tamaño de las ramas del árbol de especies (el tiempo entre los internados (*TABC–TAB*). *TSp* y los puntos representan los tiempos de especiación entre las especies *A* y *B*, y *t1*, *t2*, *t3* representan los tiempos de divergencia de los genes muestreados para las especies *A–C*.

Tradicionalmente el análisis de genes múltiples se ha llevado a cabo mediante su combinación o concatenación (Rokas *et al.,* 2003; Nylander *et al.,* 2004), de forma similar a como se analizan en inferencia filogenética. Sin embargo, la constatación de que los árboles de genes y el de especies pueden no coincidir, ha llevado en los últimos años al desarrollo de nuevas metodologías para estimar el árbol de especies en ausencia de un árbol subyacente común. Entre estos métodos podemos citar la búsqueda del árbol consenso (la topología de genes más comúnmente observada es la que se considera como árbol de especies verdadero; Bull *et al.,* 1993; Huelsenbeck *et al.,* 1996; Wiens, 1998; Gadagkar *et al.,* 2005), *gene tree parsimony* (Page & Charleston, 1997; Slowinski *et al.,* 1997; Page, 1998), *deep coalescence approach* (el árbol de especies se corresponde con aquel que minimiza el número de procesos de coalescencia necesarios para que el árbol de especies sea compatible con cada árbol de genes; Maddison, 1997; Maddison & Knowles, 2006; Carsten & Knowles, 2007a) y el modelo coalescente de especies múltiples (*multispecies coalescent model*) que se basa en métodos bayesianos para reconstruir el árbol de especies a partir de varios genes, y utiliza algoritmo de *MCMC* para estimar la distribución posterior del árbol de especies (Rannala & Yang, 2003; Liu & Pearl, 2007; Liu *et al.,* 2008; Heled & Drummond, 2010). A pesar de la

variedad de métodos existentes para inferir el árbol de especies, muchos de ellos están todavía en etapas muy inciales de su desarrollo, por lo que son necesarias investigaciones más detalladas para determinar sus propiedades y eficacia (ver Degnan & Rosenberg, 2009 para revisión de métodos).

El ADN como herramienta taxonómica

El gran desarrollo y popularización de las técnicas moleculares de amplificación del ADN ha favorecido el desarrollo de la llamada taxonomía molecular, la utilización de marcadores moleculares y secuencias de ADN para la descripción de especies o como complemento a la taxonomía clásica, principalmente basada en caracteres morfológicos (Blaxter, 2004; Hebert *et al.*, 2004a, b; Hogg & Hebert, 2004; Smith *et al.,* 2005; Monaghan *et al.,* 2006). Esta nueva aproximación ha facilitado la delimitación y diagnosis de muchas especies en un amplio rango taxonómico (Sites & Crandall, 1997; Sites & Marshall, 2003, 2004; DeSalle *et al.*, 2005) y el descubrimiento de linajes y taxones crípticos (p. ej. Goetze, 2003; Molbo *et al.*, 2003; Feulner *et al.*, 2006; Pfenninger *et al.*, 2007). Mientras que la taxonomía del ADN ha generado un intenso debate (Tautz *et al.*, 2002; 2003), ya que propone el abandono del sistema actual de tipos y la designación de unidades taxonómicas basadas exclusivamente en la información molecular, otras aproximaciones como el código de barras del ADN (*DNA barcoding*; Hebert *et al.*, 2003; Savolainen *et al.,* 2005; Hajibabaei *et al.,* 2007), que simplemente pretende facilitar y automatizar la identificación de especies mediante la secuenciación de un fragmento de ADN mitocondrial, han sido bien recibidas e incluidas en gran variedad de iniciativas a nivel mundial (p. ej. *Barcode of Life* http: //www.barcoding.si.edu).

Es indudable la gran ayuda que supone la integración de la información molecular en la delimitación de especies, aunque siempre es aconsejable su combinación con otras fuentes de información como morfología, etología, ecología y distribución geográfica de los organismos (Balakrishnan, 2005; Knowles & Carstens, 2007).

ESTIMACIÓN DE LOS TIEMPOS DE DIVERGENCIA DE LOS LINAJES

La propiedad de las secuencias nucleotídicas y aminoacídicas de acumular mutaciones de forma más o menos constante en función del tiempo de divergencia de dos organismos, lo que se conoce como Teoría del reloj molecular (Zuckerkandl & Pauling, 1965), ofrece la posibilidad de datar muchos mecanismos y procesos evolutivos, proporcionando un marco temporal del origen de los linajes, los episodios de colonización, dispersión, vicarianza, etc., permitiendo el estudio de las dinámicas de diversificación (especiación y extinción).

Los métodos de dataciones moleculares básicamente transforman la distancia genética existente entre secuencias o taxones, en el tiempo de divergencia entre estos linajes (Welch & Bromham, 2005). Los dos componentes básicos para la estima de edades de divergencia son: (a) las tasas de sustitución, que proporcionan la información temporal relativa, y (b) los puntos de calibración, que permiten la estima de edades absolutas.

a. Tasas de sustitución

En general, las tasas de sustitución de los linajes no siguen un reloj molecular estricto, imposibilitando su aplicación (Langley & Fitch, 1974; Bromham & Penny, 2003). Para contrastar si los linajes siguen una evolución basada en el reloj molecular, hay que contrastar la homogeneidad de las tasas de sustitución. El método más utilizado es el *Likelihood Ratio Test* (*LRT*, Felsenstein, 1981). Este estadístico mide la diferencia en –logL de los árboles con y sin la constricción de reloj estricto. Dicho estadístico sigue una distribución chi-cuadrado con grados de libertad correspondientes al número de taxones menos 2.

La heterogeneidad de las tasas de sustitución puede ser debida a múltiples causas: diferencias en los tamaños poblacionales efectivos (poblaciones pequeñas poseen mayores tasas de fijación de mutaciones, según las bases de la teoría neutralista de la evolución molecular); tiempos de generación cortos aceleran el reloj; tasas metabólicas altas suponen mayores tasas de síntesis de ADN que aceleran la tasa de mutación; y variación de las tasas de mutación entre diferentes

especies debido a diferencias en los niveles de fidelidad de replicación (Rutschmann, 2006).

Cuando los linajes no siguen un reloj molecular estricto, es necesario aplicar diferentes métodos para transformar las ramas y conseguir obtener un árbol ultramérico, es decir, un árbol en que todas sus ramas son proporcionales entre sí y al tiempo transcurrido. Existen dos estrategias principales: los que "corrigen" la heterogeneidad de las tasas y los que la incorporan en el proceso de estimación. Entre los primeros destacan los métodos que linearizan los árboles (*linearized trees*) (Takezaki *et al.,* 1995), a través de la detección y eliminación de las ramas significativamente diferentes, estos métodos generan un árbol "linearizado" en el cual sólo están representados los taxones con tasas de evolución similares.

Entre los métodos que incorporan la heterogeneidad de las tasas citaremos aquellos que asumen un "reloj molecular local", basado en optimizaciones de ML (Yoder & Yang, 2000; Yang & Yoder, 2003), que permite asignar diferentes tasas constantes a las diferentes partes del árbol y los que asumen una autocorrelación de las tasas (p. ej. *non-parametric rate smoothing* (*NPRS*) (Sanderson, 1997; 2003) y *Penalized Likelihood* (*PL*) (Sanderson, 2002)) que asumen que las tasas de sustitución cambian gradualmente a lo largo del árbol e introducen una función de penalización para el cambio abrupto de tasas entre linajes (*NPRS* únicamente utiliza esta información, mientras que PL las combina con estimas de máxima verosimilitud del encaje de los datos).

En los últimos años se han desarrollado métodos bayesianos que permiten incorporar la variación no correlacionada de la tasa de sustitución a lo largo del árbol como parámetro a estimar durante el análisis, y obtener mediante estrategias *MCMC* las distribuciones posteriores de tasas y tiempos de divergencias. Estos métodos asumen que las diferentes tasas de sustitución del árbol siguen una función determinada, que bien puede ser una exponencial o una log-normal. Al inferir conjuntamente las tasas de sustitución y la topología del árbol, estos métodos permiten incluir la propia incertidumbre filogenética en la estima de las

dataciones (Drummond & Ho, 2006). Para una revisión de diferentes metodologías ver por ejemplo Arbogast *et al.,* (2002), Renner (2005), Welch & Bromham (2005) o Rutschmann (2006).

b. Calibración

La estimación de las edades absolutas requiere la incorporación de puntos de calibración, que consiste en fijar o definir un intervalo de la edad de uno o varios nodos del árbol. Los fósiles, los episodios biogeográficos vicariantes y la emergencia de las islas oceánicas constituyen las principales fuentes de información para obtener puntos de calibración. Los puntos de calibración pueden incorporarse como edades fijas basadas en episodios biogeográficos de vicarianza (p. ej. apertura del estrecho de Gibraltar, separación de la placa Córcega-Cerdeña de Iberia, etc.) o como restricciones de edades, ya sean mínimas, correspondientes generalmente a fósiles, o máximas, por ejemplo la edad de formación de islas volcánicas. Dado que la incorporación de puntos de calibración requiere una serie de asunciones no siempre susceptibles de ser demostradas, es recomendable el uso de múltiples puntos de calibración independientes para reducir posibles errores en las dataciones (Renner, 2005).

A la hora de calibrar y de estimar procesos de divergencia pueden surgir ciertos problemas que conducen a una infra o una sobreestimación de las tasas de mutación, y por tanto generan errores en las dataciones calculadas. La posible existencia de saturación debido a múltiples cambios nucleotídicos en la misma posición puede infraestimar las tasas de sustitución, o sobreestimarla si se asignan erróneamente los polimorfismos en las secuencias nucleotídicas (Ho *et al.,* 2005). Las calibraciones inapropiadas, p. ej. calibración en ramas muy internas para datar procesos evolutivos recientes, pueden llevar a una infraestimación de la tasa intraespecífica (Ho *et al.,* 2008), y por tanto a una sobreestimación de las edades de divergencia. La extinción de linajes o la representación incompleta de los taxones relacionados puede derivar en sobreestimaciones de las tasas de mutación cuando calculamos edades de eventos de colonización a partir de una filogenia molecular (Emerson, 2007; Fig. 1.5). La utilización de puntos de calibración basados en el origen de islas volcánicas puede también llevar a sobreestimar las tasas de

mutación si la edad de formación geológica está infraestimada, o si la divergencia real de los linajes es anterior a la colonización de la isla (Emerson, 2007).

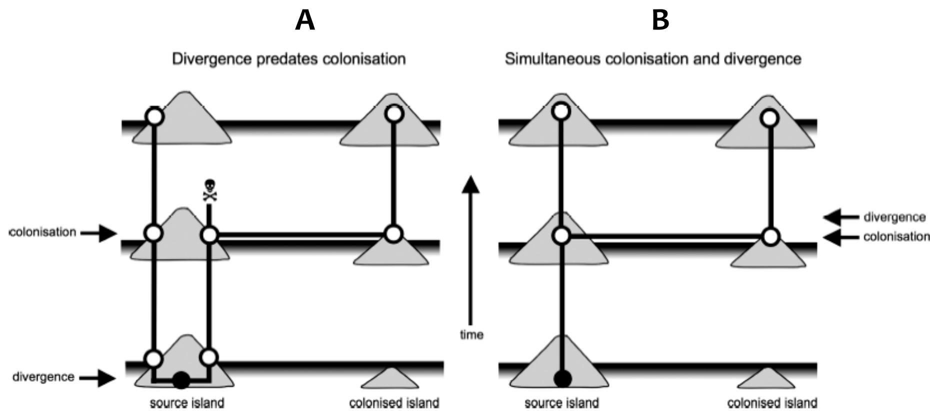

Fig. 1.5.- En el diagrama A el proceso de divergencia entre especies se produjo antes de la colonización de la isla, lo que puede sobreestimar la tasa de mutación al calibrar utilizando la edad de la isla que se colonizó posteriormente. En el diagrama B, la divergencia genética de las especies coincide con la colonización de la isla. Figura extraída de Emerson (2007).

Es frecuente en estudios filogenéticos que no se disponga de puntos de calibración fiables. En estos casos, se puede recurrir a la utilización de tasas de sustitución universales. Por ejemplo la tasa de sustitución estandar estimada para el ADN mitocondrial de artrópodos es de 2.3 de divergencia genética pareada por cada millón de años (Ma) (0.0115 mutaciones/posición nucleotídica por secuencia por Ma; Brower, 1994), lo que, en principio, permitiría obtener la datación absoluta de cualquier filogenia de artrópodos basada en genes mitocondriales por una simple regla de tres. Evidentemente, el uso inapropiado de las tasas de sustitución puede llevar a calibraciones erróneas. La principal limitación del uso de tasas universales es, por un lado, que exista una estima apropiada para los genes de estudio, y por otro, que la tasa no haya cambiado en el grupo de interés, lo cual, por definición, es indemostrable.

Estimación del tiempo de divergencia entre poblaciones

La teoría de la coalescencia proporciona las bases teóricas para la estimación del tiempo de divergencia entre poblaciones o especies recientemente diferenciadas (Arbogast *et al.,* 2002). El proceso de especiación se inicia cuando dos poblaciones divergen hasta quedar aisladas genéticamente (especiación alopátrica; p. ej. Mayr, 1963). En cierto casos, es posible que las nuevas especies sigan manteniendo un cierto flujo génico (especiación simpátrica si ocupan la misma área geográfica: p. ej. Hey, 2006; o especiación parapátrica si mantienen una zona de contacto en sus distribuciones: p. ej. Coyne & Orr, 1998; Becquet & Przeworski, 2009). En ausencia de flujo génico, la divergencia esperada entre las especies o poblaciones descendientes será proporcional al tiempo desde que se separaron de su ancestro común (Hey, 2010). En presencia de flujo génico o si el tiempo de divergencia es reciente, las especies pueden seguir compartiendo alelos porque no están totalmente diferenciadas genéticamente, es decir, siguen compartiendo polimorfismos ancestrales, que se evidencian por procesos de *incomplete lineage sorting* (Arbogast *et al.,* 2002).

Es importante entender la diferencia que existe entre el tiempo (***T***) en que comienza la diferenciación genética de los alelos, y el tiempo (***τ***) en que comienza la divergencia de las especies o poblaciones descendientes respecto a la ancestral, que se produce después de la diferenciación genética (***T***) (Wilson *et al.,* 1985) (ver Fig. 1.6). El tiempo transcurrido entre ***T*** y ***τ*** se corresponde con un periodo de coalescencia para un gen determinado, durante el cual se siguen compartiendo polimorfismos con la especie ancestral (Arbogast *et al.,* 2002), y este periodo se espera que sea de $2N_e$ generaciones, considerando una población panmítica (reproducción al azar) ancestral (Wright, 1951).

Existen varios modelos a partir de los cuales se han desarrollado diferentes metodologías para la estimación de tiempos de divergencia entre poblaciones, basados en la teoría de la coalescencia. Los primeros métodos se basan en el modelo más simple de divergencia entre poblaciones, el modelo de aislamiento *Isolation Model* (Takahata & Nei, 1985; Hey, 1994; Wakeley & Hey, 1997), a partir del cual una especie o población panmítica ancestral con tamaño efectivo

constante se escinde en dos especies o poblaciones descendientes que no intercambian flujo génico entre ellas (ausencia de migración) y que mantienen sus tamaños poblacionales constantes. Este modelo es apropiado para estimar divergencias entre poblaciones alopátricas (Hey *et al.,* 2004). El modelo se aplica sobre tres especies o poblaciones (la ancestral y las dos descendientes), para las cuales se estiman los siguientes parámetros:

$$\boldsymbol{\vartheta A = 4NeA\mu}; \quad \boldsymbol{\vartheta 1 = 4Ne1\mu}; \quad \boldsymbol{\vartheta 2 = 4Ne2\mu} \quad \text{y} \quad \boldsymbol{\tau=2\mu t};$$

ϑ: Tasa de mutación de la población; ***Ne***: tamaño efectivo de la población (*A*: ancestral; *1* y *2*: descendientes); ***t***: tiempo en generaciones desde que las poblaciones divergieron; ***μ***: tasa neutral de mutación del gen.

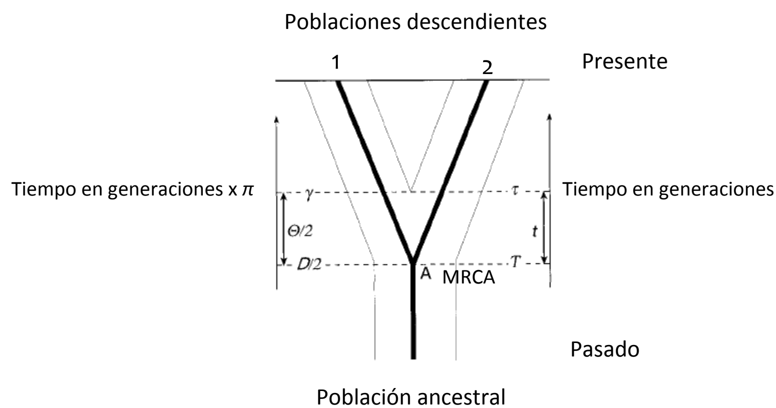

Fig. 1.6.- Diferencia entre ***T*** y ***τ***, y los parámetros implicados según Edwards & Beerli (2000). ***T***: tiempo en generaciones desde que se produjo la divergencia genética de los linajes; ***τ***: divergencia entre las poblaciones descendientes; ***γ***: divergencia poblacional (***τ***) multiplicado por la tasa de mutación (***π***); ***D***: tiempo de divergencia genética (***T***) escalada en base a ***π***; ***t***: es la diferencia entre ***τ*** y ***T***, y tiene un valor esperado determinado por ***2Ne*** generaciones, o ***ϑ/2*** eventos mutacionales, donde: ***ϑ=4Neπ***; ***A***: representa el *MRCA* (*Most Recent Common Ancestor*) donde los alelos de las especies/poblaciones descendientes coalescen.

Si existe tasa de migración pero las especies o poblaciones divergieron hace mucho tiempo, se trata de un modelo simple de *Two-island model* (Wright, 1931), en el que se asume que el patrón de variación dentro y entre poblaciones está en equilibrio en base a los procesos de mutación, deriva genética e intercambio de genes (Hey *et al.,* 2004).

Posteriormente se propuso un modelo denominado *Isolation with migration* (Fig. 1.7), el cual permite intercambio de migrantes (existencia de flujo génico) entre las poblaciones descendientes (Hey & Nielsen, 2004). Bajo este modelo se han desarrollado métodos que permiten estimar las tasas de migración y el tiempo de divergencia entre dos especies o poblaciones a partir de secuencias de ADN de un gen (Nielsen & Wakeley, 2001), o de varios genes (Hey & Nielsen, 2004), basándose en *MCMC* para obtener la distribución posterior de los parámetros. Estos métodos basados en el modelo de *Isolation with migration* incluye los mismos parámetros que el anterior y añade la tasa de migración (***m1***: proporción de la población 1 que es reemplazada por migrantes procedentes de la población 2 en cada generación; ***m2***: proporción de la población 2 que es reemplazada por migrantes procedentes de la población 1 en cada generación). Este modelo es más apropiado para el estudio de especies o poblaciones que han divergido recientemente (Hey *et al.,* 2004).

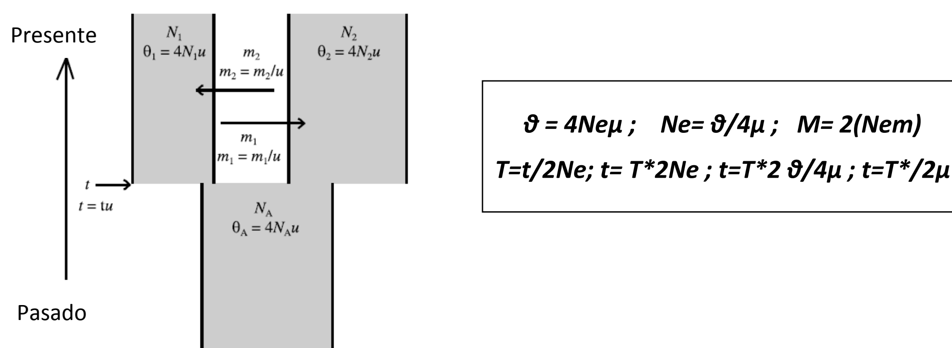

Fig. 1.7.- Los parámetros implicados en el modelo, son los mismos que en modelos anteriores (***ϑ, Ne, µ, t***), a diferencia que éste incorpora la tasa de migración (***m***). Figura extraída de Hey & Nielsen (2004).

ESCENARIO GEOGRÁFICO: EL ARCHIPIÉLAGO CANARIO

Las Islas Canarias están localizadas en el Océano Atlántico (entre 27º y 29º latitud Norte, y 13º y 18º longitud Oeste) y forman parte de la subregión biogeográfica denominada Macaronesia, a la que pertenecen además los archipiélagos de Azores, Madeira y Salvajes, y más discutiblemente Cabo Verde (Fernández-Palacios & Dias, 2001). El archipiélago canario está formado por siete islas mayores, de este a oeste Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma y El Hierro (Fig. 1.8), además de varios islotes y roques. Estas islas tienen un origen volcánico resultado de múltiples episodios geológicos, y su formación y evolución han sido objeto de debate durante las últimas décadas. Se han propuesto diversas teorías sobre el origen del archipiélago, como la evolución tectónica de la cordillera del Atlas y consiguiente creación de una fractura propagante (Anguita & Hernán, 1975; Ancochea *et al.*, 1996), la evolución de un conjunto de bloques ascensionales levantados mediante fallas inversas (Araña & Ortiz, 1986), el modelo de punto caliente o *hotspot* (Carracedo *et al.*, 1998), e incluso un modelo unificador que combina procesos de fracturas locales (fractura propagante), fuerzas tectónicas (bloques levantados) y anomalías térmicas (punto caliente) (Anguita & Hernán, 2000). Entre ellas, la variante del punto caliente (Carracedo & Day, 2002) parece ser la mejor fundamentada y actualmente es la más aceptada.

La formación de las Islas Canarias se iniciaría, según evidencias radiométricas, alrededor del Eoceno-Oligoceno (Robertson & Stillman, 1979), en torno a 70-80 Ma (Le Bas *et al.*, 1986; Balogh *et al.,* 1999), mientras que mediante dataciones K-Ar se estima su origen entre 36 Ma (Abdel-Monem *et al*., 1972) y 39 Ma (Coello *et al.,* 1992). Posteriormente, la emergencia de las islas sigue un patrón secuencial de NE-SW, comenzando con Fuerteventura hace 22 Ma (Ancochea *et al.,* 1996; Carracedo & Day, 2002), Lanzarote 15,5 Ma (Coello *et al.*, 1992), Gran Canaria 14-15 Ma (Schmincke, 1997), Tenerife 11,9 Ma (Guillou *et al*., 2004), La Gomera 11-12 Ma (Cantagrel *et al.,* 1984; Ancochea *et al.,* 1990); La Palma 1,8 Ma (Carracedo *et al.*, 2001; Carracedo & Day, 2002) y El Hierro 1,2 Ma (Guillou *et al.*, 1996; Carracedo *et al.*, 2001; Carracedo & Day, 2002; Fig. 1.8).

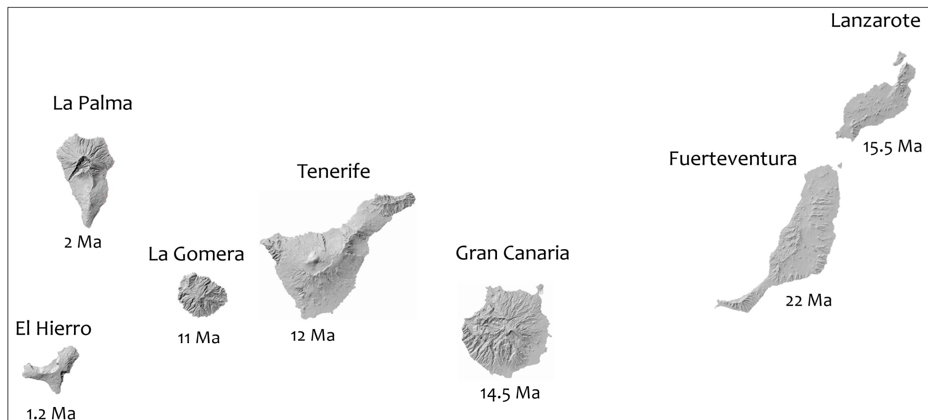

Fig. 1.8.- Mapa de las Islas Canarias en el que se muestran las edades geológicas según varios autores.

La historia geológica de las islas se caracteriza, de forma general, por la alternancia de actividad volcánica, procesos de quiescencia y erosión, deslizamientos gravitacionales y erupciones en épocas más recientes (Carracedo *et al.,* 1998; 1999; Watts & Masson, 2001; Carracedo & Day, 2002; Walter & Schmincke, 2002, entre otros). Todos estos eventos geológicos han modelado durante millones de años el relieve de las islas y, junto con la erosión, han influido en la orografía resultando en una altitud inferior en las islas orientales, y alcanzándose mayores cotas sobre el nivel del mar en las islas centrales y occidentales (Fig. 1.9). A diferencia de otros archipiélagos oceánicos como Hawai, donde las islas más antiguas acaban desapareciendo bajo el mar, las islas orientales no han sufrido una excesiva subsidencia, lo que ha permitido que con una edad superior a 20 Ma permanezcan todavía emergidas e incluso se hayan rejuvenecido parcialmente con erupciones volcánicas post-erosivas (Carracedo, 1999). Lanzarote y Fuerteventura ofrecen, por tanto, la posibilidad de inferir las etapas primigenias de los procesos de formación y diversificación de las especies que han evolucionado en ellas.

El archipiélago canario tiene un clima subtropical, con temperaturas suaves y variación estacional no muy acentuada. El clima está fuertemente influenciado por los vientos alisios del NE que, junto con los vientos secos del NW de cota superior, crean una zona de inversión térmica y forman el mar de nubes entre los

800-1500 m.s.n.m, que tiene influencia sobre los diferentes pisos bioclimáticos característicos de las islas de mayor altitud (Fig. 1.10). De esta forma, en las islas de mayor relieve el clima y la compleja orografía determinan una marcada estratificación vertical de los diversos ecosistemas y comunidades vegetales, cuya distribución altitudinal se establece de (a) 0 a 250 m: matorral costero; (b) 250 a 600/800 m: bosque termófilo; (c) 600 a 1200 m: monteverde (sólo en vertiente N); (d) 800 a 2000/2200 m: pinar; y (e) por encima de 2200 m: matorral de cumbre. En las islas orientales, de menor relieve, sólo están presentes los dos primeros pisos bioclimáticos, lo que se refleja en una escasez de precipitaciones y una mayor aridez de sus ecosistemas. Todos estos factores determinan una menor diversidad fisiográfica, y por ende una menor variedad de hábitats cuando se comparan con el resto del archipiélago.

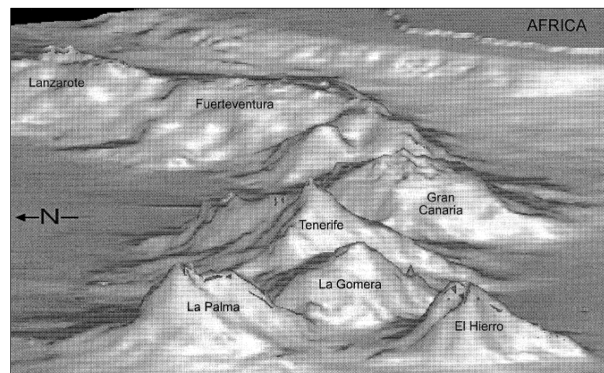

Fig. 1.9.- Imagen batimétrica en 3D de las islas Canarias, en el que se observan las diferencias de altitud entre las islas orientales y centro-occidentales. Imagen extraída de Carracedo *et al.,* (1999).

Las diferencias geológicas, ambientales y ecológicas existentes entre las islas orientales y las centro-occidentales han influido en la diversidad de su fauna y su flora. En diferentes grupos de organismos presentes en Canarias se ha observado un patrón de distribución de la diversidad, por el que las islas de edad y situación geográfica intermedias poseen el mayor número de especies y endemismos, mientras las más jóvenes y las más antiguas muestran unos niveles inferiores de diversidad (Emerson & Oromí, 2005; Cardoso *et al.,* 2010).

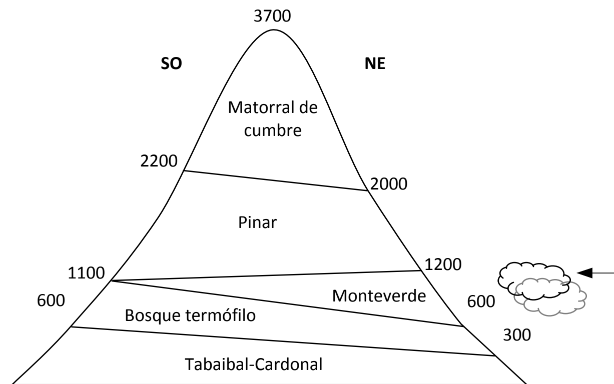

Fig. 1.10.- Esquema en el que se representan los diferentes pisos bioclimáticos y comunidades vegetales características de las islas de mayor relieve.

EL ORGANISMO MODELO: LAS ARAÑAS DEL GÉNERO *Dysdera*

La Región Macaronésica

La subregión biogeográfica de la Macaronesia se ha considerado un punto caliente (*hot spot*) de la biodiversidad mundial dada la riqueza de endemismos faunísticos y florísticos que alberga (Myers *et al.*, 2000). La mayor parte de la diversidad animal en Canarias corresponde a los artrópodos, con cerca de 7000 especies, de las cuales 465 pertenecen al grupo de las arañas (orden Araneae) (Oromí *et al.,* 2004), que además posee un elevado número de endemismos (64%). En un estudio reciente sobre el orden Araneae en la Macaronesia (Cardoso *et al.,* 2010) se determina que los factores más importantes en la predicción de la riqueza de arañas en las islas son el área, la edad geológica, la altitud y la distancia al continente. Además, en Azores la extinción y la pérdida de hábitats naturales han influido en la menor diversidad existente respecto al resto de archipiélagos macaronésicos (Cardoso *et al.,* 2010).

En Canarias los fenómenos de evolución insular han afectado a diversos géneros de arañas (*Oecobius, Scotognapha, Walckenaeria, Alopecosa, Dysdera*, *Pholcus, Spermophorides*), con la consiguiente formación de abundantes especies endémicas. Estos casos de radiación han constituido un material idóneo para

estudios filogenéticos llevados a cabo en los últimos años con *Dysdera*, *Pholcus* y *Spermophorides* (Arnedo *et al.,* 1996; 2000; 2001; Arnedo & Ribera, 1997; 1999; López-Mercader, 2005; Dimitrov & Ribera, 2006; 2007; Dimitrov *et al.,* 2008) que han permitido establecer los procesos de colonización, especiación y diversificación de estos organismos en las islas.

Morfología y biología general

Las especies pertenecientes al género *Dysdera* Latreille, 1804 tienen seis ojos y órganos copuladores sencillos, caracteres propios de las arañas araneomorfas haploginas a las que pertenece la familia Dysderidae. Son fácilmente distinguibles por la coloración rojiza del prosoma, las patas anaranjadas y el opistosoma claro. Estas arañas se encuentran preferentemente en zonas húmedas y umbrías, y no construyen tela pues son cazadoras nocturnas que acechan a sus presas resguardándose bajo piedras y troncos, entre la hojarasca o en los taludes de pistas y senderos. Normalmente se pueden encontrar dentro de capullos de seda, en los que se cobijan durante el día y donde también realizan la muda, se alimentan de sus presas, y las hembras protegen la puesta (Cooke, 1965a, b).

Muchos representantes de la familia Dysderidae han ocupado ambientes hipogeos, existiendo especies trogloxenas (que accidentalmente habitan cuevas) y especies troglobias (habitantes obligados del medio subterráneo). Algunas especies han desarrollado una serie de adaptaciones morfológicas y fisiológicas a este medio, caracterizado por la ausencia de luz, humedad elevada, temperaturas constantes, escasez de recursos tróficos y altos niveles de CO_2 (Ribera & Juberthie, 1994; Fig. 1.11). Entre los caracteres adaptativos al medio hipogeo destacan la reducción parcial o total de los ojos, el alargamiento de los apéndices, la despigmentación del tegumento, la ralentización de la actividad metabólica o la disminución del número de huevos en la puesta (Christiansen, 1992). Hay indicios que sugieren que los representantes de la familia Dysderidae están preadaptados a la vida subterránea (Arnedo *et al.,* 2007); además, hay un gran número de taxones troglobios en la familia, y algunos géneros solo están representados por especies cavernícolas (*Stalita, Minotauria, Stalagtia,* por citar los más relevantes).

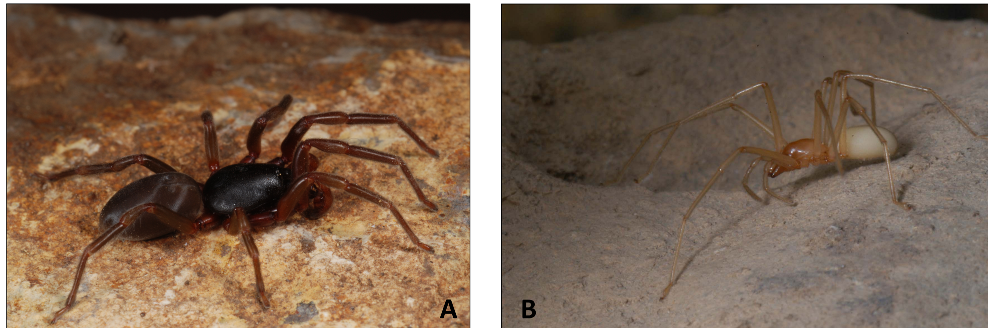

Fig. 1.11.- Imagen en la que se observan distintos aspectos entre (A) una especie epigea, *Dysdera verneaui* y (B) una troglobia, *D. unguimmanis,* la de mayor grado de troglobiomorfismo entre las especies canarias de *Dysdera* (Fotos P. Oromí).

Algunos autores (Bristowe, 1958; Pollard, 1986) han sugerido que ciertas especies de *Dysdera* son especialistas o se alimentan regularmente de isópodos. Este comportamiento ha sido confirmado tras la detección de antígenos de isópodos en el tracto digestivo de algunas especies (Sunderland & Sutton, 1980) y también por los restos de presas encontrados en los capullos de seda (Cooke, 1965a). Por otro lado, un estudio realizado con *Dysdera crocata* revela que esta especie captura isópodos, además de otros tipos de presas (Pollard, 1986). Las especies de este género poseen una gran variación morfológica asociada a las piezas bucales, sobretodo en los quelíceros (tamaño, forma y tipo de la uña; Fig. 1.12), que utilizan tanto para capturar a sus presas como para construir el capullo de seda, para el apareamiento y para la defensa (Bristowe, 1958); además estos caracteres han sido ampliamente utilizados en taxonomía (Deeleman-Reinhold & Deeleman, 1988; Arnedo *et al.,* 2001). Recientemente Řezáč y colaboradores (2008) han relacionado determinadas morfologías de quelíceros con la estrategia y las pautas de captura de las presas, y con los niveles de especialización en alimentarse de isópodos. Así, han podido constatar que las especies con quelíceros no modificados capturan gran variedad de artrópodos, mientras que las especies con quelíceros modificados se alimentan preferentemente de isópodos y rechazan el resto de presas potenciales (Řezáč *et al.,* 2008), demostrándose así una relación directa entre la morfología, el grado de especialización y la captura de un determinado tipo de presa.

Fig. 1.12.- A. Diferentes morfologías de los quelíceros y uña de *D. verneaui, D. brevisetae* y *D. hernandezi* (Foto M. A. Arnedo). B. Imagen de *Dysdera silvatica* alimentándose un isópodo (Foto P. Oromí).

Diversidad y distribución

El género *Dysdera* es el más rico en especies de la familia Dysderidae, y es uno de los grupos de arañas más diversificado dentro de la región Paleártica, representado por más de 250 especies (Platnick, 2009b). Este género se distribuye a lo largo de toda la región circum-mediterránea (Deeleman-Reinhold & Deeleman, 1988), alcanzando dentro de este rango la subregión Macaronésica como extremo más occidental y meridional. La especie *Dysdera crocata* Koch, 1838 (Simon, 1883), asociada a ambientes antropizados, es la excepción ya que tiene una distribución cosmopolita, cuya dispersión e introducción se ha visto facilitada por la actividad del hombre. Los archipiélagos de Azores, Salvajes y Cabo Verde poseen cada uno una especie endémica de *Dysdera* (Berland, 1936; Kulczynski, 1899; Arnedo *et al.*, 2000; Arnedo, *unpubl. data*), y en Madeira se conocen cinco endemismos (Blackwall, 1862; Denis, 1962; Wunderlich, 1994). Canarias constituye un caso extremo de diversificación en el grupo ya que se han descrito cerca de 50 especies

endémicas, distribuidas a lo largo de las siete islas e islotes (Arnedo *et al.*, 1996; Arnedo & Ribera, 1997; 1999; Arnedo *et al.*, 2000; 2007; Fig. 1.13).

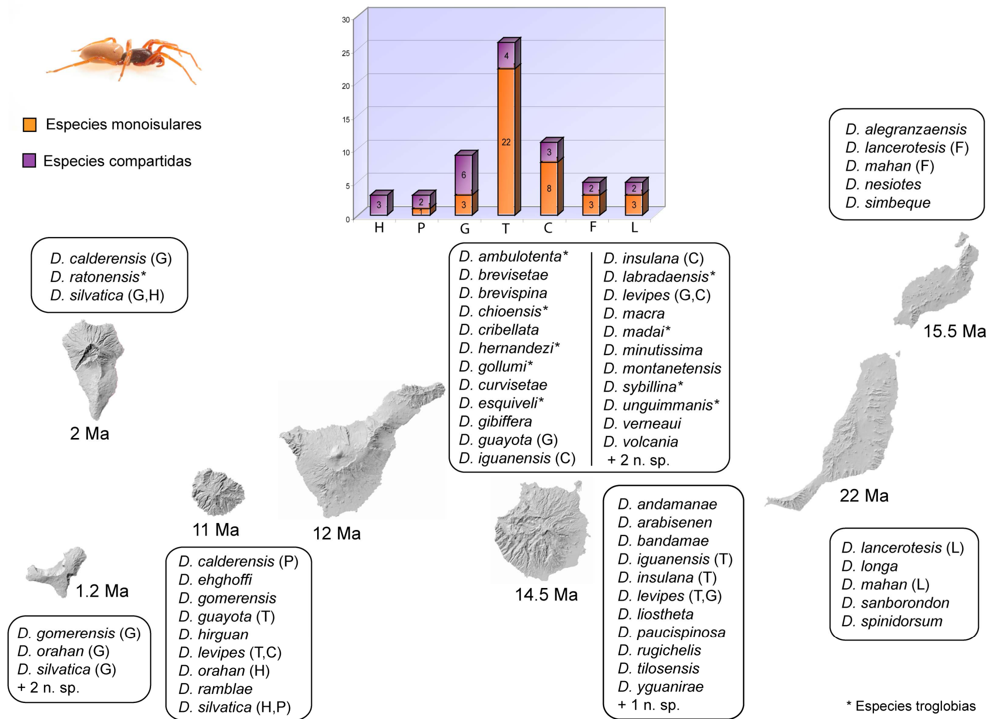

Fig. 1.13.- La diversidad de especies de *Dysdera* en Canarias. Diagrama de la parte superior representando el número de especies (monoinsulares o compartidas) de cada isla. Mapa de la parte inferior con indicación de las especies presentes en cada isla; entre paréntesis las otras islas donde también se encuentran las especies compartidas (H: El Hierro; P: La Palma; G: La Gomera; T: Tenerife; C: Gran Canaria; F: Fuerteventura; L: Lanzarote; (*) Especies troglobias).

Las especies de este género se encuentran en todo tipo de hábitats y comunidades vegetales, desde el nivel del mar hasta cotas altas, incluyendo también el medio hipogeo [cuevas y medio subterráneo superficial (MSS)]. En Canarias se concentra el mayor número de especies troglobias de todo el género: de las 16 especies conocidas con esta adaptación, 10 se encuentran en este archipiélago (9 endémicas de Tenerife y 1 de La Palma) y el resto se distribuyen por

la Península Ibérica y Marruecos. *Dysdera* es además el género de arañas que incluye un mayor número de especies troglobias en Canarias (Oromí *et al*., 2001), y muchas de sus especies epigeas han sido colectadas alguna vez en el medio subterráneo. Además, gran parte de los nuevos taxones pendientes por describir proceden del MSS, reafirmando la idea de que el medio subterráneo ha jugado un papel relevante en la diversificación de este género en Canarias. Estudios moleculares y morfológicos recientes (Arnedo *et al.*, 2007) sugieren que, en la mayoría de los casos, la colonización del medio subterráneo se ha producido de manera independiente en ocho ocasiones, ya que las especies hermanas de la mayoría de especies troglobias son epigeas. La única excepción la forman *D. hernandezi* y *D. esquiveli*, que podrían constituir un caso de especiación simpátrica dentro del medio hipogeo a partir de un ancestro ya troglobio, que generó dos especies cuya única diferencia morfológica patente está en la uña de los quelíceros, carácter probablemente relacionado con el tipo de alimentación.

Patrones de colonización y diversificación de* Dysdera *en Canarias

Estudios filogenéticos sobre *Dysdera* basados en caracteres morfológicos y secuencias de ADN mitocondrial (Arnedo *et al.,* 2001) revelaron que el conjunto de especies canarias es mayoritariamente el resultado de procesos de diversificación local. Se proponen dos posibles escenarios alternativos de colonización del género en Canarias. El primero sugiere un total de cuatro colonizaciones independientes: dos desde el continente a las islas orientales, la tercera desde el continente a las islas centrales y occidentales, donde generó la mayor parte de la diversidad actual, y una cuarta colonización de Tenerife por el ancestro de la especie cavernícola *D. unguimmanis*. El segundo escenario propone dos colonizaciones, una del ancestro de la especie oriental *D. lancerotensis*, y otra que originó todas las demás especies de Canarias. Esta última hipótesis requiere un evento de recolonización del continente. Canarias ha jugado también un papel clave en la diversificación de *Dysdera* en la Macaronesia, ya que ha servido de puente para la colonización de Salvajes, de Cabo Verde y quizás de Madeira, mientras que las Azores fueron probablemente colonizadas de forma independiente desde Europa (Arnedo *et al.,* 2001).

Dentro del archipiélago canario existen algunos casos de especiación alopátrica por colonización entre diferentes islas, aunque la mayoría de las especies de *Dysdera* han sido generadas por procesos de diversificación local, lo que sugiere que la radiación de este género en Canarias es debida principalmente a múltiples procesos de especiación dentro de islas promovidos por barreras geográficas o ecológicas (Arnedo *et al.,* 2001). El gran número de especies endémicas presentes en el archipiélago, junto con la gran variabilidad morfológica (Fig. 1.14) y los tipos de hábitat que han colonizado, hacen del género *Dysdera* un excelente modelo biológico para testar diferentes hipótesis evolutivas y para estudiar los procesos que rigen la diversificación, adaptación y especiación en archipiélagos oceánicos.

Fig. 1.14.- Imagen en la que observa la diferencia de tamaño de las especies *Dysdera longa* y *D. levipes*. En Canarias, las especies de distribución simpátrica se caracterizan por tener grandes diferencias morfológicas asociadas al tamaño corporal y a los quelíceros (Foto N. Macías).

REFERENCIAS

Abdel-Monem A, Watkins ND, Gast PW. 1972. Potassium-Argon ages, volcanic stratigraphy and geomagnetic polarity history of the Canary Islands: Tenerife, La Palma and El Hierro. *American Journal of Science* 272: 805-825.

Ancochea E, Brändle JL, Cubas CR, Hernán F, Huertas MJ. 1996. Volcanic complexes in the eastern ridge of the Canary Islands: the Miocene activity of the island of Fuerteventura. *Journal of Volcanology and Geothermal Research* 70: 183-204.

Ancochea E, Fuster JM, Ibarrola E, Cendrero A, Coello J, Hernán F, Cantagrel JM, Jamond C. 1990. Volcanic evolution of the island of Tenerife (Canary Islands) in the light of the new K-Ar data. *Journal of Volcanology and Geothermal Research* 44: 231-249.

Anguita F, Hernán F. 1975. A propagating fracture model versus a hot spot origin for the Canary Islands. *Earth and Planetary Science Letters* 27: 11-19.

Anguita F, Hernán F. 2000. The Canary Islands origin: a unifying model. *Journal of Volcanology and Geothermal Research* 103: 1–26.

Araña V, Ortiz R. 1986. Marco geodinámico del volcanismo canario. *An. Física* 82: 202–231.

Arbogast BS, Edwards SV, Wakeley J, Beerli P, Slowinski JB. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics* 33: 707-740.

Arbogast BS, Kenagy GJ. 2001. Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography* 28: 819-825.

Arnedo, Pedro Oromí, Cesc Múrria, Nuria Macías-Hernández, Ribera C. 2007. The dark side of an island radiation: systematics and evolution of troglobitic spiders of the genus *Dysdera* Latreille (Araneae: Dysderidae) in the Canary Islands. *Invertebrate Systematics* 21: 623-660.

Arnedo MA, Oromí P, Ribera C. 1996. Radiation of the genus *Dysdera* (Araneae, Haplogynae, Dysderidae) in the Canary Islands: the Western Islands. *Zoologica Scripta* 25: 241-274.

Arnedo MA, Oromí P, Ribera C. 2000. Systematics of the genus *Dysdera* (Araneae, Dysderidae) in the Eastern Canaries. *Journal of Arachnology* 28: 261-292.

Arnedo MA, Oromí P, Ribera C. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. *Cladistics* 17: 313-353.

Arnedo MA, Ribera C. 1996. *Dysdera ratonensis* Wunderlich, 1991 (Arachnida, Araneae) a troglomorphic species from La Palma, Canary Islands: Description of the male and redescription of the female. *Revue Arachnologique* 11: 109-122.

Arnedo MA, Ribera C. 1997. Radiation of the genus *Dysdera* (Araneae, Haplogynae, Dysderidae) in the Canary Islands: The island of Gran Canaria. *Zoologica Scripta*.

Arnedo MA, Ribera C. 1999. Radiation of the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. *Journal of Arachnology* 27: 604-662.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Harvard University Press: Cambridge, Mass.

Avise JC. 2004. *Molecular Markers, Natural History and Evolution*. 2nd ed. Sinauer, Sunderland, MA.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific Phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* 18: 489-522.

Balakrishnan R. 2005. Species concepts, species boundaries and species identification: A view from the tropics. *Systematic Biology* 54: 689-692.

Baldwin BG, Sanderson MJ. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences* 95: 9402-9406.

Balogh K, Ahijado A, Casillas R, Fernández C. 1999. Contribution to the chronology of the Basal Complex of Fuerteventura, Canary Islands. *Journal of Volcanology and Geothermal Research* 90: 81–101.

Ballard JWO, Whitlock MC. 2004. The incomplete natural history of mitochondria. *Molecular Ecology* 13: 729-744.

Barriel V. 1994. [Molecular phylogenies and nucleotide insertion-deletion] Phylogenies moleculaires et insertions-deletions de nucleotides. *Comptes Rendus de l Academie des Sciences. Serie III, Sciences de la Vie (Paris)* 317: 693-701.

Becquet Cl, Przeworski M. 2009. Learning about Modes of Speciation by Computational Approaches. *Evolution* 63: 2547-2562.

Beheregaray LB, Ciofi C, Geist D, Gibbs JP, Caccone A, Powell JR. 2003. Genes record a prehistoric volcano eruption in the Galapagos. *Science* 302: 75.

Berland L. 1936. Mission de M. A. Chevalier aux îles du Cap Vert (1934). 1. Araignées. *Rev. fr. ent.* 3: 67-88.

Bermingham E, Moritz C. 1998. Comparative phylogeography: concepts and applications. *Molecular Ecology* 7: 367-369.

Bird, Holland BS, Bowen BW, Toonen RJ. 2007. Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. *Molecular Ecology* 16: 3173–3186.

Birky CW. 2001. The inheritance of genes in mitochondria and chloroplasts: Laws, Mechanisms, and Models. *Annual Review of Genetics* 35: 125-148.

Blackwall J. 1862. Descriptions of newly-discovered spiders from the island of Madeira. *Annals and Magazine of Natural History* 9: 370-382.

Blaxter ML. 2004. The promise of a DNA taxonomy. *Philosophical Transactions-Royal Society of London Series B Biological Sciences*: 669-680.

Borges PAV, Brown VK. 1999. Effect of island geological age on the arthropod species richness of Azorean pastures. *Biological Journal of the Linnean Society* 66: 373-410.

Bremer K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795-803.

Bristowe WS. 1958. *The world of spiders*. Collins: London.

Brito PH, Edwards SV. 2009. Multilocus phylogeography and phylogenetics using sequence-based markers. *Genetica* 135: 439-455.

Bromham L, Penny D. 2003. The modern molecular clock. *Nature Reviews Genetics* 4: 216–224.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato*

inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences* 91: 6491-6495.

Brown WL, Wilson EO. 1956. Character displacement. *Systematic Zoology* 5: 49-64.

Brown WM. 1985. The mitochondrial genome of Animals. In: MacIntyre RJ, ed. *Molecular evolutionary genetics*. New York: Plenum Press. 95-130.

Brown WM, George M, Wilson AC. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences* 76: 1967-1971.

Bull JJ, Huelsenbeck JP, Cunningham CW, Swofford DL, Waddell PJ. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384-397.

Cantagrel JM, Cendrero A, Fuster JM, Ibarrola E, Jamond C. 1984. K-Ar chronology of the vulcanic eruptions in the Canarian archipelago: island of La Gomera. *Bulletin of Volcanology* 47: 597-609.

Cardoso P, Arnedo MA, Triantis K, Borges PAV. 2010. Drivers of diversity in Macaronesian spiders and the role of species extinctions. *Journal of Biogeography* 37: 1034-1046.

Carracedo J, Rodríguez-Badiola E, Guillou H, De La Nuez J, Pérez-Torrado F. 2001. Geology and volcanology of La Palma and El Hierro, Western Canaries. *Est. Geol.* 57: 175–273.

Carracedo JC. 1999. Growth, structure, instability and collapse of Canarian volcanoes and comparisons with Hawaiian volcanoes. *Journal of Volcanology and Geothermal Research* 94: 1-19.

Carracedo JC, Day S. 2002. *Canary Islands*. Terra: Harpenden.

Carracedo JC, Day S, Guillou H, Badiola ER, Canas JA, Torrado FJP. 1998. Hotspot volcanism close to a passive continental margin: The Canary Islands. *Geological Magazine* 135: 591-604.

Carson HL, Lockwood JP, Craddock EM. 1990. Extinction and recolonization of local-populations on a growing shield-volcano. *Proceedings of the National Academy of Sciences* 87: 7055-7057.

Carstens, Richards CL. 2007. Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution* 61: 1439–1454.

Carstens BC, Knowles LL. 2007. Estimating Species Phylogeny from Gene-Tree probabilities despite incomplete lineage sorting: An example from *Melanoplus* grasshoppers. *Systematic Biology* 56: 400-411.

Cavalli-Sforza LL, Edwards AWF. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 32: 550-570.

Coello J, Cantagrel JM, Hernán F, Fuster JM, Ibarrola E, Ancochea E, Casquet C, Jamond C, Díaz de Terán JR, Cendrero A. 1992. Evolution of the eastern volcanic ridge of the Canary Islands based on new K-Ar data. *Journal of Volcanology and Geothermal Research* 53: 251-274.

Cooke JAL. 1965a. A contribution to the biology of the British spiders belonging to the genus *Dysdera*. *Oikos* 16: 20-25.

Cooke JAL. 1965b. Spider Genus *Dysdera* (Araneae, Dysderidae). *Nature* 205: 1027-1028.

Coyne JA, Orr HA. 1998. The evolutionary genetics of speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 353: 287-305.

Cronk QC. 1992. Relict floras on Atlantic islands: patterns assessed. *Biological*

Journal of the Linnean Society 46: 91-103.

Chesson P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* 31: 343.

Christiansen K. 1992. *Biological processes in space and time: cave life in the light of modern evolutionary theory. In 'The Natural History of Biospeleology'*. (Museo Nacional de Ciencias Naturales (CSIC): Madrid, Spain.).

Darwin CR. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray: London.

Deeleman-Reinhold CL, Deeleman PR. 1988. Revision des Dysderinae. *Tijdschrift voor Entomologie* 131: 141-269.

Degnan JH, Rosenberg NA. 2006. Discordance of Species Trees with Their Most Likely Gene Trees. *PLoS Genet* 2: e68.

Degnan JH, Rosenberg NA. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* 24: 332-340.

Degnan JH, Salter LA. 2005. Gene tree distributions under the coalescent process. *Evolution* 59: 24–37.

Denis J. 1962. Les araignées de l'Archipel de Madère. *Publçôes Inst. Zool. Dr. Augusto Nobre* 79: 9-118.

DeSalle R, Egan MG, Siddall M. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions- Royal Society of London Series B Biological Sciences*: 1905-1916.

Dimitrov D, Arnedo MA, Ribera C. 2008. Colonization and diversification of the spider genus *Pholcus* Walckenaer, 1805 (Araneae, Pholcidae) in the Macaronesian archipelagos: evidence for long-term occupancy yet rapid recent speciation. *Molecular Phylogenetics and Evolution* 48: 596–614.

Dimitrov D, Ribera C. 2006. Three new species of *Pholcus* (Araneae, Pholcidae) from the Canary Islands with notes on the genus *Pholcus* in the archipelago. *Journal of Arachnology* 34: 126–134.

Dimitrov D, Ribera C. 2007. The genus *Pholcus* (Araneae, Pholcidae) in the Canary Islands. *Zoological Journal of the Linnean Society* 151: 59–114.

Donnelly P, Tavaré S. 1986. The ages of alleles and a coalescent. *Advances in Applied Probability* 18: 1-19.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4.

Edwards AWF. 1972. *Likelihood*. Oxford University Press. Oxford, U.K.

Edwards AWF, Cavalli-Sforza LL. 1963. The reconstruction of evolution. *Annals of Human Genetics* 27: 105.

Edwards SV, Beerli P. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* 54: 1839-1854.

Efron B. 1979. Bootstrap methods: Another look at the jacknife. *Ann. Atat.* 7: 1-26.

Emerson BC. 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology* 11: 951–966.

Emerson BC. 2007. Alarm Bells for the Molecular Clock? No support for Ho et al.'s model of time-dependent molecular rate estimates. *Systematic Biology* 56: 337-345.

Emerson BC, Gillespie RG. 2008. Phylogenetic analysis of community assembly and structure over space and

time. *Trends in Ecology & Evolution* 23: 619-630.

Emerson BC, Kolm N. 2005. Species diversity can drive speciation. *Nature*: 1015-1016.

Emerson BC, Oromi P. 2005. Diversification of the forest beetle genus *Tarphius* on the Canary Islands, and the evolutionary origins of island endemics. *Evolution* 59: 586-598.

Farris JS. 1970. Methods for computing Wagner trees. *Systematic Zoology* 19: 83-92.

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17: 368-376.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783-791.

Fernández-Palacios JM, Dias E. 2001. Marco biogeográfico macaronésico. In: eds Fernández-Palacios JMM-E, JL., ed. *En Naturaleza de las Islas Canarias. Ecología y Conservación* Turquesa. Santa Cruz de Tenerife, España.

Feulner PGD, Kirschbaum F, Schugardt C, Ketmaier V, Tiedemann R. 2006. Electrophysiological and molecular genetic evidence for sympatrically occuring cryptic species in African weakly electric Wshes (Teleostei: Mormyridae: *Campylomormyrus*). 39: 198-208.

Fitch WM. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 404-416.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Fu Y-X, Li W-H. 1999. Coalescing into the 21st century: An overview and prospects of coalescent theory. *Theoretical Population Biology* 56: 1-10.

Fukami Tea. 2007. Immigration history controls diversification in experimental adaptive radiation. *Nature* 446: 436–439.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* 34: 397-423.

Gadagkar SR, Rosenberg M, Kumar S. 2005. Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. *Journal of Experimental Zoology* 304B: 64–74.

Galtier N, Nabholz B, Glémin S, Hurst GDD. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* 18: 4541–4550.

Garrick RC, Rowell DM, Simmons CS, Hillis DM, Sunnucks P. 2008. Fine-scale phylogeographic congruence despite demographic incongruence in two low-mobility saproxylic springtails. *Evolution* 62: 1103-1118.

Gillespie G. 2004. Community assembly through adaptive radiation in Hawaiian Spiders. *Science* 303: 356-359.

Gillespie RG. 2002. Colonization of remote oceanic islands of the Pacific: archipelagos as stepping stones? *Journal of Biogeography* 29: 655-662.

Gillespie RG, Claridge EM, Goodacre SL. 2008. Biogeography of the fauna of French Polynesia: diversification within and between a series of hot spot archipelagos. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363: 3335–3346.

Gillespie RG, Roderick GK. 2002. Arthropods on Islands: Colonization, Speciation and Conservation. *Annual Review of Ecology and Systematics* 47: 595-632.

Goetze E. 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. *Proceedings of the Royal Society B: Biological Sciences* 270: 2321-2331.

Grant PR. 1972. Convergent and divergent character displacement. *Biological Journal of the Linnean Society* 4: 39-68.

Guillou H, Carracedo JC, Paris R, Torrado FJP. 2004. Implications for the early shield-stage evolution of Tenerife from K/Ar ages and magnetic stratigraphy. *Earth and Planetary Science Letters* 222: 599-614.

Guillou H, Carracedo JC, Pérez Torrado F, Rodríguez Badiola E. 1996. K-Ar ages and magnetic stratigraphy of a hotspotinduced, fast grown oceanic island: El Hierro, Canary Islands. *Journal of Volcanology and Geothermal Research* 73: 141–155.

Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* 23: 167-172.

Hare MP. 2001. Prospects for nuclear gene phylogeography. *Trends in Ecology & Evolution* 16: 700-706.

Hastings WK. 1970. Monte Carlo sampling methods using Markov chains and their applications. *Biometrika* 57: 97-109.

Hebert PD, Ratnasingham S, deWaard JR. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* 270 Suppl 1: S96-99.

Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences* 101: 14812-14817.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of Birds through DNA Barcodes. *Plos Biology* 2: 1657-1663.

Heled J, Drummond AJ. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* 27: 570-580.

Hennig W. 1950. *Grundzüge einer Theorie der phylogenetischen Systematik*. Deutscher Zentralverlag: Berlin.

Hennig W. 1966. *Phylogenetic Systematics*. University of Illinois Press: Urbana, Chicago, London.

Hey J. 1994. Bridging phylogenetics and population genetics with gene tree models. In: edited by B. Schierwater BS, G. Wagner and R. Desalle. Birkhäuser-Verlag, ed. *Molecular Approaches to Ecology and Evolution*. Basel, Switzerland. 435–449.

Hey J. 1997. Mitochondrial and nuclear genes present conflicting portraits of human origins. *Molecular Biology and Evolution* 14: 166-172.

Hey J. 2006. On the failure of modern species concepts. *Trends in Ecology & Evolution* 21: 447–450.

Hey J. 2010. Isolation with migration models for more than two populations. *Molecular Biology and Evolution* 27: 905-920.

Hey J, Nielsen R. 2004. Multilocus Methods for Estimating Population Sizes, Migration Rates and Divergence Time, With Applications to the Divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* 167: 747-760.

Hey J, Won YJ, Sivasundar A, Nielsen R, Markert JA. 2004. Using nuclear

haplotypes with microsatellites to study gene flow between recently separated Cichlid species. *Molecular Ecology* 13: 909-919.

Ho SYW, Phillips MJ, Cooper A, Drummond AJ. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular Biology and Evolution* 22: 1561-1568.

Ho SYW, Saarma U, Barnett R, Haile J, Shapiro B. 2008. The effect of inappropriate calibration: Three case studies in molecular ecology. *PLoS ONE* 3.

Hodges KM, Rowell DM, Keogh JS. 2007. Remarkably different phylogeographic structure in two closely related lizard species in a zone of sympatry in south-eastern Australia. *Journal of Zoology* 272: 64-72.

Hogg ID, Hebert PDN. 2004. Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 82: 749-754.

Hubbell SP. 2006. Neutral theory and the evolution of ecological equivalence. *Ecology* 87: 1387-1398.

Hudson RR. 1990. Gene genealogies and the coalescent process. In: Futuyma D and Antonovics J, eds. *Oxford Surveys in Evolutionary Biology*. Oxford: Oxford University Press. 1-44.

Hudson RR. 1992. Gene Trees, Species Trees and the Segregation of Ancestral Alleles. *Genetics* 131: 509–512.

Huelsenbeck JP, Bull JJ, Cunningham CW. 1996. Combining data in phylogenetic analysis. *Trends in Ecology & Evolution* 11: 152-158.

Huelsenbeck JP, Crandall KA. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annual *Review of* Ecology*, Evolution and Systematics* 28: 437-466.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310-2314.

Irwin DE. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution: International Journal of Organic Evolution* 56: 2383-2394.

Juan C, Emerson BC, Oromí P, Hewitt GM. 2000. Colonization and diversification: Towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution* 15: 104-109.

Kalmar A, Currie DJ. 2006. A global model of island biogeography. *Global Ecology and Biogeography* 15: 72–81.

Kingman JFC. 1982. The coalescent. *Stochastic Processes and their Applications*13: 235–248.

Kjer KM. 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: An example of alignment and data presentation from the frogs. *Molecular Biology and Evolution* 4: 314-330.

Kluge AG, Farris JS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18: 1-32.

Knowles LL. 2004. The burgeoning field of statistical phylogeography. *Journal of Evolutionary Biology*17: 1–10.

Knowles LL, Carstens BC. 2007. Estimating a geographically explicit model of population divergence. *Evolution* 61: 477-493.

Knowles LL, Maddison WP. 2002. Statistical phylogeography. *Molecular Ecology* 11: 2623-2635.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, F.X. V, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences* 86: 6196-6200.

Kozak KH, Larson AA, Bonett RM, Harmon LJ. 2005. Phylogenetic analysis of ecomorphological divergence, community structure, and diversification rates in dusky salamanders (Plethodontidae: *Desmognathus*). *Evolution* 59: 2000-2016.

Kreft H, Jetz W, Mutke J, Kier G, Barthlott W. 2008. Global diversity of island floras from a macroecological perspective. *Ecology Letters* 11: 116-127.

Kuhner MK. 2008. Coalescent genealogy samplers: windows into population history. *Trends in Ecology & Evolution* 24: 86-93.

Kulczynski W. 1899. Arachnoidea opera Rev. E. Schmitz collecta in insulis Maderianis et un insulis Selvages dictis. *Rozpr. spraw. wydz. mat. przyrod. Akad. umiej.* 36: 319-461.

Langley CH, Fitch WM. 1974. An examination of the constancy of the rate of molecular evolution. *Journal of Molecular Evolution* 3: 1432-1432

Le Bas M, Rex D, Stillman C. 1986. The Early magmatic chronology of Fuerteventura, Canary Islands. *Geological Magazine* 123: 287–298.

Lee MS. 2001. Unalignable sequences and molecular evolution. *Trends in Ecology and Evolution* 16: 681-685.

Leibold MA, McPeek MA. 2006. Coexistence of the niche and neutral perspectives in community ecology. *Ecology* 87: 1399-1410.

Lewis PO, Swofford DL. 2001. Back to the future: Bayesian inference arrives in phylogenetics. *Trends in Ecology and Evolution* 16: 600-601.

Lio P, Goldman N. 1998. Models of molecular evolution and phylogeny. *Genome Research* 8: 1233-1244.

Liu L, Pearl DK. 2007. Species trees from gene trees: Reconstructing Bayesian posterior distributions of a species phylogeny using estimated gene tree distributions. *Systematic Biology* 56: 504-514.

Liu L, Pearl DK, Brumfield RT, Edwards SV. 2008. Estimating species trees using multiple-allele DNA sequence data. *Evolution* 62: 2080-2091.

Lomolino MV. 2000. A call for a new paradigm of island biogeography. *Global Ecology and Biogeography* 9: 1–6.

López-Mercader N. 2005. *Evolutionary processes of the genus Spermophorides (Araneae, Pholcidae) in the Canary Islands*. Departamento de Biología Animal, Universitat de Barcelona, Barcelona.

Losos JB. 2000. Ecological character displacement and the study of adaptation. *Proceedings National Academy of Sciences USA* 97: 5693-5695.

Losos JB, Jackman TR, Larson A, De Queiroz K, Rodriguez Schettino L. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. *Science* 279: 2115-2118.

Losos JB, Ricklefs RE. 2009. Adaptation and diversification on islands. *Nature* 457: 830-836.

Losos JB, Schluter D. 2000. Analysis of an evolutionary species-area relationship. *Nature (London)* 408: 847-850.

Lyons-Weiler J, Milinkovitch MC. 1997. A phylogenetic approach to the problem of differential lineage sorting. *Molecular Biology and Evolution* 14: 968-975.

MacArthur RH, Wilson EO. 1963. An equilibrium theory of insular zoogeography. *Evolution* 17: 373–387.

MacArthur RH, Wilson EO. 1967. *The theory of island biogeography*. Princeton University Press: Princeton.

Maddison WP. 1997. Gene Trees in Species Trees. *Systematic Biology* 46: 523-536.

Maddison WP, Knowles LL. 2006. Inferring Phylogeny Despite Incomplete Lineage Sorting. *Systematic Biology* 55: 21-30.

Mayr E. 1963. *Animal species and evolution*. Harvard University Press: Cambridge.

Mayr E. 1967. The challenge of island faunas. *Australian Natural History* 15: 359-374.

Metropolis N, Rosenbluth AW, Rosenbluth MN, Teller AH, Teller E. 1953. Equation of state calculations by fast computing machines. *Journal of Chemical Physics* 21: 1087-1092.

Michaux JR, Libois R, Filippucci MG. 2005. So close and so different: comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. *Heredity* 94: 52-63.

Molbo D, Machado CA, Sevenster JG, Keller L, Allen Herre E. 2003. Cryptic species of fig-pollinating wasps: Implications for the evolution of the fig–wasp mutualism, sex allocation, and precision of adaptation. *Proceedings of the National Academy of Sciences* 100: 5867–5872.

Monaghan MT, Balke M, Pons J, Vogler AP. 2006. Beyond barcodes: complex DNA taxonomy of a South Pacific Island radiation. *Proceedings of the Royal Society B: Biological Sciences* 273: 887-893.

Moore J, Willmer P. 1997. Convergent evolution in invertebrates. *Biological Reviews* 72: 1-60.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. *Evolution* 49: 718-726.

Moritz C, Dowling TE, Brown WM. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics* 18: 269-292.

Moritz C, Faith DP. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology* 7: 419-430.

Myers N, Mittermeier RA, Mittermeier CC, da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature*: 853-858.

Nei M. 1987. *Molecular evolutionary genetics*: New York.

Nichols R. 2001. Gene trees and species trees are not the same. *Trends in Ecology and Evolution* 16: 358-364.

Nielsen R, Beaumont MA. 2009. Statistical inferences in phylogeography. *Molecular Ecology* 18: 1034-1047.

Nielsen R, Wakeley J. 2001. Distinguishing migration from isolation: A Markov Chain Monte Carlo approach. *Genetics* 158: 885-896.

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. 2004. Bayesian Phylogenetic Analysis of Combined Data. *Systematic Biology* 53: 47-67.

Oromí P, García A, Macías NE. 2004. Araneae. Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres). In: I. Izquierdo JLM, N. Zurita and M. Arechavaleta, ed.: Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias, Tenerife. 164–172.

Oromí P, Zurita N, Muñoz E, de la Cruz S. 2001. *Conservación de la fauna invertebrada cavernícola de las islas de Tenerife, El Hierro y La Palma.*

Page RDM. 1998. GeneTree: comparing gene and species phylogenies using reconciled trees. *Bioinformatics Applications Note* 14: 819-820.

Page RDM, Charleston MA. 1997. From gene to organismal phylogeny: reconciled trees and the gene tree/species tree problem. *Molecular Phylogenetics and Evolution* 7: 231-240.

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* 5: 568-583.

Paulay G. 1994. Biodiversity on oceanic islands: its origin and extinction. *American Zoologist* 34: 134-144.

Peck SB, Wigfull P, Nishida G. 1999. Physical correlates of insular species diversity: The insects of the Hawaiian Islands. *Annals of the Entomological Society of America* 92: 529-536.

Pfenninger M, Schwenk K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* 7.

Platnick NI. 2009. The world spider catalog, version 9.5.: American Museum of Natural History, New York. Available at: http://research.amnh.org/entomology/spiders/catalog/ index.html (accessed 20 May 2009).

Pollard SD. 1986. Prey capture in *Dysdera crocata* (Araneae: Dysderidae), a long fanged spider. *New Zealand Journal of Zoology* 13: 149-150.

Price JP, Clague DA. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society Biological Sciences Series B* 269: 2429–2435.

Rannala B, Yang Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304-311.

Rannala B, Yang Z. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164: 1645-1656.

Renner SS. 2005. Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science* 10: 550-558.

Rezac M, Pekar S, Lubin Y. 2008. How oniscophagous spiders overcome woodlouse armour. *Journal of Zoology* 275: 64-71.

Ribera C, Juberthie C. 1994. Araneae. In: Juberthie C, ed. *Encyclopaedia Biospeologica*. 197-214.

Robertson A, Stillman C. 1979. Submarine volcanic and associated sedimentary rocks of the Fuerteventura Basal Complex, Canary Islands. *Geological Magazine* 116: 203–214.

Roderick GK, Gillespie RG. 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. *Molecular Ecology* 7: 519-531 ISSN: 0962-1083.

Rokas A, Williams BL, King N, Carroll SB. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* 425: 798-804.

Rosenberg NA, Nordborg M. 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. *Nature Reviews Genetics* 3: 380-390.

Rutschmann F. 2006. Molecular dating of phylogenetic trees: A brief review of current methods that estimate divergence times. *Diversity & Distributions* 12: 35-48.

Sanderson MJ. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* 14: 1218-1231.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101-109.

Sanderson MJ. 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19: 301-302.

Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R. 2005. Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions Royal Society of London Series B Biological Sciences*: 1805-1812.

Schluter D. 2000a. Ecological character displacement in adaptive radiation. *American Naturalist* 156: S4-S16.

Schluter D. 2000b. *The Ecology of Adaptive Radiation*. Oxford University Press: Oxford.

Schmincke HU, Sumita M, Funck T. 1997. Growth and destruction of Gran Canaria: evidence from land studies, bathymetry, offshore seismic studies and drilling during ODP Leg 157 *International workshop on Immature Oceanic Islands*: La Palma, Sept. 1997. 11-12.

Schneider CJ, Cunningham M, Moritz C. 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. *Molecular Ecology* 7: 487-498.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

Simmons MP, Ochoterena H, Carr TG. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. *Systematic Biology* 50: 454-462.

Simon C. 1991. Molecular systematics at the species boundary: exploiting conserved and variable regions of the mitochondrial genome of animals via direct sequencing from enzymatically amplified DNA. In: Hewitt G, Johnston AWB and Young JPW, eds. *Molecular techniques in taxonomy*. Springer, Berlin: NATO Advanced Studies Institute, H57. 33-71.

Simon E. 1883. Études Arachnologiques XIV Mè., materiaux pour servir a la faune arachnologique des îles de l'Océan Atlantique. *Annales de la Société Entomologique de France* 6: 294-298.

Sites JW, Marshall JC. 2003. Delimiting species: a Renaissance issue in systematic biology. *Trends in Ecology and Evolution* 18: 462-470.

Sites JW, Marshall JC. 2004. Operational criteria for delimiting species. *Annual Review of Ecology Evolution and Systematics* 35: 199-228.

Sites JW, Crandall KA. 1997. Testing Species Boundaries in Biodiversity Studies. *Conservation Biology* 11: 1289-1297.

Slowinski JB, Knight A, Rooney AP. 1997. Inferring species trees from gene trees: A phylogenetic analysis of the Elapidae (*Serpentes*) based on the amino acid sequences of venom proteins. *Molecular Phylogenetics and Evolution* 8: 349-362.

Smith MA, Fisher BL, Hebert PDN. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philosophical Transactions Royal Society of London Series B Biological Sciences*: 1825-1834.

Sullivan J, Arellano E, Rogers DS. 2000. Comparative phylogeography of mesoamerican highland rodents: Concerted versus independent response to past climatic fluctuations. *American Naturalist* 155: 755-768.

Sunderland KD, Sutton SL. 1980. A seriological study of arthropod predation on woodlice in a dune grassland ecosystem. *Journal of Animal Ecology* 49: 987-1004.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453-464.

Tajima F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105: 437-460.

Takahata N. 1989. Gene genealogy in three related populations: Consistency probability between gene and population trees. *Genetics* 122: 957-966.

Takahata N, Nei M. 1985. Gene genealogy and variance of interpopulational nucleotide differences. *Genetics* 110: 325-344.

Takezaki N, Rzhetsky A, Nei M. 1995. Phylogenetic test of the molecular clock and linearized trees. *Molecular Biology and Evolution*12: 823–833.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2002. DNA points the way ahead in taxonomy. *Nature* 418: 479.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2003. A plea for DNA taxonomy. *Trends in Ecology & Evolution* 18: 70-74.

Templeton AR. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* 7: 381-397.

Templeton AR. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology* 13: 789-809.

Templeton AR, Routman E, Phillips CA. 1995. Separating population structure from population history: A cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* 140: 767-782.

Triantis KA, Nogues-Bravo D, Hortal J, Borges PAV, Adsersen H, Fernandez-Palacios JM, Araujo MB, Whittaker RJ. 2008. Measurements of area and the (island) species-area relationship: new directions for an old pattern. *Oikos* 117: 1555-1559.

Victoriano PF, Ortiz JC, Benavides E, Adams BJ, Sites JW. 2008. Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. *Molecular Ecology* 17: 2397-2416.

Wakeley J, Hey J. 1997. Estimating ancestral population parameters. *Genetics* 145: 847-855.

Walter TR, Schmincke HU. 2002. Rifting, recurrent landsliding and Miocene structural reorganization of NW Tenerife, Canary Islands. *International Journal of Earth Sciences*91: 615-628.

Watts AB, Masson DG. 2001. New sonar evidence for recent catastrophic collapses of the North flank of Tenerife, Canary Islands. *Bulletin of Volcanology* 63: 8-19.

Welch JJ, Bromham L. 2005. Molecular dating when rates vary. *Trends in Ecology and Evolution* 20: 320-327.

White EPea. 2006. A comparison of the species-time relationship across ecosystems and taxonomic groups. *Oikos* 112: 185–195.

Whittaker JR, Triantis AK, Ladle JR. 2008. A general dynamic theory of oceanic

island biogeography. *Journal of Biogeography* 35: 977-994.

Whittaker RJ, Fernández-Palacios JM. 2007. *Island Biogeography: Ecology, Evolution, and Conservation*. Oxford Biology.

Whittaker RJ, Triantis KA, Ladle RJ. 2010. A general dynamic theory of oceanic island biogeography: extending the MacArthur–Wilson theory to accommodate the rise and fall of volcanic islands. In: (ed. by Losos JBR, R.E.), ed. *The theory of island biogeography revisited*. Princeton, NJ.: Princeton University Press. 88–115.

Wiens JJ. 1998. Combining data sets with different phylogenetic histories. *Systematic Biology* 47: 568-581.

Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Higuchi RG, Palumbi SR, Prager EM, Sage RD, Stoneking M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. . *Biological Journal of the Linnean Society* 26: 375–400.

Wright DH. 1983. Species–energy theory: an extension of species–area theory. *Oikos* 41: 496–506.

Wright S. 1931. Evolution in Mendelian populations. *Genetics* 16: 97–159.

Wright S. 1951. The genetical structure of populations. *Annals of Eugenics* 15: 323-354.

Wright S. 1969. *Evolution and the Genetics of Populations. Volume 2: The Theory of Gene Frequencies*. University of Chicago Press, Chicago.

Wunderlich J. 1994. Zu Ökologie, Biogeographie, Evolution und Taxonomie einiger Spinnen der Makaronesischen Inseln. *Beitr. Araneol.* 4: 385-439.

Yang ZH, Yoder AD. 2003. Comparison of likelihood and Bayesian methods for estimating divergence times using multiple gene loci and calibration points, with application to a radiation of cute-looking mouse lemur species. *Systematic Biology* 52: 705-716.

Yoder AD, Yang Z. 2000. Estimation of primate speciation dates using local molecular clocks. *Molecular Biology and Evolution* 17: 1081-1090.

Zhang DX, Hewitt GM. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology* 12: 563-584.

Zink RM. 1996. Comparative phylogeography in North American birds. *Evolution* 50: 308-317.

Zuckerkandl E, Pauling L. 1965. Evolutionary divergence and convergence in proteins. In: Bryson V VH, ed. *Evolving Genes and Proteins*. New York: Academic Press. 97–166.

Capítulo 2

PLANTEAMIENTO, ESTRUCTURA Y OBJETIVOS DE LA TESIS DOCTORAL

Vista la oportunidad extraordinaria que brindan las Islas Canarias para el estudio de los procesos evolutivos implicados en el origen y diversificación de los seres vivos, y la notable biodiversidad que se encuentra en ellas, en la presente tesis doctoral nos planteamos combinar técnicas moleculares con metodologías de inferencia filogenética y filogeográfica con el fin de revelar los factores que han moldeado dicha diversidad. El objetivo principal de esta tesis es investigar las causas de la gran diversificación sufrida por las arañas del género *Dysdera* en el archipiélago canario y establecer su marco temporal mediante la aplicación de técnicas de sistemática molecular.

El marcado contraste geológico y ecológico entre las islas orientales y las occidentales permite determinar la importancia relativa que han tenido los diferentes factores abióticos y bióticos en la evolución del grupo. Así, las islas más antiguas (Lanzarote y Fuerteventura) proporcionan un marco idóneo para entender el efecto de la erosión y consiguiente desmantelamiento de las islas oceánicas sobre el proceso de diversificación insular (Capítulo 3). En este punto, cabe destacar la importancia de la información aportada por las secuencias de ADN para la identificación de linajes evolutivos crípticos que habían pasado desapercibidos por la taxonomía tradicional (Capítulo 4). Por otra parte, el vulcanismo recurrente y la conectividad cambiante entre las islas e islotes orientales, ofrecen la posibilidad de caracterizar el papel de las coladas de lava y los cambios eustáticos del nivel del mar sobre la estructuración de las poblaciones locales (Capítulo 5). En este sentido, la comparación de patrones filogeográficos entre especies simpátricas evolutiva y morfológicamente cercanas, permite clarificar la historia evolutiva de estas islas e investigar las causas de la coexistencia de especies (Capítulo 6). Finalmente, el gradiente altitudinal y la diversidad ecológica de las islas occidentales permite desentrañar el papel relativo de los eventos geológicos y la adaptación local sobre el flujo génico y la estructuración de las poblaciones y, en última instancia, sobre la generación de la diversidad insular (Capítulo 7).

OBJETIVOS GENERALES

1. Descifrar los patrones filogenéticos de las especies del género de arañas *Dysdera* en las islas orientales, así como los factores responsables de la diversificación y extinción de este grupo en las islas más antiguas.

2. Describir nuevos taxones de *Dysdera* a partir de información filogenética (secuencias de ADN), morfológica y ecológica.

3. Analizar la estructura poblacional y filogeográfica de *Dysdera lancerotensis* en las islas orientales, y determinar el papel de la actividad volcánica en la distribución de la diversidad genética y el flujo génico de esta especie.

4. Establecer y comparar los patrones filogeográficos de dos especies simpátricas cercanas evolutiva y morfológicamente, *Dysdera alegranzaensis* y *Dysdera nesiotes,* en Lanzarote e islotes. De esta forma, esclarecer que factores han permitido la coexistencia de especies similares.

5. Determinar el papel qué han jugado la historia geológica y la diversidad ecológica de Tenerife en la estructura filogeográfica de *Dysdera verneaui*. Complementaria-mente, establecer la posible diferenciación morfológica de esta especie a lo largo de un gradiente altitudinal.

Capítulo 3

Capítulo publicado:

Macías-Hernández, N., Oromí, P. & Arnedo, M. A. (2008). Patterns of diversification on old volcanic islands as revealed by the woodlouse-hunter spider genus *Dysdera* (Araneae, Dysderidae) in the eastern Canary Islands**.** *Biological Journal of the Linnean Society*, 94 (3): 589-615.

PATTERNS OF DIVERSIFICATION ON OLD VOLCANIC ISLANDS AS REVEALED BY THE WOODLOUSE-HUNTER SPIDER GENUS *Dysdera* (ARANEAE, DYSDERIDAE) IN THE EASTERN CANARY ISLANDS

ABSTRACT

Long-term erosion and subsidence cause dramatic alterations in the physical and ecological features of oceanic islands. Although oceanic islands have been extensively used as models for the study of speciation, little attention has been given to investigating evolutionary patterns in old volcanic islands that have suffered severe climatic degradation. The spider genus *Dysdera* has diversified across the Canary Islands and has evolved endemisms in the low-elevation, xeric eastern islands, which sharply contrast with the younger, higher and more humid western islands. A combined phylogenetic analysis of 7 mitochondrial and nuclear genes reveals that the eastern Canaries were colonized twice, although only one lineage underwent *in situ* diversification. Origins of the speciose lineage remain obscure, but probably preceded diversification of present-day Iberian and North African species. A second colonization of the eastern Canaries from North Africa has occurred in more recent times. Molecular analyses reveal several instances of geographically coherent cryptic lineages further supported by morphometric evidence. Analyses of diversification rates suggest deceleration of diversification over the course of time, and this is compatible with increasing extinction rates due to drastic yet continuous ecological changes. Extinction may also explain incongruent patterns of morphological differentiation and species coexistence. Despite a general trend towards community impoverishment, there is also evidence for recent speciation events linked to ecological shifts, which may illustrate the origins of non-speciose relic lineages on islands.

INTRODUCTION

Adaptive radiations are one of the main evolutionary features of oceanic islands (Gillespie & Roderick, 2002) and constitute fertile ground for investigating biodiversity dynamics (Losos *et al.*, 1998; Losos & Schluter, 2000, Beheregaray *et al.*, 2004; Gillespie, 2004; Emerson & Kolm, 2005). Studies on island adaptive radiations

have revealed the deterministic nature of community assembly (Losos *et al.*, 1998); similar ecomorphs evolve on different islands as a response to similar ecological regimes. It has also been shown that species assembly is dynamic, with maximum species numbers in communities of intermediate age (Gillespie, 2004; Emerson & Oromí, 2005). The chronological arrangement of islands in volcanic hot-spot archipelagos provides evolutionary snapshots of community formation and succession. Based on the pattern of diversification exhibited by the spiny-leg species of the spider genus *Tetragnatha* in the Hawaiian Islands, Gillespie (2004) suggested a series of predictable evolutionary stages in island community assembly. Younger islands display the initial stage of species buildup, probably as a result of relaxed competition due to low population numbers. Communities in ecologically similar, but geologically older islands undergo fine tuning as a result of inter- and intraspecific competition for resources, resulting in similar numbers and ecological sets of species. Similar patterns have been recovered in different groups of arthropods on the western Canary Islands (Emerson & Oromí, 2005). Islands of intermediate age show higher diversity due to the predominant role of local diversification, while in younger islands endemic species are initially the result of colonizing lineages differentiating from their source populations. The increase in the relative number of endemics of an island as a result of higher local diversification rates is small and suggests that *in situ* diversification of some lineages is occurring while others are probably going extinct (Emerson & Oromí, 2005), in agreement with the fine tuning stage identified for Hawaiian spiders.

Extinction is a key component of island ecology and lies at the very core of the classical equilibrium theory of island biogeography of MacArthur & Wilson (1967). The importance of extinction in shaping diversity on continental islands, either as a factor promoting local endemism through relictualization (paleoendemics) or through the effect of fragmentation and lost habitat (Fahrig, 2003), is well established. Nevertheless, to date, most evolutionary research conducted on oceanic archipelagoes has focused on the processes of species formation and accumulation, and has largely neglected the role of extinction, except in the context of human-mediated habitat destruction and alien species introductions (Gillespie & Roderick, 2002). However, it is well known that oceanic

islands undergo drastic physical and environmental changes as a result of geological processes that may promote high rates of extinction in the absence of human related factors (Baldwin *et al.*, 1998). The Hawaiian Islands illustrate well this pattern of geological evolution: volcanoes rapidly subside after reaching maximum height and then erode down to sea level over several million years, eventually sinking into the sea or being reduced to atolls (Price & Clague, 2002). The Canary Islands share many geological features with the Hawaiian archipelago, but they differ in one important respect: the lack of significant subsidence (Carracedo, 1999).

The eastern Canary Islands are the emerged parts of a volcanic ridge built through a series of volcanic pulses, starting about 22 million years ago (Ma). It runs roughly parallel to the African coast, which is only 100 km away at its closest point (Coello *et al.*, 1992). The ridge is presently divided into two main islands, Fuerteventura and Lanzarote, and several islets, which include the Chinijo archipelago, at the northernmost part, and Lobos, between the two main islands (Fig. 3.1). Although the islands are still emerged, their subaerial surface has been heavily transformed by mass-wasting processes. It has been estimated that Fuerteventura would have reached a maximum height of 3.300 m a.s.l. at its maximum growing stage, but now it barely reaches 800 m a.s.l. (Ancochea *et al.*, 1996). In addition to habitat destruction, linked to mass land sliding and a recurrent volcanic activity (Coello *et al.*, 1992), the eastern Canaries have experienced severe climatic degradation. The erosion-induced low elevation of the islands, which prevents them from capturing the humid NE trade winds, and the influence of pulses of climate variability and aridity in Africa starting in the Plio–Pleistocene (Demenocal, 2004), have caused a strong aridification of the islands.

The spider genus *Dysdera* includes about 250 species (Platnick, 2006) of medium size nocturnal wandering hunters that inhabit warm and wet ground habitats of the western Palaearctic. *Dysdera* has colonized all Macaronesian archipelagos, but only in the Canary Islands has undergone a major diversification process. To date, about 50 species have been recognized in the Canaries, six of which are endemic to the oldest eastern Islands (Fig. 3.2) (Arnedo, Oromí & Ribera,

1996; Arnedo, Oromí & Ribera, 2000; Arnedo & Ribera, 1997; Arnedo & Ribera, 1999). Given the prevailing winds and the sea currents, the more plausible sources of colonizers for the Canary Islands are neighbouring North Africa and the Iberian Peninsula (Juan *et al.*, 2000). The *Dysdera* fauna of the Iberian Peninsula is well-known and includes 27 species (Ferrández, 1996; Ribera, 2004). There are 36 *Dysdera* species described from the African region north to the Sahara desert (Morocco, Algeria, Tunisia, Libya and Egypt) (Simon, 1882; Simon, 1909; Simon, 1910; Denis, 1945; Denis, 1961; Ribera, 1983; Ribera, 1993), although this figure is probably an underestimation since the area has not been as thoroughly sampled as the Iberian Peninsula. Despite of the high species richness and the important level of endemism reported in Iberia and North Africa, which is in accordance to the pattern observed in other Mediterranean regions, the levels of genitalic diversity are comparatively low. Deeleman-Reinhold & Deeleman (1988) proposed to subdivide the genus *Dysdera* into 9 species groups, based on the genitalic patterns along with characters from the carapace and the chelicerae. Most *Dysdera* species from Iberian and North Africa can be easily accommodated in a few of these species groups: the group *crocata* and the group *erythrina* are by far the best represented, while the group *lata* and the group *longirostris* include the single representatives *Dysdera lata* Wider and *Dysdera scabricula* Simon 1882, respectively.

Arnedo, Oromí & Ribera (2001) investigated the phylogenetic structure of Canarian *Dysdera* using morphology and two mitochondrial genes and concluded that most of the species were the result of local diversification and that, with a single exception, eastern Canarian species formed one clade. Unfortunately, incomplete molecular sampling precluded unambiguous assessment of the number of colonization events that accounted for the observed diversity in the archipelago (N=2-4) and the relationship within Canarian clades could not be fully resolved. Most of the eastern Canarian *Dysdera* species have a distribution restricted to the highest altitudes of Lanzarote and Fuerteventura, which constitute the more humid habitats in what are typically xeric islands. These hygrophilous lineages have been interpreted to be relict inhabitants of more humid woodlands that existed before aridification (Juan *et al.*, 2000).

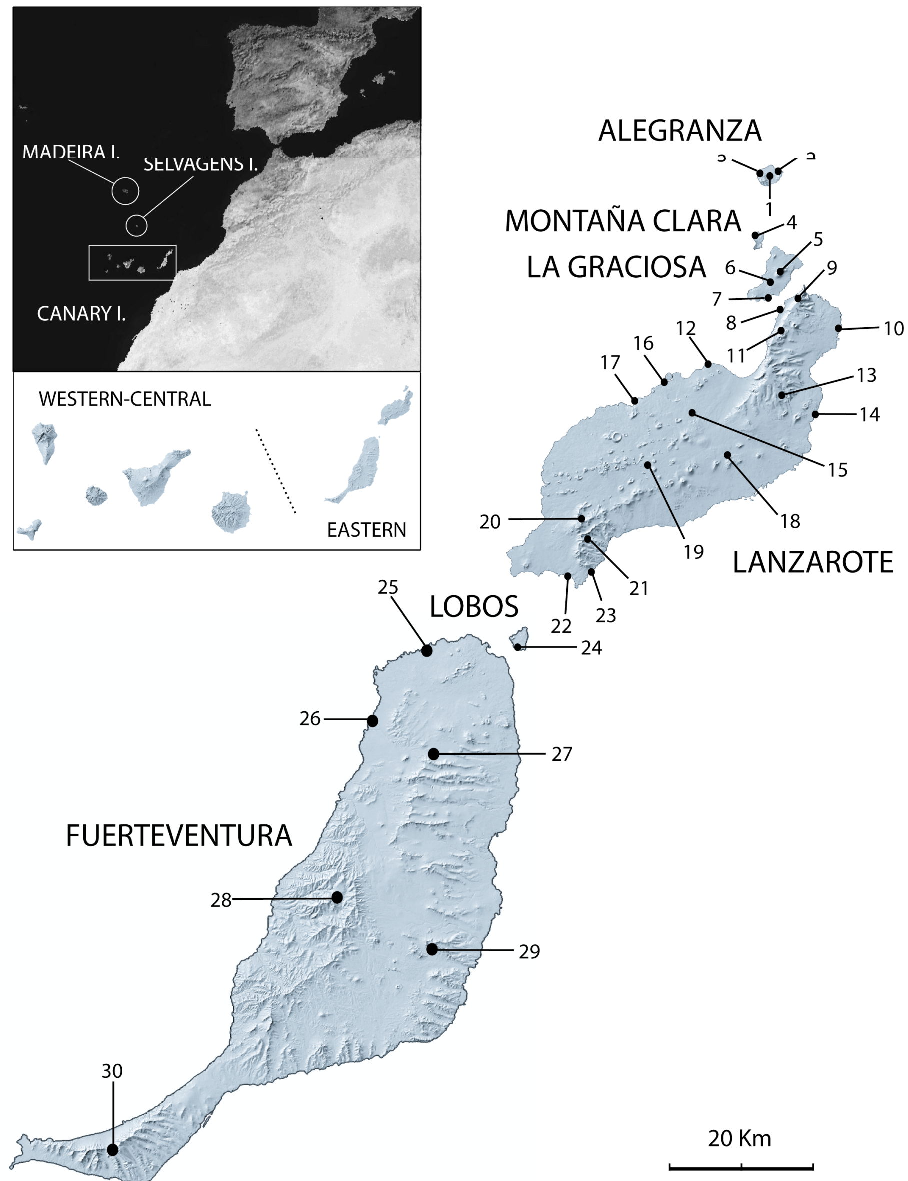

Fig. 3.1.- Maps showing the location of the Macaronesian archipelagoes (Cape Verde not included), a close up of the Canary Islands, and a finer scale map of the eastern Canary islands, showing sampling locations of *Dysdera* specimens included in the analyses. The population codes correspond to those listed in Appendix 3.2.

The relict distribution, along with the lower number of *Dysdera* endemics harbored by Fuerteventura and Lanzarote, based on island age and area, led Arnedo, Oromí & Ribera (2000) to hypothesize that extinction had played a major role in shaping diversification patterns in the Eastern Canarian *Dysdera* clade. Alternatively, the lower number of endemics of Lanzarote and Fuerteventura could also be explained by a more recent colonization of these islands by a lineage already adapted to similar arid environments from nearby north-western Africa.

The eastern Canarian *Dysdera* species offer insights into the patterns of diversification in very old volcanic islands which have undergone dramatic ecological changes and constitute an excellent model to investigate further stages in the dynamics of community assembly. In the present study, we combined molecular information from four mitochondrial and three nuclear genes to infer phylogenetic relationships in a large sample of specimens from the eastern Canaries and representatives from the western and central Canaries and continental species. The phylogenetic information was then used to assess the origins and external relationships of the eastern endemics and to examine the temporal and geographic patterns of species diversification and morphological differentiation.

Fig. 3.2.- Male of *Dydera longa* Wunderlich, 1991 (Photo: P. Oromí).

MATERIALS AND METHODS

Taxonomic sampling

Specimens included in the study were obtained in several collecting trips to Lanzarote, Fuerteventura and the islets of Alegranza, Montaña Clara, La Graciosa and Lobos during 2003-2005. For comparison, the taxonomic sampling of the non-eastern Canarian endemics followed that of Arnedo (Arnedo *et al.*, 2001), which includes representatives of all species groups found in the western Mediterranen, except *lata*, along with the group *ninnii*, found east from the island of Corsica. Representatives of two additional genera, *Harpactocrates,* and *Dysderocrates,* of the subfamily Dysderinae along with single representatives of the other two subfamilies, Harapacteinae (*Harpactea*) and Rhodinae (*Rhode*) were included as outgroups. All analyses were rooted in the branch joining *Harpactea* with the remaining taxa.

Molecular procedures

Genomic DNA was extracted from specimens using the DNeasy Tissue Kit (Qiagen) following manufacturer's guidelines. Partial fragments of the mitochondrial genes cytocrome c oxidase subunit I (*cox I*), 16S rRNA (*16S*), the complete tRNA leu UAG (*L1*), NADH dehydrogenase subunit I (*nad1*), and the nuclear genes 28S rRNA (*28S*), Histone H3 (*H3*) and the internal transcribed spacer 2 (*ITS-2*) were amplified using the following primer pairs: [*cox I*] C1-J-1490 and C1-N-2198 (Folmer *et al.*, 1994) or C1-N-2191 (Simon *et al.*, 1994), and CI-J-2183 (Simon *et al.*, 1994), or the antisense of C1-N-2198, with C1-N-2776 (Hedin & Maddison, 2001); [*16S*] LR-N-13398 (Simon *et al.*, 1994) and N1-J-12261 (Hedin, 1997) or N1-J-12373 (5´ CTTCGTATAGATCCTARTTGDCTRTATTT 3´) designed for the present study, [*28S*] 28S-B (Giribet *et al.*, 1999) and 28S-O (Hedin & Maddison, 2001), [*H3*] H3a F and H3a R (Colgan *et al.*, 1998), and [*ITS*] ITS-5.8S and ITS-28S (White *et al.*, 1990). Amplifications were carried out in 25µl reaction volume for a final concentration of 1.25 U *Taq* polymerase (Promega), 2.5mM $MgCl_2$ (Promega), 0.2mM of each dNTP, 0.2µM of each primer and about 2µl of DNA sample, and the amount of *Taq* buffer recommend by the manufacturer. PCR conditions were as follows: 5 min. at 94ºC followed by 35 cycles of denaturalization at 94ºC for 30 s, annealing at 42-52ºC for

35-45 s (depending on the primers, see below), and extension at 72ºC for 30-60 s (depending on the length of the fragment), with a final single extension step at 72ºC for 5 min. For the *cox1* and *16S-nad1* gene fragments, a successful amplification was achieved with an annealing temperature of 42ºC or 45ºC for 45 s; and for the *28S* and *ITS* an annealing temperature of 58ºC and 48-52ºC respectively, for 35 s was optimum. PCR products were purified using MultiScreen PCRµ96 cleanup filter plates from Millipore. PCR products were cycle-sequenced in both directions using one of the PCR primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem) and sequenced in an ABI 3700 automated sequencer at the Scientific and Technical Services of the University of Barcelona (http://www.sct.ub.es). Voucher specimens were stored at the *Centre de Recursos de Biodiversitat Animal* (University of Barcelona) and the Department of Animal Biology of the University of La Laguna.

Phylogenetic analyses

Chromatograms were analyzed and sequences edited using the Pregap and Gap4 programs included in the Staden Package (http://staden.sourceforge.net/) software. Sequences were manipulated and preliminary manual alignments constructed using Bioedit (Hall, 1999). Alignment of the protein-coding genes was trivial since no length polymorphism was observed. Conversely, ribosomal gene sequences showed differences in length suggesting the occurrence of insertion/deletion events during the evolution of these sequences. Automatic multiple sequence alignment programs were preferred over manual alignment due to its objectivity and repeatability (Ogden, Whiting & Wheeler, 2005) and because they provide a comparative framework to study the effect of different assumptions on the final results (Giribet, Wheeler & Muona, 2002). We explore the effect of alternative cost schemes by building several automatic multiple sequence alignments for each ribosomal gene and the *ITS-2*, with CLUSTAL X (Thompson *et al.*, 1997), using the following gap opening (GOP) and extension (GEP) costs (GOP/GEP): 8/2, 8/4, 20/2, 24/4 and 24/6 (in all cases transition weight was fixed to 0.5). Topological congruence to the elision matrix (Wheeler, Gatesy & DeSalle, 1995), as measured by the number of nodes in common between the consensus tree of each individual matrix and the elision matrix and by calculating the average symmetric-

difference distances, was used as a criterion to select the optimal alignment for each gene fragment, as suggested by Hedin & Maddison (2001). Gaps were scored as presence/absence characters, according to a set of rules based on gap overlapping and sharing of the 5´and/or the 3´termini (Simmons & Ochoterena, 2000). This coding scheme allows incorporation of indel information in phylogenetic reconstruction using not only parsimony, but also Bayesian inference methods, while minimizing the effect of increasing the weight of overlapping multiple non-homologous gaps that results from scoring gaps as an additional state (Pons & Vogler, 2006). The program GAPCODER (Young & Healy, 2002) was used to facilitate the automatic recoding of the alignments based on the simple method proposed by Simmons & Ochoterena (2000).

Combined data matrices were obtained by concatenating the selected automatic alignment of each ribosomal gene and the *ITS-2* along with the protein coding genes with the help of the WINCLADA program v.1.00.08 (Nixon, 2002). Two different data matrices were constructed. The first matrix, hereafter referred to as M1, included a more exhaustive taxonomic sampling of non-eastern Canarian endemic *Dysdera* species and was intended to resolve external relationships of the Eastern Canarian group. M1 included a shorter fragment of the *cox I* gene (471 bp) obtained with primers C1-J-1718 and C1-N-2191 (Simon *et al.*, 1994), because most of the sequences were provided by other ongoing projects in our lab, along with the *16S, L1, nad1, H3*, and *28S* gene fragments for a total of 77 terminal taxa. The second matrix, hereafter referred to as M2, was intended to resolve internal relationships of the group of eastern Canarian endemics, to infer lineage ages and to study diversification rates in the Eastern Canarian group. M2 included a subsample of the outgroup species, adding up to a total of 38 terminal taxa, but with many more characters, including sequences of the *ITS-2*, and a much longer fragment of the *coxI* (1179 bp), along with the other fragments included in M1.

Parsimony analyses of the different matrices were conducted with the programs TNT v. 1.0 (Goloboff, Farris & Nixon, 2003) and NONA (Goloboff, 2002). Heuristic searches consisted in 100 iterations of Wagner trees constructed with random addition taxa and subsequent TBR branch swapping, holding five trees per

iteration and up to a total maximum of 10000. When the number of replicates finding optimal trees was less than 10%, the number of replicates was increased to 1000. Clade support was assessed via jackknife resampling (Farris *et al.*, 1996) as implemented in TNT, based on 500 jackknife replicates with individual heuristic searches consisting on 15 iterations of Wagner tree construction using random addition of taxa, holding 5 trees per iteration and an overall maximum of 10000.

The program MODELTEST v. 3.06 (Posada & Crandall, 1998) was used to select the substitution model that best fit the data with the lower parameters, as indicated by the Akaike information criterion (AIC) (Akaike, 1973), which allows the comparison of multiple nested models and accounts for model selection uncertainty (Posada & Buckley, 2004).

Bayesian inference analyses were performed with MRBAYES v.3.1.1 (Ronquist & Huelsenbeck, 2003). An unlinked GTR+I+Γ nucleotide substitution model was specified for each gene fragment and a standard discrete model was implemented for the gaps scored as absence/presence data. The substitution estimates were allowed to vary independently between each partition. Two independent runs were performed to assess convergence of the results. Four simultaneous Markov Chain Monte Carlo chains (one cold and three heated), using random starting trees were run 1.5-2.0 million generations, sampling the Markov chain every 100 generations. The TRACER program v. 1.3 (Rambaut & Drummond, 2003) was used to ensure that the Markov chains had reached stationarity and also to determine the correct number of generations to discard as a *burn-in* for the analysis.

Maximum likelihood analyses were performed with a unix version of the PAUP* program (Swofford, 2001) run in either an 8-node Compac HPC320 at 833 Mhz or an 8-node Compaq Beowulf at 600 Mhz computers at the *Centre de Supercomputació de Catalunya* (CESCA, http://www.cesca.es). Heuristic searches involved ten random additions of taxa using the model of nucleotide substitution selected by MODELTEST and gap characters removed. The M1 matrix was reduced to 53 taxa by removing extra populations of the same species to shorten

computation times. Clade support was assessed through bootstrap resampling (Felsenstein, 1985), using the computer program PHYML v.2.4 (Guindon & Gascuel, 2003). Support values were obtained from 1000 bootstrapped matrices, implementing the same model of nucleotide substitution and using the best tree obtained in PAUP as starting tree. Alternative tree topologies were evaluated using the Templeton (1983), Winning-sites (1988), and Kishino-Hasegawa (1989), in parsimony analyses, and the Shimodaira-Hasegawa test (Shimodaira & Hasegawa, 1999) in maximum-likelihood analyses, all of them conducted with PAUP*.

Data partition congruence

Possible instances of incongruence between mitochondrial and nuclear data sets were explored using the Incongruence Length Difference Test (ILD) (Farris *et al.*, 1994). Incomplete taxa and uninformative characters were removed before performing the test as suggested in the literature (Cunningham, 1997; Arnedo *et al.*, 2001). The ILD test was performed with the program WINCLADA (Nixon, 2002) using 1000 pseudoreplicates with individual heuristic searches consisting in 15 random additions of taxa, holding 5 trees per iteration and up to a maximum of 1000 trees. The Partitioned Branch support (PBS) (Baker, Yu & DeSalle, 1998) is a useful tool to detect conflict among data sets in simultaneous analysis (Gatesy, O'Grady & Baker, 1999). PBS values were determined using PAUP* facilitated by TREEROT v.2 (Sorenson, 1999).

Biogeographic analysis

Dispersal-vicariance analysis (Ronquist, 1997), as implemented in the computer program DIVA v. 1.1 (Ronquist, 1996), was used to reconstruct ancestral distributions on the phylogeny of the eastern Canarian species. This method searches for the optimal reconstruction of ancestral distributions by assuming a vicariant explanation (i.e. allopatric speciation), while dispersal and extinction events are allowed at a cost of one per unit area added or deleted. We use islands as area units in our analysis. Every island from the Chinijo archipelago was considered separately. We used the exact search of DIVA without restricting the number of areas in which the ancestor occurred (i.e. accepting the possibility of a widespread ancestor).

Lineage age estimation

The presence of a molecular clock in the M2 matrix was rejected by a likelihood ratio test (2Δ=124.75, $P < 0.05$, d.f.= 36). The development of alternative strategies for dating phylogenies in the absence of rate constancy is a field of very active research (Welch & Bromham, 2005). The program R8S v.1.5 (Sanderson, 2003) implements several methods for estimating time and rate parameters relaxing the assumptions of the molecular clock. In addition, the program incorporates a cross-validation procedure to select the best method, given the data, as well as to find the optimal level of the smoothing required for the penalized likelihood method (Sanderson, 2002).

In the absence of fossil evidence, well-dated biogeographic events may provide the essential calibration points for estimating absolute divergence times. Island age has been successfully used as calibration point to infer clade ages in other Canarian arthropods (Emerson, Oromí & Hewitt, 2000a; Emerson, Oromí & Hewitt, 2000c; Percy, Page & Cronk, 2004). However, calibration points in volcanic archipelagos are usually based on dispersal events (colonizations), which are generally less tightly associated to geology than pure vicariant events and hence we chose to use an external, vicariant event-based, calibration point. The opening of the Strait of Gibraltar about 5.3 Ma has been used as calibration point to estimate lineage ages in other organisms, including both arthropods (Gómez-Zurita, 2004) and vertebrates (Carranza & Arnold, 2003). The marine gateways connecting the Mediterranean Sea and the Atlantic Ocean were closed about 5.9 Ma as a result of a tectonic uplift (Duggen *et al.*, 2003). This event created a land bridge between Iberia and northwest Africa that lasted for nearly 600000 years, which resulted in the Messinian Salinity Crisis. The collapse of the Gibraltar arch about 5.3 Ma reopened the connection between the Mediterranean and the Atlantic Ocean (Blondel & Aronson, 1999; Krijgsman *et al.*, 1999). The species *Dysdera inermis* Ferrández, 1984 is known from the southernmost part of the Iberian Peninsula. Several specimens with a very similar morphology to this species have been recently collected from the Rif region in Morocco (M. A. Arnedo, unpubl. data). Preliminary data show that mitochondrial genetic divergences between the Moroccan and Iberian populations (uncorrected genetic distance= 16%) are in

agreement with a post-Messinian split of these populations, assuming an approximate 2.3% pairwise divergence per million years in arthropod mitochondrial DNA (Brower, 1994). Therefore, in the present study absolute lineage ages were estimated by assuming a time of split of the southern Iberian and Rif lineages of *D. inermis* of 5.3 Ma.

The errors in the divergence estimates associated to finite character sampling were estimated through bootstrap resampling. PAUP* was used to generate 100 bootstrapped matrices from the original data set. Branch lengths were calculated for each resampled matrix constraining the preferred topology. Resulting trees with branch lengths were then processed with R8S to calculate means and standard deviations of the bootstrap distribution of divergence times.

Analysis of morphological variation

We analyzed patterns of morphological variation between and within eastern Canarian *Dysdera* species. Spiders in the genus are nocturnal hunters that use their long, protruding chelicera to catch prey, usually woodlice and other arthropods found in leaf-litter. Body size and chelicera shape most likely play a key role in resource partitioning in sympatric species of *Dysdera*. In addition, conspicuous differences have been observed in the length of walking legs (Arnedo & Ribera, 1999). The following measurements were taken from male and female specimens of the eastern Canarian endemics: maximum carapace length (P1), minimum (P2min) and maximum carapace width (P2max), length of the basal segment of the chelicera in lateral view (Q1), maximum width of the basal segment in lateral view (Q2), cheliceral fang length (F), length of the prolateral margin of the basal segment (Esc), length of the femur of leg 1 (fe1) and length of the metatarsus of leg 4 (mt4). The assumptions of normality and homogeneity of our data were rejected by a Kolmogorov-Smirnoff test. Consequently, a non parametric one-way Krustal-Wallis test was used to elucidate intraspecific sexual dimorphism and interspecific morphological differences. Significant differences in size between lineages were further assessed using a Wilcoxon matched pairs test. A species similarity matrix was estimated across a hierarchical agglomerative cluster using the Bray-Curtis distance. A Pearson test revealed a strong association between

morphological variables. Accordingly, interspecific regressions using Pearson correlations were conducted for each variable against P1 in order to remove the effect of correlations with body size, and the residual values were used in subsequent analyses (Losos *et al.*, 1998). These exploratory analyses were performed with the software packages SPSS v. 11.0 (SPSS, 2001a), Primer v 5.2.2 (Clarke & Warwick, 1994) and STATISTICA (StatSoft Inc., 1999). A principal component analysis (PCA) was conducted for all individuals using the residual variables (see above), to reduce the variability of morphological measurements to two dimensions and to assess the variance explained for each independent axis (Legendre & Legendre, 1998). The PCA analysis was performed with the STATISTICA and results plotted with SigmaPlot v. 7.0 (SPSS, 2001b).

Estimates of diversification rates

Dated phylogenies provide information on the patterns of diversification of lineages (Harvey *et al.*, 1994a; Nee *et al.*, 1994; Nee, Barraclough & Harvey, 1996) and hence can be used to test specific hypothesis about speciation and extinction processes. We first investigated whether diversification rates varied among our lineages using the B1 tree balance statistic (Kirkpatrick & Slatkin, 1993) as implemented in the TREESTAT program (Rambaut & Drummond, 2005). The Kendall-Moran estimator *b* was used to calculate speciation rate, assuming a constant model, and the Moran estimate of the variance to calculate confidence intervals (Baldwin & Sanderson, 1998; Nee, 2001; Barraclough & Vogler, 2002). Temporal shifts in diversification rates were analyzed using the γ statistic (Pybus & Harvey, 2000) and maximum likelihood methods (Paradis, 1997; Emerson *et al.*, 2000a; Barraclough & Vogler, 2002; Rabosky, 2006b). Simulation studies have shown that birth-death likelihood approaches perform as well as or better than the γ–statistic and that they provide much greater power to detect rate variation in the presence of extinction (Rabosky, 2006b). The lineage through time plot (Harvey, May & Nee, 1994b) of the eastern endemic species, the speciation and γ statistics, and the evaluation of the alternative models of diversification were computed with the APE (Paradis, Claude & Strimmer, 2004) and LASER (Rabosky, 2006a) packages, a series of routines written in R language. We test for rate variation using Birth-Death likelihood approaches of two constant (pure birth, birth-death) and four

variable models (exponential and logistic density-dependent, two-rate pure-birth and two-rate birth-death). Model selection was accomplished by comparing the difference in AIC score between the best rate constant and rate variable models as test statistic (ΔAIC_{RC}) (Rabosky, 2006b). Critical values of ΔAIC_{RC} were assessed by mean of 1000 trees generated under pure birth (*yuleSim* command in LASER), defining the same number of lineages recovered in the phylogenetic analyses and using the estimated pure speciation value (λ). Because of the relatively small and well-circumscribed area of study and the extensive fieldwork conducted, we assumed that the taxonomic sampling in the present study was exhaustive. An incomplete taxon sampling, however, would result in an apparent increase of extinction rate towards the present, lowering the value of the γ statistic (Nee *et al.*, 1994; Paradis, 1997; Barraclough & Vogler, 2002).

RESULTS

Phylogenetic and biogeographical analyses

Specimens and sequences, with corresponding Genbank accession numbers, analyzed in the present study are listed in Appendix 3.1. Localities and Universal Transverse Mercator (UTM) coordinates sampled in the eastern Canarian ridge are shown in Figure 3.1 and listed in Appendix 3.2. A total of 77 specimens were analyzed at the molecular level, representing all eastern Canarian endemic species, 21 western and central Canarian species and 20 continental taxa, along with two additional species of the subfamily Dysderinae and a single representative of each of the two additional subfamilies. Eastern Canarian endemics were represented by 32 specimens from 30 localities along the eastern ridge.

Topological congruence identified a gap opening cost of 20 and an extension gap penalty of 2 as the best parameter combination for the *16S* alignment (Table 3.1). The different parameter combination assayed, other than gap opening 8 and gap extension 2, did not have any effect on the resulting alignment of the *28S* sequences (Table 3.1). The same parameter combination as used for *16S* was arbitrarily selected for the *28S* as well. Alignments of the two ribosomal genes under the selected parameter costs and with gap scores as

presence/absence were merged with the protein coding genes, resulting in a combined data matrix of 2567 characters (*cox*1=471, *nad*1=343, *16S*= 611, *H3*= 328, and *28S*= 814) for the 77 specimens sampled (M1).

Table 3.1.- Summary of the results of the parsimony analyses with gaps as absence/presence characters and combining different parameters of gap opening/extension (GOP/GEP) of the genes *16S*, *28S* and *ITS*. Length: steps; SD: symmetric difference. Entries in bold refer to the preferred parameter combinations.

Cost	Length	Gaps	Trees	Consensus Fork	SD
16S+tRNAleu					
8/2	2043	132	1000	43	36
8/4	2042	118	828	46	41
20/2	**2084**	**88**	**9**	**60**	**18**
24/4	2094	83	6	57	25
24/6	2102	39	8	61	35
elision	10406	-	3	-	
28S					
8/2	454	40	1000	24	13
8/4	457	39	1000	30	0
20/2	**457**	**39**	**1000**	**30**	**0**
24/4	457	39	1000	30	0
24/6	457	39	1000	30	0
elision	2284	-	1000	-	
ITS					
8/2	306	70	21	15	5
8/4	**309**	**68**	**24**	**15**	**4**
20/2	299	76	12	1	30
24/4	299	76	12	1	30
24/6	299	76	12	1	30
elision	2134	-	18	-	

Parsimony analyses of M1 yielded 32 trees of 7243 steps (CI= 0.27, RI=0.55). Strict consensus of the most parsimonious trees is shown in Fig. 3.3 and jackknife supports are listed in Table 3.2. The AIC criterion implemented in MODELTEST identified the GTR+I+Γ as the model that best fit the data. Selected model parameters were as follow: base frequencies A=0.3159, C=0.1694, G=0.1830 and T=0.3317; rate matrix: A/C= 2.1420, A/G= 5.2181, A/T= 2.8264, C/G= 0.9459, C/T= 9.2285, G/T= 1.0, proportion of invariable sites= 0.4652, Γ distribution shape parameter= 0.5295. A heuristic search using maximum likelihood with ten random addition of taxa replicates found a single best tree of score –lnL=30055.01251 (not shown). Bootstrap proportions based on 100 replicates were calculated with

PHYML, implementing the same evolutionary model and using the best maximum likelihood tree as starting tree, are listed in Table 3.2. Two independent runs of 1,500,000 generations of Bayesian analyses were conducted with unlinked GTR+I+Γ models for each gene partition and a Markov k model for the gaps scored as presence/absence data. Posterior probabilities after removal of the first 50000 trees as burn-in were estimated and are listed in Table 3.2.

The results of different inference methods analyses were largely congruent, two thirds of the clades were well-supported by at least two methods, including the monophyly of the endemic species from the eastern Canaries, with the exclusion of *Dysdera lancerotensis*, and the monophyly of the endemisms from the western Canaries. Important points of disagreement include the monophyly of *Dysdera*, which was supported by the model-based methods, but rejected by parsimony, albeit with very low support, and the monophyly of the two Canarian clades, only supported under maximum likelihood. Both, parsimony and Bayesian inference analyses agree in grouping the western Canarian clade with a group of species inhabiting the Iberian Peninsula and Morocco along with the eastern Canarian endemic *D. lancerotensis*, which are shown as a clade in model-based analyses, although with very low support. The Templeton, the winning-sites, and the Kishino-Hasegawa tests revealed that trees obtained from maximum parsimony searches forcing monophyly of the two Canarian clades were not significantly different ($P > 0.05$) to unconstrained most parsimonious trees. Concomitantly, trees obtained in maximum likelihood constrained searches forcing nonmonophyly of the Canarian clade were not significantly different to the optimal trees without constraints according to the Shimodaira-Hasegawa test ($P = 0.345$). A second data matrix (M2) was constructed to examine internal relationship in the Eastern Canarian clade. This matrix included 38 terminals: all the specimens from the eastern Canaries along along with a subset of specimens from M1 (two specimens of *D. lancerotensis,* two species from the Western Canarian clade, two species from the Ibero- Moroccan clade and the more distantly related *D. scabricula*). Two more gene fragments were added to the ones analyzed in M1: 708 additional nucleotides of the *cox*1 and the full sequence of the nuclear *ITS-2* (~500 bp). The same protocol implemented to select the best parameter cost combination for the alignment of the *16S* and *28S* was applied to the *ITS-2* partition. Topological congruence selected the alignment obtained with a gap opening cost of 8 and extension gap penalty of 4.

The final concatenated matrix with gaps scored as presence/absence data yielded 3713 characters.

Table 3.2.- Clade support values for the M1 matrix.

Clade	Clade description	Jackknife MP	Bootstrap ML	PP
1		99	-	-
2		57	-	-
3		60	-	-
4		91	62	-
5		83	100	0.98
6	Eastern Canaries	95	100	0.98
7		97	-	1.00
8		99	-	-
9	Western Canaries	99	100	1.00
10		63	98	1.00
11		-	69	-
12		70	-	-
13		57	-	-
14		56	61	-
15		61	-	-
16		67	96	1.00
17		100	-	1.00
18		-	75	-
19		-	79	-
20		89	99	1.00
21		99	100	1.00
22		70	96	1.00
23		59	99	0.98
24		100	-	1.00
25		100	-	1.00
26		99	100	1.00
27		100	100	0.98
28		100	-	1.00
29		100	-	1.00
30		100	-	1.00
31		81	-	0.98
32		68	-	-
33		90	-	-
34		99	-	1.00
	Dysdera	-	74	0.96
	Canaries	-	62	-
	Ibero-Moroccan	-	93	1.00

Clade numbers as in Fig. 3.3.
Only support values above 50% for maximum parsimony (MP) Jackknife and maximum likelihood (ML) bootstraps, and above 0.95 for Bayesian posterior probabilities (PP) are reported.

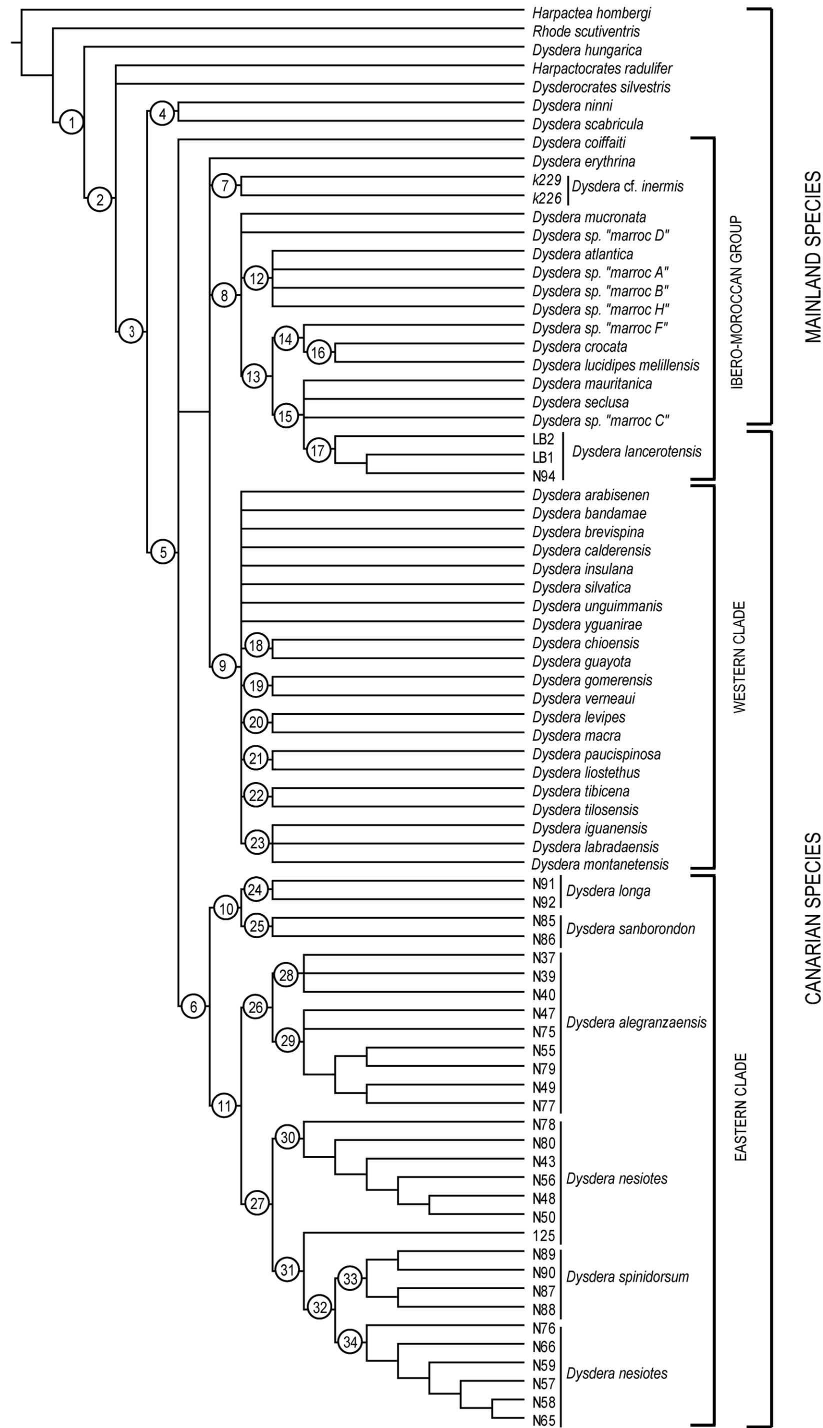

Fig. 3.3.- Strict consensus tree of 32 trees of 7243 steps (CI= 0.27, RI=0.55) resulting from uniformly weighted parsimony analysis of matrix M1 (see text for details). Refer to Table 3.2 for clade support values across all phylogenetic analyses performed.

Parsimony analyses of M2 resulted in two trees of 3363 steps, (CI= 0.54, RI=0.74). Differences between the two most parsimonious trees are restricted to the position of the species *Dysdera sanborondon*, which is shown as either a sister to the remaining Eastern Canarian clade species, or as the sister group to the Eastern Canarian clade species except *Dysdera alegranzaensis* (Fig. 3.4). Jackknife and Bremer (total and partitioned) supports are listed in Table 3.3.

Table 3.3.- Clade support values for the M2 matrix.

				PBS								
Clade	**Jackknife MP**	**Bootstrap ML**	**PP**	***cox*1**	***nad*1**	***16S***	**mtDNA**	***H3***	***28S***	***ITS***	**nDNA**	**BS**
1	100	100	100	-4.0	23.5	31.0	50.5	10.0	7.0	1.5	18.5	69.0
2	67	-	-	7.0	-2.5	0.0	4.5	0.0	-1.0	1.5	0.5	5.0
3	78	72	-	1.0	1.5	0.0	2.5	0.0	4.0	-1.5	2.5	5.0
4	85	-	-	3.0	0.5	1.0	4.5	0.0	1.0	1.5	2.5	7.0
5	99	100	100	7.0	-1.5	10.0	15.5	2.0	4.0	1.5	7.5	23.0
6	98	90	100	1.5	-1.0	9.0	9.5	0.0	3.0	3.5	6.5	16.0
7	99	100	100	8.0	4.0	1.5	13.5	0.5	6.5	0.5	7.5	21.0
8	100	100	100	3.0	3.5	11.0	17.5	-1.0	1.0	1.5	1.5	19.0
9	-	86	95	-	-	-	0	-	-	-	0	0
10	100	100	100	26.0	9.5	0.0	35.5	1.0	0.0	0.5	1.5	37.0
11	100	100	100	20.0	13.5	12.0	45.5	0.0	0.0	4.5	4.5	50.0
12	100	100	100	34.0	13.5	9.0	56.5	0.0	3.0	21.5	24.5	81.0
13	79	88	98	15.3	1.4	-10.1	6.6	-3.0	0.0	2.4	-0.6	6.0
14	100	100	100	-0.5	25.5	21.0	46.0	6.0	1.0	12.0	19.0	65.0
15	87	73	100	-7.0	7.8	1.3	2.2	1.3	0.0	3.5	4.8	7.0
16	100	100	100	16.0	1.5	16.0	33.5	0.0	0.0	-1.5	-1.5	32
17	91	100	100	3.0	0.0	5.0	8.0	0.0	0.0	0.0	0.0	8.0
18	82	89	100	3.0	-1.5	0.0	1.5	0.0	0.0	1.5	1.5	3.0
19	91	100	-	6.0	-1.5	-2.0	2.5	0.0	0.0	1.5	1.5	4.0
20	99	100	100	6.0	-1.5	4.0	8.5	0.0	0.0	1.5	1.5	10.0

Clade numbers as in Fig. 3.4.
Only support values above 50% for maximum parsimony (MP) Jackknife and maximum likelihood (ML) bootstraps, and above 0.95 for Bayesian posterior probabilities (PP) are reported. BS: total Bremer supports.

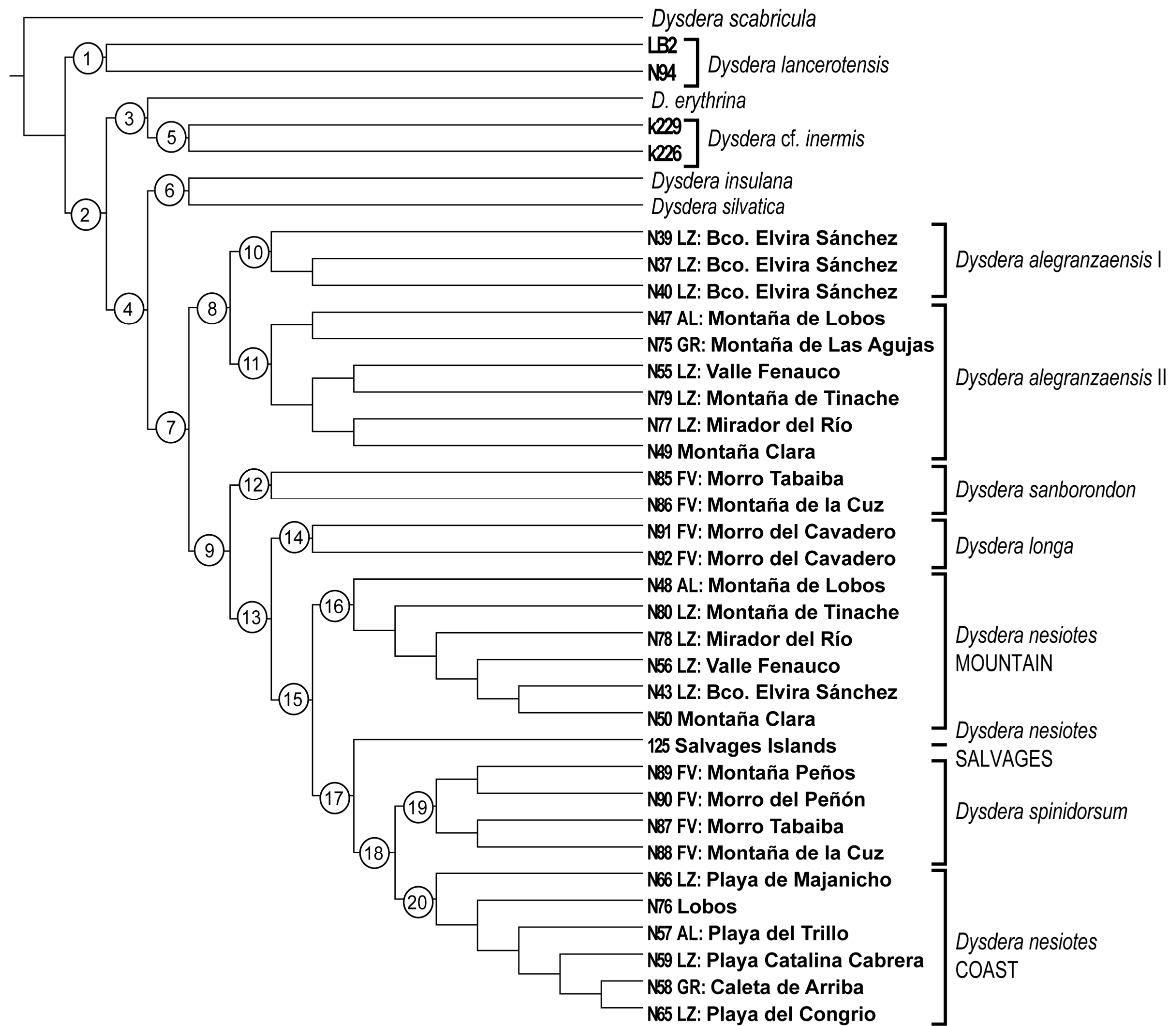

Fig. 3.4.- Strict consensus tree of two trees of 3363 steps (CI= 0.54, RI=0.74) resulting from uniformly weighted parsimony analysis of matrix M2 (see text for details). Refer to Table 3.3 for clade support values across all phylogenetic analyses performed. AL: Alegranza, FV: Fuerteventura, GR: La Graciosa, LZ: Lanzarote.

The model of evolution that best fit the data according to the AIC criterion was the GTR+I+Γ, with the following parameter values: base frequencies A=0.2602, C=0.1784, G=0.2216 and T=0.3398; rate matrix: A/C= 2.2132, A/G= 6.6654, A/T= 2.8343, C/G= 1.1445, C/T= 7.6508, G/T= 1.0), proportion of invariable sites= 0.5289, Γ distribution shape parameter= 0.8222. A heuristic search using maximum likelihood with ten random addition of taxa replicates resulted in one tree of score

-ln L=20201.76041 (not shown). Maximum likelihood bootstrap proportions were estimated following the same protocol as for M1 and are listed in Table 3.3. Two independent runs (1500000 generations each) were performed of Bayesian inference analysis with unlinked GTR+I+Γ models for each gene partition and Mk model for the gaps codes as absence/presence. Posterior probabilities after removal as burn-in of the first 60000 generations are summarized in Table 3.3. Results among different inference methods were largely congruent, and most of the clades recovered were supported by high support values. All analyses supported monophyly of the eastern Canarian species, except *D. lancerotensis*. Both model-based analyses supported *D. alegranzaensis* as the sister lineage to the remaining Eastern Canaries clade species, in agreement with one of the most parsimonious trees.

All analyses split specimens identified as *Dysdera nesiotes* into three different lineages: a clade including specimens from coastal localities sister to *Dysdera spinidorsum*, a single specimen from Salvages Islands, sister lineage to the former clade and a group of specimens from mountain localities in Lanzarote and the Chinijo archipelago, sister group to the clade including remaining *D. nesiotes*, and *D. spinidorsum*. Finally, all simultaneous analyses supported *Dysdera longa* as the sister species to the *D. nesiotes* + *D. spinidorsum* clade. Although all analyses supported monophyly of *D. alegranzaensis* samples, model-based analyses identified two deeply divergent lineages. Independent analyses of the mitochondrial and nuclear partitions revealed several instances of conflict between the two data sets, which the ILD test subsequently revealed to be significantly incongruent (P=0.01). The most parsimonious trees of the nuclear partition (645 tree of 620 steps) support *D. longa* and *D. alegranzaensis* as sister species and suggest a close relationship of two *D. nesiotes* specimens with *D. sanborondon* albeit with very low support. Topologies of most parsimonious mitochondrial trees (ten trees, 2702 steps) mostly mirrored those of the analysis of the complete data matrix. Examination of the partition Bremer supports in the combined analyses shows that only nodes 13 and 16 receive a net negative support (-0.6 and -1.5, respectively) from one of the genome partitions (nuclear), in agreement with detected incongruence.

The optimal reconstruction in DIVA, based on the assumption that all or any of the present areas may correspond to the ancestral distribution, requires eight dispersal steps (exact solution). Only two of these events involved exchanges between the two major islands, in both cases from Fuerteventura to Lanzarote. The first event resulted in the mountain lineage of *D. nesiotes* in Lanzarote, while the second event originated populations of the coastal lineage of *D. nesiotes* in Lanzarote and the Chinijo Archipelago. A vicariant split between Lanzarote, plus Archipelago Chinijo, and Fuerteventura is inferred at the base of the tree.

Lineage age estimation

The topology of the maximum likelihood analyses of M2, which was largely congruent with the trees obtained from the Bayesian analyses and compatible with one of the two most parsimonious trees, was selected to estimate time of divergence of the different eastern Canarian lineages. Branch lengths were estimated using exclusively the mitochondrial data set, which was more complete and facilitated comparison of substitution rates with other estimates available in the literature. The model that best fit the mitochondrial data forcing the selected topology was estimated with MODELTEST (GTR+I+Γ) and subsequently implemented in PAUP* to estimate maximum likelihood branch lengths with the preferred topology constrained. A cross validation procedure run in the R8S program selected the penalized likelihood as the more appropriate method to calculate the divergence time of the data and to set the smoothing factor to λ= 316.23.

The ages of the nodes were then estimated with the aforementioned method and smoothing values using the Truncated-Newton method after removal of the outgroup taxon and the zero-length branch taxa N37 and N40, and fixing the ages of divergence of the populations of *D.* cf. *inermis* on both sides of the Straits of Gibraltar to 5.3 Ma (see Material and methods). The chronogram is shown in Figure 3.5 and the absolute ages and maximum, minimum, mean and standard errors obtained from the bootstrap matrices for the different nodes are listed in Table 3.4.

The average rate of substitution estimated was 0.00873 per site per million years, corresponding to a pairwise divergence of 1.75%, slightly slower than the rates reported in the literature for arthropod mitochondrial genes, which range form 2% (DeSalle *et al.*, 1987) to 2.3% (Brower, 1994), and less than half the value reported for other spiders, including the mygalomorph genus *Aptostichus* (4 %) (Bond *et al.*, 2001) and the Hawaiian jumping spider genus *Havaika* (5.3 %) (Arnedo & Gillespie, 2006).

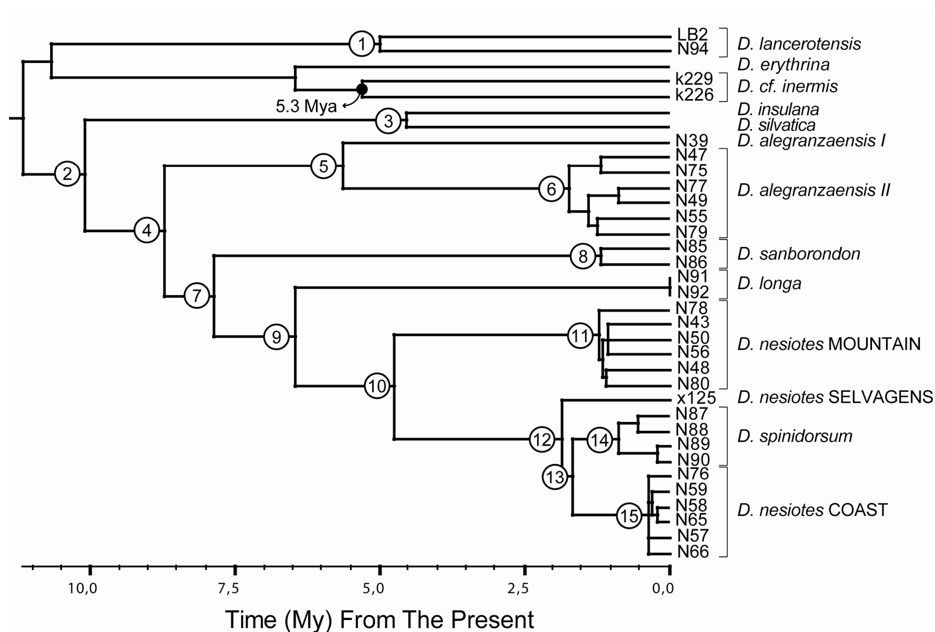

Fig. 3.5.- Chronogram of the Eastern Canarian *Dysdera* lineages and selected outgroups obtained with the penalized likelihood method (see text for details). Absolute clade ages were estimated by fixing the split of the *D.* cf. *inermis* lineages at both sides of the Straits of Gibraltar to 5.3 Ma. Refer to Table 3.3 for actual ages and confidence intervals.

Table 3.4.- Estimated lineage age (Age) and inferred confidence intervals fixing age of split of *trans*-Gibraltar populations of *D.* cf. *inermis* species to 5.3 Ma.

		Bootstrap estimates			
Lineage	**Age**	**Min**	**Max**	**Mean**	**Std. dev**
1	5.01	1.75	3.8	2.77	0.34
2	10.1	7.34	11.09	8.93	0.84
3	4.53	4.9	8.52	6.36	0.79
4	8.71	5.56	9.13	7.05	0.71
5	5.63	2.89	5.22	3.78	0.43
6	1.74	0.76	1.37	1.05	0.13
7	7.86	4.42	7.62	6.03	0.58
8	1.19	0.62	1.33	0.9	0.145
9	6.48	3.46	5.98	4.6	0.5
10	4.76	2.52	4.43	3.34	0.41
11	1.2	0.49	1.04	0.68	0.1
12	1.86	1.02	2.79	1.64	0.32
13	1.66	0.71	1.73	1.15	0.21
14	0.87	0.27	0.75	0.45	0.08
15	0.37	0.15	0.37	0.24	0.04

Lineage numbers as shown in Fig. 3.5.
SD: standard deviation.

Diversification rates

The Kendall-Moran estimate of the speciation rate from the chronogram is 0.13± 0.003 species per million years. Diversification rates are constant among the different lineages (B1= 95% confidence intervals cutoff for eight taxa 3.28-4.83). The lineage-through-time plot estimated from the chronogram is congruent with a reduction of the diversification rates in the Eastern Canarian clade towards the present (Fig. 3.6). This observation is confirmed by the negative values reported for the γ statistic (γ= -1.337), although the observed γ is above the critical value (-1.645, one-tailed test) suggesting that diversification rates do not depart significantly from a model of constant rates through time (P=0.09). On the other hand, application of the Birth-Death likelihood approach identified the rate variable logistic density-dependant as the model that best fit the data, but again failed to find significant departure (P=0.117) from a constant rate model (Table 3.5).

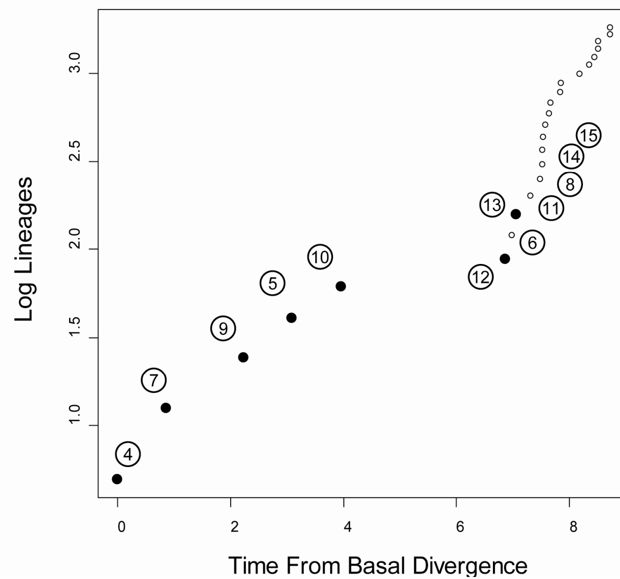

Fig. 3.6.- Lineage through time plot of the Eastern Canarian *Dysdera* lineages, numbers refer to node numbers in chronogram above. Black dots correspond to interespecific splits and open dots to within species divergences.

The lineage through time plot shows that speciation events took place more than 4 Ma, except for the split of the *D. nesiotes* lineage of Salvages and the separation of *D. spinidorsum* and the coastal lineage of *D. nesiotes*. It could be argued, however, that the colonization of the Salvages islands by *D. nesiotes* should not be included in the diversification analyses given that the event is not directly related to events driven by climatic and geological changes in the eastern Canaries. Removal of the Salvages lineage yielded marginally significant values of the γ statistic (γ= -1.658). The logistic density dependant model was the model that best fit the data as before, although this time with a significant departure from a constant rate model (*P*=0.04) (Table 3.7).

Table 3.5.- Results of fitting six birth-death models to the Eastern Canarian *Dysdera* lineages, with (eight lineages) or without (seven lineages) considering Salvages lineage.

	Model						
	Pure birth constant	**Birth-death constant**	**DDX**	**DDL**	**Yule 2 rate**	**Birth-death variable**	***p***
8 lineages							
Log-likelihood	-9.657	-9.657	-7.881	-7.210	-7.920	-7.766	
AIC	21.313	23.313	19.762	18.420	21.839	23.533	
ΔAIC	2.893	4.893	1.343	0.000	3.420	5.113	0.117
7 lineages							
Log-likelihood	-9.275	-9.275	-7.044	-4.972	-7.498	-7.334	
AIC	20.551	22.551	18.088	13.943	20.997	22.668	
ΔAIC	6.608	8.608	4.145	0.000	7.054	8.725	0.047

ΔAIC: difference in Akaike information criterion (AIC) scores between each model and the overall best fit model. DDX: exponential density-dependant model, DDL: logistic density-dependant model. *P*-values were derived from 1000 trees generated under the pure birth process, using the same number of lineages and the estimated pure speciation value.

Analysis of morphological variation

Measurements were taken for five males and five females of each of the species included in the Eastern Canarian clade, except for the species *D. sanborondon* and *D. longa* for which only 2 males of each species were available. The two divergent lineages of the species *D. alegranzaensis* and the mountain and coastal lineages of *D. nesiotes* (only one specimen of the Selvages lineage was available) revealed by the molecular phylogenetic analyses, were tentatively considered as different species in order to investigate whether molecular differentiation in these lineages was further supported by quantitative morphological differences. A Krustal-Wallis test found no significant morphological differences between sexes in most of the species. Exceptions included fe1 in *D. spinidorsum* and lineage I of *D. alegranzaensis*, and Esc and F in *D. spinidorsum.* Therefore, we rejected sexual dimorphism and interpreted significant differences among variables as random sampling variability. Consequently, all individuals of each species were pooled together for subsequent analyses. The Krustal-Wallis test also showed significant interspecific differences in all morphological variables. Differences in body size between lineages were significant in all comparisons

performed, except for the lineage pairs *D. alegranzaensis* lineage II and the mountain lineage of *D. nesiotes* (Z=0.459; *P*=0.65), *D. longa* and *D. alegranzaensis* lineage I (Z=1.859; *P*=0.063), and *D. spinidorsum* and the coastal lineage of *D. nesiotes* (Z=0.178; *P*=0.859). The first PCA axis accounted for 29.46% of the variance and was mostly associated to cheliceral measurements (Q1 and F). The second PCA axis explained 28.76% of the variance and was related to appendage length (fe1 and mt4). This second axis clearly separated the coastal *D. nesiotes* from all remaining species (Fig. 3.7).

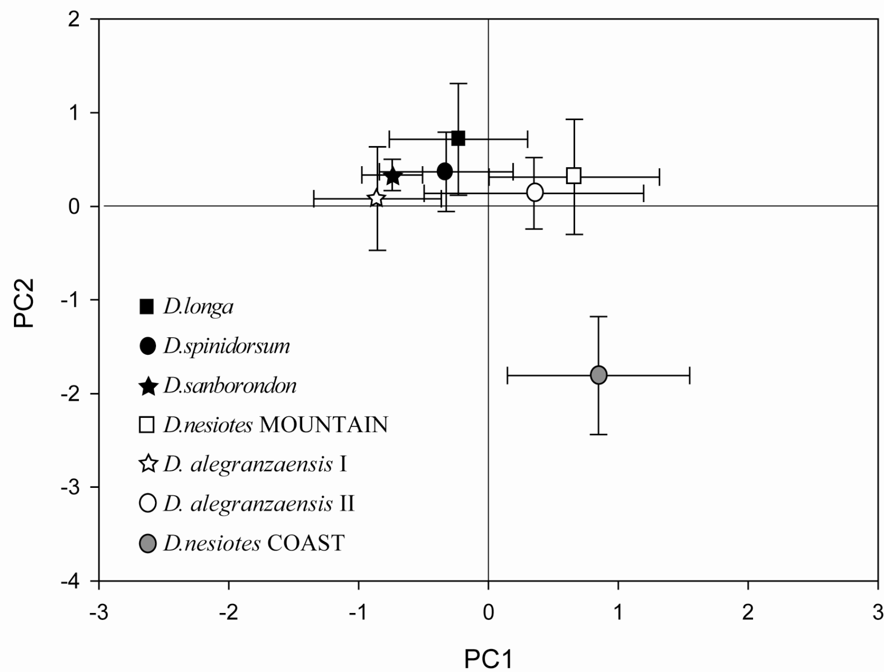

Fig. 3.7.- Principal component analysis: Plot of the first two discriminant axes with the 95% of score variance on each axes.

Eastern Canarian *Dysdera* lineages were divided into four groups based on 80% of Bray-Curtis similarity among individuals (Fig. 3.8). These groups are mostly related to body size and include the large species (*D. longa*, *D. spinidorsum*, and lineage I of *D. alegranzaensis*); the medium-size species (lineage II of *D. alegranzaensis* and *D. nesiotes* mountain populations) and the small species (*D.*

sanborondon). The coastal *D. nesiotes* constituted an independent group probably as a result of their significantly longer mt4, as revealed in the PCA analysis. Two out of the three cases of species with fully overlapping distributions (sympatric species) were included in different morphological similarity groups (*D. spinidorsum* versus *D. sanborondon* and *D*. *alegranzaensis* lineage I versus *D. nesiotes*). However, the fully sympatric lineage II of *D. alegranzaensis* and the mountain populations of *D. nesiotes* were grouped together with more than 85% of Bray-Curtis similarity.

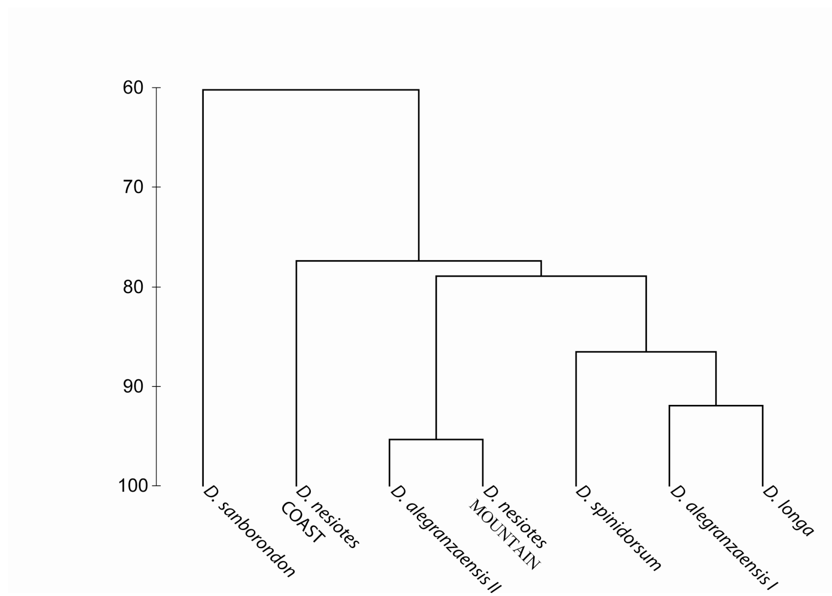

Fig. 3.8.- Dendrogram representing the species similarity scores derived from measurements estimated across a hierarchical agglomerative cluster using the Bray-Curtis distance.

DISCUSSION

On the origins of Canarian Dysdera

The results of the present study unambiguously support the hypothesis that current species of the spider genus *Dysdera* inhabiting the Canary Islands belong to three well-defined lineages: the Western-Central clade, the Eastern clade and the single species *D. lancerotensis*. The monophyly of the eastern Canarian species except *D. lancerotensis* had already been advanced by Arnedo *et al.,* (2001).

Our phylogeny also provides strong support for the monophyly of all endemic species from the central and the western Canaries, rejecting former proposals that either included some continental species in a Western-Central Canarian clade, or considered the Tenerifean species *D. unguimmanis* an independent lineage (Arnedo *et al.*, 2001). Unfortunately, our data failed to resolve relationships between the Western-Central and Eastern Canarian clades. Their monophyly is only weakly supported in the maximum likelihood analysis, but topological tests did not find significant differences between the trees that supported and the ones that rejected monophyly of the two clades. Independent colonization of the western and eastern Canary Islands has been suggested for the carabid beetles of the genus *Calathus* (Emerson, Oromí & Hewitt, 2000b), and for geckos of the genus *Tarentola* (Nogales *et al.*, 1998; Carranza *et al.*, 2000) and should not be discarded for the scincid genus *Chalcides* (Brown & Pestano, 1998).

Our results demonstrate that, with a single exception, the outstanding diversity of *Dysdera* in the Canary Islands is the result of local diversifications. Macaronesian plant communities had been traditionally considered relicts of former forests that would have extended throughout the Mediterranean region during the Tertiary and subsequently lost as a consequence of Plio-Quaternary climatic changes (Cronk, 1992). The current opinion based on molecular evidence, however, is that the Macaronesian region is a composite of paleoendemics derived from ancient Tertiary flora and a considerable proportion of neoendemics resulting from more recent colonizations (Helfgott *et al.*, 2000; Francisco-Ortega *et al.*, 2001; Carine *et al.*, 2004). In the present study, model-based analyses systematically recovered a clade that included the bulk of species sampled from the Iberian Peninsula and Morocco, which suggests that colonization of the Canaries predated diversification of most of present-day western Mediterranean *Dysdera* species. In addition, the age estimate of the most recent common ancestor of the Eastern clade points towards a Miocene origin of the group, concomitantly with subtropical conditions of weak climatic seasonality and little drought or temperature stress in the Mediterranean. In contrast, *D. lancerotensis* is part of the Ibero-Moroccan group and, according to age estimates, colonized the islands in Plio-Pleistocene times, during the onset of the present highly seasonal, summer-dry climate of the

Mediterranean Basin (Palamarev, 1989). A similar pattern of secondary recent invasion of the eastern Canaries has also been suggested for philodromid spiders (Muster, Bosmans & Thaler, 2007).

Phylogenetic structure of the Eastern Canarian* Dysdera *clade*: *cryptic diversity uncovered

The ILD test found significant incongruence between mitochondrial and nuclear partitions, although the simultaneous analysis of all genes (M2) yielded fully resolved trees with high support for relationships among eastern Canarian endemics. Only one of the incongruent clades between the separated nuclear and mitochondrial gene trees, the sister group relationship of *D. longa* and *D. alegranzaensis* reported in analyses on nuclear genes, was highly support. Stochastic sorting of ancestral polymorphisms (Pamilo & Nei, 1988; Wu, 1991), hybridization (Moore, 1995) or differences in analytical and methodological procedures (Brower, DeSalle & Vogler, 1996) may account for incongruence between nuclear and mitochondrial markers. The *ITS-2* and *H3* genes both support the sister group relationship of *D. longa* and *D. alegranzaensis*, independently (in the *28S* the position of *D. longa* is ambiguous). The fact that these two genes are unlinked in the genome suggests that incongruence with the mitochondrial data is due to differential lineage sorting. The mitochondrial trees are more congruent with morphology than the trees obtained from the nuclear partitions, especially with regard to the monophyly of species, which seems to discard the occurrence of mitochondrial introgressions. As expected, the PBS values of the combined analyses show that the net contribution of the nuclear partition is negative for the two incongruent clades, although the values are very low. In fact, most PBS values of each nuclear gene are either 0 or positive (see Table 3.3). Hence, we consider the apparent incongruence among the nuclear and mitochondrial partition an artifact, and favor the topology of the simultaneous analyses of all genes as the best current estimate of the phylogeny of the Eastern clade.

DNA sequence data is increasingly being used to test traditional, morphology-based taxonomies, and it represents a valuable tool to identify cryptic species (Wiens & Penkrot, 2002). Our study revealed a series of well-supported

haplotype groupings that are concordant with geography and hence delimit independent population lineages, overlooked in previous taxonomic studies. The paraphyly of the species *D. nesiotes* with regard to *D. spinidorsum* had already been found by Arnedo *et al.,* (2001). Thanks to the more exhaustive sampling, however, we can now identify three distinct lineages with non-overlapping distributions among *D. nesiotes* haplotypes. These lineages correspond to those haplotypes found in mountain habitats of Lanzarote and the Chinijo archipelago, those found in coastal habitats of northern Fuerteventura, Lanzarote and the Chinijo archipelago and, lastly, the single haplotype from Salvages, respectively. Unexpectedly, we also found evidence for two deeply divergent lineages in the species *D. alegranzaensis*, with between-lineage divergence being higher than interspecific divergence observed among morphologically well-differentiated *D. nesiotes* and *D. spinidorsum*. Although both lineages were collected in Lanzarote, they do not co-occur, lineage I being restricted to a single locality in the northern part of the island while lineage II is widespread in the remaining localities sampled in Lanzarote and the Chinijo archipelago.

Most of the newly identified lineages can be further diagnosed by ecological and morphological traits, which provided stronger evidence for their species status (Avise & Ball, 1990; Sites & Crandall, 1997; Wiens & Penkrot, 2002). The morphological analyses showed that the two lineages of *D. alegranzaensis* were clearly segregated in size (lineage I being significantly larger than lineage II) and that there are minor differences in male and female genitalia as well. In a recent taxonomic revision of the eastern Canarian *Dysdera* Arnedo *et al.,* (2000) pointed out the presence of an unusual genital feature in one female specimen of *D. nesiotes*. We have now confirmed that the type of vulva shape in question characterizes the coastal lineage of *D. nesiotes*. The habitat of the coastal *D. nesiotes* lineage, the intertidal zone in cobble beaches, is unique among the species of the genus. It represents the only case known so far of ecological shift reported in Canarian *Dysdera* along with the morphological adaptation to the underground environment exhibited by some species in the islands of Tenerife and La Palma (Arnedo & Ribera, 1999; Arnedo *et al.*, 2001). The morphometric analyses showed that the coastal lineage stands apart from all other eastern Canarian species by

having longer back legs (more precisely longer mt4). The length of the fourth leg is directly related to running speed (J. Moya-Laraño, pers. comm.), although the data at hand is still too sparse to address possible relationships between long back legs and the particular ecology of the lineage. The third lineage of *D. nesiotes* is represented by a single specimen collected from the Salvages Islands, a small archipelago 165 km north of the Canary Islands. The levels of morphological differentiation of this lineage remain to be investigated with the inclusion of more specimens, although neither genitalic nor morphometric differences were apparent when compared to the *D. nesiotes* mountain lineage. Close phylogenetic links between the eastern Canaries and the Selvages have been reported in the tenebrionid genus *Nesotes* (Rees *et al.*, 2001), and there are additional examples of shared endemisms in spiders and beetles between the two groups of islands (Arnedo *et al.*, 2000). Although Selvagem Grande is thought to be several million years old (Portugal-Ferreira, Macedo & Ferreira, 1998), the islands probably underwent a fairly recent period of submergence, most of the present subaerial land being the result of Quaternary volcanic activity (Bravo & Coello, 1978). Age estimates of the split of the Selvages lineage agrees with an early Pleistocene colonization of these islands by the Eastern Canarian clade.

Biogeographic patterns and community assembly in the eastern Canaries

The Canaries have been recurrently connected by eustatic sea-level changes, which probably explain the presence of the same species in Lanzarote and the Chinijo archipelago. Nevertheless, the two main islands harbor exclusively eastern Canarian *Dysdera* endemic species, suggesting that factors other than sea level may have prevented dispersal of species across the islands. For example, the species *D. longa* is restricted to the mountain tops of the Jandía peninsula, at the southernmost part of Fuerteventura, which is separated from other mountain ranges populated by sibling species by a low elevation isthmus (<100 m) covered by aeolian sands, which may have acted as an effective ecological barrier. Notably, the only species shared by the two main islands, the coastal *D. nesiotes* lineage and *D. lancerotensis*, are usually found in low elevation or seashore localities, suggesting that ecological barriers have indeed prevented dispersal of the species inhabiting higher elevations. DIVA biogeographical reconstruction suggested eight dispersal

events, only two of which involve the two major islands, along a vicariant split at the base of the tree. Similar patterns of differentiation between Lanzarote and Fuerteventura taxa have been suggested for several other invertebrate terrestrial lineages including beetles of the genera *Calathus*, *Paradromius* (Machado, 1992) and *Macrocoma* (Machado & Oromí, 2000), spiders of the genera *Spermophorides* (López-Mercader, 2005) and *Scotognapha* (Platnick, Ovtsharenko & Murphy, 2001) and *Napaeus* land snails (M. Ibáñez pers. comm.), and have also been reported in the eastern Canarian plant *Matthiola bolleana* (Brassicaceae) (Sánchez Doreste *et al.*, 2006).

In adaptive radiations the patterns of eco-morphological and behavioral divergence are usually related to the geographical overlap of lineages (Losos *et al.*, 1998; Schluter, 2000; Gillespie, 2004; Kozak *et al.*, 2005). The Eastern Canarian clade shows contrasting patterns of community composition. Some species display a positive relationship between eco-morphological divergence and geographic overlap, as would be expected if divergence promotes the coexistence of lineages (Kozak *et al.*, 2005). For example, *D. sanborondon* range fully overlaps with that of *D. spinidorsum* in Fuerteventura, and the two species are clearly separated by their body size. The same holds for the mountain lineage of *D. nesiotes* and *D. alegranzaensis* lineage I, in Lanzarote. Conversely, the lineage II of *D. alegranzaensis* and the mountain lineage of *D. nesiotes*, which are fully sympatric and mostly syntopic across Lanzarote and the northern islets, are undistinguishable on the basis of somatic morphological and the morphometric analysis failed to differentiate between them. The two species are not sister taxa and clearly diagnosable using genitalic characters. Although information on behavior and phenology of these species is still very sparse, there is at present no evidence for eco-ethological segregation of these two species. Similar patterns of absence of morphological differentiation in diagnosable species with overlapping distributions have been reported (Scarabaeidae beetles of the genus *Onthophagus:* Pizzo *et al.*, 2006; deer-mice of the genus *Peromyscus:* Zheng, Arbogast & Kenagy, 2003) and are generally interpreted as the results of secondary contact after recent population expansion. Lanzarote was originally composed by two isolated massifs subsequently connected by episodic cycles of volcanic activity that extended until

the present. This turbulent geological history has offered ample opportunities for the isolation and subsequent secondary contact of the local *Dysdera* lineages. *Dysdera longa* represents another instance of incongruent relationship between morphological divergence and species coexistence. It is the largest species of the eastern Canaries, but has a fully allopatric distribution with all the other species, except *D. lancerotensis*. *Dysdera longa*, whose geographically closest relatives share very similar if not identical habitats, which appears to discard local adaptation as an explanation for larger size. Alternatively, its larger size could have evolved as a result of former intraspecific competition, and subsequent extinction of the sympatric congeneric (see below). The clear morphological differentiation of *D. longa* and its two geographically closest species seems to support the latter explanation.

The role of extinction in shaping diversification patterns in the Eastern Canarian* Dysdera *clade

The progressive dryness of the eastern Canaries has affected the distribution of many endemic arthropods. There are many examples of lineages that have diversified in the western Canaries, but are not present in Fuerteventura and Lanzarote. These include *Steganacarus* oribatid mites, Cixiidae planthoppers, cockroaches of the genus *Loboptera*, *Anataelia* and *Guanchia* earwigs, *Hydroporus* dytiscidae beetles or *Tarphius* colydiid beetles among others (Izquierdo *et al.*, 2004). In some lineages, eastern Canarian endemics are more closely related to continental taxa than they are to western endemics (see above). The species *D. lancerotensis* supports this pattern: it has colonized the eastern Canaries independently of the remaining species and it is closely related to a highly diversified North African lineage. A possible preadaptation to more arid environments would explain the widespread distribution of this species in the eastern islands. High levels of tolerance to a wide range of environmental conditions have been reported in the closely related species *D. crocata* (Cooke, 1968). A third group of species includes those with close relatives widespread in the western Canaries, but restricted to higher altitudes in the eastern islands that have been interpreted as relict distributions (Juan *et al.*, 2000). Examples of these

lineages are the carabid *Trechus detersus* (Contreras-Díaz *et al.*, 2007) and the Eastern Canarian *Dysdera* clade (Arnedo *et al.*, 2000).

The present analysis of the diversification rates of the Eastern Canarian clade provides support for the suggestion by Arnedo *et al.,* (2000) that the relative lower number of endemics of the eastern Canaries is explained by an increase in extinction rates driven by geological and climatic changes. The speciation rate estimated for the clade (0.13) is about the average estimated rate of arthropod speciation (0.16) (Coyne & Orr, 2004), but significantly slower than values reported for spider radiations in Hawaii, including the genus *Tetragnatha* (minimum estimated value 0.35) (Gillespie, 1999), sheet-web spider *Orsonwelles* (0.41) (Hormiga, Arnedo & Gillespie, 2003) or the jumping spider *Havaika* (0.36) (Arnedo & Gillespie, 2006). Both the gamma statistic and the birth-likelihood approaches suggest that the diversification rates of the Eastern Canarian lineage have decreased through time, although significant departure from constant rates was only marginally attained under certain circumstances (birth-death likelihood approach excluding Salvages lineage). Rate variable models that assumed changes in diversification rates at specific time points were rejected in favor of logistic density dependant models, which is congruent with a more or less constant process of island destruction and climatic degradation. As suggested above, extinction provides additional explanation for the presence of non-overlapping species with segregated sizes.

It should be borne in mind, however, that confirmation that the Eastern Canarian lineage has decreased diversification rates towards the present does not necessarily imply that extinction rate, either by habitat destruction or degradation, has increased. Alternatively, speciation rate may have decelerated as a result of the reduction in suitable area, since it has been shown that species with larger geographical ranges speciate faster (Rosenzweig, 1995).

Geological and climatic changes may not be the only factors accountable for decrease in diversification. Competitive exclusion driven by the more recently arrived *D. lancerotensis* may have either prompted or worsened relictualization of the Eastern lineage. *D. lancerotensis* is more widely distributed than any other

eastern Canarian endemic, and it has been collected syntopically with all the species. We propose that these distributional patterns are more compatible with a scenario of occupancy of previously emptied areas, prompted by the wider ecological tolerance of the newcomer and by a noncompeting coexistence with other species. Experimental data will have to be gathered to further investigate the interactions of *D. lancerotensis* with the Eastern Canarian clade.

One of the most surprising findings of our study, given the apparent evolutionary decadence of the lineage, was the identification of a very recent speciation event (i.e younger than some intraspecific population divergences) corresponding to the coastal lineage of *D. nesiotes*, which constitutes one of the very few examples of ecological shift in the whole genus. This result is extremely notable in two regards. First, it constitutes yet another example of morphological differentiation with little genetic divergence in a neutral marker (Orr & Smith, 1998). This is a widespread pattern in situations of a strong selective pressure (e.g. in ecological adaptation or sexual selection; Dieckmann, 2004). The decoupling of morphological and genetic differentiation has major relevance in the context of the growing popularity of the barcode-of-life approaches (Hebert *et al.*, 2003) to species delimitation, and illustrates the deficiencies of an identification method based solely on a single molecular marker (Bond, 2004; Paquin & Hedin, 2004; DeSalle, Egan & Siddall, 2005; Meier *et al.*, 2006; Will, Mishler & Wheeler, 2005). Second, the coastal lineage of *D. nesiotes* may shed some light on the first steps towards the evolution of relictual, nonspeciose lineages. Islands are frequently considered as a refuge for relicts. For example, the spider *Doryonychus raptor* is confined to very small pockets of forest on Kauai (the oldest of the main Hawaiian Islands) and displays an extremely specialized foraging behavior (Gillespie, 1991). *Doryonychus raptor* is the only representative of a lineage distantly related to the Hawaiian species of the genus *Tetragnatha*, a clade which constitutes one of the most acclaimed examples of adaptive radiations in spiders (Gillespie, Croom & Palumbi, 1994). It has been suggested that *D. raptor* may be a relict of a previously diverse radiation of *Tetragnatha* (Gillespie *et al.*, 1994; Roderick & Gillespie, 1998). Such accumulation of autapomorphies among older clades appears to occur in a number of Hawaiian arthropod groups as well (Asquith, 1995). The coastal lineage

of *D. nesiotes* may illustrate the first stages of relictualization, as observed in present-day Hawaiian *D. raptor*. With time, continuous dismantling of the islands and increasing aridification would drive hygrophilous mountain species to complete extinction. Concomitantly, an ecological shift of coastal *D. nesiotes* to a new, more permanent environment would spare it from the fate of extinction and would help it to skip competition with congenerics arriving at a later date. Our hypothesis on the origin of relictual, nonspeciose lineages could be further tested by a thorough bibliographical search on the association of island relict taxa to ecological shifts.

In conclusion, the present study represents the first attempt to illustrate temporal and geographic patterns of diversification in old volcanic islands that have undergone severe physical and climatic modifications. We provide direct and circumstantial evidence of decreasing diversification rates in a lineage of spiders endemic to the highly transformed eastern Canaries, compatible with increasing extinction rates. Incongruent patterns of morphological differentiation and species co-occurrence offer additional support for the involvement of extinction. Despite a general trend towards species impoverishment, we identify recent speciation events related both to colonization of a separate island system and to a major ecological shift. Based on this pattern, we hypothesize that evolution of new adaptations in decaying lineages may explain the origin of nonspeciose relict lineages on islands.

APPENDIX

Appendix 3.1.- Specimens included in the present study and genbank accession numbers of the different genes sequenced. In column *16-L1-nad1*, sequence accession numbers with asterisk indicate sequences only for *16S* fragment, those with two numbers refer to complete fragments obtained in two different rounds.

Voucher number	Code	Specie	Locality	Genes				
				cox1	***16S-L1-nad1***	***28S***	***H3***	***ITS***
Eastern Canaries								
NMH429	N47	*Dysdera alegranzaensis*	Montaña de Lobos. Alegranza	EF458132	EF458087	EU139759	EU139688	EU143814
NMH364	N49	*Dysdera alegranzaensis*	Montaña Clara	EU139610	EU139637		EU139689	EU143815
NMH424	N55	*Dysdera alegranzaensis*	Valle Fenauco. Yaiza. Lanzarote	EU139611	EU139638	EU139760	EU139690	EU143816
NMH449	N75	*Dysdera alegranzaensis*	Montaña de Las Agujas. La Graciosa	EU139612	EU139639	EU139761	EU139691	EU143817
NMH73	N77	*Dysdera alegranzaensis*	Mirador del Río. Haría. Lanzarote	EU139609	EU139640	EU139762	EU139692	EU143818
NMH462	N79	*Dysdera alegranzaensis*	Montaña de Tinache. Tinajo. Lanzarote	EU139613	EU139641	EU139763	EU139693	EU143819
NMH59	N37	*Dysdera alegranzaensis*	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139614	EU139659	EU139783	EU139712	EU143838
NMH60	N39	*Dysdera alegranzaensis*	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139631	EU139660	EU139784	EU139713	EU143839
NMH55	N40	*Dysdera alegranzaensis*	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139632	EU139661	EU139785	EU139714	
NMH163	N94	*Dysdera lancerotensis*	Morro del Cavadero. Jandía. Fuerteventura	EF458120	EF458086	EU139758	EU139687	EU143813
NMH51	LB1	*Dysdera lancerotensis*	Mirador del Río. Lanzarote	EF458112	EF458079	EU139756	EU139685	
NMH441	LB2	*Dysdera lancerotensis*	Caldera. Alegranza	EF458127	EF458080	EU139757	EU139686	EU143812
NMH168	N91	*Dysdera longa*	Morro del Cavadero. Jandía. Fuerteventura	EF458134	EF458090	EU139781	EU139710	EU143836
NMH169	N92	*Dysdera longa*	Morro del Cavadero. Jandía. Fuerteventura		EU139658	EU139782	EU139711	EU143837
NMH358	N57	*Dysdera nesiotes*	Playa del Trillo. Alegranza	EU139620	EU139647	EU139769	EU139700	EU143826
NMH451	N58	*Dysdera nesiotes*	Caleta de Arriba. La Graciosa	EU139621	EU139648	EU139770		EU143827
NMH356	N59	*Dysdera nesiotes*	Playa Catalina Cabrera. Famara. Lanzarote	EU139622	EU139649	EU139771	EU139701	
NMH447	N65	*Dysdera nesiotes*	Playa del Congrio. Papagayo. Lanzarote	EU139623	EU139650	EU139772	EU139702	EU143828
NMH490	N66	*Dysdera nesiotes*	Playa de Majanicho. Fuerteventura	EU139624	EU139651	EU139773	EU139703	EU143829
NMH572	N76	*Dysdera nesiotes*	Las Salinas. Lobos	EU139625	EU139652	EU139774	EU139704	

Voucher number	Code	Specie	Locality	Genes				
				cox1	*16S-L1-nad1*	*28S*	*H3*	*ITS*
NMH57	N43	*Dysdera nesiotes*	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139615	EU139642	EU139764	EU139695	EU143820
NMH428	N48	*Dysdera nesiotes*	Montaña de Lobos. Alegranza	EU139616	EU139643	EU139765	EU139694	EU143821
NMH369	N50	*Dysdera nesiotes*	Montaña Clara	EF458133	EF458088	EU139766	EU139696	EU143822
NMH425	N56	*Dysdera nesiotes*	Valle Fenauco. Yaiza. Lanzarote	EU139617	EU139644	EU139767	EU139697	EU143823
NMH398	N78	*Dysdera nesiotes*	Mirador del Río. Haría. Lanzarote	EU139618	EU139645		EU139698	EU143824
NMH476	N80	*Dysdera nesiotes*	Montaña de Tinache. Tinajo. Lanzarote	EU139619	EU139646	EU139768	EU139699	EU143825
NHM290	125	*Dysdera nesiotes*	Selvagem Grande, Salvages Islands	EU139634	EU139683*			
NMH50	N85	*Dysdera sanborodon*	Morro Tabaiba. Vallebrón. Fuerteventura	EF458135	EF458089	EU139775	EU139705	EU143830
NMH506	N86	*Dysdera sanborodon*	Montaña de la Cruz. Betancuria.Fuerteventura	EU139626	EU139653	EU139776	EU139706	EU143831
NMH049	N87	*Dysdera spinidorsum*	Morro Tabaiba. Vallebrón. Fuerteventura	EU139627	EU139654	EU139777	EU139707	EU143832
NMH494	N88	*Dysdera spinidorsum*	Montaña de la Cruz. Betancuria. Fuerteventura	EU139628	EU139655	EU139778	EU139708	EU143833
NMH78	N89	*Dysdera spinidorsum*	Cuchillete Montaña Peños. Fuerteventura	EU139629	EU139656	EU139779		EU143834
NMH114	N90	*Dysdera spinidorsum*	Morro del Peñón. Fuerteventura	EU139630	EU139657	EU139780	EU139709	EU143835
Western Canaries								
UB3034	darak20	*Dysdera arabisenen*	La Averejilla. Vega de San Mateo. Gran Canaria	AF244291	AF244198/EU139664	EU139786	EU139715	
UB3041	dback23	*Dysdera bandamae*	Barranco de Guayadeque, Agüimes. Gran Canaria	AF244286	AF244193*	EU139787	EU139716	
UB4787	dbrptk171	*Dysdera brevispina*	Bailadero, Sta. Cruz. Tenerife	AF244316	AF244227*		EU139717	
UB4013	dcapk103	*Dysdera calderensis*	Juan Adalid, Garafía. La Palma	AF244309	AF244218/EU139665	EU139788	EU139718	
NHM286	dchtx116	*Dysdera chioensis*	Cueva Honda de Güímar. Tenerife	EU068030	EU068067	EU139789	EU139719	
UB3199	dlink3	*Dysdera gomerensis*	Pista del Derrabado, Frontera. El Hierro	AF244318	AF244229/EU139670	EU139798	EU139729	
UB3170	dgutk93	*Dysdera guayota*	Roque del Conde, Adeje. Tenerife	AF244283	AF244190/EU139667	EU139792	EU139722	
UB4935	digtk9	*Dysdera iguanensis*	Cabezo del Tejo, Sta. Cruz. Tenerife	AF244279	AF244186 /EU139668	EU139793	EU139724	
UB4785	dintk6	*Dysdera insulana*	Bailadero, Sta. Cruz. Tenerife	AF244314	AF244225/EU139669	EU139796	EU139727	EU143841
NHM288	dlbtx114	*Dysdera labradaensis*	Cueva de Felipe Reventón.Icod de los Vinos. Tenerife	EU068040	EU068074	EU139797		
UB3103	dletk67	*Dysdera levipes*	Palo Blanco, Los Realejos. Tenerife	AF244295	AF244202*		EU139728	
UB2999	dveck21	*Dysdera liostethus*	Pico del Viento, Gáldar. Gran Canaria	AF244303	AF244212*	EU139819	EU139750	

Voucher number	Code	Specie	Locality	Genes				
				cox1	*16S-L1-nad1*	*28S*	*H3*	*ITS*
UB4827	dmatk10	*Dysdera macra*	Izaña, La Orotava. Tenerife	AF244300	AF244209/EU139671	EU139800	EU139731	
UB4795	dmotk97	*Dysdera montanetensis*	Las Raíces, El Rosario. Tenerife	AF244278	AF244183/EU139672	EU139802	EU139733	
UB3009	dpack22	*Dysdera paucispinosa*	Inagua, Mogán. Gran Canaria	AF244306	AF244215/EU139673	EU139805	EU139736	
UB3058	dtbck24	*Dysdera rugichelis*	Pinar de Tamadaba, Agaete. Gran Canaria	AF244293	AF244200*	EU139816	EU139747	
UB4155	drugk94	*Dysdera silvatica*	Barranco de Juel. Hermigua. La Gomera	AF244273	AF244177/EU139674	EU139808	EU139739	EU143842
UB2974	dtlck19	*Dysdera tilosensis*	Brezal del Palmital, Sta. Mª de Guía. Gran Canaria	AF244288	AF244195/EU139679	EU139817	EU139748	
UB4829	duntk32	*Dysdera unguimmanis*	Cueva Felipe Reventón, Icod de los Vinos. Tenerife	AF244284	AF244191/EU139680	EU139818	EU139749	
UB4796	dprtk95	*Dysdera verneaui*	Las Raíces, El Rosario. Tenerife	AF244319	AF244230*	EU139806	EU139737	
UB2985	dygck18	*Dysdera yguanirae*	Brezal del Palmital, Sta. Mª de Guía. Gran Canaria	AF244290	AF244197/EU139681		EU139751	
Continental								
NHM210	dravk234	*Dysdera atlantica*	10 km from Essaouira to Marrakech. Morocco	EU139635	EU139663	EU139807	EU139738	
UB2812	dcoik131	*Dysdera coiffaiti*	Levada do Cedro, entre Rb. Janela & Fanal, 600m. Madeira	AF244251	AF244161/EU139666			
NHM040	dgM2k246	*Dysdera crocata*	Monasterio del Paular, Madrid, Iberian Peninsula	EF458136	EF458096	EU139791	EU139721	
NHM255	diM1k226	*Dysdera cf. inermis*	4 km S Tanger. Morocco	EF458142	EF458092	EU139795	EU139726	
NHM061	diI1k229	*Dysdera cf. inermis*	Sierra Aracena. Huelva. Andalucía. Iberian Peninsula	EU068039	EU068073	EU139794	EU139725	
UBery105	deryk105	*Dysdera erythrina*	Sant Llorenç del Munt, Catalunya, Iberian Peninsula	AF244252	AF244162*	EU139790	EU139720	EU143840
CRBA519	dhunk298	*Dysdera hungarica*	Mt. Massif nr. Perevalnoye Vil., Crimea, Ukraine	EU139633	EU139662		EU139723	
NHM303	dluck213	*Dysdera lucidipes*	Ras el Ma, Morocco	EU068042	EU068060	EU139799	EU139730	
NHM216	dmauk231	*Dysdera mauritanica*	Essaouira. Bushland, Morocco	EF458138	EF458093	EU139801	EU139732	
NHM253	dmuck232	*Dysdera mucronata*	4 km S Tanger. Morocco	EU068044	EU068077	EU139803	EU139734	
NHM291	dninx107	*Dysdera nini*	Slovenia	EU068045	EU068062	EU139804	EU139735	
CRBA590	dscak294	*Dysdera scabricula*	Desert de les Palmes, València, Iberian Peninsula	EU068046	EU068078	EU139809	EU139740	
UB3279	dcsecm6	*Dysdera seclusa*	Ain-Kahla (1856m) Middle Atlas. Morocco	AF244248		EU139810	EU139741	
UB3275	dsMam3	*Dysdera sp. A*	Road Marrakesh to Ouarzazate. Atlas. Morocco	AF244244	AF244155/EU139675	EU139811	EU139742	
UB3276	dsMbm4	*Dysdera sp. B*	Road Marrakesh to Ouarzazate. Atlas. Morocco	AF244245	AF244156/EU139676	EU139812	EU139743	
UB3277	dsMcm5	*Dysdera sp. C*	Road Marrakesh to Ouarzazate. Atlas. Morocco	EF458139	EF458094*	EU139813	EU139744	

Voucher number	Code	Specie	Locality	Genes				
				cox1	***16S-L1-nad1***	***28S***	***H3***	***ITS***
UB3278	dsMdm7	*Dysdera sp. D*	Tizin-n-Âit Ouirra, 1550m. Morocco	AF244247	AF244158/EU139677	EU139814	EU139745	
UB3280	dsMfm8	*Dysdera sp. F*	Azrov (1350m) Atlas Mitjá. Morocco	AF244249	AF244159/EU139678	EU139815	EU139746	
UB3281	dsMh	*Dysdera sp. H*	River Draa's mouth. Morocco	AF244250	AF244160*			
Outgroups								
NHM299	hhomk31	*Harpactea hombergi*	Montseny. Iberian Peninsula	AF244233	AF244148*	EU139820	EU139752	
NHM94	rscuk250	*Rhode scutiventris*	Sierra Grazalema, Benamahoma, Cádiz, Iberian Peninsula	EU139636	EU139684*	EU139822	EU139754	
NHM284	ysilk158	*Dysderocrates silvestris*	Gračac (Km 55 ENE de Zadari). 650m. Croatia	AF244236	AF244151*		EU139755	
NHM300	gradk60	*Harpactocrates radulifer*	Vargañón, La Rioja. Iberian Peninsula	AF244235	AF244150/EU139682	EU139821	EU139753	

Appendix 3.2.- List of localities and Universal Transverse Mercator (UTM) coordinates sampled in the eastern Canaries and *Dysdera* species collected in each site.

Cod.	**Locality**	**UTM**	**Species collected**
1	Alegranza: Montaña de Lobos	645362/3252562	*D. alegranzaensis, D. nesiotes*
2	Alegranza: Playa del Trillo.	646426/3253714	*D. nesiotes*
3	Alegranza: Caldera	642964/3253161	*D. lancerotensis*
4	Montaña Clara	642257/3241988	*D. alegranzaensis, D. nesiotes, D. lancerotensis*
5	La Graciosa: Montaña de Las Agujas	646099/3238401	*D. alegranzaensis, D. lancerotensis*
6	La Graciosa: Montaña del Mojón	644183/3235717	*D. alegranzaensis, D. lancerotensis*
7	La Graciosa: Caleta de Arriba	646115/3235124	*D. nesiotes*
8	Lanzarote: Playa Catalina Cabrera. Famara	645337/3230454	*D. nesiotes*
9	Lanzarote: Mirador del Río. Haría	647351/3232337	*D. alegranzaensis, D. nesiotes, D. lancerotensis*
10	Lanzarote: Playa La Caleta. Haría	653108/3231640	*D. nesiotes*
11	Lanzarote: Bco. Elvira Sánchez. Valle de Malpaso. Haría	644280/3223378	*D. alegranzaensis, D. nesiotes*
12	Lanzarote: Playa de Teneza. Tinajo	625000/3217500	*D. nesiotes*
13	Lanzarote: Presa de Mala. Haría	648701/3220660	*D. alegranzaensis, D. nesiotes, D. lancerotensis*
14	Lanzarote: Pta. Pasitos. Mala. Haría	650244/3220530	*D. nesiotes*
15	Lanzarote: Montaña de Tinache. Tinajo	629498/3214460	*D. alegranzaensis, D. nesiotes, D. lancerotensis*
16	Lanzarote: Caleta del Mariscadero. Tinajo	620210/3215732	*D. nesiotes, D. lancerotensis*
17	Lanzarote: Playa de la Madera. Tinajo	619287/3215482	*D. nesiotes*
18	Lanzarote: Zonzamas. San Bartolomé	639277/3210251	*D. lancerotensis*
19	Lanzarote: Montaña Blanca. Tías	633081/3207027	*D. alegranzaensis, D. nesiotes*
20	Lanzarote: Valle Fenauco. Yaiza	619527/3201168	*D. alegranzaensis, D. nesiotes*
21	Lanzarote: Atalaya de Femés. Los Ajaches. Yaiza	620513/3199699	*D. alegranzaensis, D. nesiotes*
22	Lanzarote: Playa del Congrio. Papagayo.Yaiza	618331/3190733	*D. nesiotes, D. lancerotensis*
23	Lanzarote: Las Salinas. Puerto. Calero. Yaiza	626834/3199610	*D. nesiotes*
24	Lobos: Las Salinas	614248/3180093	*D. nesiotes*
25	Fuerteventura: Playa de Majanicho. Corralejo	609973/3180239	*D. nesiotes*
26	Fuerteventura: Playa del Esquinzo. La Oliva	595244/3168097	*D. nesiotes*
27	Fuerteventura: Morro Tabaiba. Vallebrón. La Oliva	603324/3163268	*D. sanborondon, D. spinidorsum*
28	Fuerteventura: Montaña de la Cruz. Betancuria	592440/3146407	*D. sanborondon, D. spinidorsum*
29	Fuerteventura: Cuchillete Montaña Peños. Tuineje	602890/3128409	*D. spinidorsum*
30	Fuerteventura: Morro del Cavadero. Jandía	562497/3107753	*D. longa, D. lancerotensis*

Codes as shown in Fig. 3.1.

REFERENCES

Akaike H. 1973. Information theory as an extension of the maximum likelihood principle. In Petrov BN and Csaki F, eds. Second International Symposium on Information Theory. Akademiai Kiado, Budapest.

Ancochea E, Brändle JL, Cubas CR, Hernán F, Huertas MJ. 1996. Volcanic complexes in the eastern ridge of the Canary Islands: the Miocene activity of the island of Fuerteventura. Journal of Volcanology and Geothermal Research 70: 183-204.

Arnedo MA, Gillespie RG. 2006. Species diversification patterns in the Polynesian jumping spider genus Havaika Proszynski, 2001 (Araneae, Salticidae). Molecular Phylogenetics and Evolution 41: 472-495.

Arnedo MA, Oromí P, Ribera C. 1996. Radiation of the genus Dysdera (Araneae, Haplogynae, Dysderidae) in the Canary Islands: The Western Islands. Zoologica Scripta 25: 241-274.

Arnedo MA, Oromí P, Ribera C. 2000. Systematics of the genus Dysdera (Araneae, Dysderidae) in the Eastern Canaries. Journal of Arachnology 28: 261-292.

Arnedo MA, Oromí P, Ribera C. 2001. Radiation of the spider genus Dysdera (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. Cladistics 17: 313-353.

Arnedo MA, Ribera C. 1997. Radiation of the genus Dysdera (Araneae, Haplogynae, Dysderidae) in the Canary Islands: The island of Gran Canaria. Zoologica Scripta 26: 205-243.

Arnedo MA, Ribera C. 1999. Radiation of the genus Dysdera (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. Journal of Arachnology 27: 604-662.

Asquith A. 1995. Evolution of Sarona (Heteroptera, Miridae): Speciation on geographic and ecological islands. In: Wagner WL and Funk VA, eds. Hawaiian Biogeography: Evolution on a hot spot archipelago. Washington and London: Smithsoinan Institution Press.

Avise J, Ball R. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surveys in Evolutionary Biology 7: 45-67.

Baker RH, Yu X, DeSalle R. 1998. Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. Molecular Phylogenetics and Evolution 9: 427-436.

Baldwin BG, Crawford DJ, Francisco-Ortega J, Kim S-C, Sang T, Stuessy TF. 1998. Molecular phylogenetic insights on the origin and evolution of oceanic island plants. In: Soltis PS, Soltis DE and Doyle JJ, eds. Molecular Systematics of Plants II. Boston: Kluwer Academic Press. 410-441.

Baldwin BG, Sanderson MJ. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). Proceedings of the National Academy of Sciences 95: 9402-9406.

Barraclough TG, Vogler AP. 2002. Recent diversification rates in North American tiger beetles estimated from a dated mtDNA phylogenetic tree. Molecular Biology and Evolution 19: 1706-1716.

Beheregaray LB, Gibbs JP, Havill N, Fritts TH, Powell JR, Caccone A. 2004. Giant tortoises are not so slow: Rapid diversification and biogeographic consensus in the Galapagos.

Proceedings of the National Academy of Sciences 101: 6514-6519.

Blondel J, Aronson J. 1999. Biology and wildlife of the Mediterranean region. Oxford University Press: Oxford; New York.

Bond JE. 2004. Systematics of the Californian euctenizine spider genus Apomastus (Araneae: Mygalomorphae: Cyrtaucheniidae): the relationship between molecular and morphological taxonomy. Invertebrate Systematics 18: 361-376.

Bond JE, Hedin MC, Ramirez MG, Opell BD. 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider Aptostichus simus. Molecular Ecology 10: 899-910.

Bravo T, Coello J. 1978. Aportación a la geología y petrología de las islas Salvajes Contribución al estudio de la historia natural de las Islas Salvajes. Santa Cruz de Tenerife: Aula de Cultura de Tenerife. 15-35.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly Heliconius erato inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences 91: 6491-6495.

Brower AVZ, DeSalle R, Vogler A. 1996. Gene trees, species trees, and systematics: A cladistic perspective. Annual Review of Ecology and Systematics 27: 423-450.

Brown RP, Pestano J. 1998. Phylogeography of skinks (Chalcides) in the Canary Islands inferred from mitochondrial DNA sequences. Molecular Ecology 7: 1183-1191.

Carine MA, Russell SJ, Santos-Guerra A, Francisco-Ortega J. 2004. Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in Convolvulus (Convolvulaceae). American Journal of Botany 91: 1070-1085.

Carracedo JC. 1999. Growth, structure, instability and collapse of Canarian volcanoes and comparisons with Hawaiian volcanoes. Journal of Volcanology and Geothermal Research 94: 1-19.

Carranza S, Arnold EN. 2003. History of West Mediterranean newts, Pleurodeles (Amphibia: Salamandridae), inferred from old and recent DNA sequences. Systematics and Biodiversity 1: 327-338.

Carranza S, Arnold EN, Mateo JA, López-Jurado LF. 2000. Long-distance colonization and radiation in gekkonid lizards, Tarentola (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. Proceedings of the Royal Society Biological Sciences Series B 267: 637-649.

Clarke KR, Warwick RM. 1994. A framework for studying changes in community structure. Change in marine communities: An approach to statistical analysis and interpretation. Plymouth Marine Laboratory: Plymouth, UK.

Coello J, Cantagrel JM, Hernán F, Fuster JM, Ibarrola E, Ancochea E, Casquet C, Jamond C, Díaz de Terán JR, Cendrero A. 1992. Evolution of the eastern volcanic ridge of the Canary Islands based on new K-Ar data. Journal of Volcanology and Geothermal Research 53: 251-274.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46: 419-437.

Contreras-Díaz HG, Moya O, Oromi P, Juan C. 2007. Evolution and diversification of the forest and hypogean ground-beetle genus Trechus in the Canary Islands. Molecular Phylogenetics and Evolution 42: 687-699.

Cooke JAL. 1968. Factors affecting the distribution of some spiders of the genus Dysdera (Araneae, Dysderidae). Entomologist's monthly magazine 103: 221-225.

Coyne JA, Orr HA. 2004. Speciation. Sinauer Associates: Sunderland, Mass.

Cronk QC. 1992. Relict floras on Atlantic islands: patterns assessed. Biological Journal of the Linnean Society 46: 91-103.

Cunningham CW. 1997. Is congruence between data partitions a reliable predictor of phylogeny accurancy? Empirically testing an iterative procedure for choosing among phylogenetic methods. Systematic Biology 46: 464-478.

Deeleman-Reinhold CL, Deeleman PR. 1988. Revision des Dysderinae. Tijdschrift voor Entomologie 131: 141-269.

Demenocal PB. 2004. African climate change and faunal evolution during the Pliocene-Pleistocene. Earth and Planetary Science Letters 220: 3-24.

Denis J. 1945. Description d'araignées nordafricaines. Bulletin de la Société d'Histoire Naturelle de Toulouse 79: 41-57.

Denis J. 1961. Notes d'aranéologie marocaine IX. Les araignées du Maroc.1. Orthognatha et Haplogynae. Bulletin de la Société des Sciences Naturelles de Maroc 41: 141-167.

DeSalle R, Egan MG, Siddall M. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philosophical Transactions of the Royal Society of London B Biological Sciences 360: 1905-1916.

DeSalle R, Freedman T, Prager EM, Wilson AC. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian Drosophila. Journal of Molecular Evolution 26: 157-164.

Dieckmann U. 2004. Adaptive speciation. Cambridge University Press: New York.

Duggen S, Hoernle K, van den Bogaard P, Rüpke L, Morgan JP. 2003. Deep roots of the Messinian salinity crisis. Nature 422: 602-606.

Emerson BC, Kolm N. 2005. Species diversity can drive speciation. Nature 434: 1015-1016.

Emerson BC, Oromí P. 2005. Diversification of the forest beetle genus Tarphius on the Canary Islands, and the evolutionary origins of island endemics. Evolution 59: 586-598.

Emerson BC, Oromí P, Hewitt GM. 2000a. Colonization and diversification of the species Brachyderes rugatus (Coleoptera) on the Canary Islands: Evidence from mitochondrial DNA COII gene sequences. Evolution 54: 911-923.

Emerson BC, Oromí P, Hewitt GM. 2000b. Interpreting colonization of the Calathus (Coleoptera: Carabidae) on the Canary Islands and Madeira through the application of the parametric bootstrap. Evolution 54: 2081-2090.

Emerson BC, Oromí P, Hewitt GM. 2000c. Tracking colonization and diversification of insect lineages on islands: Mitochondrial DNA phylogeography of Tarphius canariensis (Coleoptera: Colydiidae) on the Canary Islands. Proceedings of the Royal Society Biological Sciences Series B 267: 2199-2205.

Fahrig L. 2003. Effects of habitat fragmentation on biodiversity. Annual

Review of Ecology, Evolution and Systematics 34: 487-515.

Farris JS, Albert VA, Kallersjo M, Lipscomb D, Kluge AG. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12: 99-124.

Farris JS, Kallersjo M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10: 315-320.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

Ferrández MA. 1996. Notas sobre los Disdéridos ibéricos VIII. Nuevas especies del género Dysdera Latreille, 1804 (Araneae, Dysderidae). Boletín de la Real Sociedad Española de Historia Natural (Sección Biológica) 92: 75-83.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294-299.

Francisco-Ortega J, Park SJ, Santos-Guerra A, Benabid A, Jansen RK. 2001. Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. Biological Journal of the Linnean Society 72: 77-97.

Gatesy J, O'Grady P, Baker RH. 1999. Corroboration among data sets in simultaneous analysis: Hidden support for phylogenetic relationships among higher level artiodactyl taxa. Cladistics 15: 271-313.

Gillespie RG. 1991. Predation through impalement of prey: The foraging behavior of Doryonychus raptor (Araneae: Tetragnathidae). Psyche: A Journal of Entomology 98: 337-350.

Gillespie RG. 1999. Comparison of rates of speciation in web-building and non-web-building groups within a Hawaiian spider radiation. Journal of Arachnology 27: 79-85.

Gillespie RG. 2004. Community assembly through adaptive radiation in Hawaiian spiders. Science 303: 356-359.

Gillespie RG, Croom HB, Palumbi SR. 1994. Multiple origins of a spider radiation in Hawaii. Proceedings of the National Academy of Sciences 91: 2290-2294.

Gillespie RG, Roderick GK. 2002. Arthropods on islands: Colonization, speciation and conservation. Annual Review of Ecology and Systematics 47: 595-632.

Giribet G, Rambla M, Carranza S, Baguñà J, Riutort M, Ribera C. 1999. Phylogeny of the arachnid order Opiliones (Arthropoda) inferred from a combined approach of complete 18S and partial 28S ribosomal DNA sequences and morphology. Molecular Phylogenetics and Evolution 11: 296-307.

Giribet G, Wheeler WC, Muona J. 2002. DNA multiple sequence alignments. In: Wheeler WC, ed. DNA Multiple Sequence Alignments. Basel: Birkhauser-Verlag. 107–114.

Goloboff PA. 2002. NONA. Program and documentation available at http:// www.cladistics.com/aboutNona.htm.2.0 ed. New York: American Museum of Natural History.

Goloboff PA, Farris JS, Nixon KC. 2003. TNT: Tree Analysis Using New Technologies. Program and documentation available at http://www.zmuc.dk/public/ phylogeny/ TNT/. 1.0 ed.

Gómez-Zurita J. 2004. Molecular systematics and time-scale for the evolution of Timarcha, a leaf-beetle genus with a disjunct Holarctic distribution. Molecular Phylogenetics and Evolution 32: 647-665.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696-704.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95 - 98.

Harvey PH, Holmes EC, Mooers AO, Nee S. 1994a. Inferring evolutionary processes from molecular phylogenies. Philosophical Transactions of the Royal Society of London B Biological Sciences 349: 25-31.

Harvey PH, May RM, Nee S. 1994b. Phylogenies without fossils. Evolution 48: 523-529.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identification through DNA barcodes. Proceedings of the Royal Society Biological Sciences Series B 270: 313-321.

Hedin MC. 1997. Molecular phylogenetics at the population/species interface in cave spiders of the Southern Appalachians (Araneae: Nesticidae: Nesticus). Molecular Biology and Evolution 14: 309-324.

Hedin MC, Maddison WP. 2001. A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). Molecular Phylogenetics and Evolution 18: 386-403.

Helfgott DM, Francisco-Ortega J, Santos-Guerra A, Jansen RK, Simpson BB. 2000. Biogeography and breeding system evolution of the woody Bencomia alliance (Rosaceae) in Macaronesia based on ITS sequence data. Systematic Botany 25: 82-97.

Hormiga G, Arnedo M, Gillespie RG. 2003. Speciation on a conveyor belt: sequential colonization of the Hawaiian Islands by Orsonwelles spiders (Araneae, Linyphiidae). Systematic Biology 52: 70-88.

Izquierdo I, Martín JL, Zurita N, Arechavaleta M. 2004. Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres). Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias.

Juan C, Emerson BC, Oromí P, Hewitt GM. 2000. Colonization and diversification: Towards a phylogeographic synthesis for the Canary Islands. Trends in Ecology & Evolution. 15: 104-109.

Kirkpatrick M, Slatkin M. 1993. Searching for evolutionary patterns in the shape of a phylogenetic tree. Evolution 47: 1171.

Kishino H, Hasegawa M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in hominoidea. Journal of Molecular Evolution 29: 170-179.

Kozak KH, Larson A, Bonett RM, Harmon LJ. 2005. Phylogenetic analysis of ecomorphological divergence, community structure, and diversification rates in dusky salamanders (Plethodontidae: Desmognathus). Evolution 59: 2000-2016.

Krijgsman W, Langereis CG, Zachariasse WJ, Boccaletti M, Moratti G, Gelati R, Iaccarino S, Papani G, Villa G. 1999. Late Neogene evolution of the Taza-Guercif Basin (Rifian Corridor, Morocco) and implications for the Messinian salinity crisis. Marine Geology 153: 147-160.

Legendre P, Legendre L. 1998. Numerical ecology. Elsevier B. V.: Amsterdam, The Netherlands.

López-Mercader N. 2005. Evolutionary processes of the genus Spermophorides (Araneae, Pholcidae) in the Canary Islands. Ph.D., Universitat de Barcelona.

Losos JB, Jackman TR, Larson A, De Queiroz K, Rodriguez Schettino L. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279: 2115-2118.

Losos JB, Schluter D. 2000. Analysis of an evolutionary species-area relationship. Nature 408: 847-850.

MacArthur RH, Wilson EO. 1967. The theory of island biogeography. Princeton University Press: Princeton.

Machado A. 1992. Monografía de los carábidos de las Islas Canarias (Insecta, Coleoptera). Instituto de Estudios Canarios: La Laguna.

Machado A, Oromí P. 2000. Catalogue of the Coleoptera of the Canary Islands. Instituto Estudios Canarios: La Laguna.

Meier R, Shiyang K, Vaidya G, Ng P. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. Systematic Biology 55: 715-728.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. Evolution 49: 718-726.

Muster C, Bosmans R, Thaler K. 2007. The Philodromus pulchellus-group in the Mediterranean: taxonomic revision, phylogenetic analysis and biogeography (Araneae: Philodromidae). Invertebrate Systematics 21: 39-72.

Nee S. 2001. Inferring speciation rates from phylogenies. Evolution 55: 661-668.

Nee S, Barraclough TG, Harvey PH. 1996. Temporal changes in biodiversity: Detecting patterns and identifying causes. In: Gaston KJ, ed. Biodiversity: A biology of numbers and difference. Osney Mead, Oxford OX2 0EL, England; 3 Cambridge Center, Cambridge, Massachusetts 02142: Blackwell Scientific Publications; Blackwell Scientific Publications. 230-252.

Nee S, Holmes EC, May RM, Harvey PH. 1994. Extinction rates can be estimated from molecular phylogenies. Philosophical Transactions of the Royal Society of London B Biological Sciences 344: 77-82.

Nixon KC. 2002. WinClada. ver. 1.00.08 ed. Ithaca, New York: Published by the Author.

Nogales M, López M, Jiménez-Asensio J, Larruga JM, Hernández M, González P. 1998. Evolution and biogeography of the genus Tarentola (Sauria: Gekkonidae) in the Canary Islands, inferred from mitochondrial DNA sequences. Journal of Evolutionary Biology 11: 481-494.

Ogden TH, Whiting MF, Wheeler WC. 2005. Poor taxon sampling, poor character sampling, and non-repeatable analyses of a contrived dataset do not provide a more credible estimate of insect phylogeny: a reply to Kjer. Cladistics 21: 295-302.

Orr MR, Smith TB. 1998. Ecology and speciation. Trends in Ecology and Evolution 13: 502-505.

Palamarev E. 1989. Paleobotanical evidences of the Tertiary history and origin of the Mediterranean sclerophyll dendroflora. Plant Systematics and Evolution 162: 93-107.

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution 5: 568-583.

Paquin P, Hedin M. 2004. The power and perils of 'molecular taxonomy': a case study of eyeless and endangered Cicurina (Araneae: Dictynidae) from Texas caves. Molecular Ecology 13: 3239-3255.

Paradis E. 1997. Assessing temporal variations in diversification rates from phylogenies: estimation and hypothesis

testing. Proceedings Royal Society of London B: 1141-1147.

Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20: 289-290.

Percy DM, Page RDM, Cronk QCB. 2004. Plant-insect interactions: Double-dating associated insect and plant lineages reveals asynchronous radiations. Systematic Biology 53: 120-127.

Pizzo A, Roggero A, Palestrini C, Cervella P, Del Pero M, Rolando A. 2006. Genetic and morphological differentiation patterns between sister species: the case of Onthophagus taurus and Onthophagus illyricus (Coleoptera, Scarabaeidae). Biological Journal of the Linnean Society 89: 197-211.

Platnick NI. 2006. The World Spider Catalog. v. 7.0 ed. New York: American Museum of Natural History, online at http://research.amnh.org/entomology/spiders/catalog81-87/INTRO1.html.

Platnick NI, Ovtsharenko VI, Murphy JA. 2001. A review of the ground spider genus Scotognapha (Araneae, Gnaphosidae), and its radiation on the Canary and Salvage Islands. American Museum Novitates 3338: 1-22.

Pons J, Vogler A. 2006. Size, frequency, and phylogenetic signal of multiple-residue indels in sequence alignment introns. Cladistics 22: 144-156.

Portugal-Ferreira M, Macedo CR, Ferreira JF. 1998. K-Ar geochronology in the Selvages, Porto Santo and Madeira islands (Eastern-Central Atlantic): a 30 m.y. spectrum of submarine and subaerial volcanism. Lunar and Planetary Institute 19: 325-326.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53: 793-808.

Posada D, Crandall KA. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14: 817-818.

Prager EM, Wilson AC. 1988. Ancient origin of lactalbumin from lysozyme: analysis of DNA and amino acid sequences. Journal of Molecular Evolution 27: 326-335.

Price JP, Clague DA. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. Proceedings of the Royal Society Biological Sciences Series B 269: 2429–2435.

Pybus OG, Harvey PH. 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. Proceedings Royal Society of London B: 2267-2272.

Rabosky DL. 2006a. LASER: A maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. Evolutionary Bioinformatics Online 2: 257-260.

Rabosky DL. 2006b. Likelihood methods for detecting temporal shifts in diversification rates. Evolution 60: 1152-1164.

Rambaut A, Drummond A. 2005. TREESTAT. v. 1.0 ed: http://evolve.zoo.ox.ac.uk.

Rambaut A, Drummond AJ. 2003. TRACER. 1.3 ed: Available from http:// evolve .zoo.ox.ac.uk/tracer/.

Rees DJ, Emerson BC, Oromí P, Hewitt GM. 2001. The diversification of the genus Nesotes (Coleoptera: Tenebrionidae) in the Canary Islands: evidence from mtDNA. Molecular Phylogenetics and Evolution 21: 321-326.

Ribera C. 1983. Aranéidos de Marruecos I. Publicaciones del Departamento de Zoología de la Universidad de Barcelona 9: 73-76.

Ribera C. 1993. Dysdera caeca n.sp. y Harpactea stalitoides n.sp. (Araneae),

dos nuevas especies cavernícolas de Marruecos y Portugal. Revue Arachnologique 10: 1-17.

Ribera C. 2004. Dysdera valentina sp. n. (Araneae, Dysderidae), a new species from Valencia province (Spain), with some additions to the cave dwelling fauna of the Iberian Peninsula. Revista ibérica de Aracnología 9: 211-215.

Roderick GK, Gillespie RG. 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. Molecular Ecology 7: 519-531.

Ronquist F. 1996. DIVA v. 1.1. Computer program for MacOS and Win32. Available from (http://morphbank.ebc.uu.se/DIVA).

Ronquist F. 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Systematic Biology 46: 195-203.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Rosenzweig ML. 1995. Species diversity in space and time. Cambridge University Press: Cambridge ; New York.

Sánchez Doreste JL, Caujapé-Castells J, Reyes-Betancort JA, Scholz S. 2006. Population genetics of Matthiola bolleana (Brassicaceae) in the Canary Islands. Plant Systematics and Evolution V262: 139-151.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Molecular Biology and Evolution 19: 101-109.

Sanderson MJ. 2003. r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19: 301-302.

Schluter D. 2000. The Ecology of Adaptive Radiation. Oxford University Press: Oxford.

Shimodaira H, Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16: 1114-1116.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49: 369-381.

Simmons MP, Ochoterena H, Carr TG. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. Systematic Biology 50: 454-462.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651-701.

Simon E. 1882. Études arachnologiques 13e mémoire. Descriptions d'espèces et de genres nouveaux de la famille des Dysderidae. Annales de la Société Entomologique de France XX: 201-240.

Simon E. 1909. Etude sur les Arachnides recueillis au Maroc par M. Martinez de la Escalera en 1907. Memorias de la Real Sociedad Española de Historia Natural 6: 1-43.

Simon E. 1910. Catalogue raisonné des Arachnides du Nord de l'Afrique, 1ere partie. Annales de la Société Entomologique de France 79: 265-332.

Sites JW, Crandall KA. 1997. Testing species boundaries in biodiversity studies. Conservation Biology 11: 1289-1297.

Sorenson MD. 1999. TreeRot. 2 ed. Boston, MA.: Boston University.

SPSS. 2001a. SPSS for Windows. 11.0.0 ed. Chicago, IL.: SPSS. Inc., .

SPSS I. 2001b. SIGMAPLOT. 7.0 ed. Chicago, IL, USA: SPSS Inc.

StatSoft Inc. 1999. STATISTICA for Windows. Tulsa, OK.: StatSoft, Inc.

Swofford DL. 2001. PAUP*: Phylogenetic analysis using parsimony (and other methods). 4.0 ed. Sunderland, MA: Sinauer Associates.

Templeton AR. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the humans and apes. Evolution 37: 221-244.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876-4882.

Welch JJ, Bromham L. 2005. Molecular dating when rates vary. Trends in Ecology & Evolution 20: 320-327.

Wheeler WC, Gatesy J, DeSalle R. 1995. Elision: A method for accommodating multiple molecular sequence alignments with alignment-ambiguous sites. Molecular Phylogenetics and Evolution 4: 1-9.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Swinsky J and White TJ, eds. PCR protocols: a guide to methods and applications. San Diego, CA: Academic Press. 315-322.

Wiens JJ, Penkrot TA. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (Sceloporus). Systematic Biology 51: 69-91.

Will KW, Mishler BD, Wheeler QD. 2005. The perils of DNA barcoding and the need for integrative taxonomy. Systematic Biology 54: 844-851.

Wu CI. 1991. Inferences of species phylogeny in relation to segregation of ancient polymorphisms. Genetics 127: 429-435.

Young ND, Healy J. 2002. GapCoder: http://www.trinity.edu/nyoung/GapCoder/Download.html.

Zheng X, Arbogast BS, Kenagy GJ. 2003. Historical demography and genetic structure of sister species: deermice (Peromyscus) in the North American temperate rain forest. Molecular Ecology 12: 711-724.

Capítulo 4

Capítulo en revisión:

Macías-Hernández, N., Oromí, P. & Arnedo, M. A. DNA sequence data uncovers hidden species diversity in woodlouse-hunter spiders (Araneae, Dysderidae) endemic to the Macaronesian archipelagos. *Systematics and Biodiversity*.

DNA SEQUENCE DATA UNCOVERS HIDDEN SPECIES DIVERSITY IN WOODLOUSE HUNTER SPIDERS (ARANEAE, DYSDERIDAE) ENDEMIC TO THE MACARONESIAN ARCHIPELAGOS[1]

ABSTRACT

The development of molecular techniques as a taxonomic tool has enhanced species discovery, facilitated species delimitation and afforded invaluable data for inferring species phylogeny. In this paper, we provide an example of how DNA sequence data helps to identify and diagnose lineages overlooked in former traditional taxonomic revisions. The nocturnal, ground-dwelling spider genus *Dysdera* has colonized all the Macaronesian archipelagos, and undergone a major species radiation in the Canary Islands. A recent molecular phylogenetic analysis of *Dysdera* species endemic to the eastern Canary Islands has revealed deep genetic divergences among populations of endemic species, suggesting the existence of cryptic taxa. Here, we use data from independent mitochondrial and nuclear loci in combination with morphological and ecological evidence to delimit and formally describe three previously overlooked new species: *D. aneris* sp. n. endemic to the Salvage Islands, *D. mahan* sp. n. distributed along coastal and sandy habitats of Lanzarote, north of Fuerteventura and islets and *D. simbeque* sp. n. restricted to two valleys in northern Lanzarote. Molecular markers provide key information to transform apparent morphological polymorphism into diagnostic features of evolutionary independent lineages. *D. mahan* sp. n. is unique among Canarian *Dysdera* in that it is found in the intertidal zone on pebbled beaches. Low genetic variability and genital differentiation associated with relatively high somatic divergence, suggest that speciation in *D. mahan* sp. n. was probably driven by a selection of phenotypic traits adaptive to this rare environment. Separate analyses and statistical tests revealed instances of phylogenetic incongruence between mitochondrial and nuclear genes, probably as a result of incomplete lineage sorting. The temporal framework for the origin and

[1] Disclaimer: Please, notice that dissertations do not meet the criteria of publication according to Article 8 of the "International Code of Zoological Nomenclature" (ICZN 1999), and therefore species names used in the following chapter are not considered valid under the current code of zoological nomenclature.

diversification of the new species inferred from the molecular data corroborates former hypotheses on the late Pliocene origin of present-day Salvage Island biota.

INTRODUCTION

Species are the basic unit of taxonomy and the subject of evolution (Wiley, 1981; Ereshefsky, 1992). Few ideas in biology, however, have revealed themselves as more elusive to define or have sparked hotter debates than the species concept (Ereshefsky, 1992). The plethora of species definitions available in the literature (Harrison, 1998) have been grouped into those that consider species as reproductive communities, and those referring to species as evolutionary lineages (Templeton, 1994). The reproductive and evolutionary perspectives on species have been reconciled by considering the lineage-based evolutionary species concept as a general theoretical definition of species, and treating remaining concepts as operational tools for species recognition and delimitation (Mayden, 1997; de Queiroz, 1998). Some authors, however, go further and suggest that species should not be defined on the basis of specific necessary properties, as implied by most definitions, but that such properties should be used instead as a line of evidence to infer species limits (Sites & Marshall, 2003; 2004). Consequently, focus on species concepts have recently shifted towards the development of methods to detect species boundaries (Wiens & Penkrot, 2002 and references therein; Morando *et al.*, 2003; Sites & Marshall, 2003; 2004).

It has been suggested that traditional species delimitation based on gross morphological features underestimates and simplifies biodiversity (Mayden, 1997; Bickford *et a.,.* 2007). The development and popularization of molecular techniques have favoured the use of DNA sequence data to test traditional, morphology-based taxonomies, (Floyd *et al.*, 2002; Blaxter, 2004; Hebert *et al.*, 2004a; Hebert *et al.*, 2004b; Hogg & Hebert, 2004; Monaghan *et al.*, 2005; Smith *et al.*, 2005; Monaghan *et al.*, 2006). The inclusion of molecular data in taxonomy has aided species delimitation and diagnosis (Sites & Crandall, 1997; Sites & Marshall, 2003; 2004; DeSalle *et al.*, 2005), enhanced the discovery of cryptic species (e.g. Goetze, 2003; Molbo *et al.*, 2003; Hebert *et al.*, 2004a; Kankare & Shaw, 2004; Feulner *et al.*, 2006;

Pfenninger *et al.*, 2007), and extended species identification beyond complete adult specimens (Hebert *et al.*, 2003). The definition of species boundaries, on the other hand, requires an integrative method that includes multiple lines of evidence (Stockman & Bond, 2007; Bond & Stockman, 2008), such as those provided by classical morphology-based taxonomy along with molecular, ecological, behavioural and geographical information.

The Macaronesian biogeographic region is included in the Mediterranean biodiversity hotspot as one of the most important areas worldwide for conservation (Myers *et al.*, 2000). Arthropods are among the most diverse organisms in Macaronesian terrestrial ecosystems, where they reach levels of endemism as high as 38% (2768 species) in the Canaries (Martín *et al.*, 2005), 25% (979 species) in Madeira and the Salvage Islands (Borges *et al.*, 2008), 23% in Cape Verde (435 species) (Arechavaleta *et al.*, 2005) and 12% (267 species) in the Azores (Borges *et al.*, 2005). In spite of their accessibility from the continent and a long tradition in taxonomy and biotic surveys that date back as far as 150 years ago, new species of arthropods continue to be found at a rate of about 25 to 200 new taxa per decade (Izquierdo *et al.*, 2004; Borges *et al.*, 2005; Borges *et al.*, 2008). Although more human resources funding for bioinventoring have enhanced species discovery within the region, the introduction of DNA-based techniques has also contributed to increasing the number of species. In the Canaries, genetic distinctiveness has been used to describe a new endemic species of the grasshopper genus *Arminda* (Hochkirch & Gözig, 2009), to detect cryptic species in the *Palmorchestia* landhoppers (Villacorta *et al.*, 2008), or to corroborate species delimitation in morphologically similar species of the *Halophiloscia* coastal woodlice (Taiti & López, 2008), to cite just some examples.

The nocturnal wandering hunter spider genus *Dysdera* Latreille, 1804, is a conspicuous component of Mediterranean ground-dwelling arthropod fauna, generally associated to warm and wet ground habitats where they can be found under stones, in the leaf-litter and, frequently, underground. *Dysdera* is one of the largest genera in the Mediterranean basin, with nearly 250 described species (Platnick, 2009), a fifth of which are endemic to the Macaronesian archipelagos. The genus is unevenly distributed across the main archipelagos: the Canary Islands

harbour almost 50 endemic species (Arnedo *et al.*, 1996; Arnedo & Ribera, 1997; 1999; Arnedo *et al.*, 2000; Arnedo *et al.*, 2007), while Madeira has five species (Blackwall, 1862; Denis, 1962; Wunderlich, 1994) and Cape Verde (Berland, 1936), the Azores (Arnedo, unpubl. data) and the Salvage Is. (Arnedo *et al.*, 2000) one each. Cladistic analyses of morphology and mitochondrial DNA sequence data (Arnedo *et al.*, 2001) suggested a close relationship of Cape Verde with the Canaries, and the independent colonization of the Azores. Relationships of Madeiran taxa remained largely unresolved. Canarian endemics, on the other hand, are more likely the result of a single colonization event, with the only exception of *D. lancerotensis* Simon, 1907, which colonized the eastern Canaries independently (Bidegaray-Batista *et al.*, 2007; Macías-Hernández *et al.*, 2008).

The eastern Canary Islands (Fig. 4.1), namely Fuerteventura, Lobos, Lanzarote and the Chinijo archipelago are home to five endemic *Dysdera* species, in addition to the aforementioned *D. lancerotensis*, one of which is also found in the Salvage Islands. A recent study of the phylogeny and evolution of these endemic species (Macías-Hernández *et al.*, 2008) based on DNA sequence data revealed the existence of deeply divergent lineages, which had gone unnoticed in previous taxonomic revisions (Arnedo *et al.*, 2000). The eastern Canaries are the oldest islands of the archipelago (Fuerteventura is 22 million years old Coello *et al.*, 1992) and the closest to the mainland (northwest Africa). These two factors have shaped their terrestrial ecosystems, which, compared to the remaining Canary Islands, are arid and xerophilic due to erosion (which prevents them from capturing the humid trade winds from the northeast) and the arid, dusty winds blowing in from the Sahara. The eastern Canaries are an exposed part of a continuous volcanic ridge, and the islands have been connected by land bridges in the past as a result of eustathic sea-level changes.

The Salvage archipelago includes two small islands (Selvagem Grande and Selvagem Pequena) and one islet (Ilhéu de Fora), located 165 km north of the Canary Islands, and 300 km south from Madeira island (Fig. 4.1). The subaerial phase of the archipelago dates back to 21 million years ago (Ma), and was followed by two post-erosional periods (12-8 and 3.4 Ma) separated by gaps in volcanic activity. The archipelago was beneath sea level during volcanic quiescence periods,

due to erosion and eustathic sea level changes (Geldmacher *et al.*, 2001). Present-day terrestrial ecosystems probably originated after the last post-erosional volcanic episode. Here, we elaborate on the phylogenetic results of Macías-Hernández, *et al.,* (2008) to re-examine morphological evidence and formally describe three new endemic species. We present new molecular and morphological data of the new species from the Salvage archipelago to facilitate delimitation and further investigate its origins.

MATERIAL & METHODS

Taxonomy

Specimens were examined under Leica MZ95 and Leica MZ16A dissection microscopes, the latter equipped with a Nixon DXM1200 digital camera. Digital microscope images were edited using the Auto-Montage software package. Digital illustrations were generated following guidelines provided in Coleman (2003) with the assistance of a WACOM digitiser board and Adobe Illustrator 10 and Photoshop 8.0.1 software. Male palps were detached, cleaned by ultrasound, critical-point dried and then coated for examination in a HITACHI S2300 scanning electron microscope at the Serveis Científico-Tècnics of the Universitat de Barcelona. Female vulva was removed with the help of needles, and muscle tissue digested with a 35% KOH solution before observation. Final plate layout and editing was done with Adobe Illustrator CS3.

Measurements were taken using an ocular measuring graticule mounted on the dissection microscope. All characters were recorded in DELTA (DEscription Language for TAxonomy) format (Dallwitz, 1980; Dallwitz *et al.,* 1993) using the DELTA editor (Dallwitz *et al.*, 1999). Taxonomic procedures and descriptions follow Arnedo & Ribera (1999) and Arnedo e*t al*., (2000). Terminology of the male palp and female vulva structures is based on Deeleman-Reinhold & Deeleman (1988) and Arnedo (2000), and have been illustrated in Arnedo & Ribera (1999) and Arnedo *et al.,* (2000). Leg spination was recorded using the codification method fully described in Arnedo *et al*., (1999). Abbreviations used in text and figures are listed in Appendix 4.1.

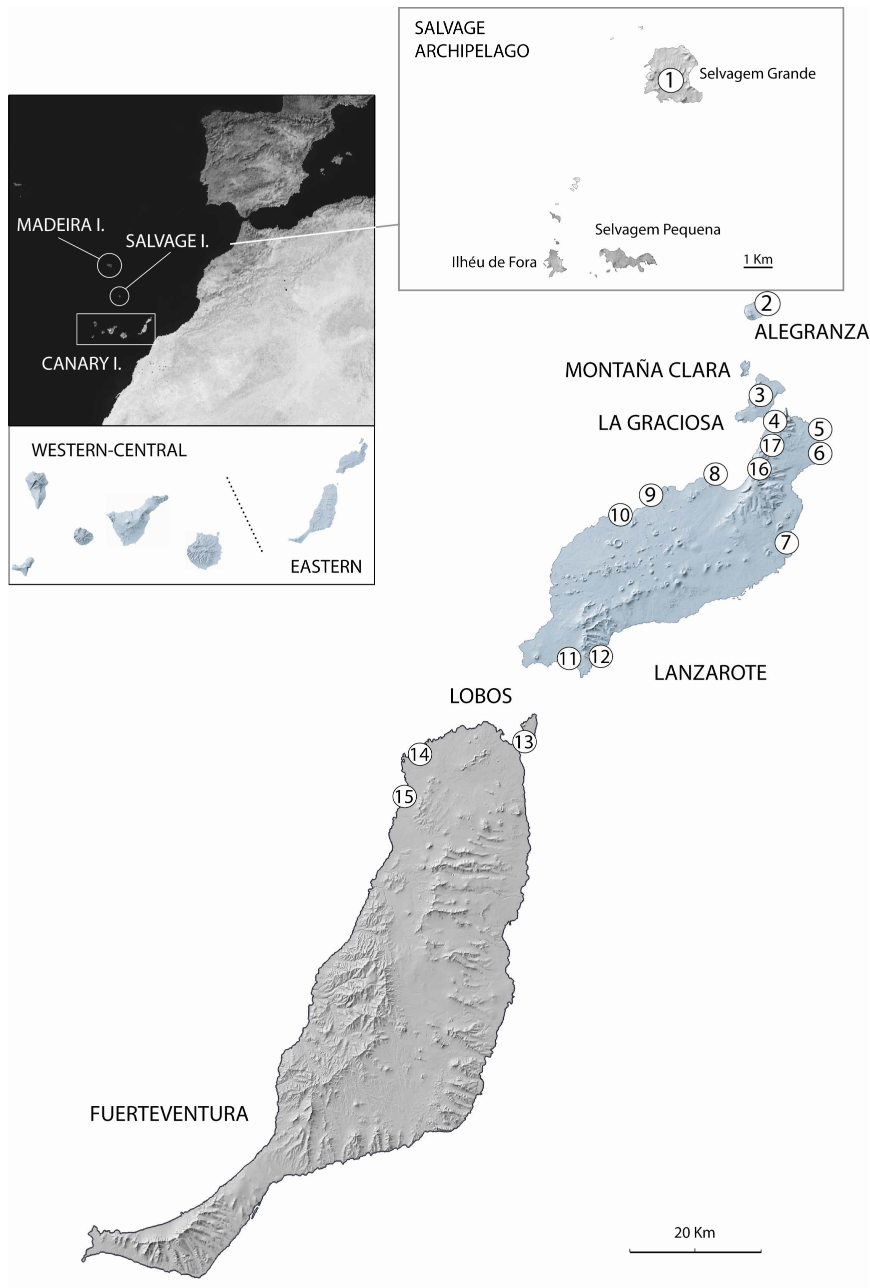

Fig. 4.1- Map with the sampling localities and the distribution of the three new species described.

Phylogenetics

Molecular analyses were based on the data matrix of Macías-Hernández *et al.,* (2008) for the eastern Canarian endemics, with the addition of 12 new individuals: 6 specimens from Selvagem Grande, 5 specimens from three western Canarian endemisms (*D. calderensis, D. silvatica* and *D. gomerensis*) and 1 from Morocco (*D.* cf. *inermis*). Specimens from the western Canaries provided additional calibration points for lineage age estimation (see below). The complete set of species, specimens and genes analysed are listed in Appendix 4.2, and sampling localities in the eastern Canaries and Salvage Islands are indicated in Fig. 4.1 and Appendix 4.3. All analyses were rooted assuming a sister group relationship of the continental species *Dysdera scabricula* Simon, 1882 to the remaining species sampled (Arnedo *et al.*, 2007; Macías-Hernández *et al.*, 2008). Samples were stored in absolute ethanol at -20ºC until DNA extractions were performed. All the eastern Canarian *Dysdera* species and additional specimens from the western Canary Islands and continental species were also sampled and included in the analyses.

Divergence time estimation

Divergence times were estimated with the computer program R8S (Sanderson, 2003). A preliminary cross-validation analysis was conducted to select the clock method that best suited the data (Sanderson, 2002). Clade age was estimated on the Bayesian topology. Each species was only represented by 1 or 2 specimens to avoid very short branches that could negatively impact time estimation algorithms (Sanderson, 2003) and to simplify calculations. Only mitochondrial data was included in the divergence time analysis to make the results comparable to former studies (Bidegaray-Batista *et al.*, 2007; Macías-Hernández *et al.*, 2008), and the partially sequenced (only mitochondrial data) specimen X125 of *D. aneris* sp. n. was added to analyses. Tree editing was performed with the computer program TREEEDIT v. 1.0 a 10. Branch lengths were reestimated on the preferred topology using the computer program RAxML and defining independent GRT+I+G models for each data partition. The outgroup taxon (*D. scabricula*) was pruned from trees before conducting clock analyses to ensure a dichotomus root node. Clade age confidence intervals were obtained from 100 trees by reestimating branch lengths after character boostrapping while keeping

the topology constant. The program TreeAnnotator (Drummond & Rambaut, 2007) assisted in summarizing confidence intervals from the sample of trees with bootstrapped branch lengths analyzed in R8S. In these analyses, we have improved divergence time estimation by including 4 additional calibration points to the single point used in former analyses, based on the time of divergence of the Iberian and Moroccan populations of *D.* cf. *inermis*, fixed to 5.3 Ma by assuming a population split following the opening of the Strait of Gibraltar (Krijgsman *et al.*, 1999). The island populations of *D. gomerensis* and *D. silvatica* from La Gomera and El Hierro, and the island populations of *D. calderensis* and *D. silvatica* from La Palma and La Gomera were set to a maximum time of divergence of 1.2 and 2 Ma. respectively, corresponding to the time of origin of the subaerial stages of the younger islands (Carracedo & Day, 2002).

Analysis of morphological variation

Morphological variation data in Macías-Hernández *et al*., (2008) was completed and reanalyzed by including 10 specimens of *D. aneris* sp. n. (no specimens were available in the former analyses) and additional material of *D. longa* (2 new male specimens). Analyses were based on the following measurements, obtained from 5 males and 5 females of each target species (only 2 *D. sanborondon* males were available): maximum carapace length (P1), minimum (P2min) and maximum carapace width (P2max), length of the basal segment of the chelicera in lateral view (Q1), maximum width of the basal segment in lateral view (Q2), cheliceral fang length (F), length of the prolateral margin of the basal segment (Esc), femur length of leg 1 (fe1), and metatarsus length of leg 4 (mt4). The assumptions of normality and homogeneity were rejected by the Kolmogorov-Smirnoff test, so we applied a non-parametric Klustal-Wallis test to detect possible interspecific morphological differences and intraspecific sexual dimorphism. A Pearson test detected high levels of autocorrelation between the morphological variables. Therefore, residual values (calculated by means of an interspecific regression using Pearson correlation for each variable against P1) were used in subsequent analyses (Losos *et al.*, 1998) to reduce the effects of the reported correlation with body size. A species similarity matrix was estimated across a hierarchical agglomerative cluster using the Bray–Curtis distance. Finally, principal

component analyses (PCA) of all individuals was conducted to asses the variance explained for each independent axis (Legendre & Legendre, 1998). The analyses were performed with the software packages SPSS v. 15, PRIMER v. 5.2.2 (Clarke & Warwick, 1994), STATISTICA (StatSoft Inc., 1999) and results plotted with SIGMAPLOT version 7.0 (SPSS, 2001).

RESULTS

Taxonomy

Dysderidae C. L. Koch, 1837

Dysdera Latreille, 1804

Type species: *Aranea erythrina* Walckenaer, 1802: 224 (unspecified sex) by original designation, unspecified number of syntype specimens from France, surroundings of Paris (C. A. Walckenaer), repository unknown, supposed lost.
Diagnosis. See Deeleman-Reinhold & Deeleman (1988)
Species included: The genus presently includes 248 species (Platnick, 2009)

Dysdera aneris Macías-Hernández & Arnedo, sp. nov.
(Figs. 4.2A-B, 4.3A-E, 4.4 A-B, Table 4.1-4.2)

Dysdera wollastoni Kulczyński, 1899: 342, pl. 6, Figs. 22-24 (3♂, 2♀, 4 juvs, Selvagens; coll. W. Kulczyński; stored at OXUM, examined) (♂, ♀ misidentified).- Simon 1912: 59-60.- Berland & Denis 1946: 224.- Wunderlich 1991: 312. Fig. 129 (♂, misidentified).

Dysdera nesiotes Denis 1963: 37-38.- Rambla 1978: 132-133.- Arnedo *et al*., 2000: 277-281, Figs. 59-61 (♂,♀ misidentified). Arnedo, 2003: 145.

HOLOTYPE: ♂ [NMH 609UBXX], 08 Oct 2005 (I. Silva) (CRBA).

PARATYPES: ♀ [NMH 612-613UBXX], same data as holotype (CRBA); 1 ♂ [NMH 608, right bulb removed for SEM], 2♀ [NMH 610-611], same data as holotype (ULL).

TYPE LOCALITY: SALVAGE IS.: Selvagem Grande (N 30.146105 W 15.864975)

ADDITIONAL MATERIAL: SALVAGE ISLANDS: 3♂, 1 sub♂, 1♀, 1 juv. [BM1897.10.18.41-46], label states; "*Dysdera verneaui* Simon" (Grant) (BMNH); 1♂, 1♀, 3 juvs [B 536] (Garreta) (MHNP); 3♂, 2♀, 4 juvs labels state: "*Dysdera wollastoni*, Selvagens" "Ins. Zool. P.A.N. coll. W. Kulczynski" (OXUM); Selvagem Grande 1♂ [NMH608], 3♀ [NMH610, 611, 613], 8 Oct 2005 (I. Silva) (UB); 1♀ [NMH1489], 3♂ [NMH1490-1492], 26 March 2009 (L. García) (ULL).

HABITAT AND DISTRIBUTION: Species found on Selvagem Grande in the Salvage Islands, 165 km north from the Canary Islands (Fig. 4.1). Denis (1963) reported the presence of *D. nesiotes* (species to which Salvage Island specimens had been transferred) also in Selvagem Pequena (Pitão) and Ilhéu de Fora. These last records, however, could not be confirmed.

ETYMOLOGY: The species epithet is a noun in apposition; the name of a female character from the book "La Pell Freda" (Cold Skin) by Albert Sánchez Piñol. Aneris belongs to a strange marine race that emerges from the sea when night falls, and wander around a desolate island.

DIAGNOSIS: *Dysdera aneris* sp. n. closely resembles *D. nesiotes* and *D. mahan* sp. n. but differs from them by vulval and DNA sequence characters. It can be distinguished from *D. nesiotes* by a rectangle–like DA (width/length ratio range 1.7-2.3, in square-like *D. nesiotes* 1.2-1.6) (Fig. 4.4 B). Sclerotization of VA is restricted to the frontal margin (it extends to the half part of the lateral margins in *D. nesiotes*) (Fig. 4.4 A). It can be distinguished from *D. mahan* sp. n. by VA tooth-like projections shorter than VA lateral margins (as long as lateral margins in *D. mahan* sp. n.) (Figs. 4.4 A, 4.7 A), and smaller body size (average carapace length 4.1 and 5.26, respectively). The three species differ in DNA sequences (divergence:

cox1, 9.4 and 7.5%; *nad1*, 14.9 and 12.7%, *16S-L1*, 8.6 and 5.5%, *ITS-2*, 0.6 and 0.8%, *H3* 0.5 and 0.05%; between *D. aneris* sp. n. and *D. nesiotes* and *D. mahan* sp. n., respectively. They can also be diagnosed by fixed nucleotide differences in the DNA barcode *cox1* as follows: position 146 (G/T/A), 257 (A/G/T) and 398 (A/T/G), in *D. aneris* sp. n., *D. nesiotes* and *D. mahan* sp. n, respectively (alignment positions correspond to a reference alignment deposited in TreeBASE, accession numbers XXXXX).

MALE (*holotype*): Figs. 4.2 A-B, 4.3 A-E. Carapace (Fig. 4.2 A) 4.08 mm long; maximum width 3.16 mm; minimum width 2.09 mm. Brownish orange, frontally darker, becoming lighter towards back; smooth with some small black grains mainly at front. Frontal border roughly triangular, from 1/2 to 3/5 carapace length; anterior lateral borders convergent; rounded at maximum dorsal width, back lateral borders straight; back margin wide, straight. AME diameter 0.22 mm; PLE 0.21 mm; PME 0.18 mm; AME on edge of frontal border, separated from one another by about 2/3 diameter, close to PLE; PME very close to each other, about 1/3 PME diameter from PLE. Labium trapezoid-shaped, base wider than distal part; longer than wide at base; semicircular groove at tip. Sternum orange, uniformly distributed; smooth; uniformly covered in slender black hairs.

Chelicerae 1.68 mm long, about 1/3 of carapace length in dorsal view; fang medium-sized, 1.53 mm; basal segment dorsal, ventral side completely covered with piligerous granulations. Chelicera inner groove short, about 1/3 cheliceral length; armed with three teeth and lamina at base; B>D>M; D round, located roughly at centre of groove; B close to basal lamina; M at middle of B and D. Legs orange. Lengths of male described above: fe1 3.31 mm (all measurements in mm); pa1 2.29; ti1 3.01; me1 3.01; ta1 0.66; total 12.29; fe2 2.91; pa2 1.99; ti2 2.55; me2 2.60; ta2 0.71; total 10.76; fe3 2.29; pa3 1.38; ti3 1.53; me3 2.29; ta3 0.61; total 8.11; fe4 3.16; pa4 1.73; ti4 2.6; me4 2.96; ta4 0.71; total 11.17; fe Pdp 2.04; pa Pdp 1.12; ti Pdp 0.97; ta Pdp 0.92; total 5.05; relative length: 1>4>2>3. Spination: leg1, 1eg2 spineless; tb3d spines arranged in two bands; proximal 1.0.1; distal 1.0.0; tb3v spines arranged in two bands; proximal 1.0.0; distal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 1-0; backward 5-3; tb4d spines

arranged in two bands; proximal 0.0.1; distal 0.0.1; tb4v spines arranged in three bands; proximal 1.0.0-1; medial-proximal 0.0.1; distal 1.0.0; with two terminal spines. Dorsal side of frontal legs covered with small piligerous grains; ventral side of pedipalp covered with hairs, lacking grains; very long hairs on back legs as well as on pedipalps. Claws with 8 teeth or less; hardly larger than claw width. Abdomen 4.9 mm long; whitish; cylindrical. Abdominal dorsal hairs 0.11 mm long; medium-sized, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

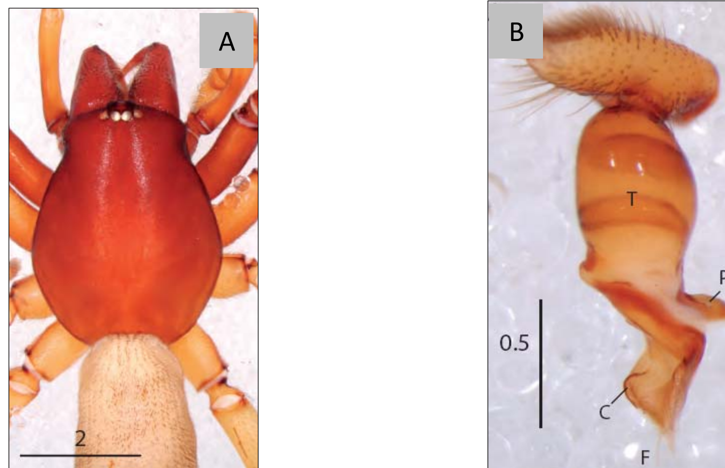

Fig. 4.2.- *Dysdera aneris* sp. n. holotype. A, Carapace, dorsal view. B, Left male palp, retrolateral view.

Male copulatory bulb (2B) T as long as DD; external distal border sloped backwards; internal sloped backwards. DD bent about 45° in lateral view; internal distal border markedly expanded. ES wider, more sclerotized than IS; IS continuous to tip. DD tip (Figs. 4.3 A-C, 4.3 D) straight in lateral view; frontal (upper) sheet internal part markedly projected above posterior (lower) sheet. C present, long; distal end close to DD internal tip; distal border rounded, smooth, markedly expanded, perpendicular to DD. AC absent. LF absent. L well-developed; external border sclerotized, laterally markedly folded, distally projected; distal border divergent, continuous. LA present, hook-like; shorter than. F present, tip divided and distally curved to external side; proximally fused to DD. AL present, well-developed; not joined to flagellum; proximal border in posterior view smooth, not fused with distal haematodoca. P (Fig. 4.3 D) fused to T; perpendicular to T in

lateral view; lateral length from 1/2 to 2/3 of T width; ridge present, perpendicular to T; distinctly expanded, rounded, upper margin slightly toothed, mainly on external side, along its extent; few teeth (4-6); not distally projected; back margin not folded.

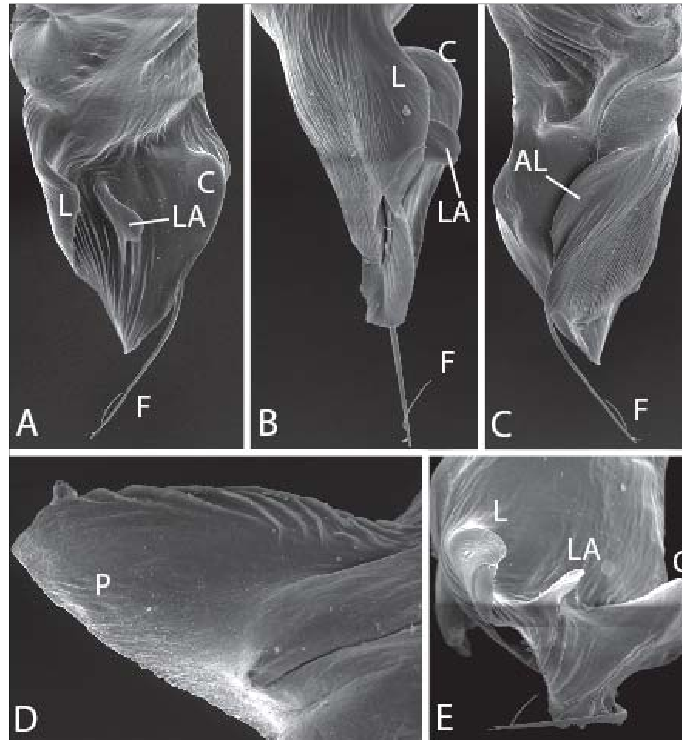

Fig. 4.3.- *Dysdera aneris* sp. n. right male bulb. A, anterior view; B, retrolateral view; C, posterior view, D, P detail, retrolateral view, E, distal tip, ventral view.

FEMALE (*paratype* NMH 612-613UBXX): Fig. 4.4 A-B. All characters as in male except: Carapace 3.88 mm long; maximum width 3.01 mm; minimum width 1.94 mm. Anterior lateral rounded at maximum dorsal width, back lateral borders straight. AME diameter 0.21 mm; PLE 0.195 mm; PME 0.17 mm. Chelicerae 1.68 mm long; fang medium-sized, 1.53 mm. Lengths of female described above: fe1 3.01 mm (all measurements in mm); pa1 2.0; ti1 2.55; me1 2.55; ta1 0.56; total 10.66; fe2 2.55; pa2 1.73; ti2 2.29; me2 2.24; ta2 0.56; total 9.38; fe3 2.0; pa3 1.22; ti3 1.48; me3 2.09; ta3 0.61; total 7.45; fe4 2.8; pa4 1.68; ti4 2.35; me4 2.75; ta4

0.71; total 10.3; fe Pdp 1.78; pa Pdp 0.87; ti Pdp 0.82; ta Pdp 0.97; total 4.44; relative length 4>1>2>3. Spination: leg1, leg2 spineless. Tb3d spines arranged in two bands; proximal 1.0.1; distal 0-1.0.0-1; tb3v spines arranged in two bands; proximal 1.0.0; distal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 1; backward 3; tb4d spines arranged in two bands; proximal 0.0.1; distal 0.0.1; tb4v with two terminal spines.

Abdomen 4.65 mm long; whitish; cylindrical. Abdominal dorsal hairs 0.1424 mm long; thin, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

Vulva (Fig. 4.4 A-B) DA not distinguishable from VA; rectangular; DA twice as wide as long; DF wide in dorsal view. MF margins fused, sheet-like, well-developed, completely sclerotized, projected backwards, shorter than DA lateral length. VA frontal region completely sclerotized; posterior region sclerotized in most anterior area; tooth-shaped expansion from internal back border; not joined to lateral sclerotization, slightly shorter than DF lateral margins; AVD absent. S attachment not projected under VA; arms as long as DA, m-shaped; ends projected forwards; neck hardly visible. TB usual shape.

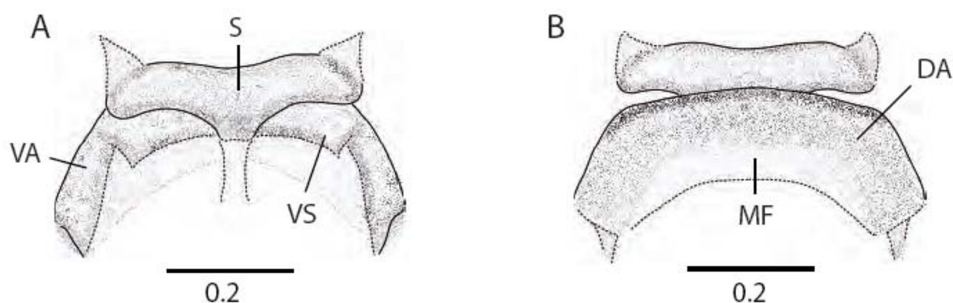

Fig. 4.4.- *Dysdera aneris* sp. n. Female vulva. A, ventral view; B, dorsal view.

VARIATION: Male carapace ranges in length from 4-4.8 mm and females' from 3.5-4.4 mm (N=5). The colour of the carapace varies between red orange to brownish orange in some individuals. The vulva shows a different degree of VA

sclerotization, and in some females S arms are smaller and rounded. Spination and leg measurement variability is listed in Table 4.1 and 4.2, respectively.

Table 4.1.- Intraspecific spination variability of *Dysdera aneris* sp. n.

	Proximal	**Medial-proximal**	**Medial-distal**	**Distal**
Tibia 3 dorsal	1.0-1-2.0-1	0	0	0-1.0.0-1
Tibia 3 ventral	0-1.0.0-1	0	0	0-1.0.0-1
Tibia 4 dorsal	0-1.0.0-1	0.0.0-1	0	0-1.0.0-1
Tibia 4 ventral	0-1.0.0-1	0-1.0.0	0	1.0.0-1
		Number of rows		**Number of spines**
Femur 3 dorsal		0-1		0-1
Femur 4 dorsal		2		1-4/4-7

Table 4.2.- *Dysdera aneris* sp. n. Leg measurements variability. Males (n=5)/Females (n=5).

	I	**II**	**III**	**IV**
Fe	3.3-3.8/2.6-3.5	2.9-3.4/2.3-3.1	2.2-2.6/1.9-2.6	3.2-3.6/2.8-3.5
Pa	2.3-2.5/1.8-1.9	1.9-2.2/1.6-2.2	1.3-1.5/1.1-1.5	1.7-1.9/1.4-1.9
Ti	3.0-3.4/2.3-3.1	2.5-3.0/1.9-2.8	1.5-1.8/1.4-1.8	2.6-2.7/2.0-2.7
Mt	3.0-3.5/2.1-3.0	2.6-3.0/1.9-2.6	2.3-2.5/1.6-2.5	2.9-3.4/2.5-3.3
Ta	0.6-0.7/0.5-0.6	0.71/0.5-0.6	0.6-0.6 /0.5-0.6	0.7-0.7/0.6-0.7
TOTAL	12.3-13.9/9.5-12.7	10.7-12.3/8.5-11.2	8.1-9.1/6.7-9.0	11.1-12.5/9.4-12.3

REMARKS: In 1864, the British naturalist John Blackwall described a new *Dysdera* species from the Salvage Islands, *Dysdera wollastoni* Blackwall, 1864. Almost 20 years later, in a taxonomic treatment of spiders from the Atlantic Ocean islands, the great French arachnologist Eugène Simon expressed his doubts about the validity of the former species, which he considered most likely to be a senior synonym of the cosmopolitan species *D. crocata* C. L. Koch, 1838 (Simon, 1883). In 1899, Polish arachnologist W. Kulczyński published a redescription with excellent illustrations of *D. wollastoni*, based on newly collected material from the Salvage

Islands. Simon examined a new batch of specimens from these islands collected by M.L. Garreta, which, according to him, closely resembled the species *D. nesiotes,* which he had recently described from the Canary Islands, except for their smaller size and fewer spines on fe IV. Therefore, he proposed downgrading the status of the Canarian specimens to subspecies and referring to it as *D. wollastoni nesiotes*, probably after observing Kulczyński's redescription (Simon, 1912).

It took more than half a century to confirm Simon's original suggestion that *D. wollastoni* was a junior synonym of *D. crocata*. Jacques Denis (1963), supported by information provided by J.A.L. Cooke, who was based at Oxford where Blackwall types were stored, considered Kulczyński's redescription of *D. wollastoni* to be based on a misidentification, and hence reinstated *D. nesiotes* to full species status and transferred all specimens from the Selvagens identified as *D. wollastoni* sensu Kulczyński to this species. Wunderlich (1991) argued against the former synonym, suggesting that a spider specialist such as Blackwall could not have possibly misidentified *D. crocata*. Arnedo and collaborators (2000) examined material from both the Salvage Islands and Lanzarote and concluded that there were not any clear diagnostic differences separating these island populations. We have now had the chance to examine the original material used by Blackwall to describe *D. wollastoni* and we can confirm that this is a junior synonym of *D. crocata*, and the material used by Kulczyński for his redescription, which belongs to *D. aneris* sp. n.

Dysdera mahan Macías-Hernández & Arnedo, sp. nov.
(Figs. 4.5 A-B, 4.6 A-E, 4.7 A-B, Table 4.3-4.4)

Dysdera nesiotes Arnedo *et al.,* 2000: 278-280, Fig. 63 (♀ misidentified).

HOLOTYPE: ♂ [NMH523XXXUB] (right bulb removed for SEM), 8 Dec 2004 (GIET) (UB).

PARATYPES: CANARY ISLANDS: ♀ [NMH 524XXXUB], same data as holotype (CRBA); 1♀ [NMH77], Fuerteventura, La Oliva, Playa de Esquinzo, 31 March 2004 (H.

López) (ULL); 1♀ [NMH 447], Lanzarote, Yaiza, Playa Caleta del Congrio, Papagayo, 7 Feb 2005, (N. Macías-Hernández) (ULL); 1♀ [NMH64], Lanzarote, Tinajo, Playa de la Madera, Timanfaya, 28 March 2004 (H. López) (ULL); 1♀ [NMH355], 2 ♂ [NMH356-357], Lanzarote, Haría, Playa Catalina Cabrera, Famara, 28 Nov 2004 (GIET) (ULL); 1♂ [NMH358], Alegranza, Playa de El Trillo; 8 Dec 2004 (GIET) (ULL).

TYPE LOCALITY: CANARY ISLANDS: Alegranza, Playa de El Trillo (N 29.404183 W 13.490834).

ADDITIONAL MATERIAL: CANARY ISLANDS: La Graciosa 4 juv. [NMH 451], Caleta de Arriba, 31 Jan 2005 (N. Macías) (ULL); Lobos: 4 juv. [NMH 572], Playa Las Salinas, Mar 2004 (N. Macías & H. López) (ULL); Lanzarote: 1♀ [NMH65], Haría, Punta Pasitos, Mala, 26 March 2004, (A.J. Pérez,) (ULL); 1♀ [2887UB], Órzola, Charcos de marea, 25 Feb 1995, (M. Arnedo, C. Ribera & P. Oromí) (CRBA); 1 juv. [NMH 113], Tinajo, Playa Caleta del Mariscadero, Timanfaya, 28 March 2004 (H. López) (ULL); 1 juv. [NMH443], Playa de Teneza, 8 Feb 2005, (N. Macías-Hernández) (ULL); 1 juv. [NMH448], Yaiza, Playa Las Salinas, Puerto Calero, 9 Feb 2005 (N. Macías- Hernández); Fuerteventura: 3 juv. [NMH490-492], Corralejo, Playa Majanicho, 5 Feb 2005 (M. Arnedo & N. Txasco) (CRBA).

HABITAT AND DISTRIBUTION: This species is found on intertidal zones of pebble beaches, on the sea-shores of Lanzarote, the northern islets, Lobos and north of Fuerteventura (Fig. 4.1).

ETYMOLOGY: The species epithet is a noun in apposition; the name refers to a giant aborigine that inhabited Fuerteventura, and it is also used to refer to the single island that formed Lanzarote and Fuerteventura during past episodes of marine regression.

DIAGNOSIS: The species closely resembles *D. aneris* sp. n. and *D. nesiotes*. It can be distinguished from the former species by its larger size, longer legs IV, anterior tooth-like projections of vulva VA as long as DA (Figs. 4.4 A, 4.7 A), rectangle-like DA (Figs. 4.4 B, 4.7 B), and inter-tidal, pebble-beach habitat. The

three species differ in DNA sequences (see *D. aneris* sp. n. diagnosis). It differs from close relative *D. spinidorsum* by copulatory bulb with shorter LA (Fig. 4.6 A), attenuated C (Fig. 4.6 D), and AL folded at its prolateral margin (Fig. 4.6 C), and vulva with shorter MF backward projections (only slightly longer than DA) (Fig. 4.7 A), and tooth-like sclerotization of frontal VA (Fig. 4.7 B). It also differs in DNA sequences (divergence: *cox1*, 3.8 %; *nad1*, 3.6%, *16S-L1*, 3.1%, *ITS-2*, 0.1%, *28S* 0.6%, *H3* 1.2). The two species can be diagnosed by fixed nucleotide differences in the DNA barcode *cox1* as follows: position 5 (A/T), 92 (T/A), 155 (C/T), 188 (T/A), 254 (G/A), 314 (A/G), 320 (G/A), 338 (G/A), 416 (A/T), 488 (C/T) and 527 (C/T) in *D. aneris* sp. n. and *D. spinidorsum*, respectively (reference alignment deposited in TreeBASE, accession numbers XXXX).

MALE (*holotype*): Figs. 4.5 A-B, 4.6 A-E. Carapace (Fig. 4.5 A) 5.3 mm long; maximum width 4.28 mm; minimum width 2.6 mm. Brownish red, frontally darker, becoming lighter towards back; smooth with some small black grains mainly at front. Frontal border roughly round, from 1/2 to 3/5 carapace length; anterior lateral borders convergent; pointed at maximum dorsal width, back lateral borders straight; back margin wide, straight. AME diameter 0.247 mm; PLE 0.234 mm; PME 0.221 mm; AME slightly back from frontal border, separated from one another by about 2/3 diameter, close to PLE; PME very close to each other, less than 1/4 PME diameter from PLE. Labium trapezoid-shaped, base wider than distal part; longer than wide at base; semicircular groove at tip. Sternum brownish orange, frontally darker, becoming lighter towards back or darkened on borders; very slightly wrinkled, mainly between legs and frontal border; uniformly covered in slender black hairs.

Chelicerae 2.7 mm long, about 1/3 of carapace length in dorsal view; fang medium-sized, 1.785 mm; basal segment dorsal, ventral side completely covered with piligerous granulations. Chelicera inner groove medium-size, about 2/5 cheliceral length; armed with three teeth and lamina at base; D=B>M; D trapezoid, located roughly at centre of groove; B close to basal lamina; M close to B. Legs dark orange-coloured. Lengths of male described above: fe1 5.56 mm (all measurements in mm); pa1 3.57; ti1 5.35; me1 5.41; ta1 1.02; total 20.91; fe2 4.7; pa2 3.06; ti2

4.59; me2 4.69; ta2 1.02; total 18.05; fe3 3.82; pa3 2.14; ti3 3.1; me3 3.67; ta3 1.02; total 13.72; fe4 4.84; pa4 2.65; ti4 4.28; me4 5.05; ta4 1.07; total 17.9; fe Pdp 2.86; pa Pdp 1.58; ti Pdp 1.53; ta Pdp 1.33; total 7.29; relative length: 1>2>4>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.0.0-1; distal 1.0.0; tb3v spines arranged in one band; proximal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 3-2; backward 5-6; tb4d spines arranged in two bands; proximal 0-1.0.1; distal 0.0.1; tb4v spines arranged in one band; proximal 1.0.0; with two terminal spines. Dorsal side of frontal legs covered with small piligerous grains; ventral side of pedipalp covered with small piligerous grains. Claws with 8 teeth or less; hardly larger than claw width. Abdomen 6.22 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

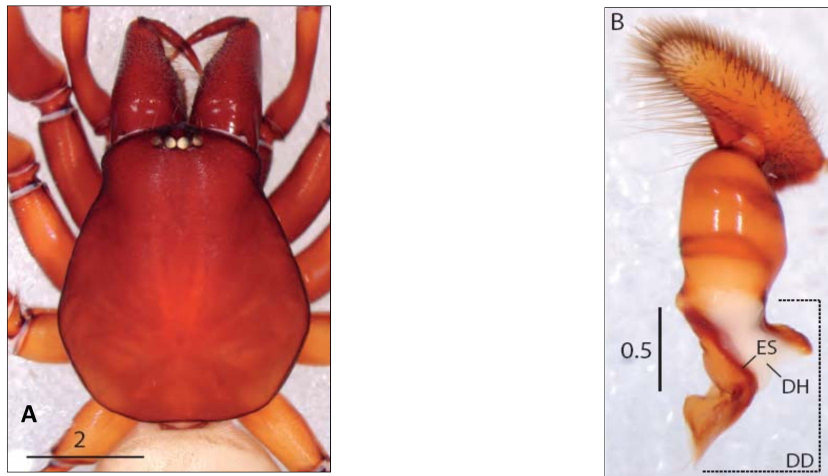

Fig. 4.5.- *Dysdera mahan* sp. n. holotype. A, Carapace, dorsal view. B, Left male palp, retrolateral view.

Male copulatory bulb T as long as DD (Fig. 4.5 B); external distal border sloped backwards; internal sloped backwards. DD bent about 45º in lateral view; internal distal border markedly expanded. ES wider, more sclerotized than IS; IS continuous to tip. DD tip (Fig. 4.6 A-E) straight in lateral view; frontal (upper) sheet internal part markedly projected above posterior (lower) sheet. C present, long; distal end beside DD internal tip; distal border rounded, smooth, markedly

expanded, perpendicular to DD. AC absent. LF absent. L well-developed; external border sclerotized, laterally markedly folded; distal border divergent, continuous. LA present, hook-like; shorter than. F present, tip divided or distally curved to external side; proximally fused to DD. AL present, well-developed; not joined to flagellum; proximal border in posterior view smooth, not fused with distal haematodoca. P fused to T; perpendicular to T in lateral view; lateral length from 2/3 to as long as T width; ridge present, perpendicular to T; distinctly expanded, rounded, upper margin slightly toothed, mainly on external side, along its extent; few teeth (4-6); not distally projected; back margin not folded.

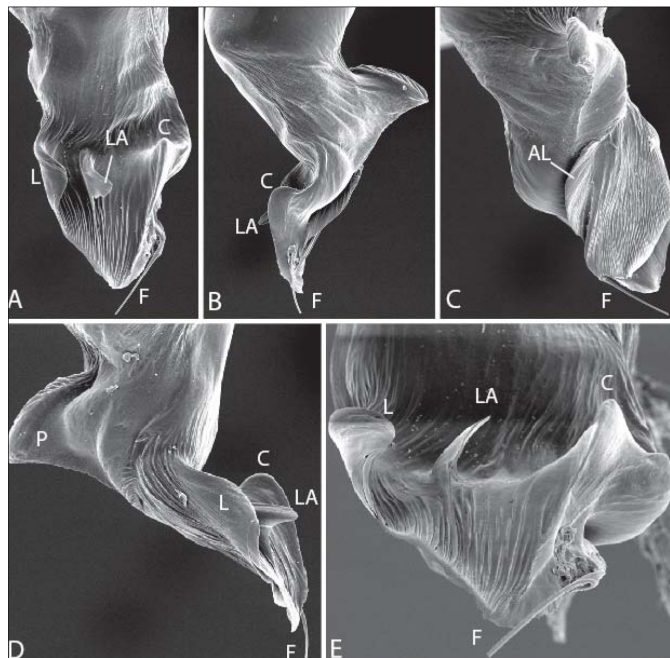

Fig. 4.6.- *Dysdera mahan* sp. n. right male bulb. A, anterior view; B, prolateral view; C, posterior view, D, retrolateral view, E, distal tip, ventral view.

FEMALE (*paratype* 524XXXUB): Fig. 4.7 A-B, D. All characters as in male except: carapace 4.84 mm long; maximum width 3.77 mm; minimum width 2.5 mm. Back lateral borders rounded. AME diameter 0.25 mm; PLE 0.23 mm; PME 0.19 mm.

Chelicerae 2.19 mm long, about 1/3 of carapace length in dorsal view; fang medium-sized, 0.34 mm. Legs orange. Lengths of female described above: fe1 4.44 mm (all measurements in mm); pa1 3.01; ti1 4.03; me1 4.03; ta1 0.87; total 16.37; fe2 3.88; pa2 2.65; ti2 3.47; me2 3.52; ta2 0.82; total 14.33; fe3 3.21; pa3 1.99; ti3 2.45; me3 3.01; ta3 0.82; total 11.47; fe4 4.23; pa4 2.5; ti4 3.67; me4 4.23; ta4 1.02; total 15.66; fe Pdp 2.55; pa Pdp 1.27; ti Pdp 1.02; ta Pdp 1.27; total 6.12; relative length 1>4>2>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.0.0-1; distal 1.0.0; tb3v spines arranged in one band; proximal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 1; backward 5-6; tb4d spines arranged in two bands; proximal 0.0-1.2; distal 0.0.1; tb4v spines arranged in two bands; proximal 0-1.2-0.0; medial-proximal 1.0.0; with two terminal spines. Abdomen 9.96 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

Vulva (Fig. 4.7 A-B) DA not distinguishable from VA; rectangular; DA twice as wide as long; DF wide in dorsal view. MF margins fused, sheet-like, well-developed, completely sclerotized, projected backwards, shorter than DA lateral length. VA frontal region completely sclerotized; posterior region sclerotized in lateral margins; tooth-shaped expansion from internal back border; not joined to lateral sclerotization, as long as DF lateral margins; AVD absent. S attachment not projected under VA; arms as long as DA, m-shaped; ends projected forwards; neck hardly visible. TB usual shape.

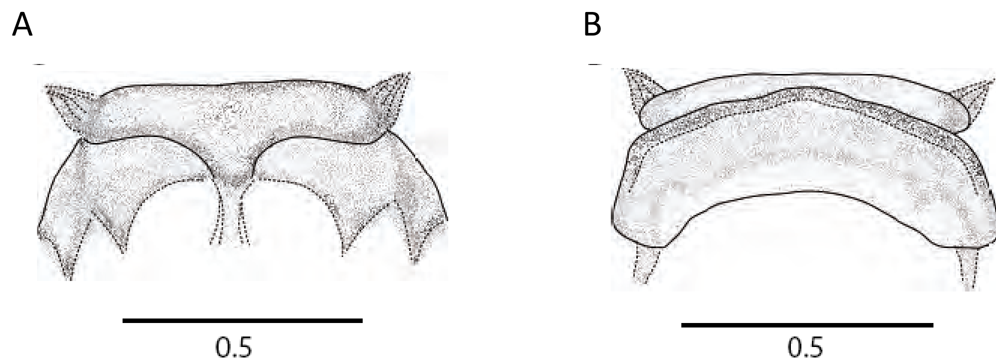

Fig. 4.7.- *Dysdera mahan* sp. n. Female vulva. A, ventral view; B, dorsal view.

VARIATION: Males' cephalothorax ranges in length from 4.9-5.3 mm (N=4), female from 4.8-6 mm (N=5). Carapace and leg colours vary from red orange to brownish orange in some specimens. The internal part of the pedipalps presents denser short black hairs in females. Spination and leg measurement is variability listed in Table 4.3 and 4.4, respectively.

REMARKS: A female specimen of the new species had already been studied by Arnedo and collaborators (2000) who rendered its particular vulva DA shape as a case of intraspecific variability in *D. nesiotes*.

Table 4.3.- Intraspecific spination variability of *Dysdera mahan* sp. n.

	Proximal	Medial-proximal	Medial-distal	Distal
Tibia 3 dorsal	1.0.0-1	0	0	0-1.0.1-0
Tibia 3 ventral	0-1.0.0	0	0	0-1.0.0
Tibia 4 dorsal	1-2.0.0	0	0	0-1.0.0-1
Tibia 4 ventral	0-1.0.0	0	0	0

	Number of rows	Number of spines
Femur 3 dorsal	0	0
Femur 4 dorsal	2	1-3/4-6

Table 4.4.- *Dysdera mahan* sp. n. Leg measurements variability. Males (n=4)/Females (n=5).

	I	II	III	IV
Fe	4.8-5.5/4.4-5.2	4.1-4.7/3.8-4.8	3.3-3.8/3.2-3.8	4.5-4.8/4.2-5.1
Pa	2.9-3.6/2.9-3.3	2.6-3.0/2.6-3.2	1.9-2.1/1.9-2.3	2.4-2.7/2.4-2.8
Ti	4.6-5.3/4.0-4.6	4.0-4.6/3.5-4.4	2.3-3.0/2.3-2.9	3.7-4.3/3.6-4.5
Mt	4.8-5.4/4.0-5.1	3.9-4.7/3.5-4.6	3.2-3.7 83.0-3.9	4.3-5.0/4.2-5.39
Ta	0.9-1.0/0.8-0.9	0.8-1.0/0.7-0.9	0.8-1.0/0.8-0.9	1.0-1.0/1.0
TOTAL	18.0-20.9/16.4-19.7	15.7-18.0/14.3-18.0	11.7-13.7/11.3-13.8	16.0-17.9/15.6-18.7

Dysdera simbeque Macías-Hernández & Arnedo, sp. nov.
(Figs. 4.8 A-B, 4.9 A-E, 4.10 A-B, Table 4.5-4.6)

TYPE: ♂ [NMH54UBXX] (right bulb removed for SEM), 29 March 2004 (GIET) (CRBA).

PARATYPE 1♀ [NMH 308UBXX], same data as holotype (CRBA); 2♂ [NMH 55-56], 3♀ [NMH42, 62, 307], same data as holotype, 29 March 2004 (GIET) (ULL).

TYPE LOCALITY: CANARY ISLANDS: Lanzarote, Cabecera del Barranco Elvira Sánchez, Haría (N 29.130723 W 13.516902).

ADDITIONAL MATERIAL: CANARY ISLANDS: Lanzarote: Haría, Fuente Ovejas, Guinate, 1 juv. [NMH 576], 26 Nov 2004 (N. Macías-Hernández) (ULL); 3 juvs [NMH1294-1296], MSS pitfall traps, 13 Jan 2007 (H. López & H. Morales) (ULL).

HABITAT AND DISTRIBUTION: This species is only known from two nearby sites on northern Lanzarote (Fig. 4.1).

ETYMOLOGY: The species epithet is an adjective in apposition; it means “big, voluminous” in the language of the aboriginal inhabitants (*Guanches*) of the Canary Islands.

DIAGNOSIS: *Dysdera simbeque* sp. n. differs from closely related *D. alegranzaensis* in its larger size (average carapace length 6.12 and 4.48, respectively), copulatory bulb with LA longer than L (Fig. 4.9 A), vulva VA not sclerotized and S attachment not projected backwards (Fig. 4.10 A). They also differ in DNA sequences (divergence: *cox1*, 10.2 %; *nad1*, 15.1%, *16S-L1*, 4.4%, *ITS-2*, 4.6%, *28S* 0.2%, *H3* 0.9). It can be easily distinguished from sympatric *D. nesiotes* by larger size, copulatory bulb with F absent (Fig. 4.9 A), vulva VA without frontal or lateral sclerotization and distal tips of S arms projected forward (Fig. 4.10 A). The three species can be diagnosed by fixed nucleotide differences in the DNA barcode *cox1* as follows: position 242 (A/G/T), 263 (G/A/T), 338 (G/A/T), 347 (T/A/G), 368

(T/G/A), 434 (G/A/T), 506 (A/T/G), 533 (G/A/T) and 596 (G/A/T) in *D. simbeque* sp. n., *D. alegranzaensis* and *D. nesiotes*, respectively (reference alignment deposited in TreeBASE, accession numbers XXXXX).

MALE (*holotype*): Figs. 4.8 A-B, 4.9 A-E. Carapace (Fig. 4.8 A) 6.12 mm long; maximum width 4.84 mm; minimum width 3.47 mm. Red orange, frontally darker, becoming lighter towards back; slightly foveate at borders, slightly wrinkled with small black grains mainly at front; hairy, uniformly covered with white hairs. Frontal border roughly round, from 1/2 to 3/5 carapace length; anterior lateral borders convergent; pointed at maximum dorsal width, back lateral borders straight; back margin wide, straight. AME diameter 0.26 mm; PLE 0.26 mm; PME 0.221 mm; AME slightly back from frontal border, separated from one another by about 1 diameter or more, close to PLE; PME very close to each other, about 1/2 PME diameter from PLE. Labium trapezoid-shaped, base wider than distal part; longer than wide at base; semicircular groove at tip. Sternum reddish orange, frontally darker, becoming lighter towards back; very slightly wrinkled, mainly between legs and frontal border; covered in hairs mainly on margin.

Chelicerae 3.57 mm long, about 2/5 of carapace length in dorsal view; fang medium-sized, 2.29 mm; basal segment dorsal, ventral side completely covered with piligerous granulations. Chelicera inner groove medium-size, about 2/5 cheliceral length; armed with three teeth and lamina at base; B>D>M; D trapezoid, located roughly at centre of groove; B close to basal lamina; M at middle of B and D. Legs dark orange-coloured. Lengths of male described above; fe1 5.508 mm (all measurements in mm); pa1 3.93; ti1 5.61; me1 4.95; ta1 0.87; total 20.86; fe2 5; pa2 3.37; ti2 4.69; me2 4.39; ta2 0.82; total 18.26; fe3 3.82; pa3 2.29; ti3 2.86; me3 3.62; ta3 0.82; total 13.41; fe4 4.84; pa4 2.75; ti4 3.82; me4 4.6; ta4 1.02; total 17.03; fe Pdp 3.6; pa Pdp 1.73; ti Pdp 1.73; ta Pdp 1.58; total 8.62; relative length: 1>2>4>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.2-1.1; distal 1.0.1.; tb3v spines arranged in one band; proximal 1.0.1; with two terminal spines. Fe4d spines in two rows; forward 1-2; backward 9-10; tb4d spines arranged in two bands; proximal 1.2.1; distal 1.0.1; tb4v spines arranged in two bands; proximal 1.0.1; distal 1.0.1; with two terminal spines. Dorsal

side of frontal legs smooth; ventral side of pedipalp smooth; Distal part of the metatarsus III and IV densely covered with short hair. Claws with 8 teeth or less; hardly larger than claw width.

Abdomen 8.38 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

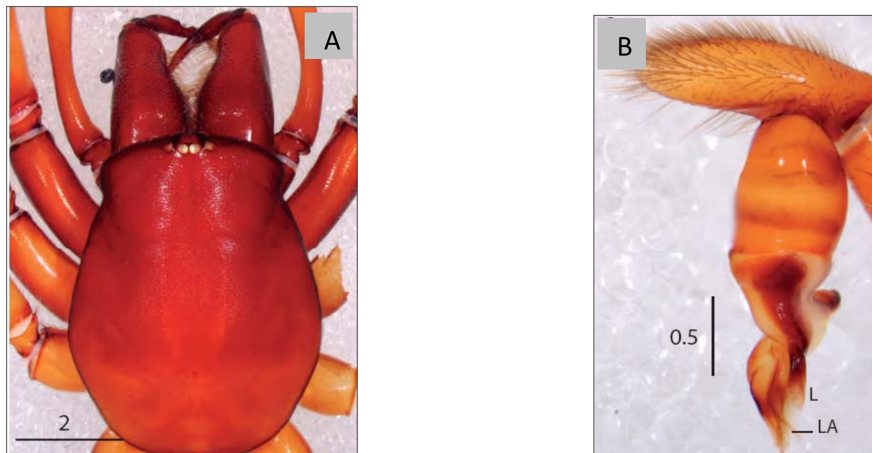

Fig. 4.8.- *Dysdera simbeque* sp. n. holotype. A, Carapace, dorsal view. B, Left male palp, retrolateral view.

Male copulatory bulb T as long as DD (Fig. 4.8 B); external distal border sloped backwards; internal sloped backwards. DD not bent, same T axis in lateral view; internal distal border markedly expanded. ES wider, more sclerotized than IS; IS continuous to tip. DD tip (Fig. 4.9 A-D) straight in lateral view. C present, short; distal end on DD internal tip; poorly developed; located close to DD distal tip; proximal border sharply decreasing; distal border truncated, upper tip not projected, rounded, external side smooth. AC absent. LF absent. L well-developed; external border sclerotized, laterally markedly folded, distally projected; distal border divergent, continuous. LA present, sheet-like; longer than; distally not fused to L. F absent. AL present, well-developed; proximal border in posterior view

smooth, not fused with distal haematodoca. P (Fig. 4.9 E) fused to T; perpendicular to T in lateral view; lateral length from 1/2 to 2/3 of T width; ridge present, perpendicular to T; distinctly expanded, right-angled, upper margin smooth; not distally projected; back margin not folded.

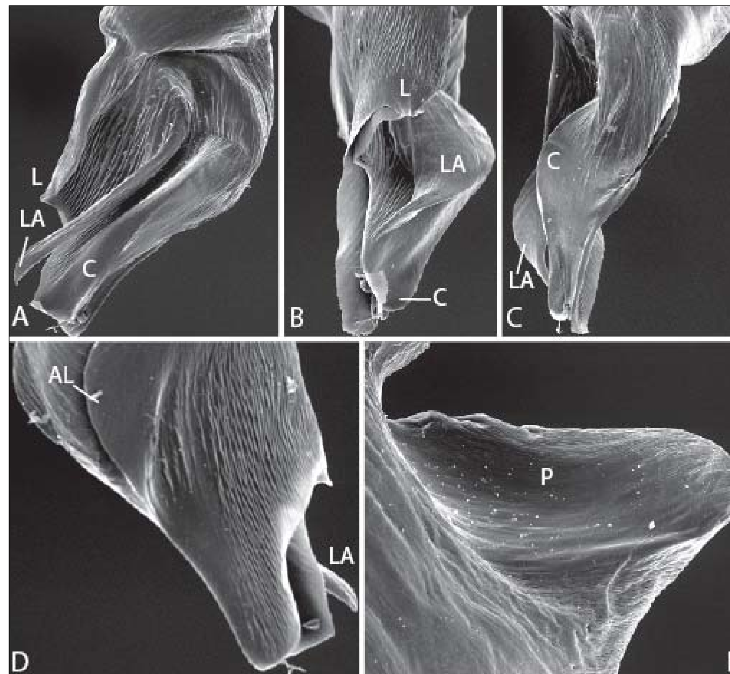

Fig. 4.9.- *Dysdera simbeque* sp. n. right male bulb (horizontal flipped images of the left bulb). A, anterior view; B, retrolateral view; C, prolateral view, D, posterior view, E, P detail, prolateral view.

FEMALE (*paratype* NMH 308UBXX): Fig. 4.10 A-B, F. All characters as in male except: Carapace 6.27 mm long; maximum width 4.9 mm; minimum width 3.26 mm. AME diameter 0.29 mm; PLE 0.25 mm; PME 0.19 mm.

Chelicerae 3.21 mm long; fang medium-sized, 2.29 mm; M close to B. Lengths of female described above: fe1 5 mm (all measurements in mm); pa1 3.47; ti1 4.28; me1 4.03; ta1 0.87; total 17.65; fe2 4.33; pa2 3.06; ti2 3.77; me2 3.52; ta2 0.82; total 15.5; fe3 3.37; pa3 2.04; ti3 2.55; me3 3.06; ta3 0.82; total 11.83; fe4 4.44; pa4 2.7; ti4 3.72; me4 4.23; ta4 0.87; total 15.96; fe Pdp 3.26; pa Pdp 1.53; ti

Pdp 1.27; ta Pdp 1.58; total 7.65; relative length 1>4>2>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.2.1; distal 1.0.0; tb3v spines arranged in one band; proximal 1.0.1; with two terminal spines. Fe4d spines in two rows; forward 2; backward 9; tb4d spines arranged in two bands; proximal 1.0.1; distal 1.0.1; tb4v spines arranged in two bands; proximal 1.0.1; distal 1.0.1; with two terminal spines.Abdomen 8.13 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

Vulva (Fig. 4.10 A-B) DA not distinguishable from VA; rectangular; DA twice as wide as long; DF wide in dorsal view. MF well-developed, completely sclerotized. VA frontal region completely sclerotized; posterior region sclerotized in most anterior area; AVD absent. S attachment not projected under VA; arms as long as DA, straight; tips dorsally projected; neck as wide as arms. TB usual shape.

VARIATION: Male cephalothorax ranges in length from 5.9-6.8 mm, females from 5.8-6.2 mm (N=4). The colour of the carapace varies between red orange to brownish orange in some specimens. Some have a larger amount of short black hairs in the sternum and in the ventral part of the chelicerae. The size of the S arms and the development of the lateral sclerotization of the VA differ among specimens. Spination and leg measurement variability is listed in Table 4.5 and 4.6, respectively.

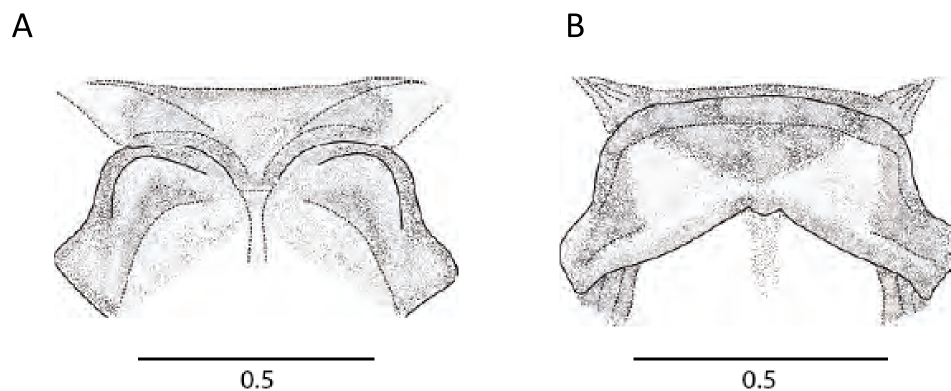

Fig. 4.10.- *Dysdera simbeque* sp. n. Female vulva. A, ventral view; B, dorsal view.

REMARKS: Specimens were collected by hand under stones as well as in MSS (Mesovoid Shallow Substratum) traps.

Table 4.5.- Intraspecific spination variability of *Dysdera simbeque* sp. n.

	Proximal	**Medial-proximal**	**Medial-distal**	**Distal**
Tibia 3 dorsal	1.1-2.1	0	0	0-1.0.0-1
Tibia 3 ventral	1.0-1.0-1	0	0	0.0.1
Tibia 4 dorsal	1.0-2.1	0	0	1.0.1
Tibia 4 ventral	1.0.1	0	0	0.2.0

	Number of rows	**Number of spines**
Femur 3 dorsal	0	0
Femur 4 dorsal	2	1-2/4-9

Table 4.6.- *Dysdera simbeque* sp. n. Leg measurements variability. Males (n=4)/Females (n=4).

	I	**II**	**III**	**IV**
Fe	5.1-5.5/4.5-5.0	4.5-5.0/4.1-4.3	3.3-4.1/3.2-3.4	4.4-4.8/4.2-4.5
Pa	3.5-3.9/3.2-3.5	3.1-3.5/2.8-3.0	2.1-2.3/1.9-2.0	2.6-2.7/2.4-2.7
Ti	5.0-5.6/4.0-4.3	4.1-4.7/3.5-4.0	2.5-2.8/2.3-2.5	3.6-3.8/3.5-3.7
Mt	4.3-4.9/3.6-4.0	4.0-4.4/3.2-3.6	3.2-3.6/2.9-3.1	4.3-4.6/3.9-4.2
Ta	0.8-1.0/0.87	0.8-0.9/0.8-0.7	0.8-0.8/0.7-0.8	0.87-1.02/0.87
TOTAL	19.1-20.8/16-17.6	16.8-18.2/14.4-15	11.9-13.4/11.3-11.8	16.1-17.0/15- 16

Phylogenetic analyses

The combined data matrix included 48 taxa, representing 15 species, and a total of 3749 characters (*cox1*: 1179 bp; *16S+L1:* 566 pb and 40 gap absence/presence characters, *nad1*: 343 pb; *28S*: 765 pb and 17 gap a/p chars., *H3*: 328 pb, *ITS-2*: 465 pb and 46 gap a/p chars.). Parsimony analyses of the combined data matrix yielded 2 trees of 4071 steps (CI: 47, RI: 74). All clades received Jackknife supports above 70%, except for the position of *D. sanborondon* as sister to *D. alegranzaensis* + *D. simbeque* (Fig. 4.11). The AIC criterion implemented in

jMODELTEST selected the following models of nucleotide substitution for each gene fragment: TIM3+I+G for *cox1*; TIM2+I+G for *16S+L1;* TrN+I+G for *nad1*; TIM2+G for *28S;* TrNef+I+G for *H3* and HKY+G for *ITS-2*. The Bayesian inference analyses were run during 4 million generations and the first 10% were discarded as burn-in. Maximum likelihood analyses yielded one tree of logL -2713.866745. All analyses agreed in supporting monophyly of eastern Canarian endemics, with the exclusion of *D. lancerotensis*, and in recovering the same internal topology. The only source of conflict across the different analyses was the position of *D. sanborondon*, sister to *D. simbeque* sp. n. + *D. alegranzaensis* in the parsimony analyses and sister to the remaining taxa in the model based analyses, although both alternative positions were poorly supported (Fig. 4.11). PBS values (Table 4.7) indicated low levels of character conflict across partitions (largest negative value for the mitochondrial data set -4 for clade 4 and largest nuclear -2 for clade 8).

Table 4.7.- Values of Partitioned Branch Support (PBS) obtained for each gene data partition. Clades numbers as shown in Fig. 4.11. BS: Bremer support.

	PBS								
Clade	***cox*1**	***nad*1**	***16S***	**mtDNA**	***H3***	***28S***	***ITS***	**nDNA**	**BS**
1	6	-1	-1	4	1	-1	0	0	4
2	13	19	21	53	13	7	0	20	73
3	2	0	-3	-1	1	2	0	3	2
4	-5	3	-2	-4	0	6	5	11	7
5	6	0	5	11	4	13	0	17	28
6	-1	10	2	11	1	16	0	17	28
7	6	16	9	31	2	3	7	12	43
8	4	7	-1	10	1	0	-3	-2	8
9	8	4	3	15	3	3	3	9	24
10	-2	2	0	0	0	0	2	2	2
11	29	11	15	55	5	3	30	38	93
12	6.5	2.5	8.5	17.5	2.5	3	3	8.5	26
13	26.5	19	4	49.5	2	0	10.5	12.5	62
14	22.5	14	9.5	46	2.5	0	8.5	11	57
15	9	4	1	14	-2	0	4	2	16
16	-2	29	15	42	9	1	8	18	60
17	-2	6	0	4	3.67	0.67	4.67	9.01	13.01
18	17.5	9.9	14.7	42.1	-0.1	0	0	-0.1	42
19	11.86	5.28	-0.57	16.57	0.28	-0.93	0.071	-0.579	16
20	31	12	12	55	1	0	1	2	57
21	13.5	12	3.5	29	0	3	1	4	33
22	9.5	-0.5	4.5	13.5	0.5	0	0	0.5	14
23	8	3.25	4.5	15.75	1.5	0.75	0	2.25	18
Total	217.86	187.43	124.63	529.92	51.85	59.49	84.74	196.08	

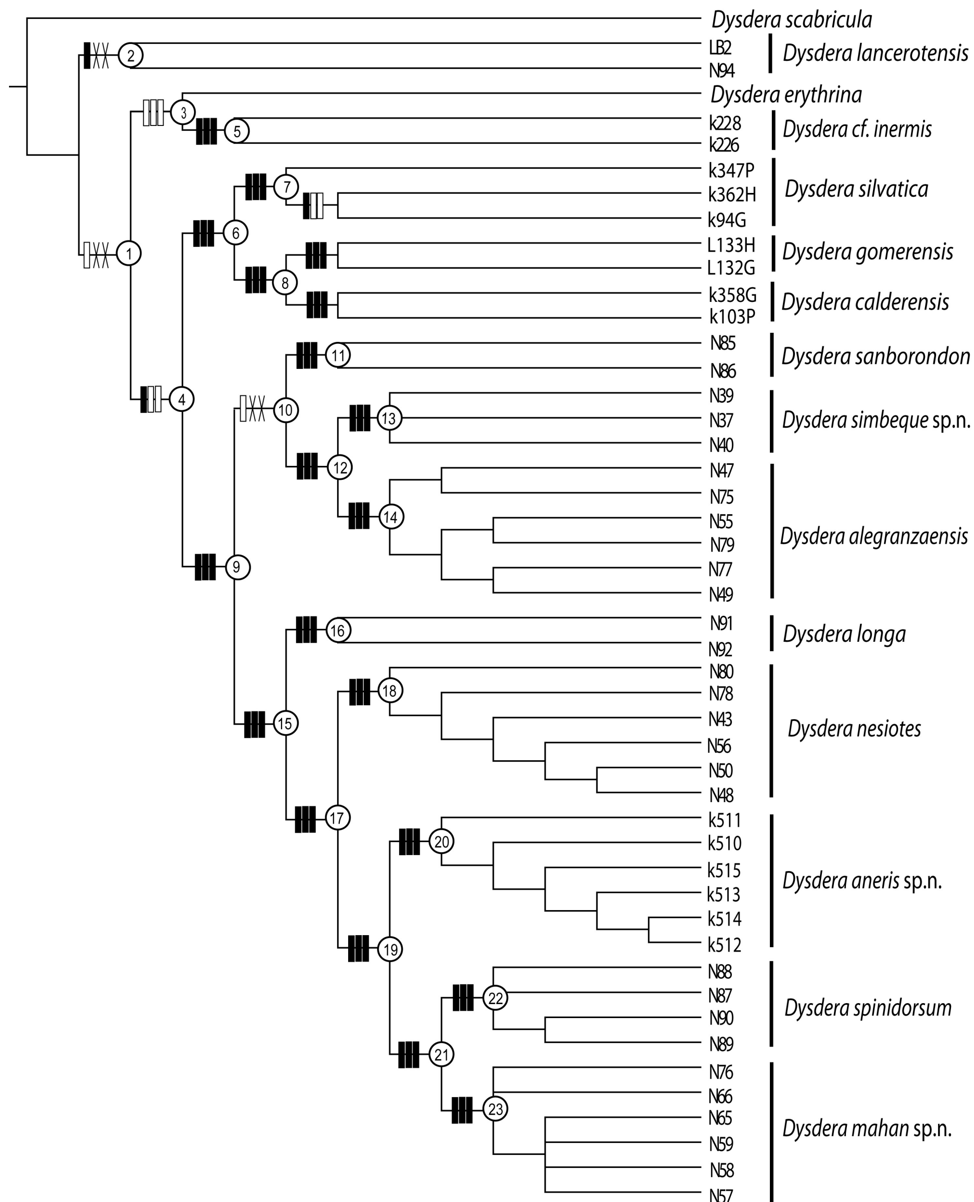

Fig. 4.11.- Strict consensus of two trees of 4071 steps (CI= 47, RI= 74) resulting from uniformly weighted parsimony analysis of the complete data set. Bars on branches denote support as follows: anterior bar refers to parsimony jackknife support, middle bar to maximum likelihood bootstrap support and posterior bar to posterior probability. Black bar: parsimony jackknife or ML bootstrap >70%, posterior probability >0.95%; white bar: parsimony jackknife or ML bootstrap <70%, posterior probability <0.95%; asterisk (*): this particular clade was not recover in the analyses.

The ILD test revealed the existence of significant incongruence between the mitochondrial and nuclear data sets (*P*=0.001). Visual inspection of the trees obtained from the independent analyses of the mitochondrial and nuclear genes revealed that the main source of conflict is the position of the species *D. longa*, which forms a monophyletic group with *D. nesiotes*, *D. aneris* sp. n., *D. spinidorsum* and *D. mahan* sp. n. based on the mitochondrial data (Fig. 4.12), but it is sister to *D. simbeque* sp. n. + *D. alegranzaensis* according to the nuclear genes (Fig. 4.13). Another relevant topological difference between the two partial analyses is the position of *D. aneris* sp. n., sister to *D. spinidorsum* + *D. mahan* sp.n. according to mitochondrial data, but sister to *D. nesiotes* based on the nuclear genes. In addition, the nuclear genes did not support monophyly of the genotypes of *D. mahan* sp. n., *D. spinidorsum, D. aneris* sp. n. and *D. nesiotes,* and some of these species did, in fact, share nuclear genotypes. Support for the conflicting topologies involving the last species, however, was in all analyses very low, and the ILD test applied only to these species failed to detect significant incongruence between both partitions (p=0.1918). Comparisons of uncorrected genetic distances (*P*-values) between eastern Canarian species indicated that *D. spinidorsum* and *D. mahan* sp. n. showed the lower levels of genetic divergence for all gene fragments. Uncorrected pairwise distances within and between species revealed higher divergence levels for the mtDNA than for the nuclear *ITS-2* gene (Table 4.8).

Divergence times

Preliminary cross-validation analyses selected the Langley-Fitch as the best clock method for analysing the mitochondrial data set. Clade age estimates and corresponding confidence intervals are summarized in Fig. 4.14. The average rate of substitution estimated was 0.04916 per site per million years, corresponding to a pairwise genetic divergence of 9.8%, five times faster than the rates estimated by Macías-Hernández *et al.,* (2008) using a single calibration point (1.75% pairwise divergence), and four times faster than universal rates reported for arthropod mitochondrial genes (DeSalle *et al.*, 1987; Brower, 1994), but similar to the rates estimated for *Dysdera lancerotensis* (10.2%) (Bidegaray-Batista *et al.*, 2007). In spite of the higher substitution rates, estimated divergence time in the present study largely overlapped with the confidence intervals of estimates by Macías-Hernández *et al*., (2008).

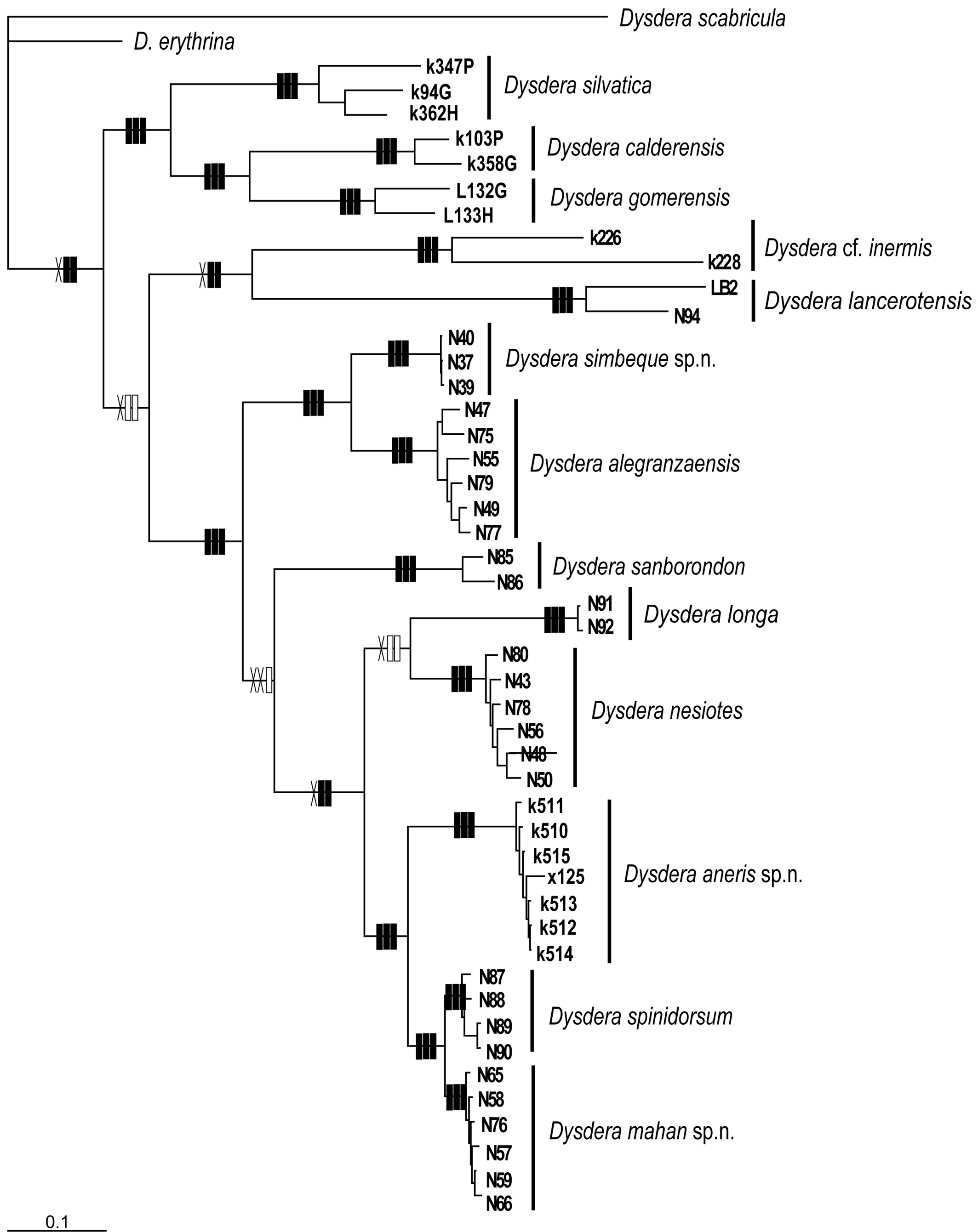

Fig. 4.12.- Bayesian majority rule consensus tree of the combined mitochondrial genes.

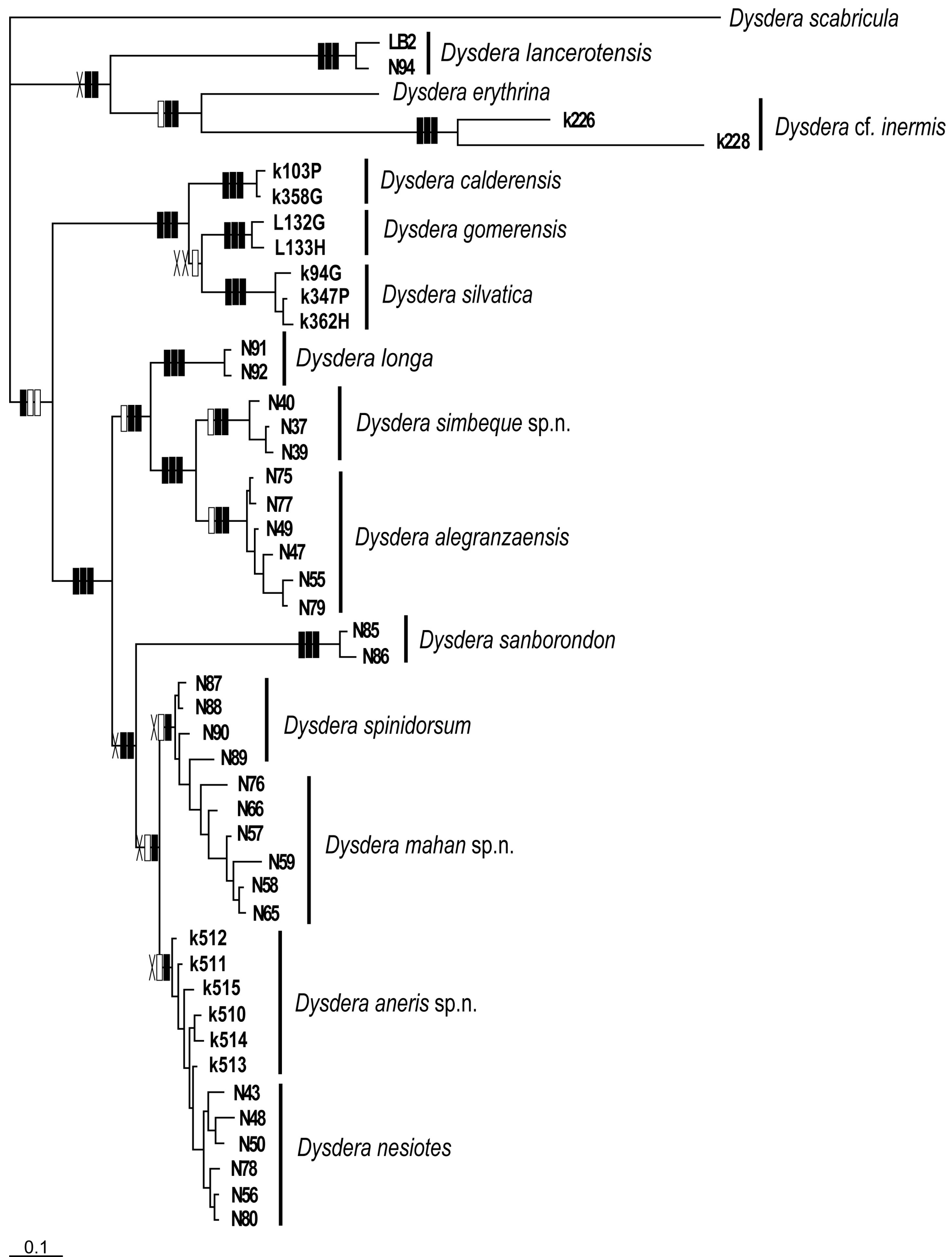

Fig. 4.13.- Bayesian majority rule consensus tree of the combined nuclear genes.

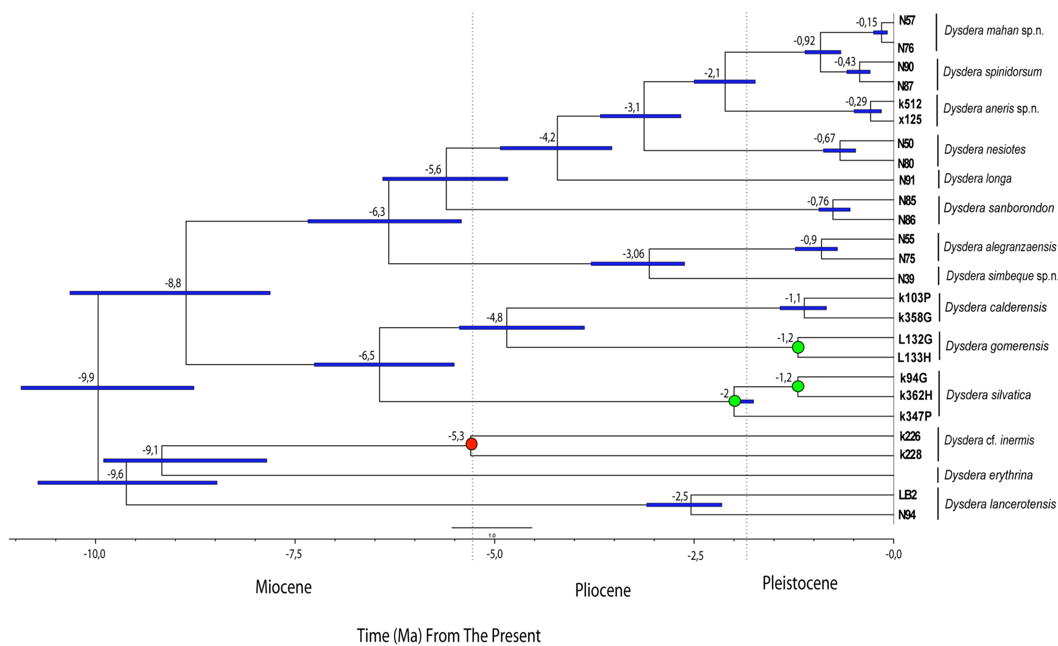

Fig. 4.14.- Chronogram obtained by the Langley-Fitch clock method, based on the preferred Bayesian topology obtained by the simultaneous analyses of the *cox1, 16S, nad1*, *28S* and *H3* partitions. Numbers on node are estimated lineage age and bars are confidence intervals based on bootstrap resampling of branch lengths. Green circles and red circle correspond to maximum and fixed calibration points, respectively (see text for details).

Analysis of morphological variation

Few variables show evidence of sex dimorphism across the species examined (Fe1, Esc and Fang in *D. spinidorsum*; Fe1 in *D. alegranzaensis*; Fe1 and Q2 in *D. aneris* sp. n.; and Fe1 and Mt4 in *D. longa*). We interpreted significant sex differences among variables as random sampling variability due to the lack of a general pattern of sex dimorphism, although some caution should be exerted regarding the Fe1 (significantly different between sexes in four species). Further analyses were conducted considering individuals of both sexes of each species. The Krustal-Wallis test revealed significant interspecific differences in all morphological variables. Wilcoxon Matched Pairs test detected significant differences in body size (P1) between all species compared except for the species pairs: *D. alegranzaensis* - *D. nesiotes* (Z=0.459; P=0.65), *D. alegranzaensis* - *D. aneris* (Z=1.172; P= 0.24) and *D. mahan* - *D. spinidorsum* (Z=0.178; P= 0.859). The first PCA axis accounted for

31.41% of the variance and was associated with the chelicera and fang lengths. The second axis accounted for 58.52% of the total variance and was associated with appendage length (Fe1, Mt4) (Fig. 4.15 A). In the PCA plot, *D. mahan* sp. n. stands clearly apart from all other species. The Bray-Curtis similarity cluster defines four main groups: small species (*D. sanborondon*), medium-size species (*D. alegranzaensis, D. nesiotes* and *D. aneris* sp. n.), large species (*D. longa*, *D. spinidorsum* and *D. simbeque* sp. n.) and *D. mahan* sp. n., a large species with long appendages (Fig. 4.15 B).

DISCUSSION

Morphology provided the single major source of evidence to delimit species boundaries until the mid 20th century (Coyne & Orr, 1998). Relying solely on morphological characters, however, tends to oversimplify and underestimate diversity (Bond *et al.*, 2001; Bickford *et al.*, 2007). Continuous or barely partitioned diversity (i.e. polymorphism), low variability (cryptic species), sex or life stage restricted diagnosability or simply lack of expertise may hinder the use of morphology for species delimitation. In recent years, the democratization of access to molecular techniques had sparked the use of DNA sequence data, particularly mitochondrial DNA, for testing traditional, morphology-based taxonomies (e.g. Caterino *et al.*, 2000).

The application of molecular techniques to taxonomy has frequently led to the discovery of overlooked lineages (e.g. Molbo *et al.*, 2003; Saez *et al.*, 2003; Hebert *et al.*, 2004a; Kankare & Shaw, 2004; Pfenninger & Schwenk, 2007), and facilitated the identification of cryptic species (Wiens & Penkrot, 2002). Success of DNA based approaches to assist in taxonomic decisions probably underpinned recent propositions for the adoption of an alternative taxonomic reference system solely based on DNA sequence information, i.e. DNA taxonomy (Tautz *et al.*, 2002; 2003). This suggestion, however, has been heavily criticized on the grounds that it seems illogical to discard a broad range of data that could potentially be used both for describing species, and generating remarkable scientific hypotheses and

predictions about the distribution of attributes among organisms (Lipscomb *et al.*, 2003; Seberg *et al.*, 2003).

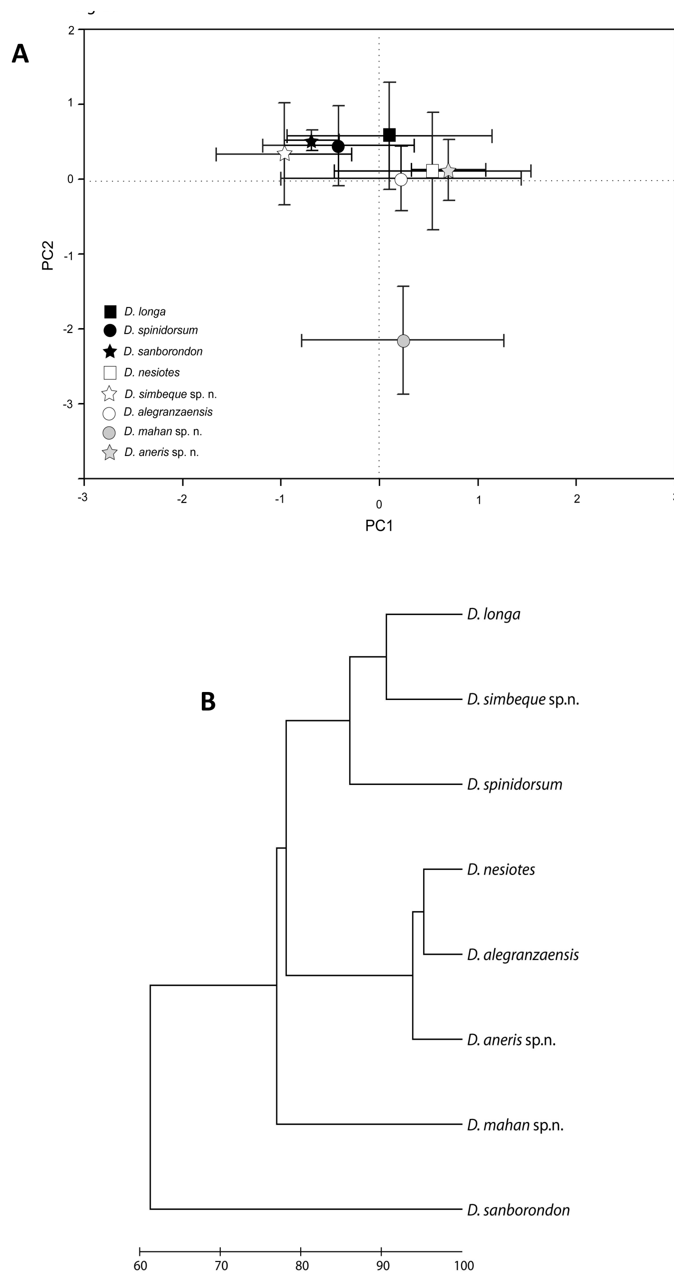

Fig. 4.15.- A. Principal Component Analysis. Plot of the first two discriminant axes, with the 95% of score variance on each axis. B. Dendrogram showing species similarity scores derived from somatic measurements estimated across a hierarchical agglomerative cluster using the Bray-Curtis distance.

There is little doubt, however, that the use of genetic data in combination with other sources of information may provide a better taxonomic knowledge of species boundaries (Balakrishnan, 2005; Knowles & Carstens, 2007), and the present study provides a good example.

A former taxonomic revision of the endemic eastern Canarian *Dysdera* highlighted an unusual variability in the vulval morphology of the species *D. nesiotes* (see Figs. 59-62 in Arnedo *et al.*, 2000), which was interpreted as intraspecific polymorphism. In the context of a molecular phylogeny of the group, Macías-Hernández *et al.,* (2008) pointed out that *D. nesiotes* might actually include three independent lineages, overlooked in the former revisionary work. The morphological study of a larger sample of specimens in combination with molecular data, geographic distribution and ecological preferences has now allowed us to reinterpret morphological polymorphism and to delimit and formally describe three new taxa.

Genetic divergence is not necessarily related to morphological differentiation (e.g. Orr & Smith, 1998). Significant genetic divergences may underlie morphologically homogeneous species, as seen in cryptic species complexes (Bond *et al.*, 2001; Hedin & Wood, 2002; Sinclair *et al.*, 2004; Boyer *et al.*, 2007). In adaptive radiations, on the other hand, remarkable phenotypic differentiation may be accomplished in the absence of clear genetic discontinuities, as exemplified by the African cichlid fishes (Nagl *et al.*, 1998). Of the three new species here described, *D. mahan* sp. n. shows the largest morphological differences with its sister taxa (see Fig. 4.15 A-B). These two species, however, display the lowest pairwise genetic divergences of any sister species pair comparison (3.6% in mtDNA), and both share common alleles in *ITS-2* and *H3*. The low genetic differentiation suggests a relatively recent time of divergence, further confirmed by the molecular clock dating (0.9 Ma, 0.6-1.6 Ma), the most recent speciation event in the group. Newly formed species achieve genealogical exclusivity in mitochondrial DNA long before they become distinct in nuclear markers (Wiens & Petrock, 2002) due to higher substitution rates (Brown *et al.*, 1979; Pamilo & Nei, 1988) and smaller effective population size (Pamilo & Nei,

1988; Tautz *et al.*, 2003). The retention of unsorted ancestral polymorphism, therefore, seems the most plausible explanation for the lack of genealogical exclusivity in the nuclear genes. The habitat occupied by *D. mahan* sp. n., the intertidal zone in pebble beaches, is unique among Canarian *Dysdera* and represents the only true case of ecological shift in this large species radiation. Decoupling of genetic divergence and morphological differentiation in this case could be the result of a natural selection acting on phenotypic traits adaptive to this unusual environment (Dieckmann, 2004). In spiders, among other animal groups, genitalia are the most important source of diagnostic characters, which implies that relative to the other structures the genitalia have diverged rapidly (Eberhard, 1985). The absence of diagnostic characters in the male copulatory bulbus of *D. mahan* sp. n. compared with *D. nesiotes* and *D. aneris* sp. n., however, provides evidence for the rapid evolution of somatic morphology in this species, and hence for the involvement of natural selection.

Dysdera aneris sp.n. lies at the other side of a putative gradient of negatively correlated genetic and morphological differentiation. This species is well characterized from a genetic standpoint, it shows high mtDNA pair-wise divergences compared with closely related taxa (7.3-10%) and all mtDNA and nuclear alleles are exclusive (except *28S*). Nevertheless, this species had been previously overlooked and misidentified as *Dysdera nesiotes*, which is not even its sister taxa according to the mitochondrial data. In fact, molecular markers provided crucial evidence to diagnose the new species based on vulval characters that were previously misinterpreted as intraspecific polymorphism. Significant molecular divergences in the absence of morphological changes are common in spider groups with poor dispersal capabilities, hence with geographically isolated populations and subject to similar environmental conditions (Bond *et al.*, 2001; Hedin, 2001). An infrequent, long range dispersal event allowed *D. aneris* sp. n. to diverge in isolation on an island with a similar environment and selective forces that preserved the original phenotype. Molecular dating puts an upper limit on the colonization of the Salvage Islands by *Dysdera* of about 2.1 Ma (1.5-2.5), similar to what has been found in the wall lizard *Lacerta dugesii* (Brehm *et al.*, 2003). This data corroborates a previous suggestion regarding the late Pliocene origin of

present-day Salvage Island biota, following subaerial volcanism after island submergence (Geldmacher *et al.*, 2001).

The third new species described in this paper, *D. simbeque* sp. n. coexists on the same island with its sister species, although they do not overlap in their distribution range. These two species significantly differ from each other in size and small genitalic features. Size segregation in these species is difficult to explain, since the two species share the same ecological regime and are both syntopic with a third endemic, *D. nesiotes*. The significant larger size of *D. simbeque* sp. n., compared with *D. nesiotes*, is in accordance with the expectations of trait segregation in sympatric species to avoid resource competition. The range size of *D. nesiotes*, on the other hand, fully overlaps with that of *D. alegranzaensis*. A comparative phylogeographic study of the two species is currently under way to investigate patterns of vicariance and secondary sympatry (Macías-Hernández *et al*., in prep) that may explain coexistence of close relatives with virtually identical morphology.

The use of DNA sequences as a complement to the traditional morphology-based taxonomy provides an invaluable resource for phylogenetics (Tautz *et al.*, 2003). In addition, the advisable use of multiple loci for species delimitation improves the chances of obtaining more reliable trees (Pamilo & Nei, 1988; Takahata, 1989; Maddison, 1997), and the patterns of congruence among multiple unlinked loci offer insights into relevant evolutionary processes, such as hybridization or introgression (Funk & Omland, 2003). Our results illustrate that the simultaneous analysis of mitochondrial and nuclear genes improves the resolution of the tree topology and increases clade support. The ILD test, however, revealed significant incongruence between mtDNA and nuDNA genes. The main areas of disagreement are the position of *D. longa* and *D. aneris* sp. n., although an ILD test run on the clade including the species *D. aneris* sp. n., *D. nesiotes*, *D. spinidorsum* and *D. mahan* sp. n. was not significant. Instances of incongruence between mtDNA and nDNA genes are not uncommon among arthropods and other organisms (Funk *et al.*, 1995; Sota & Vogler, 2001; Funk & Omland, 2003; Kroon *et al.*, 2004).

Several causes have been put forward to explain apparent incongruence between mitochondrial and nuclear markers, including incomplete lineage sorting of ancestral polymorphisms (Tajima, 1983; Takahata & Nei, 1985; Nei, 1987; Pamilo & Nei, 1988; Takahata, 1989; Wu, 1991; Hudson, 1992; Lyons-Weiler & Milinkovitch, 1997; Nagl *et al.*, 1998), introgressive hybridization (Bull *et al.*, 1993; Avise, 1994; Moore, 1995; Brower, 1996; Doyle, 1997; Maddison, 1997; Klein *et al.*, 1998; Avise, 2000; Takahashi *et al.*, 2001; van Oppen *et al.*, 2001), homoplasy in the data (Baker & DeSalle, 1997; Sota & Vogler, 2001), or differences in analytical and methodological procedures (Brower, 1996). Although shared ancestral polymorphism and hybridization are difficult to detect at the first stages of the speciation process (Sota & Vogler, 2001), hybridization is more a likely cause of incongruence if populations co-occurred in sympatry (Rokas *et al.*, 2003). All the species with present-day overlapping distributions, however, were genealogically exclusive in both mitochondrial and nuclear markers. *Dysdera nesiotes*, on the other hand, is paraphyletic with regard to *D. aneris* sp. n. based on nuclear markers, but the two species are separated by more than 165 km of open sea, which rules out the involvement of hybridization.

The patterns of non-monophyly in nuclear markers suggest that incomplete lineage sorting may be a better explanation of the incongruence between genome partitions. The independent networks of each nuclear gene showed incongruent patterns of reticulation and allele sharing among the species *D. nesiotes*, *D. aneris* sp. n., *D. spinidorsum* and *D. mahan* sp. n. The sister group relationship of *D. aneris* sp. n. and *D. nesiotes* supported by nuclear genes could hence be the result of the retention in *D. nesiotes* and *D. aneris* sp. n. of nuclear alleles already present in the common ancestor shared with closer relatives. A similar argument could be invoked to explain the incongruent position of *D. longa* in the trees recovered from each genome partition. Incomplete lineage sorting has been proposed in a variety of organisms as the main factor causing incongruence between species and gene trees, and among unlinked loci (Ohtsuka *et al.*, 1996; Hamada *et al.*, 1998; Takahashi *et al.*, 2001; Pollard *et al.*, 2006; Carstens & Knowles, 2007). The faster rate of fixation of ancestral mtDNA polymorphisms compared with nuclear genes suggests that mitochondrial gene trees are more likely to reflect the true species

relationships than a nuclear encoded gene (Moore, 1995; Moore, 1997). In the presence of gene flow between diverging populations, on the other hand, mtDNA may be homogenized between the populations more readily than nuclear DNA, so that mtDNA may appear paraphyletic when nuclear genes may be monophyletic (Ballard & Rand, 2005).

In the present study, the combined analyses resolved instances of incongruence between the genomic partitions mostly in favour of the mitochondrial partition. This may simply reflect the larger amount of variable characters in the mtDNA partition, as suggested by the higher pairwise divergences (Table 4.8). Nevertheless, the number of nodes in the combined tree with negative PBS values was low, and similar in both genomic partitions (2 and 3 for mitochondrial and nuclear genes, respectively, Table 4.7). In fact, only the *H3* dataset showed negative partitioned Bremer support values at the combined topology node 15 (Fig. 4.14), which is the major area of disagreement between partitions, while the other nuclear partitions were either positive or zero. The nuclear genes may actually not be as incongruent with mitochondrial genes as suggested by the ILD. It has been shown that the ILD test is prone to reporting significant conflict between character partitions when these differ only in the amount of noise (Quicke *et al.*, 2007), there are few characters, or the substitution rate is not homogeneous (Darlu & Lecointre, 2002). The high support of the conflicting clade between genome partitions, however, suggests otherwise (Mason-Gamer & Kellogg, 1996).

Table 4.8.- Within (diagonal) and between mean uncorrelated genetic divergences (*p*-values, s.d. in parentheses) in mtDNA and *ITS-2* genes

	D. alegranzaensis	***D. nesiotes***	***D. mahan***	***D. sanborondon***	***D. spinidorsum***	***D. longa***	***D. simbeque***	***D. aneris***
D. alegranzaensis								
mtDNA	0.028 (0.002)							
ITS	0.004 (0.002)							
D. nesiotes								
mtDNA	0.150	0.007 (0.001)						
ITS	0.049	0.001 (0.001)						
D. mahan								
mtDNA	0.143	0.097	0.007 (0.001)					
ITS	0.048	0.012	0.001 (0.001)					
D. sanborondon								
mtDNA	0.145	0.138	0.133	0.032 (0.004)				
ITS	0.121	0.098	0.099	0.011 (0.005)				
D. spinidorsum								
mtDNA	0.140	0.098	0.036	0.127	0.013 (0.002)			
ITS	0.048	0.012	0.001	0.098	0			
D. longa								
mtDNA	0.153	0.123	0.132	0.137	0.134	0.001 (0.001)		
ITS	0.052	0.039	0.037	0.116	0.036	0.005 (0.004)		
D. simbeque								
mtDNA	0.095	0.143	0.131	0.138	0.133	0.138	0.001 (0.001)	
ITS	0.046	0.046	0.041	0.096	0.041	0.056	0.005 (0.003)	
D. aneris								
mtDNA	0.145	0.100	0.076	0.130	0.073	0.132	0.133	0.008 (0.002)
ITS	0.045	0.006	0.008	0.095	0.007	0.039	0.046	0

APPENDIX

Appendix 4.1.- Abbreviations used in text and figures.

Collections

BMNH	The Natural History Museum, London, United Kingdom
CRBA	Centre de Recursos de Biodiversitat Animal, Universitat de Barcelona, Barcelona, Spain
MHNP	Muséum National d'Histoire Naturelle, Paris, France
OXUM	Hope Entomological Collections, University Museum, Oxford, United Kingdom
ULL	Departamento de Biología Animal, Universidad de La Laguna, Tenerife, Canary Islands, Spain

Eyes

AME	anterior medial eyes
PLE	posterior lateral eyes
PME	posterior medial eyes

Cheliceral teeth

B	basal tooth
D	distal tooth
M	medial tooth

Male copulatory bulb

AC	additional crest
AL	additional lateral sheet at the internal border
AR	arch-like ridge
C	crest
DD	distal division
DH	distal haematodoca
ES	external sclerite
F	flagellum
IS	internal sclerite
L	lateral sheet
LA	lateral sheet anterior apophysis
LF	lateral fold over lateral sheet between internal and external sclerites
P	posterior apophysis
T	tegulum

Female genitalia

DA	dorsal arch
DF	dorsal arch fold
MF	major fold
S	spermatheca
TB	transversal bar
VA	ventral arch
VS	ventral sclerotisation

Appendix 4.2.- Taxa examined and GenBank accession numbers (asterisks indicate that only the *16S* fragment could be amplified) XXXX new sequences obtained in the present study (will be replaced with definitive number upon acceptance)

Voucher	**DNA**	**Species**	**Locality**	***cox1***	***16S-L1-nad1***	***28S***	***H3***	***ITS-2***
Eastern Canaries								
NMH 429	N47	*D.alegranzaensis*	Montaña de Lobos. Alegranza	EF458132	EF458087	EU139759	EU139688	EU143814
NMH 364	N49	*D. alegranzaensis*	Montaña Clara	EU139610	EU139637	-	EU139689	EU143815
NMH 424	N55	*D. alegranzaensis*	Valle Fenauco. Yaiza. Lanzarote	EU139611	EU139638	EU139760	EU139690	EU143816
NMH 449	N75	*D. alegranzaensis*	Montaña de las Agujas. La Graciosa	EU139612	EU139639	EU139761	EU139691	EU143817
NMH 73	N77	*D. alegranzaensis*	Mirador del Río. Haría. Lanzarote	EU139609	EU139640	EU139762	EU139692	EU143818
NMH 462	N79	*D. alegranzaensis*	Montaña de Tinache. Tinajo. Lanzarote	EU139613	EU139641	EU139763	EU139693	EU143819
NMH 59	N37	*D. simbeque* sp.n	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139614	EU139659	EU139783	EU139712	EU143838
NMH 60	N39	*D. simbeque* sp.n	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139631	EU139660	EU139784	EU139713	EU143839
NMH 55	N40	*D. simbeque* sp.n	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139632	EU139661	EU139785	EU139714	-
NMH 163	N94	*D. lancerotensis*	Morro del Cavadero. Jandía. Fuerteventura	EF458120	EF458086	EU139758	EU139687	EU143813
NMH 441	LB2	*D. lancerotensis*	Caldera. Alegranza	EF458127	EF458080	EU139757	EU139686	EU143812
NMH 168	N91	*D. longa*	Morro del Cavadero. Jandía. Fuerteventura	EF458134	EF458090	EU139781	EU139710	EU143836
NMH 169	N92	*D. longa*	Morro del Cavadero. Jandía. Fuerteventura		EU139658	EU139782	EU139711	-
NMH 358	N57	*D. mahan* sp.n	Playa del Trillo. Alegranza	EU139620	EU139647	EU139769	EU139700	EU143826
NMH 451	N58	*D. mahan* sp.n	Caleta de Arriba. La Graciosa	EU139621	EU139648	EU139770		EU143827
NMH 356	N59	*D. mahan* sp.n	Playa Catalina Cabrera. Famara. Lanzarote	EU139622	EU139649	EU139771	EU139701	-
NMH 447	N65	*D. mahan* sp.n	Playa del Congrio. Papagayo. Lanzarote	EU139623	EU139650	EU139772	EU139702	EU143828
NMH 490	N66	*D. mahan* sp.n	Playa de Majanicho. Fuerteventura	EU139624	EU139651	EU139773	EU139703	EU143829
NMH 572	N76	*D. mahan* sp.n	Las Salinas. Lobos	EU139625	EU139652	EU139774	EU139704	-
NMH 57	N43	*D. nesiotes*	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	EU139615	EU139642	EU139764	EU139695	EU143820
NMH 428	N48	*D. nesiotes*	Montaña de Lobos. Alegranza	EU139616	EU139643	EU139765	EU139694	EU143821
NMH 369	N50	*D. nesiotes*	Montaña Clara	EF458133	EF458088	EU139766	EU139696	EU143822
NMH 425	N56	*D. nesiotes*	Valle Fenauco. Yaiza. Lanzarote	EU139617	EU139644	EU139767	EU139697	EU143823
NMH 398	N78	*D. nesiotes*	Mirador del Río. Haría. Lanzarote	EU139618	EU139645	-	EU139698	EU143824
NMH 476	N80	*D. nesiotes*	Montaña de Tinache. Tinajo. Lanzarote	EU139619	EU139646	EU139768	EU139699	EU143825
NMH 50	N85	*D. sanborodon*	Morro Tabaiba. Vallebrón. Fuerteventura	EF458135	EF458089	EU139775	EU139705	EU143830

Voucher	DNA	Species	Locality	*cox1*	*16S-L1-nad1*	*28S*	*H3*	*ITS-2*
NMH 506	N86	*D. sanborodon*	Montaña de la Cruz. Betancuria.Fuerteventura	EU139626	EU139653	EU139776	EU139706	EU143831
NMH 49	N87	*D. spinidorsum*	Morro Tabaiba. Vallebrón. Fuerteventura	EU139627	EU139654	EU139777	EU139707	EU143832
NMH 494	N88	*D. spinidorsum*	Montaña de la Cruz. Betancuria. Fuerteventura	EU139628	EU139655	EU139778	EU139708	EU143833
NMH 78	N89	*D. spinidorsum*	Cuchillete Montaña Peños. Fuerteventura	EU139629	EU139656	EU139779		EU143834
NMH 114	N90	*D. spinidorsum*	Morro del Peñón. Fuerteventura	EU139630	EU139657	EU139780	EU139709	EU143835
NMH 290	X125	*D. aneris* sp.n	Selvagem Grande, Salvage Islands	EU139634	EU139683*			
NMH608	K510	*D. aneris* sp.n	Selvagem Grande, Salvage Islands	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
NMH609	K511	*D. aneris* sp.n	Selvagem Grande, Salvage Islands	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
NMH610	K512	*D. aneris* sp.n	Selvagem Grande, Salvage Islands	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
NMH611	K513	*D. aneris* sp.n	Selvagem Grande, Salvage Islands	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
NMH612	K514	*D. aneris* sp.n	Selvagem Grande, Salvage Islands	XXXXX	XXXXX	XXXXX	XXXXX	-
NMH613	K515	*D. aneris* sp.n	Selvagem Grande, Salvage Islands	XXXXX	XXXXX	XXXXX	XXXXX	-
Western Canaries								
UB4013	K103	*D. calderensis*	Juan Adalid, Garafía. La Palma	AF244309	AF244218/EU139665	EU139788	EU139718	XXXXX
NMH 1438	N358	*D. calderensis*	Riscos de Alojera. La Gomera	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
CRBA1393	LB132	*D. gomerensis*	Cañada de Jorge, La Gomera	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
CRBA1395	LB133	*D. gomerensis*	Casa Forestal de Frontera. El Hierro	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
UB4155	K94	*D. silvatica*	Barranco de Juel, Hermigua. La Gomera	AF244273	AF244177/EU139674	EU139808	EU139739	EU143842
NMH 1395	N347	*D. silvatica*	Pinar Roque Faro. La Palma	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX
NMH 1439	N362	*D. silvatica*	Mirador de Bascos. El Hierro	XXXXX	XXXXX	XXXXX	-	XXXXX
Continental								
NHM255	K226	*D.* cf. *inermis*	4 km S Tanger. Morocco	EF458142	EF458092	EU139795	EU139726	-
NHM075	K228	*D.* cf. *inermis*	Mirador del Estrecho, Tarifa. Iberian Peninsula					-
UB-ery105	K105	*D. erythrina*	Sant Llorenç del Munt, Barcelona. Iberian Peninsula	AF244252	AF244162*	EU139790	EU139720	EU143840
CRBA590	K294	*D. scabricula*	Desert de les Palmes, València. Iberian Peninsula	EU068046	EU068078	EU139809	EU139740	NO

Appendix 4.3.- List of localities were the new species described have been collected. Codes refer to numbers in Fig. 4.1

Code	Locality	Lat long
D. aneris sp. n		
1	Selvagem Grande, Salvage Islands	N 30.146105 W 15.864975
D. mahan sp. n.		
2	Playa del Trillo. Alegranza	N 29.404183 W 13.490834
3	Caleta de Arriba. La Graciosa	N 29.236493 W 13.496500
4	Playa Catalina Cabrera. Famara. Lanzarote	N 29.194447 W 13.505115
5	Playa La Caleta. Haría. Lanzarote	N 29.202379 W 13.421033
6	Malpaís de La Corona. Charcos de Marea. Haría, Lanzarote	N 29.204139 W 13.427907
7	Playa de Teneza. Tinajo. Lanzarote	N 29.081178 W 13.715303
8	Punta Pasitos. Mala. Lanzarote	N 29.104335 W 13.455998
9	Playa de La Madera. Tinajo, Lanzarote	N 29.062068 W 13.774603
10	Caleta del Mariscadero. Timanfaya. Lanzarote	N 29.064083 W 13.766023
11	Playa del Congrio. Papagayo. Yaiza. Lanzarote	N 28.838822 W 13.787027
12	Las Salinas. Puerto Calero. Yaiza. Lanzarote	N 28.918115 W 13.698886
13	Las Salinas. Islote de Lobos	N 28.743173 W 13.829946
14	Playa de Majanicho. Corralejo. Fuerteventura	N 28.744862 W 13.873706
15	Playa del Esquinzo. La Oliva. Fuerteventura	N 28.636453 W 14.025550
D. simbeque sp. n.		
16	Fuente Ovejas. Guinate. Haría. Lanzarote	N 29.184361 W 13.501313
17	Bco. Elvira Sánchez. Valle de Malpaso. Haría. Lanzarote	N 29.130723 W 13.516902

REFERENCES

Arechavaleta M, Zurita N, Marrero M, Martín JL. 2005. *Lista Preliminar de Especies Silvestres de Cabo Verde (hongos, plantas y animales terrestres)*. Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias.

Arnedo MA, Oromí P, Múrria C, Macías-Hernández NE, Ribera C. 2007. The dark side of an island radiation: Systematics and evolution of troglobitic spiders of the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands. *Invertebrate Systematics* 21: 623–660.

Arnedo MA, Oromí P, Ribera C. 1996. Radiation of the genus *Dysdera* (Araneae, Haplogynae, Dysderidae) in the Canary Islands: The Western Islands. *Zoologica Scripta* 25: 241-274.

Arnedo MA, Oromí P, Ribera C. 2000. Systematics of the genus *Dysdera* (Araneae, Dysderidae) in the Eastern Canaries. *Journal of Arachnology* 28: 261-292.

Arnedo MA, Oromí P, Ribera C. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. *Cladistics* 17: 313-353.

Arnedo MA, Ribera C. 1997. Radiation of the genus *Dysdera* (Araneae, Haplogynae, Dysderidae) in the Canary Islands: The island of Gran Canaria. *Zoologica Scripta* 26: 205-243.

Arnedo MA, Ribera C. 1999. Radiation of the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. *Journal of Arachnology* 27: 604-662.

Avise JC. 1994. *Molecular markers, Natural History and Evolution*. Chapman & Hall: New York.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Harvard University Press: Cambridge, Mass.

Baker RH, DeSalle R. 1997. Multiple sources of character information and the phylogeny of Hawaiian *Drosophila*. *Systematic Biology* 46: 654-673.

Balakrishnan R. 2005. Species concepts, species boundaries and species identification: a view from the tropics. *Systematic Biology* 54: 689.

Ballard JWO, Rand DM. 2005. The population biology of mitochondrial DNA and its phylogenetic implications. *Annual Review of Ecology, Evolution, and Systematics* 36: 621-642.

Berland L. 1936. Mission de M. A. Chevalier aux îles du Cap Vert (1934). 1. Araignées. *Rev. fr. ent.* 3: 67-88.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22: 148-155

Bidegaray-Batista L, Macías-Hernandez N, Oromí P, Arnedo MA. 2007. Living on the edge: demographic and phylogeographical patterns in the woodlouse-hunter spider *Dysdera lancerotensis* Simon, 1907 on the eastern volcanic ridge of the Canary Islands. *Molecular Ecology* 16: 3198-3214.

Blackwall J. 1862. Descriptions of newly-discovered spiders from the island of Madeira. *Annals and Magazine of Natural History* 9: 370-382.

Blaxter ML. 2004. The promise of a DNA taxonomy. *Philosophical Transactions-Royal Society of London Series B Biological Sciences*: 669-680.

Bond JE, Hedin MC, Ramirez MG, Opell BD. 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. *Molecular Ecology* 10: 899-910.

Bond JE, Stockman AK. 2008. An integrative method for delimiting cohesion species: finding the population-species interface in a group of californian trapdoor spiders with extreme genetic divergence and geographic structuring. *Systematic Biology* 57: 628 - 646.

Borges PAV, Abreu C, Aguiar AMF, Carvalho P, Fontinha S, Jardim R, Melo I, Oliveira P, Sequeira MM, Sérgio C, Serrano ARM, Sim.Sim M, Vieira P. 2008. Terrestrial and Freshwater biodiversity of Madeira and Selvagens archipelagoes. In: Borges PAV, Abreu C, Aguiar AMF, Carvalho P, Jardim R, Melo I, Oliveira P, Sérgio C, Serrano ARM and Vieira P, eds. *A list of the terrestrial fungi, flora and fauna of Madeira and Selvagens archipelagos*. Funchal and Angra do Heroísmo: Direcção Regional do Ambiente da Madeira and Universidade dos Açores. 13-26.

Borges PAV, Cunha R, Gabriel R, Martins AMF, Silva L, Vieira V, Dinis F, Lourenço P, Pinto N. 2005. Description of the Terrestrial Azorean Biodiversity. In: Borges PAV, Cunha R, Gabriel R, Martins AMF, Silva L and Vieira V, eds. *A list of the terrestrial fauna (Mollusca and Arthropoda) and flora (Bryophyta, Pteridophyta and Spermatophyta) from the Azores* Horta, Angra do Heroísmo and Ponta Delgada: Direcção Regional de Ambiente and Universidade dos Açores. 21-68.

Boyer SL, Baker JM, Giribet G. 2007. Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread `mite harvestman' defies DNA taxonomy. *Molecular Ecology* 16: 4999-5016.

Brehm A, Jesus J, SpInola H, Alves C, Vicente L, Harris DJ. 2003. Phylogeography of the Madeiran endemic lizard *Lacerta dugesii* inferred from mtDNA sequences. *Molecular Phylogenetics and Evolution* 26: 222-230.

Brower A. 1996. Combining data in phylogenetic analysis. *Trends in Ecology & Evolution* 11: 334-335.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences* 91: 6491-6495.

Brown W, George M, Wilson A. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences* 76: 1967.

Bull JJ, Huelsenbeck JP, Cunningham CW, Swofford DL, Waddell PJ. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384-397.

Carracedo JC, Day S. 2002. Canary Islands *Classic geology in Europe*. Harpenden: Terra Publishing. 294.

Carstens BC, Knowles LL. 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* Grasshoppers. *Systematic Biology* 56: 400 - 411.

Caterino MS, Cho S, Sperling FA. 2000. The current state of insect molecular systematics: a thriving Tower of Babel. *Annual Review of Entomology* 45: 1-54.

Clarke KR, Warwick RM. 1994. *A framework for studying changes in community structure. Change in marine communities: An approach to statistical*

analysis and interpretation. Plymouth Marine Laboratory: Plymouth, UK.

Coello J, Cantagrel JM, Hernán F, Fuster JM, Ibarrola E, Ancochea E, Casquet C, Jamond C, Díaz de Terán JR, Cendrero A. 1992. Evolution of the eastern volcanic ridge of the Canary Islands based on new K-Ar data. *Journal of Volcanology and Geothermal Research* 53: 251-274.

Coyne JA, Orr HA. 1998. The evolutionary genetics of speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 353: 287-305.

Dallwitz MJ 1980. A general system for coding taxonomic descriptions. *Taxon* 29: 41-46.

Dallwitz MJ, Paine TA, Zurcher EJ. 1993. *User's guide to the DELTA system: A general sytem for processing taxonomic descriptions*. Canberra.

Dallwitz MJ, Paine TA, Zurcher EJ. 1999. User’s guide to the DELTA Editor: http://delta-intkey.com.

Darlu P, Lecointre G. 2002. When does the incongruence length difference test fail? *Molecular Biology and Evolution* 19: 432.

de Queiroz K. 1998. The general lineage concept of species, species criteria, and the process of speciation. A conceptual unification and terminological recommendations. In: Howard DJ and Berlocher SH, eds. *Endless Forms. Species and Speciation*. New York: Oxford University Press. 57-75.

Deeleman-Reinhold CL, Deeleman PR. 1988. Revision des Dysderinae. *Tijdschrift voor Entomologie* 131: 141-269.

Denis J. 1962. Les araignées de l'Archipel de Madère. *Publçôes Inst. Zool. Dr. Augusto Nobre* 79: 9-118.

Denis J. 1963. Spiders from the Madeira and Selvage Islands. *Boletim do Museu Municipal do Funchal* 17: 29-48.

DeSalle R, Egan MG, Siddall M. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society of London B Biological Sciences* 360: 1905-1916.

DeSalle R, Freedman T, Prager EM, Wilson AC. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. *Journal of Molecular Evolution* 26: 157-164.

Dieckmann U. 2004. *Adaptive speciation*. Cambridge University Press: New York.

Doyle J. 1997. Trees within trees: genes and species, molecules and morphology. *Systematic Biology* 46: 537.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.

Eberhard WG. 1985. *Sexual Selection and Animal Genitalia*. Harvard University Press: Cambridge, US.

Ereshefsky M. 1992. *The units of evolution. Essays on the nature of species*. MIT press: Massachusetts.

Feulner P, Kirschbaum F, Schugardt C, Ketmaier V, Tiedemann R. 2006. Electrophysiological and molecular genetic evidence for sympatrically occuring cryptic species in African weakly electric fishes (Teleostei: Mormyridae: *Campylomormyrus*). *Molecular Phylogenetics and Evolution* 39: 198-208.

Floyd R, Abebe E, Papert A, Blaxter M. 2002. Molecular barcodes for soil nematode identification. *Molecular Ecology* 11: 839-850.

Funk DJ, Futuyma DJ, Orti G, Meyer A. 1995. Mitochondrial DNA sequences and multiple data sets: A phylogenetic study of phytophagous beetles (Chrysomelidae: *Ophraella*). *Molecular Biology and Evolution* 12: 627-640.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: Frequency,

causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics* 34: 397-423.

Geldmacher J, Hoernle K, van den Bogaard P, Zankl G, Garbe-Schonberg D. 2001. Earlier history of the=< 70-Ma-old Canary hotspot based on the temporal and geochemical evolution of the Selvagen Archipelago and neighboring seamounts in the eastern North Atlantic. *Journal of Volcanology and Geothermal Research* 111: 55-87.

Goetze E. 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. *Proceedings of the Royal Society B: Biological Sciences* 270: 2321.

Hamada M, Takasaki N, Reist JD, Decicco AL, Goto A, Okada N. 1998. Detection of the ongoing sorting of ancestrally polymorphic SINEs toward fixation or loss in populations of two species of charr during speciation. *Genetics* 150: 301-311.

Harrison RG. 1998. *Linking evolutionary pattern and process*. In: Howard DJ and Berlocher SH, eds. Endless Forms. New York, Oxford: Oxford University Press. 19-31.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identification through DNA barcodes. *Proceedings of the Royal Society Biological Sciences Series B* 270: 313-321.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004a. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences* 101: 14812-14817.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004b. Identification of Birds through DNA Barcodes. *PLoS Biology* 2: 1657-1663.

Hedin M, Wood DA. 2002. Genealogical exclusivity in geographically proximate populations of *Hypochilus thorelli* Marx (Araneae, Hypochilidae) on the Cumberland Plateau of North America. *Molecular Ecology* 11: 1975-1988.

Hedin MC. 2001. Molecular insights into species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae: Hypochilidae). *Molecular Phylogenetics and Evolution* 18: 238-251.

Hochkirch A, Gözig Y. 2009. Colonization and speciation on volcanic islands: phylogeography of the flightless grasshopper genus *Arminda* (Orthoptera, Acrididae) on the Canary Islands. *Systematic Entomology* 34: 188-197.

Hogg ID, Hebert PDN. 2004. Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology* 82: 749-754.

Hudson R. 1992. Gene trees, species trees and the segregation of ancestral alleles. *Genetics* 131: 509.

Izquierdo I, Martín JL, Zurita N, Arechavaleta M. 2004. *Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres)*. Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias.

Kankare M, Shaw M. 2004. Molecular phylogeny of Cotesia Cameron, 1891 (Insecta: Hymenoptera: Braconidae: Microgastrinae) parasitoids associated with Melitaeini butterflies (Insecta: Lepidoptera: Nymphalidae: Melitaeini). *Molecular Phylogenetics and Evolution* 32: 207-220.

Klein J, Sato A, Nagl S, O'hUigÌn C. 1998. Molecular trans-species polymorphism. *Annual Review of Ecology and Systematics* 29: 1-21.

Knowles LL, Carstens BC. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* 56: 887-895.

Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS. 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature* 400: 652 - 655.

Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG. 2004. Phylogenetic analysis of Phytophthora species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* 41: 766-782.

Legendre P, Legendre L. 1998. *Numerical ecology*. Elsevier B. V.: Amsterdam, The Netherlands.

Lipscomb D, Platnick NI, Wheeler QD. 2003. The intellectual content of taxonomy: a comment on DNA taxonomy. *Trends in Ecology & Evolution* 18: 65-66.

Losos JB, Jackman TR, Larson A, De Queiroz K, Rodriguez Schettino L. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. *Science* 279: 2115-2118.

Lyons-Weiler J, Milinkovitch MC. 1997. A phylogenetic approach to the problem of differential lineage sorting. *Molecular Biology and Evolution* 14: 968-975.

Macías-Hernández N, Oromí P, Arnedo MA. 2008. Patterns of diversification on old volcanic islands as revealed by thewoodlouse-hunter spider genus *Dysdera* (Araneae, Dysderidae) in the eastern Canary Islands. *Biological Journal of the Linnean Society* 94: 589–615.

Maddison W. 1997. Gene trees in species trees. *Systematic Biology* 46: 523.

Martín JL, Marrero M, Zurita N, Arechavaleta M, Izquierdo I. 2005. *Biodiversidad en Gráficas. Especies Silvestres de las Islas Canarias*. DVD: Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias.

Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Systematic Biology* 45: 524-545.

Mayden RL. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. *Systematics Association Special Volume* 54: 381-424.

Meyer A, Zardoya R. 2003. Recent advances in the (molecular) phylogeny of vertebrates. *Annual Review of Ecology, Evolution and Systematics* 34: 311-338.

Molbo D, Machado C, Sevenster J, Keller L, Herre E. 2003. Cryptic species of fig-pollinating wasps: Implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. *Proceedings of the National Academy of Sciences* 100: 5867.

Monaghan M, Balke M, Gregory T, Vogler A. 2005. DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philosophical Transactions B* 360: 1925.

Monaghan M, Balke M, Pons J, Vogler A. 2006. Beyond barcodes: complex DNA taxonomy of a South Pacific Island radiation. *Proceedings of the Royal Society B* 273: 887.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. *Evolution* 49: 718-726.

Moore WS. 1997. Mitochondrial-gene trees versus nuclear-gene trees, a reply to Hoelzer. *Evolution* 51: 627-629.

Morando M, Avila LJ, Sites JW. 2003. Sampling strategies for delimiting species: genes, individuals, and

populations in the *Liolaemus elongatus-kriegi* Complex (Squamata: Liolaemidae) in Andean-Patagonian South America. *Systematic Biology* 52: 159-185.

Myers N, Mittermeier RA, Mittermeier CC, da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature*: 853-858.

Nagl S, Tichy H, Mayer WE, Takahata N, Klein J. 1998. Persistence of neutral polymorphisms in Lake Victoria cichlid fish. *Proceedings of the National Academy of Sciences* 95: 14238–14243.

Nei M. 1987. *Molecular evolutionary genetics*. Columbia Univ Pr.

Ohtsuka H, Oyanagi M, Mafune Y, Miyashita N, Shiroishi T, Moriwaki K, Kominami R, Saito N. 1996. The presence/absence polymorphism and evolution of the p53 pseudogene in the genus *Mus Molecular Phylogenetics and Evolution* 5: 548-556.

Orr MR, Smith TB. 1998. Ecology and speciation. *Trends in Ecology and Evolution* 13: 502-505.

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* 5: 568-583.

Pfenninger M, Nowak C, Kley C, Steinke D, Streit B. 2007. Utility of DNA taxonomy and barcoding for the inference of larval community structure in morphologically cryptic *Chironomus* (Diptera) species. *Molecular Ecology* 16: 1957-1968.

Pfenninger M, Schwenk K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* 7: 1471-2148.

Platnick NI. 2009. The World Spider Catalog. v. 9.5 ed. New York: American Museum of Natural History, online at http://research.amnh.org/entomology/spiders/catalog/GENERIC.IND.html, [accessed 24.II.09].

Pollard DA, Iyer VN, Moses AM, Eisen MB. 2006. Widespread discordance of gene trees with species tree in *Drosophila* evidence for incomplete lineage sorting. *PLoS Genet* 2: e173.

Quicke DLJ, Jones OR, Epstein DR. 2007. Correcting the problem of false incongruence due to noise imbalance in the incongruence length difference (ILD) test. *Systematic Biology* 56: 496-503.

Rokas A, Melika G, Abe Y, Nieves-Aldrey J, Cook J, Stone G. 2003. Lifecycle closure, lineage sorting, and hybridization revealed in a phylogenetic analysis of European oak gallwasps (Hymenoptera: Cynipidae: Cynipini) using mitochondrial sequence data. *Molecular Phylogenetics and Evolution* 26: 36-45.

Saez AG, Probert I, Geisen M, Quinn P, Young JR, Medlin LK. 2003. Pseudo-cryptic speciation in coccolithophores. *Proceedings of the National Academy of Sciences* 100: 7163-7168.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101-109.

Sanderson MJ. 2003. r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19: 301-302.

Seberg O, Humphries CJ, Knapp S, Stevenson DW, Peterson G, Scharff N, Andersen NM. 2003. Shortcuts in systematics? A commentary on DNA-based taxonomy. *Trends in Ecology & Evolution* 18: 63-65.

Simon E. 1883. Études Arachnologiques XIV Mè., materiaux pour servir a la faune arachnologique des îles de l'Océan

Atlantique. *Annales de la Société Entomologique de France* 6: 294-298.

Simon E. 1912. Arachnides recueillis par M.L. Garreta à l'île Grande-Salvage. *Bulletin Société Entomologique de France* 2: 59-61.

Sinclair EA, Bezy RL, Bolles K, Camarillo R JL, Crandall KA, Sites JW. 2004. Testing species boundaries in an ancient species complex with deep phylogeographic history: genus *Xantusia* (Squamata: Xantusiidae). *American Naturalist* 164: 396-414.

Sites JW, Crandall KA. 1997. Testing species boundaries in biodiversity studies. *Conservation Biology* 11: 1289-1297.

Sites JW, Marshall JC. 2003. Delimiting species: A renaissance issue in systematic biology. *Trends in Ecology and Evolution* 18: 462-470.

Sites JW, Marshall JC. 2004. Operational Criteria for Delimiting Species. *Annual Review of Ecology Evolution and Systematics* 35: 199-228.

Smith MA, Fisher BL, Hebert PDN. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philosophical Transactions Royal Society of London Series B Biological Sciences*: 1825-1834.

Sota T, Vogler A. 2001. Incongruence of Mitochondrial and Nuclear Gene Trees in the Carabid Beetles *Ohomopterus Systematic Biology* 50: 39–59.

SPSS I. 2001. SIGMAPLOT. 7.0 ed. Chicago, IL, USA: SPSS Inc.

StatSoft Inc. 1999. STATISTICA for Windows. Tulsa, OK.: StatSoft, Inc.

Stockman A, Bond JE. 2007. Delimiting cohesion species: extreme population structuring and the role of ecological interchangeability. *Molecular Ecology* 16: 3374-3392.

Taiti S, López H. 2008. *New records and species of Halophilosciidae (Crustacea, Isopoda, Oniscidea) from the Canary Islands (Spain)*. In: Zimmer M., Charfi Cheikhrouha F and Taiti S, eds. *Proceedings of the International Symposium of Terrestrial Isopod Biology - ISTIB-07*. Aachen: Shaker-Verlag. 43-58.

Tajima F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105: 437.

Takahashi K, Terai Y, Nishida M, Okada N. 2001. Phylogenetic relationships and ancient incomplete lineage sorting among cichlid fishes in Lake Tanganyika as revealed by analysis of the insertion of retroposons. *Molecular Biology and Evolution* 18: 2057.

Takahata N. 1989. Gene genealogy in three related populations: consistency probability between gene and population trees. *Genetics* 122: 957.

Takahata N, Nei M. 1985. Gene genealogy and variance of interpopulational nucleotide differences. *Genetics* 110: 325.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2002. DNA points the way ahead in taxonomy. *Nature* 418: 479.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2003. A plea for DNA taxonomy. *Trends in Ecology & Evolution* 18: 70-74.

Templeton AR. 1994. *The role of molecular genetics in speciation studies*. In: *Schierwater B, Streit B, Wagner GP and DeSalle R, eds. Molecualr Ecology and Evolution: Approaches and Applications.* Basel: Birkhäuser-Verlag.

van Oppen M, McDonald B, Willis B, Miller D. 2001. The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or

morphological convergence? *Molecular Biology and Evolution* 18: 1315.

Villacorta C, Jaume D, OromÌ P, Juan C. 2008. Under the volcano: phylogeography and evolution of the cave-dwelling *Palmorchestia hypogae*a (Amphipoda, Crustacea) at La Palma (Canary Islands). *BMC Biology* 6: 7.

Wiens JJ, Penkrot TA. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* 51: 69-91.

Wiley EO. 1981. *Phylogenetics. The theory and practice of phylogenetic systematic*. John Wiley & sons: New York.

Wu CI. 1991. Inferences of species phylogeny in relation to segregation of ancient polymorphisms. *Genetics* 127: 429-435.

Wunderlich J. 1991. Die Spinnen-fauna der Makaronesischen Inseln. *Beiträge zur Araneologie* 1: 1-619.

Wunderlich J. 1994. Zu Ökologie, Biogeographie, Evolution und Taxonomie einiger Spinnen der Makaronesischen Inseln. *Beiträge zur Araneologie* 4: 385-439.

Capítulo 5

Capítulo publicado:

Bidegaray-Batista, L., Macías-Hernández, N., Oromí, P. & Arnedo, M. (2007). Living on the edge: demographic and phylogeographic patterns in the woodlouse-hunter spider *Dysdera lancerotensis* Simon, 1907 on the eastern volcanic ridge of the Canary Islands*. Molecular Ecology*, 16: 3198–3214

LIVING ON THE EDGE: DEMOGRAPHIC AND PHYLOGEOGRAPHIC PATTERNS IN THE WOODLOUSE-HUNTER SPIDER *Dysdera lancerotensis* SIMON, 1907 ON THE EASTERN VOLCANIC RIDGE OF THE CANARY ISLANDS

ABSTRACT

The Eastern Canary Islands are the emerged tips of a continuous volcanic ridge running parallel to the north-eastern African coast, originated by episodic volcanic eruptions that can be traced back to the Miocene and that, following a major period of quiescence and erosion, continued from the Pliocene to the present day. The islands have been periodically connected by eustatic sea-level changes resulting from Pleistocene glacial cycles. The ground-dwelling spider *Dysdera lancerotensis* Simon, 1907 occurs along the entire ridge, except on recent barren lavas and sand dunes, and is therefore an ideal model for studying the effect of episodic geological processes on terrestrial organisms. Nested clade and population genetic analyses using 39 haplotypes from 605 base pairs of mitochondrial DNA cytochrome c oxidase I sequence data, along with phylogenetic analyses including two additional mitochondrial genes, uncover complex phylogeographic and demographic patterns. Our results indicate that *D. lancerotensis* colonized the ridge from north to south, in contrast to what had been expected given the SSW-NNE trend of volcanism and reported for other terrestrial arthropods. The occurrence of several episodes of extinction, recolonization and expansion are hypothesized for this species, and areas that act as refugia during volcanic cycles are identified. Relaxed molecular clock methods reveal divergence times between main haplotype lineages that suggest an older origin of the northern islets than anticipated based on geological evidence. This study supports the key role of volcanism in shaping the distribution of terrestrial organisms on oceanic islands and generates phylogeographic predictions that warrant further research into other terrestrial endemisms of this fascinating region.

INTRODUCTION

Volcanic islands have long captivated the attention of evolutionary biologists because of the experimental-like conditions they provide for the study of

speciation (Grant, 1998; Juan *et al.*, 2000; Mayr, 1967; Wagner & Funk, 1995). More recently, volcanic islands have become fertile ground for phylogeography, the study of the causes behind the current distribution of genetic diversity (Avise, 1998). The highly dynamic and accurately dated geology of volcanic islands make them ideal models for testing the role of historical fragmentation and recolonization in shaping contemporary patterns of the geographical distributions of species. Several studies have provided evidence of the close relationship between volcanic activity and lineage distributions. Forest fragmentation caused by recent lava flow from the Mauna Loa volcano on the island of Hawaii has affected the pattern of neutral genetic variation in several species of endemic *Tetragnatha* spiders, with a greater influence on specialist than on generalist species (Vandergast *et al.*, 2004). Research on *Brachyderes rugatus rugatus*, an endemic weevil from the island of La Palma in the Canary Islands, has revealed correlations between phylogeographic patterns and volcanic activity. However, some of the results did not match available geological evidence, which suggests that some of the volcanic terrains are older than geologists had previously assumed (Emerson *et al.*, 2006). These results emphasize the positive feedback between geological and phylogeographic inferences. Phylogeographic analysis has identified a severe bottleneck in the giant tortoise *Geochelone nigra vandenburghi* on Alcedo Volcano, in the Galapagos Islands, which it has been suggested is the result of a contraction of the tortoise population caused by the prehistoric eruption of the volcano, and the subsequent recolonization via an ancestral haplotype (Beheregaray *et al.*, 2003).

The volcanic archipelago of the Canary Islands lies in the Atlantic Ocean off the northwestern coast of Africa (Fig. 5.1). The archipelago was formed on a hot spot, hence the arrangement of its seven main islands in a linear geographic and temporal cline: islands are older to the east and become younger towards the west (Carracedo *et al.*, 1998). The Eastern Canaries comprise the islands of Lanzarote and Fuerteventura and the islet of Lobos (located between these two larger islands) as well as La Graciosa, Montaña Clara and Alegranza (to the north of Lanzarote). They are the emerged parts of a volcanic ridge that runs roughly parallel to the African coast, only 100 km away at its closest point (Coello *et al.*, 1992). Although nowadays the main islands and islets are separated, they have been periodically

connected and disconnected by marine transgressions associated with Pleistocene climate oscillations (Carracedo *et al.*, 2003). The subaerial volcanism that formed the islands started ~ 20 million years ago (Ma) and took place in two distinct volcanic cycles with a SSW-NNE temporal and spatial polarity (Coello *et al.*, 1992). The eruptive cycles were separated by a significant gap, which resulted in a long erosion period. Post-Miocene activity was restricted to Lanzarote, the northern islets and the central and northern parts of Fuerteventura. Holocene volcanic activity has been documented in the islands (Carracedo *et al.*, 1992), and both recent and historical eruptions have covered vast areas of the northeast and western central parts of Lanzarote with lava flows (Carracedo *et al.*, 2003). The Eastern Canaries are characterized by more arid and xerophilous habitats than their Western counterparts due to the joint effect of strong erosion, which prevents to capture the humid northeast trade winds, and the dry and dusty winds blowing from the nearby Sahara desert (Juan *et al.*, 2000).

The geological and climatic features make the Eastern Canary Islands an ideal model for investigating the influence of volcanism and sea-level fluctuations in shaping the geographical distribution of species, lineage diversification, population structure and demography. Lava flows and marine transgression may cause local extinctions and population fragmentations, but they also create newly available recolonization sites and subsequent population expansions. In spite of this, very few studies have dealt with phylogeographic patterns of Eastern Canarian endemics, while in recent years the Western Islands have been the object of in-depth research in this field (Contreras-Diaz *et al.*, 2003; Emerson *et al.*, 2006; Moya *et al.*, 2004; Moya *et al.*, 2006; Rees *et al.*, 2001a; Rees *et al.*, 2001b). In the only example of population level molecular analysis of an Eastern Canarian endemic, Juan *et al.,* (1998) found that the phylogeographic pattern of the darkling beetle *Hegeter deyrollei* (formerly *H. politus*) closely matched the SSW-NNE sequence of subaerial volcanic activity and suggested a sequential colonization of the eastern ridge from Jandía, in southern Fuerteventura, to the northernmost islet of Alegranza. More recently Bloor and Brown (2005) examined the effect of recent lava flows (locally known as *malpaís*) on the morphological variation of the eastern endemic lizard *Gallotia atlantica*. They concluded that the low level of

morphological differentiation identified two major groups, one spread throughout most of Lanzarote and the islets, and a second restricted to two isolated populations in recent lava flows: Malpaís de la Corona and southeast of Haria, and two localities in the southwest of Timanfaya. The authors suggested that this variation in *malpaís* populations would have developed recently, rather than being the relicts of a former widespread population.

The woodlouse hunter spider *Dysdera lancerotensis* Simon, 1907 is endemic to the Eastern Canary Islands, where it is widespread throughout Fuerteventura, Lanzarote and all the islets. Like its congeneric species, *D. lancerotensis* is a ground-dwelling, nocturnal wandering hunter. The genus *Dysdera* has undergone a major evolutionary radiation process in the Canaries, where 43 endemic species have been identified (Arnedo *et al.*, 2000; Arnedo *et al.*, 2001; Arnedo & Ribera, 1997; Arnedo & Ribera, 1999). Although the number of colonization events that account for this outstanding diversity remains a matter of debate, it has been clearly shown that *D. lancerotensis* is not closely related to any of the other Canarian endemics, and that its closest relatives are in fact found in Morocco (Arnedo *et al.*, 2001). Hence, *D. lancerotensis* is most likely to be the result of an independent colonization by African ancestors. It was originally described as a subspecies of the cosmopolitan *D. crocata* C.L. Koch, 1838 and subsequently elevated to species status (Arnedo *et al.*, 2000; Wunderlich, 1991), which has been confirmed by DNA sequence data (Arnedo *et al.*, 2001). The distribution of *D. lancerotensis* overlaps with five additional endemic species of *Dysdera*. However, the other endemics seem to have narrower ecological requirements and are mostly restricted to the highest and more humid parts of the islands, while *D. lancerotensis* has also been collected in xerophilous, sea-level habitats and should be considered a generalist (Arnedo *et al.*, 2000).

In the present study, we use mtDNA sequence data to examine genetic variability and investigate levels of genetic structure in the Eastern Canarian endemic *Dysdera lancerotensis*, in order to correlate the influence of volcanic activity and marine transgressions in shaping phylogeographic patterns in a generalist species. Based on geologic history, we anticipate the following scenarios: firstly, a sequential colonization of the eastern ridge, following the SSW-NNE trend

of post-Miocene volcanic activity, as proposed for the darkling beetle *Hegeter deyrollei* (Juan *et al.*, 1998); secondly, complex phylogeographic and demographic patterns in *D. lancerotensis*, reflecting the occurrence of frequent extinction, expansion, fragmentation and recolonization processes as a result of long-term periodic volcanism.

MATERIALS AND METHODS

Sampling

Dysdera lancerotensis specimens were collected from the Eastern Canary Islands during 2004 and 2005, and stored in absolute ethanol at 4ºC. Seventeen localities were sampled; nine in Lanzarote, three in Fuerteventura, three in La Graciosa, one in Montaña Clara and one in Alegranza, and six additional sequences of *D. lancerotensis* were obtained from GenBank (Fig. 5.1, Appendix 5.1). Samples of close morphological relatives (several haplotypes of the cosmopolitan *D. crocata* C. L. Koch, 1838 and two Moroccan species, *D. mauritanica* Simon, 1909 and *Dysdera sp.*), along with two specimens of *D. inermis* Ferrández, 1984 that provided a calibration point for estimating absolute lineage ages (see below), as well as other *Dysdera* species endemic to the Eastern Canaries were included in the analyses (Appendix 5.1).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from two legs using the REALPURE Genomic DNA extraction kit from REAL® following manufacturer's guidelines. A 649 bp region of the mitochondrial cytochrome oxidase I (*cox1*) gene was amplified for all samples using the polymerase chain reaction (PCR), using modified primers specifically designed for this study C1-J-1546 (5'-GCTATAGTGGGTACGGCTATAAG-3') and C1-N-2194 (5'-CTTCTGGATGACCAAAAAATC -3'). For a subset of samples, a region of about 1 Kb spanning fragments of the mitochondrial large ribosomal subunit (*rrnL*), tRNA leucine (L1) and the NADH dehydrogenase subunit 1 (*nad1*) genes were amplified with primers LR-N-13398: (5'-CGCCTGTTTATCAAAAACAT-3' (Simon *et al.*, 1994)) and N1-J-12261: (TCRTAAGAAATTATTTGAGC-3' (Hedin, 1997).

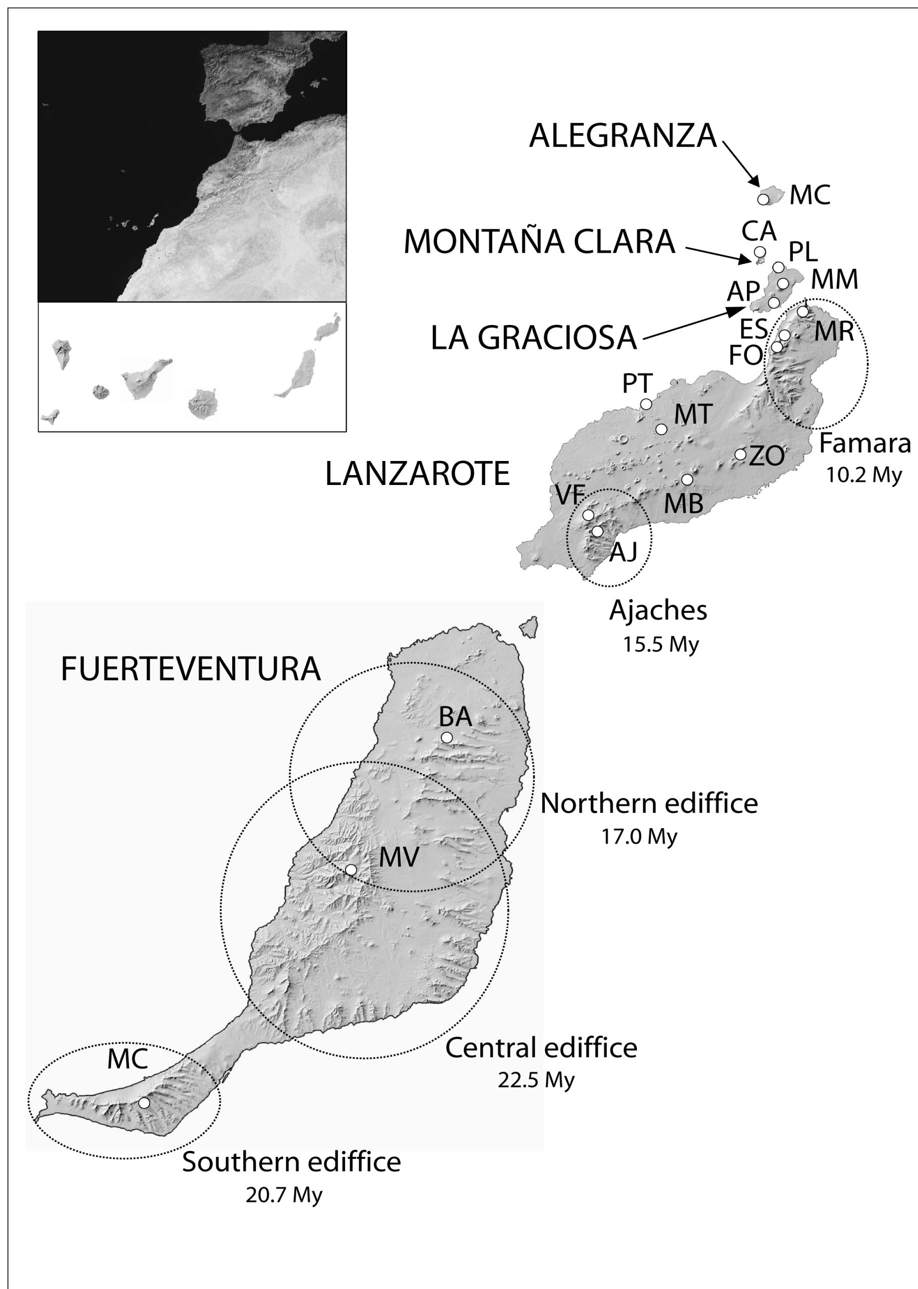

Fig. 5.1.- Map of the Eastern Canary Islands showing sampling locations of *Dysdera lancerotensis*. The population codes correspond to those listed in Appendix 5.1.

Amplifications were carried out in 25µl reaction volume in a final concentration of 1.25 U *Taq* polymerase (Promega), 1x buffer (Promega), 2.5mM $MgCl_2$ (Promega), 0.2mM of each dNTP, 0.2µM of each primer and 2µl of DNA sample. PCR conditions were as follows: initial denaturing step at 95ºC for 5 min, 35 amplification cycles (94ºC for 30s, 45ºC for 35s, 72ºC for 45s for *cox1* fragment and 94ºC for 40s, 45ºC for 1 min, 72ºC for 1 min for *rrnL-nad1* fragment) and a final step at 72ºC for 5 min. PCR products were purified using MultiScreen 96-Well Filter Plates from Millipore. PCR products were cycle-sequenced in both directions using the same PCR primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem). DNA sequences were edited using programs in the Staden package (http://staden.sourceforge.net/) and assembled and managed using Bioedit (Hall, 1999).

Phylogenetic analyses

We performed parsimony (MP), maximum likelihood (ML) and Bayesian analyses to asses the internal structure and the root of the *D. lancerotensis* haplotype tree. Two data sets were analyzed. The first data set included the haplotypes found in the 68 specimens analyzed for the *cox1* gene fragment. A second data set included a sample of 14 haplotypes (Appendix 5.1) for which the *cox1* fragment was combined with additional sequences of the genes *rrnL*, L1 and *nad1* (three sequences retrieved from GenBank: AF244152-AF244154). The aim of the second data sets was to improve the phylogenetic signal, since some of the basal relationships were ambiguous or poorly supported by the *cox1* fragment alone.

Sequence alignment of the *cox1* was trivial since no insertions/deletions were observed. In contrast, sequences of the *rrnL* and L1 gene fragments showed length polymorphism and were aligned using the automatic multiple sequence alignment algorithm implemented in CLUSTAL X (Higgins *et al.*, 1992). The effect of different gap-opening and extension values was investigated by building different multiple sequence alignments under the following parameter combinations (GOP:GEP): 8:2, 8:4, 20:2, 24:4, 24:6 (transition/transversion ratio was kept constant at 0.5). Character congruence among gene partitions (*cox1*, *nad1*, *rrnL*-L1)

as measured by the ILD index (Mickevich & Farris, 1981) was used to select one of the alignments for subsequent combined analyses. Gaps were scored as absence/presence data using the simple method of Simmons and Ochoterena (2000) as implemented in GAPCODER (Young & Healy, 2002). Absence/presence scoring allows insertion/deletion information to be incorporated into phylogenetic analyses, while minimizing the effect of increasing the weight of overlapping multiple non-homologous gaps that result from scoring gaps as a 5th character state. In addition, gaps scored as absence/presence are amenable to phylogenetic analysis in a Bayesian framework.

The program MODELTEST v. 3.6 (Posada & Crandall, 1998) was used to find the model of evolution that best fit the data with lesser parameters, as suggested by the AIC criterion (Posada & Buckley, 2004), for subsequent model-based phylogenetic analyses.

Parsimony analyses under equal weights were performed with TNT v. 1.0 (Goloboff *et al.*, 2003) using a heuristic search based on 1000 replicates of random sequence addition followed by tree-bisection-reconnection (TBR) branch swapping (5 trees retained per iteration, and final round of TBR branch swapping on all retained trees). Differential weighting (e.g. downweighting of 3rd positions) was not implemented because of the lack of an objective criterion to assess phylogenetic reliability. It has also been shown that the disadvantages of weighting schemes according to homoplasy levels may outweigh the benefits (Bjorklund, 1999; Kaellersjö *et al.*, 1999; Vogler *et al.*, 2005). Standard non-parametric bootstrap technique provided a measure of clade support based on 1000 pseudoreplicates (individual heuristic searches included 20 iterations of random sequence addition followed by TBR branch swapping and retaining 5 trees per replicate). Bayesian inference analyses were conducted with MRBAYES v.3.0 (Ronquist & Huelsenbeck, 2003). Four simultaneous MCMCMC chains (one cold, three heated) were run for 2,000,000 iterations. Two independent runs were performed. The standard deviation of the split frequencies of the two runs (< 0.01) and the effective sample size (ESS) as measured by the program TRACER v. 1.3 (Rambaut & Drummond, 2005) was used to ensure that the Markov chains had reached stationarity. TRACER was

further used to determine the correct number of generations to discard as a *burn-in* for the analysis. All analyses were run with specific and unlinked models selected by MODELTEST for each gene fragment and a standard discrete model for gaps. Posterior probabilities of each clade were used as a support measure. Maximum likelihood analyses were performed with PHYML v. 2.4.4 (Guindon & Gascuel, 2003), implementing the model and parameter values obtained with MODELTEST and using the averaged Bayesian tree as starting point. Non-parametric bootstrap supports were calculated based on 100 pseudoreplicates and using the best tree found in the former analysis as starting point.

Estimations of substitution rate & genealogical and population divergence times

It is common practice in arthropod phylogeographic studies to rely on a supposedly universal 2.3% pairwise sequence divergence per million years of arthropod mtDNA (Brower, 1994). However, it has been demonstrated that significant rate of heterogeneity exists between even closely-related taxa. Therefore, we chose to estimate a lineage-specific substitution rate instead. Because of the absence of a fossil record of the group, we use biogeographic information based on the well-dated geochronology of the Mediterranean. The vicariant events associated with the opening of the straight of Gibraltar about 5.3 Mya have been used as calibration point to estimate lineage ages in other organisms, including both arthropods (Gómez-Zurita, 2004) and vertebrates (Carranza & Arnold, 2003). The species *Dysdera inermis* Ferrández, 1984 is restricted to the southernmost part of the Iberian Peninsula, but closely related populations have been recently collected from the Rif region in Morocco (Arnedo, unpublished data). Preliminary data showed that mitochondrial genetic divergences between the Moroccan and Iberian populations are in agreement with a post-Messinian split of these populations. The *cox1* data set was reanalyzed with MRBAYES using the model selected by MODELTEST (GTR+I+G) and constraining the main clades with high support obtained in the combined analyses of multiple data sets (see Results). The averaged tree was used to estimate clade ages and substitution rates using R8S (Sanderson, 2003). The eastern clade representatives were used to root the tree but were pruned before estimating time and rates. The split between the two trans-Gibraltar sequences of *D. inermis* was fixed to 5.3 Mya.

A cross-validation analysis (Sanderson, 2002) selected the penalized likelihood with smoothing factor 316.23 as the most appropriate relaxed clock method for estimating clade ages and rates. Confidence intervals were calculated with a sample of 100 trees obtained during the Bayesian searches using the *profile* command in R8S.

We used a coalescent-based approach as implemented in MDIV (Nielsen & Wakeley, 2001) to investigate the magnitude of the difference between time of population divergence and time of the most recent ancestor of the two groups of populations that corresponded to the two main haplotype clades (northern islets vs. Lanzarote-Fuerteventura). MDIV provides a posterior distribution of the demographic parameters theta ($\theta = 2N_e u$), migration rate ($M = N_e m$), and time of population divergence ($T = t/N_e$) using a likelihood framework, and also estimates the expected time to the most recent common ancestor (*TMRCA* = *tu*) (Nielsen & Wakeley, 2001). MDIV analyses were run at Cornell's CBSU computer cluster (http://cbsuapps.tc.cornell.edu/mdiv.aspx). We ran two independent simulations to ensure the convergence of the results. Each simulation was run for 5×10^6 generations with a 10% burn-in period under the "finite site model" and using a maximum prior of 10 for the scaled migration rate (M) and divergence time (T), as recommended by the author. The values with the highest posterior probability were accepted as the best estimate. Values for *T* and *TMRCA* were calculated using a generation time of 1.5 years (Cooke, 1965) and using the lineage specific substitution rate with confidence intervals estimated in the present study (see above).

Genetic diversity and population genetic structure

Genetic diversity indices, such as nucleotide and haplotype diversity and the average number of nucleotide differences, were calculated using ARLEQUIN v 3.01 (Excoffier *et al.*, 2005). SAMOVA v. 1.0 (Dupanloup *et al.*, 2002) was used to assess population structure. Given a priori number of groups (*k*), SAMOVA implements a simulated annealing procedure to define groups of geographically homogeneous populations that maximize the proportion of total genetic variance due to differences between population groups. SAMOVA was run using 100

simulated annealing processes for *k* values from 2 to 9. We used the *Snn* statistic (Hudson, 2000) implemented in DNASP 4.0 (Rozas *et al.*, 2003) to cross-check the genetic differentiation between groups selected by SAMOVA.

Demographic analyses

The demographic history of the studied populations was investigated using mismatch distributions (Rogers & Harpending, 1992) and statistics that test for departure from the neutral mutation model. Expansion parameters were estimated using a nonlinear least-squares approach (Schneider & Excoffier, 1999) as implemented in ARLEQUIN v3.01 under two different models: the sudden expansion model (τ, θ_0 and θ_1) (Harpending *et al.*, 1998; Schneider & Excoffier, 1999) and the spatial expansion model (τ, θ, M) (Excoffier, 2004; Ray *et al.*, 2003). We estimated the approximate expansion time in generations and the number of females before and after the expansion from those parameters, assuming τ=2 *ut* is the expansion time in units of mutation time (where *u* is the mutation rate for the whole sequence and *t* the number of generations since the expansion); $\theta_0 = 2uN_0$ and $\theta_1 = 2uN_1$ the mutation parameters before and after the expansion (where N_0 and N_1 are the number of females before and after the expansion; $N_0 = N_1$ under the spatial expansion model) and M= 2N*m* the migration parameter (*m* being the migrant exchange rate). The bootstrap approach (1000 replications) implemented in ARLEQUIN was used to obtain confidence intervals for the estimated parameters and to test whether the observed data was consistent with the expansion models defined by the parameters (Schneider & Excoffier, 1999). The significance of the raggedness index (r) of the observed distribution was also investigated with ARLEQUIN to further test the validity of the model.

As an alternative approach to the study of demographic history, the Tajima's D-test statistic (Tajima, 1989) and Fu's F_S test (Fu, 1997) were also computed using ARLEQUIN. The significantly negative values for the F_S and Tajima's D-test statistics are deviations from neutrality and are associated with signatures caused by population expansion and/or direct selection (Fu, 1997; Tajima, 1989). A negative F_S value can be explained by an excess of recent mutations (Fu, 1997) while a negative Tajima's D-test value corresponds to an unequal frequency of

polymorphic variants with an excess of the most common type and a deficiency of the less common types (Hartl, 2000). Since demographic analyses are sensitive to population subdivision and haplotype frequencies, all analyses were performed in the homogeneous population groups defined by SAMOVA of considerable population size (at least 30 individuals).

Network and nested clade analyses (NCA)

Haplotype networks were inferred using the statistical parsimony analysis (Templeton *et al.*, 1992) implemented in TCS v1.21 (Clement *et al.*, 2000). Pairs of haplotypes are linked by the smallest number of differences defined by a 95% confidence limit that no homoplasic changes are included. Ambiguities in the haplotype network were resolved following the guidelines proposed by Crandall & Templeton (1993). The final haplotype network was manually converted into a nested series of clades following Templeton *et al*., (1987) and Templeton & Sing (1993) to perform a nested clade phylogeographic analysis (NCPA) (Templeton, 1998; Templeton, 2004; Templeton *et al.*, 1995). In cases where it was possible to use the tree topology resulting from the combined analyses of the *cox1*+*rrnL*+L1+*nad1* to link haplotype networks that could not be connected at the 95% level of confidence using statistical parsimony, we grouped individual networks as sister clades at equal nesting levels (Templeton *et al.*, 1987) (e.g. Kozak *et al.*, 2006). Additional criteria to establish polarity during clade distance comparisons were obtained by lowering the confidence limit of not including homoplasies provided. The NCPA was carried out using GEODIS v2.4 (Posada *et al.*, 2000) and the distance pattern was interpreted using a revised version of the inference key published by Templeton (2004) available on the Geodis web page (http://darwin.uvigo.es/software/geodis.html).

RESULTS

Sequences

We obtained 62 sequences of 605 bp of the *cox1* mitochondrial gene of *D. lancerotensis*. Six additional sequences were retrieved from GenBank, providing a

total of 68 *cox1* sequences for subsequent analyses. These sequences corresponded to 39 haplotypes (GenBank accession numbers in Appendix 5.1). All haplotypes were exclusive to a single island except haplotype 37, which was present in both Alegranza and Lanzarote (Appendix 5.1). Among these 39 haplotypes, 109 nucleotide sites were variable (69.7% at the third codon position), 75 parsimony-informative and 34 singletons (nucleotide substitution present in only one sampled sequence). The A-T bias was 62% and 68% of nucleotide substitutions were transitions. Average uncorrected pairwise genetic distance among all *D. lancerotensis* haplotypes was 4.05% (± 1.8%) and the maximum sequence divergence between any two haplotypes was 8.8%.

Phylogenetic structure

Results of the phylogentic analyses of the *cox1* data matrix are summarized in Fig. 5.2. The analyses confirm that *D. lancerotensis* is not related to the other endemic species from the eastern islands but to Moroccan species. All methods strongly support monophyly of *D. lancerotensis* haplotypes. A single haplotype from Lanzarote (32) is the sister group to the remaining haplotypes sampled, although only receives high support form the ML analyses.

The remaining haplotypes from Lanzarote are grouped in two clades: L2, which receives high support in all analyses, and L1, with low support. Haplotypes from Fuerteventura form a clade (F) that receives high support in all analyses. L2 and F are shown as sister groups but only receive high support in the ML analyses. Haplotypes sampled from the northern islets form a clade that, again, only receives high support in the ML analyses. Inside this clade, haplotypes from Montaña Clara form a highly supported clade nested within a clade that includes all haplotypes from La Graciosa and some from Alegranza (NI2).

The remaining haplotypes from Alegranza are shown as basal to the former clade. Sequences of three additional mitochondrial gene fragments (*nad1, rrnL* and L1) from outgroups and representatives of the haplotype clades supported in the *cox1* analyses were included to improve resolution (GenBank accession numbers in Appendix 5.1). Clustal alignment of the *rrnL* and L1

fragments under gap opening cost 8 and gap extension cost 2, maximized congruence among genes (Table 5.1). Results of the phylogenetic analyses of the combined *cox1*+L1+*rrnL*+*nad1* are summarized in Fig. 5.3. The combined analyses confirm some of the clades already found in the *cox1*-only analyses (NI2, L2, L1 and F), and provide strong support for some poorly resolved relationships. All methods agree in splitting haplotypes in two basal clades with high support, one that includes haplotypes from the northern islets, and another one that includes those from Lanzarote and Fuerteventura.

The northern islets clade includes, along with clade NI2, a clade exclusive to Alegranza (NI1). Haplotype 32 from Lanzarote, which is shown as basal in the *cox1*-only analyses, is shown as sister to L1 with strong support in model-based analyses. Relationships among clades L2, L1+L3 and F remain largely unresolved.

Table 5.1.- Congruence as measured by the ILD of the *rrnL+L1* ClustalX alignments under different gap opening (GOP)/gap extension (GEP) combinations. Gaps were recoded using the simple method of Simmons and Ochoterena (2000). Gaps: number of gap characters inferred from the alignments. Trees: number of most parsimonious trees. *rrnL+L1*: length of the trees of the *rrnL+L1* fragment. *cox1*: tree length of the most parsimonious tree of the *cox1* gene. *nad1*: tree length of the most parsimonious tree of the *nad1* gene. all: tree length of the simultaneous analysis of combined gene fragments. ild: incongruence length difference among fragments.

GOP:GEP	**Gap**	**Trees**	***rrnL*+L1**	***cox1***	***nad1***	**all**	**Trees**	**ILD**
8:2	51	2	527	685	432	1666	2	0.0132
8:4	44	9	516	685	432	1656	4	0.0138
20:2	37	11	553	685	432	1693	14	0.0136
24:4	31	9	576	685	432	1716	12	0.0134
24:6	31	9	576	685	432	1440	12	0.0134

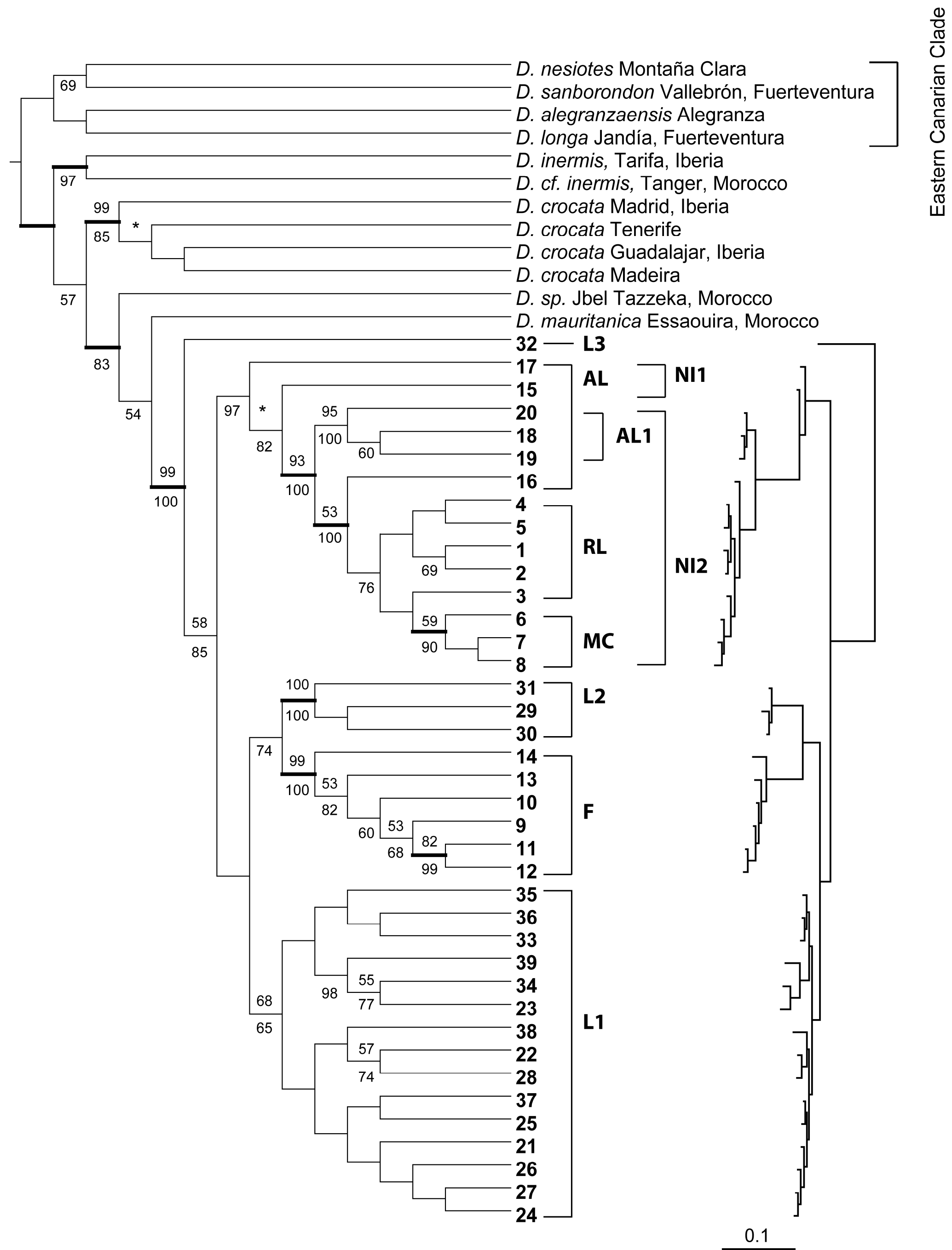

Fig. 5.2.- 50% majority rule consensus tree (left cladogram, right phylogram with outgroup branches removed) of 3,852 trees sampled from the posterior distribution of the Bayesian analysis of *cox1* after burn in. Thick branches indicate clades supported above 95% Bayesian posterior probability. MP bootstrap support indicated above branches and ML bootstrap support shown below (only values > 50% indicated). Asterisks denote contradicted groups. [MP: 1818 trees of 720 steps, CI=0.47, RI=0.73; ML: 1 tree of –ln L= -3893.76821].

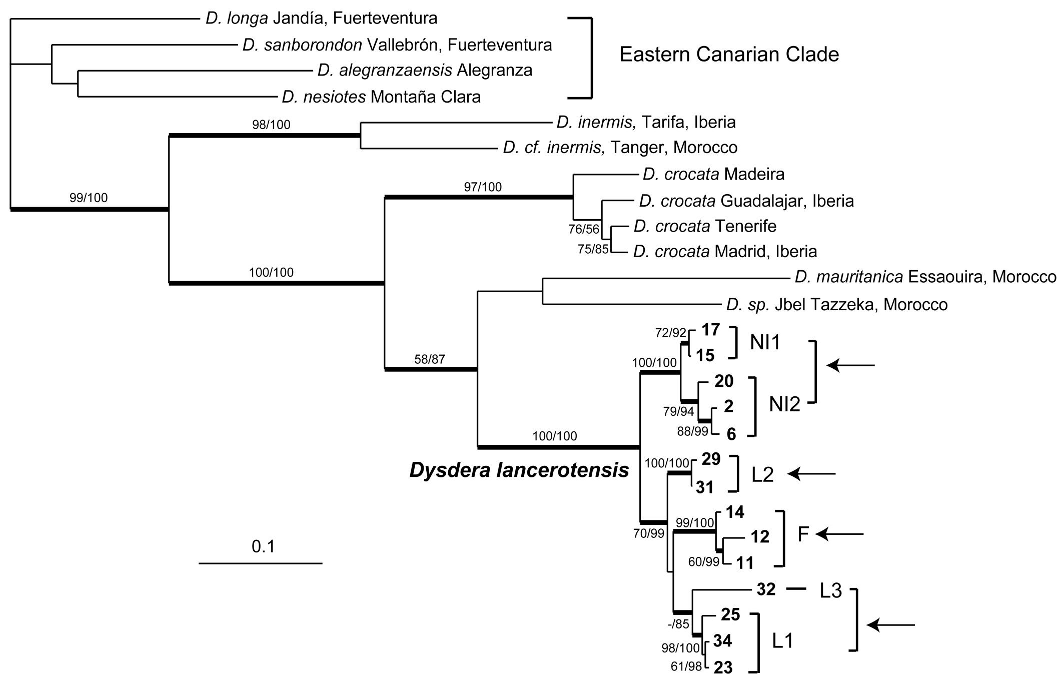

Fig. 5.3.- 50% majority rule consensus phylogram of 6,002 trees sampled from the posterior distribution of the Bayesian analysis of the combined analyses of the *cox1*, L1, *rrnL* and *nad1* partitions after burn in. Thick branches indicate clades supported above 95% Bayesian posterior probability. Numbers near internal branches correspond to MP bootstrap support and ML bootstrap support, respectively (only values > 50% indicated). Arrows indicate those clades constrained in the Bayesian analyses of the *cox1* only data set for lineage age estimation (see Material & Methods). [MP: 2 trees of length 1666, CI=0.57, RI=0.68; ML: 1 tree of –ln L= -8934.28652].

Substitution rate, haplotype clade ages and population divergence time

The substitution rate estimated with R8S using the penalized likelihood relaxed clock method for the *cox1* data set was 0.051 per lineage/million years (confidence interval 0.028-0.089, mean 0.049), about four-fold faster than the standard arthropod mtDNA rate (Brower, 1994). The TMRCA of *Dysdera lancerotensis* haplotypes is dated at ~ 1.49 Mya and the TMRCA of each of the two main haplotype lineages (Northern Islets, and Lanzarote-Fuerteventura) is ~ 1 Mya. Table 5.2 summarizes the time of MRCA of the remaining haplotype clades along with Bayesian-based confidence intervals.

The MDIV estimated divergence time between the group of populations from the northern islets and those from Lanzarote-Fuerteventura, which corresponded to the two main haplotype clades, using lineage-specific substitution rate is 0.52 Mya (0.30-0.96 Mya using the minimum and maximum substitution rate, respectively), while the time of the MRCA of their corresponding haplotypes is 0.64 Mya (0.37-1.19 Mya) and the migration rate is 7.37×10^{-7} migrants per generation (3.98×10^{-7}-1.23×10^{-6}).

Table 5.2.- Clade age estimates (in million years ago) obtained after penalized likelihood (smoothing factor= 316.23) transformation of the constrained Bayesian tree of the *cox1* data set using R8S (see Material & Methods). Mean, lower, and upper values based on 100 random trees sampled from the Bayesian MCMC runs. Clades correspond to those represented in Fig. 5.2.

	Lineage age (million years ago)			
		Confidence interval		
Clades		Minimun	Average	Maximum
D.lancerotensis	**1.49**	0.70	1.47	2.29
NI1+NI2	**1.01**	0.48	1.06	1.72
NI2	**0.40**	0.14	0.40	0.71
AL1	**0.13**	0.02	0.11	0.50
MC+RL+hap16	**0.24**	0.09	0.24	0.43
MC	**0.09**	0.02	0.08	0.19
F+L1+L2+L3	**1.18**	0.58	1.10	1.83
F+L2	**0.93**	0.38	0.87	1.55
L2	**0.13**	0.03	0.13	0.35
F	**0.31**	0.08	0.24	0.46
L1+L3	**1.05**	0.58	1.10	1.83

Population genetic structure and diversity

The fixation indices ($F_{CT,}$ $F_{SC,}$ and F_{ST}) of the SAMOVA analyses were dependent on the number of groups (*k*) defined (Fig. 5.4). The *k*= 6 explained the maximum genetic variance as differences between groups while minimizing the population variance within groups, and was selected as the best grouping for subsequent analyses. The genetic variance among groups was 77.7%, among

populations within groups 0.79% and within populations 21.51% with F_{CT}= 0.777, F_{SC}=0.035 and F_{ST}= 0.785 significant ($P<0.05$). According to SAMOVA results, populations were grouped as follows: (1) all populations from La Graciosa, (2) the population from Montaña Clara, (3) the population from Alegranza, (4) all populations from Fuerteventura, (5) the Zonzamas population from Lanzarote (Lanzarote-Zonzamas) and (6) the eight remaining populations from Lanzarote (Lanzarote-other). The *Snn* statistic provided further support for this genetic differentiation; all pairwise group comparisons yielded significant values ($P<0.05$, data not shown).

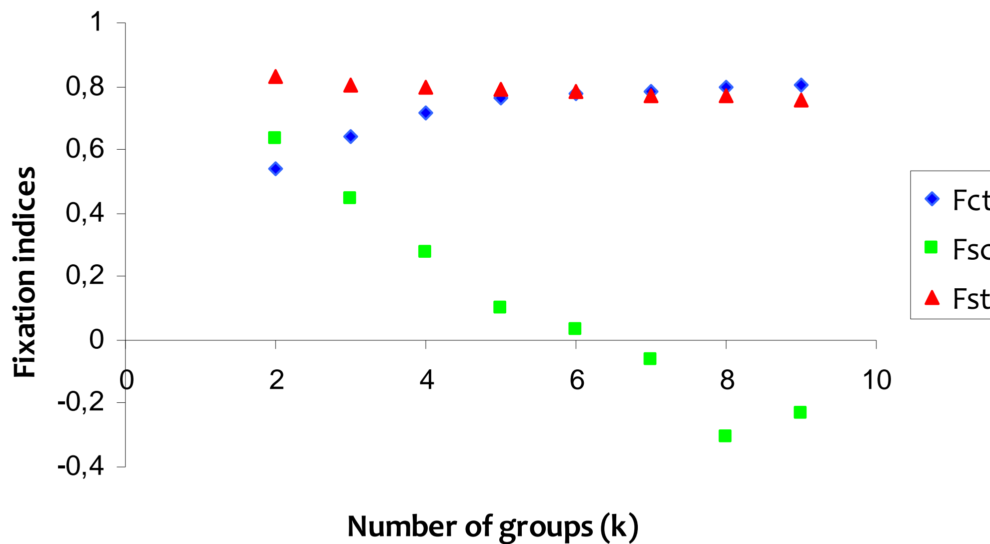

Fig. 5.4.- Values of fixation indices obtained from SAMOVA as a function of the number of groups k. F_{CT}: differentiation between groups. F_{SC}: differentiation between populations within groups. F_{ST}: differentiation between populations among groups.

Table 5.3 shows molecular diversity estimates for each group and for the whole species. The haplotype diversity ranged from 0.96 to 0.6 and the nucleotide diversity from 0.036 to 0.001. The Alegranza Islet showed the highest level of nucleotide and haplotype diversity and was the only island to share a haplotype

with another island: haplotype 37, which also appears in several localities in Lanzarote (Appendix 5.1).

Table 5.3.- Overall diversity measures estimated for *Dysdera lancerotensis* and for each of the groups defined by SAMOVA. *N*: sample size, *Nh*: number of haplotypes, *h*: haplotype diversity, π_n: nucleotide diversity. Genetic diversity values include standard deviations.

Group	*N*	*Nh*	*h*	π_n
Dysdera lancerotensis	68	39	0.9482 ± 0.0184	0.0405 ± 0.0199
Lanzarote-other	33	18	0.8617 ± 0.0549	0.0110 ± 0.0059
Lanzarote-Zonzamas	5	2	0.6000 ± 0.1753	0.0009 ± 0.0011
Fuerteventura	9	6	0.8333 ± 0.1265	0.0083 ± 0.0053
Alegranza	8	7	0.9643 ± 0.0772	0.03651 ± 0.0208
La Graciosa	7	5	0.9048 ± 0.1033	0.0058 ± 0.0040
Montaña Clara	6	3	0.6000 ± 0.2152	0.0031 ± 0.0023

Demographic patterns

The Lanzarote-other group, characterized above by SAMOVA and *Snn* statistics including 32 individuals from eight populations in Lanzarote, was the only group to provide a sufficient sample size to conduct demographic analyses (haplotype 32 was excluded because of the large amount of missing data). The mismatch distribution was unimodal and the SSD statistic and raggedness index (r) were not significant, suggesting that the curves did not differ from the distribution under the population expansion models (P_{SSD}= 0.61, P_r= 0.86 under sudden expansion model and P_{SSD}= 0.7 P_r= 0.84 under spatial expansion model). The F_S and Tajima's *D-test* statistics were significantly negative (F_S= -4.404, P= 0.05 and *D-test* = -2.17, P= 0.002) thus providing further support for a population expansion (or alternatively the result of selection). The parameter τ estimated by the sudden and spatial expansion models indicates that the expansion took place ~ 9 Kya (Table 5.4), assuming a lineage-specific substitution rate of 0.051 per lineage/million years. The difference between values of θ_0 and θ_1 indicates an increase of four orders of magnitude in the effective number of females under the sudden expansion model,

assuming a generation time of 1.5 years (Cooke, 1965). Alternatively, the mutational (θ) and migration (M) parameters under the spatial expansion model suggest a spatial expansion with high levels of migration between sub-populations.

Table 5.4.- Estimated population expansion parameters for Lanzarote population (excluding Zozamas). The values were obtained from the mismatch distribution under the sudden expansion and spatial expansion models.

	Confidence intervals (Percentil method) $\alpha = 0.05$		
Estimated parameters	Lower limit	Upper limit	Mean value
Under the sudden expansion model			
$\tau = 0.592$	0	6.499	2.109
$\theta_0 = 2.464$	0	6.864	1.378
$\theta_1 = 96.25$	3.149	20006.25	8022.287
t = 9,532 years ago	0	104,638	33,956
N_0 = 26,447	0	73,676	14,791
N_1 = 1,033,124	33,800	214,742,398	86,109,348
Under the spatial expansion model			
$\tau = 0.561$	0.375	12.321	3.517
$\theta_0 = 2.465$	0	5.146	1.503
M = 5192.2	0.448	10392.795	620.303
t = 9,032 years ago	6,038	198,376	56,625
N = 26,458	0	55,236	16,133

Network, nested design and NCA

The application of statistical parsimony yielded six independent haplotype networks separated by more than 10 mutational steps (95% confidence limit that homoplasies are not included) (Fig. 5.5). Several instances of loop connections (haplotypes with more than one alternative connection) were observed and resolved following the criteria proposed by Crandall & Templeton (1993). The different networks were composed as follows: network **NI2** includes all haplotypes from La Graciosa and Montaña Clara and four from Alegranza; network **NI1**

includes two haplotypes from Alegranza; network **L1** includes 15 haplotypes from populations across Lanzarote, one of which is also present in Alegranza; network **L2** includes three haplotypes from the Lanzarote localities Montaña Blanca and Zonzamas; network **L3** is composed of haplotype 32 from the Lanzarote locality Mirador del Río Lanzarote; and network **F** includes all haplotypes from Fuerteventura. The information provided by the tree topology obtained in the combined analyses of the *cox1+rrnL-nad1* data set (Fig. 5.2) was used to nest the independent networks at higher hierarchical levels, which yielded the following clades: 4.3 (L1+L3), 5.1 (NI1+NI2; northern islets clade) and 5.2 (Clade 4.3+L2+F) (Fig. 5.5).

Table 5.5 shows the results of the nested contingency analysis of geographic associations and the interpretations of statistically significant clades following Templeton's updated key (2004) for clades in Fig. 5. At the entire cladogram level, the separation between clades 5.1 (northern islets clade) and 5.2 (Lanzarote-Fuerteventura clade) is explained by allopatric fragmentation. However, the structure within these clades reflects different historical processes. Clade 5.2 is consistent with a contiguous range expansion, whereas the structure within clade 4.1, which includes haplotypes from the northern islets, reflects a past gradual range expansion followed by fragmentation.

Table 5.5.- Significant clades obtained from the nested contingency analyses of geographic associations and interpretation based on the inference key (see test for details). *:significant at the 0.05 level.

Clade	**Chi-squared statistic**	**Probability**	**Inference chain**	**Inference**
1.20	1.00	0.0101*	1-19-20	Inadequate geographical sampling
4.1	28.16	0.0000*	1-2-11-12-13-14 No	Past gradual range expansion followed by fragmentation
5.2	85.97	0.0000*	1-2-11-12- No	Contiguous range expansion
Total cladogram	59.61	0.0000*	1-2-3-4-9 No	Allopatric fragmentation

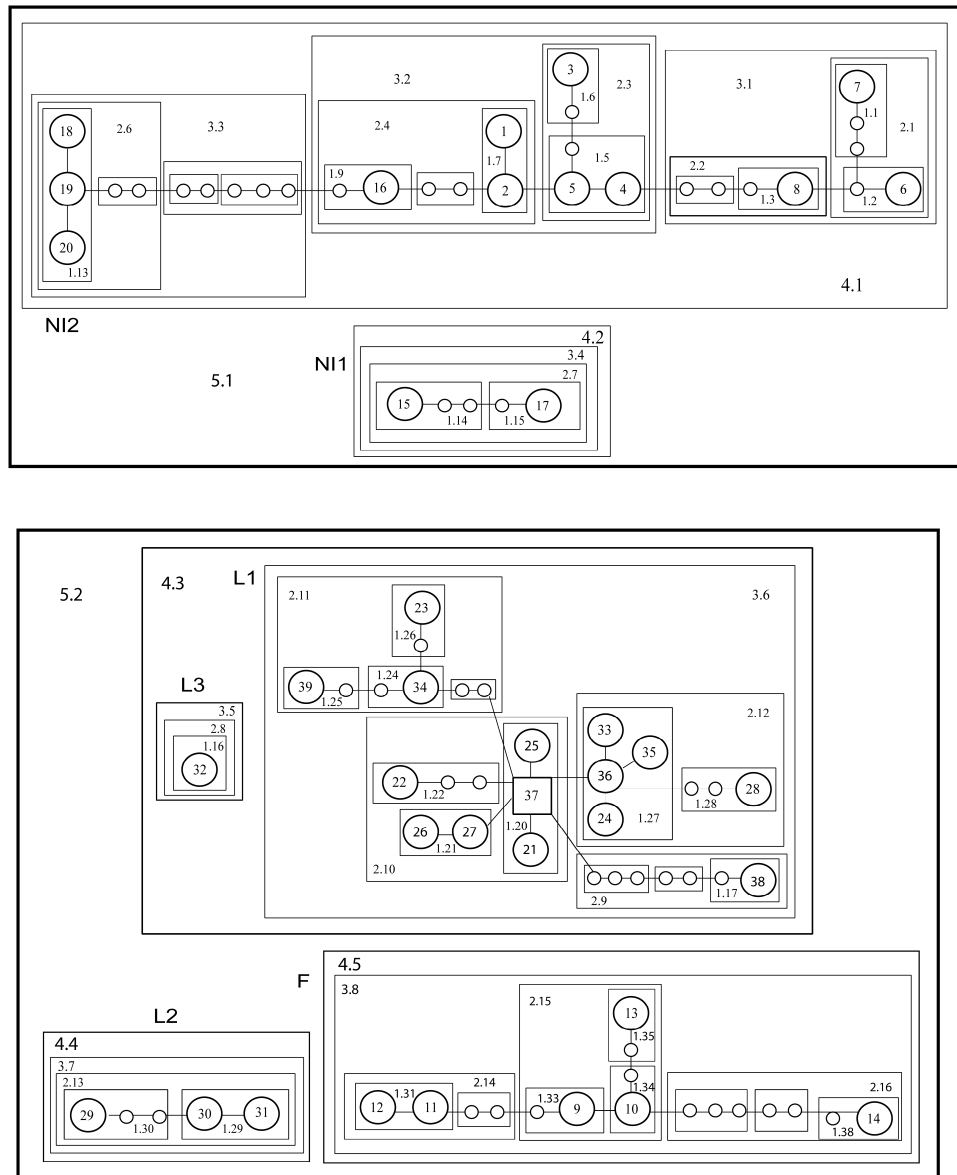

Fig. 5.5.- Nesting design for the six independent haplotype networks (NI2, NI1, L1, L2, L3 and F) obtained by statistical parsimony for *Dysdera lancerotensis*. Haplotype numbers as in Appendix 5.1. All haplotypes circled except 37, to indicate that it is the most frequent. White circles denote unsampled or extinct haplotypes.

DISCUSSION

A tale of two lineages

Phylogenetic analyses of the combined mitochondrial markers suggest a basal split of *Dysdera lancerotensis* haplotypes into two lineages, one including haplotypes from the northern islets and the other including haplotypes from Lanzarote and Fuerteventura. The MRCA estimated for the two main haplotype lineages of *D. lancerotensis* using a phylogenetic approach was 1.49 Mya (0.69-2.28 Mya) and the MRCA of each lineage was set around 1 Mya. These values are at odds with current geological evidence, which suggests that present-day subaerial volcanic terrains in the northern islets resulted from volcanic activity starting at about 45 Ka (Nuez Pestana *et al.*, 1998), although our lineage-specific substitution rate is four-fold faster than standard arthropod mtDNA estimates.

A possible explanation of this puzzle is that the split of the main haplotype clades greatly preceded the divergence between populations. However, the divergence time between populations from the northern islets and populations from Lanzarote and Fuerteventura and the time of the MRCA of their haplotypes as estimated with MDIV are actually quite similar, 0.52 Mya (0.30-0.96 Mya) and 0.64 Mya (0.37-1.19 Mya) respectively. In addition, MDIV shows a very low level of migration between populations suggesting that they diverged by isolation, which is further corroborated by the NCA interpretation of the two main haplotype networks as the result of an allopatric fragmentation event (Fig. 5.5, Table 5.5). Broad difference between the TMRCA estimated by R8S and that of MDIV is most likely the result of the deep intraspecific divergences of the *cox1* (maximum ~8.5%) in *D. lancerotensis*. R8S estimates were based on branch lengths obtained using a GTR model with invariant and rate heterogeneity. Conversely, MDIV only implements a finite sites model based on a simpler HKY model with homogenous rates, which most probably results in an underestimation of divergences among haplotypes. We tested this hypothesis by estimating TMRCA using an alternative Bayesian approach implemented in the program BEAST v.1.3. (Drummond & Rambaut, 2003), which incorporates an array of clock methods and evolutionary models. The TMRCA of the *D. lancerotensis* haplotypes using the exponential clock

method (Drummond *et al.*, 2006) and a GTR+I+Γ was 1.12 Mya (0.58 Mya-1.85 Mya), while implementing the strict clock and a HKY model with homogenous rates yielded 0.81 Mya (0.64-0.99 Mya). These results illustrate that the use of simpler models may results in more recent estimates of the TMRCA. Therefore, our population divergence estimates hints at a much older age of the northern islets, which let us to propose that they may have arisen during the volcanic series I that formed the Famara edifice. Biogeographic events older than those suggested by geologic data have also been proposed to explain phylogeographic patterns in *Brachyderes* weevils in the island of La Palma (Emerson *et al.*, 2006).

The actual processes that may in fact have generated such divergence remains unclear. The core of the northern part of Lanzarote is the Famara volcanic edifice, which was the last part of the eastern ridge to be formed (around 10 My) and has also experienced the longest and most continuous cycles of volcanic activity (Coello *et al.*, 1992). To the three initial pulses and subsequent erosive periods of the series I subaerial volcanic activity were followed by a relatively long period of quiescence from 3.8 to 1.8 My, when post-erosional volcanism started. Plio-Pleistocene activity in Lanzarote has been more or less continuous, reaching a peak in the Lower Pleistocene (Ancochea *et al.*, 2004). The estimated time of the MRCA of the two main *D. lancerotensis* lineages fits well with this final pulse of volcanic activity and suggests that severe bottlenecks during periods of volcanic activity may have played a key role in generating deep genetic divergences in *D. lancerotensis*.

The Canaries do not have any significant subsidence, which explains why islands older than 20 My are still emergent, while islands in the Hawaiian chain become submerged by subsidence after about 7 My (Carracedo, 1999). Mass-wasting processes and marine transgressions have shaped the present-day configuration of the geologically continuous eastern ridge into independent islands and islets. However, most of the islands were cyclically connected during Pleistocene glacial maxima when sea level dropped, which seems to rule out marine transgression as the main factor in generating divergence between the main lineages. This suggestion is corroborated by the internal phylogeographic

pattern of the islets (see below) and the presence of at least one haplotype widespread across Lanzarote and in Alegranza, indicative of possible recent gene flow between the two areas. The role of ecological factors in the separation of the two lineages also seems very unlikely. There are no obvious environmental differences between the collecting localities on the islets and on the main islands, neither are there clear morphological differences, although rigorous morphometric analyses have not been performed. Moreover, high levels of tolerance to a wide range of environmental conditions have been reported in the closely related species *D. crocata* (Cooke, 1968), which seem to be confirmed in *Dysdera lancerotensis* by its wider distribution range when compared to other *Dysdera* endemic to the islands (Arnedo *et al.*, 2000).

Simulation models based on coalescence predictions have shown that deep phylogeographic breaks can form within a continuously distributed species even when there are no barriers to gene flow (Irwin, 2002). However, these models require low levels of individual dispersal distance and small population sizes, which do not seem consistent with the widespread presence of *D. lancerotensis* throughout the eastern ridge.

The northern islets

The three main northern islets are separated by shallow waters (200 m below sea level at its deepest points), which hints at the existence of land bridges between the islands during Eustatic sea level changes. However, all but one of the haplotypes found in the northern islets are exclusive to the area, and each of the three islets harbour genetically differentiated populations, which suggests that such connections have not been as common as predicted by the periodical drop of sea level during Pleistocene glaciations. The pattern of genealogical relationships of the haplotypes (Figs. 5.2, 5.3), showing successive paraphyly of Alegranza exclusive haplotypes relative to La Graciosa and Montaña Clara, and paraphyly of La Graciosa haplotypes relative to Montaña Clara, supports Alegranza as the source of the current population on La Graciosa, and that Montaña Clara was colonized from La Graciosa. The successive decrease in genetic diversity from Alegranza to Montaña Clara (Table 5.3) provides further support for this pattern of colonization. The NCA

suggests that the phylogeographic pattern of *D. lancerotensis* in the northern islets is the result of a past gradual range expansion followed by fragmentation (Fig. 5.5, Table 5.5). The phylogeographic pattern observed in the northern islets could be explained by the occurrence of major population extinctions and subsequent recolonizations. The present-day subaerial terrains of the islets derive from the late Pleistocene-Holocene pulses of volcanic activity. It is not difficult to imagine a scenario in which most of the biota of a small islet like Montaña Clara, of about 1 km^2, could be completely wiped out by the effect of lava flows, and subsequently recolonized from the nearest islet, in this case La Graciosa. Our haplotype lineage age estimates are congruent with the proposed pattern of colonization, although they suggest slightly older ages that the available geological data.

The fact that Alegranza, the youngest and most remote of the islets, has the highest levels of genetic diversity and has been the source of colonization for the other islets constitutes something of a conundrum. However, these patterns are supported by further observations. Alegranza harbours by far the highest number of terrestrial invertebrate species on the islets, including several endemisms: 3 gastropods, 2 spiders, 1 pseudoscorpion, 1 silverfish and 1 beetle (also present in the Selvage Islands). The reasons for the greater diversity found on Alegranza are unclear, since no additional habitats are apparent with respect to the other islets. Remoteness may have spared Alegranza from destructive geological events that affected Montaña Clara and La Graciosa. Giant landslides had a key role in shaping the landscapes of the Canary Islands (Stillman, 1999). During the Quaternary, tsunamis caused by volcanic island mass-slides had a significant impact on the world's coastlines (Whelan & Kelletat, 2003). Evidence of such tsunamis can possibly be seen in the limestone and conglomeratic layers composed of rounded pebbles that are found in La Graciosa, although this has been disputed (Acosta *et al.*, 2003).

A reversed colonization pattern

The pattern of relationships of *D. lancerotensis* haplotypes suggests a north to south colonization of the eastern ridge, which is consistent with the higher levels of genetic diversity found in Lanzarote and Alegranza (Table 5.3). However, this

pattern opposes the SSW-NNE trend of post-Miocene volcanic activity in the ridge. It has been suggested that this temporal migration of volcanism shaped the phylogeographic patterns of the darkling beetle *Hegeter deyrollei* (Juan *et al.*, 1998). The lack of congruence between the phylogeographic patterns of *D. lancerotensis* and *H. deyrollei* is most likely to result from the colonization by these two organisms from two opposite extremes of the eastern ridge. Lanzarote was originally formed as two independent islands, the present-day massifs of Famara in the north and Ajaches in the south (Coello *et al.*, 1992), that were joined into a single mass during a new phase of volcanic activity starting at the late Pliocene and extending to the Holocene. *D. lancerotensis* may have been restricted to the northern massif until a suitable land connexion allowed them to progress south.

According to NCA the colonization of Fuerteventura from Lanzarote would have been the results of contiguous range expansion, probably facilitated by land connections associated to sea level fluctuations. The time of the MRCA of the sampled haplotypes in Fuerteventura (0.31 Mya, 0.07-0.46 Mya) is less than half that of their split from their Lanzarote sister lineage (0.93 Mya, 0.37-1.55 Mya). Population bottlenecks and/or reductions in effective population size in the past are mechanisms that may account for this pattern. A reduction in diversification rates and the lack of older lineages of the beetle *Brachyderes rugatus* in the Western Canaries have been explained by larger extinction events that would have reduced mtDNA diversity as a product of island colonization, high levels of volcanic activity and dramatic erosional events (Emerson *et al.*, 2000). All three elements probably concurred during the contiguous range expansion of *D. lancerotensis* towards Furteventura.

Population growth and expansion: the fingerprint of volcanism on demographic patterns?

Mismatch distribution and neutrality test analyses hint at a very recent population expansion in Lanzarote that would have occurred ~9 Kya (Table 5.4). Based on coalescent theory, haplotype 37 (with the highest frequency in the Famara edifice) would be the ancestral haplotype of the L1 network, because of its interior location in the network, high frequency and widespread occurrence.

Estimations for the MRCA of the main haplotype networks in Lanzarote suggest that they became differentiated after a maximum of volcanic activity in the Lower Pleistocene (Coello *et al.*, 1992). The most recent volcanic activity is still evident today in the large areas of barren lava known as *malpaís* (badlands) found in the central-west and north-east of the island, corresponding to the two more recent eruptive events: the Montaña Corona, dated 21 ka (Carracedo *et al.*, 2003), and the historical Timanfaya eruptions in 1730 and 1824 (Carracedo *et al.*, 1992). Reconciliation of the demographic and phylogeographic patterns exhibited by *D. lancerotensis* could suggest the following scenario. During short erosive stages, *D. lancerotensis* would have spread and diversified throughout the island whereas volcanic activity stages would have caused extinction of most populations and the one that survived suffered severe bottlenecks. Towards Upper Pleistocene and the Holocene the frequency of eruptive cycles was reduced becoming residual. At that point, populations from Famara would have recolonized most of the island through a range expansion as suggested by the mismatch distribution and neutrality tests. Some isolated populations that survived volcanic events in other parts of the island may have been incorporated into this late expansion, as suggested by the presence of the divergent haplotype 32, while another surviving population may have remained isolated, as represented by Zonzamas.

Conclusions

The present study reveals the complex phylogeographic and demographic patterns of Dysdera lancerotensis on the Eastern Canary Ridge as expected from the history of volcanic activity in the region. The occurrence of long branches separating groups of haplotypes with short coalescence times, and the discovery that most of the current populations in Lanzarote could be the result of a recent population expansion, suggest that the history of *D. lancerotensis* has been shaped by successive episodes of extinction, recolonization and expansion. Contrary to the colonization patterns of the ridge that had been reported for the darkling beetle Hegeter deyrolley, which closely follow the SSW-NNE migration of subaerial volcanic activity, *D. lancerotensis* has colonized the Eastern Canaries from north to south, reaching its maximum levels of genetic diversity in Alegranza, the northernmost part of the ridge. Both species seem to share certain ecological

requirements, since they occur in all areas except for recent barren lavas and sand dunes; the opposing colonization pathways of the two species should therefore be considered the result of historical contingency due to the arrival of ancestors at the extreme tips of the ridge. The presence of ancient haplotypes in Alegranza and haplotypes in Zonzamas not belonging to the main expansion in Lanzarote suggest that these areas acted as possible refuges during periods of generalized volcanic activity in the rest of the islands.

In summary, our study identifies the key role of an extremely dynamic geology in shaping historical and demographic patterns in a spider species. Future research into other terrestrial organisms in the Eastern Canaries is necessary to gain a better understanding of the historical and ecological factors that shape phylogeographic patterns and drive population-level processes.

APPENDIX

Appendix 5.1.- Summary of sequences and sampled localities of specimens analyzed. Code: locality acronym used in Fig. 5.1. UTM: locality coordinates. Hap: list of haplotypes collected in each locality with number of individuals showing the same haplotype in brackets. N: number of individuals sampled per locality. *cox1*, *rrnL/nad1*: Genbank accession numbers.

Species/Island	Locality	Code	UTM	N	Hap.	*cox1*	*rrnL/nad1*
D. lancerotensis							
Alegranza	Meseta de Concheta	MC	642964/3253161	8	15	EF458123	EF458078
					16	EF458124	
					17	EF458125	EF458077
					18	EF458126	
					19	AF244240	
					20	EF458127	EF458080
					37(2)	EF458098	
Montaña Clara	Caldera	CA	642257/3241988	6	6(4)	EF458128	EF458083
					7	EF458131	
					8	EF458129	
La Graciosa	Montaña del Mojón	MM	644183/3235717	5	1	EF458116	
					2(2)	EF458117	EF458082
					3(2)	EF458115	
	Arenal Peña	AP	644909/3234503	1	5	EF458130	
	Playa Lambra	PL	644997/3239733	1	4	AF244238	
Lanzarote	Mirador del Río	MR	647351/3232337	7	32	AF244241	AF244153
					33	EF458111	
					34	EF458112	EF458079
					35	EF458105	
					36(2)	EF458099	
					37		

Species/Island	Locality	Code	UTM	N	Hap.	*cox1*	*rrnL/nad1*
	Fuente Ovejas	FO	645721/3229341	6	37(6)		
	Bco. Elvira Sánchez	ES	644280/3223378	1	37		
	Montaña Tinache	MT	629503/3214506	5	36		
					37(3)		
					38	EF458113	EF458084
	Playa Teneza	PT	646695/3214831	3	36		
					37		
					39	EF458110	
	Zonzamas	ZO	639277/3210251	5	30(2)		
					31(3)	EF458109	EF458076
	Montaña Blanca	MB	633081/3207027	5	26	EF458108	
					27	EF458097	
					28	EF458106	
					29	EF458100	EF458075
					30	EF458107	
	Los Ajaches	AJ	620513/3199699	5	21(2)	EF458103	
					22	EF458102	
					23	EF458114	EF458081
					24	EF458104	
	Valle del Fenauco	VF	619527/3201168	1	25	EF458101	
Fuerteventura	Malpaís Bayuyo	BA	607232/3170116	1	13	EF458121	
	Morro Veloso	MV	592362/3146439	1	14	EF458122	EF458085
	Morro del Cavadero	MF	562497/3107753	7	9	EF458118	
					10	EF458119	
					11(4)	EF458120	EF458086
					12	AF244242	AF244154
			Total	68			

Species/Island	Locality	Code	UTM	N	Hap.	***cox1***	***rrnL/nad1***
Other species							
D. alegranzaensis	Canary I.: Alegranza	N47		1		EF458132	EF458087
D. longa	Canary I.: Fuerteventura	N91		1		EF458134	EF458090
D. nesiotes	Canary I.: Montaña Clara	N50		1		EF458133	EF458088
D. sanborondon	Canary I.: Fuerteventura	N85		1		EF458135	EF458089
D. crocata	Spain: Madrid	k246		1		EF458136	EF458096
	Spain: Guadalajara	k418		1		EF458137	EF458095
	Canary I.: Tenerife	k83		1		AF244237	AF244152
	Madeira	k235		1		EF458140	EF458074
D. inermis	Spain: Tarifa	k228		1		EF458141	EF458091
D. cf. inermis	Morocco: Tanger	k226		1		EF458142	EF458092
D. mauritanica	Morocco: Essaouria	k231		1		EF458138	EF458093
Dysdera. sp.	Morocco: Jbel Tazzeka	m5		1		EF458139	EF458094

REFERENCES

Acosta J, Uchupi E, Muñoz A, Herranz P, Palomo C, Ballesteros M, Group ZW. 2003. Geologic evolution of the Canarian Islands of Lanzarote, Fuerteventura, Gran Canaria and La Gomera and comparison of landslides at these islands with those at Tenerife, La Palma and El Hierro. *Marine Geophysical Researches* 24: 1-40.

Ancochea E, Barrera JL, Bellido F, Benito R, Brädle JL, Cebriá JM, Coello J, Cubas CR, De la Nuez J, Doblas M, Gómez JA, Hernán F, Herrera R, Huertas MJ, López Ruiz J, Martí J, Muñoz M, Sagredo J. 2004. Canarias y el vulcanismo neógeno Peninsular. In: Vera JA, ed. *Geología de España*. Madrid: Sociedad Geológica de España-Instituto Geológico y Minero de España. 635-682.

Arnedo MA, Oromí P, Ribera C. 2000. Systematics of the genus *Dysdera* (Araneae, Dysderidae) in the Eastern Canaries. *Journal of Arachnology* 28: 261-292.

Arnedo MA, Oromí P, Ribera C. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. *Cladistics* 17: 313-353.

Arnedo MA, Ribera C. 1997. Radiation of the genus *Dysdera* (Araneae, Haplogynae, Dysderidae) in the Canary Islands: The island of Gran Canaria. *Zoologica Scripta*.

Arnedo MA, Ribera C. 1999. Radiation of the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. *Journal of Arachnology* 27: 604-662.

Avise JC. 1998. The history and purview of phylogeography: a personal reflection. *Molecular Ecology* 7: 371-379.

Beheregaray LB, Ciofi C, Geist D, Gibbs JP, Caccone A, Powell JR. 2003. Genes record a prehistoric volcano eruption in the Galapagos. *Science* 302: 75.

Bjorklund M. 1999. Are third positions really that bad? A test using vertebrate cytochrome b. *Cladistics* 15: 191-197.

Bloor P, Brown RP. 2005. Morphological variation in *Gallotia atlantica* from the volcanic island of Lanzarote: subspecies designations and recent lava flows. *Biological Journal Linnean Society* 85: 395-406.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences* 91: 6491-6495.

Carracedo JC. 1999. Growth, structure, instability and collapse of Canarian volcanoes and comparisons with Hawaiian volcanoes. *Journal of Volcanology and Geothermal Research* 94: 1-19.

Carracedo JC, Badiola ER, Soler V. 1992. The 1730-1736 eruption of Lanzarote, Canary Islands: a long, highmagnitude basaltic fissure eruption. *Journal of Volcanology and Geothermal Research* 53: 239-250.

Carracedo JC, Day S, Guillou H, Badiola ER, Canas JA, Torrado FJP. 1998. Hotspot volcanism close to a passive continental margin: The Canary Islands. *Geological Magazine* 135: 591-604.

Carracedo JC, Singer B, Jicha B, Guillou H, Rodríguez Badiola E, Meco J, Pérez Torrado FJ, Gimeno D, Socorro S, Láinez A. 2003. La erupción y el tubo volcánico del Volcán Corona (Lanzarote, Islas

Canarias). *Estudios Geológicos* 59: 277-302.

Carranza S, Arnold EN. 2003. History of West Mediterranean newts, *Pleurodeles* (Amphibia: Salamandridae), inferred from old and recent DNA sequences. *Systematics and Biodiversity* 1: 327–338.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660.

Coello J, Cantagrel JM, Hernán F, Fuster JM, Ibarrola E, Ancochea E, Casquet C, Jamond C, Díaz de Terán JR, Cendrero A. 1992. Evolution of the eastern volcanic ridge of the Canary Islands based on new K-Ar data. *Journal of Volcanology and Geothermal Research* 53: 251-274.

Contreras-Diaz HG, Moya O, Oromí P, Juan C. 2003. Phylogeography of the endangered darkling beetle species of *Pimelia* endemic to Gran Canaria (Canary Islands). *Molecular Ecology* 12: 2131-2143.

Cooke JAL. 1965. A contribution to the biology of the British spiders belonging to the genus *Dysdera*. *Oikos* 16: 20-25.

Cooke JAL. 1968. Factors affecting the distribution of some spiders of the genus *Dysdera* (Araneae, Dysderidae). *Entomologist's monthly magazine* 103: 221-225.

Crandall KA, Templeton AR. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134: 959.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4.

Drummond AJ, Rambaut A. 2003. BEAST. 1.3 ed: Available from http://evolve.zoo.ox.ac.uk/beast/.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* 11: 2571-2581.

Emerson BC, Forgie S, Goodacre S, Oromí P. 2006. Testing phylogeographic predictions on an active volcanic island: *Brachyderes rugatus* (Coleoptera: Curculionidae) on La Palma (Canary Islands). *Molecular Ecology* 15: 449-458.

Emerson BC, Oromí P, Hewitt GM. 2000. Colonization and diversification of the species *Brachyderes rugatus* (Coleoptera) on the Canary Islands: Evidence from mitochondrial DNA COII gene sequences. *Evolution* 54: 911-923.

Excoffier L. 2004. Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology* 13: 853-864.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. 3.0 ed: *Evolutionary Bioinformatics Online* 1:47-50.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915-925.

Goloboff PA, Farris JS, Nixon KC. 2003. TNT: Tree Analysis Using New Technologies. 0.6 (Beta) ed.

Gómez-Zurita J. 2004. Molecular systematics and time-scale for the evolution of *Timarcha*, a leaf-beetle genus with a disjunct Holarctic distribution. *Molecular Phylogenetics and Evolution* 32: 647-665.

Grant PR. 1998. *Evolution on islands*. In: Grant PR, ed. *Evolution on islands.* 200 Madison Avenue, New York, New York 10016; Walton Street, Oxford OX2 6DP,

England: Oxford University Press; Oxford University Press. xiv+334p.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696-704.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Symposium on RNA biology; RNA, tool and target. Research Triangle Park, NC: Oxford University Press, 95-98.

Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherry ST. 1998. Genetic traces of ancient demography. *Proceedings of the National Academy of Sciences* 95: 1961-1967.

Hartl DL. 2000. *A Primer of Population Genetics*. Sinauer Associates: Sunderland, Massachusetts.

Hedin MC. 1997. Molecular phylogenetics at the population/species interface in cave spiders of the Southern Appalachians (Araneae: Nesticidae: *Nesticus*). *Molecular Biology and Evolution* 14: 309-324.

Higgins DG, Bleasby AJ, Fuchs R. 1992. CLUSTAL V: improved software for multiple sequence alignment. *Comput. Appl. Biosci.* 8: 189-191.

Hudson RR. 2000. A New Statistic for Detecting Genetic Differentiation. *Genetics* 155: 2011-2014.

Irwin DE. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution: International Journal of Organic Evolution* 56: 2383-2394.

Juan C, Emerson BC, Oromí P, Hewitt GM. 2000. Colonization and diversification: Towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution.* 15: 104-109.

Juan C, Ibrahim KM, Oromí P, Hewitt GM. 1998. The phylogeography of the darkling beetle, *Hegeter politus*, in the eastern Canary Islands. *Proceedings of The Royal Society of London Series B Biological Sciences* 265: 135-140.

Kaellersjö M, Albert VA, Farris JS. 1999. Homoplasy Increases Phylogenetic Structure. *Cladistics* 15: 91-93.

Kozak KH, Blaine RA, Larson A. 2006. Gene lineages and eastern North American palaeodrainage basins: phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. *Molecular Ecology* 15: 191-207.

Mayr E. 1967. The challenge of island faunas. *Australian Natural History* 15: 359-374.

Mickevich MF, Farris JS. 1981. The implications of incongruence in *Menidia*. *Sys. Zool.* 30: 351-370.

Moya O, Contreras-Diaz HG, Oromí P, Juan C. 2004. Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). *Molecular Ecology* 13: 3153-3167.

Moya O, Contreras-Diaz HG, Oromí P, Juan C. 2006. Using statistical phylogeography to infer population history: Case studies on *Pimelia* darkling beetles from the Canary Islands. *Journal of Arid Environments* 66: 477-497.

Nielsen R, Wakeley J. 2001. Distinguishing migration from isolation: A Markov Chain Monte Carlo Approach. *Genetics* 158: 885-896.

Nuez Pestana JdI, Quesada Alvarez M, Luisa, Alonso Blanco JJ. 1998. *Los volcanes de los islotes al norte de Lanzarote, Islas Canarias*. Fundación César Manrique: Teguise, Lanzarote.

Posada D, Buckley TR. 2004. Model Selection and model averaging in

phylogenetics: Advantages of Akaike Information Criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793-808.

Posada D, Crandall KA. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Posada D, Crandall KA, Templeton AR. 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Molecular Ecology* 9: 487-488.

Rambaut A, Drummond A. 2005. Tracer, A program for analysing results from Bayesian MCMC programs such as BEAST & MrBayes. 1.3 ed. Oxford: online at 663 http://evolve.zoo.ox.ac.uk /software.html?id=tracer (last accessed 12 February 2006). 664.

Ray N, Currat M, Excoffier L. 2003. Intra-deme molecular diversity in spatially expanding populations. *Molecular Biology and Evolution* 20: 76-86.

Rees DJ, Emerson BC, Oromí P, Hewitt GM. 2001a. The diversification of the genus *Nesotes* (Coleoptera: Tenebrionidae) in the Canary Islands: evidence from mtDNA. *Mol Phylogenet Evol* 21: 321-326.

Rees DJ, Emerson BC, Oromí P, Hewitt GM. 2001b. Mitochondrial DNA, ecology and morphology: interpreting the phylogeography of the *Nesotes* (Coleoptera: Tenebrionidae) of Gran Canaria (Canary Islands). *Molecular Ecology* 10: 427-434.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* 9: 552-569.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496-2497.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101-109.

Sanderson MJ. 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19: 301-302.

Schneider S, Excoffier L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152: 1079-1089.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Stillman CJ. 1999. Giant Miocene landslides and the evolution of Fuerteventura, Canary Islands. *Journal of Volcanology and Geothermal Research* 94: 89-104.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585-595.

Templeton AR. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and

population history. *Molecular Ecology* 7: 381-397.

Templeton AR. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology* 13: 789-809.

Templeton AR, Boerwinkle ES, F. C. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. *Genetics* 117: 343-351.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619.

Templeton AR, Routman E, Phillips CA. 1995. Separating population structure from population history: A cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* 140: 767-782.

Templeton AR, Sing CF. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics* 134: 659-669.

Vandergast AG, Gillespie RG, Roderick GK. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology* 13: 1729-1743.

Vogler AP, Cardoso A, Barraclough TG. 2005. Exploring rate variation among and within sites in a densely sampled tree: species level phylogenetics of North American tiger beetles (Genus *Cicindela*). *Systematic Biology* 54: 4-20.

Wagner WL, Funk VA. 1995. *Hawaiian biogeography: Evolution on a hot spot archipelago*. Smithsonia Institution Press: Washington and London.

Whelan F, Kelletat D. 2003. Submarine slides on volcanic islands - a source for mega-tsunamis in the Quaternary. *Progress in Physical Geography* 27: 198-216.

Wunderlich J. 1991. Die Spinnen-fauna der Makaronesischen Inseln. *Beitr. Araneol.* 1: 1-619.

Young ND, Healy J. 2002. GapCoder: http://www.trinity.edu/nyoung/GapCoder/Download.html.

Capítulo 6

THE ODD COUPLE: CONTRASTING PHYLOGEOGRAPHIC PATTERNS IN TWO SYMPATRIC SIBLING WOODLOUSE-HUNTER SPIDER *DYSDERA* IN THE CANARY ISLANDS

ABSTRACT

Ecological theory predicts that when two closely related species come into contact they should either evolve some sort of resource partitioning or one of the species would eventually displace the other one. The spider genus *Dysdera* has undergone intense diversification within the volcanic archipelago of the Canary Islands. Two endemic species, *Dysdera alegranzaensis* and *Dysdera nesiotes*, co-occur on most of their known localities across Lanzarote and the northern islets. Although they strongly resemble each other in body size, general appearance and foraging behavior, previous phylogenetic analyses have shown that they are not sister taxa, and came into contact secondarily. Here, we conduct a comparative phylogeographic analysis of these two species to shed light on the factors that have enabled coexistence of ecologically similar, close relatives. The mitochondrial *cox1* gene was sequenced for 53 specimens of *D. alegranzensis* and 43 of *D. nesiotes*, sampled from populations along their distribution range. Additional mitochondrial (*16S* rRNA, *nad1*) and nuclear (*28S* rRNA, Histone 3) genes were included to assess basal relationships among populations and the position of selected outgroups, as well as to infer a temporal framework. Despite their phylogenetic proximity and similar life history traits, our results reveal marked differences between the phylogeographic patterns of both endemics. While *D. alegranzensis* patterns resemble those inferred for other local taxa, *D. nesiotes* exhibits a meta-population-like structure. Although time and source of diversification of present-day haplotypes of each species may account for some of the differences observed, we propose that such differences are better explained if the two species coexist in a dynamic equilibrium. Unlimited resources would enable coexistence of both species. When those resources were depleted, however, *D. alegranzaensis* would outcompete *D. nesiotes*. When favourable conditions were restored, new migrants would restart locally extirpated populations of the later species. Our results provide additional insights on the role of lava flows and eustatic sea-level changes in

structuring local populations. In spite of frequent land connection between islands during glacial maxima, the phylogeographic patterns recovered corroborate former suggestions that populations from the northern islets, especially Alegranza, have evolved mostly in isolation and constitute an important reservoir of genetic diversity. Similarly, we identify localities in eastern-central Lanzarote that acted as refugium for populations during volcanic eruptions, and recognize instances of population expansion, probably resulting from former population extinctions by lava flows.

INTRODUCTION

Comparative phylogeography seeks to discover commonalities in the evolutionary history of populations in co-distributed species or species complexes (Cracraft, 1989; Zink, 1996; Bermingham & Moritz, 1998; Moritz, 1998; Schneider *et al.,* 1998; Avise, 2000; Riddle *et al.*, 2000; Sullivan *et al*., 2000; Arbogast *et al*., 2001). Sympatric species that share ecological requirements are expected to respond similarly to local environmental changes and geographic barriers (Irwin, 2002; Riddle *et al.,* 2000; Garrick *et al.*, 2008). Conversely, contrasting population patterns in co-distributed species may be indicative of underlying species-specific features such as habitat specificity, behaviour, life history or dispersal ability, among others (Taberlet *et al.,* 1998; Michaux *et al*., 2005; Bird, 2007; Hodges, 2007). Alternatively, co-distributed species could not have been similarly co-distributed in the past and hence they would have responded differently to different evolutionary events occurring at dissimilar times, resulting in incongruent patterns of geographic genetic diversity (Donoghue & Moore, 2003; Carstens, 2007).

The deeply eroded, dry and, relatively, species-poor eastern Canary Islands, which include the islands of Fuerteventura, Lanzarote and the northern islets of La Graciosa, Montaña Clara and Alegranza (also known as Archipelago Chinijo), have received little attention by evolutionary biologist compared with their western counterparts. The eastern Canaries, however, provide an excellent model to investigate the role of geological and climatic barriers in structuring populations of

local organisms and shaping island diversity (Juan *et al.,* 1998; Bidegaray-Batista *et al.,* 2007; Bloor *et al.,* 2008). These islands are the emerged summits of a continuous volcanic edifice that traces back to about 20 million years ago (Ma) (Coello, 1992). All the islands and islets have been connected on several occasions during glacial periods by eusthatic sea-level changes (García-Talavera, 1997; 1999), offering ample opportunities for gene flow among populations on different islands. Lanzarote was built during the Miocene as two independent islands, corresponding to the present day massifs of Ajaches (15.5-13.5 Ma), on the southwest, and Famara (10.2-3.8 Ma), on the northeast (Coello *et al.,* 1992, Carracedo & Rodríguez-Badiola, 1993). Shield stage volcanism was followed by a major gap in activity between 3.8 to 1.8 Ma, which brought about an extensive erosion of the old edifices, which may had already been connected by sand dunes (Coello *et al.,* 1992, Carracedo & Rodríguez-Badiola, 1993). Volcanic activity restarted about 1.6 Ma (post-erosional stage or rejuvenated volcanism), connecting definitively the two former islands about 0.8 Ma (Carracedo & Rodríguez-Badiola, 1993), and persisted, more or less continuously, to the present. Subhistoric and recent (1730 to 1736) eruptions have covered vast areas of the northeast and western central parts of Lanzarote with basaltic lava flows, forming volcanic badlands called *malpaís* (Carracedo *et al.,* 1992; 2003). The present day subaerial volcanic terrains in the northern islets resulted from volcanic activity starting ~0.45 Ma, (de la Nuez Pestana & Luisa Alonso Blanco, 1998).

The woodlouse hunter spider *Dysdera* is one of the most species-rich genus in the Canary Islands and presently includes about 50 endemic species. This remarkable diversity is mostly the result of local diversification, following only two or three colonization events (Arnedo *et al.,* 2001). Many Canarian *Dysdera* species occur sympatrically and often show morphological differences in prey-capture related traits (body size and/or chelicerae shape). Two of the five *Dysdera* species reported in Lanzarote, however, seem to be an exception to the former observation (Macías-Hernández *et al*., 2008). *Dysdera nesiotes* and *D. alegranzaensis* co-occur in most of the localities known from Lanzarote and the Archipelago Chinijo. Although the two species are not sister taxa, they are close relatives and hardly differ in any morphological trait other than genitalia (Arnedo *et*

al., 2000; Macías-Hernández *et al.,* 2008). The co-occurrence of close relatives with no apparent morphological or ecological differentiation, raise important evolutionary questions on the processes that may allow their coexistence.

In the present study we aim to offer insights on the factors that promote coexistence of sibling species with similar ecological preferences, by comparing the phylogeographic pattern and demographic processes of *D. alegranzaensis* and *D. nesiotes*. Our inferences are mostly based on mitochondrial genes. Although we are fully aware of the limitation of the use of mitochondrial markers to fully infer the evolutionary processes undergone by populations, we propose that in this case such limitations are largely overcome by comparing patterns in co-occurring, close relatives. In spite of the potential of the eastern Canary Islands as model system for comparative phylogeographic studies, this is the first study of this nature conducted on these islands and only the second for the whole archipelago (Moya *et al.,* 2004).

MATERIALS AND METHODS

Sampling

The specimens of *D. alegranzaensis* and *D. nesiotes* were collected on Lanzarote and its neighbouring islets during 2004 and 2005. A total of 20 localities were sampled (Fig. 6.1, Appendix 6.1). In most localities, specimens of both species were collected together, except in La Graciosa and few localities on Lanzarote (Zonzamas) where *D. nesiotes* was not found (see Appendix 6.1). Samples were stored in absolute ethanol at -20ºC until DNA extractions were performed.

Specimens of all the other *Dysdera* species reported in Fuerteventura and Lanzarote were also included in the analyses. Additional species from the western Canaries and from the continent were included as calibration points for divergence time estimations and outgroups.

Fig. 6.1.- Map of Lanzarote, showing with letters the localities where specimens of *D. alegranzaensis* and *D. nesiotes* were collected. See Appendix 6.1 for localities codes.

DNA extractions, PCR amplifications and sequencing

Protocols for DNA extractions, amplifications and sequencing of the genes included in the study followed Bidegaray-Batista *et al.*, (2007). Approximately 1100 bp of the mitochondrial gene cytocrome c oxidase subunit I (*cox1*) were amplified for all the specimens as two overlapping fragments using the primer pairs C1-J-1490 and C1-N-2198 (Folmer, 1994) or C1-N-2191 (Simon, 1994), and CI-J-2183 (Simon, 1994), or the antisense of C1-N-2198, with C1-N-2776 (Hedin & Maddison 2001). Information on additional mitochondrial and nuclear genes was retrieved for a subsample of taxa to improve phylogenetic signal at the base of the trees. The mitochondrial genes 16S rRNA (*16S*), tRNA leu UAG (*L1*) and NADH dehydrogenase subunit I (*nad1*), and the nuclear genes 28S rRNA (*28S*) and Histone H3 (*H3*) were amplified and sequenced using the following primer pairs: [*16S*] LR-N-13398 (Simon, 1994) and N1-J-12261 (Hedin, 1997) or N1-J-12373 (5´ CTTCGTATAGATCCTARTTGDCTRTATTT 3´), [*28S*] 28S-B (Giribet, 1999) and 28S-O (Hedin, 2001) and [*H3*] H3a F and H3a R (Colgan, 1998). PCR products were cycle-sequenced in both directions using BigDye terminator version 3.1 (Applied Biosystems) and sequenced in an ABI 3700 automated sequencer at the Scientific and Technical Services of the University of Barcelona (http://www.sct.ub.es). The DNA sequences obtained were edited using the PREGAP and GAP4 programs included in the Staden Package (http://staden.sourceforge.net/) software and assembled and preliminary manual alignments built using BIOEDIT (Hall, 1999).

Phylogenetics analyses

We performed maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses to resolve relationship among sampled populations of *D. alegranzaensis* and *D. nesiotes.* Two matrices were assembled and analyzed. The first one, hereafter referred as M1, included *coxI* haplotypes found in the 96 specimens of *D. alegranzaensis* and *D. nesiotes* sampled and 10 found in the remaining species analyzed. Trees recovered from M1 did not resolve basal relationships (see results) among outgroups and most divergent populations of the target taxa. Therefore, a second matrix (M2) was assembled by selecting 37 specimens from M1 (6 *D. nesiotes*, 6 *D. alegranzaensis* and 25 representatives of

the remaining species, see Appendix 6.1) and including along with the *cox1* sequences information of *16S*, *L1*, *nad1* and the nuclear genes *28S* and *H3*.

Ribosomal gene sequences were aligned with the online version of the MAFFT v. 5.8 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) automatic alignment program (Katoh, 2002; 2005), using the manual strategy option set to Q-INS-I with default options. This program has been shown to outperform other automatic algorithms in ribosomal alignments (Wilm *et al.,* 2006). Gaps were scored as presence/absence characters following Simmons & Ochoterena (2000), using the simple method (Simmons *et al.,* 2001) as implemented in the program GAPCODER (Young & Healy, 2002). Data matrices were concatenated using the program WINCLADA v.1.00.08 (Nixon, 2002). Parsimony analyses under equal weights were conducted with TNT version 1.0 (Goloboff *et al.,* 2003) using a heuristic search based on 1000 replicates of random sequence addition followed by tree-bisection–reconnection (TBR) branch swapping (five trees retained per iteration, and final round of TBR branch swapping on all retained trees). Clade support was assessed via jackknife resampling (Farris *et al.,* 1996) using 1000 replicates with individual heuristic searches consisting of 15 iterations of Wagner tree construction using random addition of taxa, holding 5 trees per iteration and an overall maximum of 10000. The program jMODELTEST, version 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) was used to select the substitution model of evolution that fit the data better with lesser parameters, as indicated by the Akaike information criterion (AIC) (Akaike, 1973) (Posada & Buckley, 2004). Bayesian inference analyses were conducted with MRBAYES v.3.1.2 (Ronquist & Huelsenbeck, 2003) and run remotely at the Bioportal computer resources of the University of Oslo (http://www.bioportal.uio.no). Unlinked nucleotide substitution models selected by jMODELTEST were specified for each gene fragment and a standard discrete model was implemented for the gaps scored as absence/presence data (Nylander *et al.,* 2004). The substitution estimates were allowed to vary independently between each partition. We conduct two independent runs with four simultaneous MCMC (Markov Chain Monte Carlo) chains (one cold and three heated), each starting with random starting trees, were performed for 10 million iterations and decreasing the temperature to 0.15 to facilitate the convergence of

the chains. The standard deviation of the split frequencies of the two runs (< 0.01) and the effective sample size (ESS) as measured by the program TRACER version 1.4 (Rambaut & Drummond, 2005) were monitored to ensure stationarity and correct mixing of Markov chains. TRACER was further used to determine the correct number of generations to discard as a *burn-in* for the analyses. Maximum likelihood analyses were conducted with the software RAxML v. 7.0.4 (Stamatakis, 2006). Independent GTR+I+G substitution models were set for each data fragment. The best likelihood tree was selected out of 10 iterations of random addition of taxa. Non-parametric bootstrap support values were drawn from 100 resampled matrices and confidence values were mapped onto the best topology. Maximum likelihood searches of M1 were conducted on RAxML by constraining the clades recovered with high support (MP jaccknife/ML bootstrap > 70%, posterior probability > 0.95) in the analyses of M2 (see Results).

Divergence time estimation

Divergence times and *cox1* substitution rates were estimated on M2 (after removal of 2 near zero-branch length taxa) with the program BEAST v.1.4.8 (Drummond & Rambaut, 2007), which implements an MCMC framework for the inference of time-measured phylogenies and testing for alternative molecular-clock model (Drummond *et al.,* 2006). Unlinked evolutionary models retrieved by jMODELTEST were defined for each gene partition and the prior on the distribution node heights was set to follow a Birth-Death process. Time constraints on selected nodes were defined based on available biogeographic information. The opening of the Strait of Gibraltar (5.3 Ma) was used as a fixed calibration point corresponding to the divergence between the Iberian and Morocco populations of *Dysdera inermis.* The time of emergence of several Canary Islands provided maximum time constrains for additional nodes. The emergence of La Palma (2 Ma) (Carracedo & Day, 2002) provide a maximum estimate age of the divergence between La Palma and La Gomera populations of *Dysdera calderensis* and *Dysdera silvatica;* and the age of El Hierro (1.2 Ma) (Carracedo & Day, 2002) provide a maximum estimated age of the divergence between El Hierro and La Gomera populations of *Dysdera gomerensis* and *D. silvatica*. A preliminary tree including these time constrains was calculated with the program R8S to ensure that the starting tree of the chains

satisfy or calibration constraints. Analyses were run under different clock models (strict clock, relaxed uncorrelated lognormal and relaxed uncorrelated exponential) and the best model was selected based on Bayes Factor (Suchard *et al.,* 2001). Two independent runs of 50 million generations each were conducted for each analysis, sampling every 1000 generations and removing the first 10% of samples as burn-in. Results were visualized and convergence and mixing assessed with TRACER.

Haplotype network

Relationships among populations at the intraspecific level are better estimated by means of multifurcated networks (Posada & Crandall, 2001; Cassens *et al.*, 2005). Haplotype networks were constructed using statistical parsimony (Templeton *et al.,* 1992), with a confidence limit of 95% with the software TCS version 1.21 (Clement *et al.,* 2000) and the Median-joining methods (Bandelt *et al.,* 1999) as implemented in the program NETWORK v.4.2.0.1 (www.fluxusengineering.com).

Genetic diversity, population structure and demographic analyses

Diversity indices (nucleotide π_n, and haplotype *h* diversity), pairwise genetic distance F_{ST} (Wright 1951) between populations and the analysis of molecular variance (AMOVA) (Excoffier *et al.,* 1992) were calculated with the software ARLEQUIN 3.01 (Excoffier *et al.,* 2005). Significance of F_{ST} was tested by running 10000 permutations. Populations represented by single sequenced individuals were excluded from F_{ST} calculations (localities Z, FA, PM, F, MA for *D. alegranzaensis*, see Table 6.1 A). AMOVA was conducted on different sets of populations, defined on the basis of geographical and geological background information: Lanzarote *vs.* northern islets (two groups); northern islets, northeast, central and southwest Lanzarote (four groups); and Alegranza, Montaña Clara, La Graciosa, northeast, central and southwest Lanzarote (six groups, only five in *D. nesiotes* since it was not found on La Graciosa). The significance of the variance components (P<0.01) was computed using a nonparametric permutation test (10000 permutations). The group that maximized the variance among groups (Φct), minimized the variance within gropus (Φsc) and gave statistically significant Φst,

Φsc and Φct was assumed to be the most plausible geographical subdivision (Paulo *et al.,* 2002).

Star-like networks may be indicative of recent demographic expansion (Slatkin & Hudson, 1991; Garrick & Sunnucks, 2006). Several neutrality tests were conducted to confirm population's growth in star-like haplotype networks recovered in the analyses (see Results). The program DnaSP vs. 4.0 (Rozas *et al.,* 2003) was used to compute the following neutrality tests (selecting coalescent simulations options): Tajima's D-test (Tajima, 1989), Fu's F_S test (Fu, 1997) and R_2 test (Ramos-Onsins & Rozas, 2002).

RESULTS

Phylogenetic analyses

Specimens and sequences analysed in the present study, with corresponding GenBank accession numbers, are listed in Appendix 6.1. Parsimony (2 trees of 3710 steps, CI= 44, RI= 67), Bayesian inference and maximum likelihood analyses conducted on M2, converged in very similar topologies (data not shown) that corroborate (1) monophyly of the populations of *D. alegranzensis* and *D. nesiotes* and (2) that the two former species are not sister taxa, although both are part of a clade that includes all eastern Canarian endemic *Dysdera* species, with the exclusion of *D. lancerotensis*.

Results of the maximum likelihood analyses of M1 constraining M2 high-support clades (logL= -20118.560420) are shown in Fig. 6.2. In *D. alegranzaensis*, there is a basal split in two main haplotype lineages, one including all Alegranza haplotypes plus additional ones from La Graciosa and Lanzarote (bootstrap support 82%) and a second one comprising the remaining haplotypes (bootstrap support 79%). Several haplotype lineages with high support are recovered in *D. nesiotes*, almost all of them exclusive from single localities.

Table 6.1. Fst values for mtDNA *cox1* of *D. alegranzaensis* (A) and *D. nesiotes* (B) based on Tamura & Nei model. Population codes correspond to codes in Appendix 6.1. Values in bold represent significant comparisons for *P*<0.05. Localities represented by single individuals were excluded of the analysis.

A. *Fst _D. alegranzaensis*

	ML	**MCA**	**MC**	**MBG**	**MM**	**BH**	**M**	**MR**	**BT**	**MT**	**MB**	**VF**	**AF**
ML	0.0000												
MCA	0.1246	0.0000											
MC	**0.7086***	**0.98147***	0.0000										
MBG	-0.2990	0.0647	**0.5519***	0.0000									
MM	0.6357	**0.97925***	**0.8551***	0.2751	0.0000								
BH	0.7580	**0.98016***	**0.9700***	0.6691	0.9658	0.0000							
M	0.6008	0.9837	**0.9757***	0.5384	0.9681	0.9510	0.0000						
MR	**0.3450***	**0.59171***	0.2619	0.2814	0.2194	0.3573	0.0021	0.0000					
BT	0.6413	0.9927	**0.9856***	0.5651	0.9824	0.9660	0.4008	0.0413	0.0000				
MT	**0.4857***	**0.7395***	**0.6323***	**0.4805***	**0.5753***	**0.6963***	0.0276	0.0330	0.0493	0.0000			
MB	**0.8063***	**0.99367***	**0.9883***	**0.8077***	**0.9879***	**0.9797***	0.6232	**0.3152***	-0.2903	**0.3184***	0.0000		
VF	**0.5903***	**0.84268***	**0.7724***	**0.5575***	**0.7464***	**0.7787***	-0.0174	0.0405	-0.0303	-0.1404	0.2483	0.0000	
AF	**0.7718***	**0.99511***	**0.9897***	0.7632	**0.9894***	**0.9795***	0.6625	0.2455	0.0000	0.2524	-0.0526	0.1829	0.0000

B. *Fst_D. nesiotes*

	ML	**MC**	**CES**	**BH**	**M**	**MR**	**FO**	**MT**	**MB**	**VF**	**AF**
ML	0.0000										
MC	**0.9572***	0.0000									
CES	**0.9861***	**0.988***	0.0000								
BH	**0.8890***	**0.9056***	**0.9791***	0.0000							
M	0.9678	0.9727	1.0000	0.9411	0.0000						
MR	**0.9784***	**0.9804***	**1.0000***	**0.9643***	0.0000	0.0000					
FO	0.9678	**0.9727***	1.0000	0.9411	0.0000	0.0000	0.0000				
MT	**0.9177***	**0.9444***	**0.9092***	**0.9193***	**0.6771***	**0.7619***	**0.6771***	0.0000			
MB	**0.8077***	**0.8606***	**0.8136***	**0.7321***	0.3471	**0.5023***	0.3471	**0.7340***	0.0000		
VF	**0.9467***	**0.9507***	**0.9343***	**0.9245***	**0.8550***	**0.894***	**0.8550***	**0.8504***	**0.7260***	0.0000	
AF	**0.9340***	**0.9526***	**0.5991***	**0.9229***	0.8170	**0.8751***	**0.8170**	**0.8397***	**0.7262***	**0.8539***	0.0000

Fig. 6.2. Maximum likelihood tree recovered for M1 analyses with high suport clades of the M2 constrained (marked with an asterisk). Grey bars and circles on branches denote ML bootstrap > 60% at species and intraspecific level, respectively. Divergence times obtained with BEAST are included on relevant nodes.

Lineage age estimates

Bayes Factor (BF) indicate that the relaxed lognormal clock model (ln -20025.023) provided a better fit to the data than the relaxed exponential model (ln -20028.677), or the strict clock model (ln -20056.812). The *cox1* substitution rate estimate was 0.035 per lineage/million years (confidence intervals 0.022-0.05). This value correspond with a pairwise sequence divergence of 7% per Ma, about three times faster than the 2.15% standard mtDNA substitution rate obtained for arthropods (Brower, 1994), but slower than the substitution rate obtained for the eastern Canarian *Dysdera lancerotensis,* 0.051 per lineage/million years (pairwise divergence of 10%). Divergence time estimate for relevant nodes are sown in Fig. 6.2. The TMRCA of the *D. alegranzaensis* haplotypes was dated at ~0.716 Ma (0.42-1.04 Ma) and the TMRCA of the *D. nesiotes* haplotypes at ~0.535 Ma (0.3- 0.7 Ma).

Haplotype networks

Statistical parsimony and the median joining method resulted in the same *cox1* haplotype network for *D. nesiotes* (Fig. 6.3). Haplotypes from the same locality clustered together and were separated by few mutations, while many mutational steps separated haplotype clusters from different localities. The most represented haplotype (10MR) is the only one shared among localities (3, northern Lanzarote). Both coalescent theory (Crandall & Templeton, 1993; Posada & Crandall, 2001) and the phylogenetic tree (Fig. 6.2) identified 10MR as the most ancestral haplotype, suggesting that present day *D. nesiotes* haplotypes could have originated in northern Lanzarote. Statistical parsimony yielded four independent haplotype networks, separated by 18-23 mutational steps (Fig. 6.4), in *D. alegranzaensis*, which largely corresponded to the main haplotype lineages in Fig. 6.2. This network only differed from the median-joining network by the position of Montaña Clara and La Graciosa haplotypes, and the number of mutational steps and connection points of the four independent networks (see Fig. 6.4). The most represented haplotype (21CS.L) was shared between five localities distributed on southern and central Lanzarote. Other haplotypes were shared among localities within islets (e.g. 3MCA in Alegranza, 8MAG and 9MBG in La Graciosa). The remaining haplotypes were restricted to single localities. The statistical network 1 included two haplotypes from Lanzarote (Zonzamas) and Alegranza, network 2 included four haplotypes from Alegranza and one from La Graciosa, network 3 included four haplotypes from three localities on northern Lanzarote and network 4 included 18 haplotypes from Lanzarote and the islets (except Alegranza). The complexity of the networks and the uncertainty of their connections hampered assigning the source of colonization.

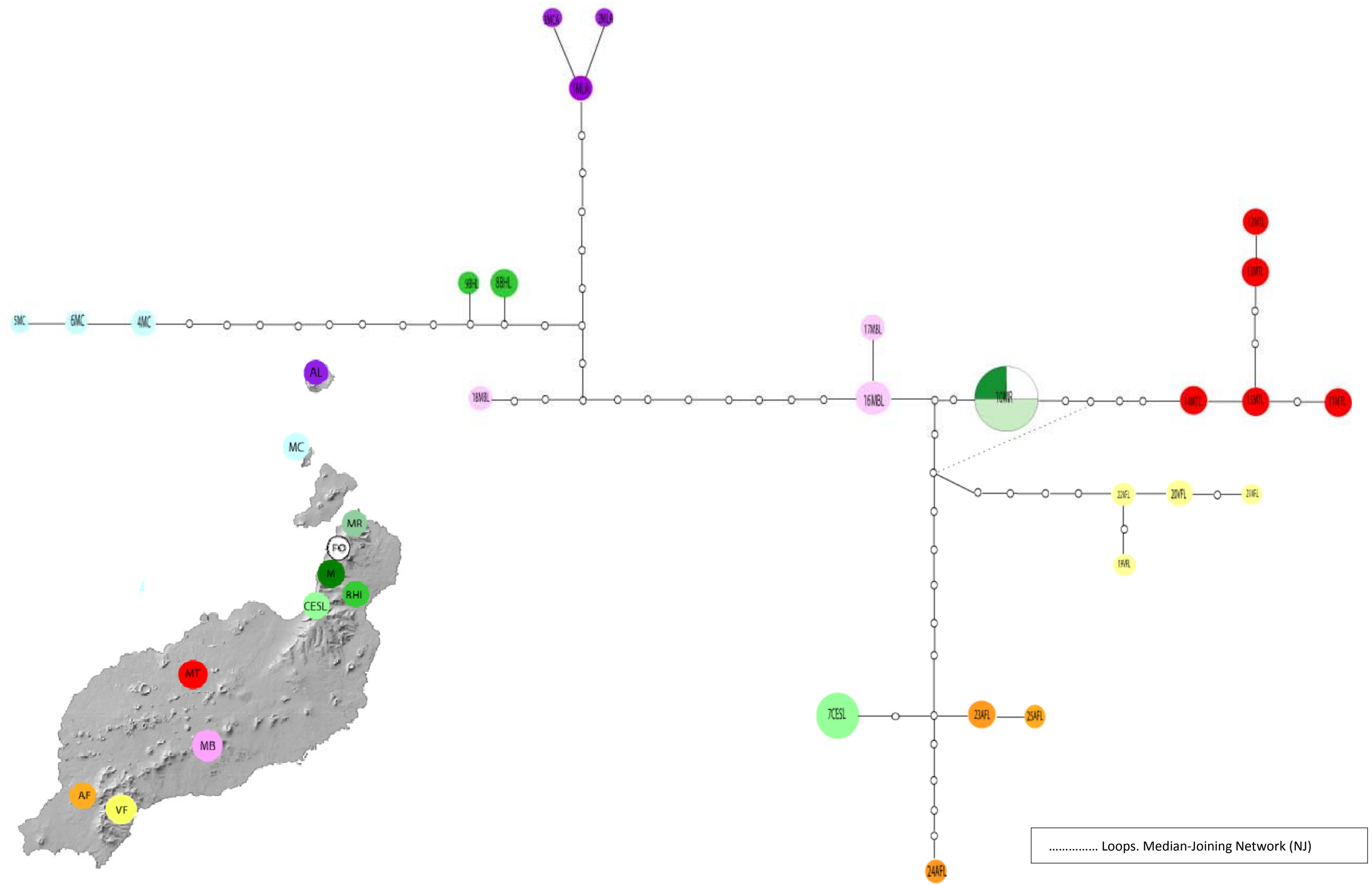

Fig. 6.3. Statistical parsimony and median-joining network of mtDNA haplotypes of *D. nesiotes.* Haplotype numbers as in Appendix 6.1 and small white circles - indicate missing or extinct haplotypes. The size of the circle is equivalent with their frequency.

Fig. 6.4. Statistical parsimony and median-joining network of mtDNA haplotypes of *D. alegranzaensis.* Haplotype numbers as in Appendix 6.1 and small white circles indicate missing or extinct haplotypes. The size of circle is roughly proportional to haplotype frequency.

Genetic diversity, population structure and demographic analyses

Dysdera nesiotes and *D. alegranzaensis* showed high levels of haplotype diversity (0.9524±0.0198 and 0.9296± 0.0261, respectively) and low levels of nucleotide diversity (0.012085±0.006151, 0.021184±0.010506), although the last values were almost twofold higher in *D. alegranzaensis* than *D. nesiotes*.F_{ST} values obtained for the pairwise comparison between localities suggested contrasting patterns of gene flow in both species. In *D. nesiotes*, pairwise F_{ST} values among all populations were greater than 0.7, and most of them were significant (P> 0.05, except M, MR and FO, see Table 6.1 A-B), indicating strong geographic structure in this species. In *D. alegranzaensis*, most F_{ST} were low and non-significant. Significant values of F_{ST} were concentrated in comparisons involving the central-south part of Lanzarote and the north of Lanzarote and the northern islets. F_{ST} values reveal lower and more localized population structure in *D. alegranzaensis* compared with *D. nesiotes*.

In all populations groups defined for *D. nesiotes*, the maximum genetic variance was explained by differences among populations within groups (52-73%), which corroborates the high levels of population structure in this species (Table 6. 2 A). A small percentage of genetic variance was allocated to intra-populations variation (7.35-9.5%). The major variance among groups (40.23%) was observed when populations were grouped between northern islets and Lanzarote (2 groups). For the remaining groups (4 and 5), variance was lower and not significant (P <0.05). In *D. alegranzaensis*, the variance explained among populations within groups varied 28-37% (Table 6.2 B), much lower that the values retrieved for *D. nesiotes* (52-73%), while within population genetic variance (25-31%) was much higher than in *D. nesiotes* (7.35-9.5%), regardless of the treatment, indicating lower population structure and higher population heterogeneity in this species. The highest genetic variance observed was attributed to differences among groups, when populations were divided between northern islets and Lanzarote (47%). A star-like network was identified in the network of *D. alegranzaensis* (centered around haplotype 21). Two neutrality tests (Tajima´s D and Fu´s F_s) reported significant values (P <0.01 and P <0.002, respectively, data not shown), while the R_2 test did not.

Table 6.2. AMOVA for mtDNA *cox1* of *D. alegranzaensis* (A) and *D. nesiotes* (B) based on pairwise nucleotide differences. Significant tests at *P*< 0.05 after 10000 permutations. F_{SC}, F_{ST} y F_{CT} are the *F*-statistics. (AL: Alegranza; MC: Montaña Clara; GR: La Garciosa; L: Lanzarote; LN: Lanzarote North; LC: Lanzarote centre; LS: Lanzarote South).

A. AMOVA_*D. nesiotes*

Source of variation	df	Sum of squares	Variance components	% of variation	Fixation indices
AMOVA 2 Groups (AL+MC / L)					
Among groups	1	131.39	6.49460 Va	40.23*	*Fct:* 0.40233
Among populations within groups	9	301.138	8.46162 Vb	52.42*	*Fsc*: 0.87703
Within populations	32	37.965	1.18642 Vc	7.35*	*Fst*: 0.92650
Total	42	470.497	16.14264		
AMOVA 4 Groups (AL+MC / LN / LC / LS)					
Among groups	3	191.104	2.13841 Va	17.21	*Fct*: 0.17213
Among populations within groups	7	241.427	9.09842 Vb	73.24*	*Fsc*: 0.88464
Within populations	32	37.965	1.18642 Vc	9.55*	*Fst*: 0.90450
Total	42	470.497	12.42325		
AMOVA 5 Groups (AL/ MC / LN / LC / LS)					
Among groups	4	243.704	2.69397 Va	21.62	*Fct*: 0.21620
Among populations within groups	6	188.828	8.58018 Vb	68.86*	*Fsc*: 0.87852
Within populations	32	37.965	1.18642 Vc	9.52*	*Fst*: 0.90479
Total	42	470.497	12.46056		

B. AMOVA_*D. alegranzaensis*

Source of variation	df	Sum of squares	Variance components	% of variation	Fixation indices
AMOVA 2 Groups (AL+MC+GR / L)					
Among groups	1	587.509	21.70167 Va	47*	*Fct*: 0.46997
Among populations within groups	16	774.244	12.93616 Vb	28.01*	*Fsc*: 0.52854
Within populations	35	403.872	11.53920 Vc	24.99*	*Fst*: 0.75011
Total	52	1765.624	46.17703		
AMOVA 4 Groups (AL+ MC+GR/ LN / LC / LS)					
Among groups	3	687.632	12.62614 Va	33.29*	*Fct*: 0.33290
Among populations within groups	14	674.12	13.76245 Vb	36.29*	*Fsc*: 0.54393
Within populations	35	403.872	11.53920 Vc	30.42*	*Fst*: 0.69576
Total	52	1765.624	37.92779		
AMOVA 6 Groups (AL/ MC /GR/ LN / LC / LS)					
Among groups	5	899.517	14.74483 Va	39.8*	*Fct*: 0.39801
Among populations within groups	12	462.236	10.76279 Vb	29.05*	*Fsc*: 0.48259
Within populations	35	403.872	11.53920 Vc	31.15*	*Fst*: 0.68852
Total	52	1765.624	37.04683		

DISCUSSION

Contrasting phylogeographic patterns in codistributed sibling species

Co-distributed species have most likely been subjected to similar geological and environmental changes and are thus expected to show similar phylogeographic patterns (Irwin, 2002; Riddle *et al.,* 2000; Garrick *et al.,* 2008). Differences in factors such as habitat preferences, dispersal ability or historical ranges, however, can result in contrasting patterns of genetic structure among co-occurring species (Arbogast, 2001; Bird, 2007; Hodges, 2007).

The two species examined in the present study co-occur in most of their known localities along the island of Lanzarote and the northern islets, show no clear differences in life-history traits and are almost identical in somatic morphology (Arnedo, 2000; Macías-Hernández, 2008). Consequently, we hypothesized that they would have undergone similar historical processes (e.g. population extinction/isolation due to volcanic activity or changes in climatic and environmental conditions) and hence they would show similar phylogeographic and demographic patterns. Contrary to the expectation, however, our results reveal striking differences in phylogeography and population structure between the two species. *Dysdera nesiotes* displays an almost metapopulation-like structure (Levins, 1969; Hanski, 1991). The F_{ST} pairwise comparisons indicated high levels of population differentiation. Similarly, AMOVA revealed that most of the genetic variance is explained by among population within group comparisons, while differences within populations are small (7-9%). The haplotype network shows high levels of phylopatry, most haplotype clades being exclusive of particular localities. *D. alegranzaensis,* on the other hand, displayed little population structure. The F_{ST} pairwise comparison values were low and many of them were not significant. AMOVA results showed, in general, high levels of genetic variance within population, and in two of the three groupings tested, most of the genetic variance corresponded to among group comparisons. The haplotype network of *D. alegranzaensis* indicated weak geographical structure of the haplotypes, and higher genetic divergences among main haplotype clades that those reported in *D. nesiotes* (4 independent networks versus 1 single network, respectively). These

results are reflected in the TMRCA estimates, which are older for *D. alegranzaensis* haplotypes (~0.71 Ma, 0.42-1.04 Ma) than for the *D. nesiotes* ones (~0.53 Ma, 0.3-0.7 Ma), although their confidence intervals partially overlapped. All in all, *D. alegranzaensis* show a more complex population dynamic, which would involve allopatric fragmentations (involving the northern islets), local population demographic expansions (as suggested by star-like haplotype topologies and some tests), along with high levels of gene flow among most populations.

Causes of incongruent phylogeographic and demographic patterns

Non-common phylogeographic and demographic patterns in sympatric species have been reported in a variety of organism. Such differences have been attributed to differences in dispersal abilities (Pizzo *et al.,* 2006; Steele *et al.,* 2009), habitat preferences (Bird *et al.,* 2007; Hodges *et al.,* 2007; Papadopoulou *et al.,* 2009), response to climatic changes (Taberlet *et al.,* 1998; Sullivan *et al.,* 2000; Michaux *et al.,* 2005), life history attributes (Lamb *et al.,* 1992; Zink *et al.,* 1996; Hewitt, 1999), niche segregation (Beavis *et al.,* 2006), prey specialization (Hoelzel *et al.,* 1998; Adams & Rohlf, 2000; Carmichael *et al.,* 2001; Dalén *et al.,* 2005; Pilot *et al.,* 2006), or ecological strategies (e.g. generalist *vs*. specialist, Vandergast *et al.,* 2004), among others. None of the former explanations seems to explain the differences observed in the two *Dysdera* species, since they hardly differ in any of these features.

Like other ground-dweller spiders, the family Dysderidae performs cursorial dispersal, a method of dispersal that could potentially restrict gene flow and generate population structure (Bond *et al*., 2001; Woodman *et al*., 2006). In spiders, larger bodies and leg lengths increase running performance (Foelix, 1996; Moya-Laraño, 2008) and hence body size differences may result in different cursorial capabilities. There are no direct observations or experiments conducted on the cursorial capabilities of *Dysdera*, although close similarities in body size and leg length between *D. alegranzaensis* and *D. nesiotes* (Macías-Hernández *et al.,* 2008) suggest that both species have similar roving performances.

Life history traits such as sex-biased phylopatry and specificity of reproductive timing may affect dispersal ability and ultimately the patterns of phylogeographic subdivision (Avise, 2004). For example, sex-biased dispersal with female philopatry had been proposed to explain strong phylogeographic structure in the absence of geographical barriers to dispersal in some reptilians (Thorpe & Richard, 2001; Gübitz *et al.,* 2005; Bloor *et al.,* 2008). Comparison of collections of both *Dysdera* species from the sampling period 1995 to 2006 (285 adult specimens; 36 male and 75 female *D. nesiotes* and 24 male and 57 female *D. alegranzaensis*, 143 juveniles) fail to find any relevant difference in the distribution of the number of males and females between the species throughout the year.

Differences in the time and source of colonization of the ancestral populations could also explain incongruent phylogeographic patterns. The two species differ with regards to the location of their respective sister-groups, while *D. alegranzaensis* sister species is also a Lanzarote endemic, *D. nesiotes* relatives are found in Fuerteventura. Biogeographic reconstruction suggested that the ancestor of each species originated in different islands (Macías-Hernández *et al.,* 2008): *D. alegranzaensis* was already a resident lineage in Lanzarote, while *D. nesiotes* ancestors colonized the island from Fuerteventura. The older TMRCA of the haplotypes and the presence of independent haplotype networks corroborate the longer residence of *D. alegranzaensis* in Lanzarote. The complex geological history of Lanzarote, which originated as two independent islands and endured several cycles of volcanic activity, could have facilitated survival of both species on different parts of the island, where they would have evolved in isolation until a connection was established between the islands and volcanic activity decreased. Although our data suggest that *D. nesiotes* haplotypes probably originated in northern Lanzarote and colonized the island southwards, they do not allow discriminating the ancestral source of *D. alegranzaensis* haplotype diversity. On the other hand, the timing of connection and the cycles of volcanic activity reported for Lanzarote (Carracedo & Rodríguez-Badiola, 1993) are much older than the estimated TMRCA and would not support the former scenario.

As stated above, the TMRCA of present-day haplotypes of both species are slightly different, *D. alegranzaensis* being older than *D. nesiotes* (~0.71 and ~0.53, respectively) although confidence intervals actually overlap. Differences in the TMRCA of both species, however, are probably the consequence not the explanation of their contrasting phylogeographic and demographic pattern, since the youngest age of *D. nesiotes* haplotype could be the results of historical smaller population sizes and migration rates (Kingman, 1982), as suggested by the high level of phylopatry of its populations and the close relatedness of local haploypes.

Sympatric coexistence of sibling species

Niche differentiation has long been the focus for explaining species coexistence (Gauze, 1934), and the co-occurrence of close relatives has been interpreted as being due to non-equilibrium situations. The development of the neutral theory, however, has stressed the fact that communities containing very similar species far from being the exception may be the rule.

We have not found compelling evidence of obvious niche differentiation in our model system. Although no explicitly designed, quantitative analyses have been conducted, co-occurrence of both species in most of the sampled localities and the similar distribution of adult and immature stages throughout the year suggest close ecological preferences in both species. The lack of morphological differentiation between *D. nesiotes* and *D. alegranzaensis* (Macías-Hernández *et al.,* 2008) is particularly striking, as *Dysdera* is remarkable among spider genera in showing a wide range of variation in body size and mouthpart size and shape (Deeleman-Reinhold & Deeleman, 1988; Arnedo, *et al.,* 2001), which has been related to prey specialization and prey capture strategies (Řezáč *et al.,* 2008). In fact, differences in body size and cheliceral modifications in co-occurring cave-dwelling *Dysdera* species in the Canaries have been argued in favour of the key role of prey segregation in shaping these underground communities (Arnedo *et al.,* 2007).

Although the most obvious explanation for the contrasting phylogeographic patterns and demographic processes revealed in both species is the existence of an overlooked microhabitat niche preference (Connell, 1978) or spatial environmental

variation of the species (Chesson, 1985; 2000; Muko & Iwasa, 2000), the striking differences in the population dynamics of both species, however, points towards a more complex scenario: the two species may coexist in a dynamic equilibrium. The meta-population-like pattern inferred for *D. nesiotes*, which shows very low levels of within-population divergence and among-population geographic structure, suggests recurrent extinction and recolonization of local populations. The extinction events could have been driven by environmental changes, but this would not explain the resilience of *D. alegranzaensis* in these localities, unless some kind of physiological or behavioural difference exists between the two species. Alternatively, *D. alegranzaensis* could have led *D. nesiotes* populations to extinction by competitive exclusion. Under unfavourable conditions (e.g. prey shortage due to climatic changes or lava flows restricting hunting grounds) *D. alegranzaensis* would outcompete *D. nesiotes*, either through direct (preying upon) or indirect (prey monopolization) means. Conversely, during favourable times unlimited resources would warrant species coexistence by avoiding competition. Admittedly, there is little evidence at hand to support this dynamic equilibrium hypothesis, other than the phylogeographic patterns and *D. alegranzaensis* showing a slightly wider distribution than *D. nesiotes* and being found in more localities. Conducting simple direct competition experiments under conditions of limited prey availability, however, could readily test this hypothesis.

Emerging phylogeographic patterns in the Eastern Canary Islands

Recurrent connectivity among islands, dramatic climatic changes and episodic volcanism have provided with ample opportunities for the generation of deep phylogeographic patterns in local species of the Eastern Canaries. The availability of several studies on different species allows investigation of the commonalities of those patterns. Another eastern Canarian endemic, *Dysdera lancerotensis*, has been the subject of a recent phylogeographic study, which is especially relevant in the present context. Unlike, *D. nesiotes* and *D. alegranzaensis*, *D. lancerotensis* closer relatives are not Canarian, but northern African, which suggests that the species colonized the archipelago independently. It also differs from the former species in that it has a wider distribution, which also includes Fuerteventura island. The TMRCA of the *cox1* haplotypes of *D. lancerotensis* are

much older than those found in the present study. *D. lancerotensis* has been considered as generalist species (Arnedo *et al.,* 2000) due to its overspread distribution in xerophilous, sea-level and anthropized habitats, which could explain the higher resilience of its populations. Population structure of *D. alegranzaensis* closely resembles that of *D. lancerotensis*. Both species included several independent mtDNA haplotype networks and showed high levels of within population differentiation.

The northern islets and Lanzarote are surrounded by shallow waters and they were probably joined during the glacial periods on several occasions (García-Talavera, 1997; 1999). Evidence for recent contact between *Purpuraria* grasshoppers populations from Lanzarote and Montaña Clara, has been attributed to land bridges connections due to Pleistocene sea level oscillations (López *et al.,* 2006). Despite the recurrent opportunities for gene flow, the three *Dysdera* species show high levels of genetic differentiation between the archipelago Chinijo (northern islets) and Lanzarote. Clusters of related haplotypes exclusive to each of the northern islets, in some cases forming independent networks, were found in all three species. The relationships among populations on each islet, however, differed among the three species. Alegranza populations are mostly isolated, although there is evidence of secondary gene flow from northern Lanzarote in *D. lancerotensis*. Alegranza was most likely the source of colonist of the extant populations of *D. lancerotensis* from La Graciosa, while populations of *D. alegranzaensis* seem to be an admixture from northern Lanzarote and Alegranza. Current populations of *D. lancerotensis* and *D. alegranzaensis* from Montaña Clara probably originated in La Graciosa, while *D. nesiotes* haplotypes are more closely related to northern Lanzarote (*D. nesiotes* was not found in La Graciosa). These results corroborate the evolutionary distinctiveness of the archipelago Chinijo populations, emphasizing its relevance as wildlife refuge, but also reflect the idiosyncratic nature of inter-island colonization.

The pervasive role of recurrent volcanic activity on the phylogeographic and demographic patterns of island taxa has been well documented (Carson *et al.,* 1990; Beheregaray *et al.,* 2003; Moya *et al.,* 2004; Vandergast *et al.,* 2004; Emerson

et al., 2005; Brown *et al*., 2006; Papadopoulou *et al.,* 2009). Population expansion during periods of volcanic activity quiescence following population extinction and fragmentation by lava flows have been identified in *D. lancerotensis* (Bidegaray-Batista *et al.,* 2007) and the endemic lizard *Gallotia atlantica* (Bloor *et al.,* 2008), although they overlap neither in time nor in space. A recent demographic expansion was also found in *D. alegranzaensis* (star-like network around haplotype 21). The fact that these expansions seems to coincide with neither time nor space, may indicate that these phenomena may be frequent but easily erased by additional processes as admixture of new migrant with surviving local populations, which would reduce difficult the possibility of detection to a handful of non related cases.

Of particular interest for the phlogeography of Lanzarote is the finding in *D. alegranzaensis* of an isolated, divergent haplotype in the Centre-East region of Lanzarote (Zonzamas). A similar pattern has been found in *D. lancerotensis* and also in the endemic lizard *Gallotia atlantica* (Bloor *et al.,* 2008), and has been interpreted as evidence for local populations that survived Lanzarote's recent and subhistoric phase of volcanism (Carracedo & Rodríguez-Badiola, 1993) in isolated small refugia. This multiple line of evidence confirms the relevance of the area around Zonzamas as a volcanic refuge and warrants further studies to closely delimit its area and assess their relevance across taxonomically and ecologically unrelated taxa.

Conclusions

Our study confirms that sibling, codistributed species with similar life history traits (habitat requirements, dispersal abilities, ecology, etc), may exhibit contrasting phylogeographic patterns. We hypothesize that such differences are indicative of a cryptic, dynamic competition in a fluctuating environment. Further experimental studies under controlled conditions will have to be conducted to confirm the plausibility of this hypothesis. In addition, our results corroborate former suggestions that in spite of the ample opportunities for gene flow through land bridges induced by sea-level changes, the northern islets, and Alegranza in particular, have remained mostly isolated and constitute an important reservoir of

haplotype diversity. Similarly, localities on the eastern-central Lanzarote (Zonzamas) seem to have acted as a refugium during volcanic eruptions. In this regard, we also recognize instances of population expansion, probably related to population extirpation by lava flows, as shown for other Lanzarote taxa. Further studies on endemic taxa with very limited vagility may offer more detailed insights on the role of lava flows on demography and population structure and the limits and the location of additional volcanic refugia.

APPENDIX

Appendix 6.1.- Summary of specimens and sequences included in the study with details of the collection localities, UTM coordinates and sample sizes. GeneBank accession numbers of the different genes sequenced. (*) Genes amplified for each specimen.

Island	Locality	Code	Coordinates	N	*cox1* Haplotypes	GeneBank accesion number			
Dysdera alegranzaensis						***cox*1**	***16S-L1-nad1***	***28S***	***H3***
Alegranza	Montaña de Lobos	ML	N 29.39392 W -13.50194	3	1A (1)	*	*	*	*
					2A (1)	*			
					3A (1)	*			
	Meseta de Concheta	MCA	N 29.39959 W -13.52657	4	3A (3)	*			
					4A (1)	*			
	El Faro	FA	N 29.40335 W -13.48870	1	5A (1)	*			
Montaña Clara	Montaña Clara	MC	N 29.29886 W -13.53530	5	6A (4)	*	*		*
					7A (1)	*			
La Graciosa	Montaña de Las Agujas	MA	N 29.26606 W -13.49623	1	8A (1)	*	*	*	*
	Montaña Bermeja	MB	N 29.27820 W -13.50739	2	8A (1)	*			
					9A (1)	*			
	Montaña del Mojón	MM	N 29.24206 W -13.51629	3	9A (1)	*			
					10A (1)	*			
					11A (1)	*			
Lanzarote	Barranco Hondo del Valle	BH	N 29.14080 W -13.48320	3	12A (2)	*			
					13A (1)	*			
	Máguez. Haría	M	N 29.15441 W -13.51944	2	14A (1)	*			
					15A (1)	*			
	Presa de Mala	PM	N 29.10569 W -13.47183	1	16A (1)	*			
	Mirador del Río	MR	N 29.21120 W -13.48415	5	14A (2)	*			

Island	Locality	Code	Coordinates	N	*cox1* Haplotypes	GeneBank accesion number			
					17A (1)	*			
					18A (1)	*	*	*	*
					19A (1)	*			
	Famara	F	N 29.18436 W -13.50131	1	20A (1)	*			
	Barranco Teguereste. Guatiza	BT	N 29.05328 W -13.49332	2	21A (2)	*	*	*	*
	Zonzamas	Z	N 29.01283 W -13.56995	1	22A (1)	*			
	Montaña Tinache. Tinajo	MT	N 29.05185 W -13.66985	5	21A (1)	*			
					23A (1)	*			
					24A (1)	*			
					25A (1)	*	*	*	*
					26A (1)	*			
	Montaña Blanca. Tías	MB	N 28.98440 W -13.63393	5	21A (4)	*			
					27A (1)	*			
	Valle Fenauco. Yaiza	VF	N 28.93287 W -13.77366	5	21A (2)	*			
					28A (2)	*			
					29A (1)	*			
	Atalaya de Femés. Los Ajaches	AF	N 28.91952 W -13.76370	4	21A (4)	*			
			Total	**53**					
Dysdera nesiotes									
Alegranza	Montaña de Lobos	ML	N 29.39392 W -13.50194	3	1N (2)	*	*	*	*
					2N (1)	*			
	Meseta de Concheta	MCA	N 29.39959 W -13.52657	1	3N (1)	*			
Montaña Clara	Montaña Clara	MC	N 29.29886 W -13.53530	5	4N (2)	*	*	*	*
					5N (1)	*			
					6N (2)	*			
Lanzarote	Cab. Barranco Elvira Sánchez. Haría	CES	N 29.13072 W -13.51690	4	7N (4)	*	*	*	*

Island	Locality	Code	Coordinates	N	*cox1* Haplotypes	GeneBank accesion number			
	Barranco Hondo del Valle	BH	N 29.14080 W -13.48320	3	8N (2)	*			
					9N (1)	*			
	Máguez. Haría	M	N 29.15441 W -13.51944	2	10N (2)	*			
	Mirador del Río	MR	N 29.21120 W -13.48415	4	10N (4)	*			
	Fuente Ovejas	FO	N 29.18436 W -13.50131	2	10N (2)	*	*	*	*
	Montaña Tinache. Tinajo	MT	N 29.05185 W -13.66985	5	11N (1)	*			
					12N (1)	*			
					13N (1)	*	*	*	*
					14N (1)	*			
					15N (1)	*			
	Montaña Blanca. Tías	MB	N 28.98440 W -13.63393	5	16N (3)	*			
					17N (1)	*			
					18N (1)	*			
	Valle Fenauco. Yaiza	VF	N 28.93287 W -13.77366	5	19N (1)	*	*	*	*
					20N (2)	*			
					21N (1)	*			
					22N (1)	*			
	Atalaya de Femés. Los Ajaches	AF	N 28.91952 W -13.76370	4	23N (2)	*			
					24N (1)	*			
					25N (1)	*			
			Total	**43**					
Dysdera longa	Canary I. Fuerteventura			1	N91	EF458134	EF458090	EU139781	EU139710
***Dysdera mahan* sp.n**	Canary I. Alegranza			1	N57	EU139620	EU139647	EU139769	EU139700
***Dysdera mahan* sp.n**	Canary I. Lanzarote			1	N59	EU139622	EU139649	EU139771	EU139701
***Dysdera mahan* sp.n**	Canary I. Lanzarote			1	N65	EU139623	EU139650	EU139772	EU139702
Dysdera sanborodon	Canary I. Fuerteventura			1	N85	EF458135	EF458089	EU139775	EU139705
Dysdera sanborodon	Canary I. Fuerteventura			1	N86	EU139626	EU139653	EU139776	EU139706
Dysdera simbeque	Canary I. Lanzarote			1	N37	EU139614	EU139659	EU139783	EU139712

Island	Locality	Code	Coordinates	N	*cox1* Haplotypes	GeneBank accesion number			
Dysdera simbeque	Canary I. Lanzarote			1	N39	EU139631	EU139660	EU139784	EU139713
Dysdera spinidorsum	Canary I. Fuerteventura			1	N87	EU139627	EU139654	EU139777	EU139707
Dysdera spinidorsum	Canary I. Fuerteventura			1	N88	EU139628	EU139655	EU139778	EU139708
Dysdera aneris	Salvages I. Selvagem Grande			1	k510				
Dysdera lancerotensis	Canary I. Fuerteventura			1	N94	EF458120	EF458086	EU139758	EU139687
Dysdera lancerotensis	Canary I. Alegranza			1	LB2	EF458127	EF458080	EU139757	EU139686
Dysdera calderensis	Canary I. La Palma			1	dcaPk103	AF244309	AF244218/EU139665	EU139788	EU139718
Dysdera calderensis	Canary I. La Gomera			1	dca358G	*	*	*	*
Dysdera gomerensis	Canary I. La Gomera			1	dgoGl132	*	*	*	*
Dysdera gomerensis	Canary I. El Hierro			1	dgoHl133	*	*	*	*
Dysdera silvatica	Canary I. La Gomera			1	dsiGk94	AF244273	AF244177/EU139674	EU139808	EU139739
Dysdera silvatica	Canary I. La Palma			1	dsi347P	*	*	*	*
Dysdera silvatica	Canary I. El Hierro			1	dsi362H	*	*	*	NO
Dysdera scabricula	Iberian Peninsula: Valencia			1	dscak294	EU068046	EU068078	EU139809	EU139740
Dysdera erythrina	Iberian Peninsula: Barcelona			1	deryk105	AF244252	AF244162*	EU139790	EU139720
Dysdera* cf. *inermis	Morocco: Tanger			1	diM1k226	EF458142	EF458092	EU139795	EU139726
Dysdera inermis	Iberian Peninsula: Cádiz			1	dil3k228	EF458141	EF458091	*	*

REFERENCES

Adams DC, Rohlf FJ. 2000. Ecological character displacement in *Plethodon*: Biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences* 97: 4106-4111.

Akaike H. 1973. *Information theory as an extension of the maximum likelihood principle*. Budapest: Akademiai Kiado.

Arbogast BS, Kenagy GJ. 2001. Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography* 28: 819-825.

Arnedo, Pedro Oromí, Cesc Múrria, Nuria Macías-Hernández, Ribera C. 2007. The dark side of an island radiation: systematics and evolution of troglobitic spiders of the genus *Dysdera* Latreille (Araneae: Dysderidae) in the Canary Islands. *Invertebrate Systematics* 21: 623-660.

Arnedo MA, Oromí P, Ribera C. 2000. Systematics of the genus *Dysdera* (Araneae, Dysderidae) in the Eastern Canaries. *Journal of Arachnology* 28: 261-292.

Arnedo MA, Oromí P, Ribera C. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. *Cladistics* 17: 313-353.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Harvard University Press: Cambridge, Mass.

Avise JC. 2004. *Molecular Markers, Natural History and Evolution*. 2nd ed. Sinauer, Sunderland, MA.

Bandelt, Peter Forster, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37-48.

Beavis AS, Rowell DM. 2006. *Phylogeography of two australian species of funnel web spider (Araneae: Mygalomorphae: Hexathelidae) in Tallaganda state forest, New South Wales*. In Grove SJH, James L., eds., ed. Insect biodiversity and dead wood: proceedings of a symposium for the 22nd International Congress of Entomology. Gen. Tech. Rep. SRS-93. Asheville, NC: U.S. Department of Agriculture Forest Service, Southern Research Station, 109.

Beheregaray LB, Ciofi C, Geist D, Gibbs JP, Caccone A, Powell JR. 2003. Genes record a prehistoric volcano eruption in the Galapagos. *Science* 302: 75.

Bermingham E, Moritz C. 1998. Comparative phylogeography: concepts and applications. *Molecular Ecology* 7: 367-369.

Bidegaray-Batista L, Macías-Hernández N, Oromí P, Arnedo MA. 2007. Living on the edge: demographic and phylogeographical patterns in the woodlouse-hunter spider *Dysdera lancerotensis* Simon, 1907 on the eastern volcanic ridge of the Canary Islands. *Molecular Ecology* 16: 3198-3214.

Bird, Holland BS, Bowen BW, Toonen RJ. 2007. Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. *Molecular Ecology* 16: 3173–3186.

Bloor P, Kemp SJ, Brown RP. 2008. Recent volcanism and mitochondrial DNA structuring in the lizard *Gallotia*

atlantica from the island of Lanzarote. *Molecular Ecology* 17: 854–866.

Bond JE, Hedin MC, Ramirez MG, Opell BD. 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. *Molecular Ecology* 10: 899-910.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences* 91: 6491-6495.

Brown RP, Hoskisson PA, Welton JH, Baez M. 2006. Geological history and within-island diversity: a debris avalanche and the Tenerife lizard *Gallotia galloti*. *Molecular Ecology* 15: 3631-3640.

Carmichael LE, Nagy JA, Larter NC, Strobeck C. 2001. Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Molecular Ecology* 10: 2787-2798.

Carracedo JC, Badiola ER, Soler V. 1992. The 1730-1736 eruption of Lanzarote, Canary Islands: a long, highmagnitude basaltic fissure eruption. *Journal of Volcanology and Geothermal Research* 53: 239-250.

Carracedo JC, Day S. 2002. *Canary Islands*. Terra: Harpenden.

Carracedo JC, Rodríguez-Badiola E. 1993. Evolución geológica y magmática de la isla de Lanzarote (Islas Canarias). *Revista de la Academia Canaria de la Ciencia* 4: 25–58.

Carracedo JC, Singer B, Jicha B, Guillou H, Rodríguez Badiola E, Meco J, Pérez Torrado FJ, Gimeno D, Socorro S, Láinez A. 2003. La erupción y el tubo volcánico del Volcán Corona (Lanzarote, Islas Canarias). *Estudios Geológicos* 59: 277-302.

Carson HL, Lockwood JP, Craddock EM. 1990. Extinction and recolonization of local-populations on a growing shield-volcano. *Proceedings of the National Academy of Sciences* 87: 7055-7057.

Carstens, Corinne LR. 2007. Integrating coalescent and ecological niche modelling in comparative phylogeography. *Evolution* 61: 1439–1454.

Cassens I, Mardulyn P, Milinkovitch MC. 2005. Evaluating intraspecific "Network" construction methods using simulated sequence data: Do existing algorithms outperform the global maximum parsimony approach? *Systematic Biology* 54: 363-372.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660.

Coello J, Cantagrel JM, Hernán F, Fuster JM, Ibarrola E, Ancochea E, Casquet C, Jamond C, Díaz de Terán JR, Cendrero A. 1992. Evolution of the eastern volcanic ridge of the Canary Islands based on new K-Ar data. *Journal of Volcanology and Geothermal Research* 53: 251-274.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* 46: 419-437.

Connell JH. 1978. Diversity in tropical rain forests and coral reefs. *Science* 199: 1302-1310.

Cracraft J. 1989. *Speciation and its ontology: the empirical consequences of*

alternative species concepts for understanding patterns and processes of differentiation. In: Otte D and Endler JA, eds. *Speciation and its consequences*. Sunderland, Mass.: Sinauer Assoc. 28-57.

Crandall KA, Templeton AR. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134: 959.

Chesson P. 2000. General theory of competitive coexistence in spatially-varying environments. *Theoretical Population Biology* 58: 211-237.

Chesson PL. 1985. Coexistence of competitors in spatially and temporally varying environments - A look at the combined effects of different sorts of variability. *Theoretical Population Biology* 28: 263-287.

Dalén L, Fuglei E, Hersteinsson P, Kapel CMO, Roth JD, Samelius G, Tannerfeldt M, Angerbjorn A. 2005. Population history and genetic structure of a circumpolar species: the arctic fox. *Biological Journal of the Linnean Society* 84: 79-89.

de la Nuez Pestana J, Quesada Alvarez ML, Alonso Blanco JJ. 1998. *Los volcanes de los islotes al norte de Lanzarote, Islas Canarias*. Fundación César Manrique: Teguise, Lanzarote.

Deeleman-Reinhold CL, Deeleman PR. 1988. Revision des Dysderinae. *Tijdschr. Entomol* 131: 141-269.

Donoghue MJ, Moore BR. 2003. *Toward an integrative historical biogeography*. Annual Meeting of the Society for Integrative and Comparative Biology. Anaheim, California, 261-270.

Drummond A, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4.

Emerson BC, Forgie S, Goodacre S, Oromí P. 2005. Testing phylogeographic predictions on an active volcanic island: *Brachyderes rugatus* (Coleoptera: Curculionidae) on La Palma (Canary Islands). *Molecular Ecology* 15: 449-458.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. 3.0 ed: *Evolutionary Bioinformatics Online* 1:47-50.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes - Application to human mitochondrial-DNA restriction data. *Genetics* 131: 479-491.

Farris JS, Albert VA, Kallersjo M, Lipscomb D, Kluge AG. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99-124.

Foelix RF. 1996. *Biology of Spiders*. New York, Oxford University Press.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915-925.

García-Talavera F. 1997. Las Canarias orientales y la vecina costa africana en el Holoceno. *Eres* 7: 55–63.

García-Talavera F. 1999. *La Macaronesia. Consideraciones geológicas, biogeográficas y paleoecológicas*. In: Fernández-Palacios JM, Bacallado, J.J. & Belmonte, J.A., ed. *Ecología y cultura en Canarias: Museo d*e la Ciencia, Cabildo Insular de Tenerife, Santa Cruz de Tenerife, Canary Islands, Spain. 39–63.

Garrick R, Sunnucks P. 2006. Development and application of three-tiered nuclear genetic markers for basal Hexapods using single-stranded conformation polymorphism coupled with targeted DNA sequencing. *BMC Genetics* 7: 11.

Garrick RC, Rowell DM, Simmons CS, Hillis DM, Sunnucks P. 2008. Fine-scale phylogeographic congruence despite demographic incongruence in two low-mobility saproxylic springtails. *Evolution* 62: 1103-1118.

Gauze GF. 1934. *The struggle for existence*. The Williams & Wilkins company: Baltimore.

Giribet G, Carranza S, Riutort M, Baguna J, Ribera C. 1999. Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences. *Philosophical Transactions of the Royal Society of London B Biological Sciences* 354: 215-222.

Goloboff PA, Farris JS, Nixon KC. 2003. TNT: Tree Analysis Using New Technologies. 0.6 (Beta) ed.

Gübitz T, Thorpe RS, Malhotra A. 2005. The dynamics of genetic and morphological variation on volcanic islands. *Proceedings Royal Society of London B* 272: 751-758.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696-704.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Symposium on RNA biology; RNA, tool and target. Research Triangle Park, NC: Oxford University Press, 95-98.

Hanski I. 1991. Single species metapopulation dynamics: concepts models and observations. *Biological Journal of the Linnean Society* 42: 17-38.

Hedin MC. 1997. Speciational history in a Diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): Inferences from geographic-based sampling. *Evolution* 51: 1929-1945.

Hedin MC, Maddison WP. 2001. A Combined Molecular Approach to Phylogeny of the Jumping Spider Subfamily Dendryphantinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution* 18: 386-403.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biological Journal- Linnean Society* 68: 87-112.

Hodges KM, Rowell DM, Keogh JS. 2007. Remarkably different phylogeographic structure in two closely related lizard species in a zone of sympatry in south-eastern Australia. *Journal of Zoology* 272: 64-72.

Hoelzel AR, Dahlheim M, Stern SJ. 1998. Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity* 89: 121-128.

Irwin DE. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution: International Journal of Organic Evolution* 56: 2383-2394.

Juan C, Ibrahim KM, Oromí P, Hewitt GM. 1998. The phylogeography of the darkling beetle, *Hegeter politus*, in the eastern Canary Islands. *Proceedings Of The Royal Society Of London Series B Biological Sciences* 265: 135-140.

Katoh K, Kuma K-i, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*33: 511-518.

Katoh K, Misawa K, Kuma K-i, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059-3066.

Kingman JFC. 1982. The coalescent. *Stochastic Processes and their Applications* 13: 235–248.

Lamb T, Thomas RJ, Avise JC. 1992. Phylogeographic histories of representative herpetofauna of the southwestern U.S.: mitochondrial DNA variation in the desert iguana (*Dipsosaurus dorsalis*) and the chuckwalla (Sauromalus obesus). *Journal of Evolutionary Biology* 5: 465-480.

Levins R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of Entological Society of America* 15: 237-240.

López H, Contreras-Diaz HG, Oromi P, Juan C. 2006. Delimiting species boundaries for endangered Canary Island grasshoppers based on DNA sequence data. *Conservation Genetics* 8: 587-598.

Macías-Hernández N, Oromí P, Arnedo MA. 2008. Patterns of diversification on old volcanic islands as revealed by the woodlouse-hunter spider genus *Dysdera* (Araneae, Dysderidae) in the eastern Canary Islands. *Biological Journal of the Linnean Society* 94: 589-615.

Michaux JR, Libois R, Filippucci MG. 2004. So close and so different: comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. *Heredity* 94: 52-63.

Moritz C, Faith DP. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology* 7: 419-430.

Moya-Laraño J, Vinkovic´ D, De Mas E, Corcobado G, Moreno E. 2008. Morphological evolution of spiders predicted by pendulum mechanics. *PLoS ONE* 3.

Moya O, Contreras-Diaz HG, Oromí P, Juan C. 2004. Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). *Molecular Ecology* 13: 3153-3167.

Muko S, Iwasa Y. 2000. Species Coexistence by permanent spatial heterogeneity in a lottery model. *Theoretical Population Biology* 57: 273-284.

Nixon KC. 2002. WinClada. ver. 1.00.08 ed. Ithaca, New York: Published by the Author.

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. 2004. Bayesian phylogenetic analysis of combined data. *Systematic Biology* 53: 47-67.

Papadopoulou A, Anastasiou I, Keskin B, Vogler AP. 2009. Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: the effect of dispersal ability and habitat preference. *Molecular Ecology* 18: 2503-2517.

Paulo O, Jordan W, Bruford M, Nichols R. 2002. Using nested clade analysis to assess the history of colonization and the persistence of populations of an Iberian Lizard. *Molecular Ecology* 11: 809-819.

Pilot M, Jedrzejewski W, Branicki W, Sidorovich VE, Jedrzejewska B, Stachura K, Funk SM. 2006. Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology* 15: 4533-4553.

Pizzo A, Roggero A, Palestrini C, Cervella P, Del Pero M, Rolando A. 2006. Genetic and morphological differentiation patterns between sister species: the case of *Onthophagus taurus* and *Onthophagus illyricus* (Coleoptera, Scarabaeidae). *Biological Journal of the Linnean Society* 89: 197-211.

Posada D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*25: 1253-1256.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike Information Criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793-808.

Posada D, Crandall KA. 2001. Selecting the best-fit model of nucleotide substitution. *Systematic Biology* 50: 580-601.

Rambaut A, Drummond A. 2005. Tracer, A program for analysing results from Bayesian MCMC programs such as BEAST & MrBayes. 1.3 ed. Oxford: online at 663 http://evolve.zoo.ox.ac.uk /software.html?id=tracer (last accessed 12 February 2006). 664.

Ramos-Onsins SE, Rozas J. 2002. Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* 19: 2092-2100.

Rezac M, Pekar S, Lubin Y. 2008. How oniscophagous spiders overcome woodlouse armour. *Journal of Zoology* 275: 64-71.

Riddle BR, Hafner DJ, Alexander LF. 2000a. Comparative phylogeography of baileys' pocket mouse (*Chaetodipus bailey*) and the *Peromyscus eremicus* species group: Historical vicariance of the Baja California Peninsular desert. *Molecular Phylogenetics and Evolution* 17: 161-172.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496-2497.

Schneider CJ, Cunningham M, Moritz C. 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. *Molecular Ecology* 7: 487-498.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

Simmons MP, Ochoterena H, Carr TG. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. *Systematic Biology* 50: 454-462.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution,

weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Slatkin M, Hudson, R. R. 1991. Pairwise comparisons of mitochondrial DNA Sequences in stable and exponentially growing populations. *Genetics* 129: 555-562.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

Steele CA, Baumsteiger J, Storfer A. 2009. Influence of life-history variation on the genetic structure of two sympatric salamander taxa. *Molecular Ecology* 18: 1629-1639.

Suchard MA, Weiss RE, Sinsheimer JS. 2001. Bayesian selection of continuous-time markov chain evolutionary models. *Molecular Biology and Evolution*18: 1001-1013.

Sullivan J, Arellano E, Rogers DS. 2000. Comparative phylogeography of mesoamerican highland rodents: Concerted versus independent response to past climatic fluctuations. *American Naturalist* 155: 755-768.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453-464.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585-595.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619.

Thorpe RS, Richard M. 2001. Evidence that ultraviolet markings are associated with patterns of molecular gene flow. *Proceedings of the National Academy of Sciences* 98: 3929-3934.

Vandergast AG, Gillespie RG, Roderick GK. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology* 13: 1729-1743.

Wilm A, Mainz I, Steger G. 2006. An enhanced RNA alignment benchmark for sequence alignment programs. *Algorithms for Molecular Biology* 1: 19.

Woodman JD, Ash JE, Rowell DM. 2006. Population structure in a saproxylic funnelweb spider (Hexathelidae: *Hadronyche*) along a forested rainfall gradient. *Journal of Zoology* 268: 325-333.

Wright S. 1951. The genetical structure of populations. *Annals of Eugenics* 15: 323-354.

Young ND, Healy J. 2002. GapCoder: http://www.trinity.edu/nyoung/GapCoder/Download.html.

Zink RM. 1996. Comparative phylogeography in North American birds. *Evolution* 50: 308-317.

Capítulo 7

THE ROLE OF GEOLOGY AND HABITAT DIVERSITY IN SHAPING WITHIN-ISLAND DIVERSIFICATION OF ARTHROPODS: THE CASE OF THE SPIDER *Dysdera verneaui* IN TENERIFE, CANARY ISLANDS

ABSTRACT

Geological processes and ecological adaptation are major drivers of diversification in oceanic islands. The spider *Dysdera verneaui* is endemic to the island of Tenerife, where it is widely distributed along an altitudinal range of about 3000 m, including most local habitats, providing an excellent model to investigate the role of geological and ecological factors in shaping local diversity. Our results suggest that the phylogeographic patterns of this species are mostly the result of historical factors, such as the existence of independent precursor islands that gave rise to present day Tenerife and the dramatic volcanic activity endured by the central part of Tenerife during the last 3 My. Molecular markers identify a highly distinct evolutionary lineage circumscribed to the Teno region, which according to its mitochondrial divergence and nuclear sorting, as well as the possession of diagnostic genitalic characters, may deserve independent species status. A second lineage includes the population of the remaining part of the island. There is some evidence to support the hypothesis that the lineage may have also been originated in former populations on the precursor islands, which exchange migrants following interconnection. Alternatively, populations on present day Anaga massif may have acted as source for the colonization of the central part of the islands. Episodic introgression events between the two main evolutionary lineages of *D. verneaui* may have contributed through homogenization of their *ITS-2* sequence types to the observed incongruence between mitochondrial and nuclear markers. *D. verneaui* shows a positive correlation between body size and elevation, that according to our results is not due to historical factors, but to adaptation to local environmental conditions.

INTRODUCTION

Oceanic archipelagos have served as natural model systems for studying patterns and processes of diversification (Gillespie, 2004; Emerson & Kolm, 2005; Emerson & Oromí, 2005; Losos & Ricklefs, 2009 and references therein). Although island hopping accounts for a large part of speciation events on oceanic islands, within-island speciation has also played a relevant role in the generation of oceanic island biotas (Juan *et al.,* 2000; Gillespie & Roderick, 2002). Factors promoting within-island diversification include vicariant geological events and ecological shifts (Roderick & Gillespie, 1998). Lava flows, for instance, seem to have favoured speciation (Carson *et al.,* 1990; Pestano & Brown, 1999; Holland & Cowie, 2007), severing gene flow through population fragmentation and promoting recurrent colonization/extinction processes of local populations (e.g. Hawaiian *Drosophila* Kaneshiro & Boake, 1987; *Cephalops* Pipunculids DeMeyer, 1996; Canarian *Chalcides* skinks Pestano & Brown, 1999). Several studies have revealed a strong geographically signal in the distribution of divergent lineages (Carson *et al.,* 1990; Beheregaray *et al.,* 2003; Vandergast *et al.,* 2004; Gübitz *et al.,* 2005), pointing towards the involvement of vicariant processes in generating those patterns. In some case, however, within-island diversification is better explained by ecological shifts, as suggested for Canarian *Calathus* (Emerson *et al.,* 1999) and *Pimelia* beetles (Juan *et al.,* 1996), Hawaiian *Tetragnatha* spiders (Gillespie *et al.,* 1997) and *Megalagrion* damselflies (Polhemus, 1997), Caribbean lizards (Losos, 1992; Losos, *et al.,* 1994), Galápagos *Galapaganus* weevils (Sequeira *et al.,* 2008) or island cave fauna (Howarth, 1981; Howarth, 1993; Arnedo, 2007).

Island size, isolation and geological age are major drivers of island diversity (Willis, 1922; MacArthur & Wilson, 1967). Climatic variables and niche availability (Parent & Crespi, 2006; Losos & Parent, 2009) have also been identified as factors promoting diversification on islands. At the small scales like those of oceanic archipelagos, however, both variables are strongly correlated with elevation, which has been frequently use as surrogate for both climatic and habitat diversity. Area and elevation together were also found to be the best predictors of species richness of Hawaiian insects (Peck *et al.,* 1999). In general, large islands with complex topography and higher elevation favour within-island speciation thanks to

their broad habitat diversity and ecological niche availability (Whittaker & Palacios, 2007). In oceanic archipelagos, elevation, area and, ultimately, species richness frequently shows a hump-shaped relationship with island age due to the loss of area and topographical complexity of the older islands, as subsidence and erosion take their toll (Price & Clague, 2002; Whittaker *et al*., 2008, 2010). The general dynamic model of oceanic island biogeography (GDM) has been recently proposed to take into account this time related pattern (Whittaker *et al.,* 2008, 2010). The GDM model provides the best explanation for the distribution of species richness in endemic spiders from the Canary Islands (Cardoso *et al.,* 2010), and similar patterns of higher species richness in island of intermediate age have also been reported in *Laparocerus, Attalus, Hegeter* and *Tarphius* beetles or *Dolichoiulus* millipedes, among others (see examples in Enghoff & Báez, 1993; Izquierdo *et al.,* 2004).

The island of Tenerife lies at the centre of the Canary Islands, a chain of volcanic islands 110 km off the northwest coast of Africa, spanning 475 km roughly east to west, and about 20 My of subaerial volcanic activity. Tenerife has a complicated and dynamic geological history. It originated in the late Miocene as three independent island volcanoes, roughly corresponding to present-day Anaga (NE), Teno (NW) and Roque del Conde (SW) massifs, dated around 4.9-3.9 Ma, 6.2-5.1 Ma and 11.9-8.9 Ma, respectively (Guillou *et al.,* 2004). The large, central Cañadas volcanic edifice, which joined together the former islands, started building approximately 3.5 Ma, and underwent three cycles of volcanic activity until 0.2 Ma (Abdel-Monem *et al.,* 1972; Ancochea *et al.,* 1999; Cantagrel *et al.,* 1999). The last active phase in the Cañadas matched a period when the volcanic activity was also intense in the 'Dorsal Edifice' situated in the easterly wing of Tenerife (Ancochea *et al.,* 1999). The eruption of the present day active Teide-Pico Viejo complex occurred approximately 0.15 Ma, and volcanism persisted through historical times (Ancochea *et al.,* 1999; Carracedo *et al.,* 2007). The present day geomorphology of Tenerife has been additionally shaped by six major debris avalanches that occurred on both slopes of the island during the last 1 My (Ancochea *et al.,* 1990; Watts & Masson, 1995, 2001; Cantagrel *et al.,* 1999) (see Fig. 7.1 for more details).

Tenerife is the third largest volcano in the world, and the largest and highest island of the Canarian archipelago. Tenerife is also the most habitat-rich island in the whole Macaronesia region, thanks to the joint effect of elevation and trade winds. As in most oceanic islands, Tenerife main ecological zones and habitats are defined along altitudinal and latitudinal clines. The northern slope is affected by the humid and cool NE trade winds between 400-1200 m a.s.l., and by the dry NW trade winds above 2000 m, causing a temperature inversion. There are dramatic habitat differences between the dryer southern slopes and the more humid and rainy northern slopes. Along the altitudinal gradient different bioclimatic and vegetation communities characterize both slopes of the island. Five main ecological zones can be recognized on the more humid northern slope: (1) from the seashore up to 250 m is characterized by xerophytic shrub communities; (2) from 250–600 m features the thermo-sclerophilous woodland; (3) from 600–1000 m is present the subtropical, humid laurel forest covered by the cloud belt; (4) from 1000–2000 m is characterized by an endemic *Pinus canariensis* forest; (5) from 2000 m to the top (3718 m) is characterized by a dry subalpine scrub. The laurel forest is missing on the southern slopes, where it is replaced pine forest. Tenerife size, elevation and habitat diversity provides ample opportunities for ecological differentiation of local organisms.

The spider genus *Dysdera* has undergone a major diversification process in the Canary Islands, where more than 50 endemic species have been reported (Arnedo *et al.,* 2001). Species richness in Canarian *Dysdera* is positively correlated to area, elevation, geological age and ecological complexity (Arnedo *et al.,* 1999 and 2000; Cardoso *et al.,* 2010). Most of the 25 species of *Dysdera* occurring in Tenerife have restricted geographical distributions, probably as a result of narrow ecological requirements, as shown by the nine cave dwelling species described in the island. Few species, however, seems to have more relaxed habitat preferences and are broadly distributed on the island. *Dysdera verneaui* is the most widespread *Dysdera* species on Tenerife, where it can be found in a wide range of habitats, including caves, along an altitudinal gradient spanning from the coastal areas (200 m a.s.l.), up to the highest elevations on the Teide volcano (>3100 m).

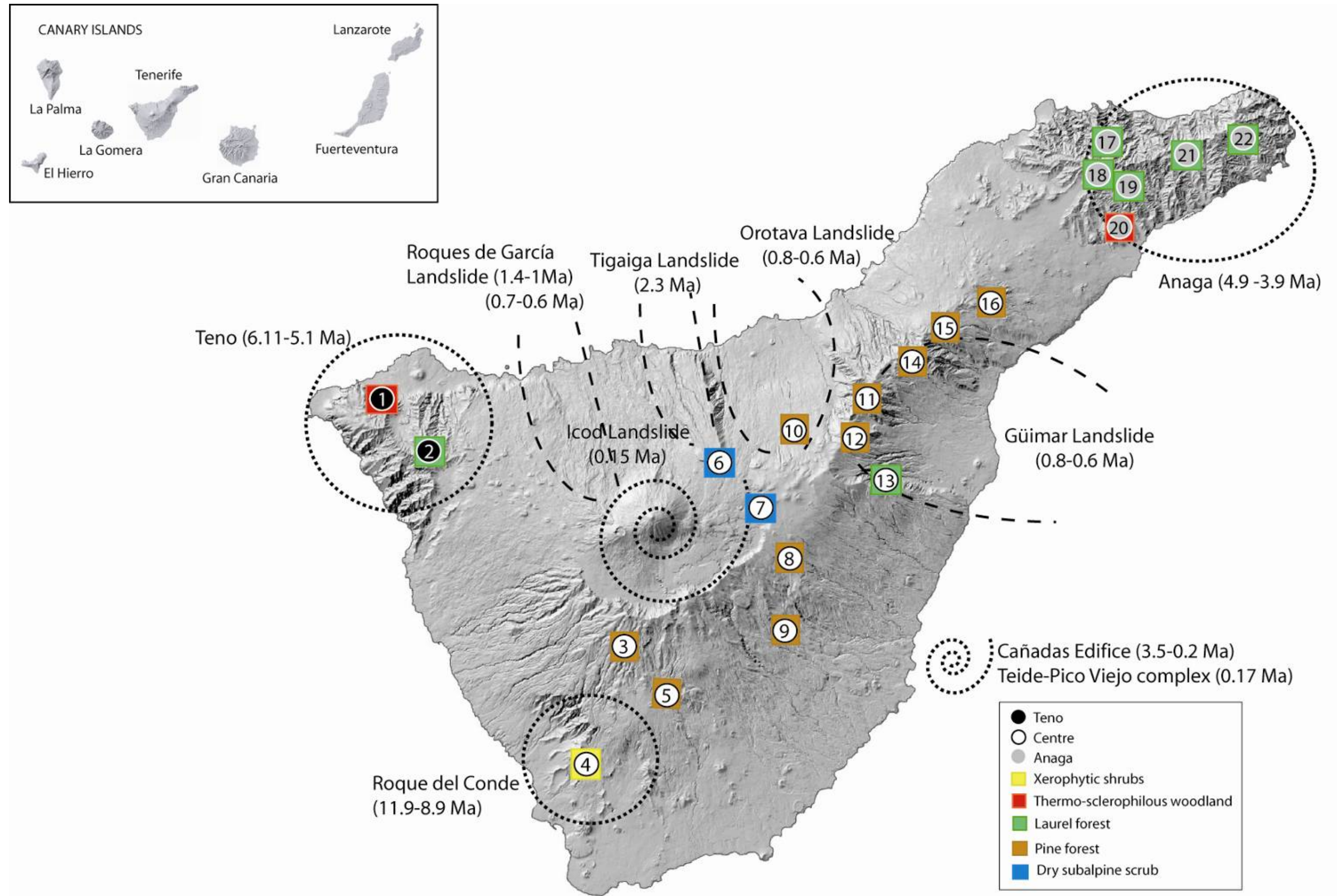

Fig. 7.1. Map of Tenerife, showing with numbers the localities where specimens of *D. verneaui* were collected. Colours of the circles refer to the three main ancient protoislands (black: Teno; white: central shield; grey: Anaga). Colours of localities refer to vegetation type (yellow: xerophytic shrubs; red: humid to semi-arid tropical shrubs and woods; green: laurel forest; brown: pine forest; blue: dry subalpine scrub). The main geologic events are indicated.

Dysdera verneaui is less frequent on the southern, dryer slopes. *Dysdera verneaui* is an excellent candidate to characterize phylogeographic patterns of terrestrial arthropods on Tenerife and to investigate the factors promoting within-island diversification. The widespread distribution along a habitat diversity gradient may affect the population genetic structure of the species. Selection of different types of habitats and adaptation to a particular niche may reduce gene flow between populations, as demonstrated for several island organisms (Losos & DeQueiroz*,* 1997; Ogden & Thorpe, 2002). Similarly, Tenerife's dynamic geological history may also have contributed to shaping phylogeographical structure and ultimately driving speciation.

The present study aims to characterize the role of geological processes and ecological adaptation in shaping phylogeographic patterns and genetic diversity of the endemic, widespread species *D. verneaui* in Tenerife. We investigated population structure of this species using a combination of mitochondrial (*cox1*) and nuclear genes (*ITS-2*). Firstly, we will test whether the recover phylogeographic and population structure of *D. verneaui* can be traced back to the precursor islands (Anaga, Teno and Roque del Conde). Secondly, we will investigate correlations between population structure, morphology and altitudinal gradient (as a surrogate of habitat diversity) to test the role of ecological adaptation on genetic and morphological differentiation. This study will help to decipher the factors promoting the generation of biodiversity in oceanic islands, and will provide important clues to a better understanding of the radiation undergone by *Dysdera* spider in the Canarian archipelago.

MATERIAL AND METHODS

Taxonomic sampling

Specimens of *Dysdera verneaui* were collected on Tenerife, sampling in different habitats on the north and south slopes of the island. *D. verneaui* was found in 22 out of 40 visited localities (see Appendix 7.1). The mainland species *D. inermis,* and the Canarian endemics *D. silvatica, D. calderensis* and *D. gomerensis*

were included in the analyses to provide calibration points for estimating absolute lineage ages (see below). Additionally, *D. gomerensis* is *D. verneaui* closest relative (see Arnedo *et al.,* 2001). The mainland species *Dysdera erythrina* was used as outgroup to root phylogenetic trees.

DNA extractions, PCR amplifications and sequencing

To date, most phylogeographic studies have inferred patterns of genetic distribution in species and populations relying only on mitochondrial markers, because of practical and methodological considerations. Several features of the mtDNA, however, may compromise its ability to reconstruct the complete evolutionary history of populations and species (Ballard *et al.,* 2004; Galtier *et al.,* 2009). The search for nuclear markers to infer intraspecific relationships in non-model organisms, like spiders, has been hampered by the lack of genomic information. As a first step towards the incorporation of nuclear data to infer *D. verneaui* phylogeography, we investigated the potential of few nuclear markers previously used on spiders, which included the internal transcribed spacer 2 (*ITS-2*) of the ribosomal cluster (Hedin, 1997; Zhang, *et al.,* 2004; Bond & Stockman, 2008); Elongation factor 1-alpha (*EF-1α*) (Maddison & Hedin, 2003), Elongation factor 1-gamma (*EF-1*γ) (Ayoub *et al.,* 2007), Actin 5C (Vink *et al.,* 2008), the signal recognition particle 54-kDa subunit intron (*SRP54*) (Jarman *et al.,* 2002, Bidegaray-Batista *et al.* pers. comm) and wingless (*wg*) (Brower & DeSalle, 1998). Only the *ITS-2* yielded reliable amplifications and was polymorphic enough to resolve intraspecific relationships in our study model.

Genomic DNA was extracted from specimens using the DNeasy Tissue Kit (Qiagen) following manufacturer's guidelines. Fragments of the mitochondrial cytochrome oxidase I (*cox1*) gene (1008 bp), the 16S rRNA (*16S*) and the complete tRNA leu UAG (*L1*) (571 bp), NADH dehydrogenase subunit I (*nad1*) (343 bp), and the nuclear genes internal transcribed spacer 2 (*ITS-2*) (455 bp) and 28S rRNA (*28S*) (803 bp) were amplified and sequenced following Macías-Hernández *et al.* (2008) (look herein for primers information). Amplifications were carried out in 25µl reaction volume for a final concentration of 1.25 U *Taq* polymerase (Promega), 2.5mM $MgCl_2$ (Promega), 0.2mM of each dNTP, 0.2µM of each primer and about

2µl of DNA sample, and the amount of *Taq* buffer recommend by the manufacturer. PCR conditions were as follows: 5 min. at 94ºC followed by 35 cycles of denaturalization at 94ºC for 30 s, annealing at 42-52ºC for 35-45 s (depending on the primers, see below), and extension at 72ºC for 30-60 s (depending on the length of the fragment), with a final single extension step at 72ºC for 5 min. For the *cox1* and *16S-nad1* gene fragments, a successful amplification was achieved with an annealing temperature of 42ºC or 45ºC for 45 s; while for the *28S* and *ITS-2* an annealing temperature of 58ºC and 48-52ºC, respectively, for 35 s yielded the best results.

Some *ITS-2* sequences showed evidence of multiple copies. Multiple alleles were separated by cloning the gel-purified PCR product with pGEM-T Easy Vector cloning kit (Promega). Three to eight colonies per individual were sequenced using bacterial colonies directly as template for PCR amplification with vector primers T7 and SP6, using the following PCR conditions: 94ºC for 5 min, 30 cycles of 94ºC for 1 min, 50ºC for 30 s, and 72ºC for 3 min, followed by a final extension of 72ºC for 5 min. PCR products were purified using MultiScreen PCRµ96 cleanup filter plates from Millipore. PCR products were cycle-sequenced in both directions using one of the PCR primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem) and sequenced in an ABI 3700 automated sequencer at the Scientific and Technical Services of the University of Barcelona (http://www.sct.ub.es). DNA sequences were assembled and edited using the STADEN software package (http://staden.sourceforge.net/) and managed using the computer program BIOEDIT (Hall, 1999).

Phylogenetic and phylogeographic analyses

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted to resolve phylogenetic relationships among evolutionary lineages (see Appendix 7.1). Several matrices were analyzed. The first one (M1) included 2725 bp of four genes (*cox1, 16S*, *nad*1 and *28S*) for 37 taxa, corresponding to a geographically diverse subsample of 27 *D. verneaui* specimens with unique mtDNA haplotypes, along with 10 specimens of six additional species (see above). Separated analyses of the mtDNA and nDNA

partitions of M1 were conducted to investigate incongruence between data sets. A second matrix (M2) was obtained by adding to M1, 31 unique *cox1* haplotypes of *D. verneaui* and 6 of *D. gomerensis*. Phylogeographic and populations analyses were conducted on the *cox1* and *ITS-2* data sets. The *cox1* matrix included 85 specimens of *D. verneaui* (57 haplotypes), while the *ITS-2* included 32 specimens of *D. verneaui* and 6 of *D. gomerensis* (15 sequence types).

Intraspecific relationships are better estimated by means of multifurcated networks (Posada & Crandall, 2001; Cassens *et al.*, 2005). Haplotype network of the *cox1* and *ITS-2* gene were estimated using the software TCS v. 1.21 (Clement *et al.,* 2000), which implements the statistical parsimony procedure (Templeton *et al.,* 1992; Crandall, 1994), which provides a 95% confidence limits that no homoplasic changes are included.

Unlike protein coding genes, the ribosomal gene sequences showed length polymorphism and were aligned with the online version of the MAFFT v.5.8 automatic alignment program (Katoh, 2002; 2005) (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/). This method outperforms other approaches for ribosomal alignments (Wilm, 2006). The alignment was constructed using the manual strategy option set to Q-INS-i, the most accurate multiple sequence alignment method available, with default options. Gaps were treated as single mutational events and were scored as presence/absence characters following Simmons & Ochoterena (2000). The program GapCoder (Young & Healy, 2002) was used to facilitate the automatic recoding of the alignments based on the simple method proposed by Simmons *et al.* (2001). Data matrices were concatenated with the help of the program WINCLADA v.1.00.08 (Nixon, 2002). Parsimony analyses under equal weights were performed with TNT v. 1.1 (Goloboff *et al.,* 2003) using a heuristic search based on 1000 replicates of random sequence addition, followed by tree-bisection–reconnection (TBR) branch swapping (five trees retained per iteration, and final round of TBR branch swapping on all retained trees). Clade support was assessed by means of jackknife resampling (Farris, 1996), using 1000 replicates with individual heuristic searches consisting on 15 iterations of Wagner tree construction using random addition of taxa, holding 5 trees per iteration and

an overall maximum of 10000. The program jMODELTEST v.0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) was used to select the substitution model of evolution that better fit the data with lesser parameters, as indicated by the Akaike information criterion (AIC) (Akaike, 1973). Bayesian inference analyses were conducted with MRBAYES v.3.1.2 (Ronquist, 2003) and run remotely at the Bioportal computer resources of the University of Oslo (http://www.bioportal.uio.no). Independent substitution models selected by jMODELTEST were specified for each gene fragment and a standard discrete model was implemented for the gaps scored as absence/presence data. The substitution estimates were allowed to vary independently between each partition Two independent runs with eight simultaneous MCMC (Markov Chain Monte Carlo) chains (one cold and seven heated), each starting with random starting trees, were performed for 10 million iterations. Decreasing temperature to 0.15 facilitated the convergence of the chains. Analyses were run for 4 million generations, discarding the first 10% as burn-in. The standard deviation of the split frequencies between runs (< 0.01) and the effective sample size (ESS), as measured by the program TRACER version 1.4 (Rambaut & Drummond, 2005), were used to ensure that the Markov chains had reached stationarity and the correct mixing of the parameters. Maximum likelihood analyses were conducted with the software RAxML v. 7.0.4 (Stamatakis, 2006). Independent GTR+G+I substitution models were set for each data fragment. The best likelihood tree was selected out of 10 iterations of random addition of taxa. Non-parametric bootstrap support values were drawn from 100 resampled matrices and confidence values mapped onto the best topology. Uncorrected genetic distances among lineages were calculated for the *cox1* mitochondrial and *ITS-2* nuclear gene fragments with the software MEGA v. 4.0 (Tamura *et al.,* 2007) using nucleotide p-distances.

Genetic diversity and population genetic structure

Standard diversity indices, including nucleotide diversity (π_n), number of haplotypes (*Nh*) and haplotypic diversity (*h*) for the complete *cox1* and *ITS-2* matrices were calculated with the software ARLEQUIN 3.01 (Excoffier *et al.,* 2005) (see Appendix 7.2). ARLEQUIN 3.01 was also used to estimate pairwise F_{ST} genetic distances (Wright, 1951) between populations for the *cox1* gene, based on pairwise

nucleotide differences, and their significance ($P<0.01$) assessed by performing 10000 permutations. Populations represented by single sequenced individuals were excluded from the analyses (localities 4 and 9).

The computer program SAMOVA 1.0 (Dupanloup *et al.,* 2002) was used to identify geographically homogeneous populations that maximize genetic variance between groups of populations. Analyses were performed for *k* values ranging from 2 to 13 groups for *cox1*, and from 2 to 6 for *ITS-2,* using 100 simulated annealing procedures.

Lineage age and population divergence times

Well-dated biogeographical events provided four calibration points for estimating absolute divergence times. The opening of the Strait of Gibraltar approximately 5.3 Ma (Krijgsman, 1999) was used as a fixed calibration point for the divergence between the Iberian and Moroccan populations of *Dysdera inermis.* The oldest subaerial datation of La Palma (2 Ma) (Carracedo & Day, 2002) provided a maximum age estimate for the divergence between La Palma and La Gomera populations of *D. calderensis* and *D. silvatica*, while that of El Hierro (1.2 Ma) (Carracedo & Day, 2002) provided a maximum age estimate of the divergence between El Hierro and La Gomera populations of *D. gomerensis* and *D. silvatica*.

The computer program BEAST v.1.4.8 and v.1.5.3 (Drummond & Rambaut, 2007) were used to estimate divergence times and substitution rates for gene trees and the species tree. Three different strategies were implemented. First, clade ages were estimated based on a concatenated data matrix of all genes, except *ITS-2*, and representatives of all sampled species, including 11 *D. verneaui* haplotypes belonging to independent mitochondrial networks. Bayes Factors provided strong evidence for choosing the uncorrelated lognormal relaxed clock as the best clock model for the data (BF=2.59 and 2.52 vs. exponential and strict clock models, respectively) (Suchard, 2001), and it was used for all subsequent analyses, except the "*multispecies coalescent*" analyses (see below). The tree prior was set to speciation Birth-Death process, and calibration points incorporated as node priors. Second, we applied a "*multi-demographic coalescent model"* (Ho *et al.,* 2008) to

the *cox1* haplotype matrix, constraining those clades that received high support (PP>0.95, MP jackknife or ML bootstrap > 70%) in the concatenated analysis. This method combined a Yule tree prior model with a coalescent demographic model of exponential growth, allowing the join estimation of divergence times and substitution rates in trees that include species and population level divergences. Third, we used the "*multispecies coalescent model*" (Heled & Drummond, 2010) implemented in BEAST v.1.5.3 (*BEAST), which co-estimates multiple gene trees embedded in a shared species tree, using multi-locus data from multiple individuals per species. This approach allowed the *ITS-2* gene to be included in the species tree estimation, and its substitution rate inferred. The "*multispecies coalescent model*" was applied to the Canarian taxa, because mitochondrial and the two nuclear gene partitions were only available for these taxa. Analyses were rooted by assuming that *D. silvatica* is the sister group of the remaining taxa, as recovered in all former analyses. Substitution rates and clock models were unlinked for each gene. Independent evolutionary models, as selected by jMODELTEST, were specified for each gene partition. All mitochondrial genes were set to share the same partition tree, while independent trees were allowed for the *28S* and *ITS-2* genes. Haplotypes and sequence types were assigned to each nominal species, except *D. verneaui* populations from Teno and the rest of the island that were defined as two different species. The mitochondrial haplotype 53MAT found in Teno but phylogenetically related to the Central lineage (see below) was assigned to *D. verneaui* rest of the island. A relaxed lognormal clock was specified for all genes except *ITS-2*, to which a strict clock was assigned. Preliminary runs with relaxed lognormal clock assigned to *ITS-2* reported infinite values for the posterior and likelihood scores. To simplify calculations, the priors for the ucdl.mean of each gene were set to include the 95% highest posterior density substitution rate values obtained in the combined (first) analyses. The species tree prior was set to Yule process and the "share the same tree prior" option checked.

All analyses were run for 50 million generations (10^8 and $5*10^7$ generations for the "*multispecies coalescent model*" analysis), sampling every 1000 generations and removing the first 10% of samples as burn-in. Two independent runs were run for each analysis to assess convergence. Results of the two MCMC runs were

analyzed in TRACER v. 1.4 (Rambaut & Drummond, 2007) and ESS values monitored to assess correct mixing of the chains. The accompanying programs LogCombiner and Tree Annotator were used to combine the parameter values and trees of each run in a single data set and to summarize tree information, respectively.

Coalescent-based methods as implemented in MDIV (Nielsen & Wakeley, 2001), were used to estimate time of population divergence and time of the most recent common ancestor (TMRCA) between pairs of populations based on the mtDNA *cox1* and nDNA *ITS-2* genes. The following populations pairs were analysed: Teno vs. Centre-Anaga, Centre vs. Anaga, and Anaga E vs. Anaga W. MDIV estimates a posterior distribution of the parameters theta ($\vartheta = 2Ne\mu$ or $\vartheta = 4Ne\mu$, depending on the maternal or biparental inheritance of genes, respectively), scaled migration rate ($M = Nem$ or $M = 2Nem$) and scaled time of population divergence ($T = t/Ne$ or $T = t/2Ne$), were Ne (effective population size), μ (mutation rate per sequence per generation) and m (migration rate), using a likelihood framework, and also estimates the expected time to the most recent common ancestor (TMRCA=tu) (Nielsen & Wakeley, 2001). MDIV assumes that there is no recombination within loci. The Hudson and Kaplan's (1985) four-gamete test, as implemented in DNAsp (Rozas *et al.,* 2003), was applied to the mitochondrial and nuclear data sets, using 1000 replicates. In all cases, the values reported were non significant (P= 1.000), and resulted in a lower minimum number of recombination events for the *ITS-2* (Rm=7) than for the *cox1* (Rm=38), which confirms prior expectations of low intra-sequence recombination at population level comparisons. MDIV analyses were run at Cornell's CBSU computer cluster (http://cbsuapps.tc.cornell.edu/mdiv.aspx). Three independent simulations were run to ensure convergence of the results. Each simulation was run for 5000000 generations with a 10% burn-in period under the 'finite site model' (HKY), and using a maximum prior of 10 for the scaled migration rate (M) and divergence time (T), as recommended by the authors. The values with the highest posterior probability were accepted as the best estimate. Values for T and TMRCA were calculated using the lineage-specific substitution rates with confidence intervals estimated in the present study (see below) and a generation time of 1.5 years (Cooke, 1965).

Analyses of morphological variation

Patterns of morphological differentiation associated to ecological gradients were investigated by analyzing morphological variation among individuals collected along an elevation cline. Thirteen localities distributed along an altitudinal gradient spanning from 200-3100 m a.s.l. were included in the analyses (see (*) in Appendix 7.2), measuring 6 individuals (3♀ and 3♂) per population (more females were measured in those localities where fewer males were collected). Previous studies have shown that there is no significant sexual dimorphism in *Dysdera* for the measurements analyzed (Macías-Hernández *et al.,* 2008), and hence males and females were pooled together. The following measurements were taken: maximum carapace length (P1), minimum (P2min) and maximum carapace width (P2max), length of the basal segment of the chelicera in lateral view (Q1), maximum width of the basal segment in lateral view (Q2), cheliceral fang length (F), length of the prolateral margin of the basal segment (Esc), length of the femur of leg 1 (Fe1), and length of the metatarsus of leg 4 (Mt4). A Pearson test detected high level of autocorrelation between the morphological variables ($P<0.05$). Therefore, residual values (calculated by means of an interspecific regression using Pearson correlation for each variable against P1) were used in subsequent analyses to reduce the effect of correlation with the body size (Losos *et al.,* 1998). A linear regression analysis was conducted for each measurement to test for correlation between altitude and each morphological measurement. Additionally, the non-parametric Kendall´s tau correlation test, which measures the degree of correspondence between two rankings, was calculated for the variable P1 (body size) against altitude. Analyses were performed with the software packages SPSS v. 15 and STATISTICA (StatSoft Inc., 1999) and the results plotted with SigmaPlot, version 7.0 (SPSS, 2001b).

Correlation between morphological differentiation and both genetic divergence and elevation was investigated by means of Mantel tests (Thorpe *et al.,* 1996). Genetic (*cox1*), morphological and altitude matrices were tested for significance using permutation tests (10000 runs) with ARLEQUIN 3.01. Genetic distances and locality elevation of samples were expressed as Slatkin`s linear [F_{ST}/ (1- F_{ST})] over the logarithm of altitude distances for all pairs of populations. A morphological pairwise distance matrix was calculated using Euclidean distances

between the locality average of P1 measurement using the software PRIMER (Clarke & Warwick, 1994).

RESULTS

Sequence variation

Specimens and sequences analysed in the present study, with corresponding GenBank accession numbers, are listed in Appendix 7.1. About 1 kb of the mitochondrial *cox1* gene was obtained from 85 specimens of *D. verneaui* from 22 localities yielded 57 haplotypes, including 249 polymorphic sites, 226 of which were parsimoniously informative. The A-T bias was 64.29%, and 67.58% of nucleotide substitutions were transitions. Among the 57 haplotypes the average pairwise difference was 7.5% ± 0.4% and the maximum sequence divergence was 14.4%.

The complete nuclear *ITS-2* (442 bp, and 13 additional gap characters) was obtained from 43 specimens, 32 *D. verneaui* individuals from 16 localities, and 6 individuals of the closely related species *D. gomerensis*. Five heterozygotic individuals were cloned. Three of them showed two different alleles of different length. Five sequences differing only in singleton mutations were found in two individuals. These singleton mutations may represent either true mutations or errors introduced by the *Taq* polymerase during amplification or cloning (Pääbo & Wilson, 1998). Singleton mutations that appear in only one clone of the alignment were replaced to match the consensus sequence (Villablanca *et al.,* 1998, Calderón *et al.,* 2009). This approach could slightly underestimate the genetic diversity of the gene, but it is unlikely that modified our general conclusions.

The final alignment of *ITS-2* yielded 10 polymorphic sites, the G-C content was 53.1%, and 40% of nucleotide substitutions were transitions. Most of the variability in these sequences is found in the central region (from position 135 to 240), which includes single base substitutions and some insertion/deletion events of 3-8 bp. A total of 9 sequences types were detected in *D. verneaui* and 5 in *D.*

gomerensis. Among the 5 sequences types of *D. gomerensis,* one is found in two individuals from El Hierro and the rest are from individuals from La Gomera. Average pairwise difference among the 5 *ITS-2* sequences types of *D. gomerensis* and among the 9 *ITS-2* sequences types of *D. verneaui* was the same (0.8% ± 0.3%). The mean number of pairwise differences among the 9 sequences types was 3.1488± 1.6526.

Phylogenetic analyses

Results of the parsimony, maximum likelihood and Bayesian inference analyses are summarized in Fig. 7.2. Parsimony analyses of M1 and M2 yielded 2 trees of 2355 steps (CI: 50, RI: 71) and 2 trees of 2478 steps (CI: 49, RI: 79), respectively. The AIC criterion implemented in jMODELTEST selected the following models of nucleotide substitution for each of the gene fragments: TIM3+G for *cox1* (M2); TIM2+G for *16S;* HKY+I+G for *nad1*; TIM2+G for *28S;* and HKY+G for *ITS-2* (M3). Maximum likelihood analyses yielded single trees of logL -13875.306008 and logL -14687.607514 for M1 and M2, respectively.

Topologies recovered for the M1 (not shown) and M2 matrices (Fig. 7.2) indicate paraphyly of *D. verneaui*, which includes two divergent lineages (13.2% *cox1* pairwise divergence), one lineage comprises haplotypes exclusively from the Teno region, (hereafter referred as the Teno lineage) and the other lineage includes haplotypes from the rest of the islands, and one from Teno (hereafter referred as the Anaga-Centre lineage). *Dysdera gomerensis* is the sister group of the Anaga-Centre lineage (M1= 0.98 PP support, M2= 70% MP jackknife, 62% ML bootstrap and 0.99 PP support). The phylogenetic positions of the Teno lineage is unresolved, in both matrices MP and ML suggest a sister group relationship with *D. calderensis*, and in BI analysis of M1 as sister taxa of *D. silvatica*, but none of the locations received any support.

The Anaga-Centre lineage of *D. verneaui* includes two main, well-supported clades (hereafter referred as clade A and B). Clade A is formed by two well-supported lineages, one includes all individuals from localities on eastern Anaga (localities 21 and 22, see Fig. 7.2) and the second lineage includes individuals from

two central localities close to Anaga (16 and 20) and individuals from other localities in central Tenerife (6, 8 and 13). Clade B was formed by four well-supported lineages that roughly matched particular geographical areas: western Anaga (grey circles), northern slope, and southern and central ridge (see Fig. 7.1 and 7.2). Individuals from clade A and B coexisted in several localities on the central ridge (e.g. 6, 7, 8, 11, 13, 14 and 15). One single individual collected in Teno (N186) was included in one of the clade B lineages. Separated analyses of the mtDNA and nDNA partitions revealed that N186 is a putative case of introgression, as it combines a Teno nuclear sequence type with an Anaga-Centre clade B mtDNA haplotype (53MAT). In summary, some of the intraspecific lineages of *D. verneaui* roughly correspond geographically with the three precursor islands (Teno, Anaga and central Tenerife).

MtDNA and nDNA networks

The statistical parsimony analysis of the *cox1* haplotypes consisted of 10 unlinked networks plus two single haplotypes (data not shown). The independent networks obtained mostly match the lineages detected in the phylogenetic analyses (see Fig. 7.2), with some cases where two unlinked networks or single haplotypes corresponded with the same clade. Sixty-one steps (missing haplotypes) separate the network of *D. gomerensis* from *D. verneaui* Anaga-Centre networks, while 76 and 137 steps separated the “Teno” network from the *D. gomerensis* and *D. verneaui* Anaga-Centre networks, respectively.

The statistical parsimony analysis of the nuclear *ITS-2* gene yielded two independent networks (see Fig. 7.3), one for the sequence types of *D. gomerensis* and one for those of *D. verneaui.* Fourteen steps separated the two networks. All Teno individuals (n=7), including the specimen with *cox1* haplotype 53MAT, bear the same sequence type (9ITS), which is more closely related to the sequence types found in the rest of Tenerife (6 steps) than to the *D. gomerensis* ones. The pairwise *ITS-2* genetic divergence between Teno and Anaga-Centre lineages ranges from 1.4 to 2.2%, while largest divergences within the Anaga-Centre lineage are 0.7%. Pairwise genetic distances between *D. gomerensis* and any of the *D. verneaui* sequence types ranges from 3.6 to 6.1%.

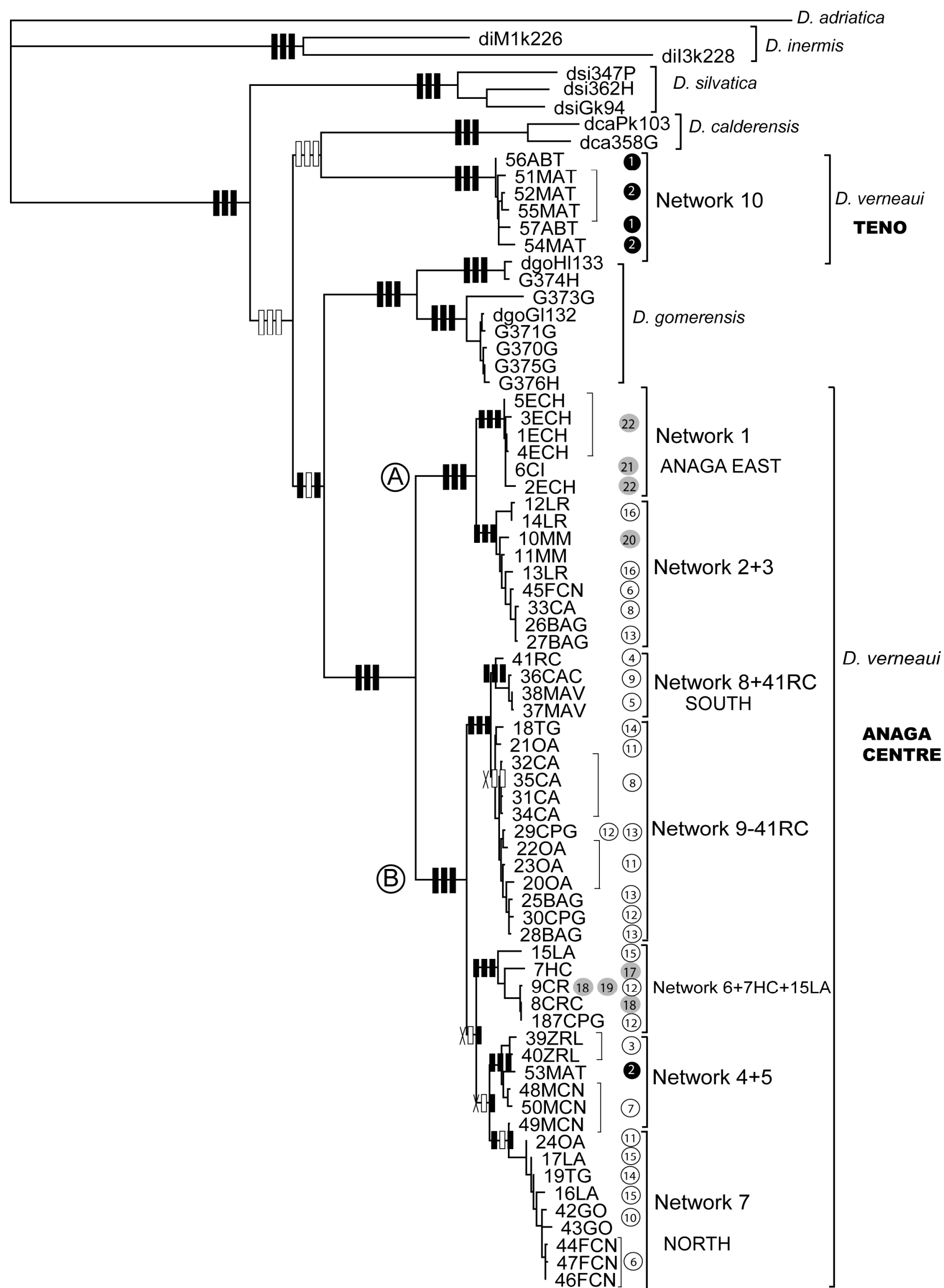

Fig. 7.2.- Maximum likelihood tree topology of the combined mitochondrial and nuclear genes (*cox1, 16S*, *nad*1 and *28S*) plus the 57 *cox1* haplotypes of *D. verneaui.* Bars on branches denote support as follows: left bar refers to parsimony jackknife support, middle bar to maximum likelihood bootstrap support, and right bar to posterior probability. Black bar: parsimony jackknife or ML bootstrap >70%, posterior probability >0.95; white bar: parsimony jackknife or ML bootstrap <70%, posterior probability <0.95%; X: the clade was not recovered in the analyses. Circles refer to localities according to geographical haplotype distribution in Tenerife. The *cox1* haplotype networks obtained with statistical parsimony are indicate.

The sequence types 3ITS and 4ITS, shared by 11 and 10 individuals respectively, were widespread across Tenerife except Teno. Sequence type 3ITS was found in localities from Anaga (western and eastern), central and southern Tenerife. Sequence type 4ITS is found in central Tenerife and western Anaga and overlaps with 3ITS in four localities (8, 13, 17 and 18) (see Fig. 7.3). Sequences types 1-2ITS and 5-8ITS are represented by one or two individuals, and are mainly found in localities from central and southern Tenerife (localities 3, 5, 7, 8 and 11), and one in western Anaga (17) (see Fig. 7.3).

ITS-2 sequence type network analyses agreed with that of *cox1* in revealing a clear differentiation between individuals from Teno and Anaga-Centre, although they differed in *ITS-2* Teno sequence types being more closely related to the ones from Anaga-Centre, while *cox1* haplotypes from Anaga-Centre are more closely related to *D. gomerensis* ones. Unlike *cox1* haplotypes, western and eastern Anaga populations did not show exclusive *ITS-2* sequence types.

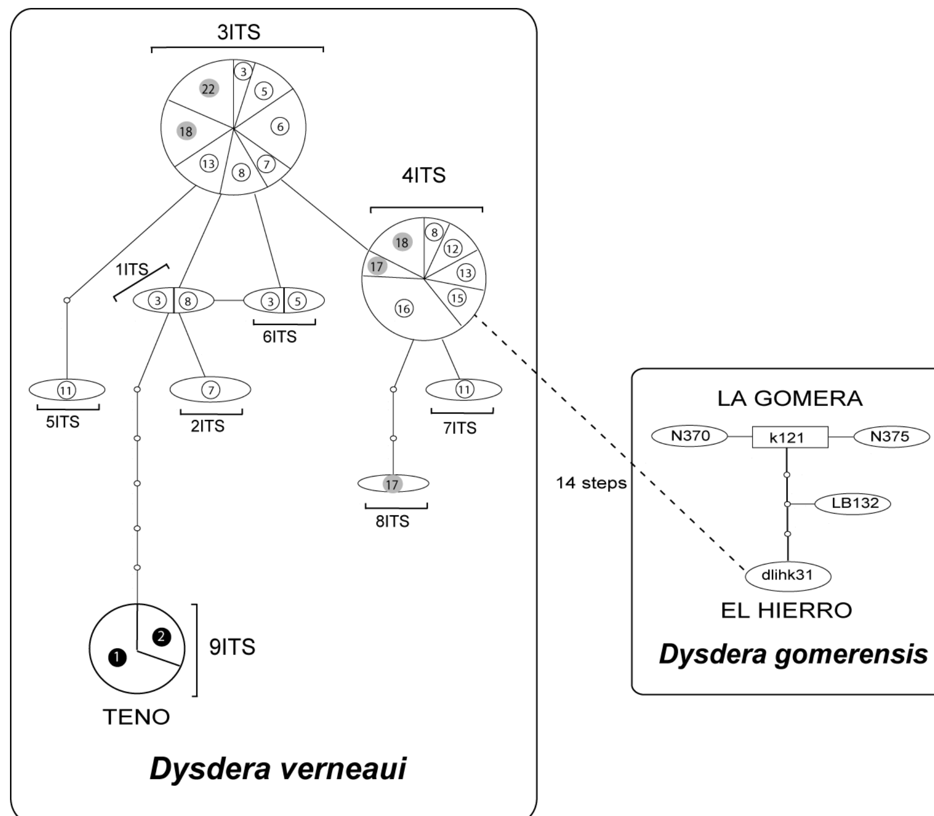

Fig. 7.3.- Statistical parsimony network of the *ITS-2* sequences types of *D. verneaui* and *D. gomerensis.* Circles refer to localities according to geographical haplotype distribution in Tenerife (see Fig. 7.1).

Genetic diversity and population genetic structure

Nucleotide diversity (π_n), number of haplotypes (*Nh*) and haplotypic diversity (*h*) within populations of the *cox1* and *ITS-2* genes are summarized in Appendix 7.2. Most localities show high *cox1 h*-values (in 7 populations, *h*=1.0, all individuals within the population carried different haplotypes). Three nearby localities from western Anaga show *h*=0, i.e. all individuals bear the same haplotype. All *cox1* haplotypes were exclusive of single populations, except the widespread haplotype 8vCR, shared by 9 individuals collected in two close localities on western Anaga (18 and 19) and in central Tenerife (12), and the haplotype v29CPG, found in two close localities on the central Tenerife Dorsal mountain range (12 and 13). Nucleotide diversity within populations ranged from 0 to 0.0547, with the highest value reported from a high elevation locality on the northern side of the Dorsal range (6).

Localities with high *h* values in both *cox1* and *ITS-2* genes are distributed along the central shield (3, 7, 8, 11 and 13). Other populations, distributed along the island, show high *h* values for the *cox1* but only one *ITS-2* sequence type (e.g. localities 2, 16 and 22), while others localities found on western Anaga, show low *h*-values for *cox1* but several *ITS-2* sequence types (e.g. localities 17 and 19). All former comparisons were restricted to localities with similar sample sizes.

Population pairwise F_{ST} values are generally high for the *cox1* gene (see Appendix 7.3), indicating that genetic variation is larger between than within locations. All F_{ST} comparisons between Teno and eastern Anaga and the remaining localities are significant ($P < 0.05$). One single locality on southern Tenerife (5) also shows significant F_{ST} values when compared with all other localities (except 6).

According to SAMOVA, pooling *cox1* samples into 7 groups ($K = 7$) maximized the variance between groups while minimizing the population variance within groups. The genetic variance among groups was 63.73%, among populations within groups 9.03% and within populations 27.23% with $\Phi_{CT} = 0.637$, $\Phi_{SC} = 0.249$ and $\Phi_{ST} = 0.72$ respectively, all comparisons being significant ($P < 0.05$). In all *K* groupings, Φ_{ST} values were high (0.8-0.71), indicating high within population genetic variability. Populations were grouped as follows: (I) Teno (localities 1 and 2);

(II) eastern Anaga (21 and 22); (III) western Anaga (17, 18 and 19); (IV) eastern-Centre (16 and 20); (V) northern Centre and Dorsal ridge (6, 10, 14 and 15); (VI) central ridge (3 and 7); (VII) southern Centre and Dorsal ridge (4, 5, 8, 9, 11, 12 and 13).

The best grouping defined by SAMOVA for the nuclear *ITS-2* was *K*= 5, and populations grouped as follows: (I) Teno (localities 1 and 2); (II) Centre and Anaga (6, 8, 13, 17, 19 and 21); (III) southern Centre and Dorsal ridge (3, 5 and 7); (IV) Dorsal ridge localities 12, 15 and 16 and (V) Dorsal ridge locality 11. Genetic variance among groups was 84.69%, among populations within groups 2.5% and within populations 12% with Φ_{CT} = 0.876, Φ_{SC} = 0.0053 and Φ_{ST} = 0.877 respectively, ($P < 0.05$).

Lineage age and species tree estimation

The lineage age estimations obtained with the concatenated (M1) and the *cox1*-only matrices, were roughly similar when confidence intervals are taking into account. Therefore, only times inferred from the *cox1*-only matrix will be further detailed (Fig. 7.4). The time of the split of the *D. verneaui* Teno lineage from the clade including *D. gomerensis* and Anaga-Centre lineage was estimated at ~3.9 Ma (5.1-2.7 Ma). The TMRCA of *D. gomerensis* and *D. verneaui* Anaga-Centre lineage was ~3.2 Ma (4.3-2.3Ma), and the TMRCA of *D. verneaui* Anaga-Centre clade A and clade B was ~2.3 Ma (3.4-1.5Ma) (Fig. 7.4). The substitution rate estimated for the *cox1* was lower for the *cox1-only* matrix, than the obtained with the concatenated matrix, 0.034 (0.024-0.045) and 0.049 (0.034-0.067) per lineage/million years, respectively. The geometric mean of the *cox1* substitution rate obtained for each matrix was used for subsequent analyses (0.0415 per lineage/million years).

The species tree inferred under the "*multispecies coalescent model*" (see Fig. 7.5), recovered *D. gomerensis* as the sister group of *D. verneaui* Anaga-Centre lineage, although with low posterior probability (0.88). The Teno lineage is shown as sister to the *D. gomerensis*+Anaga-Centre lineage, with even lower PP. The estimated TMRCA of Teno and *D. gomerensis*+Anaga-Centre lineages, was ~3.9 Ma, while the TMRCA of *D. gomerensis* and Anaga-Centre lineage was ~1.8 Ma (4.6-1.7 Ma). The substitution rate of the *ITS-2* gene was estimated at 0.00171 (0.00086-0.0027) per lineage/million years.

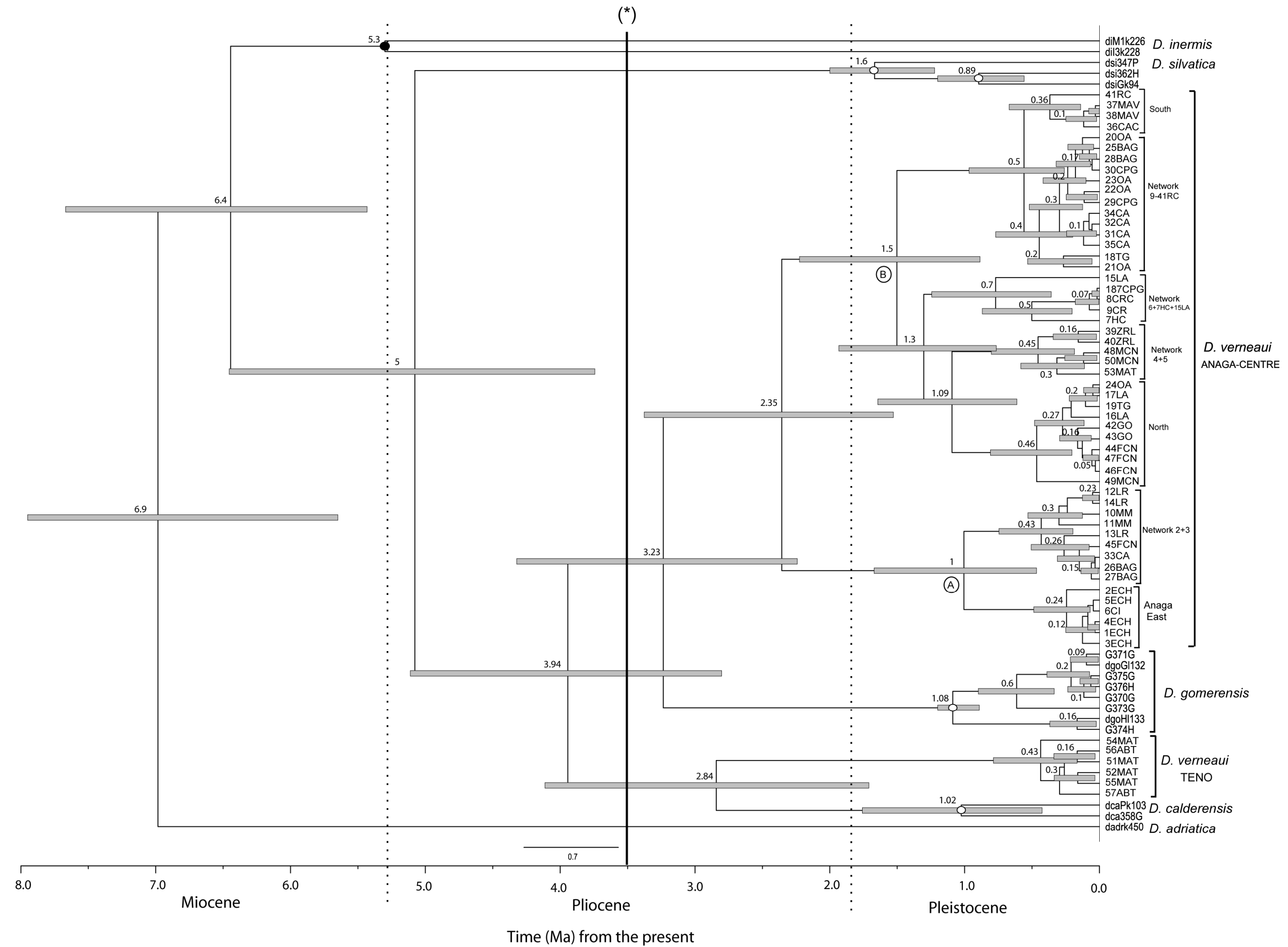

Fig. 7.4.- Chronogram obtained by the multi-demographic coalescent model of the *cox1* haplotype matrix, based on the preferred maximum likelihood topology obtained by the simultaneous analyses of the all genes matrix. Numbers on node are estimated lineage ages, and bars are confidence intervals based on bootstrap resampling of branch lengths. Open circles and fill circles correspond to maximum and fixed calibration points, respectively (see text for detail).
(*) Start of volcanic activity that joined the three ancient protoislands.

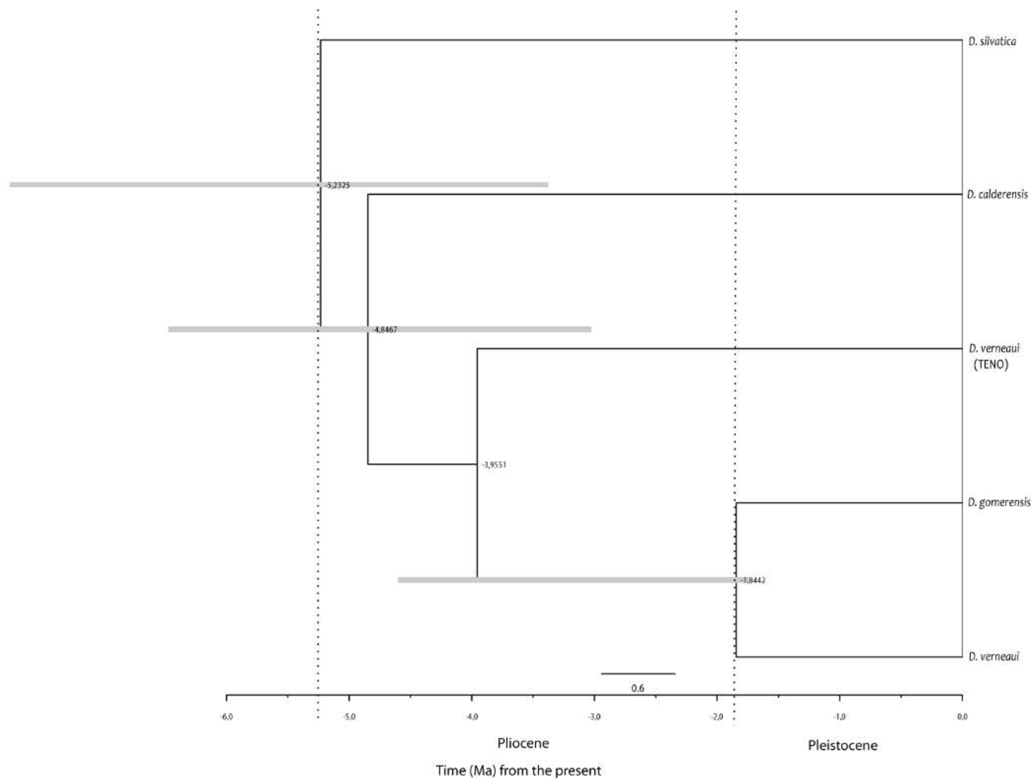

Fig. 7.5.- Chronogram of the species tree obtained with the "*multispecies coalescent model*" method. Numbers on node are estimated lineage ages, and bars are confidence intervals based on bootstrap resampling of branch lengths.

Population divergences

Inferred substitution rates for the *cox1* and *ITS-2* were further used to estimate time of population divergence with the program MDIV. Pairwise population comparisons were defined as follows: Teno (localities 21-22) vs. Anaga-Centre (remaining localities); Anaga (17-22) vs. Centre (remaining localities except Teno); eastern Anaga (21-22) vs. western Anaga (17-20). The haplotype 53MAT was excluded from the analyses as it represents a single event of introgression and could mislead time estimation.

The divergence time between Teno and Anaga-Centre populations estimated for the *cox1* was 1.72 Ma (2.46-1.3 Ma), and the TMRCA 2.11Ma (3.02-1.56Ma), which is younger than the estimates inferred by BEAST. Lower TMRCA estimates in MDIV have also been reported in other studies (e.g. Bidegaray-Batista *et al.*, 2007), and have been interpreted as underestimations due to the use of simpler models of evolution (e.g. the HKY model implemented by MDIV). The same values estimated for the *ITS-2* were 3.4 Ma (6.7-2.1 Ma) and 4.05 Ma (8-2.5 Ma), respectively, more similar to the estimations obtained with the "*multispecies coalescent model"*. The divergence time between Anaga and Centre populations for the *cox1* was 0.72 Ma (1.02-0.5Ma) and the TMRCA 1.4 Ma (2.01-1.04Ma), and for the *ITS-2* 0.16 Ma (0.37-0.09Ma) and 1.4 Ma (3.38-0.88 Ma), respectively. Finally, divergence time between eastern and western Anaga for the *cox1* was 0.76 Ma (1.09-0.5 Ma) and the TMRCA was 1.19 Ma (1.7-0.88 Ma).

Migration rate between Teno and Anaga-Centre was very low, based on either the *cox1* (9.58×10^{-8} migrants per generation) or the *ITS-2* (5.108×10^{-8}) and a bit higher between Anaga and Centre populations (*cox1*, 1.27×10^{-6} and *ITS-2*, 4.18×10^{-6}). Migration between Anaga's eastern and western populations was lower than that observed between Anaga and the Centre (*cox1*, 7.19×10^{-7}).

Analyses of morphological variation

All dependent variables tested (measurements) showed significant correlations with elevation except for P2min and Q2 (see Table 7.1). In all cases, a positive lineal correlation with altitude is observed (Fig. 7.6), except for the variable P2max, which shows a negative correlation. Kendall´s tau correlation test also reveals significant positive correlation between P1 (body size) and elevation (Fig. 7.6). Mantel test shows no significant correlation between genetic distance and either elevation (r=0.024663, P=0.4508) or morphological distance (r=-0.074063, P=0.643). Fig. 7.7 illustrates the range of morphological differentiation associated to a 1500 m elevation gap.

Table 7.1.- Lineal regression summary of the all measurement tested, all variables showed significant correlations with altitude except P2min and Q2 (Significant values at the $p < 0.05$ were marked in bold) (See text for details).

Lineal Regression Summary (y=β1x + β0; y = 0.000453x + 3.52)				
		B	**St. Err. of B**	**p-level**
P1				
	Intercpt	3.52214041	0.084630483	**0**
	X	0.00045269	5.28074E-05	**8.0065E-13**
P2Max				
	Intercpt	0.05728029	0.026653427	**0.03468961**
	ALT	-3.9622E-05	1.64858E-05	**0.018588**
P2min				
	Intercpt	0.03203507	0.019612386	0.10636224
	ALT	-2.2159E-05	1.21308E-05	0.07152328
Q1				
	Intercpt	-0.03620085	0.018437108	**0.05310932**
	ALT	2.5041E-05	1.14038E-05	**0.03103865**
Q2				
	Intercpt	-0.01466728	0.012332995	0.23789512
	ALT	1.0146E-05	7.62827E-06	0.18734574
Fang				
	Intercpt	-0.03040584	0.015666068	**0.05584064**
	ALT	2.1032E-05	9.68986E-06	**0.03296941**
Esc				
	Intercpt	-0.02136637	0.011879019	**0.07589127**
	ALT	1.4779E-05	7.34748E-06	**0.04768248**
F1				
	Intercpt	-0.17061268	0.040840953	**7.5414E-05**
	ALT	0.00011802	2.52612E-05	**1.2066E-05**
M4				
	Intercpt	-0.1055196	0.041195224	**0.01232649**
	ALT	7.299E-05	2.54803E-05	**0.00534738**

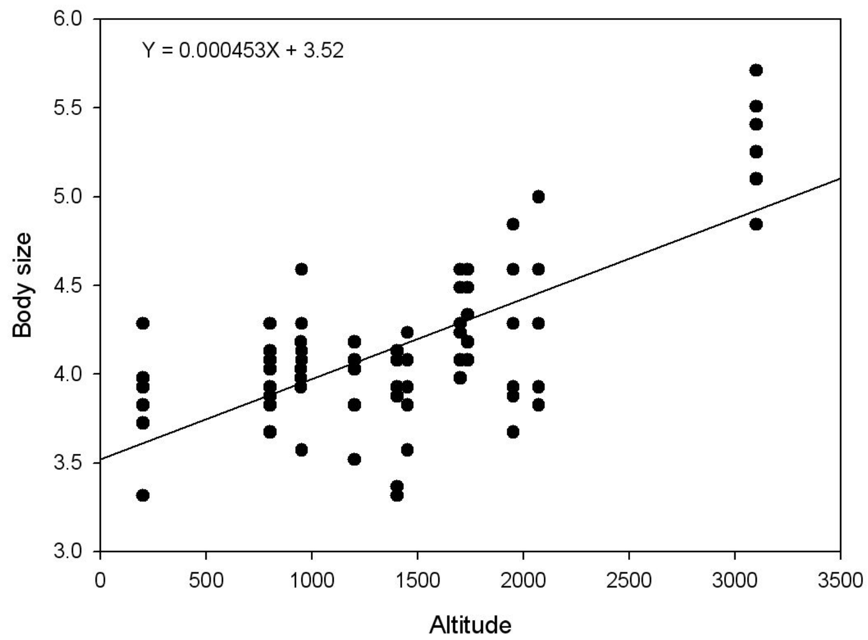

Fig. 7.6.- Graphic of the linear regression analysis, showing a positive correlation of body size (P1) and altitude (y=0.000453x+3.52).

Fig. 7.7.- Two individuals of *D. verneaui* collected at different altitudinal sites. Left specimen from Las Lagunetas (1400 m); right specimen from Las Cañadas (>3100 m).

DISCUSSION

The phylogeographic fingerprint of past geological events

The phylogeographic structure observed in *D. verneaui* has a strong geographical signal: divergent lineages are grossly circumscribed to particular areas of Tenerife. Conversely, ecological adaptation, as represented by local adaptation along an altitudinal gradient, does not seem to have had an impact on the population structure of the species.

One of the main findings of the present study is the identification of an old, mostly isolated lineage of *D. verneaui* in the Teno region. According to our different estimates, the split of this lineage most likely preceded the volcanic phase that gave rise to the Cañadas edifice, which started about 3.5 Ma and eventually joined the three island volcanoes that formed present-day Tenerife.

Individuals sampled from the rest of the island form a lineage that may have or not originated before the connection of the precursor islands depending on the preferred time estimate. Unlike Teno, populations from the Anaga region are not homogeneous and show close genetic affinities with populations from central Tenerife. The eastern and western populations from Anaga are clearly distinct, at least based on mtDNA data (9.2% pairwise genetic divergence of *cox1* gene). The eastern populations bear exclusive mtDNA haplotypes, which form an evolutionary lineage (clade A) with other haplotypes found in one western Anaga population and in few central Tenerife ones. Western Anaga includes, along the population cited above, populations with haplotypes belonging to a different evolutionary lineage (clade B) that are also shared by populations from central Tenerife. The western and eastern Anaga populations are not differentiated at the nuclear level, suggesting either ongoing male-mediated gene flow or, most likely, recent mitochondrial divergence in formerly continuous populations. Nuclear data also suggest close links between Anaga and Central populations. Genetic differentiation among Anaga populations has also been reported in the ground beetles *Eutrichopus canariensis* (Moya *et al.,* 2004) and *Calathus arbaxoides* (Emerson, 1999), and has been explained as the result of habitat discontinuity (Moya *et al.,*

2004). Major landslides have been invoked to explain phylogeographic breaks in Tenerife's Güimar region in both the lizard *Gallotia galloti* (Thorpe *et al*., 1996; Brown *et al*., 2006) and the gecko *Tarentola delalandii* (Gübitz *et al.,* 2000). Interestingly, a giant landslide occurring 1-0.5 Ma (Watts & Masson, 2001; but see also Watts & Masson, 1998; Masson *et al.,* 2002) has been documented in the central Anaga region of Taganana, roughly corresponding to the estimated age of Anaga's western and eastern populations (~0.76Ma, 1.09-0.5 Ma). Alternatively, Anaga's eastern and western populations may have different origins, the first being formed by the original local residents and the second being the results of colonizations from Central Tenerife (see below). Western and eastern Anaga populations, however, share *ITS-2* sequence types, suggesting either nuclear gene flow or common origin with subsequent mitochondrial divergence.

Evidence for phylogeographic structure tracing back to Tenerife precursor islands has been found in the gecko *Tarentola delalandii* (Gübitz *et al.,* 2000), the skink *Chalcides viridanus* (Brown *et al.,* 2000), the lizard *Gallotia galloti* (Thorpe *et al.,* 1996) and the darkling beetle *Pimelia* (Juan *et al.,* 1996). These organisms, however, differ in the pattern of relationships among populations on precursor islands. In *Gallotia galloti* and *Pimelia*, Anaga populations are the most divergent, while Teno and South are closely related or undifferentiated. Conversely, the Teno region harbours the most divergent populations in *Tarentola delalandii*, *Chalcides viridanus* and, as shown in the present study, *D. verneaui*. Unlike *Tarentola* and *Chalcides*, however, *D. verneaui* show evidence of gene flow between Anaga and Central populations. Individuals from Roque Conde, the only present day remnants of the once isolated Central shield, analyzed in this study do not form a basal lineage, but are closely related to other populations form the southern slope of central Tenerife. Several evolutionary scenarios could account for these results. Anaga could have served as source for several, independent waves of colonist that would have repopulated the central part of the island, after lava flow-driven extinctions. The fact that Anaga has not experience volcanic activity since the middle Pliocene, while Central Tenerife has been almost completely covered by recurrent lava flows until as recently as 0.2 My, supports this scenario. Alternatively, some older population could have survived in Central Tenerife refugia, as

suggested by exclusive *ITS-2* sequence types in the area, and hence Anaga and Central Tenerife could have both acted as sources and exchanged migrants. This scenario would account for the observation that each one of the two main clades is mostly formed by haplotypes from one of the two areas: Anaga for clade A and Central Tenerife for clade B, suggestive of the two lineages having originated on different precursor island.

Central Tenerife populations show the lowest *Fst* values but highest levels of haplotype and nucleotide diversity, suggesting ongoing gene flow and admixture populations. The outstanding geological activity endured by the region almost uninterruptedly for the last 3 My, including volcanic eruptions and large debris avalanches, have most likely shaped local *D. verneaui* populations by causing extinctions, bottlenecks episodes and subsequent recolonizations from different sources.

A tale of two lineages: uncovered cryptic species?

Patterns of deep mitochondrial divergence in allopatric populations are common in many organisms (Avise, 2000), and are usually interpreted as the result of long isolation periods due to extrinsic barriers (e.g. Hayes & Harrison, 1992; Zarza *et al.,* 2008). Our results revealed almost complete isolation of Teno populations of *D. verneaui* from the rest of the island, a pattern also reported in the endemic beetle *Tarphius canariensis* (Emerson *et al.,* 2000; Emerson & Oromí, 2005). Volcanism may explain this isolation pattern, since the Teno massif has been surrounded by recurrent lava flows during the last 3.5 My, following the phases of vocanic activity that gave rise to Las Cañadas edifice (Ancochea *et al*., 1999). Reasons other than volcanism may have account for deep genetic divergences between Teno and the rest of Tenerife. The characteristic steep cliffs of the Teno massif have been singled out as the reason behind the limited spatial expansion of the Teno clades of the gecko *T. delalandii* (Gübitz *et al.,* 2000) and the lizard *G. galloti* (Thorpe *et al.,* 1996). In this regard, it has been demonstrated that the rough topography of La Gomera island, similar to that of Teno, has restricted gene flow in the ground-beetle *Paraeutrichopus harpaloides* (Moya *et al.,* 2007).

Mitochondrial and nuclear data disagree on the phylogenetic position of the Teno lineage, the first suggesting a paraphyletic *D. verneaui* with the species *D. gomerensis* being the sister-group of the Anaga-Centre lineage, while *ITS-2* supports the sister group relationship of the two main *D. verneaui* lineages. Incongruence between mitochondrial and nuclear genes is commonly found when examining relationships at the population/species interphase due to the different effective population sizes (*Ne*), recombination and substitutions rates of both markers. Unlinked genes may support distinct, yet correct topologies that may in turn differ from the species tree (Brito & Edwards, 2009). It has been suggested that mitochondrial gene trees are more likely to reflect the true species relationships than nuclear encoded genes (Moore, 1995 ; Moore & Willmer, 1997), because of their faster rate of fixation of ancestral polymorphisms, due to higher substitution rates (Brown *et al.,* 1979; Pamilo & Nei, 1988) and smaller effective population size (Pamilo & Nei, 1988; Tautz *et al.,* 2003).

On the other hand, under certain circumstances mitochondrial markers may not accurately reflect population history (Irwin 2002; Funk & Omland, 2003; Ballard & Whitlock, 2004; Lin & Danforth, 2004). In a recent review, Galtier and collaborators (2009) provide evidence for the high incidence of recombination, positive selection and erratic evolutionary rate in mtDNA, which may lead to misleading inferences of the population history. Ancient introgression may also led mtDNA genes to support erroneous species trees, as shown for New Zealand *Galaxias* fishes.

In the case of *D. verneaui*, the particularities of the *ITS-2* marker may lay behind the observed incongruence between mitochondrial and nuclear genes. This non-coding region has been one of the most popular nuclear markers for inferring species (e.g Gonzalez *et al.,* 1990; Pleyte *et al.,* 1992; Ritland, *et al.,* 1993; Wesson *et al.,* 1993; Miller *et al.,* 1996; Manos, *et al.,* 1999, Mukabayire *et al.,* 1999; Weekers *et al.,* 2001; Wörheide *et al.,* 2004) and population (Fritz *et al.,* 1994; Vogler & DeSalle, 1994; Zhuo *et al.,* 1994; King *et al.,* 1999; Wörheide *et al.,* 2002; Muster *et al.,* 2009) relationships, because it is easy to amplify, due to high copy numbers in the genome, has a relatively high substitution rate within species. One

of the main limitations of the *ITS-2* as phylogenetic marker is that, although the ribosomal cluster is assumed to be homogenized trough concerted evolution, the process is not always complete and multiple, paralogous copies may be found in a single individual (Williams *et al.,* 1988). The closest similarity of the two *D. verneaui* lineages as inferred from the *ITS-2*, may be the results of homogenization of *ITS-2* copies exchanged by the two lineages following sporadic occurrence of gene flow. Limitations to gene flow between islands, on the other hand, preserved distinctiveness of *D. gomerensis ITS-2* sequence copies. The detection of at least one instance of mtDNA introgression, from Anaga-Centre (clade B) into Teno populations, supports the occurrence of limited gene flow between the two lineages. The deep divergences observed between the two *D. verneaui* lineages, on the other hand, favour episodic mitochondrial introgression across divergent lineages populations of *D. verneaui*, rather than unsorted ancestral mtDNA polymorphism as explanation to the observed pattern.

Deep mitochondrial divergences and sorted nuclear differentiation suggest that Teno's populations of *D. verneaui* are an independent evolutionary lineage. Levels of genetic divergence in the mtDNA and *ITS-2* observed between the two lineages of *D. verneaui* are higher, for instance, than those reported for closely related *Dysdera* species on the eastern Canary Islands (Macías-Hernández, *et al*., in prep). Although individuals from Teno and Anaga-Centre lineages are undistinguishable in their somatic morphology, they seem to differ in small genitalic features. Arnedo *et al.,* (1999, see Fig. 167) pointed out the existence of a well-developed additional lateral fold in the male bulb of some *D. verneaui* individuals. Re-examination of a large series of specimens confirms that this feature is restricted to Teno males. Further mating experiments may clarify the role of this feature as prezigotic barrier, although our data already shows that this difference did not prevent cross mating between Teno males and females from the centre of the island.

Patterns of congruence among mitochondrial and nuclear genetic divergence, along with allopatric geographic ranges, and male genitalic diagnostic characters support the species status of the Teno populations of *D. verneaui*.

Morphological variation along an altitudinal gradient

During the last decades, many studies have focused on the geographic variation of body size across latitudinal and altitudinal gradients in a variety of organisms, and several hypotheses have been put forward to explain the patterns observed. The Bergmann's rule (Bergmann, 1847) was originally proposed to explain patterns of clinal geographic variation in endothermic organisms (Brown & Lee, 1969; Ashton *et al.,* 2000; Ashton & Feldman, 2003; Ashton, 2004), which tend to increase in body size at higher latitudes and colder climates, as a result of physiological and thermoregulatory adaptations to metabolic heat preservation. Subsequently, Bergmann's rule was extended to ectothermic organisms (Ray, 1960; Cushman *et al.,* 1993; Huey *et al.,* 2000), and explained either a an adaptive response to temperature (Atkinson, 1994; Atkinson & Sibly, 1997; Partridge & Coyne, 1997), or as a developmental response at cellular level to lower temperatures, which would increase the number and size of cells (Partridge *et al.,* 1994; VanVoorhies, 1996) or eggs (Azevedo *et al.,* 1996; Blanckenhorn, 2000). In addition, other factors as food availability may affect clines as well (Chown & Klok, 2003), as suggested by species with higher body sizes resisting starvation in unfavourable conditions for longer periods (Cushman *et al.,* 1993). The counter gradient variation, or latitudinal compensation hypothesis (Levinton, 1983; Conover & Present, 1990) explains the increase in body size at higher latitudes or elevations as a result of faster growth and higher metabolic rates in compensation for shorter seasonal time compared with lower-elevation conspecifics. More complex hypothesis based on interactions between factors such as temperature, growing season length and generation time has also been proposed to explain clines in insect body size (Chown & Klok, 2003). The reverse pattern, the converse Bergmann's cline, i.e. decreasing body size with increasing latitude or altitude, has been explained as the result of shorter favourable seasons at higher elevations, which would limit the available time for growth and development (Dingle *et al.,* 1990; Blanckenhorn, 1997; Fischer & Fiedler, 2002; Chown & Klok, 2003).

In arthropods, both trends of body size increase or decrease with latitude or elevation are equally common, although larger organisms with longer development times tend to show a converse Bergmann's cline, while smaller

organisms with shorter development times tend to follow the Bergmann's clines (Blanckenhorn & Demont, 2004). A recent study on European spiders, identified physiological traits as the main drivers of a pattern of increasing body size going from cool/moist to warm/dry environments (Entling *et al.,* 2009), while a similar inverse Bergman´s cline in the spider *Waitkera waitakerensis* has been attributed to reproductive success (Opell *et al.,* 2006).

Dysdera verneaui follows the Bergmann's cline along an elevation ranging from 200 to 3100 m. The same trend has also been observed in other Tenerife arthropods, like *Dolichoiulus* millipedes (Enghoff & Báez, 1993), *Alopecosa* spiders (Txasco pers. comm) or the harvestmen *Bunochelis spinifera* (pers. obs.). High elevation habitats in Tenerife are characterized by strong seasonality, high aridity, daily thermal stress, and contrasting changes of snow and freezing periods in winter and drier strong heatstroke in summer. The environmental conditions at this elevation are very harsh and many organisms have developed some sort of adaptations to live in these habitats (Ottesen & Sømme, 1987). This trend do not seems to be explained by historical factors, as has been reported for other organisms (Mosseau & Roff, 1989; Arnett & Gotelli, 1999; Huey *et al.,* 2000), but rather as adaptation to local environmental conditions, as suggested by the non significant correlation of genetic differentiation and either morphological variation or elevation. A similar pattern, in this case related to latitude, has also been reported in the lizard *Gallotia galloti* (Thorpe *et al.,* 1994; 1996).

Conservation issues

A large gap in the distribution of the species in the northern slope of the island has been made evident during the course of the present study. The original forest on this part of the island, which probably included *Pinus canariensis* and laurel forest, was drastically deforested from the XVth to XVIIIth centuries, and subsequently reforested in the early 50's of the last century with the introduced species *Pinus radiata* (Del Arco *et al.,* 1992). Although few endemic *Dysdera* have been collected in this area, the synanthropic species *Dysdera crocata* can be easily found. Dramatic habitat changes may have favoured the spreading of *D. crocata*, either by promoting extinction of local fauna or by facilitating dominance of the

alien species. In this regard, *D. crocata* has been pointed out as the main cause of extinction of the endemic *Dysdera* species from the Azores archipelago, most likely as a result of competitive exclusion (Cardoso *et al.,* 2008).

APPENDIX

Appendix 7.1.- Summary of sequences and sampled localities of *Dysdera* specimens analysed. Code: number of locality used in Fig. 7.1.; *N*: number of individuals sampled per locality; list of *cox1* haplotypes and *ITS-2* sequences types collected in each locality with number of individuals showing the same haplotype in brackets; *cox1*, *ITS-2, 16S*, *rrnL/nad1*and *28S* column entries are GenBank accession numbers.

				GeneBank accesion number				
Locality	**Code**	**N**	**Haplotypes *cox1***	***cox1***	**Sequences types. *ITS-2***	***ITS-2***	***16S-L1-nad1***	***28S***
Dysdera verneaui								
El Aderno. Buenavista	1	5	56ABT (4)		9ITS (2)			
			57ABT (1)					
Monte del Agua.Teno	2	6	51MAT (2)		9ITS (5)			
			52MAT (1)					
			53MAT (1)					
			54MAT (1)					
			55MAT (1)					
Las Lajas.Vilaflor	3	2	39ZRL (1)		1ITS (1)			
			40ZRL (1)		3ITS (1)			
					6ITS (1)			
Roque del Conde	4	1	41RC (1)					
Madre del Agua. Vilaflor	5	5	37MAV (4)		3ITS (1)			
			38MAV (1)		6ITS (1)			
La Fortaleza. Las Cañadas	6	5	44FCN (1)		3ITS (2)			
			45FCN (2)					
			46FCN (1)					
			47FCN (1)					
Mña. Chusqueros. 7 Cañadas	7	3	48MCN (1)		2ITS (2)			
			49MCN (1)		3ITS (1)			

				GeneBank accesion number				
Locality	**Code**	**N**	**Haplotypes *cox1***	***cox1***	**Sequences types. *ITS-2***	***ITS-2***	***16S-L1-nad1***	***28S***
			50MCN (1)					
Cumbres de Arico	8	5	31CA (1)		1ITS (1)			
			32CA (1)		3ITS (1)			
			33CA (1)		4ITS (1)			
			34CA (1)					
			35CA (1)					
Cumbres de Arico.Contador	9	1	36CAC (1)					
El Guanche.La Orotava	10	3	42GO (2)					
			43GO (1)					
Orticosa	11	5	20OA (1)		5ITS (1)			
			21OA (1)		7ITS (1)			
			22OA (1)					
			23OA (1)					
			24OA (1)					
Caldera de Pedro Gil	12	3	8CR (1)		4ITS (1)			
			29CPG (1)					
			30CPG (1)					
Bco. del Agua. Güímar	13	6	25BAG (1)		3ITS (1)			
			26BAG (2)		4ITS (1)			
			27BAG (1)					
			28BAG (1)					
			29CPG (1)					
Torre del Gaitero	14	3	18TG (1)					
			19TG (2)					
Las Lagunetas	15	4	15LA (2)		4ITS (1)			
			16LA (1)					

Locality	Code	N	Haplotypes cox1	GeneBank accesion number				
				cox1	Sequences types. *ITS-2*	*ITS-2*	*16S-L1-nad1*	*28S*
			17LA (1)					
Las Raíces	16	5	12LR (1)		4ITS (3)			
			13LR (2)					
			14LR (2)					
Las Hiedras-Carboneras	17	3	7HC (3)		4ITS (1)			
					8ITS (1)			
Batán-Cruz del Carmen	18	3	8CR (2)					
			9CR (1)					
Cruz Carmen	19	6	8CR (6)		3ITS (2)			
					4ITS (2)			
Monte de las Mesas	20	2	10MM (1)					
			11MM (1)					
Camino a Ichires	21	4	6CI (4)					
Ensillada-Chamorga	22	5	1ECH (1)		3ITS (2)			
			2ECH (1)					
			3ECH (1)					
			4ECH (1)					
			5ECH (1)					
TOTAL		**85**						
Dysdera gomerensis								
Puntallana. La Gomera		1	G370G (1)		10ITS(1)			
Noruegos. La Gomera		1	G371G (1)					
Mña. Las Pilas. La Mérica. La Gomera		1	G373G (1)					
Enchereda. La Gomera		1	G375G (1)		11ITS (1)			
Cañada de Jorge. La Gomera		1	G132G (1)	>dgoGl132	12ITS (1)			

				GeneBank accesion number				
Locality	**Code**	**N**	**Haplotypes *cox1***	***cox1***	**Sequences types. *ITS-2***	***ITS-2***	***16S-L1-nad1***	***28S***
					14ITS (1)			
Pista Garoé. El Hierro		1	G374H (1)					
Pista Mercader. El Hierro		1	G376H (1)					
Casa Forestal de Frontera. El Hierro		1	G133H (1)	>dgoHl133	13ITS (1)			
Dysdera calderensis								
Juan Adalid, Garafía. La Palma			>dcaPk103	AF244309			AF244218/EU139665	EU139788
Riscos de Alojera. La Gomera			>dca358G					
Dysdera silvatica								
Barranco de Juel, Hermigua. La Gomera			>dsiGk94	AF244273		EU143842	AF244177/EU139674	EU139808
Pinar Roque Faro. La Palma			>dsi347P					
Mirador de Bascos. El Hierro			>dsi362H					
Dysdera inermis								
Tangier-Tétouan. Morocco			>diM1k226	EF458142		NO	EF458092	EU139795
Andalucía, Cadiz, Tarifa. Iberian Peninsula			>diI3k228			NO		
Dysdera adriatica								
Kozina. Slovenia			>dadrk450			NO		

Appendix 7.2.- Diversity measures of *cox1* and *ITS-2* genes for populations of *Dysdera verneaui* sampled in this study. Asterisks indicate localities which provided specimens for morphological analyses. (N) sample size, (*H*) number of *cox1* haplotypes, (πn) nucleotide diversity, (*h*) haplotype diversity.

Dysdera verneaui					***cox1***				***ITS-2***			
	Locality	**Habitat types**	**Altitude**	**Latitude/longitude**	**N**	**H**	***πn***	***h***	**N (Exx.)**	**H**	***πn***	***h***
1	El Aderno. Buenavista	Semi-arid tropical shrubs	200 m*	28.3582 -16.8644	5	2	0.004762 +/- 0.003267	0.4000 +/- 0.2373	4 (2)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
2	Monte del Agua.Teno	Laurel forest	950 m*	28.3238 -16.8172	6	5	0.052579 +/- 0.030755	0.9333 +/- 0.1217	10 (5)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
3	Las Lajas.Vilaflor	Pinus forest	2050m	28.1903 -16.6691	2	2	0.005964 +/- 0.006442	1.0000 +/- 0.5000	4 (2)	3	0.00123 +/- 0.00152	0.8333 +/- 0.2224
4	Roque del Conde	Xerophytic scrubs	525 m	28.0931 -16.6988	1	1	NC	NC			NC	NC
5	Madre del Agua. Vilaflor	Pinus forest	1700 m*	28.1694 -16.6306	5	2	0.000397 +/- 0.000505	0.4000 +/- 0.2373	4 (2)	2	0.00164 +/- 0.00184	0.6667 +/- 0.2041
6	La Fortaleza. Las Cañadas	Subalpine shrubs	2070 m*	28.3167 -16.5912	5	4	0.054762 +/- 0.033567	0.9000 +/- 0.1610	4 (2)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
7	Mña. Chusqueros. 7 Cañadas	Subalpine shrubs	2100-3100 m*	28.2922 -16.5590	3	3	0.029431 +/- 0.022376	1.0000 +/- 0.2722	4 (2)	2	0.00234 +/- 0.00232	0.5000 +/- 0.2652
8	Cumbres de Arico	Pinus forest	1950 m*	28.2492 -16.5287	5	5	0.037897 +/- 0.023354	1.0000 +/- 0.1265	4 (2)	3	0.00236 +/- 0.00233	0.8333 +/- 0.2224
9	Cumbres de Arico.Contador	Pinus forest	1050 m	28.1977 -16.5312	1	1	NC	NC			NC	NC
10	El Guanche.La Orotava	Pinus forest	1425 m	28.3472 -16.5140	3	2	0.006614 +/- 0.005355	0.6667 +/- 0.3143			NC	NC
11	Orticosa	Pinus forest	1450 m*	28.3845 -16.4474	5	5	0.025595 +/- 0.015903	1.0000 +/- 0.1265	4 (2)	2	0.00663 +/- 0.00528	0.6667 +/- 0.2041
12	Caldera de Pedro Gil	Pinus forest	1790 m	28.3484 -16.4717	3	3	0.041997 +/- 0.031744	1.0000 +/- 0.2722	2 (1)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
13	Bco. del Agua. Güimar	Dry-Laurel forest	800 m*	28.3078 -16.4481	6	5	0.054497 +/- 0.031862	0.9333 +/- 0.1217	4 (2)	2	0.00157 +/- 0.00176	0.6667 +/- 0.2041
14	Torre del Gaitero	Pinus forest	1735 m*	28.3947 -16.4319	3	2	0.032407 +/- 0.024595	0.6667 +/- 0.3143			NC	NC
15	Las Lagunetas	Pinus forest	1400 m*	28.4185 -16.4100	4	3	0.038525 +/- 0.025561	0.8333 +/- 0.2224	2 (1)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
16	Las Raíces	Pinus forest	1200 m*	28.4297 -16.3808	5	3	0.012103 +/- 0.007728	0.8000 +/- 0.1640	6 (3)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
17	Las Hiedras-Carboneras	Laurel forest	950 m	28.5400 -16.2737	3	1	0.000000 +/- 0.000001	0.0000 +/- 0.0000	2 (1)	2	0.00707 +/- 0.00817	1.0000 +/- 0.5000
18	Batán-Cruz del Carmen	Laurel forest	880 m	28.5353 -16.2968	3	2	0.001323 +/- 0.001359	0.6667 +/- 0.3143			NC	NC
19	Cruz Carmen	Laurel forest	945 m*	28.5319 -16.2799	6	1	0.000000 +/- 0.000001	0.0000 +/- 0.0000	6 (3)	2	0.00141 +/- 0.00149	0.6000 +/- 0.1291
20	Monte de las Mesas	Semi-arid tropical shrubs	390 m	28.4813 -16.2636	2	2	0.008929 +/- 0.009412	1.0000 +/- 0.5000			NC	NC
21	Camino a Ichires	Laurel forest	830 m	28.5400 -16.2319	4	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000			NC	NC
22	Ensillada-Chamorga	Laurel forest	800 m*	28.5562 -16.1798	5	5	0.005357 +/- 0.003630	1.0000 +/- 0.1265	4 (2)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
				Total	85	57	0.075024 +/- 0.036146	0.9815 +/- 0.0068	32	9	0.00769 +/-0.00448	0.7970 +/- 0.0257

Appendix 7.3.- Pairwise F_{ST} values among locations for *cox1* based on pairwise difference method. Significant comparisons at the P < 0.05 were marked in bold.

	1	**2**	**3**	**5**	**6**	**7**	**8**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
1	0.0000																			
2	0.0446	0.0000																		
3	**0.9613**	**0.6221**	0.0000																	
5	**0.9817**	**0.7769**	0.9734	0.0000																
6	**0.7722**	**0.5564**	0.3310	**0.6017**	0.0000															
7	**0.9002**	**0.6117**	0.1671	**0.8162**	0.2355	0.0000														
8	**0.8408**	**0.6302**	**0.4739**	**0.4585**	0.2903	**0.4275**	0.0000													
10	**0.9581**	**0.6778**	0.8773	**0.9540**	0.1312	0.5557	**0.5766**	0.0000												
11	**0.8858**	**0.6690**	**0.5870**	**0.5355**	**0.3421**	**0.4402**	-0.0076	**0.5775**	0.0000											
12	**0.8680**	**0.5966**	0.4227	**0.5635**	0.2587	0.2987	-0.0105	0.5406	-0.0949	0.0000										
13	**0.7588**	**0.5655**	0.3507	**0.4822**	0.1042	**0.3470**	0.0415	**0.4738**	0.2222	0.1183	0.0000									
14	**0.8911**	**0.6149**	0.5274	**0.7220**	0.0476	0.2560	**0.2742**	0.1238	0.1588	0.1390	0.2713	0.0000								
15	**0.8546**	**0.6133**	0.4311	**0.7058**	0.1598	0.2120	**0.3726**	0.2923	**0.3419**	0.1925	**0.3360**	0.0403	0.0000							
16	**0.9328**	**0.7130**	**0.8634**	**0.9303**	**0.4529**	**0.7939**	**0.6537**	**0.8900**	**0.7843**	**0.7409**	0.3317	**0.7825**	**0.7347**	0.0000						
17	**0.9762**	**0.7072**	0.9638	**0.9961**	**0.5034**	0.7201	**0.6453**	0.9405	**0.7194**	0.5767	**0.5275**	0.7066	**0.5190**	**0.9119**	0.0000					
18	**0.9725**	**0.6960**	0.9407	**0.9892**	**0.4993**	0.6844	**0.6344**	0.9241	**0.7012**	0.4480	**0.5191**	0.6806	**0.4709**	**0.9089**	0.9762	0.0000				
19	**0.9838**	**0.7779**	**0.9792**	**0.9973**	**0.6358**	**0.8136**	**0.7435**	**0.9630**	**0.7947**	0.6473	**0.6386**	**0.8100**	**0.6356**	**0.9415**	**1.0000**	0.2500	0.0000			
20	**0.9560**	**0.6426**	0.9032	**0.9766**	0.2846	0.7450	0.5798	0.9182	0.7543	0.6570	0.1761	0.7241	0.6633	0.2019	0.9663	0.9568	**0.9832**	0.0000		
21	**0.9778**	**0.7306**	0.9812	**0.9973**	**0.5617**	**0.8618**	**0.7144**	**0.9706**	**0.8308**	**0.8005**	**0.4821**	**0.8499**	**0.7831**	**0.8254**	**1.0000**	**0.9942**	**1.0000**	0.9386	0.0000	
22	**0.9509**	**0.7362**	**0.9322**	**0.9664**	**0.5689**	**0.8425**	**0.7136**	**0.9362**	**0.8212**	**0.7914**	**0.4954**	**0.8332**	**0.7767**	**0.7830**	**0.9620**	**0.9567**	**0.9741**	**0.8652**	-0.0179	0.0000

***P<0.05**

Populations represented by one sequenced individual were excluded (localities 4 and 9).

REFERENCES

Abdel-Monem A, Watkins ND, Gast PW. 1972. Potassium-Argon ages, volcanic stratigraphy and geomagnetic polarity history of the Canary Islands: Tenerife, La Palma and El Hierro. *American Journal of Science* 272: 805-825.

Akaike H. 1973. Information theory as an extension of the maximum likelihood principle. Budapest: Akademiai Kiado.

Ancochea E, Fuster JM, Ibarrola E, Cendrero A, Coello J, Hernán F, Cantagrel JM, Jamond C. 1990. Volcanic evolution of the island of Tenerife (Canary Islands) in the light of the new K-Ar data. *Journal of Volcanology and Geothermal Research* 44: 231-249.

Ancochea E, Huertas MJ, Cantagrel JM, Coello J, Fúster JM, Arnaud N, Ibarrola E. 1999. Evolution of the Cañadas edifice and its implications for the origin of the Cañadas Caldera (Tenerife, Canary Islands). *Journal of Volcanology and Geothermal Research* 88: 177-199.

Arnedo, Pedro Oromí, Cesc Múrria, Nuria Macías-Hernández, Ribera C. 2007. The dark side of an island radiation: systematics and evolution of troglobitic spiders of the genus *Dysdera* Latreille (Araneae: Dysderidae) in the Canary Islands. *Invertebrate Systematics* 21: 623-660.

Arnedo MA, Oromí P, Ribera C. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. *Cladistics* 17: 313-353.

Arnedo MA, Ribera C. 1999. Radiation of the genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: The island of Tenerife. *Journal of Arachnology* 27: 604-662.

Arnett AE, Gotelli NJ. 1999. Geographic variation in life history traits of the ant lion, *Myrmeleon immaculatus*: evolutionary implications of Bergmann’s rule. *Evolution* 53: 1180–1188.

Ashton KG. 2004. Sensitivity of intraspecific latitudinal clines of body size for tetrapods to sampling, latitude and body size. *Integrative and Comparative Biology* 44: 403-412.

Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: Turtles follow it, lizards and snakes reverse it. *Evolution* 57: 1151-1163.

Ashton KG, Tracy MC, de Queiroz A. 2000. Is Bergmann's rule valid for mammals? *American Naturalist* 156: 390-415.

Atkinson D. 1994. Temperature and organism size: a biological law for ectotherms? *Advances in ecological research* 25: 1-58.

Atkinson D, Sibly RM. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology & Evolution* 12: 235-239.

Avise JC. 2000. *Phylogeography: the history and formation of species.* Harvard University Press: Cambridge, Mass.

Ayoub, Garb JE., Hedin M., Hayashi CY. 2007. Utility of the nuclear protein-coding gene, elongation factor-1 gamma (EF-1 Γ), for spider systematics, emphasizing family level relationships of tarantulas and their kin (Araneae: Mygalomorphae). *Molecular Phylogenetics and Evolution* 42: 394–409.

Azevedo RBR, French V, Partridge L. 1996. Thermal evolution of egg size in *Drosophila melanogaster*. *Evolution* 50: 2338-2345.

Ballard JWO, Whitlock MC. 2004. The incomplete natural history of

mitochondria. *Molecular Ecology* 13: 729-744.

Beheregaray LB, Ciofi C, Geist D, Gibbs JP, Caccone A, Powell JR. 2003. Genes record a prehistoric volcano eruption in the Galapagos. *Science* 302: 75.

Bergmann C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Gött. Stud. 1: 595–708.

Bidegaray-Batista L, Macías-Hernández N, Oromí P, Arnedo MA. 2007. Living on the edge: demographic and phylogeographical patterns in the woodlouse-hunter spider *Dysdera lancerotensis* Simon, 1907 on the eastern volcanic ridge of the Canary Islands. *Molecular Ecology* 16: 3198-3214.

Blanckenhorn WU. 1997. Altitudinal life history variation in the dung flies *Scathophaga stercoraria* and *Sepsis cynipsea*. *Oecologia* 109: 342-352.

Blanckenhorn WU. 2000. Temperature effects on egg size and their fitness consequences in the yellow dung fly *Scathophaga stercoraria*. *Evolutionary Ecology* 14: 627-643.

Blanckenhorn WU, Demont M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integrative and Comparative Biology* 44: 413-424.

Bond JE, Stockman AK. 2008. An integrative method for delimiting cohesion species: finding the population-species interface in a group of californian trapdoor spiders with extreme genetic divergence and geographic structuring. *Systematic Biology* 57: 628 - 646.

Brito PH, Edwards SV. 2009. Multilocus phylogeography and phylogenetics using sequence-based markers. *Genetica* 135: 439-455.

Brower AV, DeSalle R. 1998. Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of wingless as a source of characters for phylogenetic inference. *Insect Molecular Biology* 7: 73-82.

Brown JH, Lee AK. 1969. Bergmann´s rule and climatic adaptation in woodrats (*Neotoma*). *Evolution* 23: 329-338.

Brown RP, Campos-Delgado R, Pestano J. 2000. Mitochondrial DNA evolution and population history of the Tenerife skink *Chalcides viridanus*. *Molecular Ecology* 9: 1061-1067.

Brown RP, Hoskisson PA, Welton JH, Baez M. 2006. Geological history and within-island diversity: a debris avalanche and the Tenerife lizard *Gallotia galloti. Molecular Ecology* 15: 3631-3640.

Brown WM, George M, Wilson AC. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences* 76: 1967-1971.

Calderón I, Turón X, Lessios HA. 2009. Characterization of the sperm molecule bindin in the sea urchin genus *Paracentrotus*. *Journal of Molecular Evolution* 68: 366-376.

Cantagrel JM, Arnaud NO, Ancochea E, Fuster JM, Huertas MJ. 1999. Repeated debris avalanches on Tenerife and genesis of Las Cañadas caldera wall (Canary Islands). *Geology* 27: 739-742.

Cardoso P, Arnedo MA, Triantis K, Borges PAV. 2010. Drivers of diversity in Macaronesian spiders and the role of species extinctions. *Journal of Biogeography* 37: 1034-1046.

Cardoso P, Borges PAV, Macías-Hernández N. 2008. *Dysdera crocata* C.L. Koch, 1838. In: ed. by L. Silva ELOaJLR-L, ed. Invasive terrestrial flora and fauna of Macaronesia. TOP 100 in Azores, Madeira and Canaries. ARENA, Ponta Delgada. 415-417.

Carracedo JC, Badiola ER, Guillou H, Paterne M, Scaillet S, Torrado FJP, Paris R, Fra-Paleo U, Hansen A. 2007. Eruptive and structural history of Teide Volcano and rift zones of Tenerife, Canary Islands. *Geological Society of America Bulletin* 119: 1027-1051.

Carracedo JC, Day S. 2002. Canary Islands. Terra: Harpenden.

Carson HL, Lockwood JP, Craddock EM. 1990. Extinction and recolonization of local-populations on a growing shield-volcano. *Proceedings of the National Academy of Sciences* 87: 7055-7057.

Cassens I, Mardulyn P, Milinkovitch MC. 2005. Evaluating intraspecific "Network" construction methods using simulated sequence data: Do existing algorithms outperform the global maximum parsimony approach? *Systematic Biology* 54: 363-372.

Clarke KR, Warwick RM. 1994. A framework for studying changes in community structure. Change in marine communities: an approach to statistical analysis and interpretation.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660.

Conover DO, Present TMC. 1990. Countergradient variation in growth-rate - compensation for length of the growing-season among atlantic silversides from different latitudes. *Oecologia* 83: 316-324.

Cooke JAL. 1965. A contribution to the biology of the British spiders belonging to the genus *Dysdera*. *Oikos* 16: 20-25.

Crandall KA. 1994. Intraspecific cladogram estimation: Accuracy at higher levels of divergence. *Systematic Biology* 43: 222-235.

Cushman JH, Lawton JH, Manly BFJ. 1993. Latitudinal patterns in european ant assemblages variation in species richness and body-size. *Oecologia* 95: 30-37.

Chown S, Klok CJ. 2003. Altitudinal body size clines: latitudinal effects associated with changing seasonality. *Ecography* 26: 445-455.

De Meyer M. 1996. Cladistic and biogeographic analyses of Hawaiian pipunculidae (Diptera) revisited. *Cladistics* 12: 291-303.

Del Arco Aguilar MJ, Pérez de Paz PL, Rodríguez Delgado O, Salas Pascual M, Wildpret de la Torre W. 1992. Atlas Cartográfico de los pinares canarios II. Tenerife. Viceconsejería de Medio Ambiente. Consejería de Política Territorial Gobierno de Canarias: Santa Cruz de Tenerife.

Dingle H, Mousseau TA, Scott SM. 1990. Altitudinal variation in life-cycle syndromes of californian populations of the grasshopper, *Melanoplus sanguinipes* (F). *Oecologia* 84: 199-206.

Drummond A, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* 11: 2571-2581.

Emerson BC, Kolm N. 2005. Species diversity can drive speciation. *Nature*: 1015-1016.

Emerson BC, Oromi P. 2005. Diversification of the forest beetle genus *Tarphius* on the Canary Islands, and the evolutionary origins of island endemics. *Evolution* 59: 586-598.

Emerson BC, Oromi P, Hewitt GM. 1999. MtDNA phylogeography and recent intra-island diversification among Canary Island *Calathus* beetles.

Molecular Phylogenetics and Evolution 13: 149-158.

Emerson BC, Oromi P, Hewitt GM. 2000. Tracking colonization and diversification of insect lineages on islands: Mitochondrial DNA phylogeography of *Tarphius canariensis* (Coleoptera: Colydiidae) on the Canary Islands. *Proceedings of the Royal Society Biological Sciences Series B* 267: 2199-2205.

Enghoff H, Báez M. 1993. Evolution of distribution and habitat patterns in endemic millipedes of the genus *Dolichoiulus* (Diplopoda: Julidae) on the Canary Islands, with notes on distribution patterns of other Canarian species swarms. *Biological Journal of the Linnean Society* 49: 277-301.

Entling W, Schmidt-Entling MH, Bacher S, Brandl R, Nentwig W. 2009. Body size-climate relationships of European spiders. *Journal of Biogeography* 37: 477-485.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. 3.0 ed: Evolutionary Bioinformatics Online 1:47-50.

Farris JS, Albert VA, Kallersjo M, Lipscomb D, Kluge AG. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99-124.

Fischer K, Fiedler K. 2002. Reaction norms for age and size at maturity in response to temperature: a test of the compound interest hypothesis. *Evolutionary Ecology* 16: 333-349.

Fritz GN, Conn J, Cockburn A, Seawright J. 1994. Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of *Anopheles nuneztovari* (Diptera: Culicidae). *Molecular Biology and Evolution* 11: 406-416.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics* 34: 397-423.

Galtier N, Nabholz B, Glémin S, Hurst GDD. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* 18: 4541-4550.

Gillespie G. 2004. Community assembly through adaptive radiation in hawaiian spiders. *Science* 303: 356-359.

Gillespie G, Croom Henrietta B, Hasty GL. 1997. Phylogenetic relationships and adaptive shifts among major clades of *Tetragnatha* spiders (Araneae: Tetragnathidae) in Hawaii. *Pacific Science* 51: 380-394.

Gillespie RG, Roderick GK. 2002. Arthropods on islands: Colonization, speciation and conservation. *Annual Review of Ecology and Systematics* 47: 595-632.

Goloboff PA, Farris JS, Nixon KC. 2003. TNT: Tree Analysis Using New Technologies. 0.6 (Beta) ed.

Gonzalez IL, Sylvester JE, Smith TF, Stambolian D, Schmickel RD. 1990. Ribosomal-RNA gene sequences and hominoid phylogeny. *Molecular Biology and Evolution* 7: 203-219.

Gübitz T, Thorpe RS, Malhotra A. 2000. Phylogeography and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypotheses. *Molecular Ecology* 9: 1213-1221.

Gübitz T, Thorpe RS, Malhotra A. 2005. The dynamics of genetic and morphological variation on volcanic islands. *Proceedings Royal Society of London B* 272: 751-758.

Guillou H, Carracedo JC, Paris R, Torrado FJP. 2004. Implications for the early

shield-stage evolution of Tenerife from K/Ar ages and magnetic stratigraphy. *Earth and Planetary Science Letters* 222: 599-614.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696-704.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Symposium on RNA biology; RNA, tool and target. Research Triangle Park, NC: Oxford University Press, 95-98.

Hayes JP, Harrison RG. 1992. Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the eastern United States. *Systematic Biology* 41: 331-344.

Hedin MC. 1997. Molecular phylogenetics at the population/species interface in cave spiders of the Southern Appalachians (Araneae: Nesticidae: *Nesticus*). *Molecular Biology and Evolution* 14: 309-324.

Heled J, Drummond AJ. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* 27: 570-580.

Ho SYW, Larson G, Edwards CJ, Heupink TH, Lakin KE, Holland PWH, Shapiro B. 2008. Correlating Bayesian date estimates with climatic events and domestication using a bovine case study. *Biology Letters* 4: 370-374.

Holland BS, Cowie RH. 2007. A geographic mosaic of passive dispersal: population structure in the endemic Hawaiian amber snail *Succinea caduca* (Mighels, 1845). *Molecular Ecology* 16: 2422-2435.

Howarth FG. 1981. Community structure and niche differentiation in Hawaiian lava tubes. In: Mueller-Dombois D, Bridges KW and Carson HL, eds. Island Ecosystems; Biological Organization in Selected Hawaiian Communities. New York: Academic Press. Ch. 11.

Howarth FG. 1993. High stress subterranean habitats and evolutionary change in cave-inhabiting arthropods. *American Naturalist* 142: 565-577.

Hudson RR, Kaplan NL. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics* 111: 147-164.

Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L, iacute. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287: 308-309.

Izquierdo I, Martín JL, Zurita N, Arechavaleta M. 2001. Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres) 2001. Consejería de Política Territorial y Medio Ambiente Gobierno de Canarias.

Jarman SN, Ward RD, Elliott NG. 2002. Oligonucleotide primers for PCR amplification of coelomate introns. *Marine Biotechnology* 4: 347-355.

Juan C, Emerson BC, Oromí P, Hewitt GM. 2000. Colonization and diversification: Towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution* 15: 104-109.

Juan C, Ibrahim KM, Oromi P, Hewitt GM. 1996. Mitochondrial DNA sequence variation and phylogeography of *Pimelia* darkling beetles on the Island of Tenerife (Canary Islands). *Heredity* 77: 589-598.

Kaneshiro KY, Boake CRB. 1987. Sexual selection and speciation: issues raised by Hawaiian *Drosophila*. *Trends in Ecology & Evolution* 2: 207-212.

Katoh K, Kuma K-i, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33: 511-518.

Katoh K, Misawa K, Kuma K-i, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059-3066.

King T, L. , Michael SE, Branimir G, Walter RH. 1999. Intraspecific phylogeography of *Lasmigona subviridis* (Bivalvia: Unionidae): conservation implications of range discontinuity. *Molecular Ecology* 8: S65-S78.

Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS. 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature* 400: 652 - 655.

Levinton JS. 1983. The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. I. Interspecific comparison of *Ophryotrocha* (Polychaeta: Dorvilleidae). *Biological Bulletin* 165: 686-698.

Lin, CP, Danforth, BN. 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. *Molecular Phylogenetics and Evolution* 30: 686-702.

Losos JB. 1992. The evolution of convergent structure in Caribbean *Anolis* communities. *Systematic Biology* 41: 403-420.

Losos JB, DeQueiroz K. 1997. Evolutionary consequences of ecological release in Caribbean *Anolis* lizards. *Biological Journal of the Linnean Society* 61: 459-483.

Losos JB, Irschick DJ, Schoener TW. 1994. Adaptation and constraint in the evolution of specialization of Bahamian *Anolis* lizards. *Evolution* 48: 1786-1798.

Losos JB, Jackman TR, Larson A, De Queiroz K, Rodriguez Schettino L. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. *Science* 279: 2115-2118.

Losos JB, Parent CE. 2009. The Theory of Island Biogeography Revisited. Princeton University Press.

Losos JB, Ricklefs RE. 2009. Adaptation and diversification on islands. *Nature* 457: 830-836.

MacArthur RH, Wilson EO. 1967. The theory of island biogeography. Princeton University Press: Princeton.

Macías-Hernández N, Oromí P, Arnedo MA. 2008. Patterns of diversification on old volcanic islands as revealed by the woodlouse-hunter spider genus *Dysdera* (Araneae, Dysderidae) in the eastern Canary Islands. *Biological Journal of the Linnean Society* 94: 589-615.

Maddison W, Hedin M. 2003. Phylogeny of *Habronattus* jumping spiders (Araneae: Salticidae), with consideration of genital and courtship evolution. *Systematic Entomology* 28: 1-22.

Manos PS, Doyle JJ, Nixon KC. 1999. Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). *Molecular Phylogenetics and Evolution* 12: 333-349.

Masson DG, Watts AB, Gee MJR, Urgelés R, Mitchell NC, Le Bas TP, Canals M. 2002. Slope failures on the flanks of the western Canary Islands. *Earth-Science Reviews* 57: 1-35.

Miller BR, Crabtree MB, Savage HM. 1996. Phylogeny of fourteen *Culex* mosquito species, including the *Culex pipiens* complex, inferred from the internal transcribed spacers of ribosomal DNA. *Insect Molecular Biology* 5: 93-107.

Moore J, Willmer P. 1997. Convergent evolution in invertebrates. *Biological Reviews* 72: 1-60.

Moore WS. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. *Evolution* 49: 718-726.

Moya O, Contreras-Díaz HG, Oromí P, Juan C. 2007. Phylogeography of a ground beetle species in La Gomera (Canary Islands): the effects of landscape topology and population history. *Heredity* 99: 322-330.

Moya O, Contreras-Diaz HG, Oromí P, Juan C. 2004. Genetic structure, phylogeography and demography of two ground-beetle species endemic to the Tenerife laurel forest (Canary Islands). *Molecular Ecology* 13: 3153-3167.

Mukabayire O, Boccolini D, Lochouarn L, Fontenille D, Besansky NJ. 1999. Mitochondrial and ribosomal internal transcribed spacer (ITS2) diversity of the African malaria vector *Anopheles funestus*. *Molecular Ecology* 8: 289-297.

Muster C, Maddison WP, Uhlmann S, Berendonk TU, Vogler AP. 2009. Arctic-alpine distributions—Metapopulations on a continental scale? *The American Naturalist* 173.

Nielsen R, Wakeley J. 2001. Distinguishing migration from isolation: A Markov Chain Monte Carlo approach. *Genetics* 158: 885-896.

Nixon KC. 2002. WinClada. ver. 1.00.08 ed. Ithaca, New York: Published by the Author.

Ogden R, Thorpe RS. 2002. Molecular evidence for ecological speciation in tropical habitats. *Proceedings of the National Academy of Sciences* 99: 13612-13615.

Opell BD, Berger AM, Shaffer RS. 2007. The body size of the New Zealand orb-weaving spider *Waitkera waitakerensis* (Uloboridae) is directly related to temperature and affects fecundity. *Invertebrate Biology* 126: 183-190.

Ottesen P, Sømme L. 1987. Adaptations to high altitudes in beetles from Tenerife. *Vieraea* 17: 217-226.

Pääbo S, Wilson AC. 1988. Polymerase chain reaction reveals cloning artefacts. *Nature* 334: 387-388.

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* 5: 568-583.

Parent CE, Crespi BJ. 2006. Sequential colonization and diversification of Galapagos endemic land snail genus *Bulimulus* (Gastropoda, Stylommatophora). *Evolution* 60: 2311-2328.

Partridge L, Barrie B, Fowler K, French V. 1994. Evolution and development of body-size and cell-size in *Drosophila melanogaster* in response to temperature. *Evolution* 48: 1269-1276.

Partridge L, Coyne JA. 1997. Bergmann's rule in ectotherms: Is it adaptive? *Evolution* 51: 632-635.

Peck SB, Wigfull P, Nishida G. 1999. Physical correlates of insular species diversity: The insects of the Hawaiian Islands. *Annals of the Entomological Society of America* 92: 529-536.

Pestano J, Brown RP. 1999. Geographical structuring of mitochondrial DNA in *Chalcides sexlineatus* within the island of Gran Canaria. *Proceedings of the Royal Society of London Series B: Biological Sciences* 266: 805-812.

Pleyte KA, Duncan SD, Phillips RB. 1992. Evolutionary relationships of the Salmonid fish genus *Salvelinus* inferred from DNA sequences of the first internal transcribed spacer (ITS 1) of ribosomal DNA. *Molecular Phylogenetics and Evolution* 1: 223-230.

Polhemus DA. 1997. Phylogenetic analysis of the Hawaiian damselfly genus *Megalagrion*(Odonata: Coenagrionidae) Implications for biogeography, ecology, and conservation biology. *Pacific Science* 51: 395-412.

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256.

Posada D, Crandall KA. 2001. Selecting the best-fit model of nucleotide substitution. *Systematic Biology* 50: 580-601.

Rambaut A, Drummond A. 2005. Tracer, A program for analysing results from Bayesian MCMC programs such as BEAST & MrBayes. 1.3 ed. Oxford: online at 663 http://evolve.zoo.ox.ac.uk /software.html?id=tracer (last accessed 12 February 2006). 664.

Ray C. 1960. The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology* 106: 85-108.

Ritland CE, Ritland K, Straus NA. 1993. Variation in the ribosomal internal transcribed spacers (ITS1 and ITS2) among eight taxa of the *Mimulus guttatus* species complex. *Molecular Biology and Evolution* 10: 1273-1288.

Roderick GK, Gillespie RG. 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. *Molecular Ecology* 7: 519-531.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496-2497.

Sequeira AS, Lanteri AA, Albelo LR, Bhattacharya S, Sijapati M. 2008. Colonization history, ecological shifts and diversification in the evolution of endemic Galápagos weevils. *Molecular Ecology* 17: 1089-1107.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

Simmons MP, Ochoterena H, Carr TG. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. *Systematic Biology* 50: 454-462.

SPSS. 2001a. SPSS for windows Version 11.0.0. Chicago, IL: SPSS Inc.

SPSS I. 2001b. SIGMAPLOT Version 7.0. Chicago, IL: SPSS Inc.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

StatSoft I. 1999. STATISTICA for windows. In: Tulsa OS, Inc., ed.

Suchard MA, Weiss RE, Sinsheimer JS. 2001. Bayesian Selection of Continuous-Time Markov Chain Evolutionary Models. *Molecular Biology and Evolution* 18: 1001-1013.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP. 2003. A plea for DNA taxonomy. *Trends in Ecology & Evolution* 18: 70-74.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619.

Thorpe RS, Black H, Malhotra A. 1996. Matrix correspondence tests on the DNA phylogeny of the Tenerife lacertid elucidate both historical causes and morphological adaptation. *Systematic Biology* 45: 335-343.

Thorpe RS, Mc Gregor DP, Cumming AM, Jordan WC. 1994. DNA evolution and colonization sequence of island lizards

in relation to geological history: mtDNA RFLP, cytochrome B, cytochrome oxidase, 12S rRNA sequence, and nuclear RAPD analysis. *Evolution* 48: 230-240.

Vandergast AG, Gillespie RG, Roderick GK. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology* 13: 1729-1743.

vanVoorhies WA. 1996. Bergmann size clines: A simple explanation for their occurrence in ectotherms. *Evolution* 50: 1259-1264.

Villablanca FX, Roderick GK, Palumbi SR. 1998. Invasion genetics of the Mediterranean fruit fly: variation in multiple nuclear introns. *Molecular Ecology* 7: 547-560.

Vink CJ, Hedin M, Bodner MR, Maddison WP, Hayashi CY, Garb JE. 2008. Actin 5C, a promising nuclear gene for spider phylogenetics. *Molecular Phylogenetics and Evolution* 48: 377-382.

Vogler AP, DeSalle R. 1994. Evolution and phylogenetic information content of the ITS-1 region in the tiger beetle *Cicindela dorsalis*. *Molecular Biology and Evolution* 11: 393-405.

Waters JM, Rowe DL, Burridge CP, Wallis GP. 2010. Gene trees versus species trees: Reassessing life-history evolution in a freshwater fish radiation. *Systematic Biology*, syq031.

Watts AB, Masson DG. 1995. A giant landslide on the north flank of Tenerife, Canary Islands. *Journal of Geophysical Research* 100: 24,487–424,498.

Watts AB, Masson DG. 1998. Reply (to comment on "A gigant landslide on the north flank of Tenerife", Canary Islands by J. Marti). *Journal of Geophysical Research* 103: 9949-9952.

Watts AB, Masson DG. 2001. New sonar evidence for recent catastrophic collapses of the North flank of Tenerife, Canary Islands. *Bulletin of Volcanology* 63: 8-19.

Weekers PHH, De Jonckheere JF, Dumont HJ. 2001. Phylogenetic relationships inferred from ribosomal ITS sequences and biogeographic patterns in representatives of the genus *Calopteryx* (Insecta: Odonata) of the West Mediterranean and adjacent West European zone. *Molecular Phylogenetics and Evolution* 20: 89-99.

Wesson DM, Porter CH, Collins FH. 1993. Sequence and secondary structure comparisons of ITSrDNA in mosquitoes (Diptera: Culicidae). *Molecular Phylogenetics and Evolution* 1: 253-269.

Whittaker JR, Triantis AK, Ladle JR. 2008. A general dynamic theory of oceanic island biogeography. *Journal of Biogeography* 35: 977-994.

Whittaker RJ, Fernández-Palacios JM. 2007. Island Biogeography: Ecology, Evolution, and Conservation. Oxford Biology.

Whittaker RJ, Triantis KA, Ladle RJ. 2010. A general dynamic theory of oceanic island biogeography: extending the MacArthur–Wilson theory to accommodate the rise and fall of volcanic islands. In: (ed. by Losos JBR, R.E.), ed. *The theory of island biogeography revisited*. Princeton, NJ.: Princeton University Press. 88–115.

Wilm A, Mainz I, Steger G. 2006. An enhanced RNA alignment benchmark for sequence alignment programs. *Algorithms for Molecular Biology* 1: 19.

Williams SM, Debry RW, Feder JL. 1988. A commentary on the use of ribosomal DNA in systematic studies. *Systematic Zoology* 37: 60-62.

Willis J. 1922. Age and area: A study in geographical distribution and origin of

species. Cambridge University Press: Cambridge.

Wörheide G, Hooper JNA, Degnan BM. 2002. Phylogeography of western Pacific *Leucetta 'chagosensis'* (Porifera: Calcarea) from ribosomal DNA sequences: implications for population history and conservation of the great barrier reef world heritage area (Australia). *Molecular Ecology* 11: 1753-1768.

Wörheide G, Nichols SA, Goldberg J. 2004. Intragenomic variation of the rDNA internal transcribed spacers in sponges (Phylum Porifera): implications for phylogenetic studies. *Molecular Phylogenetics and Evolution* 33: 816-830.

Wright S. 1951. The genetical structure of populations. *Annals of Eugenics* 15: 323-354.

Young ND, Healy J. 2002. GapCoder: http://www.trinity.edu/nyoung/GapCoder/Download.html.

Zarza E, Reynoso VH, Emerson BC. 2008. Diversification in the northern neotropics: mitochondrial and nuclear DNA phylogeography of the iguana *Ctenosaura pectinata* and related species. *Molecular Ecology* 17: 3259-3275.

Zhang D, Cook W, Horner N. 2004. ITS2 rDNA variation of two black widow species, *Latrodectus mactans* and *Latrodectus hesperus* (Araneae, Theridiidae). *Journal of Arachnology* 32: 349-352.

Zhuo L, Sajdak SL, Phillips RB. 1994. Minimal intraspecific variation in the sequence of the transcribed spacer regions of the ribosomal DNA of lake trout (*Salvelinus namaycush*). *Genome* 37:664-671.

Capítulo 8

GENERAL CONCLUSIONS

1. Canarian species of the genus *Dysdera* belong to three well-supported, independent evolutionary lineages: the Central-Western clade, the Eastern clade, and the species *Dysdera lancerotensis*, which is more closely related to northern African species. Relationships among the Central-Western and Eastern lineages are ambiguous, although former suggestions on the independent origin of the Tenerife endemic *Dysdera unguimmanis* are rejected.

2. Lineage age estimations based on relaxed molecular clock models suggest a late Miocene origin of the Eastern clade, and a younger origin, from the Plio-Pleistocene, for the colonization of the ancestor of *Dysdera lancerotensis*.

3. Biogeographical reconstruction based on phylogenetic relationships suggests eight dispersal events among the eastern islands, two of which involved Fuerteventura and Lanzarote, probably favored by eustatic sea-level changes during glacial cycles.

4. Diversification rate analyses suggest a deceleration of net speciation through time in the Eastern clade, which is compatible with increasing extinction rates due to loss of suitable habitats induced by island erosion.

5. Low correlation between morphological differentiation and species coexistence observed in eastern *Dysdera* species may be explained by the extinction of previously codistributed species, corroborating the former conclusion on increasing extinction rates in the clade.

6. The phylogenetic analyses of the Eastern clade reveal three previously overlooked evolutionary lineages that can be delimited on the basis of molecular, morphological, ecological and geographical diagnostic traits. Three new species are here described: *Dysdera simbeque* (endemic to northern Lanzarote), *Dysdera mahan* (endemic to the eastern islands and islets) and

Dysdera aneris (endemic to the Salvage archipelago). These results constitute an example of integrative taxonomy.

7. The intertidal habitat of the new species *Dysdera mahan* represents a unique case of habitat shift in the genus. Moreover, this is the most recent speciation event in the Eastern clade and, probably an example of ecological speciation.

8. The lack of correlation between genetic and morphological differentiation in the eastern species suggests a different involvement of natural selection in each speciation event.

9. Post-erosional volcanic activity in the eastern islands has shaped phylogeographic and demographic patterns in *Dysdera lancerotensis*. The proposed evolutionary scenario involves several bottlenecks due to population extinction by lava flows, followed by population expansions. Several geographical areas are identified as volcanic refugia for local populations.

10. Contrary to expectations based on the time of origin of the main volcanic edifices, *Dysdera lancerotensis* shows a North to South colonization pattern. Colonization from Lanzarote to Fuerteventura was the result of a contiguous range expansion, probably favored by land-bridge connections induced by eustatic sea-level changes.

11. Phylogenetic analyses of *Dysdera lancerotensis* detect two independent and divergent mtDNA haplotype lineages, the northern islets and Lanzarote-Fuerteventura, which are the result of isolation and allopatric fragmentation involving quaternary volcanic activity on northern Lanzarote.

12. In spite of the ample opportunities for gene flow during island connections induced by sea-level changes, the northern islets show clear genetic differentiation. The reconstructed colonization pattern identifies Alegranza as the source of colonization of La Graciosa, from where Montaña Clara was populated. These populations movements are inferred to be the result of past

gradual range expansion followed by fragmentation, probably driven by recolonizations following extinction processes.

13. The species *Dysdera alegranzaensis* and *Dysdera nesiotes*, which coexist in Lanzarote and the northern islets and have similar morphological and ecological features, exhibit marked differences in their phylogeographic and demographic patterns.

14. The phylogeographic pattern found in *Dysdera alegranzaensis* mtDNA haplotypes reveals low geographic structure, high levels of gene flow and high genetic diversity within populations. Population expansion events are identified, which are compatible with bottlenecks following lava flow driven extinctions. These phylogeographic patterns resemble those of *Dysdera lancerotensis*, which also share the genetic isolation of the northern islets, and similar locations that served as volcanic refugia.

15. Phylogeographic and demographic patterns in *Dysdera nesiotes* differ from those of the aforementioned species (*Dysdera alegranzaensis* and *Dysdera lancerotensis*), in being more similar to a meta-population type. High levels of phylopatry are observed within localities, but nearby locations show low genetic affinity.

16. Despite the apparent lack of biological and ecological differences between *Dysdera alegranzaensis* and *Dysdera nesiotes*, their underlying phylogeographic patterns allow us to hypothesize that the two species coexist in a dynamic equilibrium. Unlimited resources would enable coexistence of both species. If those resources were depleted, however, *Dysdera alegranzaensis* would outcompete *Dysdera nesiotes*. If favorable conditions were restored, new migrants would restart locally extirpated populations of the later species. Further experimental studies under controlled conditions will be necessary to confirm this hypothesis.

17. MtDNA haplotypes of the Tenerife endemic *Dysdera verneaui* are paraphyletic with regard to *Dysdera gomerensis*, an endemism from La Gomera and El Hierro. Two divergent mitochondrial lineages have been detected in *Dysdera verneaui*. One is restricted to the Teno region and probably originated before the three protoislands that formed Tenerife joined together. The second lineage is distributed throughout the rest of the island, including the terrains that belonged to two of the protoislands.

18. Mitochondrial and nuclear gene trees of *Dysdera verneaui* are incongruent. Nuclear gene trees support monophyly of *Dysdera verneaui* populations. Identification of, at least, one instance of mitochondrial introgression from the rest of Tenerife to Teno suggests that the higher nuclear similarity between Teno and the rest of the island is due to genetic homogenization following the split of *Dysdera gomerensis*. Infrequent, but recurrent introduction of nuclear genes from the rest of the island to the population of Teno will increase genome similarity between island lineages.

19. The geological history of Tenerife played a key role in shaping the distribution of genetic diversity and population structure of *Dysdera verneaui*. The origin of some populations probably preceded the consolidation of the present-day island. The high levels of diversity and gene flow detected between the populations of central Tenerife and Anaga, as well as the identification of population expansion events, is probably the result of the intense volcanism that built the central part of the island.

20. The presence of exclusive mitochondrial haplotypes and nuclear lineages in Teno, their high genetic divergence with regard to the individuals from the rest of the island, and the identification of a diagnostic morphological character on the male bulb point towards the existence of an overlooked, cryptic species. Genetic isolation between the Teno species and *Dysdera verneaui*, however, is not complete and at least one case of mitochondrial introgression has been detected.

21. In agreement with Bergmann's rule, a significant correlation was observed between elevation and body size in *Dysdera verneaui*. Body size does not seem to be related to genetic similarity, suggesting that differences in body size are driven by adaptation to local environmental conditions.

CONCLUSIONES GENERALES

1. Se confirma que las especies canarias de *Dysdera* se agrupan en tres linajes evolutivos independientes: el clado centro-occidental, el oriental y la especie *Dysdera lancerotensis* que está más relacionada filogenéticamente con especies del continente africano. Las relaciones entre los dos linajes principales son ambiguas, pero los datos permiten rechazar que la especie de Tenerife *Dysdera unguimmanis* esté más relacionada con especies continentales, tal y como se había sugerido anteriormente.

2. Las dataciones de la filogenia mediante métodos de relojes moleculares relajados indican que el origen del clado oriental se remonta al Mioceno tardío, mientras que la colonización que dio origen a *Dysdera lancerotensis* es posterior, durante el Plio-Pleistoceno.

3. La reconstrucción del patrón biogeográfico a partir de las relaciones filogenéticas indica la existencia de ocho eventos de dispersión/colonización en la islas orientales, dos de ellos desde Fuerteventura a Lanzarote, que pudieron verse favorecidos por los cambios eustáticos del nivel del mar, cuyo descenso conectó islas e islotes durante las glaciaciones acaecidas durante el Pleistoceno.

4. Los análisis de diversificación de los linajes muestran un descenso de la tasa de diversificación a lo largo del tiempo en el clado oriental, lo que es compatible con un incremento de los procesos de extinción, probablemente favorecidos por la pérdida de hábitat adecuado por efecto de la erosión.

5. La baja correlación entre diferenciación morfológica y coexistencia en las comunidades de *Dysdera* orientales, puede explicarse por la extinción de taxones anteriormente codistribuidos, corroborando el punto anterior.

6. Los análisis filogenéticos del clado oriental revelan tres linajes evolutivos, no reconocidos previamente, con caracteres diagnósticos de tipo molecular, morfológico, ecológico y geográfico que permiten su delimitación como nuevas

especies. Se describen tres nuevas especies: *Dysdera simbeque* (endémica del norte de Lanzarote), *Dysdera mahan* (endémica de ambas islas orientales e islotes) y *Dysdera aneris* (endémica del archipiélago de Salvajes). Estos resultados constituyen un ejemplo de taxonomia integrativa.

7. La nueva especie *Dysdera mahan* es un caso único de cambio ecológico (*habitat shift*) dentro del género, ya que se especializa en la vida en el intermareal. Además se trata del evento de especiación más reciente del clado oriental, y posiblemente un ejemplo de especiación ecológica.

8. No existe correlación entre diferenciación genética y morfológica en las nuevas especies del clado oriental, lo que sugiere papeles distintos de la selección natural en los diferentes eventos de especiación.

9. La estructura filogeográfica y demográfica de *Dysdera lancerotensis* se ha visto influenciada y modelada por los eventos volcánicos acaecidos en las islas orientales durante los últimos millones de años. El escenario general sugiere cuellos de botella poblacionales producto de la extinción por acción directa del vulcanismo, seguida de procesos de expansión poblacional. Se identifican diferentes zonas que actuaron como refugios.

10. *Dysdera lancerotensis* muestra un patrón de colonización de las islas orientales de norte a sur, opuesto al esperado en función de la formación geológica de estas islas. La estructura filogeográfica sugiere que la dispersión de esta especie desde Lanzarote a Fuerteventura es el resultado de un rango de expansión continuo, probablemente favorecido por las conexiones entre ambas islas asociados a las fluctuaciones en el nivel del mar.

11. Los análisis filogenéticos de *Dysdera lancerotensis* detectan dos linajes haplotípicos independientes (islotes y Lanzarote-Fuerteventura), cuya diferenciación es el resultado de procesos de aislamiento y fragmentación alopátrica, coincidentes con la actividad volcánica más reciente acaecida en la zona norte de Lanzarote.

12. La diferenciación genética encontrada en cada uno de los islotes sugiere que las conexiones entre Lanzarote y los islotes favorecidas por los cambios eustáticos del nivel del mar pudieron no ser tan frecuentes. El patrón de colonización detectado indica que Alegranza es la fuente de colonización de La Graciosa, y ésta de Montaña Clara, resultado de una expansión gradual pasada de rango de gradual seguida de fragmentación, provocado por procesos de extinción, seguidos de recolonización.

13. Las especies *Dysdera alegranzaensis* y *Dysdera nesiotes,* codistribuidas en Lanzarote e islotes, poseen características morfológicas y ecológicas similares pero muestran patrones filogeográficos y demográficos distintos.

14. El patrón filogeográfico encontrado en *Dysdera alegranzaensis* refleja poca estructuración geográfica de los haplotipos con elevada diversidad genética dentro de las poblaciones, y altos niveles de flujo génico. Se detectan episodios de expansión poblacional, compatibles con cuellos de botella debido a extinciones por vulcanismo. En general los patrones filogeográficos son muy parecidos a los de *Dysdera lancerotensis*, reconociéndose también una fuerte estructuración y aislamiento del archipiélago Chinijo, así como áreas de refugio volcánico comunes.

15. Los patrones filogeográficos y demográficos de *Dysdera nesiotes* difieren de las especies anteriores (*Dysdera alegranzaensis* y *Dysdera lancerotensis*), y son de tipo más metapoblacional. Se observa una elevada filopatría de sus poblaciones con baja afinidad genética por proximidad geográfica.

16. A pesar de la imposibilidad de detectar diferencias biológicas y ecológicas entre *Dysdera alegranzaensis* y *Dysdera nesiotes*, los patrones filogeográficos de ambas especies permiten hipotetizar que la coexistencia de ambas especies es fruto de un equilibrio dinámico. En situaciones ecológicas óptimas ambas especies podrían convivir evitando la competencia por los recursos. Sin embargo, en periodos de escasez de alimento, debido a cambios climáticos o episodios volcánicos, *Dysdera alegranzaensis* desplazaría a *Dysdera nesiotes* (bien por

competencia directa o indirecta). Se propone contrastar esta hipótesis en el futuro mediante experimentos de laboratorio con ambas especies.

17. Los haplotipos mitocondriales de la especie endémica de Tenerife *Dysdera verneaui* son parafiléticos respecto a *Dysdera gomerensis* (endémica de La Gomera y El Hierro). Se detectan dos linajes mitocondriales divergentes de *D. verneaui*, uno restringido a la región de Teno (cuyo origen es probablemente anterior a la unión de las protoislas) y un segundo distribuido por el resto de la isla.

18. Se detecta incongruencia entre los árboles de genes mitocondriales y los nucleares. En estos últimos las poblaciones de *Dysdera verneaui* si aparecen como monofiléticas. La detección de un caso de introgresión mitocondrial desde el resto de la isla a Teno, sugiere que la mayor similitud nuclear entre Teno y el resto de la isla pueda ser debida a la homogeneización genética posterior a la separación de *Dysdera gomerensis,* mediada por la introducción infrecuente, pero recurrente de genes nucleares del resto de la isla en la población de Teno.

19. La historia geológica de Tenerife es un factor clave en la distribución de la diversidad genética y la estructuración de las poblaciones de *Dysdera verneaui*. El origen de algunas poblaciones probablemente precedió la consolidación de la isla actual. La elevada diversidad y flujo génico detectado entre las poblaciones centrales y de Anaga, así como la identificación de fenómenos de expansión poblacional es probablemente indicativo de la intensa actividad volcánica acaecida durante la formación de la parte central de la isla.

20. La presencia de linajes mitocondriales y nucleares exclusivos en Teno, su elevada divergencia genética respecto a las poblaciones del resto de la isla y la presencia de un carácter morfológico diagnóstico en el órgano copulador de los machos, sugieren la existencia de una especie críptica. Sin embargo, el aislamiento genético de ambas especies no es total, ya que se ha detectado al menos un caso de introgresión mitocondrial.

21. En *Dysdera verneaui* se detecta una correlación significativa entre el incremento del tamaño corporal y la altitud, acorde con la regla de Bergmann. No se observa un componente genético asociado a esta diferenciación morfológica, lo que permite sugerir que dicha diferenciación morfológica podría ser resultado de la adaptación a las condiciones ambientales locales.