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Histological and ultrastructural features of the peculiar seta and stem of *Leucodon canariensis* (Leucodontaceae)

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ABSTRACT

The seta and stem in *Leucodon canariensis* are examined by means of light microscopy, transmission and scanning electron microscopy. Their anatomies reveal relevant differences although both show a remarkable structural complexity, as their conducting tissues are equally effective in carrying out essential processes. Five types of cells have been recognized in the transverse sections: (i) in both seta and stem: stereids in the external region with a peripheral cuticle; (ii) in the stem only: parenchymatous cells with plasmodesmata underlying the stereids; in the seta only: (iii) an unusual area of nacreous-walled cells without live protoplasm, surrounding (iv) the food-conducting cells (leptoid-like), and again both in seta and stem: (v) hydroids in the internal region. The seta in this species has a peculiar organization and shares some characteristics with polytrichaceous mosses; the ultrastructural similarities and differences and functional significance of these cells are discussed, both systematically and in relation to the habit of the moss itself.

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Introduction

The gametophyte is the dominant generation in mosses and generally consists of leafy haploid shoots with a stem that configurates the plant architecture and allows the colonization of its habitat and occupation of a wider space, while the simple, unbranched sporophyte is dependent upon the gametophyte. This diploid phase presents a foot embedded in gametophyte tissue, a seta or stalk for food-conduction and connection of both extremes, and an apical capsule. The junction complex between gametophyte-sporophyte is a crucial region for bryophyte life strategy (Uzawa and Higuchi 2010), as the diploid generation is matrotrophic (Graham and Wilcox 2000; Haig 2013). Mosses, as non-lignified plants, are considered as lacking a true vasculature (Ligrone et al. 2000, 2008), although recently, Brodribb et al. (2020) showed that mosses (*Polytrichum commune* Hedw.) exhibit functional parallels with the vascular system of higher plants.

Several studies have dealt with the anatomy of the moss gametophyte in recent years, but the anatomy of the sporophytic seta remains comparatively little studied.

A summary of the main works on the anatomy of moss gametophytes and the setae in Bryophytina sensu Liu et al. (2019) are listed in Table 1. In essence, the stem consists of three main regions: (1) an epidermis, usually with thick-walled cells, but in some taxa, sometimes corresponding with stereids, (2) a cortex, divided into an outer, thick-walled sclerodermis and an inner zone of parenchymatous cells (often conducting parenchyma), and (3) a central strand, sometimes absent, consisting of hydroids [water-conducting cells (WCCs) with no cytoplasm], usually with very thin walls. In Polytrichopsida, hydroids have thick walls and are surrounded by specialized, food-conducting leptoids.

The seta anatomy parallels that of the stem, although it is often more complex: the wax-covered epidermis is more specialized as an isolating tissue, and the hydroid central strand is generally well developed and more often associated with a phloem-like tissue: true leptoids in Polytrichopsida or specialized, parenchymatous food-conducting cells (FCCs) of controversial identity in other taxa (sometimes regarded also as leptoids *s.l.*, see Glime 2017a; Woudenberg et al. 2022).

Most of these studies have focused on acrocarpous rather than pleurocarpous mosses. This last group includes the genus *Leucodon* Schwägr., as the five Mediterranean and Macaronesian species of this genus (Hodgetts et al. 2020) show a similar gametophyte morphology, and they fructify only rarely, their identification is usually difficult (Akiyama 1988, 1994). Although some recent molecular studies on North Atlantic *Leucodon* species (Stech et al. 2011) have yielded useful information on phylogenetic and biogeographic relationships, several questions remain unsolved, notably the taxonomic status of *Leucodon immersus* Lindb. and the affinities of *Leucodon canariensis* (Brid.) Schwägr.

The study of the anatomy and ultrastructure could be useful both in identification and in assessing systematic affinities in the genus. However, the scarce histological observations refer only to a few gametophytic characters in *Leucodon*

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Table 1. Conducting tissues of stems and setae in a diversity of bryoid moss genera (only taxa with TEM-observed conducting tissues are included, although not necessarily all the references include TEM microcraphs).

	Author	Conducting tissues (outwards to inwards)
Gametophyte stem		
Oedipodium Schwägr.	Ligrone and Duckett 2011	No data on FCCs, central strand of thin-walled hydroids.
Atrichum P.Beauv.	Hébant 1967, 1968, 1969, 1977; Stevenson 1974, Schofield and Hébant 1984; Scheirer 1990; Ligrone and Duckett 1994; Ligrone et al. 2002	True leaf traces, endodermal-like tissue; true leptoids with nacreous walls as FCC, well-developed central strand of thick-walled hydroids.
Dawsonia R.Br.	Hébant 1969, 1975, 1977; Scheirer 1990; Ligrone et al. 2002; Glime 2017a	True leaf traces, endodermal-like tissue; true leptoids as FCC, well-developed central strand of thick-walled hydroids.
Dendroligotrichum (Müll.Hal.) Broth.	Hébant 1973, 1976, 1977; Scheirer 1980; Hébant in Scheirer 1990; Ligrone et al. 2002	True leaf traces, endodermal-like tissue; true leptoids as FCC, well-developed central strand of thick-walled hydroids.
Polytrichadelphus (Müll.Hal.) Mitt.	Hébant 1974, 1977	True leaf traces, endodermal-like tissue; true leptoids as FCC, well-developed central strand of thick-walled hydroids.
Polytrichum s. l. (including *Polytrichum* Hedw. and *Polytrichastrum* G.L.Sm.)	Hébant 1975, 1977; Schofield and Hébant 1984; Scheirer 1990; Ligrone and Duckett 1996; Ligrone et al. 2000; Ligrone et al. 2002; Pressel et al. 2006	True leaf traces, endodermal-like tissue; true leptoids, sometimes with nacreous walls, as FCC, well-developed central strand of thick-walled hydroids.
Pogonatum P.Beauv.	Hébant 1974, 1977; Ligrone and Duckett 1994	True leaf traces, endodermal-like tissue; true leptoids as FCC, well-developed central strand of thick-walled hydroids.
Buxbaumia Hedw.	Ligrone et al. 1982	No explicit data on FCC; rudimentary stem with lipid-rich, highly vacuolated cells, no central strand of hydroids.
Funaria Hedw.	Hébant 1969, 1977; Schulz and Wiencke 1976, Ligrone and Duckett 1994	Both true and false leaf traces, conducting parenchyma of polarized cells as FCC, central strand of thin-walled hydroids.
Timmiella (De Not.) Limpr.	Ligrone et al. 1980	Conducting parenchyma of polarized cells as FCC, central strand of thin-walled hydroids.
Grimmia Hedw.	Kawai 1965; Estébanez 1995	Conducting parenchyma with porose walls (with primary pit fields) as FCC; small strand of thin-walled hydroids (lacking in some species).
Leucophanes Brid.	Favali and Bassi 1978	Homogeneous in cross section, conducting parenchyma with primary pit fields as FCC, no hydroids.
Bryum s.l. (including *Bryum* Hedw. and *Ptychostomum* Hornsch.)	Ligrone and Duckett 1994	False leaf traces, conducting parenchyma of polar cells as FCC, central strand of thin-walled hydroids.
Mnium s.l. (including *Mnium* Hedw. and *Plagiomnium* T.J.Koponen)	Hébant 1967, 1968; Ligrone and Duckett 1994, 1996; Ligrone et al. 2000; Ligrone et al. 2002; Glime 2017a, 2017b	False leaf traces, conducting parenchyma of polar cells as FCC, central strand of thin-walled hydroids.
Aulacomnium Schwägr.	Ligrone and Duckett 1994; Ligrone et al. 2000	Conducting parenchyma of polarized cells as FCC, central strand of thin-walled hydroids.
Hookeria Sm.	Hébant 1975; Cortella et al. 1994	Conducting parenchyma with porose walls (with primary pit fields) as FCC; small central strand of thin-walled hydroids.
Hylocomium Schimp.	Bonnot 1967; Sokolowska et al. 2017	Conducting parenchyma in inner cortical layers as FCC, consisting of elongated cells with numerous thin-walled areas (probable primary pit fields), with no central strand of hydroids.
Pleurozium Mitt.	Noailles 1974; Sokolowska et al. 2017	Conducting parenchyma in both inner and outer cortical layers as FCC, cells with oval, thin-walled areas (probable primary pit fields), small central strand of thin-walled hydroids.
Isothecium Brid.	Alfayate 1995	Conducting parenchyma with primary pit fields in transversal and longitudinal walls) as FCC; small central strand of thin-walled hydroids.
Thuidium Schimp.	Finocchio 1967; Bonnot (in Hébant 1977)	Inner cortical cells with pitted transverse walls as possible FCC, no central strand of hydroids.
Neckera Hedw.	Ligrone and Duckett 1994, Alfayate 1995; Ligrone et al. 2000	Conducting parenchyma of polarized cells (with primary pit fields in transversal and longitudinal walls) as FCC; no central strand of hydroids.
Cryptoleptodon Renauld & Cardot	Alfayate 1995	Conducting parenchyma with porose transversal and longitudinal walls (with primary pit fields) as FCC; no central strand of hydroids.
Sporophyte seta		
Oedipodium Schwägr.	Ligrone and Duckett 2011	No explicit data on FCC (apparently conducting parenchyma), central strand of thin-walled hydroids.
Dawsonia R.Br.	Hébant 1975, 1977	Lacunar ring of parenchymatous cells with plasmodesmata-rich walls, and inner layer of leptoids with oblique transverse walls, as FCC; central strand of thin-walled hydroids.
Dendroligotrichum (Müll.Hal.) Broth.	Hébant 1975, 1976, 1977;	Lacunar ring of parenchymatous cells with plasmodesmata-rich walls, and inner layer of leptoids with oblique transverse walls, as FCC; central strand of thin-walled hydroids.
Pogonatum P.Beauv.	Favali and Gianni 1975; Ligrone and Duckett 1994	Lacunar ring of parenchymatous cells with plasmodesmata-rich walls, and inner layer of leptoids with oblique transverse walls, as FCC; central strand of thin-walled hydroids.
Polytrichum s.l. (including *Polytrichum* Hedw. and *Polytrichastrum* G.L.Sm.)	Favali and Bassi 1974; Hébant 1977; Ligrone and Duckett 1994; Ligrone et al. 2000	Lacunar ring of parenchymatous cells with plasmodesmata-rich walls, and inner layer of leptoids with oblique transverse walls, as FCC; central strand of thin-walled hydroids.
Buxbaumia Hedw.	Ligrone et al. 1982	Extensively lacunar cortex as probable FCC, with plasmodesmata-rich transverse walls; small central strand of few thin-walled hydroids.

(*Continued*)

Table 1. Continued.

	Author	Conducting tissues (outwards to inwards)
Funaria Hedw.	Schulz and Wiencke 1976; Hébant 1977; Ligrone and Duckett 1994	Tibia-like, polarized cells as FCC, central strand of thin-walled hydroids.
Timmiella (De Not.) Limpr.	Ligrone and Duckett 1994	Conducting parenchyma of polarized cells as FCC, central strand of thin-walled hydroids.
Grimmia Hedw.	Estébanez 1995	Conducting parenchyma with thin transversal walls with plasmodesmata as FCC; very small central strand of thin-walled hydroids.
Leucophanes Brid.	Favali and Bassi 1978	Both thick-walled peripheral layers and intermediate layers probably acting as FCC (with plasmodesmata); central strand of thin-walled hydroids.
Tortula Hedw.	Favali and Gianni 1973	Conducting parenchyma with transversal walls rich in plasmodesmata, central strand of thin-walled hydroids.
Mnium s.l. (including *Mnium* Hedw. and *Plagiomnium* T.J.Koponen)	Bassi and Favali 1973, Ligrone & Duckett 1994, 1996, Ligrone et al. 2000	Conducting parenchyma with transversal walls rich in plasmodesmata as FCC, central strand of thin-walled hydroids.
Isothecium Brid.	Alfayate 1995	Parenchyma with thin walls and scarce plasmodesmata (FCC?), small central strand of ca. 20 thin-walled hydroids.
Neckera Hedw.	Alfayate 1995	Parenchymatous cortex with no plasmodesmata observed, small central strand (*ca.* 15 cells) of thin-walled hydroids.
Cryptoleptodon Renauld & Cardot	Alfayate 1995	Parenchymatous cortex with no plasmodesmata observed, small central strand (*ca.* 15 cells) of thin-walled hydroids.

sciuroides (Hedw.) Schwägr., *L. sciuroides* var. *morensis* Schwägr. (Fuertes et al. 1977), *L. canariensis* (Velázquez 1994) and the European–Asian species of *Leucodon* (Werner et al. 2015).

L. canariensis (Brid.) Schwägr. (Leucodontaceae) is a moss endemic to the Canary Islands and Madeira, a mesophilous pleurocarpous and plagiotropic moss, epiphyte on tree bark, with a crawling-shoot type of growth. Its life-form type is a 'tail' (shade-loving; radially leafed; creeping, shoots stand away from substrate) according to Magdefrau (1982). The sporophyte grows slowly and presents a very long seta (14–24 mm) supporting a subspherical capsule with arthrodontous peristome teeth.

In this paper we examine the detail of the histology and ultrastructure of seta and stem of *L. canariensis* from a laurel forest of Tenerife (Canary Islands).

Material and methods

Plant material

Samples of *L. canariensis* (Brid.) Schwägr., (Leucodontaceae Schimp., Hypnales; Hill et al. 2006), were collected from a wet laurisilva at Monte de las Mercedes (948 masl, 28RCS566749; Tenerife, Canary Islands), a several million-year-old relict forest (Pliocene 6 my; Fernández-Palacios et al. 2011). Voucher specimens were deposited at the Herbarium of the Biology Faculty (TFC), La Laguna University, with numbers: TFCBry no. 9566, TFCBry no. 9567 and TFCBry no. 17531.

Sample preparation

The seta and stem were excised and processed for light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM), using standard protocols as follows.

The same number of individuals was taken from each of the populations (3) mentioned above (TFCBry nos. 9566, 9567, 17531). The sample size for each technique was: for fresh sections (LM) and histochemistry: seta $n=3$, fertile stem $n=3$, sterile stem $n=3$; for samples embedded in resin (LM and TEM): seta $n=3$, fertile stem $n=3$, sterile stem $n=3$; and SEM: seta $n=3$, fertile stem $n=3$, sterile stem $n=3$. The seta development stage is post-meiotic, applied to all techniques.

LM

Observations were made with a LM Leica DM4000B using a Leica QWin computer image apprehension system on semithin sections (1 µm) of resin-embedded material (seta and stem), obtained with glass knives and stained with toluidine blue, and fresh sections (5 µm) of samples of setae and stems obtained with a frozen-microtome.

TEM

Middle portions of setae and stems were cut into pieces 2 mm in length and fixed for 2 h in 3% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4, at room temperature. After washing in 0.1 M in PB, they were postfixed in 1% OsO_4 in PB 0.1 M and washed again in buffer solution. The samples were dehydrated in a graded ethanol series beginning at 30% ethanol, treated with absolute ethanol and propylene oxide and embedded in Spurr's (1969) resin. Semithin (1 µm) and ultrathin sections (70–90 nm) were produced using a Reichert-Jung Ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate. They were studied using a Zeiss 902 at Microscopy and Cytometry Centre, Complutense University, Madrid, or a JEOL JEM-1010 microscope at the Interdepartament Research Service, Universidad Autónoma de Madrid, operating a 100 kV.

SEM

The setae and stems were fixed in 3% glutaraldehyde buffered in Na-cacodylate 0.1 M; then rinsed in 3% sucrose-Na-cacodylate 0.1 M buffer and dehydrated in an acetone series. Critical point drying was carried out after substituting acetone with liquid CO_2. Samples were sputtered with a gold coat (ca. 300 Å). They were observed with a JOEL-JSM-T-330A microscope (CSIC, Royal Botanical Garden, Madrid).

Histochemical studies

Some samples were also prepared for histochemical studies using LM. Tests were performed on frozen-microtome sections of setae and stems obtained from fresh material with a Reichert-Jung 1130/Biocut.

The following histochemical tests were performed: toluidine blue (Sakai 1973; proteins), IKI (Johansen 1940; starch), Zn–Cl_2–I (Rawlins and Takahashi 1952; cellulose), phloroglucinol (Johansen 1940; Siegel 1953; lignin), ruthenium red (Johansen 1940; pectic substances), Sudan III (Johansen 1940; neutral lipids), Sudan Black B (Jensen 1962; total lipids), Nile blue (Jensen 1962; phospholipids), OsO_4 (Parducz 1967; unsaturated lipids) and aniline blue (Johansen 1940; callose). In all cases, the appropriate positive controls were used.

Results

The seta in the post-meiotic sporophyte (Figures 1 and 2) is organised into four concentric histological layers (Figure 1(A)), not so the stem (Figure 3). Both the outermost layer, consisting of stereids (thus a stereome: ST; Figures 1(A,B)), and the central strand of hydroids (thus a hydrom: Hy; Figures 1(A) and 2(E,F)) are similar to the corresponding layers in the leafy stem (Figure 3(A)). The two intermediate layers of the seta do not have an equivalent in the gametophyte, where this space is filled by homogeneous parenchyma (Figures 1(A) and 3(A)). In the seta, the layer underlying the stereome consists of nacreous thickening cells and the layer adjacent to the central hydrom contains FCCs [leptoids and specialized parenchyma cells as defined by Ligrone et al. (2000) and Pressel et al. (2006); Figures 1(A,F) and 2(A–C)].

Seta of the sporophyte

The peripheral stereome (with orange cell walls in fresh sections) is formed by two to three layers of thick electron-dense-walled living cells (Figure 1(A,B)). The cytoplasm of these stereids contains large vacuoles, lipid inclusions, mitochondria and chloroplasts showing well-developed grana, starch granules and plastoglobuli (Figure 1(B,C)). A thin electron-lucent cuticle is observed in the outermost region of the tangential walls (Figure 1(B)). The cuticle comprises visually distinct layers, defined following Jeffree (2006). The cell wall projections (CWPs) appear as reticulations on the outside edge of the cells (Figure 1(B,Bi)). The cuticle proper (CP) lies exterior to the projections. The CP is a uniform medium electron density layer just outside the dark edge of the cell wall matrix (Figure 1(Bi)).

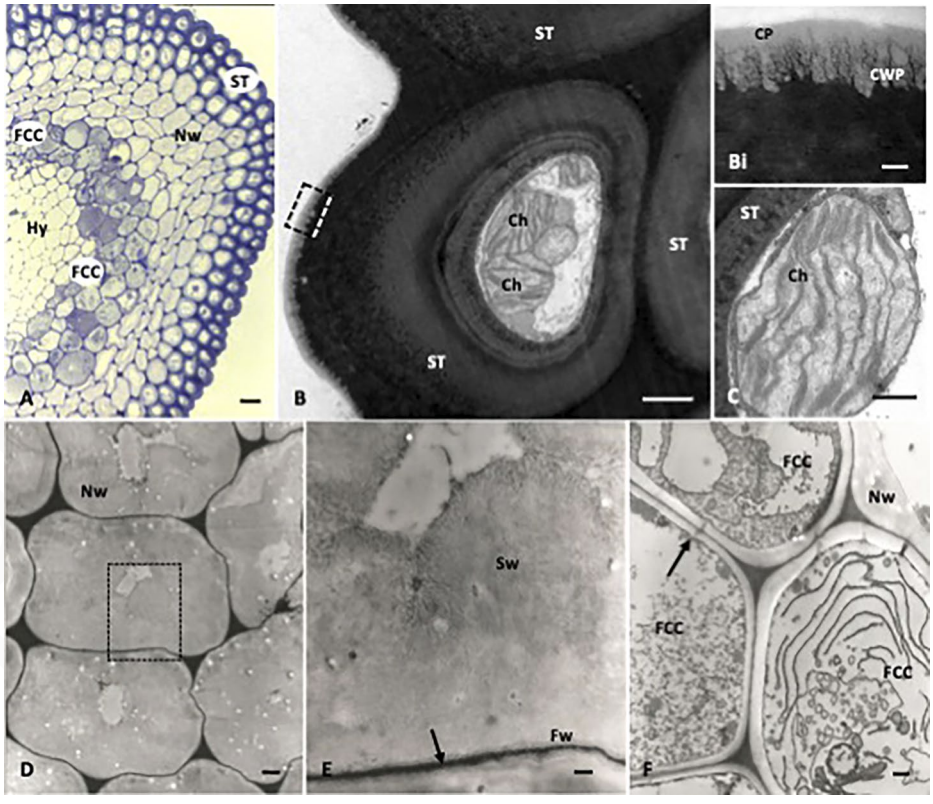

Figure 1. Sporophyte seta of *L. canariensis* (Brid.) Schwägr. (A) Light micrograph of a transverse section of the seta. Four cell types are recognized from the outside to the inside of the section: stereids (ST) in the outer region, underlying an unusual area of nacreous cells (Nw), contiguous are the FCCs/leptoids (FCC) and the hydroids (Hy) in the inner region. (B–F) Transmission electron micrographs of transverse sections of seta. (B) Cell of the outermost layer of the seta showing thick-walled peripheral, plastids (Ch), mitochondria and vacuoles. (Bi) Detail of the framed region in (B). Cuticle of bi-layered structure that coat on the external surface, CP: cuticle proper, CWPs: cell wall projections. (C) Detail of chloroplasts (Ch) with well-developed grana and thylakoids. (D) Several layers of cells with thick walls and narrow lumen, similar to nacreous-walled sieve elements of vascular plants. (E) Detail of framed region in (D). The first wall (Fw) is thin and electron-dense and, in contrast, the second wall (Sw) of the nacreous-walled cell has a low electron-dense appearance and contains microfibrils. Middle lamina: arrow. (F) FCCs/leptoids (FCC) that show different cytoplasmic appearances and plasmodesmata (arrow) in their walls. These cells show dense granular material, irregular plastids, endoplasmic reticulum, lipid inclusions, numerous vesicles and membrane profiles throughout the electron-transparent cytoplasm. Scale bar: A = 10 µm; B,D,F = 1.1 µm; E = 0.6 µm; C = 0.5 µm; Bi = 100 nm.

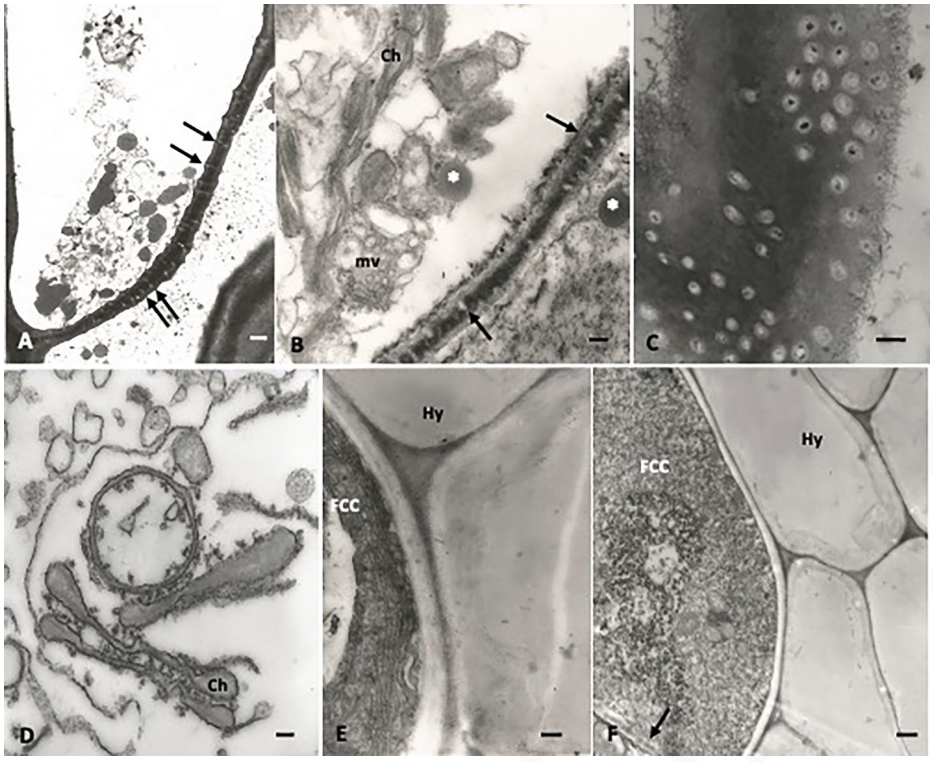

Figure 2. Sporophyte seta of *L. canariensis* (Brid.) Schwägr. (A–F) Transmission electron micrographs of seta. (A) Two FCCs/leptoids cells of the middle region in longitudinal section, separated by a perforated oblique end-wall (arrows). (B) Detail of the wall of two cells connected by abundant plasmodesmata (arrows) in transverse section. The content of these cells consists of chloroplasts (Ch), multivesicular body (mv), cytoplasmic remnants as membranous structures, granular material and lipid-like inclusions (white asterisk). (C) Numerous plasmodesmata in oblique end-wall of FCCs/leptoid. (D) Pleomorphic plastids (Ch), vesicles and remnants of membranes inside FCCs/leptoid cell. (E,F) Transverse section of FCCs/leptoids (FCC) and adjacent hydroids (Hy) in a seta. Note the FCCs/leptoids (FCC) with a cytoplasmic content consisting of a degenerating nucleus, dense granular material, mitochondria and sheets of endoplasmic reticulum adjacent to the inner wall layer, and hydroids (Hy), as empty cells with their thin and low electron-dense walls. Scale bar: A = 2.5 µm; F = 1.1 µm; B,C,E = 0.6 µm; D = 0.4 µm.

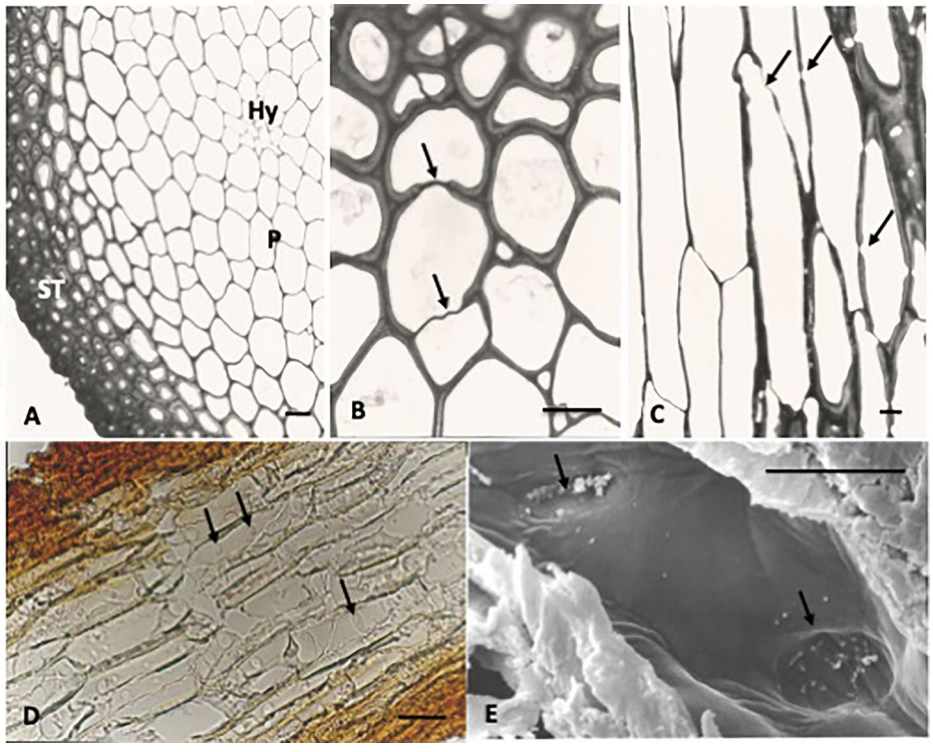

Figure 3. Stem of *L. canariensis* (Brid.) Schwägr. (A–D) Light micrographs of stem. (A) Transverse section of the mature stems, illustrating the external layers of stereids (ST), the middle parenchymatous cortex (P) and the small central strand of hydroids (Hy). (B,C) Stems in transverse (B) and longitudinal sections (C), details of the parenchymatous cells showing thin-walled areas (arrows), representing pit-like areas. (D,E) Longitudinal sections of the stems with parenchymatous cells showing plasmodesmata in pit fields (arrows) on the anticlinal walls, by LM of fresh material (D) and SEM (E). Scale bar: D = 25 µm; A–C = 10 µm; E = 5 µm.

In the next zone innerwards, the cells with nacreous wall thickenings are arranged in three layers (Figure 1(A)) with few intercellular spaces. Ultrastructurally (Figures 1(D,E)) they lack whole protoplasms but may contain degenerated remains, such as fragments of the plasmalemma. The medial lamina is thin and electrondense, the primary wall is thin and of medium electron density, while the secondary wall is thickened, showing an irregular internal surface of the cell lumen. The lumen is quite reduced, often totally occluded (Figure 1(D)). This layer has lower electron density than the medial lamina and the primary wall and is composed of a loose fibrillar matrix with irregularly oriented microfibrils, mainly perpendicular to the long radial axis of the cell (Figure 1(E)). Plasmodesmata have not been observed in these cells.

Mature FCCs form the layer to adjacent to the nacreous cells, with three-four layers of living cells (Figures 1(A,F) and 2(A–F)), rich in primary pit fields and plasmodesmata in all walls between contacting FCCs (Figures 1(F) and 2(A,B); arrows, Figure 2(C)). In longitudinal section, they are observed as elongated elements with oblique end walls (Figure 2(A,C)). At this post-meiotic stage of sporophyte development, the cytoplasm of these cells is variable in cross section and contain a nucleus, sometimes in partial degeneration, dense granular material and various organelles, including pleiomorphic plastids, starchless, spherical vesicles, sheets of endoplasmic reticulum close to the plasmalemma, numerous mitochondria, vacuoles and lipid inclusions (Figures 1(F) and 2(B,D–F).

The hydrom forms a large central strand in the seta (Figure 1(A)), noticeable for containing a large number of cells (60–80 cells in cross section; sample size: $n = 9$). These cells have thin, fibrillar, electron translucent walls although some of them show thickenings in the corners. These walls are unperforated and retain a thin primary wall and medial lamina, with few intercellular spaces (Figure 2(E,F)). These cells lack any living protoplasm, but may contain some lipid inclusions.

Stem of the gametophyte

The structure of the gametophyte stem of *L. canariensis* is similar in both fertile and sterile plants, is circular in cross section and has three concentric regions with well-differentiated cells according to their wall thickness and coloration in fresh sections (Figure 3(A–D)).

The mature outer region or stereome comprises three–four layers of cells with reddish, thick walls and narrow lumen (Figure 3(A,D)). An external cuticle was also observed, similar to that of the seta of the sporophyte described above. Innerwards, and gradually, the stereome cell walls become thicker and their cytoplasm disappears, the innermost ones showing an almost empty cytoplasm with lipid inclusions and protoplasmic remains.

The intermediate region (Figure 3(A)) comprises six-seven layers of parenchymatous cells with thinner walls and abundant plasmodesmata (Figure 3(B–E)). At mature stages, their walls are intact and show corner thickenings, with no intercellular spaces; these cells have a sparse cytoplasm with some endomembranes remains of and lipid inclusions.

The internal strand, or hydrom, is of small diameter and consists of few (5–6) hydroids (Figure 3(A)). These cells are devoid of cytoplasm but conserve some lipid inclusions. Their walls are thinner not only than those of adjacent, parenchymatous cells, but also than the sporophytic hydroids. In longitudinal sections, they are observed as elongate cells with transverse or slightly oblique end walls.

The results of the histochemical tests for a qualitative approximation of the composition of the cell walls of the different histological layers are shown in Table 2. The walls of the stereids, nacreous cells, parenchymatous cells, FCCs and hydroids reacted positively to tests for proteins, cellulose, pectin, phospholipids and unsaturated lipids; and negatively for starch, lignin, neutral and total lipids and callose. These tests also confirmed the presence of a lipidic cuticle in both setae and stem. On the other hand, for the tests which the nacreous wall cells are positive, no differences showed in the staining intensity of the primary and secondary wall.

Discussion

The anatomy of seta and stem *L. canariensis* revealed histological differences between the stem and the seta, with maximum complexity in the sporophytic structure. The differentiation into four types of cellular regions in the seta, as observed in this study (stereids, nacreous cells, FCCs and hydroids), has only been described previously in polytrichaceous species (Ligrone and Duckett 1994; Ligrone et al. 2000), never before in other mosses (see Table 1). A more complex structure in the seta than in the stem has already been reported from several species of acrocarpous mosses, such as

Table 2. Results of the histochemical tests on transverse sections of setae (Se) and stems (Sm).

		Hydroids	FCCs/Leptoids	Nacreous cells	Stereids	Cuticle
Test	Compound	Se/Sm	Se	Se	Se/Sm	Se/Sm
Toluidine blue	Protein	+	+	+	+	–
IKI	Starch	–	–	–	–	–
$Zn–Cl_2–I$	Cellulose	+	+	+	+	–
Phloroglucinol	Lignin	–	–	–	–	–
Ruthenium red	Pectins	+	+	+	+	–
Sudan III	Neutral lipids	–	–	–	–	–
Sudan Black	Total lipids	–	–	–	–	+
Nile blue	Phospholipids	+	+	+	+	–
OsO_4	Unsaturated lipids	+	+	+	+	+
Aniline blue	Callose	–	–	–	–	–

+ indicates a positive reaction.
– indicates no reaction.

Funaria hygrometrica Hedw. and *Splachnum luteum* Hedw. (Hébant 1977), but in few pleurocarpous species: *Neckera crispa* Hedw. (Hébant 1977; Ligrone and Duckett 1994; Alfayate 1995; Ligrone et al. 2000) and *Cryptoleptodon longisetus* (Mont.) Enroth (Alfayate 1995, as *Leptodon longisetus* Mont.).

In both seta and stem there are three to four layers of peripheral stereids with thick walls providing support, and with well-developed chloroplasts and mitochondria. The stereome is outlined by a cuticle, common to both, seta and stem. The presence of a cuticle in bryophytes has been known for a long time, as pointed out by Strunk (1914), Hébant (1977) or, more recently, by Koch et al. (2009), Budke et al. (2011), Busta et al. (2016) or Glime (2017c). Here, we follow Jeffree (2006) and recognize a bi-layered structure with thin CP and CWP, as observed by Sack and Paolillo (1983) in *Funaria hygrometrica*, while Budke et al. (2011) pointed it out as a multi-layered structure. The histochemical analyses with Sudan B on cross sections (seta and stem) confirm its lipidic composition (Table 2). Parallel chemical analysis in the same species (Alfayate et al. 1997) showed the presence of triterpenoids (ursolic acid) and a lineal long-chain hydrocarbon with an odd C-number ($C_{29}H_{60}$), nonacosane, that might be part of the cuticle. This fits very well with the observations of Neinhuis and Jetter (1995), who described nonacosan-10-ol wax tubules in the sporophyte of Polytrichaceae, while in the sporophyte of *Buxbaumia viridis* (Moug. ex Lam. & DC.) Brid. ex Moug. & Nestl wax was observed forming platelets and granules (Koch et al. 2009). Besides, it has been long suggested (Proctor 1979) that *n*-alkanes, considered as part of the cuticular wax in vascular plants, could have a similar role in bryophytes. The n-alkanes of bryophyte origin have been used as biomarkers in paleoenvironmental reconstructions in mires (see, for instance, Ortiz et al. 2011, 2016), although they could contribute to slow down water loss (Budke et al. 2011, 2013). Meanwhile, their true role in mosses remains to be determined. The function of the epidermis as a transpiration barrier is connected with the presence of waxes (Riederer and Schreiber 2001), but the easy dehydration of the bryophytes would suggest that water retention is not the main function of the cuticle, although Buda et al. (2013) provide evidence that the deficiency in cuticular wax accumulation in a mutant of *Physcomitrium patens* (Hedw.) Mitt. (as *Physcomitrella patens* (Hedw.) Bruch & Schimp.) reduces its stress tolerance to desiccation. Epidermal waxes are common in mosses (Proctor 1979; Glime 2017d), and probably play an important role in keeping balance between the need of external water storage and conduction, and of gas exchange for photosynthesis (Proctor 2008).

The cuticle can also provide a barrier against microorganisms. This is consistent with the observation that microbial attacks lead to a loss of esters and wax hydrocarbons in mosses (Karunen and Ekman 1981), and with more recent research on the different functions of plants cuticle (Tafolla-Arellano et al. 2013; Busta et al. 2016). The role of triterpenes as components of the cuticles, and in protection and defence from pathogens or herbivores is known (Chen et al. 2021). The presence of ursolic acid, as reported before (Alfayate et al. 1997), could support this role for the cuticle in *L. canariensis*.

The very unusual thickening cells of the intermedial region of the seta, according to their morphology and their positive reactions to the cellulose, pectin and protein tests (following Esau and Cheadle 1958; Esau 1969) are similar to those of the nacreous-walled sieve elements of the vascular plants (Warmbrodt and Evert 1974; Perry and Evert 1975; Kuo and Stewart 1995). These cells are present in vascular cryptogams, as well as in seed plants (Esau 1969), and in both groups their walls have a low electron density and contain numerous microfibrils. In contrast, the walls of normal, non-nacreous sieve elements in vascular plants are uniformly and moderately electrondense. The functional significance of the nacreous-walled sieve elements in vascular plants is not clear. The apparent reduction of their cell lumen in certain portions suggests that their translocation ability may be restricted (Kuo et al. 1988, 1990). In mosses, leptoids with nacreous walls have been described by Schofield and Hébant (1984) in the gametophytes of *Atrichum* P.Beauv., of several species in the section *Juniperina* (Brid.) I.Hagen of the genus *Polytrichum* Hedw., and as an additional feature of FCCs (leptoids) in the seta of polytrichaceous mosses by Ligrone et al. (2000). In these cases, the cell walls are somewhat thicker than those of the neighbouring parenchymatous cells, but in many other cases the thickness does not differentiate the leptoids from them. On the contrary, in *L. canariensis* the difference in thickness with normal FCCs/leptoids is striking. The composition of these walls (i.e. cellulose, pectins, proteins and phospholipids in a loose fibrillar matrix) is similar to that of the leptoids of the gametophore of *Atrichum undulatum* (Hedw.) P.Beauv. (Stevenson 1977) and in the nacreous cells of the seta of *L. canariensis* (Table 2). This author noted that the formation of the nacreous wall occurs after the cell enlargement, and that the wall microfibrils were perpendicular to the long axis of the cell, as we have observed in *L. canariensis*.

It is possible that in earlier stages of the sporophyte development in *L. canariensis,* these cells correspond to normal FCCs/leptoids, becoming later pachydermatous dead cells following the thickening of the walls and the autolysis of their protoplast. The function of these cells in the early stages of the sporophyte of *L. canariensis* remains to be determined.

In the post-meiotic stages observed here, the presence of a tissue where the wall ingrowth invades the cell lumen could be involved with an increase of mechanical support in the seta.

The average length of the seta in this species, up to 24 mm, is remarkable for a pleurocarpous moss species and comparable to the setae of *Thuidium delicatulum* (Hedw.) Schimp. (20–38 mm), *Thuidium tamariscinum* (Hedw.) Schimp. (30–35 mm), *Hypnum polypterum* (Mitt.) Broth. (30 mm) and *Hylocomiadelphus triquetrus* (Hedw.) Ochyra & Stebel [as *Rhytidiadelphus triquetrus* (Hedw.) Warnst.): 25–30 mm]. A long seta supporting the capsule vertically would facilitate spore dispersal over a wide area (Niklas 2000; Raven 2002) and increase the probability of the new generation growing away from the maternal, tail-type gametophyte of creeping shoots. Besides, its elongation also elevates the position of the

stomata present in the neck of capsule (Alfayate 1995). Haig (2013) considers it to increase the transpirational pull that draws nutrients that are needed for sporogenesis, but recently some new observations point at a non-crucially functional, spendable nature of bryophyte stomata (Renzaglia et al. 2020). It seems likely that the nacreous-walled cells of the *L. canariensis* seta may contribute structurally to the reproductive success of this species, considering that *L. canariensis* capsules contain viables spores of two types—uni/multi-cellular medium-sized spores and multicellular, large spores (Alfayate et al. 2013). Although the reduction of the seta is a common adaptative strategy in saxicolous and epiphytic pleurocarps (Hedenäs 2012; Huttunen et al. 2018), the humid environment in the laurisilva, where *L. canariensis* lives, may require a more elevated capsule for an efficient spore dispersal. This need of a long seta could associate with highly developed conducting tissues, resulting in the complex histology here described.

The position and morphology of the leptoids have already been described in the setae of Polytrichaceae species (see Table 1 for references). In those mosses, both seta and stem, with leptoids (FCCs) and hydroids, present a similar structure, whereas *L. canariensis* here is shown to present a greater tissue differentiation in the sporophyte than in the gametophyte, where FCCs/leptoids are absent.

Although some reviews extend the use of the term leptoid to relatively unspecialised parenchymatous cells (Glime 2017a, Woudenberg et al. 2022), most authors working on bryophyte ultrastructure restrict it to the specialized FCCs of polytrichaceous mosses (Ligrone et al. 2000, 2012; Pressel et al. 2006), in which ultrastructural studies have revealed a distinctive cytological organization that include plasmodesmata in the end walls, plastids, mitochondria and endoplasmic reticulum-derived vesicles along longitudinal arrays of endoplasmic microtubules, breakdown of the tonoplast, mixing of the vacuolar and cytoplasmic contents and nuclear breakdown. Some of these characteristics are also present in the FCCs of *L. canariensis*, although no endoplasmic microtubules were observed, a common feature with sieve elements that was also reported by Pressel et al. (2006) in the FCCs (leptoids) of *Polytrichastrum formosum* (Hedw.) G.L.Smith (as *Polytrichum formosum* Hedw.) subjected to desiccation. The cytological organization of the FCCs can change and depends on different influences (Ligrone and Duckett 1996; Pressel et al. 2006); these could be related to the fact that the samples studied here were post-meiotic specimens.

The presence of an endoplasmic reticulum, autophagic and multivesicular vacuoles in the cytoplasm of the FCCs, and the strong positive reaction to toluidine blue appear to confirm an active conducting activity in these cells. These observations support previous work on endomembranes (Hébant 1974; Pais and Carrapiço 1979a, 1979b) and symplastic transport in mosses (Eschrich and Steiner 1967, 1968a, 1968b; Hébant, 1970, 1973, 1977; Scheirer 1978; Ligrone et al. 2000).

The well-developed FCCs and numerous imperforated hydroids in the *L. canariensis* seta indicate an efficient system of internal conduction towards the active tissues in the capsule and may be needed in this long-stalked sporophyte, as has been considered for polytrichaceous mosses equipped with an internal system of specialized WCCs (Ligrone et al. 2002; Brodribb et al. 2020). A strand of hydroids (60–80 cells in cross section, in the distal part) also appear in the foot of this species (Alfayate et al. 2000), in accordance with Ligrone et al. (1993) who suggested that in Bryidae foot and seta usually have a similar histological structure. Presuming that more hydroids conduct more water, this large central strand would represent that the endohydric mode of water movement is predominant in the seta, while in the leafy stem (5–6 hydroids) both endo- and ectohydric conduction would concur (Ligrone et al. 2000; Glime 2017a, 2017e).

The type of stem present in *L. canariensis*, differentiating from the epidermis inwards into three cellular regions: stereome, parenchyma and hadrom, is common in both acrocarpous (Kawai 1971a, 1971c; Scheirer 1972; Ligrone et al. 1980; Glime 2017a) and pleurocarpous mosses (Kawai 1971b, 1976, 1977, 1978). It also agrees with the results reported for *L. sciuroides* (Hedw.) Schwägr. both typical and var. *morensis* Schwägr. (Fuertes et al. 1997), but not with those reported by Velázquez (1994) for the same species, who described a stem without hydroids or solely vestigial ones. In pleurocarps, the stem central strand is held as an ancestral character state, whereas its absence would be a common reduction in epiphytic mosses (Hedenäs 2001, 2007, 2012; Huttunen et al. 2018). In the genus *Leucodon*, the plasmodesmata of the parenchymatous cells would contribute, together with the hydroids, to water conduction (Finocchio 1967; Hébant 1977; Ligrone et al. 1980; Cortella et al. 1994). This conducting parenchyma is common in other mesophytic mosses (Finocchio 1967; Caputo and Castaldo 1968), and in maintaining cell-to-cell communication compatible with the function of internal transport (Trebacz and Fensom 1989; Ligrone et al. 2000; Pressel et al. 2006; Glime 2017c).

The histological study of the moss *L. canariensis* proves a unique internal structure, notably with respect to its conducting tissues and their adjacent nacreous cells. More studies searching for the presence of this tissue in other moss groups, especially allied taxa, are needed to assess its possible taxonomical and functional value.

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Author contributions

CA and ER planned and designed the research; CA contributed plant material, performed the experiments and collected data. CA and BE wrote the manuscript and CA, BE and ER supervised the writing. All coauthors reviewed the manuscript before submission.

Disclosure statement

The authors declare that they have no conflict of interest.

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