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## Morpho-anatomical Studies and Evolutionary Interpretations of the Rhizomes of Extant Dennstaedtiaceae

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**ABSTRACT.**—Dennstaedtiaceae is a monophyletic, extant family of ferns with sub-cosmopolitan distribution and ca. 265 species. Rhizome morphology in the Dennstaedtiaceae is relatively well known, yet its anatomy has been poorly explored. We performed morpho-anatomical studies on 26 taxa from eight genera, mostly from Brazil, Japan, and New Zealand. Among the studied species, the main rhizome type is long-creeping with alternate phyllotaxy and a solenostele *s. str.* The exception is *Blotiella lindeniana*, which has ascending rhizomes with radial phyllotaxy and a dictyostele. A polycyclic solenostele was found in *Dennstaedtia* (“*Patania*”) *cornuta*, *D.* (“*Patania*”) *dissecta*, and two subspecies of *Pteridium arachnoideum*. Lateral-line aerenchyma characterized *Blotiella lindeniana*, *Histiopteris incisa*, *Hypolepis* spp., *Paesia* spp., and *Pteridium arachnoideum* subsp. The position of sclerified parenchyma in the cortex and pith varied among species. Our data enabled us to hypothesize evolutionary patterns of rhizome evolution in extant lineages of the Dennstaedtiaceae. The “Monachosoridae” has retained ancestral conditions: short-creeping to ascending rhizomes with dictyostele *s. str.* In the “Hypolepidoideae”, two notable changes occurred in *Blotiella* and *Pteridium* (in which the solenostele evolved into a polycyclic solenostele). In the “Dennstaedtiodeae”, one notable change occurred in *Dennstaedtia* (“*Patania*”), namely parallel evolution with *Pteridium* (in which the polycyclic solenostele has also evolved). We present illustrations, morpho-anatomical descriptions, evolutionary inferences based on the available phylogeny, and supporting tools for studying the taxonomy, systematics, phylogeny, and paleontology of the group.

**KEY WORDS.**—ancient ferns, bracken fern, amphiphloic siphonostele, solenostele

Dennstaedtiaceae Lotsy is a monophyletic family of extant leptosporangiate ferns containing 10 genera and ca. 265 living species (PPG I, 2016). The family has a subcosmopolitan distribution, primarily due to the widely-distributed bracken ferns, *Pteridium* Gled ex Scop. (Der *et al.*, 2009; Tryon, 1941).

Although the Cretaceous diversification of the Polypodiales (*sensu* PPG I, 2016) has been controversial, Schneider, Schmidt, and Heinrichs (2016) just recently described a leaf from the mid-Cretaceous, *Krameropteris resinatus* H.Schneid *et al.*, with an undeniable cathetogyrate sporangium, and the authors ascribed it to the Dennstaedtiaceae. This discovery supports the molecular clock estimates for the origin of the family, of ca. 115 mya (Schneider *et al.*, 2004). Three other fossil genera are also ascribed to the

family: *Microlepiopsis* Sebert & Rothwell, also from the Cretaceous; *Dennastra* McIver & Basinger; and *Dennstaedtiopsis* Arnold & Daugherty (Collinson, 2001; Fossilworks, 2016; Sebert and Rothwell, 2003; Shi, Schopf, and Kudryavtsev, 2013).

As currently defined (Smith *et al.*, 2008; PPG I, 2016), most extant genera of Dennstaedtiaceae are probably monophyletic, except possibly for *Hypolepis* Bernh. (Brownsey, 1983) and for the clearly paraphyletic *Dennstaedtia* Bernh. (Perrie, Shepherd, and Brownsey, 2015; Schneider, Schmidt, and Heinrichs 2016; Schuettpelz and Pryer, 2007).

Stems in Dennstaedtiaceae are generally long-creeping rhizomes, and both their external morphology and internal anatomy are fairly well known (Bower, 1926; Gruber, 1981; Gwynne-Vaughan, 1903; Kaur, 1971; Keating, 1968; Kramer, 1990; Nair and Sem, 1974; Navarrete and Øllgaard, 2000; Ogura 1972; Schwartzburd and Prado, 2015; Troop and Mickel, 1968; Tryon and Tryon, 1982; Watt, 1940). However, research focusing on rhizome anatomy in the family has addressed only a few genera and species, and some features of the species, such as their lateral-line aerenchyma, have been poorly analyzed.

The anatomy of stems, especially of their vascular system, is considered to be a conserved character, as these organs are affected by only few environmental influences and have undergone few evolutionary modifications (Srivastava and Chandra, 2009). It has been hypothesized that the diversity of primary vascular systems was established early in the evolution of vascular plants (Beck, Schmid, and Rothwell, 1982). Studies on stem morpho-anatomy may yield important information on the relationships between extinct and extant taxa (Cantrill, 1997; Matsumoto *et al.*, 2006) as well as on the evolution of monophyletic lineages (Ebihara, 2007; Pryer, Smith, and Skog, 1995; Roux and Wyk, 2000; Smith, 1995).

In this paper, we provide an initial database on morphological features combined with anatomical characteristics of selected species from some extant Dennstaedtiaceae genera, in order to contribute complementary morphological data for circumscription of genera and to infer the evolutionary history of rhizomes in the family.

#### MATERIAL AND METHODS

We sampled 26 taxa (23 species, two subspecies, and one variety) belonging to eight genera of the Dennstaedtiaceae (*sensu* PPG I, 2016), mostly from Brazil, Japan, and New Zealand (Appendix 1). Sixty two specimens were analyzed, of which 35 were freshly collected and 27 were obtained from exsiccates (from herbaria CRH, TNS, and VIC). Rhizome external morphology was analyzed by naked eye and under a stereo microscope (model SMZ 068, Motic, China).

For the anatomical analysis, fragments were sampled from the internode mid regions of freshly collected rhizomes, fixed in FAA<sub>50</sub> (Johansen, 1940), dehydrated in an ethanol series, and embedded in methacrylate resin (Leica Histoiresin, Germany). Transversal and longitudinal sections 5–7  $\mu\text{m}$  thick were obtained using an advance rotary microtome (model Spencer 820,

American Optical Corporation, USA). Sections were stained with toluidine blue, pH 7.2 (O'Brian and McCully, 1981), and mounted in synthetic resin (Permunt-Fischer). For samples from herbarium materials, rhizome fragments were first rehydrated (Smith and Smith, 1942) and then subjected to the method described above. We also performed a Phloroglucinol-HCl test for lignin (Johansen, 1940).

Tracheal elements were isolated through maceration and then identified. Rhizome fragments were fixed in Jeffrey solution (Johansen, 1940) for ca. 20 h, washed with distilled water and 50% ethanol, and stained with 1% safranin in 50% ethanol for five h. Samples were then washed in 30% ethanol and mounted on temporary microscopic glass slides. Samples were documented and photographed using a photomicroscope (model AX70TRF, Olympus Optical, Japan) coupled with a digital camera (AxioCam HR3, Zeiss, Germany).

Classification of the vascular system followed Schmid (1982). Our evolutionary interpretations were based on the available phylogeny of the Dennstaedtiaceae, which was constructed using *rbcL* sequences (Perrie, Shepherd, and Brownsey, 2015; and Schneider, Schmidt, and Heinrichs, 2016), excluding *Saccoloma* species from the former study. Sequences of *Saccoloma* spp. (analyzed by Wolf, 1995), which nested within Dennstaedtiaceae, were later found to be contaminated (Wolf, pers. comm.; see also PPG I, 2016). In the phylograms of Perrie, Shepherd, and Brownsey (2015) and Schneider Schmidt, and Heinrichs (2016), we inserted the species we analyzed, which did not have available *rbcL* sequences in GenBank (Fig. 1). Our insertions, while speculative (dotted lines), were based on morphological criteria and unpublished *trnL-trnF* sequences of *Hypolepis rugosula* subsp. *pradoana* Schwartsb. and *H. stolonifera* Fée var. *stolonifera*, which were compared with other *Hypolepis* spp. (Schwartzburd and Perrie, *unpubl. data*). To avoid bias, we inserted most species in polytomies. A dual hypothesis was elaborated for *Dennstaedtia globulifera* (Poir.) Hieron. (indicated by an asterisk).

We named the three main clades of Dennstaedtiaceae (Perrie, Shepherd, and Brownsey, 2015) as: “Monachosoroideae” (containing only *Monachosorum* Kunze), “Hypolepidoideae” (cont. *Paesia* A.St.-Hil., *Histiopteris* J.Agardh, *Blotiella* R.M.Tryon, *Hypolepis* Bernh., and *Pteridium*), and “Dennstaedtioidae” (cont. paraphyletic *Dennstaedtia* Bernh., *Oenotrichia* Copel., *Leptolepia* Kuhn, and *Microlepia* C.Presl). We also put alternative generic names for some *Dennstaedtia* species in brackets, similar to Schneider, Schmidt, and Heinrichs (2016). One sensitive difference from Schneider, Schmidt, and Heinrichs (2016) regards the placement of “*Patania*”. The type of *Patania* C.Presl is *P. obtusifolia* (= *D. obtusifolia*), which forms a species complex with *D. dissecta* (Sw.) T. Moore and *D. cornuta* (Kaulf.) Mett. Thus, we named this clade “*Patania*”; and not *D. scabra*, as Schneider, Schmidt, and Heinrichs (2016) did. We polarized our tree using some basal Pteridaceae genera, such as *Llavea* Lag. and *Cryptogramma* R. Br. (e.g., Pabón-Mora and González, 2016). That baseline information was used to describe the rhizome of our hypothetical ancestor of the Dennstaedtiaceae.

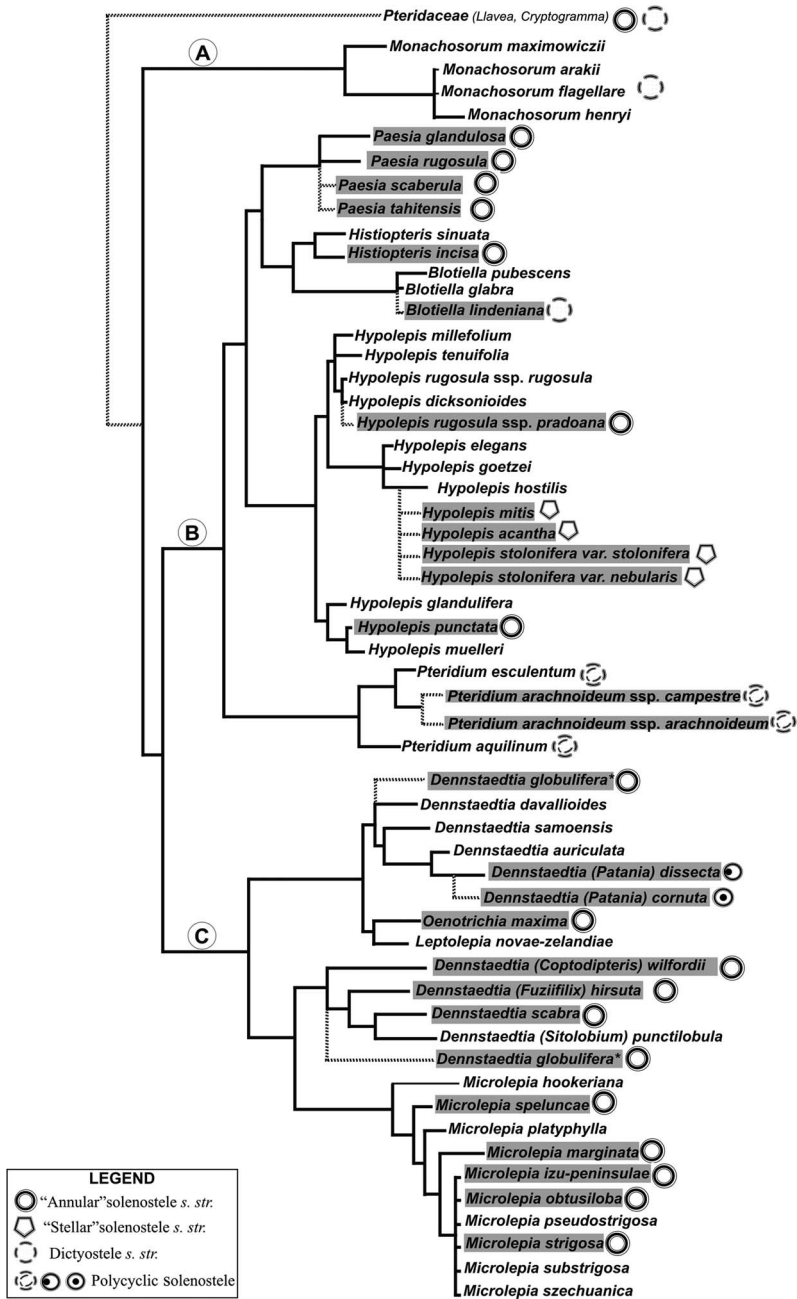


FIG. 1. Phylogram of Dennstaedtiaceae, according to Perrie, Shepherd, and Brownsey (2015) and Schneider, Schmidt, and Heinrichs (2016). Species sampled in our study are highlighted in gray. Dotted lines show tentative positioning of species not sampled in the original phylogenies. Asterisks indicate alternate hypotheses of the relationships in *Dennstaedtia globulifera*.

## RESULTS

Rhizome morphology is relatively homogeneous among the studied species. Among the species studied, the main rhizome type was the creeping rhizome (Fig. 2C, 2D, 3A, 3D, 3F, 4A, 4B, 4D, 4F, 4G) with alternate phyllotaxy (e.g., Fig. 4G), except in *Blotiella lindeniana* (Fig. 2A), which has ascending rhizomes with radiate phyllotaxy. With a few exceptions of either glabrous or glabrescent rhizomes (*Dennstaedtia (Patania) cornuta*), most species are furnished with catenate hairs (Figs. 3E, 4C, E, 7H). *Histiopteris incisa* has also comose proto-scales (Figs. 3B, 7G). Epipetiolar buds (e.g., Fig. 3F), secondary roots (e.g., Fig. 3F), and catenate hairs are found at the base of petioles in most species.

All species have an uniseriate epidermis. The lateral-line aerenchyma is present in *Blotiella lindeniana* (Figs. 2B, 8C, E), *Histiopteris incisa* (Figs. 3A, C), *Hypolepis* spp. (Figs. 3G, 8D, F), *Paesia* spp. (Figs. 3E, 8B), and *Pteridium arachnoideum* subspp. (Figs. 2E - arrows, 8A). Lines of aerenchyma run laterally on rhizomes and have stomata on their surface (Figs. 8D, E). The aerenchyma below the epidermis is characterized by conspicuous intercellular gaps and by intercellular protuberances (IP) of cell walls (Fig. 8F). *Blotiella lindeniana*, *Paesia* spp., and *Pteridium arachnoideum* subspp. have “neck-like” extensions from the inner cortex to the aerenchyma (Figs. 8A–C). Neither *Histiopteris incisa* nor *Hypolepis* spp. have such “neck-like” extensions (e.g., Fig. 8D).

The location of sclerified parenchyma layers in the cortex varies among species. This tissue is composed by slightly longer cells with thick cell walls, but without lignin deposit (thus not composing a true sclerenchyma). Those layers may be located in the outer, medium, and/or inner cortex. *Hypolepis* spp., *Histiopteris incisa*, and *Oenotrichia maxima* have sclerified parenchyma in the inner cortex (next to the endodermis) (e.g., Fig. 7E). On the other hand, the inner cortex of *Blotiella lindeniana*, *Paesia* spp., *Dennstaedtia* spp., *Microlepia* spp., and *Pteridium arachnoideum* subspp. has unmodified parenchyma (e.g., Fig. 7F).

The vascular systems of all studied species are classified into the major group of amphiphloic siphonostele *sensu* Schmid (1982). In this group, steles are enclosed by both an inner and outer endodermis. In all species we studied, the endodermis is formed by tabular cells without lignin deposit. The pericycle below it is formed by two layers of isodiametric cells (e.g., Fig. 7A). We found three types of steles (*sensu* Schmid 1982): 1. solenostele *s. str.*, 2. polycyclic solenostele, and 3. dictyostele *s. str.*

Solenostele *s. str.* is, by far, the most common stele type in the Dennstaedtiaceae (Figs. 5A–C, E, 6C–H). This stele type was found in *Dennstaedtia globulifera* (Fig. 6D), *D. (Fuziifilix) hirsuta* (Fig. 6E), *D. scabra*, *D. (Coptodipteris) wilfordii* (Fig. 6C), *Histiopteris incisa* (Fig. 5C), *Hypolepis acantha* (Fig. 5A), *Hyp. mitis*, *Hyp. punctata* (Fig. 5B), *Hyp. rugosula* subsp. *pradoana*, *Hyp. stolonifera* var. *stolonifera*, *Hyp. stolonifera* var. *nebularis*, *Microlepia izu-peninsulae*, *M. marginata*, *M. obtusiloba* (Fig. 6G), *M.*

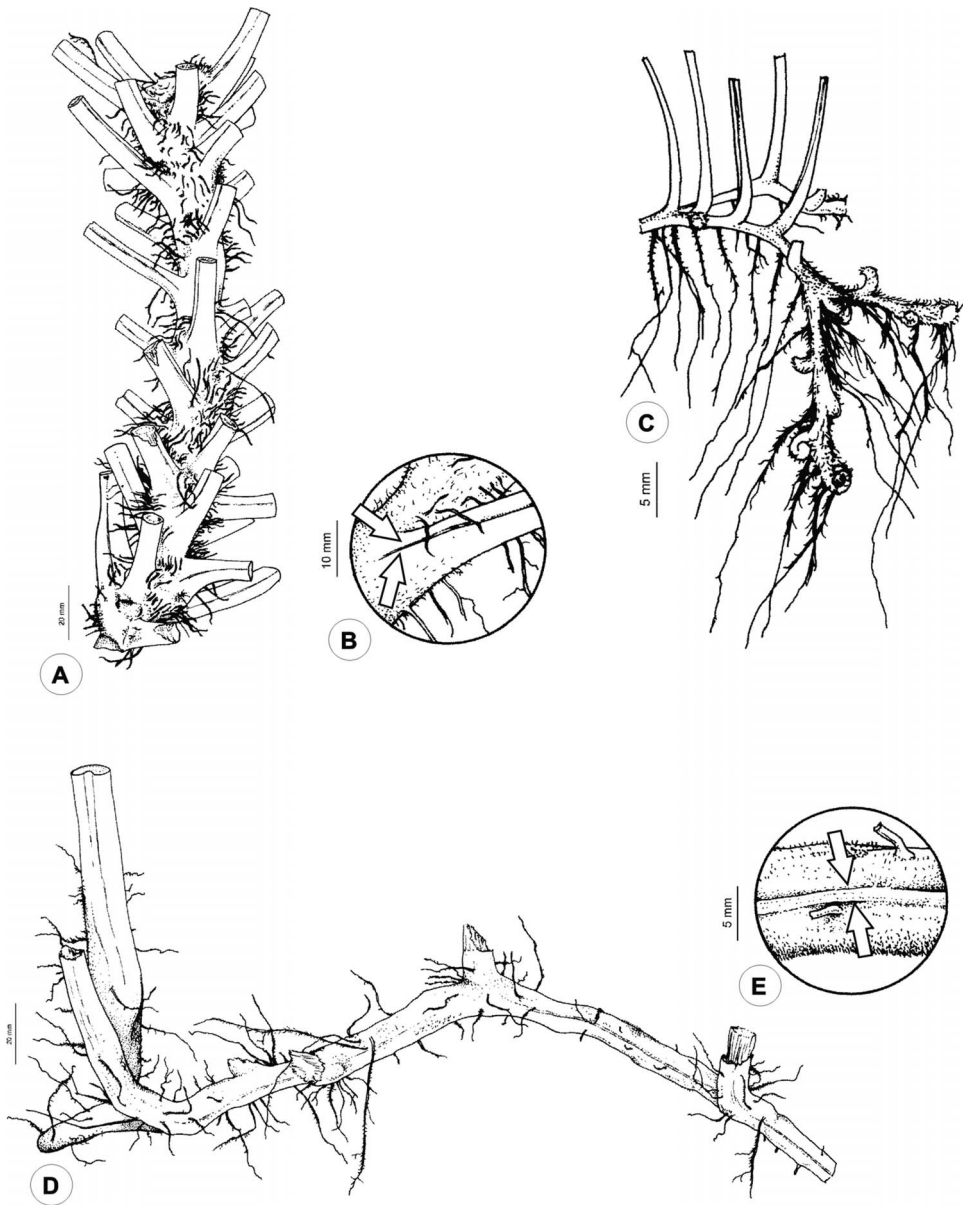


FIG. 2. Habit and rhizome morphology in the Dennstaedtiaceae. **A, B.** *Blotiella lindeniana*. **A.** Ascending rhizome. **B.** Arrows indicate the lateral-line aerenchyma, which extends from the rhizome to the petiole. **C.** Creeping rhizome of *Dennstaedtia (Coptodipteris) wilfordii*. **D, E.** *Pteridium arachnoideum* subsp. *campestre*. **D.** Creeping rhizome. **E.** Arrows indicate the lateral-line aerenchyma.

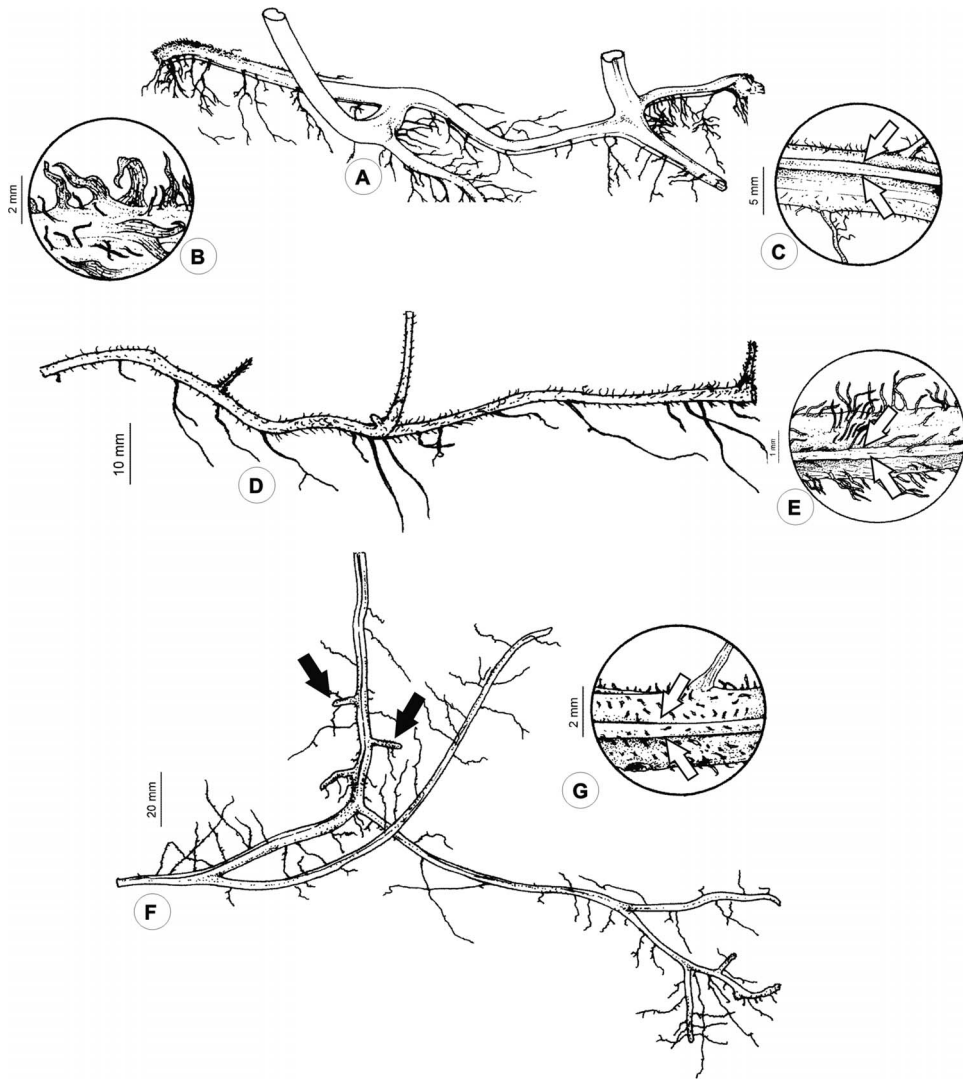


FIG. 3. Habit and rhizome morphology in the Dennstaedtiaceae. A–C. *Histiopteris incisa*. A. Creeping rhizome. B. Detail of comose proto-scales and hairs. C. Arrows indicate the lateral-line aerenchyma. D, E. *Paesia glandulosa*. D. Creeping rhizome. E. Arrows indicate the lateral-line aerenchyma. F, G. *Hypolepis stolonifera* var. *nebularis*. F. Creeping rhizome (Arrows indicate epipetiolar buds). G. Arrows indicate the lateral-line aerenchyma.

*speluncae* (Fig. 6H), *M. strigosa*, *Oenotrichia maxima* (Fig. 6F), *Paesia glandulosa* (Fig. 5E), *P. rugosula*, *P. scaberula*, and *P. tahitensis*. All these species have annular solenosteles, except for *Hyp. acantha*, *Hyp. mitis*, *Hyp. stolonifera* var. *stolonifera*, and *Hyp. stolonifera* var. *nebularis*, which have stellar solenosteles with well-defined protoxylem poles (e.g., Fig. 5A).



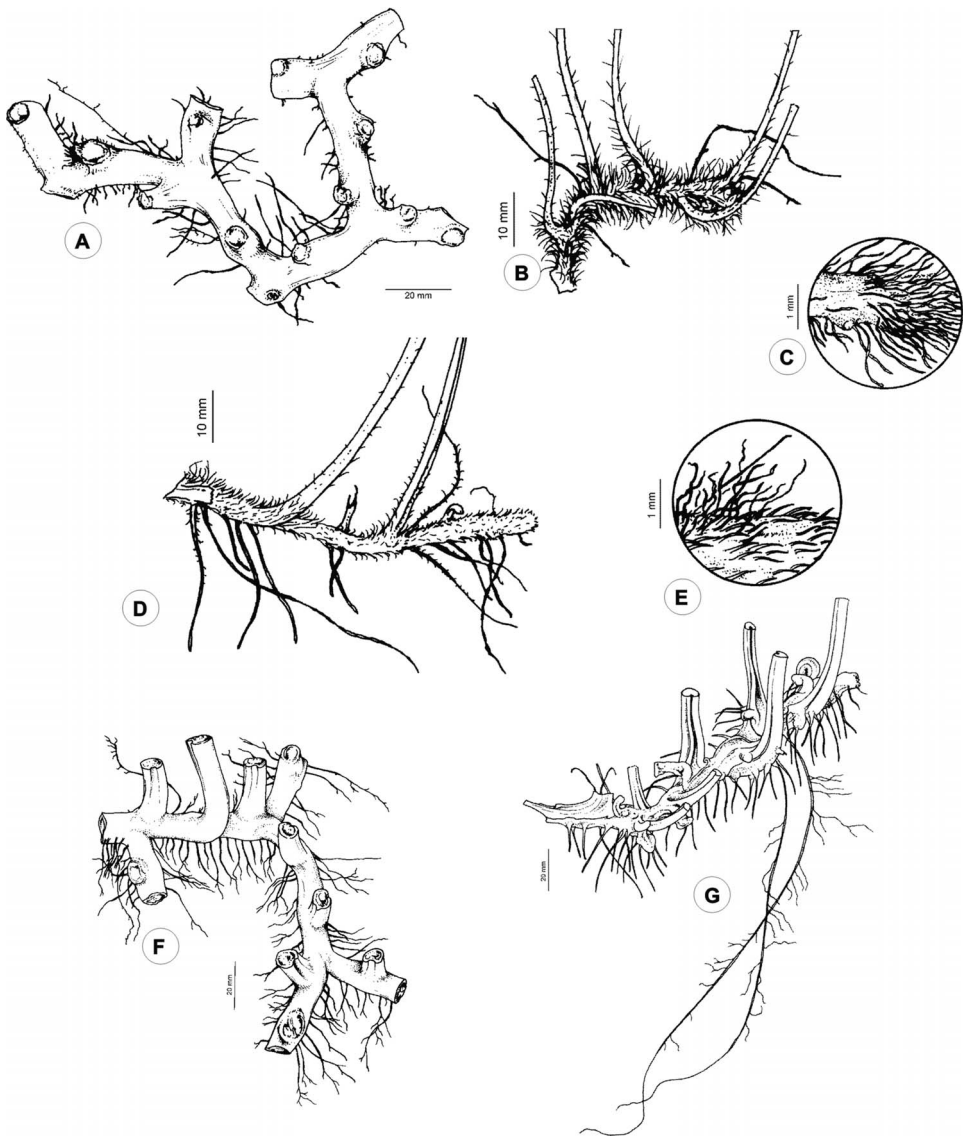


FIG. 4. Habit and rhizome morphology in the Dennstaedtiaceae. A. Creeping rhizome in *Dennstaedtia globulifera*. B, C. *Dennstaedtia (Fuziifilix) hirsuta*. B. Creeping rhizome. C. Detail of catenate hairs. D, E. *Microlepia marginata*. D. Creeping rhizome. E. Detail of catenate hairs. F. Creeping rhizome in *Microlepia speluncae*. G. Short-creeping rhizome in *Dennstaedtia (Patania) cornuta*.

The two other stele types are rare in the family. *Blotiella lindeniana* has a dictyostele *s. str.* (Fig. 5D), while *Dennstaedtia* (“*Patania*”) *dissecta*, *D.* (“*Patania*”) *cornuta*, *Pteridium arachnoideum* subsp. *arachnoideum*, and *P. arachnoideum* subsp. *campestre* have a polycyclic solenostele (Figs. 5F, 6A–

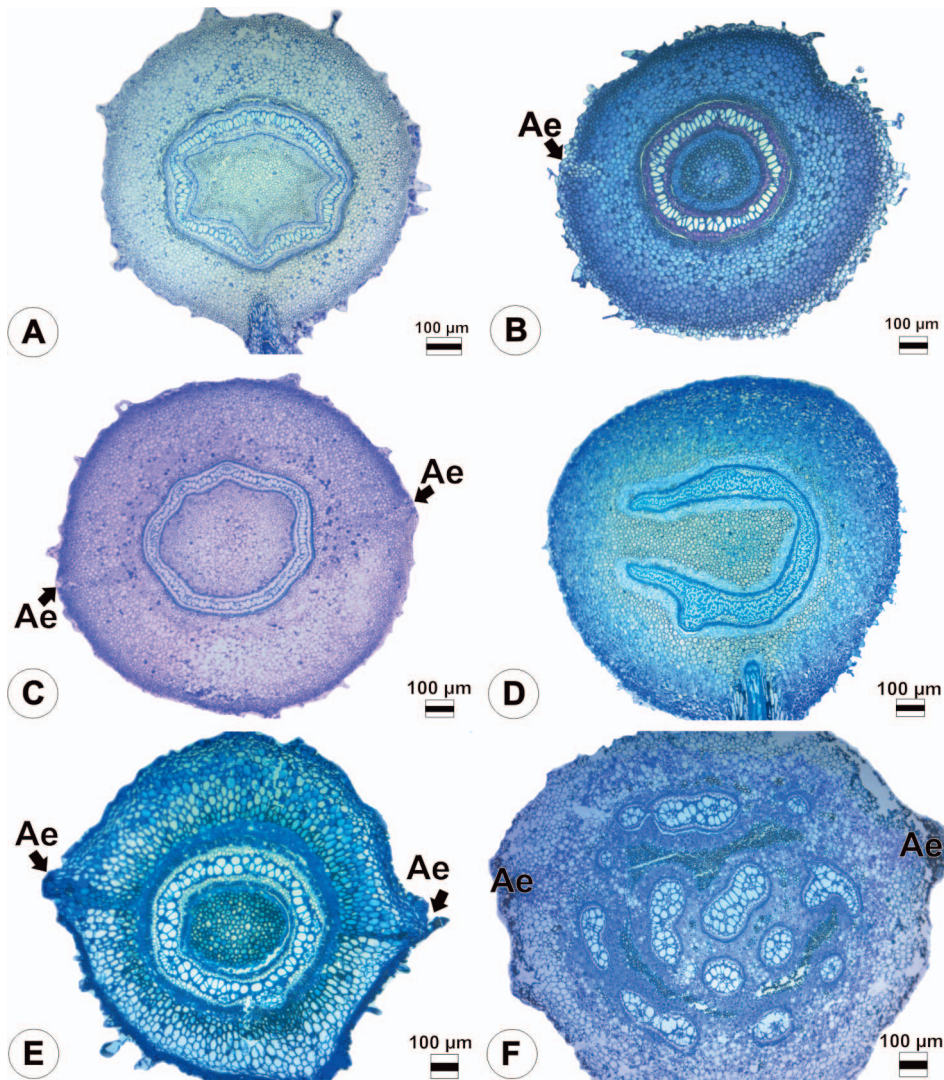


FIG. 5. Anatomical overview of rhizomes in the Dennstaedtiaceae (in cross section). A. *Hypolepis acantha*. B. *Hypolepis punctata*. C. *Histiopteris incisa*. D. *Blotiella lindeniana*. E. *Paesia glandulosa*. F. *Pteridium arachnoideum* subsp. *campestre*. “Ae” = lateral-line aerenchyma.

B). The polycyclic solenostele differed among these four taxa. In *D. dissecta*, there is a vascular protuberance inward the annular solenostele (Fig. 6B). In *D. cornuta*, there are either two concentric annular solenosteles (Fig. 6A), or occasionally the inner solenostele may have no pith, thus resembling a protostele. In *Pteridium arachnoideum* subsp., there are two concentric rows of meristeles (Figs. 5F, 7B).

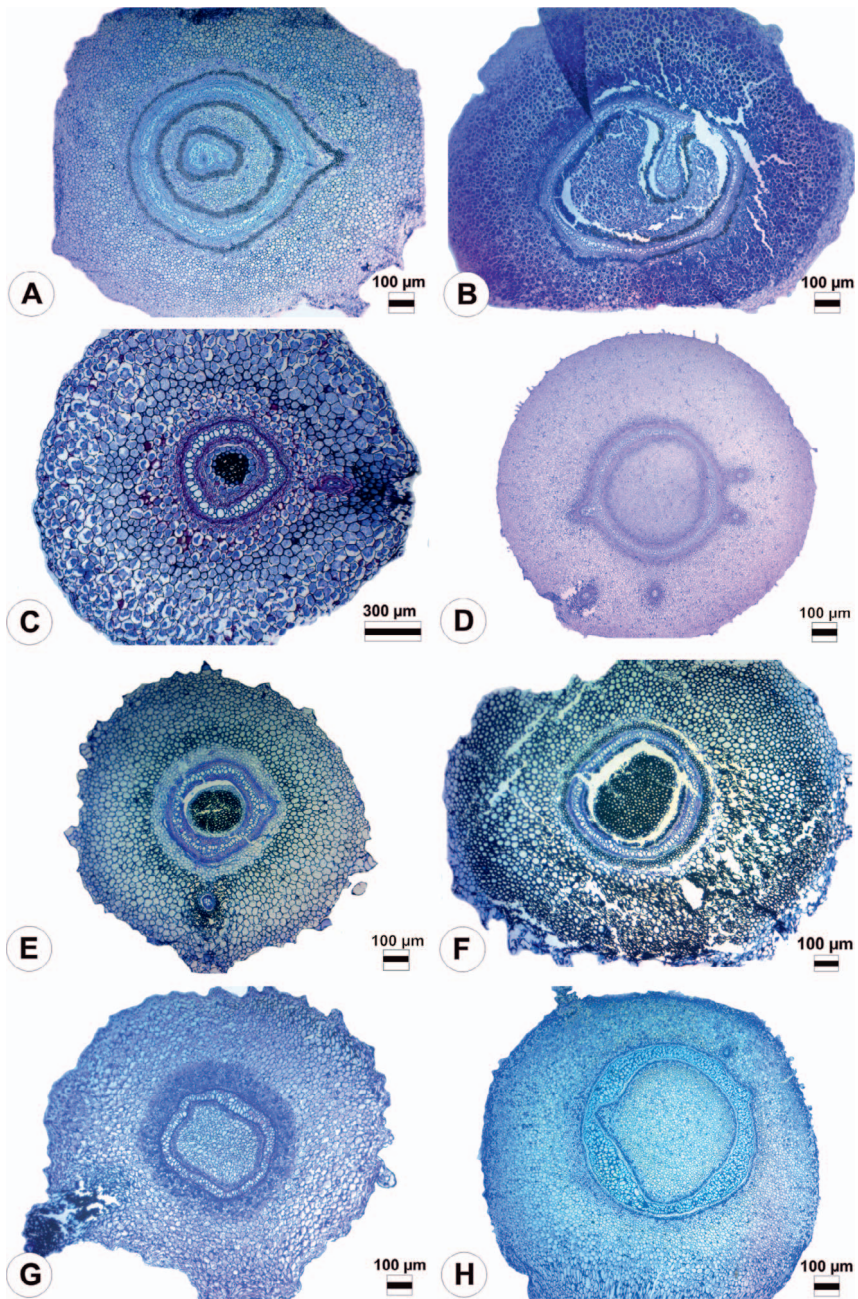


FIG. 6. Anatomical overview of rhizomes in the Dennstaedtiaceae (in cross section). A. *Dennstaedtia (Patania) cornuta*. B. *Dennstaedtia (Patania) dissecta*. C. *Dennstaedtia (Coptodipteris) wilfordii*. D. *Dennstaedtia globulifera*. E. *Dennstaedtia (Fuziifilix) hirsuta*. F. *Oenotrichia maxima*. G. *Microlepia obtusiloba*. H. *Micropelia speluncae*.

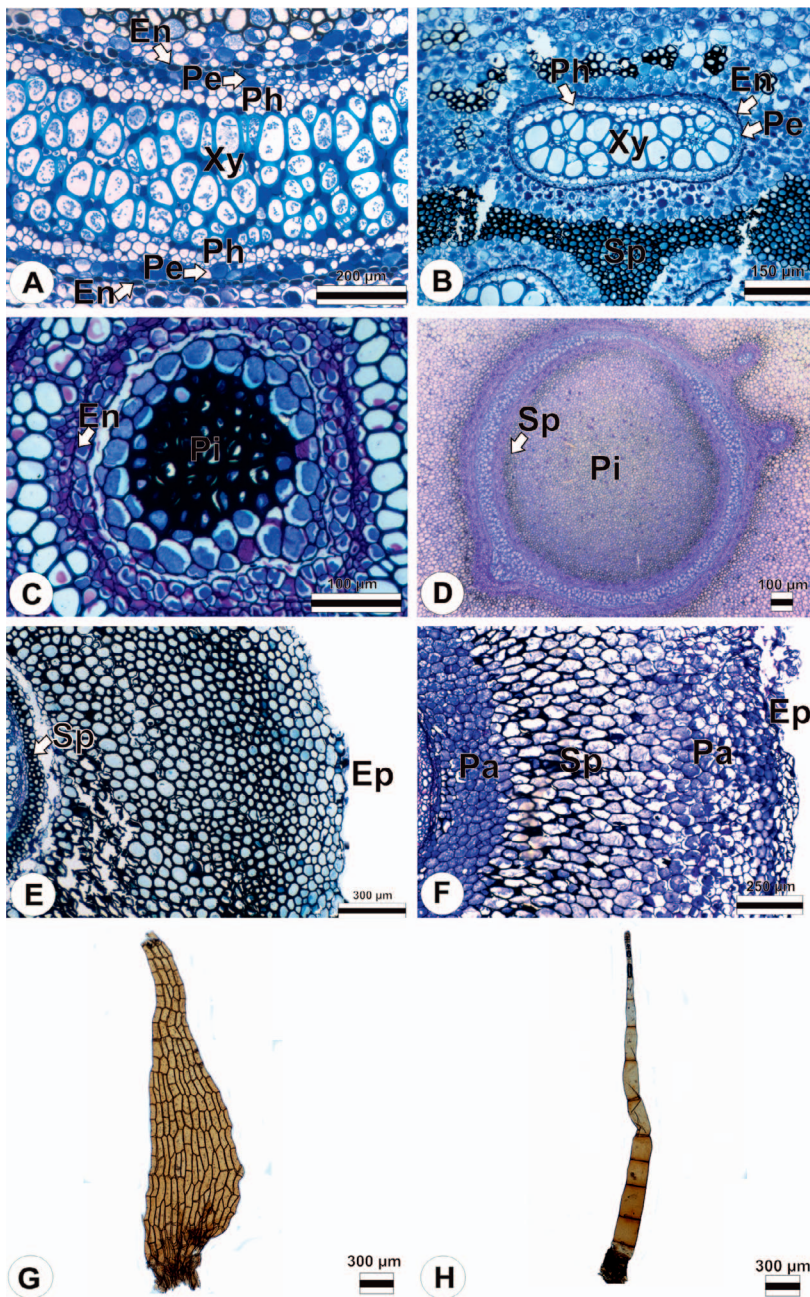


FIG. 7. Anatomical details of rhizomes in the Dennstaedtiaceae (A–F in cross section). A. Vascular bundle with inner and outer endodermis in *Microlepia speluncae*. B. Meristele of *Pteridium arachnoideum* subsp. *campestre*. C. Inner pith with sclerified parenchyma in *Dennstaedtia (Coptodipteris) wilfordii*. D. Pith with only its outer layer having a sclerified parenchyma in *Dennstaedtia globulifera*. E. Cortex of *Oenotrichia maxima*. F. Cortex of *Microlepia marginata*. G. Comose proto-scales of *Histiopteris incisae*. H. Catenate hair of *Hypolepis acantha*. “En”= endodermis; “Ep”= epidermis; “Pa”= non-sclerified parenchyma; “Pe”= pericycle; “Ph”= phloem; “Pi”= pith; “Sp”= Sclerified parenchyma; Xy= xylem.

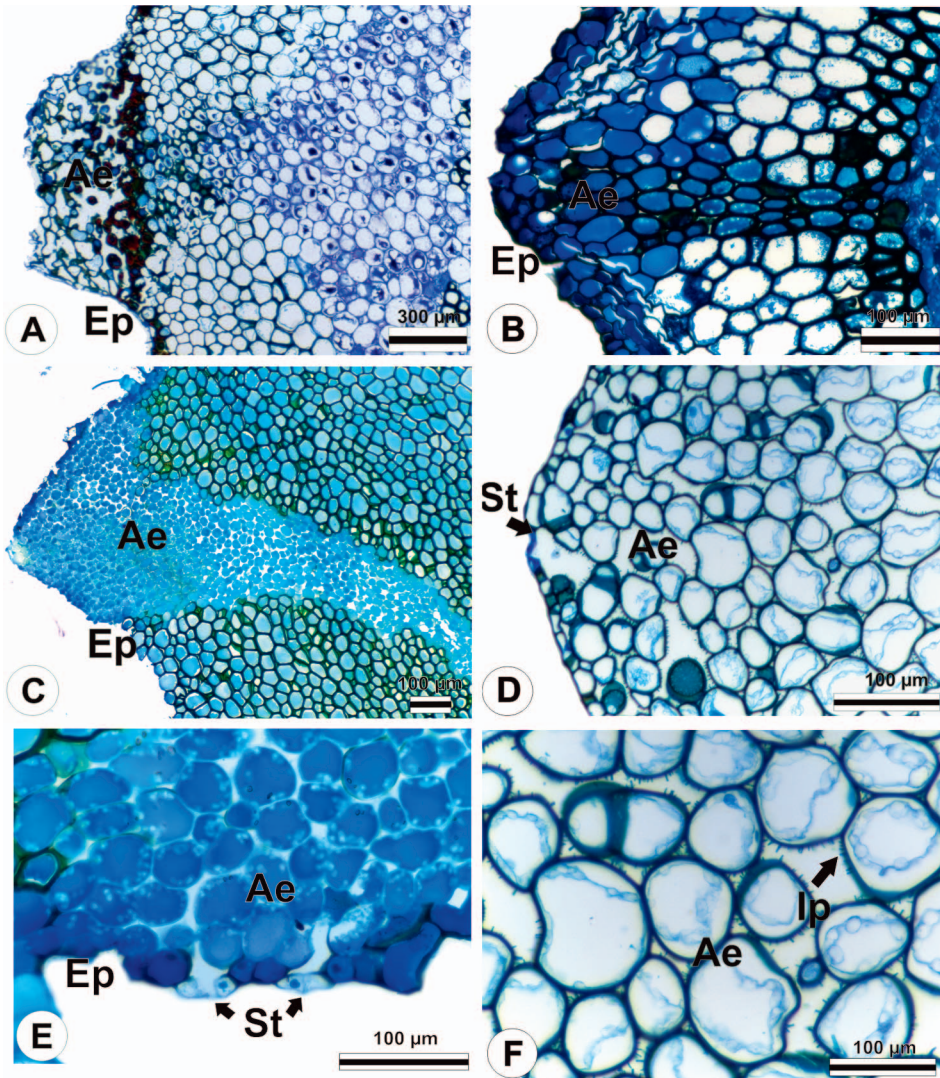


FIG. 8. Lateral-line aerenchyma in rhizomes of the Dennstaedtiaceae (in cross section). A. *Pteridium arachnoideum* subsp. *campestre*. B. *Paesia glandulosa*. C. *Blotiella lindeniana*. D. *Hypolepis acantha*. E. Detail of stomata on the epidermis of *Blotiella lindeniana*. F. Detail of inter-cellular protuberances in *Hypolepis acantha*. “Ae”= lateral-line aerenchyma; “Ep”= epidermis; “Ip”= inter-cellular protuberances; “St”= stomata.

All species have only scalariform tracheids as conducting cells in the xylem, except for *Pteridium arachnoideum* subsp., which also has vessel elements. Phloem in the species is formed by sieve cells.

Pith composition also varies among species. Some of them have sclerified parenchyma occupying almost the entire pith, while others have sclerified

parenchyma only in the outermost pith layers (i.e., those next to the endodermis). *Blotiella lindeniana*, *Dennstaedtia hirsuta*, *D. scabra*, *D. wilfordii*, *Microlepia marginata*, *M. izu-peninsulae*, *M. obtusiloba*, *M. strigosa*, *Oenotrichia maxima*, and *Paesia* spp. have sclerified parenchyma occupying almost the entire pith (e.g., Fig. 7C). On the other hand, in *D. globulifera*, *Histiopteris incisa*, *Hypolepis* spp., and *M. speluncae* only the outermost layers are occupied by sclerified parenchyma (e.g., Fig. 7D).

TENTATIVE KEY TO GENERA OF DENNSTAEDTIACEAE BASED ON THE MORPHO-ANATOMY OF THE STUDIED SPECIES (SOME DENNSTAEDTIA AND MICROLEPIA SPECIES CANNOT BE PRECISELY DISTINGUISHED):

- 1. Rhizomes solenostelic *s. str.*
- 2. Rhizomes bearing catenate hairs and comose scales . . . . . *Histiopteris*
- 2. Rhizomes bearing catenate hairs only
- 3. Rhizomes with stellar solenostele *s. str.* . . . . . *Hypolepis* (in part)
- 3. Rhizomes with annular solenostele *s. str.*
- 4. Lateral-line aerenchyma present
- 5. Pith mostly sclerified . . . . . *Paesia*
- 5. Pith with only the peripheral region sclerified . . . . . *Hypolepis* (in part)
- 4. Lateral-line aerenchyma absent
- 6. Inner cortex sclerified adjacent to the outer endodermis . . . . . *Oenotrichia*
- 6. Inner cortex not sclerified
- 7. Pith with only the peripheral region sclerified . . . . .  
 . . . . . *Dennstaedtia* (*D. globulifera*) and *Microlepia* (*M. speluncae*)
- 7. Pith mostly sclerified . . . . .  
 . . . . . *Dennstaedtia* (*D. scabra*, *D. hirsuta*, *D. wilfordii*) and  
*Microlepia* (*M. marginata*, *M. izu-peninsulae*, *M. obtusiloba*, *M. strigosa*)
- 1. Rhizomes with dictyostele *s. str.* or polycyclic solenostele
- 8. Rhizomes ascending, with dictyostele . . . . . *Blotiella*
- 8. Rhizomes medium- to long-creeping, with polycyclic solenostele
- 9. Rhizomes long-creeping; polycyclic solenostele subdivided into meristemes. . . . .  
 . . . . . *Pteridium*
- 9. Rhizomes medium-creeping; polycyclic solenostele not subdivided into meristemes . . . . . *Dennstaedtia* (*Patania*)

DISCUSSION

*Evolutionary interpretations.*—Based on the outgroup (e.g., *Llavea*, *Cryptogramma*), we established a hypothesis on the rhizome of the family ancestor (Fig. 1). This hypothetical ancestor had a short-creeping to ascending rhizome, with dorsiventral symmetry, short internodes, either a solenostele *s. str.* or a transitional dictyostele *s. str.*, and indumentum formed by scales. The first divergent lineage (“Monachosorideae”, Fig. 1 – clade A) from the ancestor retained its conserved features, which is similar to the basal Pteridaceae (Nair and Sem, 1974; Kramer, 1990). The exception is the indumentum, which consisted of hairs.

In the other lineage (a large clade containing “Hypolepidoideae” and “Dennstaedtioideae”, Fig 1 – clades B and C, respectively), a higher morpho-

anatomical diversification occurred in rhizomes. In this large clade, the main rhizome type is long-creeping with extended internodes, solenostele *s. str.*, and indumentum formed also by hairs.

Within the “Hypolepidoideae” (Fig. 1 – clade B), the long-creeping rhizome with solenostele *s. str.* is maintained in *Paesia* (Figs. 3D, 5E), *Histiopteris* (Figs. 3A, 5C), and *Hypolepis* (Figs. 3F, 5A, B), but two notable changes occurred within this clade. In *Blotiella*, the rhizome became truly ascending with radial symmetry, but also with a dictyostele *s. str.*, similar to the one of the ancestor (Figs. 2A, 5D). The other change occurred in *Pteridium*. The rhizome remained long-creeping, but the vascular system evolved into a polycyclic solenostele (Figs. 2D, 5F). The polycyclic solenostele of *Pteridium* is considered to be an advanced stage of the dictyostele *s. str.*, having probably derived from two concentric rings of dictyostele (Ogura, 1972). *Pteridium* has also vessel elements, which is a unique feature in the Dennstaedtiaceae. From the points of view of survival and colonizing success, the new features in *Pteridium* were extremely advantageous, in light of its current distribution and biomass, when compared with those of other Dennstaedtiaceae genera (e.g., Der *et al.*, 2009; Schwartsburd, Moraes, and Lopes-Mattos, 2014; Tryon, 1941).

The presence of comose proto-scales in rhizomes of *Histiopteris* (Figs. 3B, 7G) may be considered the result of reverse evolution. Within *Hypolepis*, there was also a slight change in shape of the solenostele *s. str.* Some species retained the annular solenostele (Fig. 5B), while others evolved the stellar solenostele with protoxylem poles (Fig. 5A).

In the “Dennstaedtioideae” clade (Fig. 1 – clade C), the long-creeping rhizomes with solenostele *s. str.* also remained as the main rhizome type, being present in *Microlepia* (Figs. 4D, F, 6G, H), *Oenotrichia* (Fig. 6F), and most species of *Dennstaedtia* (Figs. 4A, B, 6C–E), including “*Coptodipteris*” and “*Fuziifilix*”. Within “Dennstaedtioideae”, one notable change occurred. The rhizomes of *Dennstaedtia* “*Patania*” became medium-creeping, and the vascular system evolved from solenostele *s. str.* into a polycyclic solenostele (Figs. 4G, 6A, B). The polycyclic solenostele represents a parallel evolution between *Dennstaedtia* “*Patania*” (“Dennstaedtioideae” clade) and *Pteridium* (“Hypolepidoideae” clade). The shape of the polycyclic solenostele in *D. (Patania) dissecta* (Fig. 6B) is probably an intermediary condition between the solenostele *s. str.* and the shape found in *D. (Patania) cornuta* (Fig. 6A).

The three fossil genera with rhizomes currently ascribed to the Dennstaedtiaceae (*Dennastra*, *Dennstaedtiopsis*, and *Microlepiopsis*) are similar to the extant genera of the family, especially in their vascular system. This also indicates the conservation of the main characters along lineages of the Dennstaedtiaceae. The notable aerenchyma in *Dennstaedtiopsis aerenchymata* (Shi, Schopf, and Kudryavtsev, 2013) may either be only an isolated acclimatization to marshy places or represent a feature from another lineage of Dennstaedtiaceae that is now extinct.

*Contributions to taxonomy and systematics.*—Some variation in rhizomes of the Dennstaedtiaceae may be valuable to the taxonomy and systematics of the family. Differential distribution of the sclerified parenchyma in the pith and cortex (Figs. 7C–F) is an important feature to distinguish *Dennstaedtia s.l.*, *Hypolepis*, *Histiopteris*, and *Paesia*. This tissue type has taxonomic value in other fern families, and is responsible for protection and support of the stele (Ogura, 1972).

The lateral-line aerenchyma (Fig. 8) present in *Blotiella*, *Hypolepis*, *Histiopteris*, *Paesia*, and *Pteridium* also has taxonomic value. Such a lateral line has been described in many fern families, but primarily in petioles and not in rhizomes (Davies, 1991; Schwartsburd and Prado, 2015). Lateral-line aerenchyma has been reported in *Histiopteris incisa* and *P. aquilinum* (Ogura, 1972, as “pneumatic stripes”), and to a few species of *Dennstaedtia* and *Hypolepis* (Heidelberg, 1976). The occurrence of stomata and IP in the lateral-line aerenchyma has been reported by Barton, Overall, and Thomson (2015) in *P. aquilinum* subsp. *aquilinum*, *P. aquilinum* subsp. *wightianum*, and *P. esculentum* subsp. *esculentum*. On the other hand, IP had never been reported from any other Dennstaedtiaceae genus. These structures seem to be intimately related to the aerenchyma in the family.

*Microlepia* and *Dennstaedtia s.l.* (except “*Patania*”) have indistinguishable rhizome anatomies (Figs. 6C–E, G, H). *Dennstaedtia* was demonstrated to be paraphyletic by Perrie, Shepherd, and Brownsey (2015) and Schneider, Schmidt, and Heinrichs (2016), with most of its species nesting within *Microlepia* and other species nesting within another clade. *Dennstaedtia* will probably be split into two to several smaller genera so that a monophyletic classification may be established. Our anatomical data agree with the phylogenetic conclusions. Probably, “*Patania*” will be resurrected as a valid genus, and most diagnostic features will be based on rhizomes (medium-creeping, polycyclic solenostele, etc.).

Lastly, this work also aimed to provide the first database on rhizomes of the Dennstaedtiaceae. We hope our data will be useful for paleontologists to compare extinct and extant genera and help them classify fossil specimens into extant genera.

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## APPENDIX 1: LIST OF SPECIES AND ANALYZED MATERIALS

Species	Vouchers	Locality
<i>Blotiella lindeniana</i> (Hook.) R.M.Tryon	<i>Schwartsburd &amp; Becari-Viana</i> 3348 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3401 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3414 (VIC)	Brazil Brazil Brazil
<i>Dennstaedtia (Patania) dissecta</i> (Sw.) T.Moore	<i>Yañez &amp; Marquez</i> 104 (VIC)	Argentina
<i>Dennstaedtia (Patania) cornuta</i> (Willd.) T.Moore	<i>Schwartsburd &amp; Becari-Viana</i> 2981 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3070 (VIC) <i>Becari-Viana &amp; Pereira</i> 10 (VIC) <i>Becari-Viana &amp; Pereira</i> 11 (VIC) <i>Becari-Viana &amp; Pereira</i> 12 (VIC)	Brazil Brazil Brazil Brazil Brazil
<i>Dennstaedtia globulifera</i> (Poir.) Hieron.	<i>Schwartsburd &amp; Becari-Viana</i> 3069 (VIC) <i>Becari-Viana &amp; Pereira</i> 06 (VIC) <i>Becari-Viana &amp; Pereira</i> 07 (VIC) <i>Becari-Viana &amp; Pereira</i> 08 (VIC)	Brazil Brazil Brazil Brazil
<i>Dennstaedtia scabra</i> (Wall.) T.Moore	<i>Yamamoto</i> 2636 (VIC,TNS) <i>Yamanaka</i> (VIC-44.535, TNS)	Japan Japan
<i>Dennstaedtia (Fuziifilix) hirsuta</i> (Sw.) Mett. ex Miq.	<i>Takesako</i> 4188 (VIC,TNS) <i>Shimozono</i> (VIC-44.536, TNS)	Japan Japan
<i>Dennstaedtia (Coptodipteris) wilfordii</i> (T.Moore) Christ.	<i>Yuzawa</i> 3494 (TNS) <i>Saito</i> (VIC- 44.526, TNS) <i>Nagase</i> (VIC-44.538, TNS)	Japan Japan Japan
<i>Histiopteris incisa</i> (Thunb.) J.Sm.	<i>Becari-Viana &amp; Pereira</i> 19 (VIC) <i>Becari-Viana &amp; Pereira</i> 20 (VIC) <i>Becari-Viana &amp; Pereira</i> 20 (VIC) <i>Enzat</i> 141 (CRH)	Brazil Brazil Brazil New Zealand
<i>Hypolepis acantha</i> Schwartsb. <i>Hypolepis mitis</i> Kunze ex Kuhn	<i>Schwartsburd et al.</i> 2264 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3021 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3097 (VIC) <i>Becari-Viana &amp; Pereira</i> 03 (VIC) <i>Becari-Viana &amp; Pereira</i> 04 (VIC) <i>Becari-Viana &amp; Pereira</i> 05 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3316 (VIC)	Brazil Brazil Brazil Brazil Brazil Brazil Brazil
<i>Hypolepis punctata</i> (Thunb.) Mett. ex Kuhn	<i>Tsutsui</i> 5135 (VIC, TNS)	Japan
<i>Hypolepis rugosula</i> subsp. <i>pradoana</i> Schwartsb.	<i>Becari-Viana &amp; Pereira</i> 01 (VIC)	Brazil
<i>Hypolepis stolonifera</i> var. <i>nebularis</i> Schwartsb.	<i>Becari-Viana &amp; Pereira</i> 13 (VIC) <i>Becari-Viana &amp; Pereira</i> 15 (VIC) <i>Becari-Viana &amp; Pereira</i> 14 (VIC)	Brazil Brazil Brazil
<i>Hypolepis stolonifera</i> Fée var. <i>stolonifera</i>	<i>Labiak et al.</i> 4269 (VIC)	Brazil
<i>Microlepia marginata</i> (Panz.) C.Chr.	<i>Iwatsuki &amp; Kato</i> 79 (CRH) <i>Wang et al.</i> 368 (CRH) <i>Haruda</i> 366 (VIC,TNS) <i>Takesako</i> 5597 (VIC, TNS)	Japan China Japan Japan
<i>Microlepia izu-peninsulae</i> Sa.Kurata	<i>Nakaike</i> (CRH- 239661)	Japan
<i>Microlepia obtusiloba</i> Hayata <i>Microlepia speluncae</i> (L.) T.Moore	<i>Iwatsuki &amp; Kato</i> 51 (CRH) <i>Schwartsburd &amp; Becari-Viana</i> 3290 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3310 (VIC) <i>Schwartsburd &amp; Becari-Viana</i> 3315 (VIC)	Japan Brazil Brazil Brazil

## APPENDIX 1: Continued.

Species	Vouchers	Locality
<i>Microlepia strigosa</i> (Thunb.) C.Presl	Croft 836 (CRH) Braithwaite 2491 (CRH) Hovell (CRH-465258 A) Kido 12966 (VIC, TNS)	Papua New Guinea New Zealand New Zealand Japan
<i>Oenotrichia maxima</i> (E.Fourn.) Copel.	Brownlie 200 (CRH)	New Caledonia
<i>Paesia glandulosa</i> (Sw.) Kuhn	Schwartsburd & Fortuna-Perez 2929 (VIC) Schwartsburd & Fortuna-Perez 2930 (VIC) Schwartsburd & Fortuna-Perez 2931 (VIC)	Brazil Brazil Brazil
<i>Paesia rugosula</i> (Labill.) Kuhn	More & Brownlie 463 (CRH)	New Caledonia
<i>Paesia scaberula</i> (A.Rich.) Kuhn	Smith 84 (CRH) Moore (CRH-533179)	New Zealand New Zealand
<i>Paesia tahitensis</i> Copel.	Sykes 446 (CRH)	French Polynesian
<i>Pteridium arachnoideum</i> (Kaulf.) Maxon subsp. <i>arachnoideum</i>	Schwartsburd et al. 2838 (VIC, NSW) Schwartsburd et al. 2837 (VIC) Schwartsburd et al. 3383	Brazil Brazil Brazil
<i>Pteridium arachnoideum</i> subsp. <i>campestre</i> (Schrad.) Schwartsb. & P.L.R. Moraes	Alves da Silva et al. 01 (VIC, NSW).	Brazil