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Structure and chemical nature of intercellular protuberances in Dennstaedtiaceae rhizomes (Polypodiopsida)

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ABSTRACT. Intercellular protuberances (IPs) are projections on the cell surface and have been reported for families of ferns, Gymnosperms, and Angiosperms. Data on the type, chemical composition, and distribution of these structures among vascular plants are still scarce. Here, we investigate the occurrence, distribution, type of IPs among species of eight Dennstaedtiaceae genera and verify the taxonomic significance of these protuberances in the family. Rhizomes of 23 species of Dennstaedtiaceae from field and herbaria collections were analyzed by light microscopy and scanning electron microscopy. Histochemical tests were performed to identify the main classes of IPs chemical compounds. Two types of IPs were observed in Dennstaedtiaceae species: strand and filament types. They were observed in the intercellular spaces of the parenchyma in the cortex and pith regions. Overall, protuberances are irregularly shaped, with angustate or spheroidal apices. Their polysaccharide nature and pectic constitution were confirmed by histochemical tests. Concerning *Pteridium arachnoideum* (Kaulf.) Maxon subsp. *arachnoideum,* IPs have confirmed phenolic composition. Evidence indicates that IPs in Dennstaedtiaceae originate from the fragmentation of the middle lamella and that they have a structural function as well as protection against pathogens. In lateral-line aerenchyma, the occurrence of filament-type IPs may be related to the larger intercellular spacing in the cortex region, providing greater mechanical resistance. We have expanded the data on the occurrence of IPs in the Dennstaedtiaceae, which appear to be notable characters for the family. Moreover, the data presented herein confirmed the polysaccharide and pectic nature of these structures. However, we were unable to find links between IPs and taxonomy and evolution of the Dennstaedtiaceae. On the other hand, different IPs types were identified between the clades Dennstaedtioideae (strand-type IPs) and Hypolepidoideae (filament-type IPs, with exceptions).

Keywords: filaments; intercellular space; lateral-line aerenchyma; strands; taxonomic value.

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Introduction

Intercellular protuberances (IPs) are projections on the cell wall surface (Potgieter & Van Wyk, 1992) that have reported occurrence in cells of different organs such as root, stem, and leaf (Barton, Overall, & Thomson, 2015; Becari-Viana & Schwartsburd, 2017; Carr & Carr, 1975; Leroux et al., 2007; Miller & Barnett, 1993; Potgieter & Van Wyk, 1992) in families of ferns, Gymnosperms, and Angiosperms (Potgieter & Van Wyk, 1992). Different names have been attributed to IPs according to their structure and chemical composition (Leroux et al., 2007; Potgieter & Van Wyk, 1992) and these exert important roles in cell adhesion, cell wall hydration, apoplastic transport, and defense against pathogens (Tiné, Cortelazzo, & Buckeridge, 2000). However, currently, there are still few studies about the distribution of IPs and their functions among vascular plants, which is a knowledge gap.

Regarding their origin, evidence suggest that IPs are formed through fragmentation of the middle lamella pectin (Paiva & Machado, 2008; Potgieter & Van Wyk, 1992; Tiné et al., 2000) during the formation of intercellular spaces (Evert, 2006) or incorporation of substances deposited after intercellular spaces are formed (Leroux et al., 2007). Stress factors such as nutritional deficiency (Veys, Lejeune, & Van Hove, 2002), injury (Davies & Lewis, 1981), grafting (Jeffree, Dale, & Fry, 1986) and fungal infection (Scheidegger, Günthardt-Goerg, Matyssek, & HatVani, 1991) have also been pointed out to contribute to IPs formation.

Different types of IPs - filaments, scalae, strands, and warts (Potgieter & Van Wyk, 1992) - along with other morpho-anatomical characteristics, have been useful in taxonomic studies at different hierarchical levels (Lavalle, 2003; Machado & Sajo, 1996; Mengascini, 2002; Prada & Rolleri, 2005). Yet, the lack of knowledge about the distribution of IPs between vascular plants limits the use of these structures by taxonomy (Potgieter & Van Wyk, 1992); thus, IPs taxonomic value remains unclear (Machado & Sajo, 1996).

In ferns, IPs have been reported so far mainly for the petiole and leaf blade of 16 families (Potgieter & Van Wyk, 1992) and the occurrence of IPs in the rhizome of this group is still poorly explored. In rhizomes, IPs have been identified in species of the Davalliaceae, Polipodiaceae, Pteridaceae, Oleandraceae, (Potgieter & Van Wyk, 1992) and Aspleniaceae (Leroux et al., 2007) families. In the Dennstaedtiaceae, IPs were found in the rhizome of *Pteridium esculentum* (Carr & Carr, 1975) and *Pteridium aquilinum* subsp. *aquilinum* (Barton et al., 2015). Becari-Viana and Schwartsburd (2017) conducted a morpho-anatomical study on the Dennstaedtiaceae rhizome and found IPs mainly in lateral-line aerenchyma regions of some species. However, the type, distribution, and chemical composition of IPs in Dennstaedtiaceae are not known.

Here, we provide a detailed study on the occurrence of IPs in rhizomes of 23 species in the Dennstaedtiaceae, also presenting an anatomical and histochemical characterization of IPs to verify their taxonomic relevance for the family.

Material and methods

We sampled 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 (Table 1). Sixty-two specimens were analyzed, 35 of which were collected in natural field and 27 were obtained from herbaria CRH (Landcare Research New Zealand Limited), TNS (National Museum of Nature and Science), and VIC (*Herbário da Universidade Federal de Viçosa*).

Internodal segments from field-collected rhizomes were obtained from two fully-expanded leaves, fixed in FAA 50 (formaldehyde 37%, glacial acetic acid, ethyl alcohol 50%) (Johansen, 1940), dehydrated through a graded ethanol series, and embedded in methacrylate resin (Leica Historesin, Nussloch/Heidelberg, Germany). Transversal and longitudinal sections (5-7 µm thick) were obtained using an advance rotary microtome (model Spencer 820, American Optical Corporation, Buffalo, NY, USA). Sections were stained with toluidine blue, pH 4.7 (O'Brien & McCully, 1981), and mounted onto slides with synthetic resin (Permount-Fischer). The samples from herbarium material were rehydrated (Smith & Smith, 1942) prior to the processing steps described above.

The chemical nature of IPs was evidenced from the histochemical tests: ruthenium red (Johansen, 1940) for pectin; Schiff reagent/Periodic Acid Solution - PAS - (McManus, 1948) for polysaccharides; Sudan black B (Pearse, 1972) for lipids; and formalin-ferrous sulfate solution (Johansen, 1940) for phenolic compounds. Histochemical tests were performed only using field-collected material.

Internodal samples (0.2 cm thick) fixed in FAA 50 (Johansen, 1940) were dehydrated through a graded ethanol series and subjected to critical point drying (CPD 030, Bal-tec, Balzers, Liechtenstein). Samples were then mounted onto gold-coated stubs with double-sided sticky carbon tabs using the equipment Sputter Coater (SCD 050 Bal-tec, Balzers, Liechtenstein) and analyzed on a scanning electron microscope (LEO 1430VP, Zeis, Cambridge, England) of the Center for Microscopy and Microanalysis of the Federal University of Viçosa.

Classification of IPs types was performed according to Potgieter and Van Wyk (1992).

Results

Two types of IPs - strands and filament - were observed in the rhizomes (Figures 1A-F, Figure 2) of all studied species of the genera within the Dennstaedtiaceae (Table 2).

In the rhizomes of these species, the distribution of IPs in the pith and cortex is very homogeneous, occurring between the cells of parenchyma. In *Hypolepis* spp., *Paesia* spp., *Pteridium* spp., and *Histiopteris incisa*, IPs occur mainly in lateral-line aerenchyma cells. Despite the presence of lateral-line aerenchyma in *Blotiella lindeniana*, no IPs was observed in this region.

Table 1. List of analyzed species.

Species	Vouchers	Locality
Blotiella lindeniana (Hook.) R.M.Tryon	Schwartsburd & Becari-Viana 3348 (VIC)	Brazil
	Schwartsburd & Becari-Viana 3401 (VIC)	Brazil
	Schwartsburd & Becari-Viana 3414 (VIC)	Brazil
Dennstaedtia dissecta (Sw.) T.Moore	Yañez & Marquez 104 (VIC)	Argentina
Dennstaedtia cornuta (Kauf.) Mett.	Schwartsburd & Becari-Viana 2981 (VIC)	Brazil
	Schwartsburd & Becari-Viana 3070 (VIC)	Brazil
	Becari-Viana & Pereira 10 (VIC)	Brazil
	Becari-Viana & Pereira 11 (VIC)	Brazil
	Becari-Viana & Pereira 12 (VIC)	Brazil
Dennstaedtia globulifera (Poir.) Hieron.	Schwartsburd & Becari-Viana 3069 (VIC)	Brazil
	Becari-Viana & Pereira 06 (VIC)	Brazil
	Becari-Viana & Pereira 07 (VIC)	Brazil
	Becari-Viana & Pereira 08 (VIC)	Brazil
Dennstaedtia scabra (Wall.) T.Moore	Yamamoto 2636 (VIC, TNS)	Japan
	Yamanaka (VIC-44.535, TNS)	Japan
Dennstaedtia hirsuta (Sw.) Mett. ex Miq.	Takesako 4188 (VIC, TNS)	Japan
	Shimozono (VIC-44.536, TNS)	Japan
Dennstaedtia wilfordii (T.Moore) Christ.	Yuzawa 3494 (TNS)	Japan
	Saito (VIC- 44.526, TNS)	Japan
	Nagase (VIC-44.538, TNS)	Japan
Histiopteris incisa (Thunb.) J.Sm.	Becari-Viana & Pereira 19 (VIC)	Brazil
	Becari-Viana & Pereira 20 (VIC)	Brazil
	Becari-Viana & Pereira 20 (VIC)	Brazil
	Enzat 141 (CRH)	New Zealand
Hypolepis acantha Schwartsb.	Schwartsburd et al. 2264 (VIC)	Brazil
Hypolepis mitis Kunze ex Kuhn	Schwartsburd & Becari-Viana 3021 (VIC)	Brazil
	Schwartsburd & Becari-Viana 3097 (VIC)	Brazil
	Becari-Viana & Pereira 03 (VIC)	Brazil
	Becari-Viana & Pereira 04 (VIC)	Brazil
	Becari-Viana & Pereira 05 (VIC)	Brazil
	Schwartsburd & Becari-Viana 3316 (VIC)	Brazil
Hypolepis punctata (Thunb.) Mett. ex Kuhn	Tsutsui 5135 (VIC, TNS)	Japan
Hypolepis rugosula subsp. *pradoana* Schwartsb.	Becari-Viana & Pereira 01 (VIC)	Brazil
Hypolepis stolonifera var. *nebularis* Schwartsb.	Becari-Viana & Pereira 13 (VIC)	Brazil
	Becari-Viana & Pereira 15 (VIC)	Brazil
	Becari-Viana & Pereira 14 (VIC)	Brazil
Hypolepis stolonifera Fée var. *stolonifera*	Labiak et al. 4269 (VIC)	Brasil
Microlepia marginata (Panz.) C. Chr.	Iwatsuki & Kato 79 (CRH)	Japan
	Wang et al. 368 (CRH)	China
	Haruda 366 (VIC, TNS)	Japan
	Takesako 5597 (VIC, TNS)	Japan
Microlepia izu-peninsulae Sa. Kurata	Nakaike (CRH- 239661)	Japan
Microlepia obtusiloba Hayata	Iwatsuki & Kato 51 (CRH)	Japan
Microlepia speluncae (L.) T. Moore	Schwartsburd & Becari-Viana 3290 (VIC)	Brazil
	Schwartsburd & Becari-Viana 3310 (VIC)	Brazil
	Schwartsburd & Becari-Viana 3315 (VIC)	Brazil
	Croft 836 (CRH)	Papua New Guinea
Microlepia strigosa (Thunb.) C. Presl	Braithwaite 2491 (CRH)	New Zealand
	Hovell (CRH-465258 A)	New Zealand
	Kido 12966 (VIC, TNS)	Japan
Oenotrichia maxima (E. Fourn.) Copel.	Brownlie 200 (CRH)	New Caledonia
Paesia glandulosa (Sw.) Kuhn	Schwartsburd & Fortuna-Perez 2929 (VIC)	Brazil
	Schwartsburd & Fortuna-Perez 2930 (VIC)	Brazil
	Schwartsburd & Fortuna-Perez 2931 (VIC)	Brazil
Paesia rugosula (Labill.) Kuhn	More & Brownlie 463 (CRH)	New Caledonia
Paesia scaberula (A. Rich.) Kuhn	Smith 84 (CRH)	New Zealand
	Moore (CRH-533179)	New Zealand
Paesia tahitensis Copel.	Sykes 446 (CRH)	French Polynesian
Pteridium arachnoideum (Kaulf.) Maxon subsp. *arachnoideum*	Schwartsburd et al. 2838 (VIC, NSW)	Brazil
	Schwartsburd et al. 2837 (VIC)	Brazil
	Schwartsburd et al. 3383	Brazil
Pteridium arachnoideum subsp. *campestre* (Schrad.) Schwartsb. & P.L.R. Moraes	Alves da Silva et al. 01 (VIC, NSW).	Brazil

Table 2. Intercellular Protuberances (IPs) types and distribution among species in the Dennstaedtiaceae.

Genera	Species	Ip types	Distribution
Blotiella R.M.Tryon	*B. lindeniana* (Hook.) R.M.Tryon	Strands	Cortex, except lateral-line aerenchyma
Dennstaedtia Bernh.	*D. dissecta* T.Moore, D. *cornuta* (Kauf.) Mett., *D. globulifera* (Poir.) Hieron., *D. scabra* (Wall.) T. Moore, *D. hirsuta* (Sw.) Mett. ex Miq., *D. wilfordii* (T.Moore) Christ.	Strands	Cortex and pith
Histiopteris J.Agardh	*H. incisa* (Thunb.) J.Sm.	Filaments	Cortex and pith
Hypolepis Bernh.	*H. acantha* Schwartsb., *H. mitis* Kunze ex Kuhn, *H. punctata* (Thunb.) Mett. ex Kuhn, *H. rugosula* subsp. *pradoana* Schwartsb., *H. stolonifera* var. *nebularis* Schwartsb., *H. stolonifera* Fée var. *stolonifera*	Filaments	Cortex and pith
Microlepia C.Presl	*M. marginata* (Panz.) C.Chr., *M. izu-peninsulae* Sa. Kurata, *M. obtusiloba* Hayata, *M. speluncae* (L.) T. Moore, *M. strigosa* (Thunb.) C. Presl	Strands	Cortex and pith
Oenotrichia Copel.	*O. máxima* (E. Fourn.) Copel.	Filaments	Cortex and pith
Paesia A.St.-Hil.	*P. glandulosa* (Sw.) Kuhn, *P. rugosula* (Labill.) Kuhn, *P. scaberula* (A. Rich.) Kuhn, *P. tahitensis* Copel.	Filaments	Cortex and pith
Pteridium Gled. ex Scop.	*P. arachnoideum* (Kaulf.) Maxon subsp. *arachnoideum, P. arachnoideum* subsp. *campestre* (Schrad.) Schwartsb. & P.L.R. Moraes	Filaments	Cortex and pith

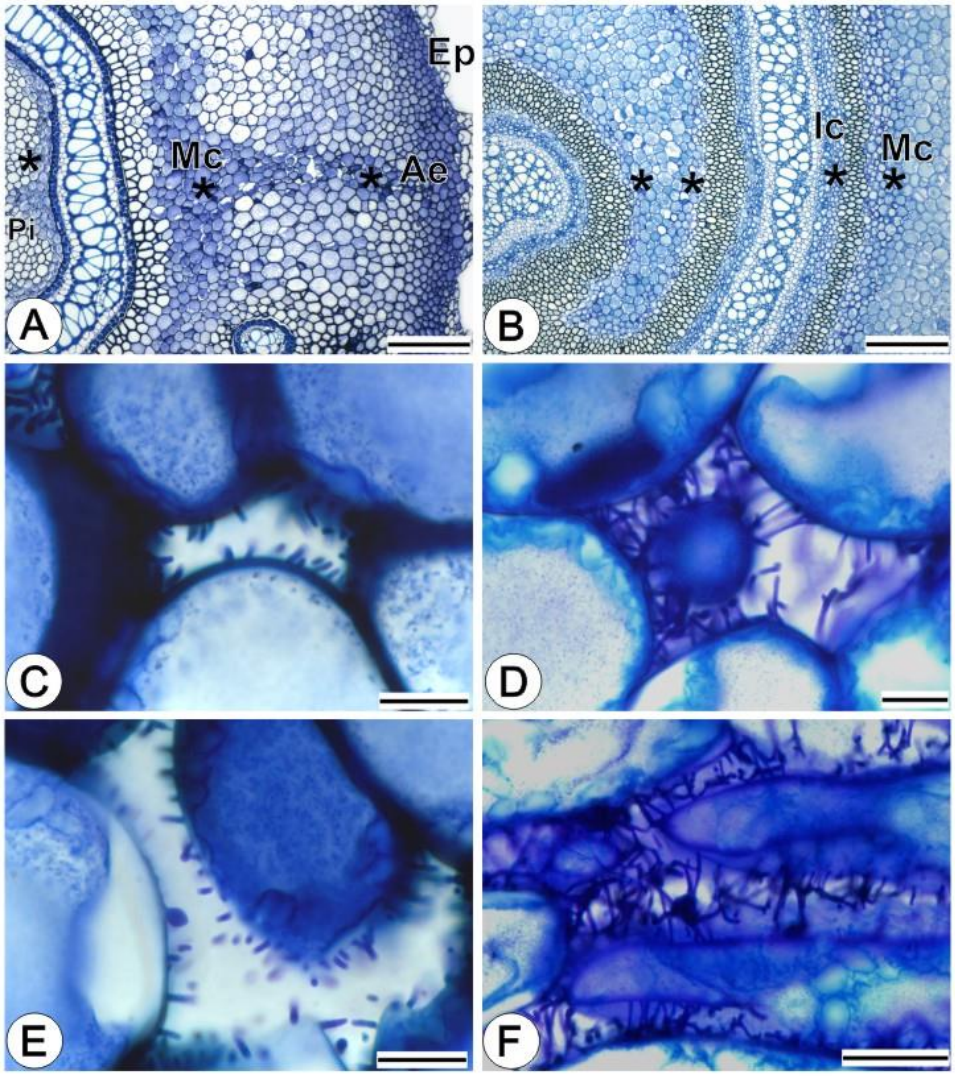

Figure 1. Distribution and anatomy of intercellular protuberances (IPs) in rhizomes of Dennstaedtiaceae spp. Transverse (A, B, C, D) and longitudinal (E and F) sections. A, C, E - *Histiopteris incisa*. B, D, F – *Dennstaedtia cornuta*. Asterisks indicate IPs distribution in the rhizome. Ae = lateral-line aerenchyma; Ic = inner cortex; Mc = medium cortex; Ep = epidermis; Pi = pith. Scale bar: 10 µm (C, D, E), 20 µm (F), 400 µm (A, B).

Intercellular filamentous protuberances have spheroidal or angustate apices and face the intercellular region, but are not connected to neighboring cells (Figures 1C,1E, 3A-B). IPs apices vary within the same species, such as *Histiopteris incisa*, which presents IPs with angustate and spheroidal apices (Figure 3D). In addition, strand-type IPs (Figure 3C - arrows) might be mixed with filament-type IPs.

Strand-type IPs form a connection between cells (Figures 1D, 1F, 4A-B) and can be branched or unbranched. The branched strand-type IPs have irregular ramifications and connect cells at different points in the cell wall (Figure 4B) and they also have a few free branches (Figure 4B - arrow). Unbranched strand-type IPs are directly connected to the neighboring cell wall (Figure 4C). Some of these protuberances have areas that resemble rupture points (Figure 4D – white arrow). Mixed with strand-type IPs, filament-type IPs (Figure 4D - asterisk) can also be observed in smaller quantities.

Overall, most IPs are irregularly shaped, often nodulated and with spheroidal apices (Figure 4D - black arrow). No evidence that IPs are hollow, or that the cell wall shows porosity, was observed.

The polysaccharide nature of both IPs types was evidenced by PAS (Figures 5A-B) and their pectic constitution by ruthenium red (Figures 5C-D). Sudan black B test was negative for lipid.

In *Hypolepis* spp., *Paesia* spp., *Pteridium* spp., and *Histiopteris incisa*, IPs reacted positively to toluidine blue, resulting in a greenish color in the lateral-line aerenchyma, as shown in *Pteridium arachnoideum* subsp. *arachnoideum* (Figures 2A, 2C, 6C), indicating the presence of phenolic compounds in these structures. The same was not observed in the region of the inner cortex (Figure 6A). The phenolic nature of IPs in the lines of aerenchyma was confirmed in *Pteridium arachnoideum* subsp. *arachnoideum* through the ferrous-sulfate formalin test (Figure 6D), which was also positive for inner cortex IPs (Figure 6B). We also found that lateral line aerenchyma IPs in *Pteridium arachnoideum* subsp. *arachnoideum* were not heavily stained with Schiff's reagent (Figure 7C) or ruthenium red (Figure 7D) as those of the inner cortex (Figure 7A-7B, respectively).

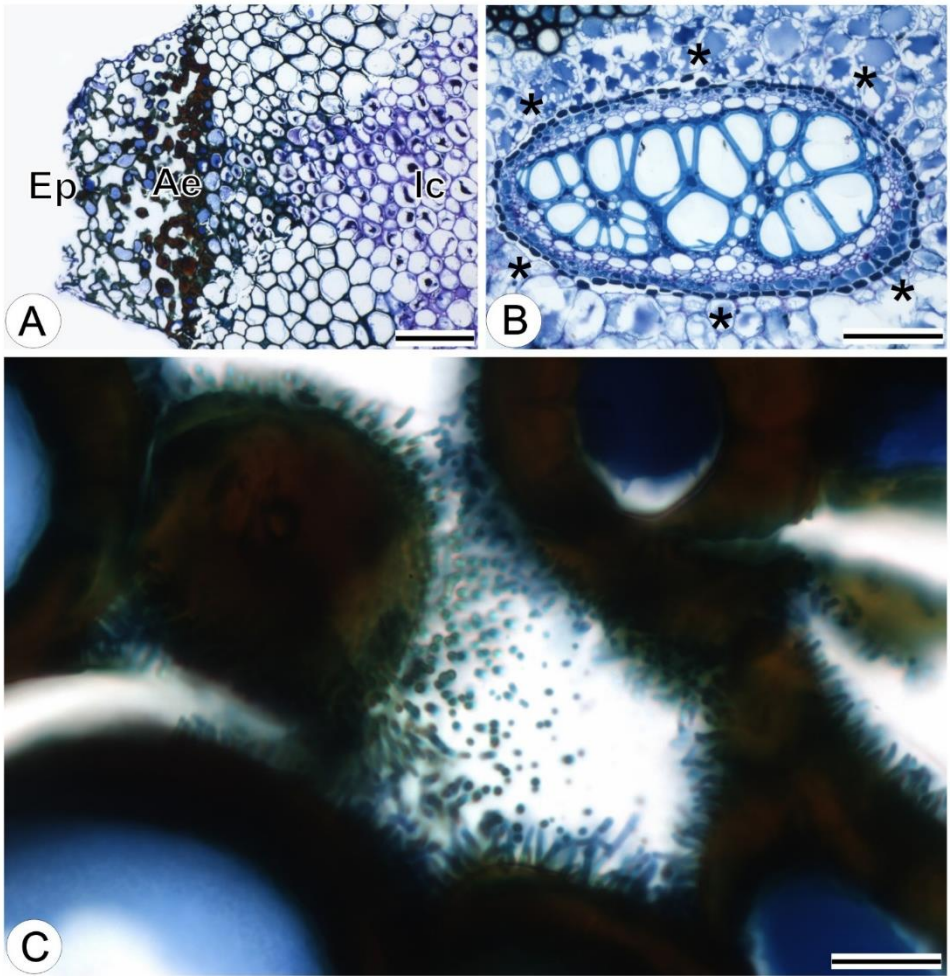

Figure 2. Distribution and anatomy of intercellular protuberances (IPs) in rhizome of *Pteridium arachonoideum* subsp. *arachnoideum* in transverse sections. A - overview of lateral-line aerenchyma in the outer cortex region. B - Inner cortex region. C - Detail of IPs on the lateral-line aerenchyma. Asterisks indicate IPs distribution in the rhizome. Ae = lateral-line aerenchyma; Ic = inner cortex; Ep = epidermis. Scale bar: 250 µm (A), 200 µm (B), 10 µm (C).

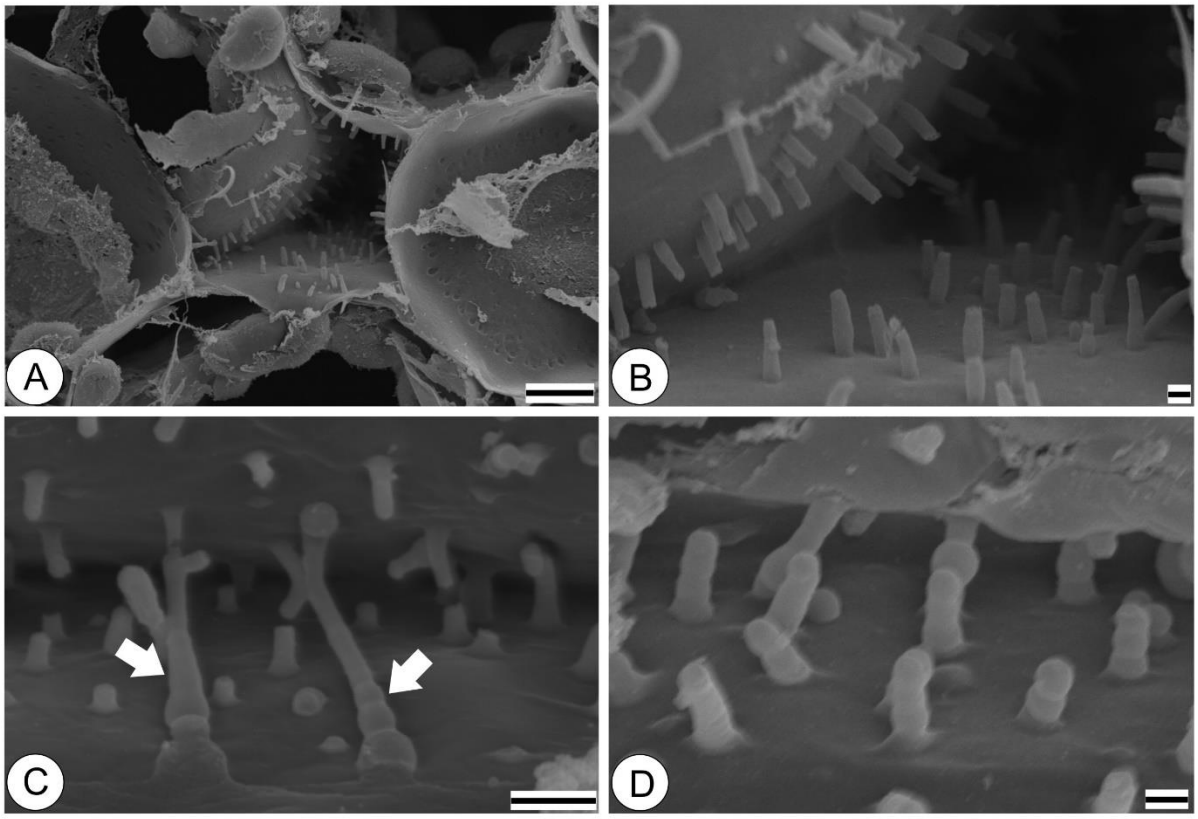

Figure 3. Types of intercellular protuberances (IPs) in *Histiopteris incisa* (scanning electron microscopy). A-B - overview and detail of the intercellular space, respectively, in the cortical region with filament-type IPs. C - Detail of strand-type IPs (white arrows) mixed with filament-type IPs. D - filament-type IPs with spheroidal apices. Scale bar: 1 µm (B, D), 2 µm (C), 10 µm (A).

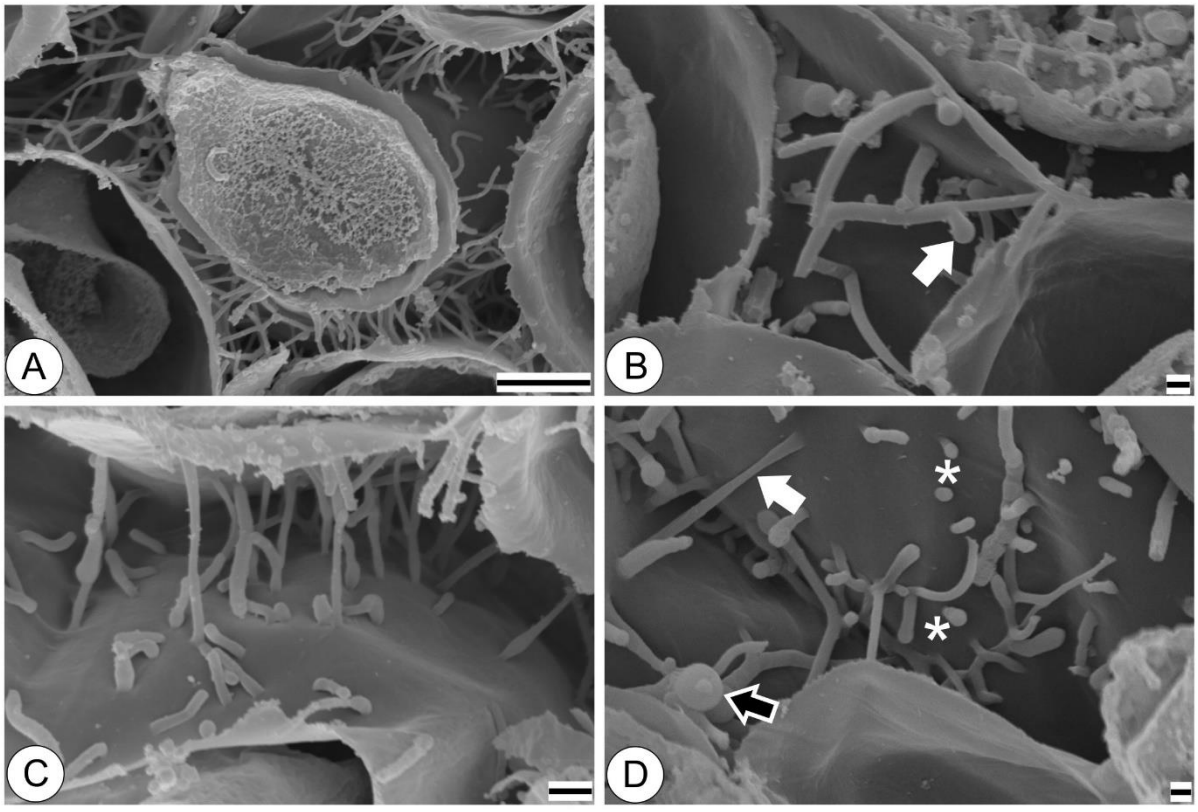

Figure 4. Types of intercellular protuberances (IPs) in *Dennstaedtia cornuta* (scanning electron microscopy). A - overview of intercellular spaces in the cortical region with strand-type IPs. B - detail of branched strand-type IPs (arrow indicating spheroidal branching). C - unbranched strand-type IPs. D - rupture point of a strand-type IPs (white arrow), filament-type IPs mixed with strand-type IPs (asterisks), and an IPs with spheroidal apex (black arrow with white outline). Scale bar: 1 µm (B, D), 2 µm (A, C).

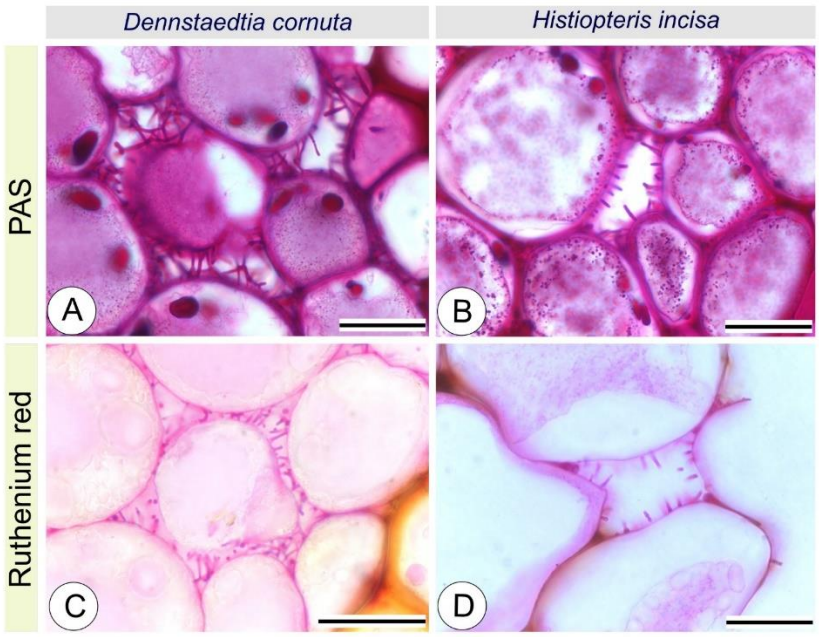

Figure 5. Chemical nature of intercellular protuberances (IPs) of *Dennstaedtia cornuta* (A, C) and *Histiopteris incisa* (B, D). Positive results for pectin with ruthenium red test (C, D), and for polysaccharides with PAS test (A, B). A, C - strand-type IPs. B, D - filament-type IPs. Scale bar: 20 µm.

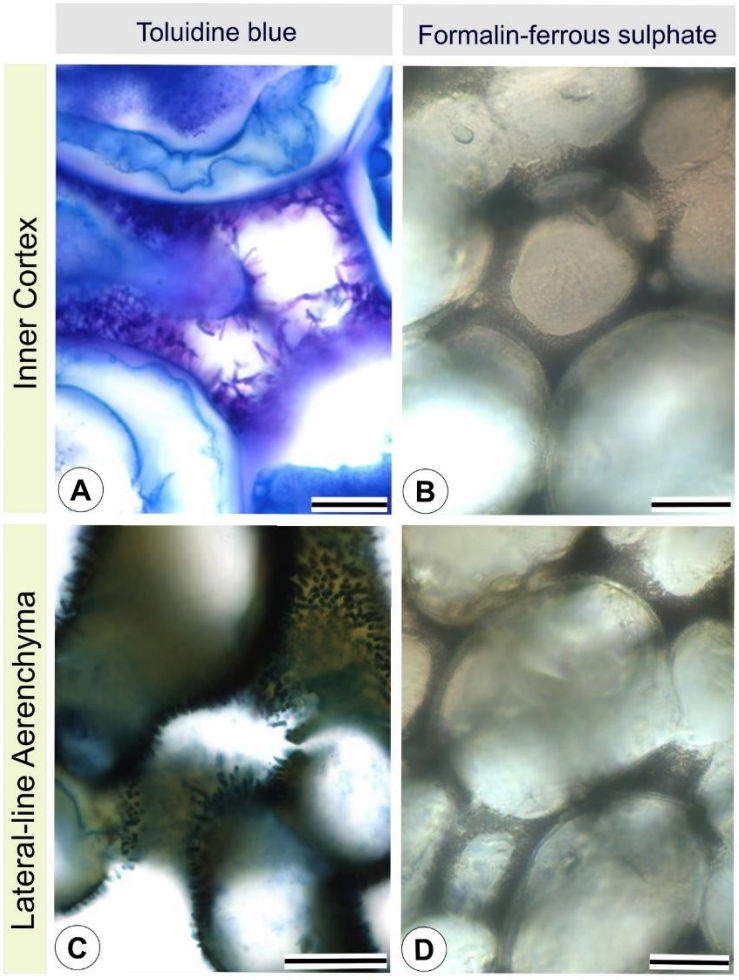

Figure 6. Intercellular protuberances (IPs) from the outer cortex (lateral-line aerenchyma region) and inner cortex of *Pteridium arachnoideum* subsp. *arachnoideum*. Histochemical reactions phenolic compounds (formalin-ferrous sulphate), and toluidine blue stain. A, C - toluidine blue staining showing the presence of phenolic compounds. B, D - positive results for phenolic compounds. Scale bar: 20 µm (A, B, C), 100 µm (D).

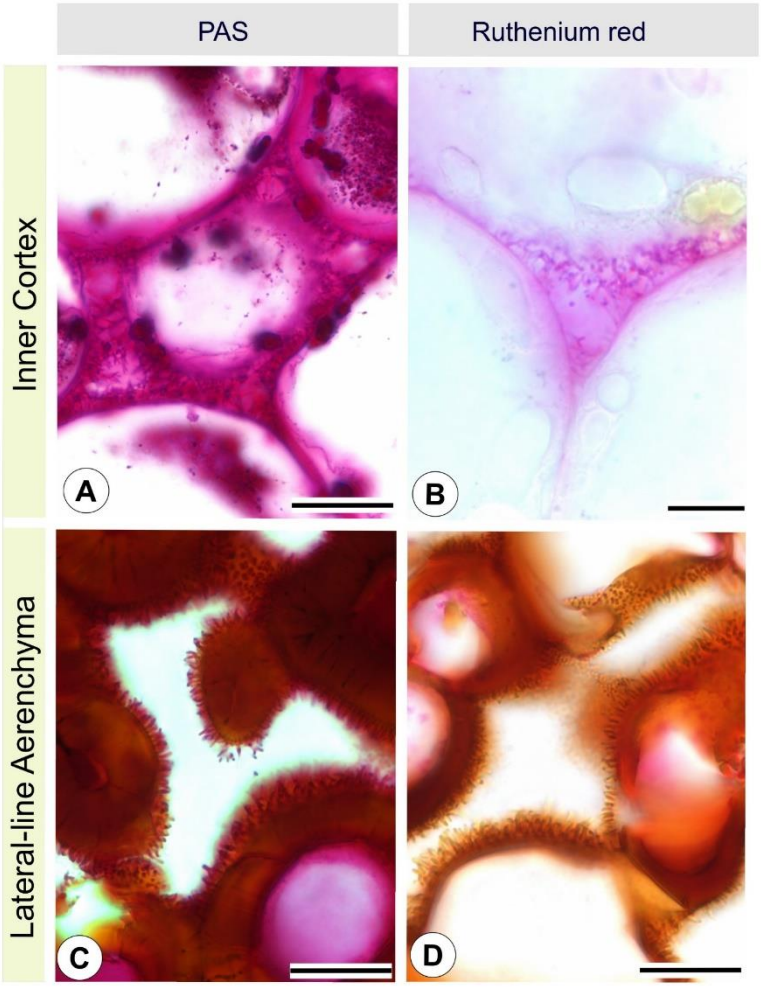

Figure 7. Intercellular protuberances (IPs) from the outer cortex (lateral-line aerenchyma region) and inner cortex of *Pteridium arachnoideum* subsp. *arachnoideum*. Histochemical reactions for polysaccharides (PAS) and pectin (ruthenium red). A, C - results positive and negative, respectively, for neutral polysaccharides. B, D - results positive and negative, respectively, for pectin. Scale bar: 10 µm (B, C), 20 µm (A, E), 100 µm (D).

Discussion

We hypothesize that the two types of IPs – strand and filament - observed in species of the Dennstaedtiaceae are formed by fragmentation of the middle lamella during the formation of intercellular spaces as a natural process, especially because the plants collected in the natural field presented healthy aspect. This reasoning is corroborated by the position that IPs occupy, the intercellular space of the middle lamella, as well as by the pectic composition and the structural similarity between IPs and cell wall. This type of origin has also been suggested for other species by Potgieter and Van Wyk (1992), Tiné et al. (2000), and Paiva and Machado (2008).

The polysaccharide nature and pectic composition of IPs in the Dennstaedtiaceae demonstrated herein agree with the data presented by Potgieter and Van Wyk (1992). It can be observed that, regardless of origin, whether it is the middle lamella as inferred here or protoplasmic activity after intercellular space formation, IPs composition does not differ (Butterfield, Meylan, & Exley, 1981; Veys et al., 2002).

Variations in IPs shape such as nodulations, spheroidal, or angustate and intertwined apices, as observed in Dennstaedtiaceae, were also reported for *Asplenium* petiole (Leroux et al., 2007), *Christensenia* leaf blade (Rolleri, 1993), and *Cocos nucifera* stem (Butterfield et al., 1981). These variations are presumed to originate from rupture events and/or contractions of strand-type IPs under high tension (Butterfield et al., 1981; Carr & Carr, 1975; Potgieter & Van Wyk, 1992). According to Potgieter and Van Wyk (1992), during the formation of intercellular spaces, strand-type IPs can rupture and form filament-type IPs or warts. This hypothesis can be applied to IPs occurring in Dennstaedtiaceae spp., such as *Dennstaedtia cornuta*, in which filament-type IPs mixed with strand-type IPs are observed.

The occurrence of filament-type IPs in the lateral-line aerenchyma in the genera *Histiopteris*, *Hypolepis*, *Pteridium*, and *Paesia* can be explained by the conspicuous intercellular gaps that are formed in this region of the cortex. In the inner cortex, where smaller intercellular spaces occur, filament-type IPs predominate but strand-type IPs are also present. These observations reinforce the hypothesis that, in Dennstaedtiaceae, IPs originate from the middle lamella rupture and also evidencing how far the cells move apart during the formation of the intercellular space. Prada and Rolleri (2005) demonstrated that the coexistence of strand and filament-like IPs appear to be common among ferns.

Although several functions are ascribed to IPs, such as apoplastic transport, cell wall hydration, carbohydrate reserve, and pathogen defense (Rolleri, 1993), we believe that these structures play a mechanical role in rhizomes of Dennstaedtiaceae spp. by promoting adhesion between parenchyma cells that have large intercellular spaces. Therefore, IPs exert a relevant function regarding structural maintenance of this tissue.

In addition to the mechanical contribution, IPs occurring in the lateral-line aerenchyma, as observed in *Pteridium arachnoideum* subsp. *arachnoideum*, *Histiopteris incisa,* and the species *Hypolepis* and *Paesia,* may be associated with defense against pathogens indicated by the accumulation of phenolic compounds, as shown by toluidine blue and ferrous-sulfate formalin staining in *Pteridium arachnoideum* subsp. *arachnoideum*. The accumulation of phenolic compounds in IPs was also demonstrated in *Pteridium aquilinum* subsp. *aquilinum* by Barton et al. (2015), who emphasized the importance of these compounds against microorganisms in the lateral-line aerenchyma.

Cells are loosely organized in the lateral-line aerenchyma (Davies, 1991; Watt, 1979). As a result, while these allow the aeration of inner tissues, they can also represent an entry point to pathogens, especially in underground organs such as Dennstaedtiaceae rhizomes.

The lack of knowledge regarding the distribution of IPs among the different groups of plants, as well as the difficulties related to their identification limit the use of these structures in taxonomy. Still, some studies highlight that along with other characters, intercellular protuberances have been useful in taxonomic studies and aided in species diagnosis (Lavalle, 2003; Mengascini, 2002; Prada & Rolleri, 2005).

This work was able to identify links between IPs and taxonomy and evolution in Dennstaedtiaceae. However, some trends were observed:

1. Dennstaedtiaceae presents two major large clades, 'Hypolepidoideae' and 'Dennstaedtioideae' – see phylograms by Becari-Viana and Schwartsburd (2017) and Perrie, Shepherd and Brownsey (2015). With a few exceptions, filament-type IPs predominate in 'Hypolepidoideae', whereas the strand type prevails in 'Dennstaedtioideae'.

2. IPs were not found only in the lateral-line aerenchyma of *Blotiella lindeniana*. This can be explained by the fact that, among the studied species, only *B. lindeniana* has ascending and aerial rhizomes – see images in Becari-Viana and Schwartsburd (2017). This reinforces the idea that IPs present in the lateral-line aerenchyma play a protective role in species bearing underground rhizomes.

Conclusion

Our results expand the IPs record for Dennstaedtiaceae and vascular plants and histochemical data confirm their polysaccharide and pectic nature. We emphasize that ontogenetic, ultrastructural, and immunohistochemical studies on protuberances and the lateral-line aerenchyma are essential to elucidate their development, as well as the composition of IPs and cell wall. We suggest that studies with larger numbers of species of the Dennstaedtiaceae may shed light on whether the presence and type of IPs may be valuable to the taxonomy of the family.

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