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Spore morphology and ornamentation in the genus *Blechnum* (Blechnaceae)

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Abstract

A study of the spores of 64 neotropical and palaeotropical taxa of the genus *Blechnum* was performed using scanning electron microscopy (SEM). Two basic types of perispores were recognised: perispore smooth or only slightly ornamented (laevigate, with micro-granules or micro-lamellae, venulose and rugulate), and perispore ornamented (rugate, low reticulate, cristate-reticulate, orbiculate, and colliculate). SEM observations allow for distinguishing differences in the structure of perispores, but the character does not correlate with the ornamentation. The smooth exospore predominates among the studied species. Results suggest that perispore characters have important diagnostic value, since different combinations of ornamentation/structure were found among the studied species. Moreover, when spore characters are considered together with other morphological traits of the sporophytes and the habit of plants, ornamentation of spores becomes a useful complementary feature at the specific level. The results are discussed in light of the recent systematic studies and emphasise that any eventual subdivision of the genus *Blechnum* must include characters of the sporophyte as well as spore features to ensure a more robust classification than the previously proposed. The spores also show a significant affinity between *Blechnum* and other genera of Blechnaceae, which was also suggested by recent phylogenetic studies.

Keywords: *Perispore ornamentation, perispore structure, micro-ornamentation, laevigate, rugulate, foliose, rugate, reticulate, cristate-reticulate, exospore*

The Blechnaceae are a cosmopolitan group of ferns including eight (de la Sota, 1977), nine (Copeland, 1947; Tryon & Tryon, 1982; Proctor, 1985; Lellinger, 1989; Kramer et al., 1990; Tryon & Stolze, 1993; Moran, 1995) or twelve genera (Pichi Sermolli, 1977; Cranfill & Kato 2003). Six genera are confined to the Old World: *Brainea*, *Doodia*, *Pteridoblechnum*, *Sadleria*, *Stenisioblechnum* and *Stenochlaena*; *Salpichlaena* is exclusively American, while *Blechnum* and *Woodwardia* occur both in the Old and New World. *Anchistea*, *Chieniopteris* and *Lorinseria* are considered additional well-defined genera by Pichi Sermolli (1977) or they are included in *Woodwardia* by most authors.

Based on recent phylogenetic analyses, Kenrick and Crane (1997) and Pryer et al. (2001) concluded that leptosporangiate ferns represent part of a monophyletic group, the Monilophyta, which also includes the eusporangiate ferns, the Equisetaceae and the Psilotaceae. This configuration was supported by Schuettpelz et al. (2006) and Schuettpelz and Pryer (2007, 2008), who suggested a rearrangement of the families and genera in the largest order of the leptosporangiate ferns, the Polypodiales. According to this concept, the Blechnaceae (including Stenochlaenaceae) would consist of twelve genera (i.e. *Blechnum*, *Brainea*, *Doodia*, *Pteridoblechnum*, *Sadleria*, *Salpichlaena*,

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Steenisiolechnum, *Stenochlaena*, *Woodwardia*, *Anchistea*, *Chieniopteris* and *Lorinseria*).

As currently circumscribed, *Blechnum* is a cosmopolitan genus comprising around 200 species, of which about 138 occur in the Southern Hemisphere and some 78 species in the Northern Hemisphere (Kasmirczak, 1999) with species in America (North America, Central America, the Caribbean and South America), Europe to the Ural, Africa, Madagascar, Asia (eastward to Japan), Melanesia, Australia, New Zealand and some Pacific Islands (Polynesia to Tahiti, Marquesas Islands and Rapa Iti). The genus is adventitious in Hawaii. Its sporophytes are terrestrial, erect or scandent, epiphytic, lithophytic or amphibious plants, adapted to tropical and sub-tropical rainforests, lowland or montane forests, tepuis, along the edges of forests, thickets, savanna or disturbed areas, wetlands, meadows, bogs and swamps from cold sub-Antarctic regions, at altitudes ranging from sea level to 4500 m. The rhizomes are decumbent, creeping, scandent or erect to arborescent, scaly, with monomorphic or dimorphic fronds that bear entire, pinnatifid or once-pinnate laminae and free, rarely anastomosing venation. Cenosori are formed on a vascular commissure or at the base of the indusium, without paraphyses. Spores are monolete, with a perispore.

The number of taxa is not established with certainty. Rolleri and Prada (2006a) recorded 92 taxa in the Neotropics, including species and hybrids, but new species were described later (e.g., Moran & Smith, 2005; Rojas-Alvarado, 2006, 2008a, 2008b; Kessler et al., 2007; Ramos Giacosa, 2010) and partial revisions (e.g., Rolleri and Prada, 2006b; Gabriel y Galán et al., 2008; Prada et al., 2008; Rolleri et al., 2008, 2010) have been published that include detailed studies of subsets of species. These studies may eventually result in a revision of the species number. Further, recent molecular analyses of the Blechnaceae resolved *Blechnum* as a paraphyletic genus and indicate that most of the genera currently recognised in the Blechnaceae are nested within *Blechnum* s. l. (Cranfill and Kato, 2003; Smith et al., 2006; Shepherd et al., 2007). An expansion of the genus *Blechnum* as suggested by molecular studies requires, however, a thorough revision of the family (Smith et al., 2006) that is not possible with the current, insufficient knowledge of the Blechnaceae. Most importantly, the delimitation of species is not yet conclusive. To achieve better understanding of the family at species level, studies in the context of regional floras such as those of Schelpe (1952), de la Sota (1972a, 1972b, 1972c, 1977), Murillo (1968), Tryon and Tryon (1982), Proctor (1985), Duncan and Isaac (1986), Lellinger (1989), Tryon and Stolze (1993), Marticorena and Rodríguez Ríos (1995), Moran (1995), Smith (1995), de la Sota et al.

(1998) and Mickel and Smith (2004) are needed, and must be integrated and updated with new sets of diagnostic characters that will allow clear distinction of the species.

Any revision attempting to reach a well defined sub-generic treatment, provided that sub-generic division would be justified, should be made on the basis of diagnostic characters, a more complete cytological knowledge of the species, and a selection of the diagnostic traits that could determine with reasonable certainty, the limits, affinities and relationships of species. The systematics conducted in floristic treatments appears to be based on too variable characters, a problem that results both from the variation and adaptive phenotypic plasticity of the species (some characters, such as indumentum, vary with altitude), the different size and ecology of plants, and also the cytology of species, yet poorly known.

Among potential important features for taxonomic delimitations at the species level are also features of the spores. While several studies have focused on the morphology and structure of the spores of Blechnaceae, and particularly of the genus *Blechnum* (e.g. Lugardon, 1965, 1974; Morbelli, 1976, 1980; Tryon & Lugardon, 1991; Ramos Giacosa et al., 2006, 2009; de la Sota et al., 2006), most of the studies are mainly descriptive and do not discuss the systematic implications of the features in any detail. However, these contributions provide important information on spore morphology and structure that may eventually be included in more comprehensive taxonomic/systematic studies. Some attempts to relate spore types within *Blechnum* to systematic grouping of species have been made recently. These studies include a review of the *B. penna-marina* (Poir.) Kuhn group by Rolleri and Prada (2006b), who proposed a comprehensive set of defining features that included spores. Passarelli (2007) also analysed the group based on spore morphology and suggested relationships with other groups and spore types of *Blechnum*, Blechnaceae and related families. Further studies of different species in a broader context including the morphology of the spores, their relationships with other characters of the sporophytes, and the geographical distribution of species was carried out by Rolleri et al. (2008), Prada et al. (2008) and Gabriel y Galán et al. (2008). These studies clearly show that spores may provide critical diagnostic features.

While preparing a comprehensive review of both arborescent and epiphytic species of *Blechnum* (unpublished data), the importance of spores in both neotropical and palaeotropical taxa became apparent. However, as no broad spore survey was available, we initiated this detailed study of spore morphology of 64 taxa of *Blechnum* from both the Neotropics and the Palaeotropics. The study represents 32% of the

described species of the genus and includes both light microscopy (LM) and scanning electron microscopy (SEM). This allows easy access to the data of ornamentation to most taxonomists and the results can easily be compared when many samples are studied. Furthermore, the aim of this work was to obtain a wide and representative selection of the variety of ornamentation found in spores, in order to prospect possible variations in the genus and to test whether variations may be matched with groups of species proposed by previous systematic analysis.

Materials and methods

Sixty-four species were selected for this study (see 'Specimens investigated' list). Species identification was made based on Burrows (1990), Tryon and Stolze (1993), Moran (1995) and Chambers and Farrant (1998), and also by determination by the authors after consulting the type material. The selection of species was made to include as many species as possible, covering all groups of *Blechnum* recognised in earlier studies, such as the informal classification of Tryon and Tryon (1982), revised by Oliveira Dittrich (2005), as well as our own data suggesting groups of affinity (Rolleri & Prada, 2006b; Passarelli, 2007; Prada et al., 2008; Rolleri et al., 2008, 2010, and unpublished work on the genus). Species not previously included in any pre-existing group were also added. Efforts were made to represent the diversity of the genus, both in types of sporophytes (habits) and the different environments they inhabit.

For each species 6–10 fertile specimens were studied, although only one representative specimen is listed at the end of this work. The exception was made in the case of *Blechnum tabulare*, for which one African and two American specimens of the species are cited. Spore samples were taken from adult medium pinnae from each individual. For LM observations spores were mounted in a drop of DePex mounting medium (Gurr, 1966), which does not influence the exospore size, but clears the perispore slightly and gives a superior outline of the exospore, allowing it to be measured more accurately (Viane, 1993). The measurements of major and minor equatorial diameters as seen in polar view were taken on 30 spores from each spore sample; the minimum, average and maximum are given in micrometres (Table I). In spores where the perispore is present as a thin layer down to 1 µm thick, evenly adhered to the exospore, the given measurements include both spore walls. In spores with thick and/or ornamented easily detaching perispore, the thickness of this wall above the exospore was measured where the most prominent areas occur (Table I).

For SEM observations, spores were mounted without treatment on metal stubs with adhesive double sided tape, covered with gold/palladium under vacuum, and examined by a Jeol T100 microscope of the Electronic Microscopy Service from the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Argentina, and a Jeol JSM-T330A at 15 kV, at the Real Jardín Botánico de Madrid, Spain.

In the illustrations, photographs of the spores of the species are arranged by the increasing complexity observed in the ornamentation of the perispore. The terminology is the same used in previous pteridological works of the authors and, with some modifications, is also found in Lellinger and Taylor (1997) and Lellinger (2002).

Results

Spores of *Blechnum* are monolete with ellipsoid outline in polar view, plane-convex to concavo-convex (reniform) in equatorial longitudinal view; media of equatorial diameters range 31–89 µm × 22–66 µm (Table I). The thickness of the perispore varies from 1 µm to 10 µm. The outer surface of the perispore of the studied species may be nearly smooth or with different types of ornamentation. Two main types of perispore have been recognised: perispore smooth or with inconspicuous ornamentation (laevigate with micro-granules or micro-lamellae, venulose and rugulate), and perispore distinctly ornamented (rugate, low reticulate, cristate-reticulate, orbiculate, and colliculate).

Perispore smooth or with inconspicuous ornamentation

This group includes spores with laevigate perispore that bears micro-granules, either scattered or grouped in areas without a definite pattern; spores with laevigate perispore with very thin, smooth, superficial micro-lamellae; spores with venulose perispore with a more or less regular pattern of micro-areolae; and spores with rugulate perispore with rounded, very low, non-anastomosing rugulae.

Laevigate perispore with micro-granules were found in *Blechnum andinum* (Figure 1C), *B. asplenoides*, *B. attenuatum* (Figure 1B), *B. austrobrasilianum*, *B. corralense* (Figure 1D), *B. divergens* (Figure 1E), *B. glandulosum*, *B. gracile*, *B. laevigatum*, *B. lanceola*, *B. lherminieri* (Figure 1F), *B. meridense*, *B. mochaenum* subsp. *achalense*, *B. occidentale* (Figure 1A) and *B. stoloniferum*. Venulose perispores were found in *B. auriculatum* (Figure 2A), *B. brasiliense* (Figure 2B), *B. chambersii*, *B. hastatum*, *B. polypodioides* and *B. sprucei* (Figure 2C). Rugulate perispores were found in *B. arcuatum* (Figure 2E), *B. blechnoides* (Figure 2F) and *B. mochaenum* subsp. *mochaenum* (Figure 2D).

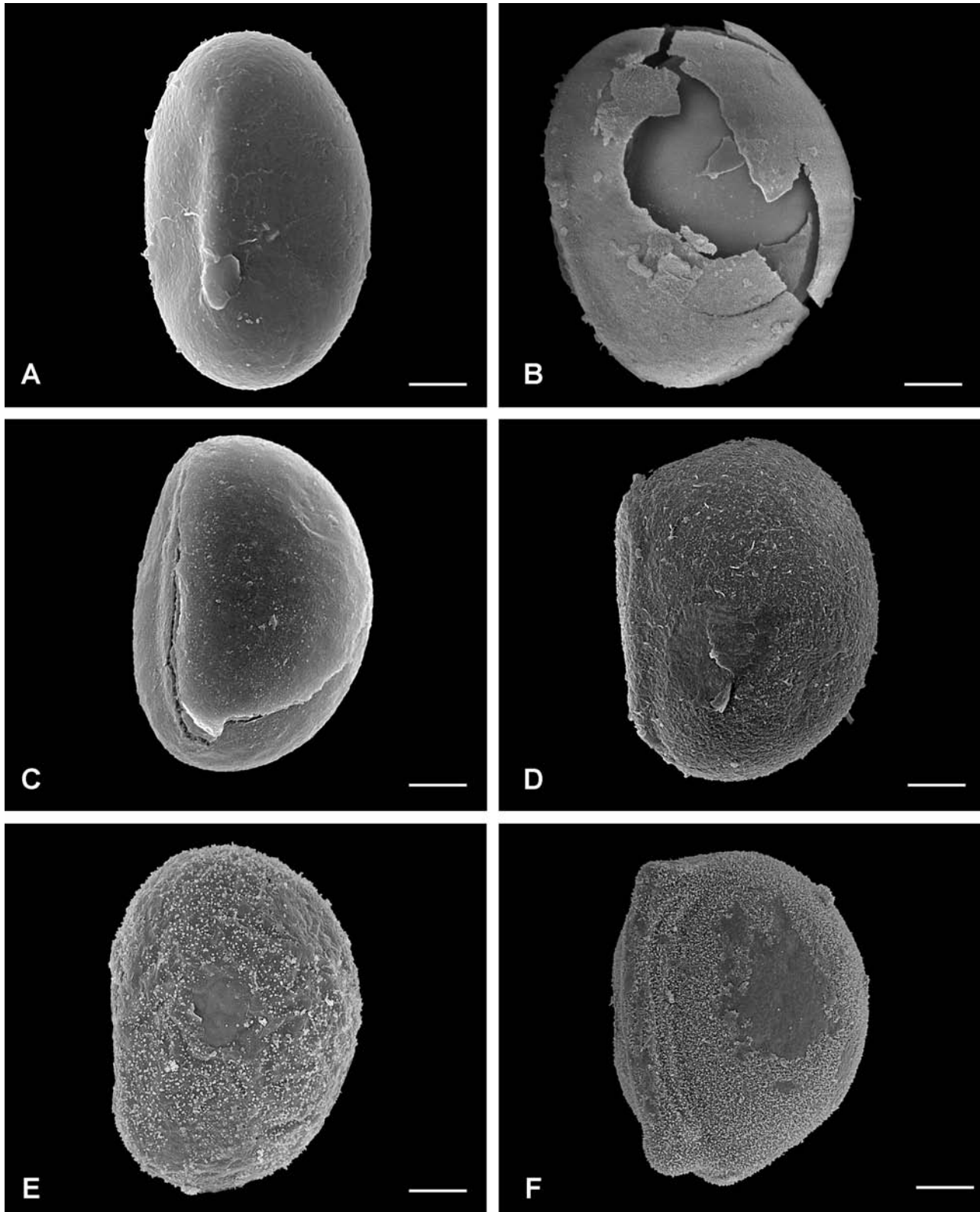


Figure 1. Laevigate perisporangia with micro-granules in *Blechnum* species. **A.** *Blechnum occidentale*. **B.** *Blechnum attenuatum*. **C.** *Blechnum andinum*. **D.** *Blechnum corralense*. **E.** *Blechnum divergens*. **F.** *Blechnum lherminieri*. Scale bars – 7 μm (A–E), 11 μm (F).

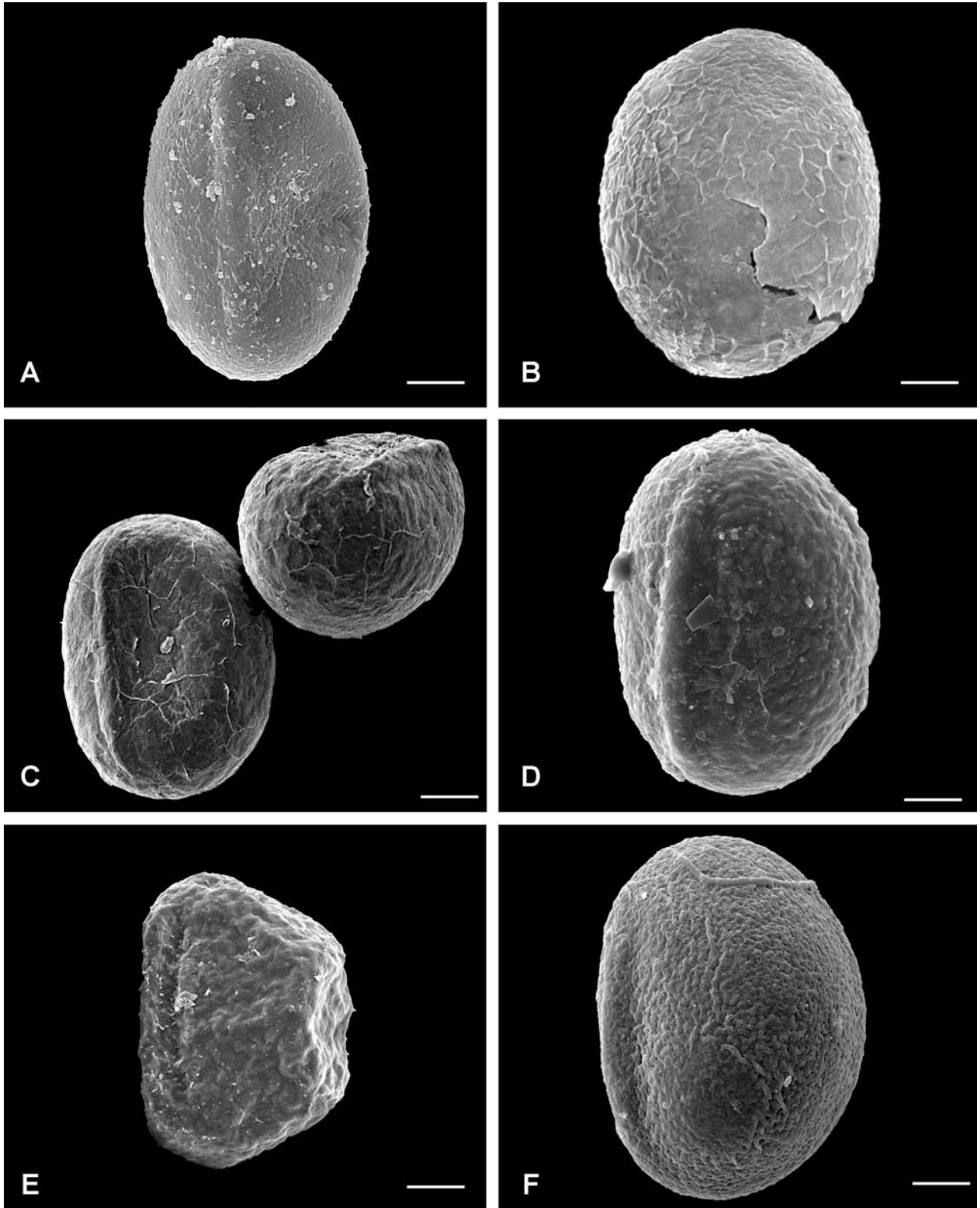


Figure 2. Venulose and rugulate perispores in *Blechnum* species. **A.** *Blechnum auriculatum*. **B.** *Blechnum brasiliense*. **C.** *Blechnum sprucei*. **D.** *Blechnum mochaenum* subsp. *mochaenum*. **E.** *Blechnum arcuatum*. **F.** *Blechnum blechnoides*. Scale bars – 7 μm (A–D), 9 μm (E, F).

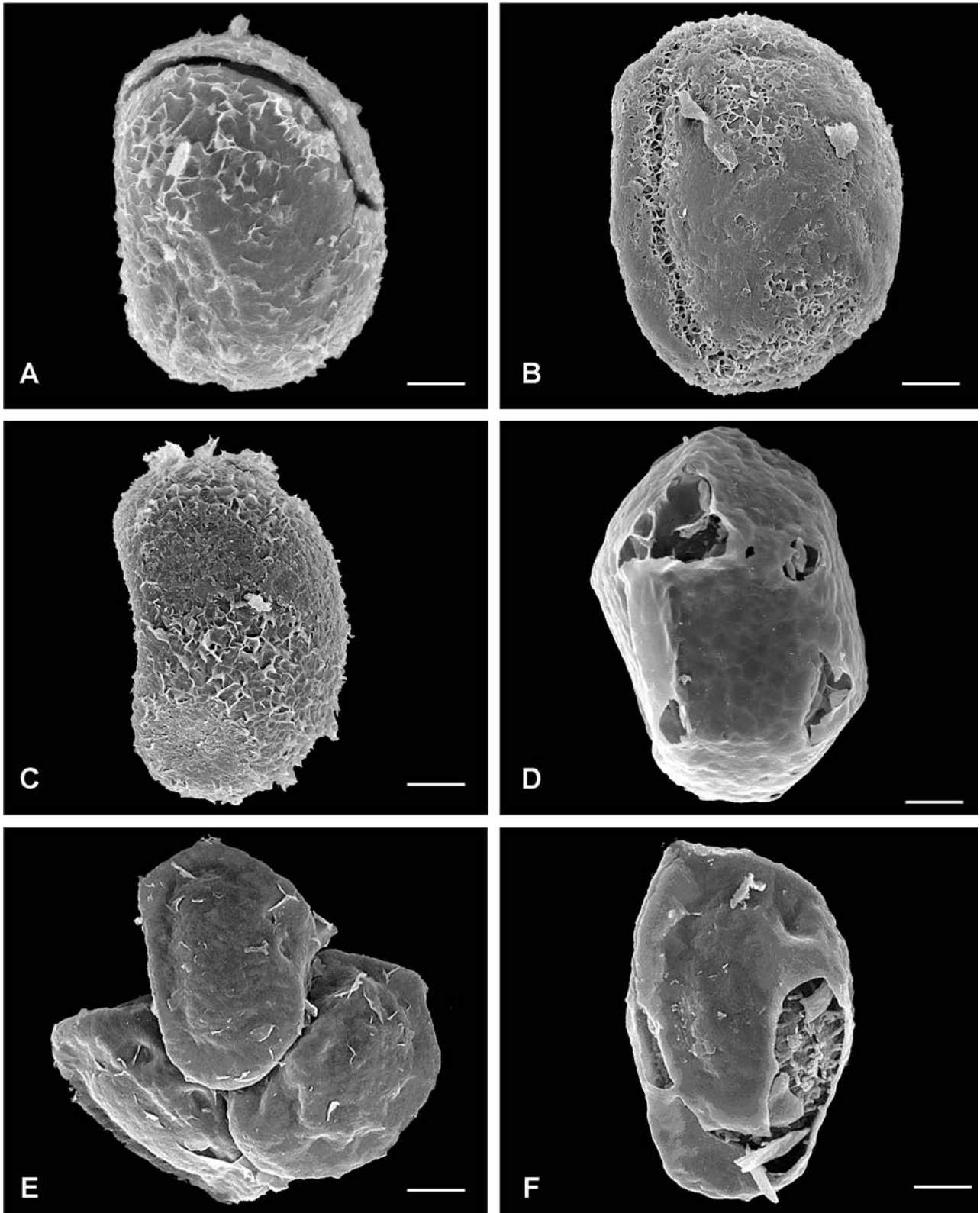


Figure 3. Perispores in *Blechnum* species. **A–C.** Perispore with thin, discontinuous superficial stratum: **A.** *Blechnum cartilagineum*; **B.** *Blechnum fluviatile*; **C.** *Blechnum penna-marina*. **D–F.** Sparsely folded perispore: **D.** *Blechnum spicant*; **E, F.** *Blechnum orientale*. Scale bars – 5 μm (A), 8 μm (B–D, F), 10 μm (E).

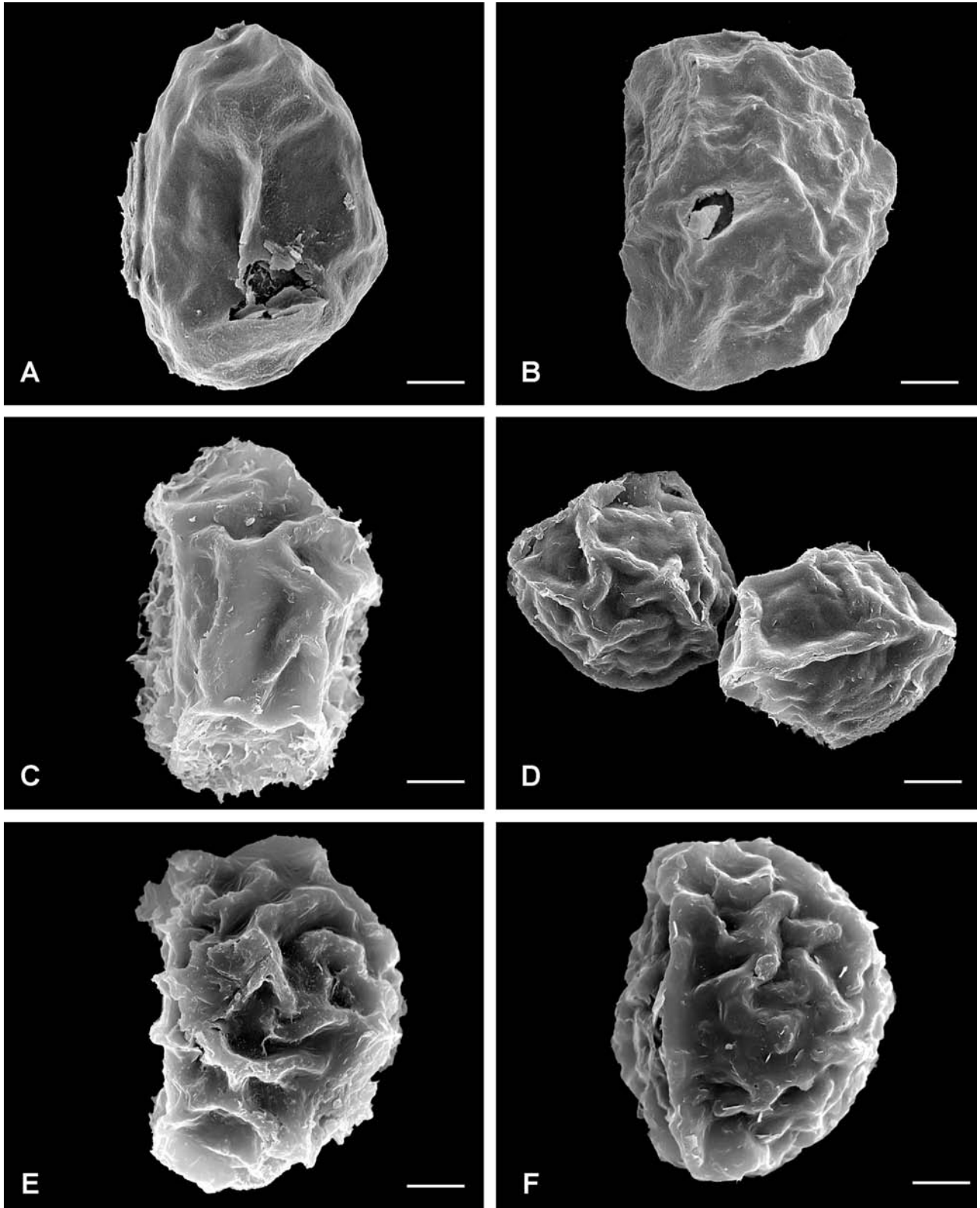


Figure 4. Perispores in *Blechnum* species. **A, B.** Sparsely folded perispores: **A.** *Blechnum madagascariense*; **B.** *Blechnum tabulare*. **C–F.** Rugate, densely folded perispores: **C.** *Blechnum schomburgkii*; **D.** *Blechnum yungense*; **E.** *Blechnum auratum*; **F.** *Blechnum werkleanum*. Scale bars – 8 μm (A), 9 μm (C, E, F), 10 μm (D), 14 μm (B).

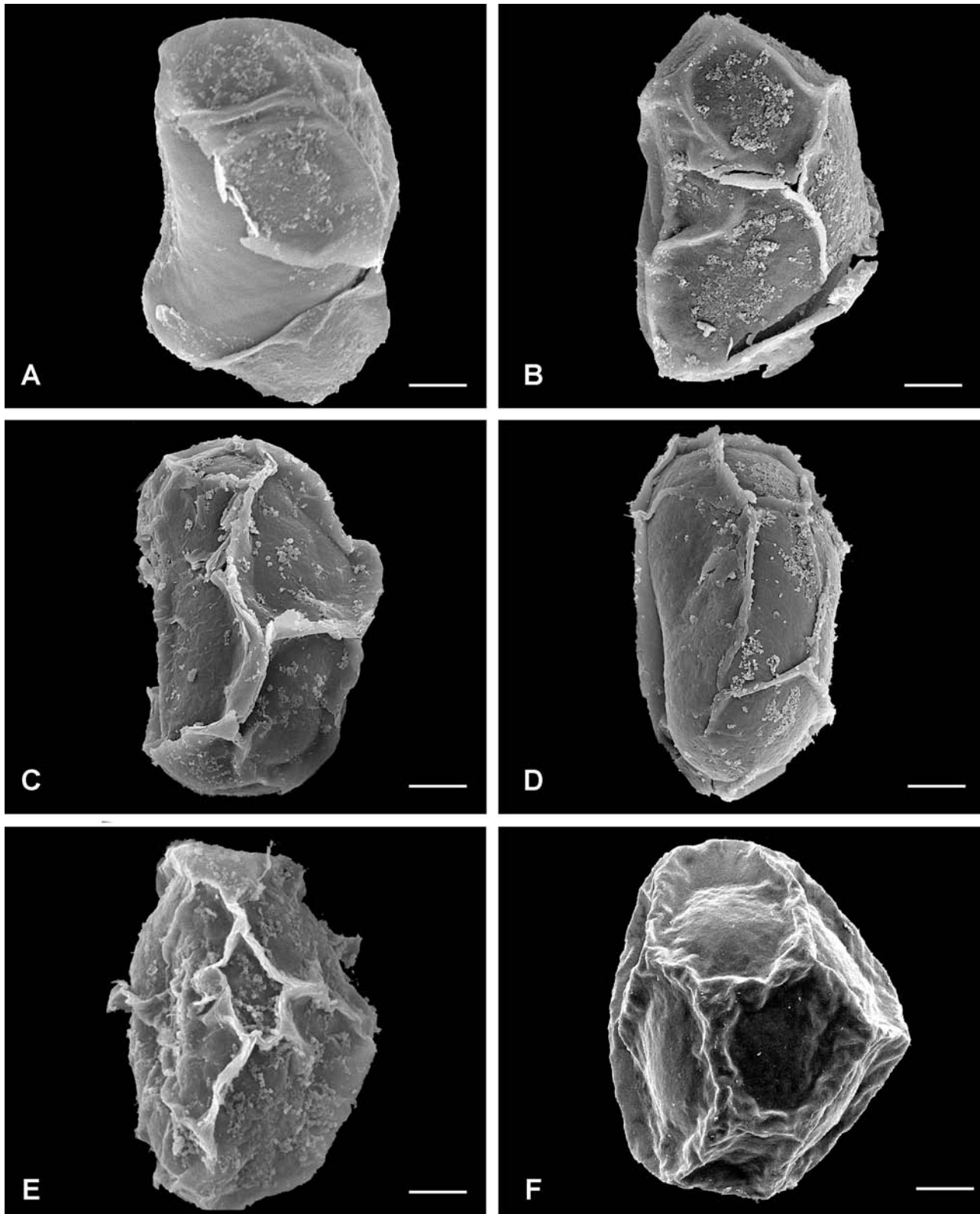


Figure 5. Low reticulate perispores in *Blechnum* species. **A.** *Blechnum schottii*. **B.** *Blechnum kunthianum*. **C.** *Blechnum fragile*. **D.** *Blechnum ensiforme*. **E.** *Blechnum giganteum*. **F.** *Blechnum sampaioanum*. Scale bars – 9 μm (B, D, F), 10 μm (A, C, E).

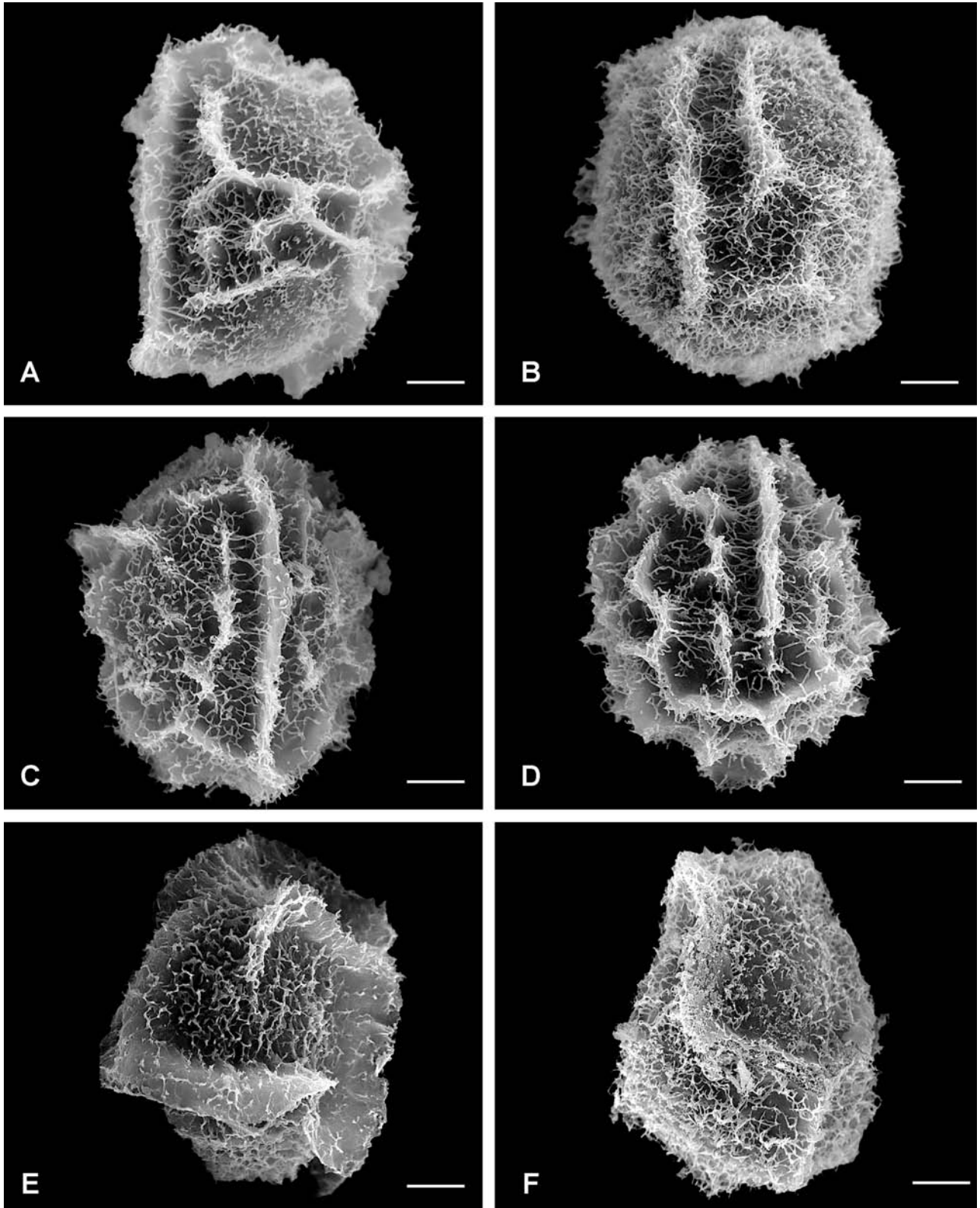


Figure 6. Cristate-reticulate perispores in *Blechnum* species. **A–D.** Muri or cristae delimiting polygonal areolae with long filamentous processes: **A.** *Blechnum atropurpureum*; **B.** *Blechnum falciforme*; **C.** *Blechnum costaricense*; **D.** *Blechnum lechleri*. **E, F.** Muri or cristae delimiting polygonal areolae with short filamentous processes: **E.** *Blechnum loxense*; **F.** *Blechnum stipitellatum*. Scale bars – 8 μm (E), 9 μm (A–D), 11 μm (F).

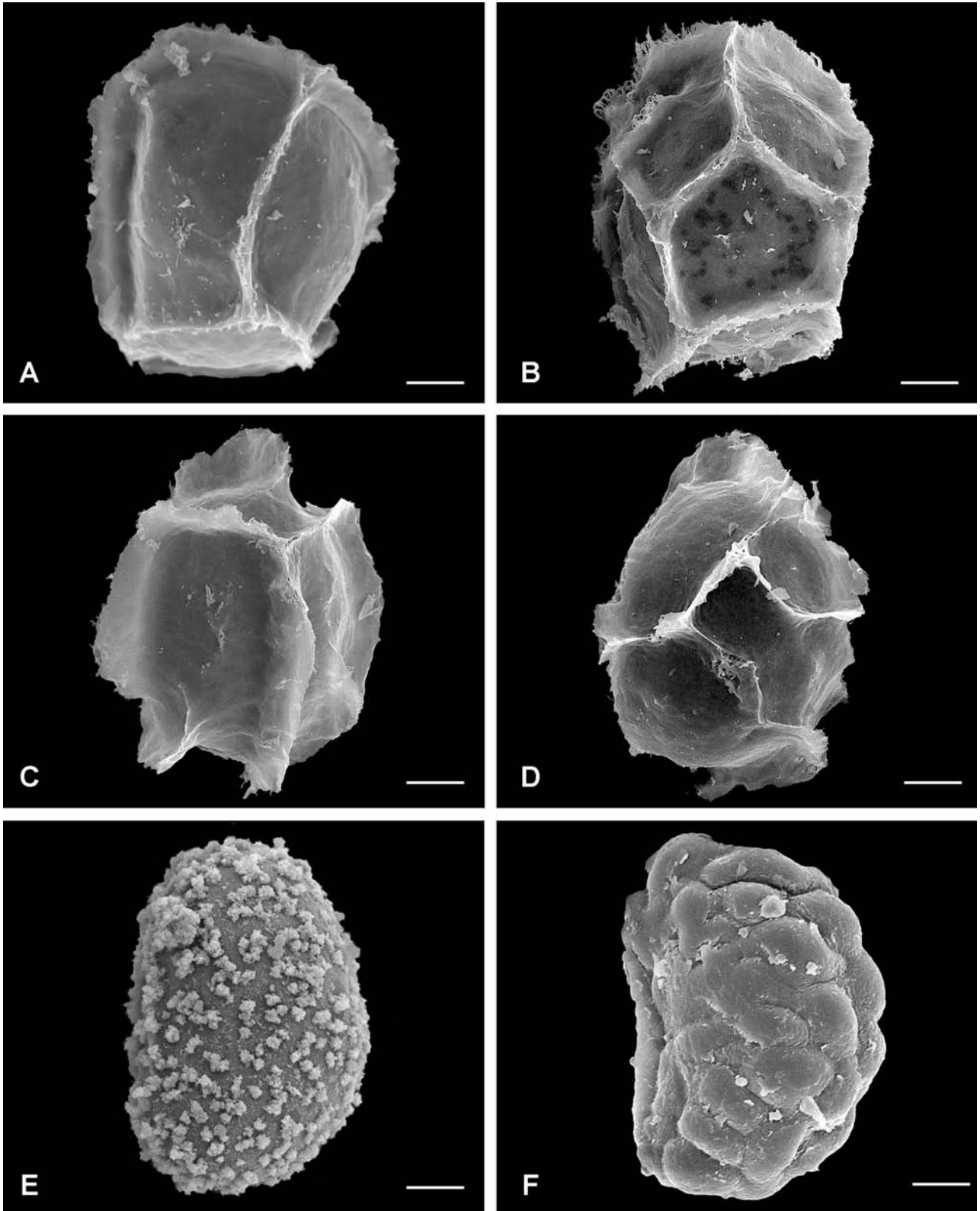


Figure 7. Perispores in *Blechnum* species. **A–D.** Cristate-reticulate perispores with muri or cristae delimiting polygonal smooth areolae: **A.** *Blechnum rheophyticum*; **B.** *Blechnum proliferum*; **C, D.** *Blechnum stuebelii*. **E.** Orbiculate perispore in *Blechnum serrulatum*. **F.** Colliculate perispore in *Blechnum fraseri*. Scale bars – 6 μm (E), 7 μm (F), 9 μm (B), 12 μm (A), 13 μm (C, D).

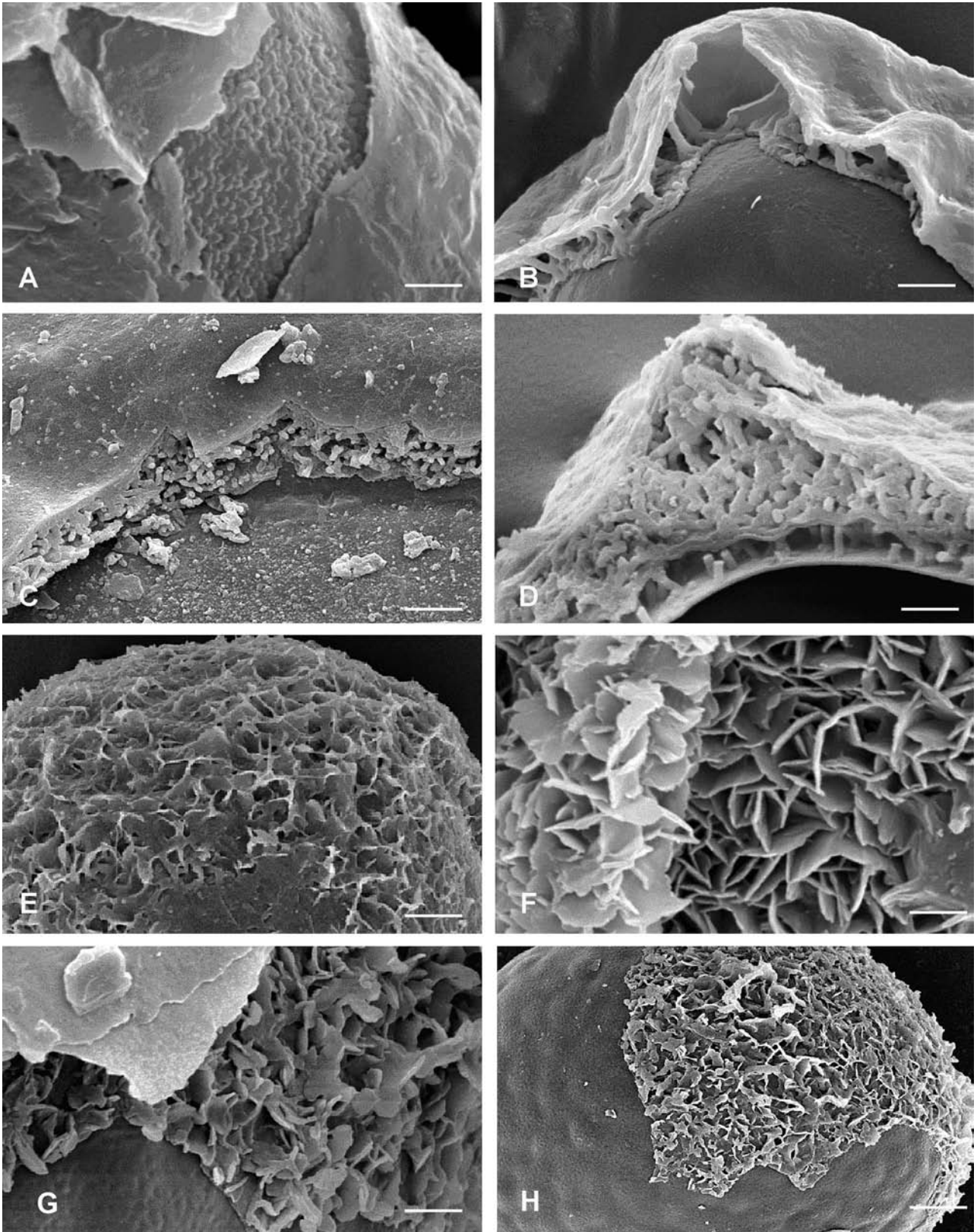


Figure 8. Structure of the perispores in *Blechnum* species. **A.** *Blechnum auriculatum*. **B.** *Blechnum orientale*. **C.** *Blechnum proliferum*. **D.** *Blechnum arcuatum*. **E.** *Blechnum fluviatile*. **F.** Detail of micro-lamellae in *Blechnum tabulare* from Argentina. **G.** Detail of micro-lamellae in *Blechnum tabulare* from Africa. **H.** Bulliform exospore in *Blechnum tabulare* from Africa. Scale bars – 1.5 μm (A, F), 2 μm (G), 3 μm (B, D, E), 5 μm (C, H).

Table I. Characters of spores in species of *Blechnum*.

Perispore ornamentation	Species and informal groups	Major aequatorial diameter (µm)	Minor aequatorial diameter (µm)	Perispore thickness (µm)		
Smooth or with little ornamentation: laevigate with micro-granules; laevigate with thin, superficial micro-lamellae; venulose, with micro-areolae, and rugulate	1	<i>B. andinum</i>	(31) 34 (38)	(22) 24 (28)	1.5–2.5	
		<i>B. asperum</i>	(40) 53 (65)	(32) 41 (48)	1.2–3.6	
		<i>B. blechnoides</i>	(40) 49 (60)	(26) 40 (45)	1.0–2.0	
		<i>B. chambersii</i> (+)	(36) 41 (46)	(25) 27 (30)	1.0–2.5	
		<i>B. corralense</i>	(55) 57 (60)	(32) 36 (40)	0.4–0.5	
		<i>B. fluviatile</i> (+)	(49) 47 (50)	(33) 37 (40)	2.5–5.0	
		<i>B. microphyllum</i>	(35) 48 (55)	(26) 39 (36)	3.0–3.2	
		<i>B. mochaenum</i> subsp. <i>achalense</i>	(50) 52 (55)	(30) 33 (35)	1.0–2.0	
		<i>B. mochaenum</i> subsp. <i>mochaenum</i>	(40) 44 (48)	(26) 30 (32)	3.0–5.0	
		<i>B. mochaenum</i> subsp. <i>squamipes</i>	(37) 45 (48)	(27) 32 (40)	3.0–3.1	
	<i>B. penna-marina</i>	(37) 44 (48)	(26) 31 (38)	2.0–3.0		
	<i>B. stoloniferum</i>	(45) 51 (54)	(30) 36 (46)	1.5–2.0		
	2	<i>B. arcuatum</i>	(42) 52 (60)	(22) 30 (50)	6.0–8.0	
		<i>B. asplenioides</i>	(32) 38 (45)	(30) 32 (34)	0.3–0.4	
		<i>B. auriculatum</i>	(51) 58 (64)	(40) 45 (50)	0.7–2.0	
		<i>B. austrobrasilianum</i>	(40) 42 (47)	(27) 29 (32)	2.0–4.0	
		<i>B. glandulosum</i>	(42) 45 (47)	(25) 26 (30)	0.5–1.0	
		<i>B. gracile</i>	(31) 33 (38)	(18) 22 (24)	1.0–2.0	
		<i>B. hastatum</i>	(33) 38 (43)	(23) 25 (28)	0.5–1.5	
		<i>B. laevigatum</i>	(35) 38 (40)	(22) 25 (30)	0.2–0.5	
		<i>B. lanceola</i>	(46) 48 (54)	(25) 30 (37)	0.2–1.0	
		<i>B. meridense</i>	(29) 34 (45)	(16) 22 (31)	1.0–2.5	
		<i>B. occidentale</i>	(40) 45 (55)	(26) 31 (35)	0.4–0.8	
		<i>B. polypodioides</i>	(30) 43 (47)	(22) 32 (42)	0.4–0.5	
		3	<i>B. brasiliense</i>	(40) 49 (60)	(26) 40 (45)	0.4–0.6
			<i>B. cartilagineum</i> (+)	(28) 31 (37)	(20) 24 (27)	1.0–4.5
	<i>B. divergens</i>		(28) 36 (44)	(28) 32 (40)	0.75–2.0	
	<i>B. lherminieri</i>		(64) 69 (77)	(52) 57 (67)	2.5–4.7	
	<i>B. sprucei</i>		(29) 34 (44)	(21) 25 (30)	2.0–4.0	
Rugate, sparsely to densely folded; low, rounded, non anastomosing rugae	4	<i>B. auratum</i>	(51) 58 (64)	(40) 45 (50)	3.0–5.0	
		<i>B. cycadifolium</i>	(47) 51 (56)	(35) 39 (42)	7.0–8.0	
		<i>B. madagascariense</i>	(45) 49 (53)	(32) 36 (41)	2.5–7.0	
		<i>B. magellanicum</i>	(64) 67 (78)	(48) 51 (55)	5.0–9.0	
		<i>B. orientale</i>	(37) 41 (45)	(25) 28 (32)	3.0–5.5	
		<i>B. schomburgkii</i>	(50) 56 (62)	(40) 45 (55)	3.5–5.0	
		<i>B. spannagelii</i>	(55) 62 (72)	(30) 31 (42)	4.0–5.0	
		<i>B. spicatum</i> (+)	(46) 50 (52)	(32) 35 (37)	1.0–2.0	
		<i>B. tabulare</i>	(84) 89 (96)	(48) 54 (60)	0.9–1.0	
		<i>B. werkleanum</i>	(43) 55 (59)	(49) 43 (46)	0.8–1.0	
		<i>B. yungense</i>	(57) 65 (75)	(42) 46 (55)	5.0–16.5	
Low reticulate	5	<i>B. attenuatum</i> (+)	(47) 51 (56)	(32) 37 (42)	0.5–0.7	
		<i>B. ensiforme</i>	(47) 54 (62)	(28) 34 (41)	2.5–8.0	
		<i>B. fragile</i>	(50) 58 (53)	(26) 38 (33)	1.0–4.0	
		<i>B. giganteum</i> (+)	(45) 51 (58)	(30) 37 (42)	3.5–5.0	
		<i>B. ivohibense</i> (+)	(45) 51 (55)	(39) 37 (42)	7.5–12.5	
		<i>B. kunthianum</i>	(37) 40 (45)	(23) 26 (39)	0.6–2.5	
		<i>B. microbasis</i> (+)	(49) 44 (50)	(24) 26 (30)	2.5–3.0	
		<i>B. sampaioanum</i>	(42) 47 (50)	(32) 36 (41)	5.0–10.0	
		<i>B. schottii</i> (+)	(45) 49 (55)	(21) 26 (30)	1.0–3.5	
Cristate-reticulate; muri delimiting polygonal areolae with short/long filamentous processes	6	<i>B. atropurpureum</i>	(42) 50 (57)	(30) 36 (41)	2.5–6.0	
		<i>B. chiriquanum</i>	(40) 47 (55)	(27) 34 (40)	2.5–3.5	
		<i>B. cordatum</i>	(60) 62 (74)	(44) 47 (57)	3.0–4.5	
		<i>B. costaricense</i>	(47) 51 (56)	(35) 39 (42)	3.0–7.5	
		<i>B. falciforme</i>	(45) 53 (60)	(35) 42 (47)	4.0–7.5	
		<i>B. lechleri</i>	(50) 53 (55)	(38) 49 (43)	6.0–8.0	
		<i>B. rheophyticum</i>	(50) 54 (58)	(35) 41 (43)	5.5–12.5	
		<i>B. loxense</i> (+)	(45) 47 (50)	(35) 38 (43)	1.9–2.0	
		<i>B. stipitellatum</i> (+)	(52) 63 (79)	(49) 45 (55)	4.0–7.0	

Table I. (Continued)

Perispore ornamentation	Species and informal groups	Major aequatorial diameter (μm)	Minor aequatorial diameter (μm)	Perispore thickness (μm)
Cristate-reticulate; muri delimiting smooth polygonal areolae	7 <i>B. proliferum</i>	(50) 54 (58)	(35) 41 (43)	6.0–8.0
	<i>B. stuebelii</i> (+)	(61) 69 (75)	(39) 49 (54)	3.0–7.5
	<i>B. tuerkheimii</i> (+)	(50) 59 (65)	(41) 45 (59)	3.0–5.0
Smooth, with orbicules	8 <i>B. serrulatum</i>	(33) 35 (36)	(22) 25 (27)	0.7–0.8 (orbicules 0.5)
	<i>B. fernandezianum</i> (+)	(40) 50 (60)	(34) 36 (38)	0.3–0.4 (orbicules 1.0)
Colliculate	9 <i>B. fraseri</i>	(35) 39 (45)	(23) 25 (29)	2.5–4.0

Groups:

1. Species of *Blechnum penna-marina* group (Tryon & Tryon, 1982; Rolleri et al., 2006b) and others (+) with similar ornamentation not previously assigned to any group.
2. Species of *Blechnum occidentale* group (Tryon & Tryon, 1982; Rolleri et al., unpublished data).
3. Species with ornamentation similar to 1–2, belonging to other groups (Tryon & Tryon, 1982; Oliveira Dittrich, 2005) or not being assigned to any previous group (*Blechnum cartilagineum*, here considered close to *B. brasiliense*).
4. Species of *Blechnum butchianii* or *B. tabulare* group (Tryon & Tryon, 1982; Oliveira Dittrich, 2005; Rolleri et al., 2008), and others (+) with similar ornamentation not previously assigned to any group.
5. Species of *Blechnum fragile* group (Tryon & Tryon, 1982), *B. divergens* group (Oliveira Dittrich, 2005) and others, and others (+) with similar ornamentation not previously assigned to any group.
- 6–7. Species of *Blechnum lineatum* group (Tryon & Tryon, 1982), *B. cordatum* group (Oliveira Dittrich, 2005; Prada et al., 2008), *B. loxense* group (Tryon & Tryon, 1982) or not being assigned to any group (*B. stipitellatum*, here considered close to *B. loxense*).
8. Species of *Blechnum serrulatum* group (Tryon & Tryon, 1982; Rolleri et al., 2010), and others with similar ornamentation (+).
9. Species with colliculate perispores.

In some species such as *B. auriculatum*, *B. blechnoides*, *B. gracile*, *B. hastatum*, *B. lanceola*, *B. meridense*, *B. occidentale* and *B. polypodioides*, very low, long, erratically distributed and easily noticeable wrinkles may occur. Laevigate perispore with very thin, smooth, discontinuous surface, which tends to rise or separate as micro-lamellae were found in *B. cartilagineum* (Figure 3A), *B. fluviatile* (Figure 3B), *B. microphyllum* and *B. penna-marina* (Figure 3C).

Perispore ornamented

Spores with rugate, sparsely folded perispore with low, rounded, non-anastomosing rugae, which do not form areolae, were found in *Blechnum asperum*, *B. spicant* (Figure 3D), *B. orientale* (Figure 3E, F), *B. madagascariense* (Figure 4A) and *B. tabulare* (Figure 4B); in *B. cycadifolium* and *B. magellanicum*, the rugae are higher and may anastomose partially. Rugate, densely folded perispore with coarse, wide, sinuous, thick, discontinuous folds running more or less parallel, are present in *B. auratum* (Figure 4E), *B. schomburgkii* (Figure 4C), *B. spannagelii*, *B. werkleanum* (Figure 4F) and *B. yungense* (Figure 4D). The perispore in these spores break partially and sometimes the innermost stratum remains on the exospore.

Spores with low reticulate perispore show a reticulum of low, narrow, sharp or rounded muri, delimiting broad, more or less defined, polygonal areolae, with or without micro-ornamentation. They were found in *Blechnum ensiforme* (Figure 5D), *B. fragile* (Figure 5C), *B. giganteum* (Figure 5E), *B. ivohibense*, *B. kunthianum* (Figure 5B), *B. microbasis*, *B. mochaenum* subsp. *squamipes*, *B. schottii* (Figure 5A) and *B. sampaiouanum*

(Figure 5F). They are fragile in some species, and peel off and break very easily in mature spores, exposing a smooth exospore below, as seen in *B. ensiforme*, *B. fragile*, *B. ivohibense*, *B. kunthianum* and *B. schottii*. Folds are higher in *B. sampaiouanum* than in those of the other species, slightly wavy and do not break easily.

Spores with cristate-reticulate perispore have high, narrow, sharp edged, conic in section muri, which tend to anastomose delimiting polygonal areolae. Areolae with short to long filamentous processes are present in *Blechnum atropurpureum* (Figure 6A), *B. chiriquianum*, *B. cordatum*, *B. costaricense* (Figure 6C), *B. falciforme* (Figure 6B), *B. lechleri* (Figure 6D), *B. loxense* (Figure 6E) and *B. stipitellatum* (Figure 6F), while the spores of *B. rheophyticum* (Figure 7A), *B. proliferum* (Figure 7B), *B. stuebelii* (Figures 7C–D) and *B. tuerkheimii* have smooth areolae.

Spores with orbiculate perispore show a thin network carrying rugose orbicules densely and irregularly arranged. Orbicules are as large as 2 μm , and occur in *Blechnum fernandezianum* and *B. serrulatum* (Figure 7E). Spores with colliculate perispores with large, coarse, broadly attached, wider than high, with an obtuse apex, contiguous bullae covering the whole spore, are characteristic of *B. fraseri* (Figure 7F).

Perispore structure

Regardless of ornamentation, the perispores have variable structure and consist of different layers, as observed by SEM. The perispore is apparently compact and thin, with an outer layer that may peel off or crack from the spore, leaving a smooth or granular inner surface below in *Blechnum asplenoides*, *B.*

auriculatum (Figure 8A), *B. ensiforme*, *B. hastatum*, *B. glandulosum*, *B. gracile*, *B. kunthianum*, *B. laevigatum*, *B. lanceola*, *B. meridense*, *B. occidentale*, *B. poly-podioides* and *B. schottii*; in *B. mochaenum* subsp. *achalense* this layer is somewhat thicker.

In other species, the perispore has an outer, compact layer that can appear stratified, a middle layer varying in thickness and structure, and an inner layer next to the exospore, which can be easily separated from it in some species, but not in others. The variation of the middle layer is the most obvious when using SEM. The middle layer is formed by more or less discrete, straight, cylindrical rodlets, constituting a structure of lacunar aspect, not thicker than 1–2 µm in *Blechnum attenuatum*, *B. brasiliense*, *B. magellanicum* and *B. orientale* (Figure 8B). The middle layer is formed by a complex lacunose structure of fused, intersecting rodlets, 2.5–10 µm thick, in *B. andinum*, *B. cordatum*, *B. costaricense*, *B. divergens*, *B. falciforme*, *B. lechleri*, *B. loxense*, *B. proliferum* (Figure 8C), *B. rheophyticum*, *B. sprucei*, *B. stipitellatum*, *B. stuebelii* and *B. tuerkheimii*. In some species a basal double stratum can be observed, from which emerge the rodlets and an innermost granular stratum that remains attached to the exospore. The structure of the perispore of *B. arcuatum* (Figure 8D) combines the two previous types of middle layer, with a basal stratum close to the observed in *B. orientale* and an upper stratum made of fused, densely intermingled rodlets.

The middle layer appears to be formed by a complex lacunose structure of fused intersecting lamellae, with an alveolate or foliose appearance, covered by the outermost perispore layer. In surface view, differences in thickness of the outer layer covering the lamellae and in the thickness of the lamellae themselves can be observed: they are very delicate and thin in *Blechnum cartilagineum*, *B. fluviatile* (Figure 8E), *B. microphyllum* and *B. penna-marina*, and compact, thicker and somewhat brittle in *B. tabulare* (Figures 8F–H), *B. auratum*, *B. schomburgkii*, *B. spannagelii*, *B. werkleanum* and *B. yungense*.

Exospores

The exospores are smooth as in *Blechnum attenuatum* (Figure 1B) and *B. orientale* (Figure 8B), slightly rugulate, or bear a bulliform surface, with or without microornamentation, as in *B. auratum*, *B. magellanicum*, *B. schomburgkii*, *B. spannagelii*, *B. stuebelii*, *B. tabulare* (Figures 8G–H), *B. werkleanum* and *B. yungense*.

Discussion

The characters of the spores suggest that they possess an important diagnostic value at the specific

level, particularly in ornamentation and structure of the perispores that vary considerably among the species studied. Also, the distribution of ornamentation types among the species indicates some agreement with previous subdivision of the genus in informal and/or taxonomic groups. Spores with a smooth perispore are the most diversified in the genus and may have any type of structure; these smooth spores belong to two of the informal species groups, the *Blechnum occidentale* and *B. penna-marina* groups, which were originally separated based on specialisation of the sporogenous fronds. Spores with folded perispore are characteristic of species of *Lomaria*, *Lomaria* sect. *Lomariocycas* or *Lomariocycas* that have been variously treated as genera, sub-genera or sections; although the folded type can show specific morphological features, species of these categories, in any hierarchy that they might be considered, can be grouped together if the spore morphology is taken into consideration. If other characters of the sporophyte are added to the spore morphology, support for the relationship increases. However, spores of some species of *Lomaria* are similar to most of the species attributed to *Parablechnum* s. l., which lacks folds, but instead has cristate or cristate-reticulate perispore. Within *Parablechnum*, the differences between species are the shape and outline of areoles, the absence or presence of microfilaments and, when appropriate, the shape, length and partial fusion of the microfilaments. The low reticulate perispore characterises species included in *Lomaridium*. The colliculate and the orbiculate perispore are infrequent in the studied species. In each group it is possible to find some species related with species of other groups, with differences that might well be bridged by intermediates.

This study suggests that *Blechnum* is a genus comprising large groups of related species and any sub-generic subdivision needs to be defined by several characters, including features from the sporophyte as well as from the spores. All types of surfaces described in previous studies of spores of Blechnaceae (9 genera of Blechnaceae, including 30 species of *Blechnum*: Tryon & Lugardon, 1991; 18 taxa of *Blechnum* from New Zealand: Large & Braggins, 1991; 20 taxa of *Blechnum* from Malesia: Chambers & Farrant, 2001; spore wall of *B. australe* L. subsp. *auriculatum* (Cav.) de la Sota, *B. austrobrasiliense*, *B. brasiliense*, *B. laevigatum* and *B. occidentale*: Ramos Giacosa et al., 2009) were identified in our study, except for the echinate type surface present in *Pteridoblechnum acuminatum* (C.T. White & Goy) Hennipman and *B. diversifolium* Mett., and the coarsely tuberculate surface present in *Stenochlaena* from the Palaeotropics (Tryon & Lugardon, 1991). Only Tryon and Tryon (1982) commented

on the relationship between ornamentation types and species grouping of *Blechnum*, as well as *Blechnum* type spores and those of other close genera, such as *Doodia* and *Woodwardia*.

The simplest perispore ornamentation found in our study includes the laevigate, venulose and rugulate type of ornamentation; micro-granules and micro-lamellae appearing in laevigate ornamentation. Laevigate spores with micro-granules were illustrated for *Doodia*, laevigate spores with micro-lamellae and rugulate spores for *Brainea* and *Sadleria* by Tryon and Lugardon (1991). Large and Braggins (1991) concluded that in most *Blechnum* and *Doodia* spores, exospores are not ornamented and perispores present three types (i.e. folded, tuberculate and granulate), the latter being the most common in the palaeotropical species analysed. Molecular phylogenetic studies of DNA sequences indicate that the genus *Doodia* is monophyletic and is embedded in *Blechnum*, which is resolved as paraphyletic (Smith et al., 2006; Shepherd et al., 2007). Although not yet conclusive, the most common types of ornamentation in *Blechnum* also appear in *Doodia* [cf. for example Chambers and Farrant (2001) who illustrated eight species with laevigate micro-granulate perispores] which would also support the close relationship suggested by the molecular studies.

Our study suggests that the neotropical and palaeotropical species of the *Blechnum occidentale* and *B. penna-marina* groups are united by the most simple perispore ornamentation. Other uniting features include small to medium plants, occasionally large sporophytes, prostrate, sometimes stoloniferous rhizomes, monomorphic, sub-dimorphic and dimorphic fronds with adnate or sessile pinnae and herbaceous habit; their axes are internally dyctiostelic, with 5–10 vascular bundles in the rhizomes, 5–6 vascular bundles in the stolons and three vascular strands in the stipes (Rolleri & Prada, 2006b; Gabriel y Galán et al., 2008; unpublished data). Some species with this spore type exhibit a wide geographical distribution and have certain tolerance to exposition, altitude and latitude. Their habitats range from low, pedemontane or montane rainforests to cold, hyper-humid woodlands. Spores with rugulate perispore are uncommon: *Blechnum arcuatum* is a monomorphic species of humid, cold woodlands from Argentina and Chile and *B. mochaenum* subsp. *mochaenum* is usually found in similar latitudes, while *B. blechnoides* grows in Chile and New Zealand in salty coastal wetland areas. Spores with laevigate perispore with micro-lamellae are also infrequent in the genus, only found in *B. cartilagineum*, *B. fluviatile*, *B. microphyllum* and *B. penna-marina*. With the exception of *B. fluviatile* from damp shady lowlands, the other three species grow in open forested areas. In spite of

the lack of macro-ornamentation, the perispore exhibits varied structures: from simple, apparently compact to complex structures of interwined rodlets and lamellae as illustrated by Passarelli (2007) for *B. mochaenum* subsp. *mochaenum* and Gabriel y Galán et al. (2008) for *B. sprucei*.

Spores with a rugate perispore were found in terrestrial plants with large, arborescent or sub-arborescent, less commonly herbaceous, sporophytes with dimorphic or, rarely, monomorphic fronds, inhabiting pedemontane and montane forests, disturbed deciduous montane woodlands, cold high altitude and low latitude humid grasslands, volcanic slopes, paramos and 'paramillos' (the paramos without *Espeletia* from Costa Rica) in the Neotropics and Palaeotropics. The arborescent species included by Tryon and Tryon (1982) in the *Blechnum buchtienii* Rosenst. group differ in the morphology of the perispore, suggesting heterogeneity in this group regarding spore ornamentation, venulose in *B. brasiliense*, sparsely folded in *B. cycadifolium*, *B. madagascariense*, *B. magellanicum*, *B. orientale* and *B. tabulare*, densely and coarsely folded in *B. auratum*, *B. schomburgkii*, *B. spannagelii*, *B. werkleanum* and *B. yungense*. Large and Braggins (1991) illustrated rugate perispore in an undescribed species from New Zealand, while Chambers and Farrant (2001) found a perispore type close to the rugate studied here only in spores of one Malaysian species, *B. revolutum* (Alderw.) C. Chr. The perispore of *B. tabulare* has a sparsely folded surface, both in the African, Brazilian and Argentinean material (Rolleri et al., 2008), and is close to that of *B. madagascariense*, while the perispore of *B. yungense* is similar to the type found in spores of *B. werkleanum*. Ramos Giacosa et al. (2009) illustrated the spores of what they called *B. tabulare*, but their plant material was lately assigned to *B. yungense* and our current observations on this latter species also suggest the material illustrated by Ramos Giacosa et al. (2009) belongs to *B. yungense*. Spores of *B. auratum* and *B. schomburgkii* are extremely similar both in size and ornamentation.

The low reticulate perispore type was found in hemi-epiphytic or epiphytic plants with medium to large sporophytes, usually dimorphic fronds and adnate pinnae, living in low rainforests and hyper-humid, sometimes cold woodlands, both in the Neotropics and Palaeotropics. Except in spores of *Blechnum sampaioanum*, the perispore of these species is a fragile, thin, apparently compact layer, which easily separates from the exospore. The perispore of the hemi-epiphytic species *B. whelanni* F. M. Bailey, as illustrated by Chambers and Farrant (2001), could be included in this type.

The reticulate-cristate perispore with superficially smooth areolae characterises spores of medium to

large plants, with sub-erect rhizomes, large dimorphic, pinnate fronds with sessile to peciolulate pinnae, living in pedemontane to montane rainforests. The reticulate-cristate perispore with filamentous areolae was found in terrestrial species with dimorphic, pinnate fronds, sessile or peciolulate pinnae, growing in lowlands, pedemontane or montane forests, borders of rainforests and cold, hyper-humid woodlands at low latitudes. The great morphological similarity between spores of some species, such as *Blechnum atropurpureum*, *B. chiriquanum*, *B. cordatum*, *B. costaricense*, *B. falciforme* and *B. lechleri*, coincides with remarkable similarities in several vegetative features of their sporophytes. Large and Braggins (1991) illustrated a similar type of perispore for *B. procerum* (Forst. f.) Sw. with very distant filamentous processes on areolae. *Blechnum loxense* and *B. stipitellatum*, with shorter filaments and a perispore almost spongy throughout, are sub-arborescent plants with mucilaginous axes, dimorphic or sub-dimorphic, stiff and coriaceous fronds, sessile or shortly peciolulate pinnae, which inhabit paramos, montane disturbed forests and 'paramillos'. Similar reticulate types of perispores were illustrated by Tryon and Lugardon (1991) for species of *Woodwardia*. The perispore is variable in thickness, with the spongy layer formed by intermingled or fused rodlets.

The orbiculate perispores are infrequent in the genus and occur in *Blechnum fernandezianum*, an insular small plant with dimorphic fronds that grows in wet rock soils near streams, in *B. serrulatum*, a pantropical amphibious or palustrine, sometimes terrestrial species, and also in *B. indicum*, a palaeotropical species resembling *B. serrulatum*. In these species broken spores are absent or have not been observed; the perispore appears to be an extremely thin, almost transparent layer like a network, overlaid by diversely scattered, rugose orbicules. Tryon and Lugardon (1991) illustrated an apparently similar type for *Salpichlaena volubilis* (Kaulf.) Hook., considering the exospore as a partially exposed wall below an underdeveloped perispore. The spores of *B. fraseri* were considered by Tryon and Lugardon (1991) as similar to those of *Pteridoblechnum neglectum* (F. M. Bail.) Hennieman and the authors suggested that the perispore of the latter species would be a very thin wall deposited over a macro-ornamented exospore. Large and Braggins (1991) showed that the external ornamentation of *B. fraseri* actually corresponds to the perispore.

In ferns, a relationship is often found between spore size and ploidy level (Barrington et al., 1986). The known *Blechnum* diploids fall in the range of 30–40 µm in spore length, and the tetraploids in the range of 40–60 µm (Mehra & Bir, 1958; Walker, 1966,

1973, 1985; Smith & Mickel, 1977; de la Sota & Cassá de Pazos, 1983; Manickam & Irudayaraj, 1988; Kawakami et al., 1997; Jara-Seguel et al., 2006; González et al., 2010). A higher level of ploidy is only known for *B. lherminieri*, reported as a dodecaploid by Smith and Mickel (1977), with spore length above 60 µm. Our results show that the spore length of 73% of the taxa fall between 40–60 µm, with the rest between 30–40 µm (nine species) and 60–90 µm (eight species). Although it might be tempting to draw conclusions from these data, still too little is known about chromosome counts in *Blechnum* to extrapolate information.

Conclusions

The spores of 64 *Blechnum* species exhibit two principal types of perispore: perispore smooth or only slightly ornamented, and perispore with macro-ornamentation. Spores with perispore lacking macro-ornamentation were found to be the most common in the genus, in plants of herbaceous habit, with monomorphic and dimorphic fronds, adnate pinnae, with a wide geographical range of distribution, living in the Neotropics and Palaeotropics.

Although the number of species studied represent more or less a third of all described species of *Blechnum*, ornamented perispores, such as rugate perispores that are predominant, but not exclusively occurring in arborescent plants, low reticulate perispores mostly related to epiphytic and hemi-epiphytic plants, and reticulate-cristate perispores found in terrestrial, sub-arborescent plants seem to be restricted to a lower number of species than the smooth or only slightly ornamented types. The most uncommon are the orbiculate and colliculate perispore types.

The structure of the perispore does not correlate to the ornamentation. Spores with simple ornamentation may have either the simplest to the more complex type of middle layer. The structure of a middle layer with thick, intersecting lamellae seems to be characteristic of some spores with rugate perispore of arborescent plants.

The exospore does not vary as much as the perispore and the smooth types are the most common in the species studied, associated to all types of perispore with the exception of the rugate spores, which usually have rugulate or bullate exospore.

The most remarkable finding of this study is that the spores show distinct diagnostic value, little used and often omitted in many previous systematic studies on *Blechnum*. The characters of the spores have an interesting and promising diagnostic value at the specific level, especially since the combination of ornamentation and structure of perispore relate to each other differently in almost every species studied.

Furthermore, independently of the differentiation of sporogenous fronds (mono- or di-morphism), the spore ornamentation indicates some kind of agreement, yet not complete, with previous subdivisions of the genus in informal and/or taxonomic groups. A preliminary conclusion suggests that an excessive subgeneric fragmentation of the genus is unsupported by the spore data, and that instead larger groups of species that can be defined both by features of the sporophytes and the spores. When spore characters are considered together with morphological features of the sporophytes they become a useful complementary tool that could lead to the establishment of more natural groups in the genus.

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Specimens investigated

- Blechnum andinum* (Baker) C. Chr., Brazil, Itatiaia, Agulhas Negras, Brade 14540 (CTES 19372).
- Blechnum arcuatum* J. Rémy ex Gay, Chile, Palena: Chaitén: Expediitio Suecica 1907–190, en el curso inferior del río Yelcho, Skottsberg s. n. (BA 152).
- Blechnum asperum* (Klotzsch) J. W. Sturm, Chile, Concepción, Arauco, Isla de Mocha, Weldt-Rodríguez 1085/378 B (CONC).
- Blechnum asplenoides* Sw., Brazil, Rio de Janeiro: Guanabara, Alto de Boa Vista, Estrada Das Canoas, Trinta & Fromm 29502 (LP).
- Blechnum atropurpureum* A. R. Smith, Venezuela, Amazonas: Atabapo, Cerro Marahuaca, Liesner 25215 (UC 1557245).
- Blechnum attenuatum* (Sw.) Mett., French Polynesia, Austral Islands: Rapa, Tautautu, Fosberg 568 (UC 542542).
- Blechnum auratum* (Fée) R.M. Tryon & Stolze, Costa Rica, San José: Villa Mills, hacia el Cerro de la Muerte, Gabriel y Galán & Sundue s.n. (MA).
- Blechnum auriculatum* Cav., Uruguay, Rocha: Maturrango, entre Castillos y la Sierra del Oratorio, Fuertes & Prada s. n. (MA).
- Blechnum austrobrasilianum* de la Sota, Argentina, Salta: Santa Victoria, Los Toldos, Casa de LIES, de la Sota 7115 (LP).
- Blechnum blechnoides* Bory ex Keyserl., Chile: Concepción: Talcahuano, Rocoto, en acantilados, de la Sota 6024 (LP).
- Blechnum brasiliense* Desv., Brazil, Rio de Janeiro: Guanabara, Serra Carioca, Carauta N-638 (LP).
- Blechnum cartilagineum* Sw., Australia, New South Wales, Mount Wilson, below Cathedrals of Ferns, N.S.W. Blue Mountains, Constable NSW P 8023 (LIL 462590).
- Blechnum chambersii* Tindale, Fidji Islands, Viti Levu: Tholo North: vicinity of Nandarivatu, Degener s. n. (LIL 168394).
- Blechnum chiriquanum* (Broadh.) C. Chr., Costa Rica, Limón: Cordillera de Salamanca, Atlantic slope, Valle de Silencio, area just N of Cerro Hoffman, 4 1/2 airline km W of the Costa Rican-Panamanian border, Davidse et al. 28674 (UC 1509051).
- Blechnum cordatum* (Desv.) Hieron., Brazil, Rio Grande Do Sul: Santa Cruz, Jürgens & Ittier 69 (BA).
- Blechnum corralense* Espinosa, Chile, Corral San Juan, Gunckel 1961 (LIL).
- Blechnum costaricense* (H. Christ) C. Chr., Panamá, Chiriquí: Pila, Las Nubes, Cerro Punta, González 13 (MA 702487).
- Blechnum cycadifolium* (Colla) J. W. Sturm, Chile, Valparaíso: Masatierra, Cumberland bay, camino al Portezuelo, Looser 408 (LP).
- Blechnum divergens* (Kunze) Mett., Haití, Middel Commissaires, Holdridge 2247 (LIL 399813).
- Blechnum ensiforme* (Liebm.) C. Chr., Colombia, Antioquia: Municipio Frontino, road to Murri, 15 km W of Nutibara, Altos de Cuevas, Brant & Martínez 1325 (UC 1546354).
- Blechnum falciforme* (Liebm.) C. Chr., Mexico, Oaxaca: along Highway 175, through Sierra de Juárez, between Tuxtepec and Oaxaca, 21.5 miles S of bridge at Valle Nacional, just south of Village of La Esperanza, Croat 48085 (UC 1505460).
- Blechnum fernandezianum* (Looser) Prada & Rolleri, Chile, Valparaíso: Más Afuera, Quebrada de la Mata Maqui, on trail to Correspondencia Camp, Meyer 9745 (LP).
- Blechnum fluviatile* (R. Br.) Lowe ex Salomon, New Zealand, Auckland: Kauri Glen Road, Northcote, coll. illegible (LIL 371087).
- Blechnum fragile* (Liebm.) C. V. Morton & Lellinger, Jamaica, Portland: east slope of the John Crow Mts., ca. 2 miles southwest of Ecclestown, Proctor 5686 (LIL 409018).
- Blechnum fraseri* (A. Cunn.) Luerss., New Zealand, Auckland: Titirangi, North Island, Somerville s. n. (LIL 300618).
- Blechnum giganteum* (Kaulf.) Schldl., South Africa, Transvaal: about 15 miles from Sibasa to Zoutpansberg Mountains, toward Lake Funduzi, Rodin 4093 (UC 802033).
- Blechnum glandulosum* Kaulf ex Link, Costa Rica, Puntarenas: San Vito, Estación Biológica Las Cruces, Gabriel y Galán s.n. (MA).
- Blechnum gracile* Kaulf., Argentina, Misiones: Iguazú, Parque Nacional Iguazú, Sendero Jacaratá, ex ruta 12, 2 km del CIES, Vanni et al. 3254 (LP).
- Blechnum hastatum* Kaulf., Argentina, Neuquén, Isla Victoria, Cascada de Chachín, senda a Chachín, desvío del camino a Hua Hum, Prada A 38 (MA).
- Blechnum ivohibense* C. Chr., Tanganika, Bezirk Morogoro: Uluguro-Gebirge, Northwestseite, Lupanga Berg, Nebelwald, Schlieben 2959 (MA 389177).
- Blechnum kunthianum* C. Chr., Argentina, Misiones: Parque Provincial Cruce Caballero, Prada s. n. (MA).
- Blechnum laevigatum* Cav., Argentina, Córdoba: sin. loc., Castellanos 23157 (LP).
- Blechnum lanceola* Sw., Argentina, Misiones: Libertador Gral. San Martín: Puerto Rico, Bridarolli 3033 (LP).
- Blechnum lechleri* Mett., Ecuador, Morona-Santiago: Cordillera del Cóndor, Morales & Embrey 1374 (UC 1869110).
- Blechnum lherminieri* Hieron., Colombia, Chocó: principal ridge and slopes 2 km E of San José del Palmar, Lellinger & de la Sota 757 (LP).
- Blechnum loxense* (Kunth) Hook. ex Salomon, Venezuela, Trujillo, Municipio Boconó, Laguna Larga, vía laguna Las Parias, to laguna Eco, Páramo de Motumbo, Monumento Natural Teta de Niquitao-Guirigay, Stergios 20421 (UC 1785091).
- Blechnum madagascariense* Tardieu, Madagascar, Antananarivo: R. N. 1, 50 km W of Antananarivo, Tapia forest, Barnett et al. 457 (UC 1549512).
- Blechnum magellanicum* (Desv.) Mett., Argentina, Neuquén: Isla Victoria, Laguna Frías, Meyer 7338 (LIL 113135).
- Blechnum meridense* Klotzsch, Brazil, São Paulo: Toledo, Albricht 165 (LP).
- Blechnum microbasis* (Baker) C. Chr., Madagascar, Toamasina: near Andisabe, forest of Mantadia, van der Werff et al. 13736 (UC 1604665).

Blechnum microphyllum (Goldm.) C. V. Morton, Argentina, Neuquén: Río Agrío, Lago Caviahué, Diem 3366 (LP).

Blechnum mochaenum G. Kunkel subsp. *mochaenum*, Argentina, Chubut: Futaleufú: Parque Nacional Los Alerces, Lago Cisnes, Roig et al. 13907 (LP).

Blechnum mochaenum subsp. *achalense* (Hieron.) Prada & Rolleri, Argentina, Salta: Santa Victoria, Los Toldos, Finca El Nogalar, Río Huaico, Cassá et al. 235 (LP).

Blechnum mochaenum subsp. *squamipes* (Hieron.) Prada & Rolleri, Argentina, Tucumán: Quebrada Pueblo Viejo, de la Sota 4070 (LP).

Blechnum occidentale L., Panamá, Veraguas: Isla Coiba, Distrito de Montijo, N de la isla, manglar adyacente a Gambute, Galdames et al. 2096 (MA 563577).

Blechnum orientale L., Japan, Ryūkyū, Island Tokunoshima, interior of Boma, Tokunoshima – Chō, Ooshima-Gun, Kagoshima Prefecture, Iwatsuki et al. (LP).

Blechnum penna-marina, Argentina, Tierra del Fuego: Puerto Cook, Torres 1122 (LP).

Blechnum polypodioides Raddi, Nicaragua: Nueva Segovia, ca. 5.2 km W of San Fernando, NE to portillo Los Coyoles, SW up narrow quebrada to Cerro El Peñascal, Stevens & Krukoff 3236 (LP).

Blechnum proliferum Rosenst., Colombia, Chocó-Antioquia: at and on both sides of the principal ridge W of La Mansa, at ca Km 105.5 of the ciudad Bolívar-Quibdo Road. Lellinger & de la Sota 919 (LP).

Blechnum rheophyticum R. C. Moran, Colombia, Nariño: Municipio de Ricaurte, Resguardo Indígena Nulpe Medio, orillas del río Nulpe, Ramírez & González 9438 (UC 1732585).

Blechnum sampaoianum Brade, Brazil, Paraná: Prefeitura Curitiba, Municipio Piraquara, Serra Piramirim, Estrada Itupava, Silva et al. 1651 (MA 655870).

Blechnum schomburgkii (Klotzsch) C. Chr., Brazil, São Paulo: Campos de Jordão, Leite & Leite 3504 (LIL 162347).

Blechnum schottii (Colla) C. Chr., Chile, Valparaíso: Masatierra, subida al Mirador Selkirk desde San Juan Bautista, Agua del Guindal, Marticorena et al. 9000 (MA).

Blechnum serrulatum Rich., Brazil, Rio de Janeiro: Guanabara, Tijuca, Recreio dos Bandeirantes, 'Casuarinas', Strang 342 (LP).

Blechnum spannagelii Rosenst., Brazil, Minas Gerais: Serra do Cipó, Palacio Hotel, de la Sota 2616 (LIL 473356).

Blechnum spicant (L.) Roth., Spain, Galicia: Coruña, Fraga de Caaveiro, Prada s/n (MACB).

Blechnum sprucei C. Chr., Argentina. Tucumán: Tafi, carretera de Tafi del Valle, El Nogalar, Prada & Gallardo s. n. (MA).

Blechnum stipitellatum (Sodirol) C. Chr., Ecuador, Napo: Llanganates, steep along Río Topo, immediately SE of Aucacocho, Øllgaard & Holm-Nielsen 38772 (UC 1480966).

Blechnum stoloniferum (Mett. ex Fourn.) C. Chr., México, Oaxaca: Mixes, NW slopes of Cerro Zempaltepetl, trail from Yacoche to Tototepec, Mickel & Leonard 4661 (LP).

Blechnum stuebelii Hieron., Colombia, Cauca: Parque Nacional Munchique, El Tambo, vereda La Romelia, camino a Nueva Granada, González et al. FG2950 (MA 610969).

Blechnum tabulare (Thunb.) Kuhn, Africa. Tanganiika, Lupembe: Stromgebiet des oberen Ruhuge, Landschaft Lupembe, 1600 m nördlich des Flusses, Schlieben 1068 A (MA 389243). South America. Argentina, Misiones: Iguazú, Paraje Aguaray, Lote P, bañado en rodal 5 y 6, sector anegado con *Eryngium* sp., Keller, Colcombet & Ferreira 1774 (MA 710843). Brazil, Rio de Janeiro: RS-Jaquirana, Fazenda Boa Vista, 900 m, Watsum et al. 3995 (MA 422162).

Blechnum tuerkheimii Brause, Dominican Republic, La Vega: Cordillera Central, little N of La Nuez, 40,4 km N of San José de Ocoa on Road to Constanza, Mickel 8236 (UC 1733553).

Blechnum werkleanum (H. Christ) C. Chr., Panamá, Bocas del Toro: Oleoducto Road, near Continental Divide, Fortuna Dam area, Churchill et al. 4510 (UC 1506934).

Blechnum yungense Ramos Giacosa, Argentina, Salta: Santa Victoria, cerca del Pueblo de Baritú, Prada et al. s. n. (MA).

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