Review of Palaeobotany and Palynology 269 (2019) 55–63

Contents lists available at ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

Spore morphology and wall ultrastructure of *Lomariocycas* (Blechnaceae) species from America

J.P. Ramos Giacosa

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Cátedra de Morfología Vegetal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Boulevard 120 s/n° entre 61 y 62, 1900 La Plata, Argentina

ARTICLE INFO

Article history:
Received 23 April 2019
Accepted 19 June 2019
Available online 22 June 2019

Keywords:
Lomariocycas
Blechnaceae
Spores
Morphology
Ultrastructure

ABSTRACT

The spores of 11 species of *Lomariocycas* that grow in America were studied: *Lomariocycas aurata*, *L. columbiensis*, *L. cycadifolia*, *L. insularis*, *L. magellanica*, *L. moritziana*, *L. rufa*, *L. schomburgkii*, *L. shaferi*, *L. underwoodiana* and *L. werckleana*. The study was performed with light microscope (LM), scanning electron microscope (SEM) and transmission electron microscope (TEM). The spores are monolete of 61–100 μm in equatorial diameter and 40–79 μm in polar diameter. The exospore is 1.5–4.8 μm thick and in section is two-layered. The perispore is folded, two types of folds were observed in the different species analyzed and also they could be fused in different ways. In section, it is 0.5–13.8 μm thick and is two-layered. The outer layer is single stratified and the inner layer is three-stratified. The spores of some species are illustrated and described for the first time. Moreover, the sporoderm ultrastructure of *L. underwodiana* was not studied until now. The differences found in perispore morphology and ultrastructure could be valuable characters for systematic and phylogenetic purposes.

© 2019 Elsevier B.V. All rights reserved.

1. Introduction

Lomariocycas (J.Sm.) Gasper & A.R. Sm. (Blechnaceae) is a genus recently described that was traditionally considered as *Blechnum* sect. *Lomariocycas* (J. Sm.) C.V. Morton. This genus is characterized by erect, arborescent rhizome, stiff, thickened curved stipe base and rhizome apex scales and dimorphic fronds. The erect trunk-like rhizomes and the rosetted arrangement of the pinnate leaves of the *Lomariocycas* is similar to the *Cycas* (gymnosperm) and that is the reason for the genus name. About 19 species are distributed in America, Africa and Madagascar (Gasper et al., 2016, 2017; PPGI, 2016).

In America the genus grows from Costa Rica to the subantarctic forests of Argentina and Chile, including other areas like the Antilles, Juan Fernandez and Malvinas Islands. The species are mainly terrestrial and a few of them have epiphytic habit. They grow in montane forest, gallery forest, savannahs and paramos (de la Sota, 1977; Tryon and Tryon, 1982).

Palynological contributions about the Blechnaceae, was made by Lugardon (1965, 1966, 1972, 1974) who studied the spores of *Blechnum spicant* (L.) Sm. with scanning electron microscope (SEM) and transmission electron microscope (TEM). Morbelli (1974, 1976) analyzed with light microscope (LM) the spores of the species and hybrids of the subgenera *Blechnum*. Passarelli et al. (2010) studied with SEM the spores of several species of *Blechnum*. Regarding the species of *Lomariocycas*, the spores of some taxa were analyzed. The spores of *L. magellanica* Mett. from Chile were studied with LM by Heusser (1971). Kurita and Nishida (1985) studied with SEM the spores of *L. magellanica* and *L. cycadifolia* (Colla) J. W. Sturm. Tryon and Lugardon (1991), illustrated with SEM the spores of *L. aurata* and *L. schomburgkii* (Klotzsch) C. Chr and mentioned them as rugated. Later, Ramos Giacosa et al. (2009) studied with LM, SEM and TEM the spores of *L. moritziana* (Klotzsch) Gabriel y Galán & Vicent (under *Blechnum tabulare*). Recently, Moran et al. (2018) analyzed with SEM some exospore and perispore characters of the Blechnaceae in a phylogenetic context. They described the exospore of a few species of *Lomariocycas* (*L. aurata*, *L. columbiensis*, *L. schomburgkii* and *L. werckleana*) as low verrucate, sometimes inconspicuously and a densely folded perispore for the Neotropical species.

The spores of some of the American species of *Lomariocycas* were not studied yet and a few of them were not even described or illustrated. The aim of the work is to study with LM, SEM and TEM the spores of the following species of *Lomariocycas* that grow in America in order to contribute to the palynological knowledge of the genus and to give characters for future phylogenetic or systematic purposes. The species are: *Lomariocycas aurata* (Fée) Gasper & A.R.Sm., *L. columbiensis* (Hieron.) Gasper & A.R.Sm., *L. cycadifolia* (Colla) Gasper & A.R.Sm., *L. insularis* (C.V.Morton & Lellinger) Gasper & A.R.Sm., *L. magellanica* (Desv.) Gasper & A.R.Sm., *L. moritziana* (Klotzsch) Gabriel y Galán & Vicent, *L. rufa* (Spreng.) Gasper & A.R.Sm., *L. schomburgkii*

E-mail address: jpramosgiacosa@hotmail.com.

https://doi.org/10.1016/j.revpalbo.2019.06.010
0034-6667/© 2019 Elsevier B.V. All rights reserved.

Table 1
Spore morphological data for *Lomariocycas* species from America. Dimensions in μm.

Taxa	Equatorial diameter	Polar diameter	Laesurae length	Exospore thickness	Perispore thickness	
					Between folds	Folds
L. aurata	62–72	46–58	31–46	2.7–3.6	0,6–1	0.7–2
L. columbiensis	63–70	44–59	29–41	2.2–3.6	0.7–1.4	0.6–2
L. cycadifolia	75–100	58–75	31–47	2–3.4	0.6–0.9	3.9–7.6
L. insularis	66–85	43–56	41–58	3.1–3.6	0.6–0.8	7.2–9.6
L. magellanica	61–79	43–53	29–41	2–2.8	0.7–1.2	5.5–6.9
L. moritziana	82–95	61–79	42–66	3.7–4.8	0.6–0.9	6.2–9.3
L. rufa	73–86	48–64	41–50	2.2–3.5	0.5–0.9	3.3–6.5
L. schomburgkii	68–99	40–78	25–58	3.1–4.2	0.6–1	3.6–8.3
L. shaferi	61–83	46–56	35–41	1.5–1.9	0.7–0.9	8.9–13.8
L. underwoodiana	61–87	46–58	29–41	2.5–4.3	0.8–1	4.8–11.4
L. werckleana	66–76	43–51	33–46	2.4–3.6	0.7–1.4	3.8–9

Table 2
Ornamentation of the perispore in *Lomariocycas* species from America.

Taxa	Fold type	Reticulum
L. aurata	Ridge	Incomplete
L. columbiensis	Ridge	Incomplete
L. cycadifolia	Crest	Incomplete
L. insularis	Ridge	Complete
L. magellanica	Ridge	Incomplete
L. moritziana	Ridge	Incomplete
L. rufa	Crest	Complete
L. schomburgkii	Crest	Incomplete
L. shaferi	Ridge	Complete
L. underwoodiana	Crest	Complete
L. werckleana	Ridge	Complete

(Klotzsch) Gasper & A.R.Sm., *L. shaferi* (Broadh.) Gasper & A.R.Sm., *L. underwoodiana* (Broadh.) Gasper & A.R.Sm. and *L. werckleana* (Christ) Gasper & A.R.Sm.

2. Materials and methods

Spores were obtained from herbarium specimens from the following Institutions (abbreviations according to Thiers, 2019): BA, BAB, GH, LP, MVFA, NY, SI and US.

The spores were studied using light (LM), scanning (SEM) and transmission (TEM) electron microscopy. For LM, the spores were treated with hot 3% sodium carbonate for 2 min and acetolyzed according to the method of Erdtman (1960). For SEM the material was treated with hot 3% sodium carbonate, washed, dehydrated, suspended in 96%

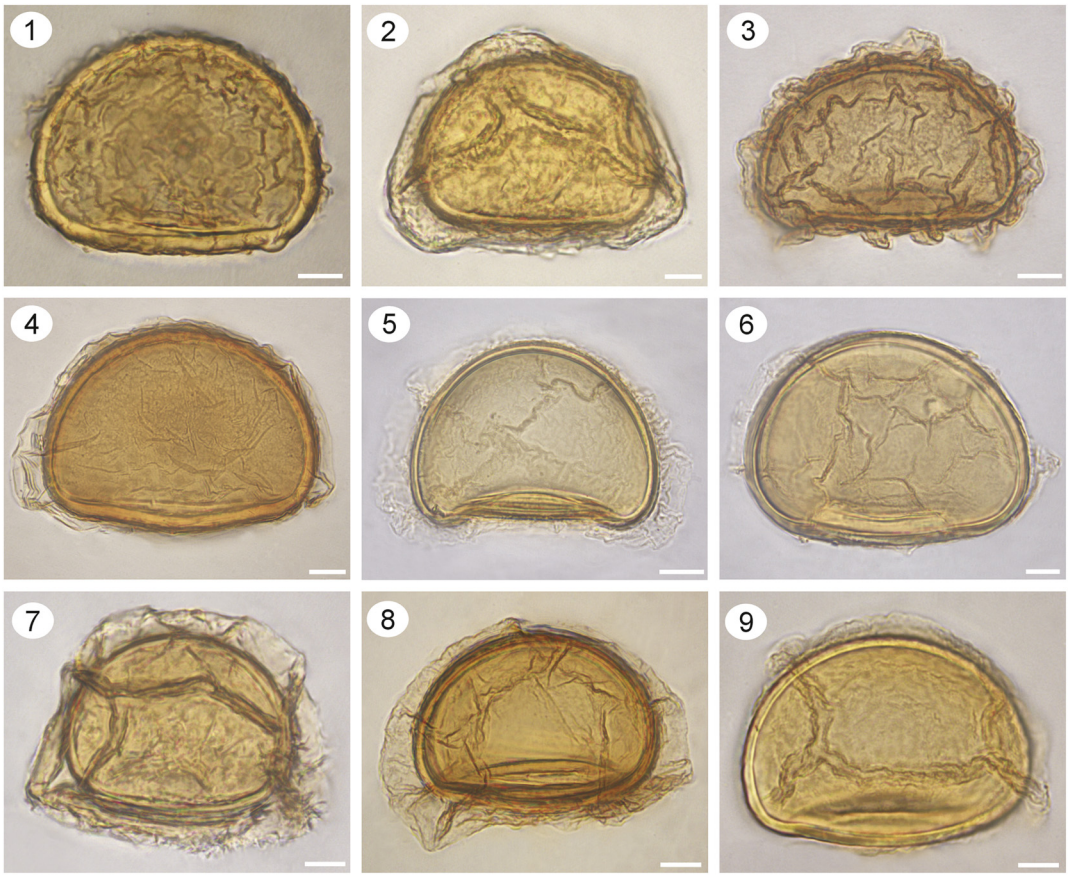

Plate I. Spores of *Lomariocycas* with LM. 1. *L. aurata*. Scale bar = 10 μm. 2. *L. insularis*. Scale bar = 10 μm. 3. *L. magellanica*. Scale bar = 10 μm. 4. *L. moritziana*. Scale bar = 10 μm. 5. *L. rufa*. Scale bar = 10 μm. 6. *L. schomburgkii*. Scale bar = 10 μm. 7. *L. shaferi*. Scale bar = 10 μm. 8. *L. underwoodiana*. Scale bar = 10 μm. 9. *L. werckleana*. Scale bar = 10 μm.

ethanol and then transferred to acetate plates. After drying they were coated with gold.

For TEM, dry material from herbarium specimens was hydrated following the technique by Rowley and Nilsson (1972), that consists of the use of phosphate buffer and alcian blue (AB). Then, the material was fixed with Glutaraldehyde + 1% Alcian Blue in phosphate buffer for 12 h and post-fixed with 1% OsO4 in water plus 1% Alcian Blue. The spores were dehydrated in an acetone series and then embedded in Spurr soft mixture. Sections 3 μm thick were stained with toluidine blue and observed with LM. Ultrathin sections were stained with 1% uranyl acetate for 15 min followed by lead citrate for 3 min.

The observations were performed with an Olympus BH2, Nikon E200 light microscope, a JEOL JSMT-100 scanning electron microscope at the Museo de Ciencias Naturales de La Plata, and a Zeiss T-109 transmission electron microscope at the Instituto de Biología Celular, Facultad de Medicina, Universidad de Buenos Aires.

After the LM and SEM analysis, *Lomariocycas moritziana* and *L. underwoodiana* were selected as representative for the study with TEM.

For spore descriptions, the terms proposed by Tryon and Lugardon (1991) and Punt et al. (2007) were used.

2.1. Material studied

Lomariocycas aurata: COSTA RICA: San José, entre Cartago y Cerro de la Muerte, Ruta Panamericana km 73, de la Sota 5102 (LP); Cartago road from Cartago to San Isidro del Generla, Smith 2092, (NY). ECUADOR: Tungurahua, Rimbach s/n° (SI).

Lomariocycas columbiensis: PERU: Dpto. Amazonas, 20 km. de Balsas, Madison 1.138 (GH). COLOMBIA: Dpto. Santander, Nlle. Grenade, Schlim 307 (GH).

Lomariocycas cycadifolia: CHILE: Islas Juan Fernández, Isla Robinson Crusoe (más a tierra), Cumberland Bay, Meyer 9686 (LP); camino al Portezuelo, Looser 408 (LP).

Lomariocycas insularis: PUERTO RICO: Municipio de Naguabo, Sierra de Naguabo, Quebrada Grande o Cuchilla Firme, Shafer 3.581 (US).

Lomariocycas magellanica: ARGENTINA: Neuquén, Dpto. Los Lagos, Quetrihué, Diem 715 (BAB); Tierra del Fuego, Isla de los Estados, Dudley et al. 1526 (LP); Islas Malvinas, Port Harriet, East Falkland, Rumboll 68.073 (BA), Puerto Argentino, Ulibarri, Dimitri & Orfila 196 (BAB). CHILE: Prov. Magallanes, Pto. Nuevo, Parra 140 (LP).

Lomariocycas moritziana: ARGENTINA: Prov. Jujuy, Dpto. Ledesma, Abra de las Cañas, de la Sota 4439 (LP); Camino a Valle Grande, 4 km

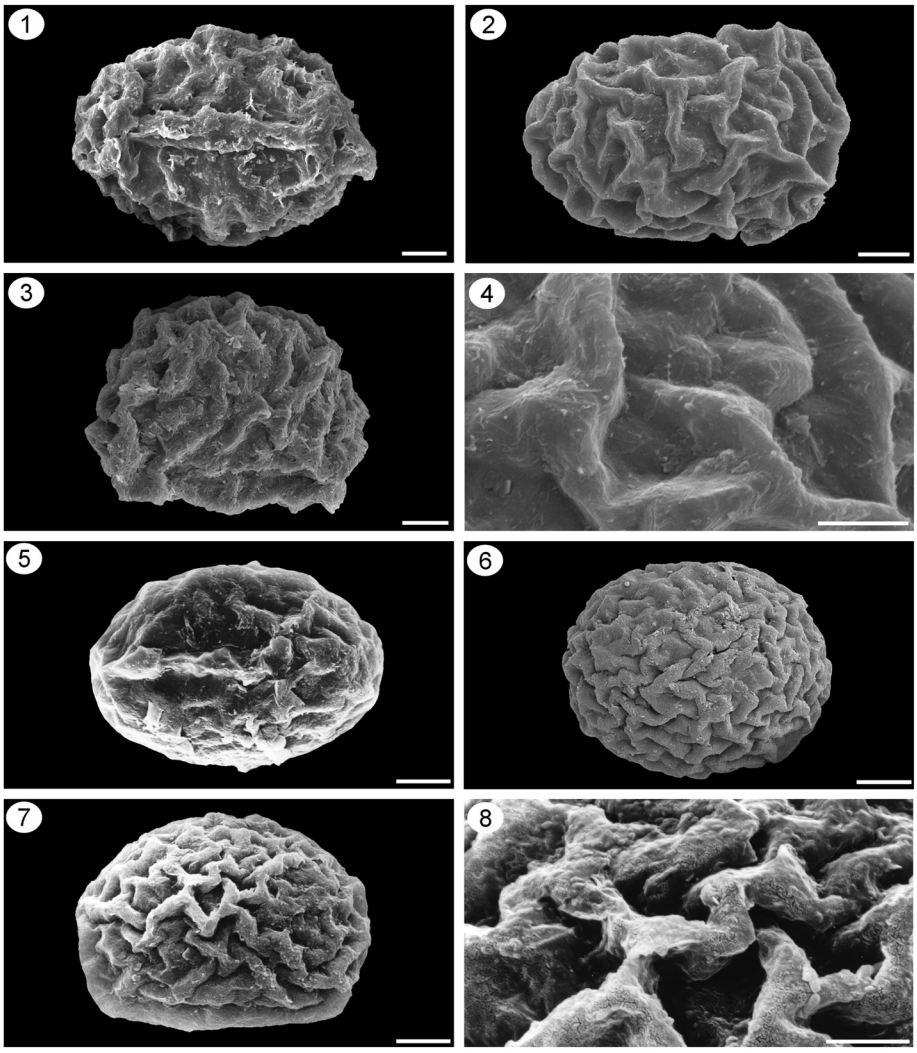

Plate II. Spores of *Lomariocycas aurata* and *L. columbiensis* with SEM. 1–4. L. aurata 1. Proximal view of an elliptic spore. Scale bar = 10 μm. 2. Distal view of a folded spore. The folds are robust and are partially fused forming an incomplete reticulum. Scale bar = 10 μm. 3. Equatorial view of a spore. Scale bar = 10 μm. 4. Detail of the folded perispore. Scale bar = 5 μm. 5–8. *L. columbiensis* 5. Proximal view of an elliptic spore. Scale bar = 10 μm. 6. Distal view of a spore with abundant folds. Scale bar = 10 μm. 7. Equatorial view of a spore. The folds are robust and are partially fused forming an incomplete reticulum. Scale bar = 10 μm. 8. Detail of the perispore. Scale bar = 5 μm.

antes de Abra de las Cañas, Legname & Cuezzo 8.219 c (LP); Legname & Cuezzo 8.609 (LP); Prov. Salta, Dpto. Santa Victoria, San José, Martínez, Ganem & de la Sota 603 (LP).

Lomariocycas rufa: GUADELOUPE (LESSER ANTILLES): Duss 4164 (BA); L Herminier s/n°, NY 127.315 (NY).

Lomariocycas schomburgkii: BRAZIL: Estado de Goiás, Serra dos Cristais, Anderson 8148 (LP); Chapada dos Veadeiros, Anderson 6403 (LP); Estado de Rio Grande do Sul, São Leopoldo, Dutra 278 (SI); Estado de San Pablo, Estação Biologica Alto da Serra, 23° 47' S, 46° 19' W, Smith 1942 (GH); Estado de Paraná, 13 km de Horizonte, Krapovickas & Cristóbal 39,690 (LP). PARAGUAY: localidad indeterminada, 1920, Jorgensen 4606 (LP); Dpto. Misiones, Santiago, Estancia La Soledad, Pedersen 4404 (SI). PERU: Dpto. Cuzco, Prov. La Convención, 12° 36′ S, 73° 30′ W, Dudley 10.975 (GH). URUGUAY: Dpto. Tacuarembó, Sierra del Tacuarembó chico, Herter 3525 (GH); Dpto. San José, Barra de Santa Lucía, Berro 2376 (MVFA).

Lomariocycas shaferi: CUBA: Prov. Oriente: Crest of Sierra Maestra between Pico Turquino and La Bayamesa, Morton & Acuna 3773 (US); Morton & Acuna 3761 (US).

Lomariocycas underwoodiana: JAMAICA: New Haven Gap, Underwood 985 (NY). DOMINICAN REPUBLIC: Ciénaga de la Culata, Constanza, Liogier 17.056 (NY); Prov. La Vega, Cordillera Central, 5,4 km. al sur de la ciudad de Constanza, 18° 50′ N, 70° 45′ W; Zanoni, Mejía, Pimentel & Mickel 19.354 (NY).

Lomariocycas werckleana: COSTA RICA: Werckle s/n, (NY); El Gallito, Valerio 59 (US), Prov. Alajuela, Pacific side of Alto Palomo, Lent 1.839 (GH).

3. Results

3.1. Morphology

The palynological characteristics of the analyzed taxa are summarized in Tables 1 and 2.

The spores of the genus *Lomariocycas* are monolete, the spore shape in equatorial view is plane-convex and elliptic in polar view. The equatorial diameter is 47–100 μm and the polar diameter is 35–79 μm. The laesurae are 19–66 μm long. Analyzed with LM (Plate I, 1–9), the exospore is smooth and the perispore is folded. The folds are very conspicuous and translucent in some species, like *L. insularis*, *L. rufa*, *L. shaferi* and *L. werckleana* (Plate I, 2, 5, 7, 8). They can reach up to 13.8 μm height depending on the species analyzed (Table 1).

Analyzed with SEM the exospore is verrucated (Plate III, 3, 6). Two morphological types of folds were observed in the spores of *Lomariocycas*:

Type 1: The folds are narrow and resemble the morphology of a crest. They could be named "crest shaped." These types of folds are partially fused forming an incomplete reticulum in *Lomariocycas cycadifolia* (Plate III, 1) and *L. schomburgkii* (Plate IV, 6–8) or totally fused forming a complete reticulum with evident lumina in *L. rufa* (Plate IV, 4, 5) and *L. underwoodiana* (Plate V, 4–6). Some perforations can be observed on the perispore of *L. cycadifolia* (Plate III, 2).

Type 2: The folds are wide and robust and resemble the morphology of a ridge. They could be named "ridge shaped." In *L. aurata* (Plate II, 1–4), *L. columbiense* (Plate II, 5–8), *L. magellanica* (Plate IV, 1–3) and

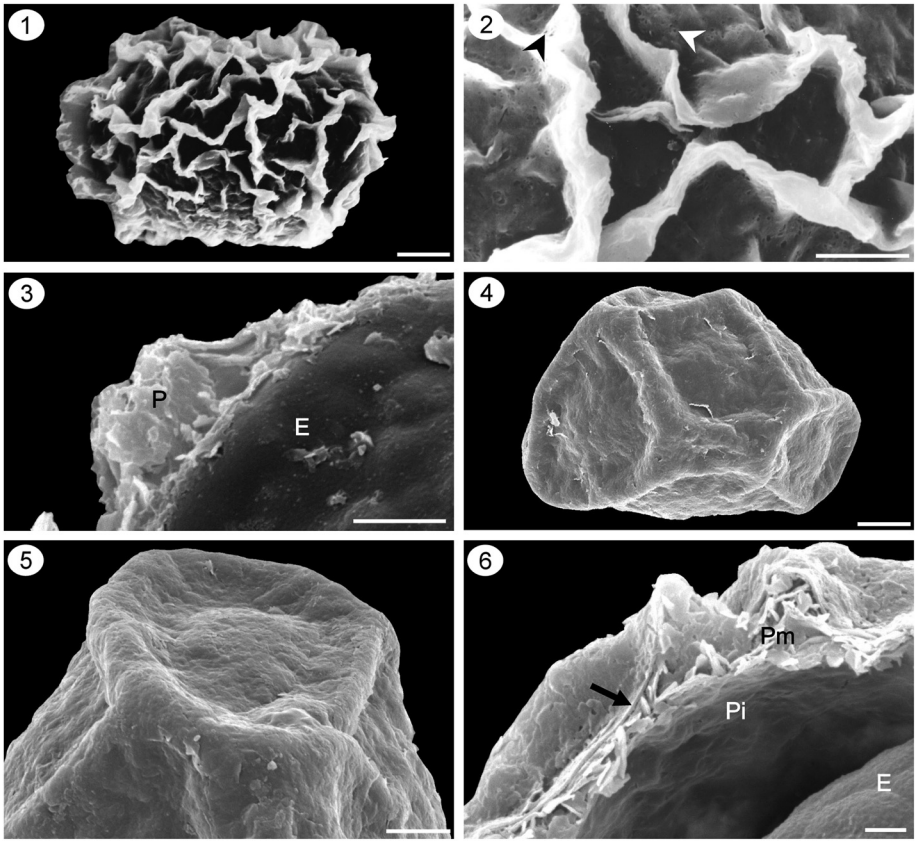

Plate III. Spores of *Lomariocycas cycadifolia* and *L. insularis* with SEM. 1–3. *L. cycadifolia* 1. Equatorial view of a spore. The ornamentation is composed of narrow folds. Scale bar = 10 μm. 2. Detail of the perispore. The folds are narrow and some perforations are observed (arrowheads). Scale bar = 2 μm. 3. Fold of the perispore (P) and exposed verrucated exospore (E). Scale bar = 2 μm. 4–6. *L. insularis* 4. Equatorial view of a spore with robust folds totally fused forming a complete reticulum. Scale bar = 10 μm. 5. Detail of one lumen of the perispore. Scale bar = 2 μm. 6. Section through the perispore. The perispore is composed of three strata (Pi, Pm, arrow) of different thickness. The exospore (E) is also evident. Scale bar = 2 μm.

L. moritziana (Plate VI, 3–5), the folds are partially fused forming an incomplete reticulum. In *L. aurata* and *L. columbiense* the folds are more densely distributed than in the other species. Likewise, in *L. insularis* (Plate III, 4, 5), *L. shaferi* (Plate V, 1–3) and *L. werckleana* (Plate VI, 1, 2) are totally fused forming a complete reticulum. Some perforations can also be observed on the perispore of *L. magellanica* (Plate IV, 2).

In fractured spores analyzed with SEM (Plate III, 6; Plate VI, 6), three strata of the perispore with variable thickness can be distinguished: the inner (Pi) is thin and adhered to the exospore (E), the middle (Pm) with rodlets, is the thickest and the outer stratum (Po) has undulated margin and constituted the spore surface.

3.2. Ultrastructure

Based on the two morphological types of perispore observed, the following species were selected for the ultrastructural studies:

Type 1: Lomariocycas underwoodiana (Plate VII)

The exospore is 1.6–2.6 μm thick and two-layered: the inner layer (Ei) is 0.1–0.4 μm thick and the outer layer (Eo) is 1.5–2.2 μm thick, less contrasted than the inner one (Plate VII, 4). The two layers are sometimes difficult to distinguish.

The perispore is two-layered: an inner layer (P1) and an outer layer (P2). The inner layer P1 is tree-stratified: an inner stratum (P1i) is 30–100 nm thick with some portion of membranes (scales) (Plate VII, 4); the middle stratum (P1m) is 2300–11,000 nm thick and is composed of some threads (Plate VII, 2, 3); the outer stratum (P1o) is 70–170 nm thick. The outer stratum is the one which forms the narrow and high folds (Plate VII, 1). The layer P2 is 30–45 nm thick and is slightly differentiated from P1 layer as a lesser contrasted area (Plate VII, 2).

A few spheroids can be observed inside P1m stratum. Their central structure and contrast are similar to the exospore and are surrounded by several scales covers all the external surface of the spheroid (Plate VII, 4).

Type 2: Lomariocycas moritziana (Plate VIII)

The exospore is two-layered: the inner layer (Ei) is 0.1–0.6 μm thick and the outer layer (Eo) is 1.8–2.2 μm thick, less contrast than the inner one. The apertural area consists of a strongly protruding fold (Plate VIII, 1).

The perispore is two-layered: an inner layer (P1) and an outer layer (P2). The layer P1 is three-stratified: the inner stratum (P1i) is 30–70 nm thick with some short portion of membranes (scales) (Plate VIII, 3); the middle stratum (P1m) is 1000–5000 nm thick, and is

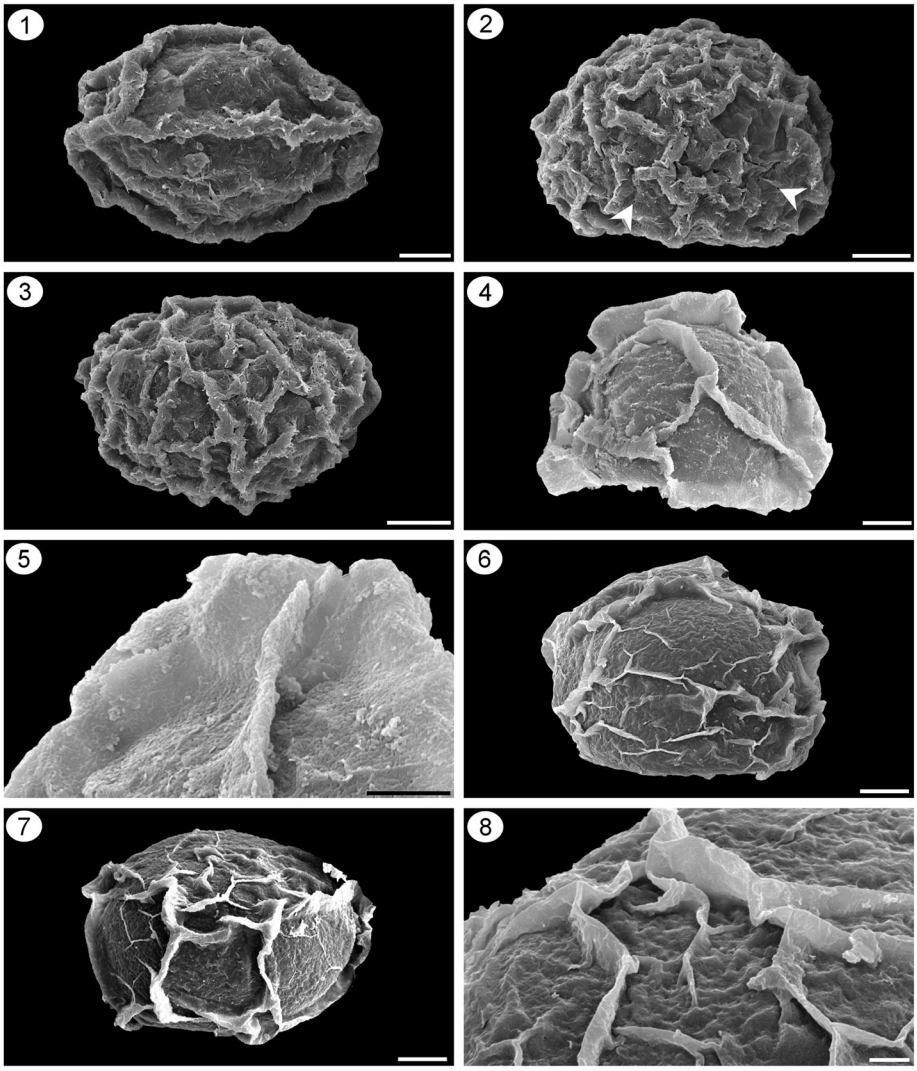

Plate IV. Spores of *Lomariocycas magellanica*, *L. rufa* and *L. schomburgkii* with SEM. 1–3. *L. magellanica*. 1. Proximal view of a folded spore. Scale bar = 10 μm. 2. Equatorial view of a spore. The folds are partially fused forming an incomplete reticulum. Some perforations are observed (arrowheads). Scale bar = 10 μm. 3. Distal view of a spore. Scale bar = 10 μm. 4–5. *L. rufa*. 4. Equatorial view of a spore. The folds are narrow, high and are totally fused forming a complete reticulum. Scale bar = 10 μm. 5. Detail of the perispore. Scale bar = 5 μm. 6–8. *L. schomburgkii*. 6. Equatorial view of a folded spore. The folds are partially fused. Scale bar = 10 μm. 7. Distal view. Scale bar = 10 μm. 8. Detail of the perispore. The folds are narrow and have different sizes. Scale bar = 2 μm.

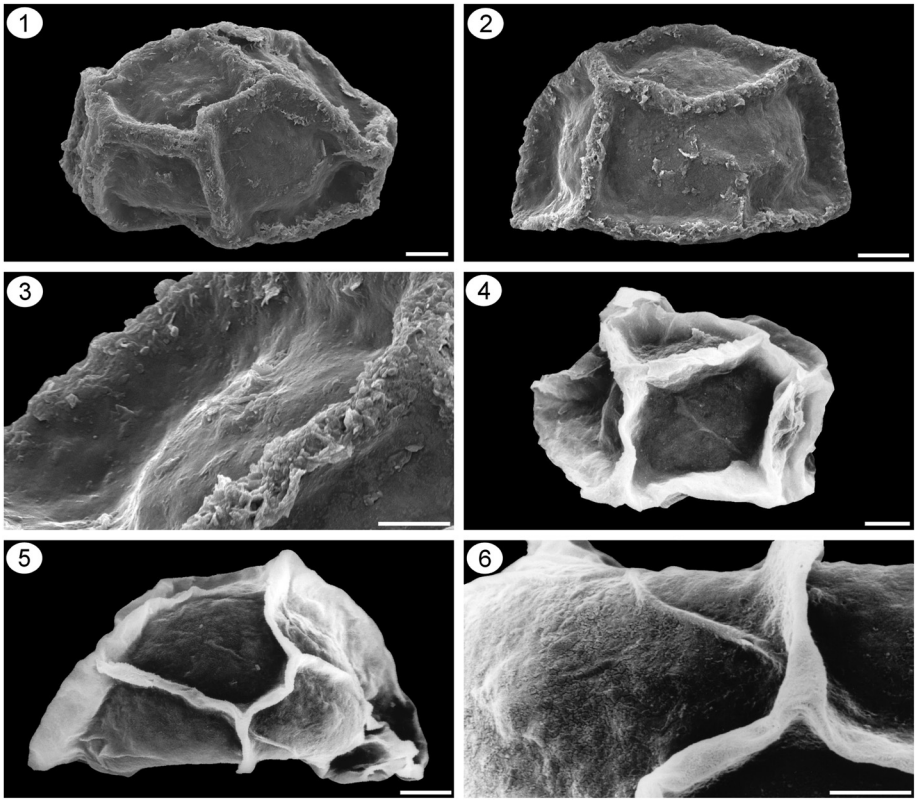

Plate V. Spores of *Lomariocycas shaferi* and *L. underwoodiana* with SEM. 1–3. *L. shaferi.* 1. Distal view of a spore with robust folds totally fused forming a complete reticulum. Scale bar = 10 μm. 2. Equatorial view of a spore, three lumina are evident. Scale bar = 10 μm. 3. Detail of the perispore. Scale bar = 5 μm. 4–6. *L. underwoodiana.* 4, 5. Folded spores with narrow folds totally fused forming a complete reticulum. Scale bar = 10 μm. 6. Detail of the perispore. Scale bar = 5 μm.

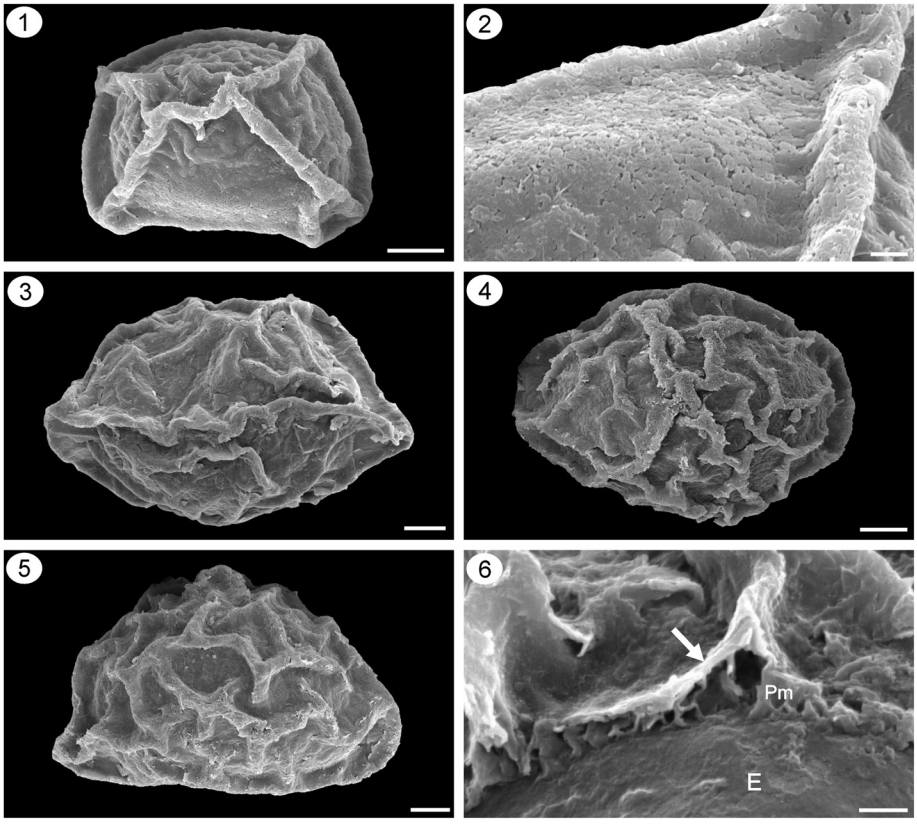

Plate VI. Spores of *Lomariocycas werckleana* and *L. moritziana* with SEM. 1–2. *L. werckleana.* 1. Equatorial view of a spore with robust folds totally fused forming a complete reticulum. Scale bar = 10 μm. 2. Detail of the perispore. Scale bar = 2 μm. 3–6. *L. moritziana.* 3. Proximal view of an elliptic spore. Scale bar = 10 μm. 4. Distal view of a spore. The folds are robust and are partially fused forming an incomplete reticulum. Scale bar = 10 μm. 5. Scale bar = 10 μm. 6. Section through the perispore. The middle stratum (Pm) and the outer strata (arrow) of the perispore are observed. The exospore (E) is also evident. Scale bar = 2 μm.

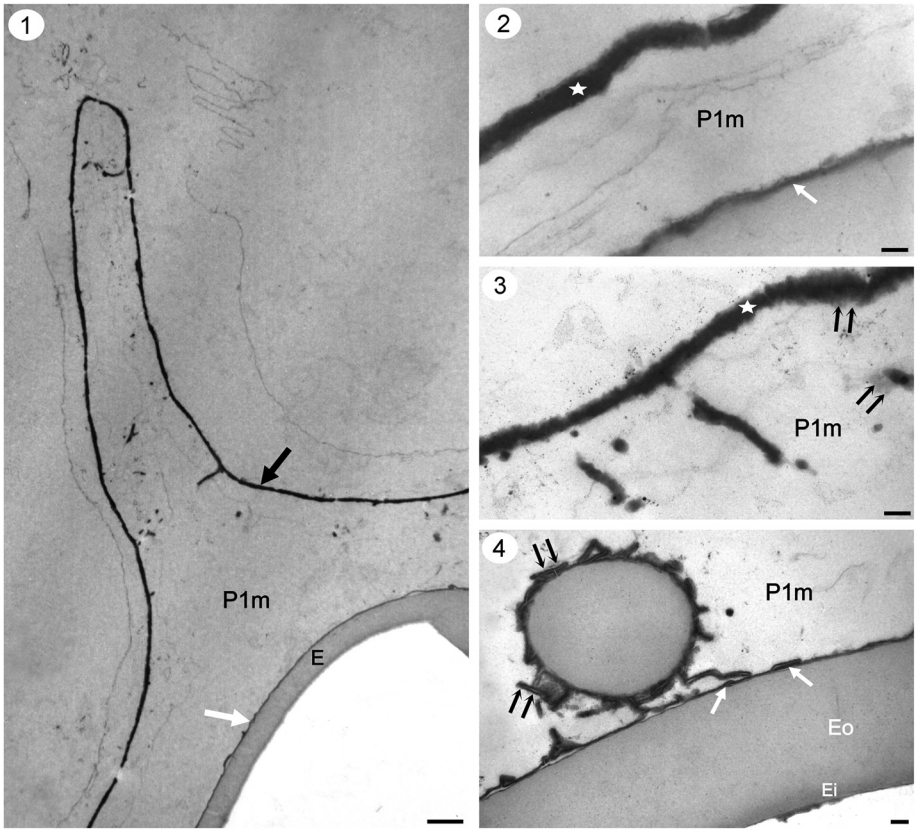

Plate VII. Spores of *Lomariocycas underwoodiana* with TEM. 1. Section through the sporoderm. A narrow and high fold of the perispore is observed. The layer P1 of the perispore is composed by the inner stratum (white arrow), the middle stratum (P1m) with a few threads and an outer stratum (black arrow). Exospore (E). Scale bar = 1 μm. 2, 3. Section through the perispore. It is two-layered: an inner layer (P1) and an outer layer (P2). The layer P1 is three-stratified: the inner stratum (white arrow) is the thinnest, the middle stratum (P1m) is composed of a few threads and an outer stratum (star). The layer P2 (double arrow) is difficult to differentiate; less contrasted than P1 layer and is coating all around the different elements forming P1 layer. Scale bar 2 = 0.1 μm, Scale bar 3 = 200 nm. 4. The exospore consists of two layers: an inner exospore layer (Ei) more contrasted and thinner than the outer exospore layer (Eo). A globule is immersed in the P1m stratum of the perispore. Its centre has a similar structure and contrast to the exospore and are surrounding by abundant short portion of membranes (scales) which are distributed tangentially (double arrow). A few of them are also observed in the inner stratum of the perispore (white arrow). Scale bar = 0.2 μm.

composed of abundant threads distributed in several directions (Plate VIII, 2, 3); the outer stratum (P1o) is 120–170 nm thick. The layer P2 is 50–60 nm thick, less contrasted than P1 layer and is coating all around the different elements forming P1 layer (Plate VIII, 4). This layer in some sections is difficult to differentiate.

A few spheroids with same structure and contrast as the exospore and perispore can be observed immersed in the perispore (Plate VIII, 1).

4. Discussion

Based on the different dimensions observed in the spores of *Lomariocycas* studied here, the spores of *L. cycadifolia* are the largest with an equatorial diameter that can reach 100 μm. The height of the folds in the species analyzed is variable and is another aspect to take into account. The highest folds are the ones of *L. shaferi* that can reach 13.8 μm high and the lowest are the ones of *L. aurata* and *L. columbiensis* that are only 2 μm high.

As regards spore morphology, in this study the exospore of the *Lomariocycas* can be defined as verrucated. This observation is particularly clear when spores with detached perispore are seen with SEM. The same type of exospore was observed by other authors in the spores of *L. cycadifolia* (Kurita and Nishida, 1985) and *L. tabularis* (Passarelli et al., 2010). The perispore in all the species is folded. This result is in agreed with other authors (Tryon and Lugardon, 1991; Passarelli et al., 2010; Moran et al., 2018) that described the same type of ornamentation for some species of the genus. When Tryon and Lugardon (1991) illustrated the spores of *L. aurata*, they described them as coarsely rugate and defined this term as a surface of short folds. Thus, this description is also in agreed with the one defined here.

As it was showed in the present work, the variations in the morphology of the folds and degree of fusion of them are important characters to consider when comparing the spores of different species of *Lomariocycas*. These characters are not usually mention by other authors and could contribute to the systematic of the genus. Observations by SEM are better than LM to define which type of fold has a particular species and can define properly the morphology of the folds. Nevertheless, the spores of *L. insularis*, *L. shaferi* and *L. werckleana* which have few "ridge shaped" folds can also be correctly described using LM.

When Moran et al. (2018) analyzed the evolution of the spore morphology in the Blechnaceae, studied the spores of four American species of *Lomariocycas* (*L. aurata*, *L. columbiensis*, *L. schomburgkii* and *L. werckleana*) and also one Old World species (*L. tabularis*). They suggested that a densely folded perispore might be a synapormorphy for the Neotropical species of *Lomariocycas*. However, according to the results presented in the present paper it can be confirmed that some Neotropical species have a densely folded perispore (*L. aurata*, *L. columbiensis*, *L. cycadifolia*, *L. magellanica*, *L. schomburgkii* and *L. moritziana*) whereas others have only a few folds forming the ornamentation of the spore (*L. insularum*, *L. rufum*, *L. shaferi*, *L. underwoodiana* and *L. werckeana*). Thus, the presence of a densely folded perispore should not be considered as a synapomorphy for the Neotropical species of *Lomariocycas*.

The ultrastructural studies reveal that the exospore is two-layered in *L. moritziana* and *L. underwodiana* and this coincides with a "blechnoid" type of exospore described by Lugardon (1972). Comparing the

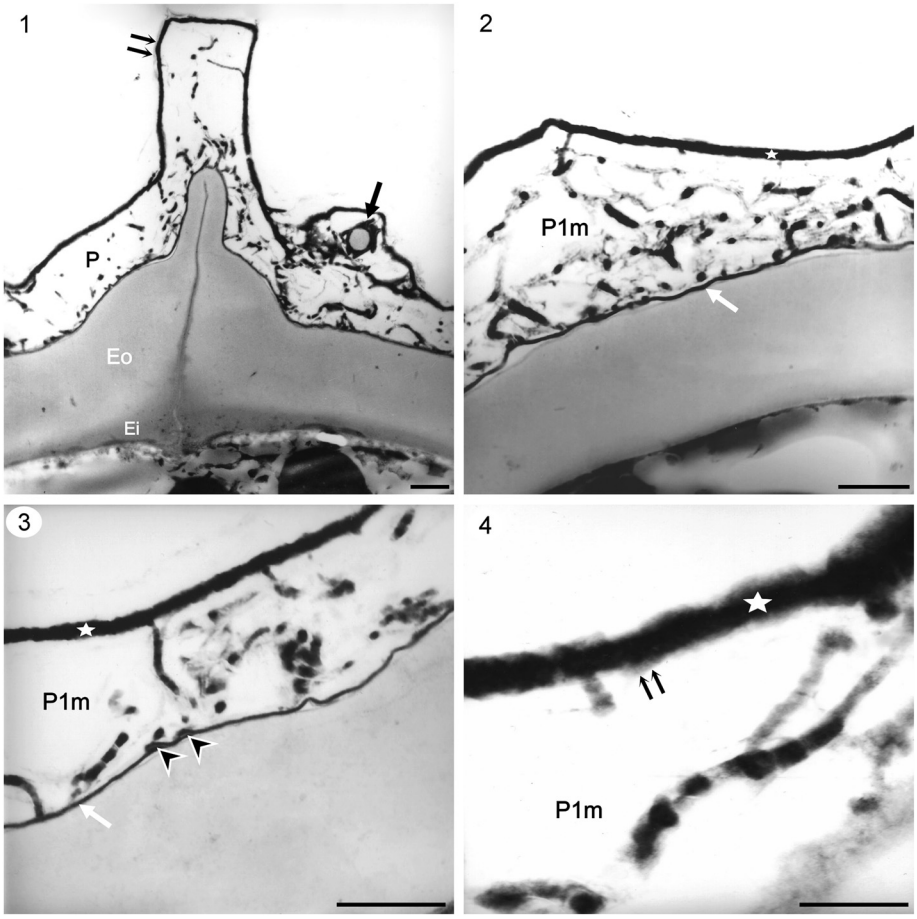

Plate VIII. Spores of *Lomariocycas moritziana* with TEM. 1. Section through the laesura. The exospore consists of two layers. The inner exospore layer (Ei) is more contrasted and thinner than the outer exospore layer (Eo). A supralesural fold is observed (double arrow). Inside the perispore (P) a globule are evident (arrow). Scale bar = 1 μm. 2, 3, 4. The perispore is two-layered: an inner layer (P1) and an outer layer (P2). The layer P1 is three-stratified: the inner stratum (arrow) is the thinnest with some short portion of membranes (scales) (arrowheads), the middle stratum (P1m) is composed of abundant threads and an outer stratum (star). The layer P2 (double arrow) is less contrasted than P1 layer and is coating all around the different elements forming P1 layer. Scale bars 2, 3 = 1 μm, 4 = 0.5 μm.

perispore, the stratification is also similar in the two species. A slight difference was observed in the density of threads of the middle stratum of the perispore. Thus, a higher density of threads was observed in *L. moritziana*. The description of the perispore coincides with the one illustrated by Tryon and Lugardon (1991) for *L. palmiformis* (Thouars) Gasper & A. R. Sm. a species that grows in Tristan de Cunha Island in the South Atlantic Ocean.

The two species observed with TEM have scales in the perispore inner stratum and surrounding the globules. These scales could be related to membrane unit which are responsible for wall deposition. Tryon and Lugardon (1991) explained that the globules are originated in the tapetum, develop at the same time as the exospore and they are enveloped by perispore. The presence of scales surrounding the globules was also cited in Blechnaceae by Tryon and Lugardon (1991) and in other fern families such as Anemiaceae (Ramos Giacosa, 2014) and Schizaeaceae (Ramos Giacosa and Barakat, 2018).

5. Conclusions

The spores of *Lomariocycas* from America are folded and two types of perispore ornamentation are defined: (1) narrow and high folds (crest shaped), (2) wide and robust folds (ridge shaped). Moreover, these two types of folds could be partially fused forming an incomplete reticulum or totally fused forming a complete reticulum with evident lumina.

The ultrastructural analysis confirmed that the perispore is the wall that forms the ornamentation of the spores in the genus. In addition, it was revealed that the two types of perispore ornamentation are very similar in the sporoderm stratification: a two layered exospore and a two layered perispore with three strata. Furthermore, short portion of membranes (scales) located on the exospore surface and a few globules surrounding by scales which are immersed in the perispore middle stratum (P1m) are present in the two species analyzed. The only slightly difference observed between the species is a higher density of threads in the P1m of *L. moritziana*. These results show that the morphological variability observed in the spore ornamentation are not corresponded with the ultrastructural morphology which is very similar in the species studied here. Additional TEM studies in other species of *Lomariocycas* are needed to confirm the same sporoderm stratification in the genus.

The data presented in the present work could be useful for systematic and phylogenetic purposes of the genus *Lomariocycas*, the Blechnaceae family and additionally, could provide valuable palynological information to make systematic assignments in paleobotanical studies.

Acknowledgments

The author thanks the curators of the institutions that sent the herbarium material used in this study.

References

de la Sota, E.R., 1977. Pteridophyta. En Flora de la Provincia de Jujuy. In: Cabrera, A.L. (Ed.), Col. Cient. INTA. 13 (2).

Erdtman, G., 1960. The acetolysis method. A revised description. Sven. Bot. Tidskr. 54, 561–564.
Gasper, A.L. de, Dittrich, V.A.O., Smith, A.R., Salino, A., 2016. A classification for Blechnaceae (Polypodiales: Polypodiopsida): New genera, resurrected names, and combinations. Phytotaxa 275, 191–227.
Gasper, A.L. de, Almeida, T.E., Dittrich, V.A.O., Smith, A.R., Salino, A., 2017. Molecular phylogeny of the fern family Blechnaceae (Polypodiales) with a revised genus-level treatment. Cladistics 33, 429–446.
Heusser, C.J., 1971. Pollen and Spore of Chile. Modern Types of the Pteridophyta, Gimnnospermae and Angiospermae. The University of Arizona Press.
Kurita, S., Nishida, H., 1985. Spore morphology in the subgeneric groups of *Blechnum* -a preliminary report. In: Nishida, M. (Ed.), A Report of the Botanical Survey to Bolivia and Southern Chile. Japan, Faculty of Science, Chiba University.
Lugardon, B., 1965. Structure des parois de la spore de *Blechnum spicant* (L.) Roth. Pollen Spores 7, 409–428.
Lugardon, B., 1966. Formation de l'exospore Chez *Blechnum spicant* (L.) Roth. C.R. Acad. Sc. Paris, D 262, 2029–2031.
Lugardon, B., 1972. La structure fine de l'exospore et de la perispore des Filicinées isosporées. I. Généralités. Eusporangiées et osmundales. Pollen Spores 14, 227–261.
Lugardon, B., 1974. La structure fine de l'exospore et de la perispore des Filicinées isosporées. II. Filicales. Commentaires. Pollen et Spores 16, 161–226.
Moran, R.C., Hanks, J.G., Labiak, P.H., 2018. Evolution of spore morphology in the Blechnaceae. Int. J. Plant Sci. 179, 712–729.
Morbelli, M.A., 1974. Análisis palinológico en híbridos interespecíficos del género *Blechnum* L. subgénero *Blechnum* (Blechnacaee–Pteridophyta). Bol. Soc. Argent. Bot. 15, 447–465.
Morbelli, M.A., 1976. Estudio palinológico de las especies austrosudamericanas del género *Blechnum* L. subgénero *Blechnum* (Blechnaeace–Pteridophyta). Bol. Soc. Argent. Bot. 17, 155–175.
Passarelli, L.M., Gabriel y. Galán, J.M., Prada, C., Rolleri, C., 2010. Spore morphology and ornamentation in the genus *Blechnum* (Blechnaceae). Grana 49, 243–262.
PPGI, 2016. A community-derived classification for extant lycopods and ferns. J. Syst. Evol. 54, 563–603.
Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A., 2007. Glossary of pollen and spore terminology. Rev. Palaeobot. Palynol. 143, 1–81.
Ramos Giacosa, J.P., 2014. Abnormal spore morphology and wall ultrastructure in *Anemia tomentosa* var. *anthriscifolia* and *A. tomentosa* var. *tomentosa* (Anemiaceae). Plant Syst. Evol. 300, 1571–1578.
Ramos Giacosa, J.P., Barakat, M.C., 2018. Spore morphology and wall ultrastructure of *Actinostachys pennula* (Sw.) Hook. and *A. subtrijuga* (Mart.) C. Presl. (Schizaeaceae). Palynology 42, 483–491.
Ramos Giacosa, J.P., Morbelli, M.A., Giudice, G.E., 2009. Spore morphology and wall ultrastructure of *Blechnum* L. species from North West Argentina. Rev. Palaeobot. Palynol. 156, 185–197.
Rowley, J.R., Nilsson, S., 1972. Structural stabilization for electron microscopy of pollen from herbarium specimens. Grana 12, 23–30.
Thiers, B., 2019. Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium http://sweetgum.nybg.org/science/ih/.
Tryon, A.F., Lugardon, B., 1991. Spores of Pteridophyta. Springer- Verlag, New York.
Tryon, R.M., Tryon, A.F., 1982. Fern and Allied Plants with Special Reference to Tropical America. Springer Verlag, Berlin, Heidelberg, New York.