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Palynological analysis of the genus *Dryopteris* Adans. (Dryopteridaceae) in Argentina

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Abstract: The spore morphology and wall ultrastructure of *Dryopteris filix-mas*, *D. patula* and *D. wallichiana* from Argentina were studied using light microscope, scanning and transmission electron microscope. The study was carried out with herbarium material from Argentine institutions. Equatorial diameters, polar diameters and laesura length were measured. The spores are monoletes with a rugate ornamentation. The folds are short to long, inflate, irregular in shape and size, and varying from subglobose to elongate. The perispore surface is rugulate. The exospore of all the species analyzed is two-layered in section. Simple and branched channels are also present. The perispore is composed of two layers, the inner one forms the ornamentation and the outer covers all the outer and inner surfaces. Some abnormalities, such as globose, triangular or twisted spores were observed. The morphology and ultrastructure of the species are very similar. The differences observed are related to the length and thickness of the perispore folds. The characteristics of these spores would not provide relevant information to differentiate species or sections within the genus, but can provide information for phylogenetic studies as well as for alterations in the biological cycles.

Key words: Argentina, *Dryopteris*, ornamentation, spore, ultrastructure.

INTRODUCTION

The Dryopteridaceae family is one of the most diverse among leptosporangiate ferns, with 26 genera and an estimated 2115 species of cosmopolitan distribution (PPG I 2016). The genus *Dryopteris* Adans. is one of the most complex within the family and one of the richest in species, with about 225-300 species throughout the world except Antarctica (Geiger & Ranker 2005, Sessa et al. 2012b). About 160 of them are found in Southeast Asia, which seems to be their center of diversity (Sessa et al. 2012b), about 15 species are distributed in Central and South America (Prado et al. 2014) and 3 species are cited by Ponce & Arana (2016): *D. filix-mas* (L.) Schott, *D. patula* (Sw.) Underw. and *D. wallichiana* (Spreng.) Hyl., for continental Argentina.

In tropical America, they usually grows in humid mountain forests, cloud forests and lowland rain forests. They grow in areas ranging from sea level to 4000 m, preferably within the 1000 - 2500 m (Tryon & Tryon 1982, Narváez et al. 2008).

Classifications within the genus have been established on the basis of morphology (Sessa et al. 2012b): Ito (1935, 1936) has treated the species of Japan and Taiwan whereas Ching (1938) has done so with those of China, the Himalayas, India and Sri Lanka. Fraser-Jenkins (1986) has made the most accepted classification which includes 225 species, subdivided in 4 subgenera and 16 sections, several *incertae sedis* and about 90 hybrids. Within the last classification,

the 3 species growing in Argentina belong to the subgenus *Dryopteris* although they are located in different sections: *D. filix-mas* correspond to Sect. Dryopteris, *D. patula* to Sect. Cinnamomeae and *D. wallichiana* to Sect Fibrillosae.

The geographic distribution of *D. filix-mas* corresponds to North America, Europe, Asia, Madagascar and it was introduced in South America. In Argentina, it was cultivated and naturalized in the Patagonian region (Sessa et al. 2012a, Ponce & Arana 2016). *D. patula* grows in Central and South America. In Argentina it is found in the Northwestern region (Prado et al. 2014). *D. wallichiana* is a species that grows in the Himalayas, Africa, India, China, Japan, Malaysia and America, along the Andes, from Mexico to the northwest of Argentina (Tryon & Lugardon 1991, Narváez et al. 2008, Ponce & Martínez 2012, Prado et al. 2014, Ponce & Arana 2016).

The *Dryopteris* genus presents one of the most difficult fern complexes in America due to its tendency to hybridize (Walker 1959, Whittier & Wagner 1971, Hoshizaki & Wilson 1999) thus creating problems in the definition of species in the group (Barrington et al. 1989).

In addition to its tendency to hybridize, the family often has apogamic species (Nayar & Kaur 1971), including *D. wallichiana* (Loyal 1959, Fraser-Jenkins 1986, 1989, Pérez-García et al. 2001, Geiger & Ranker 2005, Narváez et al. 2008, Sessa et al. 2012b).

As well as hybridity, the ploidy level has also been studied by different authors in the following species: *D. wallichiana* has been registered as diploid, apogamic triploid and hexaploid (Tryon & Tryon 1982, Sessa et al. 2012a, 2015); *D. filix-mas* is considered a sexual allotetraploid (Manton 1950, Manton & Walker 1954, Fraser-Jenkins 1976, Lovis 1977, Xiang et al. 2006) and *D. patula* is a diploid species (Tryon and Tryon 1982, Sessa et al. 2012a).

Several authors have reported that smallest spores are diploid while the largest ones are tetraploid and hexaploid (Mehra & Loyal 1965, Kanamori 1969, Mitui 1972, Tryon & Lugardon 1991, Quintanilla & Escudero 2006). Regarding hybrids, their chromosomes tend to be irregularly distributed so the spores differ genetically among them and in size (Witthier & Wagner 1971). Thus, differences in spore sizes are related to ploidy level and hybridity (Manton 1950, Wagner 1966, Kanamori 1971, Nakato & Mitui 1979, Moran 1982, Pryer & Britton 1983, Barrington et al. 1986).

The morphology of the spores of several *Dryopteris* species has been examined in worldwide in various places such as Africa, Asia, Europe and, North and Central America (Crane 1953, 1955, 1956, 1960, Nayar & Devi 1964, Britton 1972a, b, Mitui 1972, Belling & Heusser 1974, Britton & Jermy 1974, Tryon & Tryon 1982, Tryon & Lugardon 1991, Lee & Park 2014).

Crane was the first who studied spores of the *Dryopteris* genus from North America (1953, 1955, 1956, 1960). He made a key for North American species based on the characters of the spores. Some of these characters, such as the presence or absence of spines, spore size and the size and number of folds are very valuable, since they help to easily differentiate some species from others. Britton & Jermy (1974), on the other hand, showed that the spores of *D. filix-mas* from the United States, Canada and Mexico have a background pattern of the perispore which is anastomosed or reticuled.

Only a few studies using SEM have been carried out on the spores of the genus in South America. The spores of *D. filix-mas* have only been illustrated from Argentina by de la Sota et al. (1998) and they have been characterized as greenish brown and with crestate perispore. The spores of *D. patula* have been described by Prado et al. (2014) with material from Brazil as

ellipsoidal, rugose and having long broad folds. Tryon & Lugardon (1991) have observed the spores of this species with material from Mexico as inflated tubercles with fine, superficial ridges. The spores of *D. wallichiana* from Mexico and Peru have been described by Tryon & Lugardon (1991) as long inflated folds, forming the rugate surface. Narváez et al. (2008) have mentioned the spores of this species from Argentina as dark brown and rugulate, having wide rounded usually anastomosed folds and forming short rounded reticles.

However, TEM studies of the genus have not been carried out in America yet. The spores of *D. filix-mas* from France have been studied with TEM by Tryon & Lugardon (1991). These authors have described the spores as having a plain exospore and a perispore of one cavate layer usually showing short scales below.

The spores of the species growing in Argentina have scarcely been studied with SEM and have not been studied with TEM yet. Besides, the sporoderm demands for a thorough study in order to define its ultrastructure and complexity. The aim of this work was to analyze the morphology and ultrastructure of the spores of the genus *Dryopteris* in continental Argentina with LM, SEM and TEM.

MATERIALS AND METHODS

The study was done with herbarium material from the following Argentine institutions: BA, BAB, BCRU, CTES and LP (Thiers 2016).

The spores were studied with Light Microscope (LM) and Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). After LM and SEM analysis, *D. patula* and *D. wallichiana* were selected as representative for the study with TEM.

For the analysis with LM, the material was acetolized according to the method of Erdtman (1960). For the study with SEM, the spores without treatment were placed into stubs with adhesive double-faced tape and coated with gold. For the TEM analysis, the material was treated following the technique of Rowley & Nilsson (1972): it was rehydrated with 0.1 M buffer and with 1% Alcian Blue (AB) for 2 hours; then the material was fixed with 1% glutaraldehyde + 1% AB in phosphate buffer for 12 hours; then it was washed for 15 minutes in phosphate buffer and postfixed with 1% Osmium tetraoxide in water + AB for 2 hours. The spores were dehydrated in an acetone series (30-100%) and subsequently embedded in a mixture of Spurr resin. The semi-thin sections 3 µm thick were stained with Toluidine blue and observed with LM. The ultrathin sections were stained with Uranyl acetate for 15 minutes followed by Lead citrate for 3 minutes.

The observations were made with a Zeiss EM 109T with Gatan ES1000W digital camera from the Instituto de Biología Celular, Facultad de Medicina, Universidad de Buenos Aires, a JEOL JSMT-100 SEM from the Museo de Ciencias Naturales de La Plata and a Nikon E200 from Cátedra de Morfología Vegetal, Facultad de Ciencias Naturales and Museo, Universidad Nacional de La Plata.

The following characteristics were analyzed: shape, equatorial and polar diameters, laesura, ornamentation and ultrastructure. For the description of the spores, the terms proposed by Tryon & Lugardon (1991) were used.

Material studied

D. filix-mas: Argentina, Neuquén, Los Lagos, Villa Puerto Manzano, 1975, Diem 3606 (BAB); 04/05/1973, Diem 3596 (LP); Argentina, Neuquén, Península Quetrihué, Arroyo from Laguna Hua-Huán, 2005, Puntieri, Grosfeld and Passo 547 (BCRU).

D. patula: Argentina, Salta, Orán, path of the Bermejo River to Pescado River passing by Yaculika, 27/05/1971, Legname and Cuezzo 8299 (LP); Argentina, Salta, Santa Victoria, ravine road from Baritú to Lipeo, 14/07/1999, Martínez and Ganem 153 (CTES); Argentina, Tucumán, Monteros, Los Morteritos, 20/07/1920, Schreiter 1188 (BA).

D. wallichiana: Argentina, Catamarca, Andalgalá, Laguna Grande, 02/05/1915, Jorgensen 1493 (BA); Andalgalá, Esquina Grande, 1920, Schreiter 1180 (BA); Argentina, Tucumán, Tafí Viejo, La Lagunita, 10/04/1912, Rodríguez 481 (BA); Argentina, Salta, Anta, Parque Nacional El Rey, Arroyo Los Puestos, 17/07/1979, Brown 969 (LP); Argentina, Jujuy, Ledesma, Parque Nacional Calilegua, 2016, Luna, Arana and Ganem 1905a (LP); Argentina, Jujuy, Santa Bárbara, Sierra de Santa Bárbara, 13/12/1962, de la Sota 2922 (LP).

RESULTS

Morphology

Dryopteris filix-mas: the spores are monolete, bilateral, light brown and ellipsoidal in polar view (Fig. 1b, c). In equatorial view, they are plane-concave to convex (Fig. 1a). The major equatorial diameter is 43-63 µm, the minor equatorial diameter is 29-40 µm, the polar diameter is 28-45 µm, and the laesura is of 31-42 µm long. The perispore is the ornamented wall and the sculpture is rugate. The folds are inflated and variable in shape and size, from subglobose (Fig. 1c) to elongate (Fig. 2c) in the

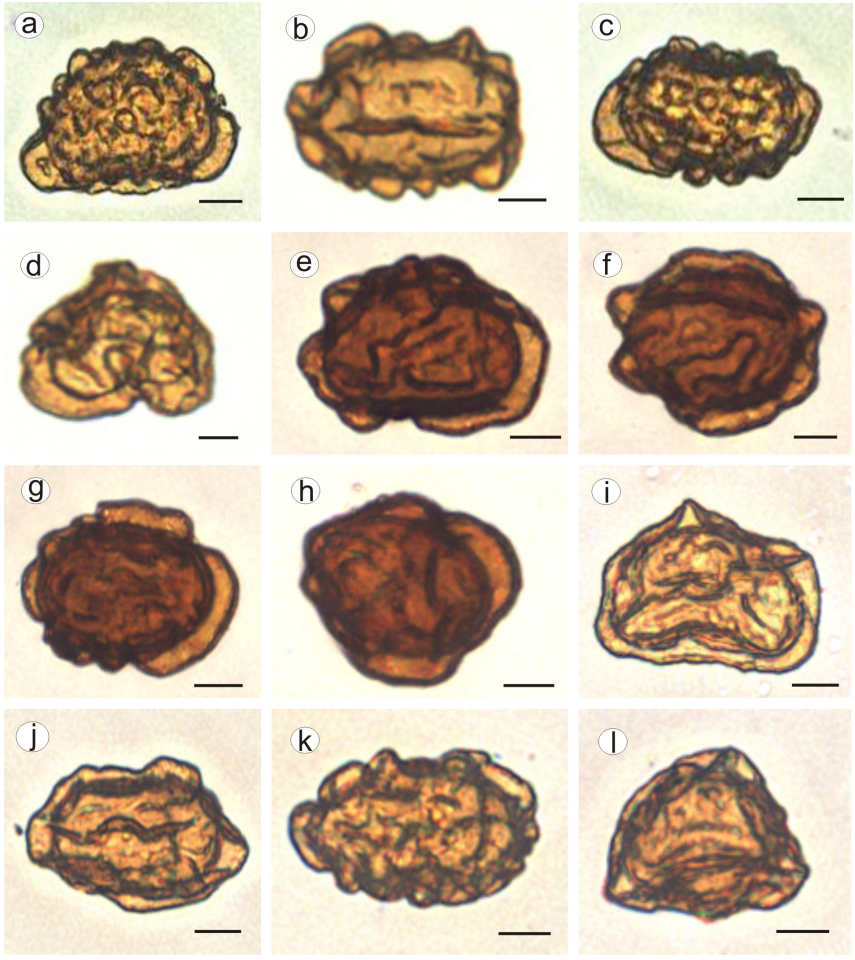

Figure 1. Spores of *Dryopteris* with LM. a-d: *D. filix-mas.* a. Spore in equatorial view. Large folds are observed at the edges. b. Spore in proximal view. c. Spore in distal view. Large folds are observed at the edges and subglobose folds in the center. d. Collapsed spore, twisted, with big folds. e-h: *D. patula.* e. Spore in equatorial view. Long folds in the edges are evident. f. Spore in proximal view with wide folds. g. Spore in distal view with coarse folds. h. Globose spore with large folds. i-l: *D. wallichiana.* i. Spore in equatorial view with long and branched folds. j. Spore in proximal view with long folds in the edges. k. Spore in distal view. Inflated, subglobose folds are observed. l. Abnormal spore with triangular shape. Scale bars: a-l = 10 µm.

distal polar view, with rounded apices 2.1-7.1 µm high. In equatorial view, large folds are observed at the edges (Figs. 1a, 2a) while in proximal polar view (SEM) these folds are seen adjacent with the laesura (Fig. 2b). The perispore surface is rugulate with small folds which are partially or totally fused forming an irregular reticulum (Fig. 2d). The perispore is sometimes seen detached or broken (Fig. 2b).

Dryopteris patula: The spores are monolete, bilateral, dark brown and ellipsoidal in polar view (Fig. 1f, g). In equatorial view, they are plane-concave to convex (Fig. 1e). The major equatorial diameter is 40-54 µm, the minor equatorial diameter is 28-40 µm, the polar diameter is 29-39 µm, and the laesura is of 17-32 µm long. The perispore is the ornamented wall. The sculpture is rugate. The folds are coarse, slightly low and long or sometimes high and short. Partially to totally fused. They are 0.9-5.1 µm high and 1.7-11.9 µm width (Figs. 1e-g, 2e-g). The perispore surface is finely rugulate (Fig. 3c) or rugulate-foveolate (Fig. 3a, b) with usually small rounded depressions. Often, this surface looks broken (Fig. 2h). A few spheroids are observed on the perispore surface (Fig. 3a).

Dryopteris wallichiana: The spores are monolete, bilateral, light brown and ellipsoidal in polar view (Fig. 1j, k). In equatorial view, they are plane-concave to convex-hemispheric (Fig.

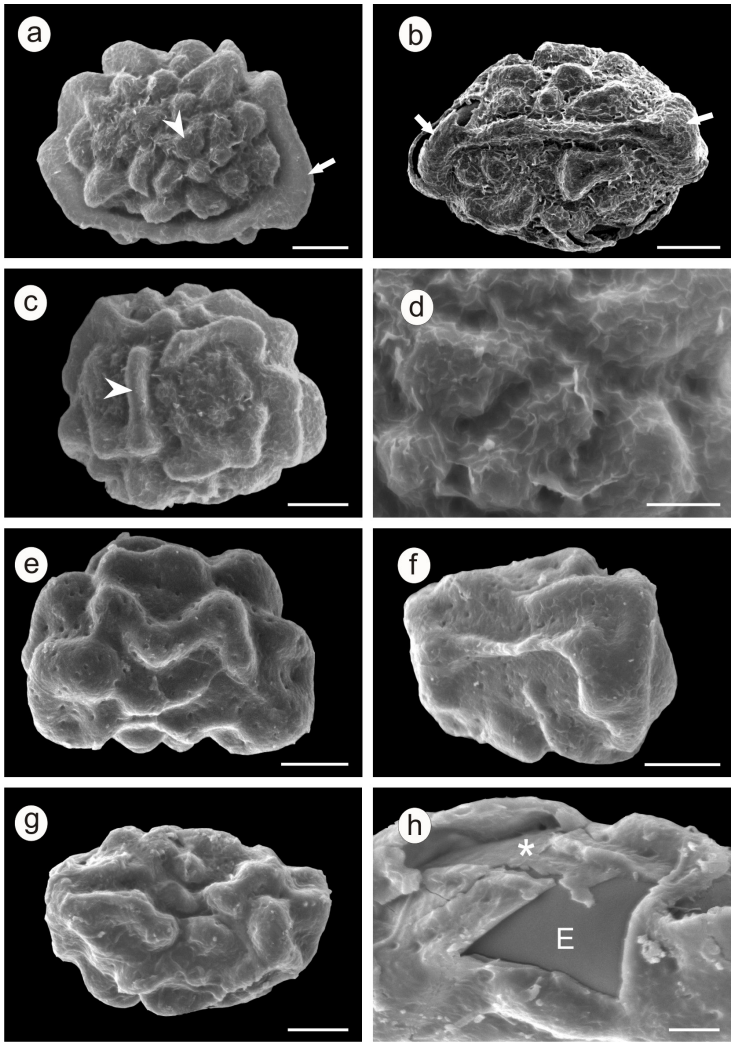

Figure 2. Spores of *Dryopteris* with SEM. a-d: *D. filix-mas*: a. Spore in equatorial view with inflated, subglobose folds at the center (arrowhead) and large folds at the edges (arrow). b. Spore in proximal view. Large folds at the edge are associated to the laesura (arrows). c. Spore in distal view with elongated folds (arrowhead). d. Rugulate perispore surface. e-h: *D. patula* e. Spore in equatorial view. Coarse, elongated folds are observed. f. Spore in proximal view. g. Spore in distal view. Coarse folds are observed. h. Sporoderm fracture. Smooth exospore (E) below, base of the fold (asterisk) above. Scale bars: a-c, e-g = 10 µm; d, h = 5 µm.

1i). The major equatorial diameter is 39-57 µm, the minor equatorial diameter is 27-37 µm, the polar diameter is 28-39 µm, and the laesura is of 22-36 µm long. The perispore is the ornamented wall. The sculpture is rugate. The folds are lax to compact, short and subglobose in proximal polar view (Fig. 3e), and they are elongated, linear, sinuous or branched in Y-shape in distal polar view and equatorial view (Fig. 3d, 3f). The folds are 3.1-5.4 µm high and 1.9-5.6 µm wide. They are partially or totally fused forming reticulum (Figs. 1i-k, 3d-f). The perispore surface is rugulate (Fig. 3g), with very small folds fused partially or totally forming a complete and incomplete reticulum. The perispore is sometimes seen detached or broken (Fig. 3h).

Observations: some abnormalities such as aborted, immature, globose, triangular, twisted or collapsed spores were observed in some specimens of *D. filix-mas* (Fig. 1d), in a very few specimens of *D. patula* (Fig. 1h) and in most specimens of *D. wallichiana* (Fig. 1l). What these abnormalities have in common is that they show larger folds than the typical ones.

Ultrastructure

D. patula: the exospore is 0.36-0.64 µm thick and composed of two layers, the inner one which is 0.03-0.15 µm thick is more contrasted than

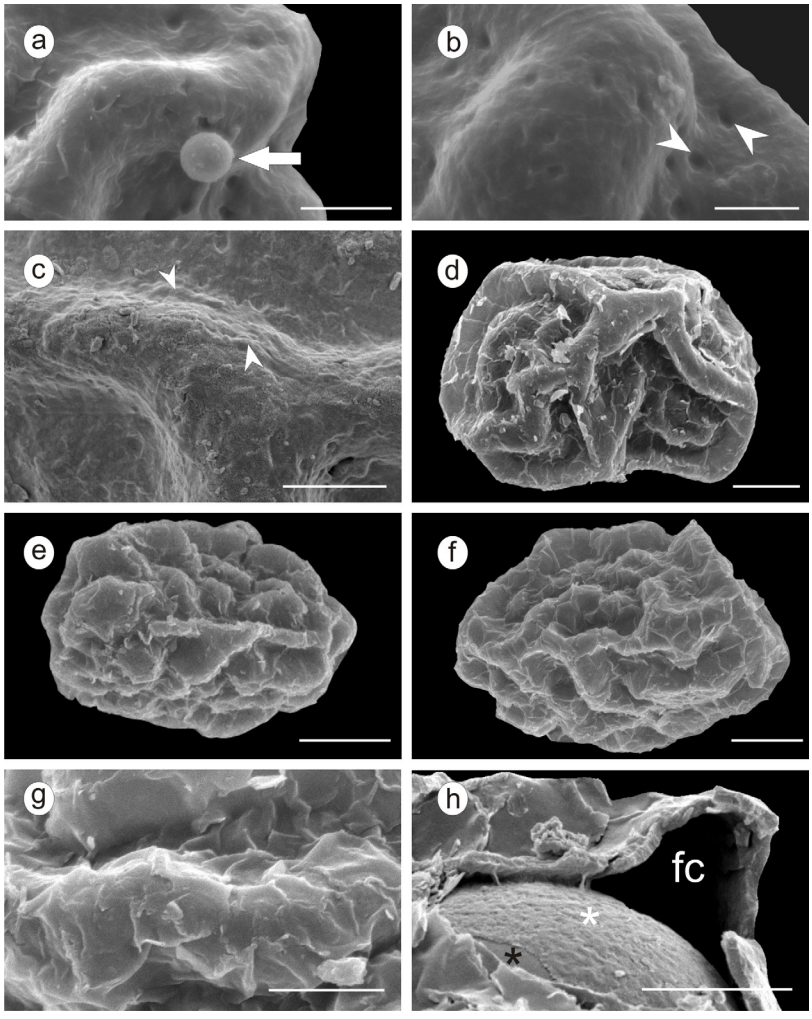

Figure 3. Spores of *Dryopteris* with SEM. a-c: *D. patula* a. Spheroid at the perispore surface is observed (arrow). b. Foveolate perispore surface. Small depressions (arrowheads) are seen. c. Rugulae (arrowhead) are seen in the perispore surface. A coarse fold branched is observed. d-h: *D. wallichiana* d. Spore in equatorial view with elongated folds forming irregular reticulum. e. Spore in proximal view. Inflated, subglobose folds are seen. f. Spore in distal view. Subglobose, inflated, elongated folds are observed. g. Rugulate perispore surface. h. Sporoderm fracture. The base of fold (white asterisk) covers the smooth exospore (black asterisk). The fold cavity (fc) is observed. Scale bars: a-c, g-h = 5 µm; d-f =10 µm.

the outer which is 0.25-0.52 µm thick (Fig. 4b). It widens at the base of the laesura. Simple and branched channels were observed in the outer (Fig. 4d) and inner exospore (Fig. 4e).

The perispore is 0.07-1.04 µm thick and is composed of two layers (Fig. 4a-d). The inner layer 0.05-1 µm thick is more contrasted than the exospore. The outer layer, 0.01-0.2 µm thick, is less contrasted than the inner one and it covers the folds on their outside and inside (Fig. 4a-d).

The laesura usually has an associated supralaesural fold (Fig. 4c).

D. wallichiana: The exospore is 0.79-1.32 µm thick and it is composed of two layers: the inner, 0.06-0.51 µm thick, is more contrasted that the outer one which is 0.42-1.26 µm thick (Fig. 5a, b). It widens at the base of the laesura (Fig. 5b). Simple and branched channels were observed in the inner exospore (Fig. 5d).

The perispore is 0.28-2.22 µm thick and it is composed of two layers. The inner layer is 0.25-2.2 µm thick and it is more contrasted than the exospore (Fig. 5c). The outer layer is 0.02-0.25 µm thick, it is less contrasted than the inner one (Fig. 5b, 5e) and it covers the folds on the outside as well as the inside (Fig. 5c). Several small portions of membranes (scales) are on the perispore (Fig. 5f). Spheroids with similar contrast to the perispore can also be observed on the perispore surface (Fig. 5e).

The laesura usually has an associated supralaesural fold (Fig. 5b).

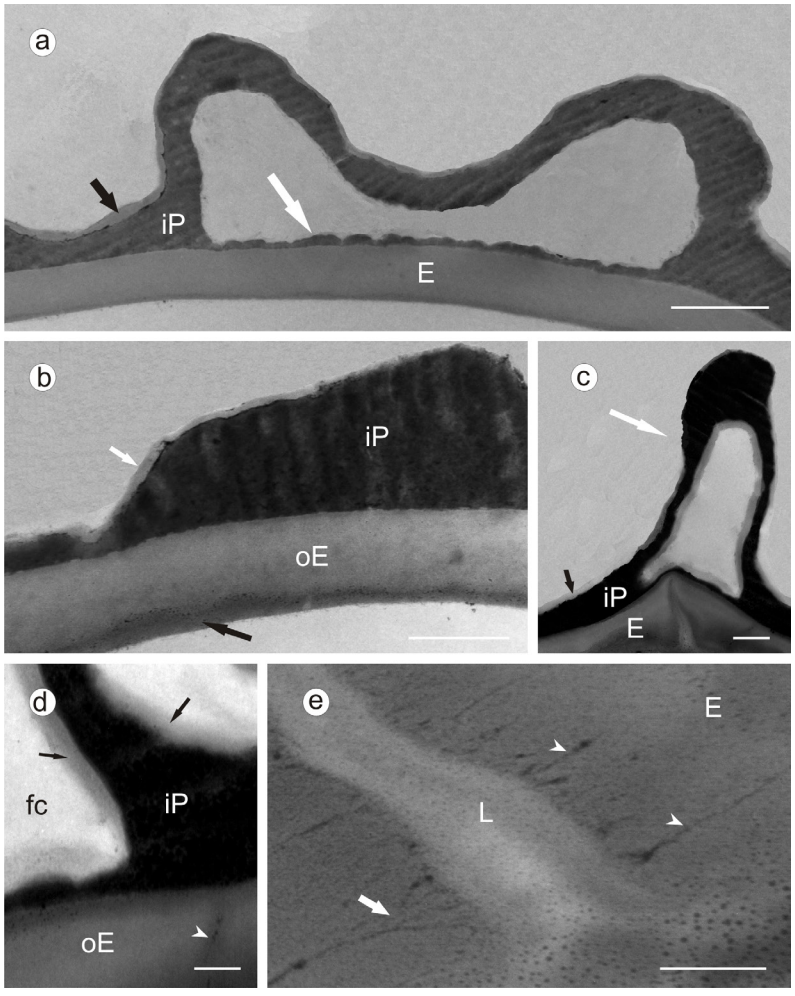

Figure 4. Spores of *Dryopteris patula* with TEM. a. The folded perispore is observed. The white arrow shows the base of the fold and the black arrow shows the outer perispore. E: exospore. iP: perispore. b. Wall stratification. The outer exospore (oE) is less contrasted than the inner exospore (white arrow). The outer perispore (black arrow) is less contrasted that the inner perispore (iP) and covers it. c. Section through the laesura. The exospore (E) is less contrasted than the inner perispore (iP). The black arrow shows the outer perispore. A supralaesural fold (white arrow) is observed. d. The sporoderm is seen in section. The outer perispore (arrows) covers the inner perispore (iP) both outside the fold as fold cavity (fc). Simple channel (arrowhead) in the outer exospore (oE) is observed. e. Section through the laesura (L). Simple (arrowheads) and branched (arrow) channels are observed in the inner exospore (E). Scale bars: a = 1 µm; b, c = 0.5 µm; d, e = 0.2 µm.

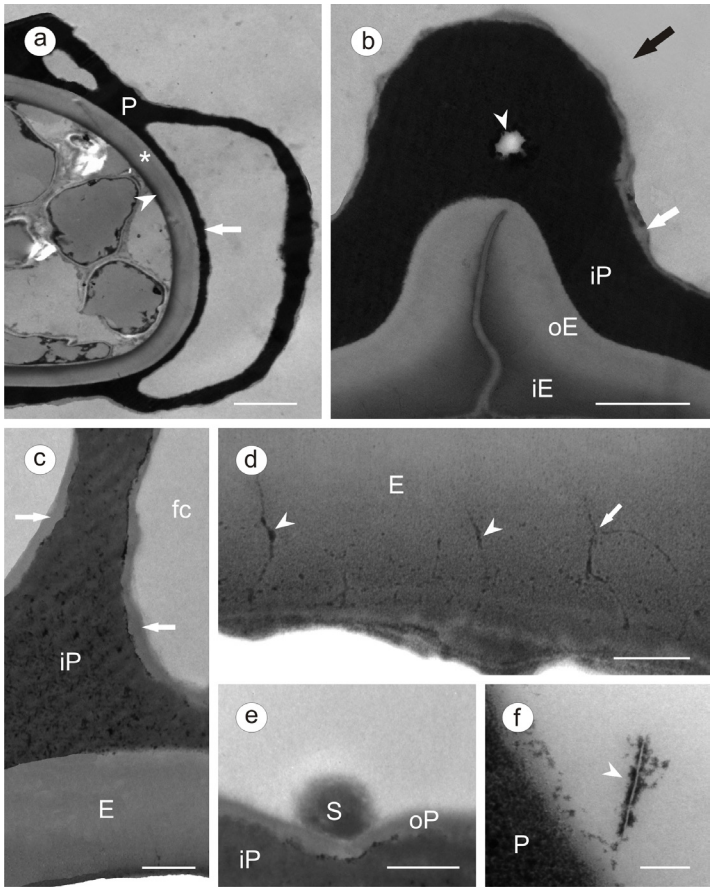

Figure 5. Spores of *Dryopteris wallichiana* with TEM. a. The folded perispore is observed. The arrowhead shows the base of the fold. The asterisk shows outer exospore and the arrow shows inner exospore. P: perispore. b. The laesura is seen in section. The outer exospore (oE) is less contrasted than the inner exospore (iE). The inner perispore (iP) is more contrasted than the outer perispore (black arrow). A supralaesural fold (white arrow) with fold cavity (arrowhead) is observed. c. Section through the sporoderm. The outer perispore (arrows) covers the inner perispore (iP) both outside the fold as fold cavity (fc). E: exospore. d. Simple (arrowheads) and branched (arrow) channels is observed in the inner exospore (E). e. A spheroid (S) is seen in the perispore surface. It has contrast and structure as the perispore. iP: inner perispore. oP: outer perispore. f. A scale (arrowhead) is seen on the perispore. P: perispore. Scale bars: a = 3 µm; b, e = 1 µm; c, d, f = 0.2 µm.

DISCUSSION

According to Crane (1960), the spores of *D. filix-mas* are easily distinguishable from other North American species by their large size, few, small, rounded and scattered folds and lack of spinules. However, the same characters do not serve to distinguish *D. filix-mas* from others species analyzed here, since they have very similar characters, regarding the size of the spore and shape of the folds.

The spores of *D. patula* analyzed by Hernández-Hernández et al. (2009) from Mexico differ from the specimens studied in this work by about 20 µm less in equatorial major diameter. Prado et al. (2014) have described the spores of this species with material from Brazil as ellipsoidal, rugose and with long broad folds. Tryon & Lugardon (1991) observed the spores of this species with material from Mexico and described them as inflated tubercles with fine, superficial ridges. However, in our opinion the ornamentation of this species is rugate not tuberculated since, according to our analysis with TEM, the ornamentation is formed by hollow folds and not by tubercles, a projection that we considered having an inner solid structure.

Narváez et al. (2008) analyzed the spores of *D. wallichiana* from Northwest Argentina with SEM and described them as monolete, dark brown, rugate, with wide and rounded usually anastomosed folds forming a short rounded reticulum, with similar characteristics to those studied in this work.

The perispore surface of *D. patula* analyzed here, is finely rugulate or rugulate-foveolate while for *D. wallichiana* is only finely rugulated with very small folds forming complete and

incomplete reticulum, unlike Tryon & Tryon (1982) which only mentions a finely rugulose pattern for both species.

Lee & Park (2014) who analyzed the spore morphology of *Dryopteris* from Korea, described three perispore types (rugate, echinate and spinose) and three surface types (reticulate, granular and smooth) in contrast with spores analyzed in this work, with only one perispore type (rugate) and two surface types (rugulate and foveolate). Therefore, none of the authors who have previously studied the genus have observed a foveolated surface as we have done so in *D. patula* spores.

When comparing the spores of the species studied, it is evident that it is difficult to differentiate them at first sight. With LM, the color is the only significant characteristic which differentiates *D. patula* from the other species. Regarding the ultrastructure, species studied in this work are very similar in their stratification and structure.

Thus, in our opinion, the most appropriate way to evidence their different characteristics is to analyze the spores with SEM, since their main differences lie in the size of the folds and the perispore surface. In *D. filix-mas*, large folds are observed at the edges and these folds are seen adjacent with the laesura. The folds of *D. patula* are slightly low, long and finely rugulate or rugulate foveolate while those of *D. wallichiana* are short, subglobose, branched with a perispore surface rugulate with very small folds fused partially or totally forming a complete and incomplete reticulum.

When Tryon & Lugardon (1991) studied the spores of *Dryopteris,* they mentioned that in mature spores, the surface is often partially fragmented. Likewise, we frequently observed broken folds in the spores analyzed with SEM. This condition may be due to the thickness of the perispore wall and the fact that the folds are hollow inside not offering enough resistance to the compression suffered in the SEM methodology.

Tryon & Lugardon (1991) analyzed with TEM the spores of *D. affinis* (Lowe) Fraser-Jenk., *D. carthusiana* (Vill.) H. P. Fucks, *D. filix-mas* and *D. sabaei* (Franch. & Sav.) C. Chr. from France and determined that the perispore is composed of one cavate layer, while Tryon & Tryon (1982) analyzed with SEM the spores of *D. cinnamomea* (Cav.) C. Chr. from Mexico and determined that the perispore is formed of two strata, a slightly rugose inner part and the inflated outer perispore layer. However, the ultrastructural studies which were carried out for this work, allowed us to determinate that the perispore is composed of two layers. The inner part described by these authors corresponds to the outer and inner perispore layers at the base of the fold. The perispore outermost layer covers all the outer and inner surfaces.

Spore abnormalities are frequents in ferns. They were reported in genera like *Ceratopteris* (Hickok & Klekowski 1973), *Gymnocarpium* (Pryer & Britton 1983), *Thelypteris* (Nakato et al. 2012) and *Anemia* (Ramos Giacosa 2014).

Crane (1953) was among the first authors who studied the hybrids in *Dryopteris* and he observed that hybrids are easily recognized for their particular production of spores. Wagner & Chen (1965) proposed that the largest abnormalities of spore morphology of sterile hybrids in *Dryopteris* are: 1) size (large size, with large numbers of small and unusual spores present); 2) shape (some spores are not typically kidney-lined, there are also spherical, twisted, square or triangular ones); 3) color (some spores are not transparent enough to show the exospore, they are too dark). Taking into account the abnormalities mentioned by Wagner & Chen (1965), we found that some specimens of *D. filix-mas*, very few specimens of *D. patula* and

most specimens of *D. wallichiana* have spores with different sizes (some very large and others very small), with irregular shapes (some are triangular or twisted and others globose) or collapsed. Additionally, larger folds than the typical ones were observed.

With all evidence previously mentioned regarding the tendency to hybridize, apogamic species and the ploidy level recorded in the genus, it could be possible that some of these issues are occurring in some of the studied species which would be causing the abnormalities observed in this work. Nevertheless, would be important carry out cytological studies on these species to try to explain their difference in size and to verify the hybridity and/or polyploidy in this region.

CONCLUSIONS

The spore morphology and ultrastructure of the species are very similar. The exospore is smooth and the folded perispore forms the ornamented wall. The ornamentation similarities among the spores of the genus *Dryopteris* in continental Argentina are evident, even when the folds are very variable in size and length. Differences observed are regarding the length and thickness of the perispore folds, hence, the shape of the folds vary from short and small to elongated and coarse. Although these characteristics are mainly evidenced with SEM, with LM the outstanding feature is *D. patula* dark brown color.

The exospore as well as the perispore are two-layered. The inner layer is more contrasted both in the exospore and the perispore. The inner perispore is the layer that forms the ornamentation and the outer covers all the outer and inner surfaces.

Although there are no significant differences in size, the greatest differences are established between *D. filix-mas* and *D. patula*. These differences are given in the major equatorial diameter and the length of the laesura, where the spores of *D. filix-mas* are 10 µm larger in both characteristics; but more specimens must be analyzed to check the differences.

The characteristics of these spores would not provide relevant information to differentiate species or sections within the genus, however, they would do so to analyze phylogenetic relationships with other groups as well as to detect alterations in the biological cycles.

The morphology of these taxa is highly variable within each species and may be due to the frequent occurrence of apogamy, hybridity and/or polyploidy. Therefore, cytogenetic studies are required for elucidate this intraspecific variability.

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