

## PALYNOLOGICAL STUDY IN ASPLENIACEAE FROM NORTH-WEST ARGENTINA

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**Summary:** The spores of *Asplenium* L. species from North- West Argentina were analysed under light and scanning electron microscopes. The spores are monolete and 40-50  $\mu\text{m}$  in diameter; the exospore is 1.2- 2.5  $\mu\text{m}$  thick, one-layered in section and smooth. The perispore is 1 to 10  $\mu\text{m}$  thick, seemingly one layered with three-strata in section and camerate. The elements of the sculpture are echinulae, cristae, rugulae and ridges. Correlation between spore size and ploidy levels was found, since species referred as tetraploids and hexaploids produce larger spores. On the basis of the combination of characters of the outer perispore stratum, such as folds (type and distribution), degree of perforation, and sculpture (type, location and combination), the species present in the area of study were categorised into two main groups: winged and ridged. Species can be determined by their spore ornamentation. Most species fall into the winged, scarcely perforate type, with varied ornamentation between folds. No congruence was found between spore characteristics and vegetative characteristics, with the exception of *Asplenium serra*, in which both characteristics make its determination possible.

**Key words:** *Asplenium*, spores, perispore, exospore, Argentina.

**Resumen:** Estudio palinológico en las Aspleniaceae del Noroeste de Argentina. Se analizaron con microscopio óptico y electrónico de barrido las esporas de las especies de *Asplenium* L. que crecen en el Noroeste de Argentina. Las esporas son monoletes, de 40-50  $\mu\text{m}$  de diámetro, con exosporio de 1.2- 2.5  $\mu\text{m}$  de espesor, de una capa en sección y liso. El perisporio, de 1 a 10  $\mu\text{m}$  de espesor, posee aparentemente una capa con tres estratos en sección y es camerado. Los elementos de la escultura son espinulas, crestas, rúgulas y lomos. Se ha observado correlación entre el tamaño de las esporas y el nivel de ploidía, ya que especies citadas como tetraploides y hexaploides poseen las esporas de mayor tamaño. En base a la combinación de caracteres del estrato externo del perisporio, tales como: pliegues (tipo y distribución), grado de perforación y escultura (tipo, ubicación y combinación), las especies presentes en el área de estudio pueden ser reunidas en dos grupos: aladas y lomadas. La mayoría de las especies se encuentran dentro del tipo alado con escasas perforaciones y ornamentación variada ubicada entre pliegues. No se ha observado congruencia entre los caracteres de las esporas y los vegetativos, con excepción de *Asplenium serra*, en la que la combinación de ambos caracteres permite su determinación.

**Palabras clave:** *Asplenium*, esporas, perisporio, exosporio, Argentina.

### INTRODUCTION

The spores of the Aspleniaceae are analysed as part of the project Palynological Flora of the Pteridophyta of North- West Argentina. This family is represented by *Asplenium* L.: *A. achalense* Hieron., *A. argentinum* Hieron., *A. auritum* Sw., *A. depauperatum* Fée, *A. formosum* Willd., *A. gilliesii* Hook, *A. laetum* Sw., *A. lilloanum* de la Sota, *A. lorentzii* Hieron., *A. monanthes* L., *A. palmeri* Willd., *A. praemorsum* Sw., *A. pumilum* Sw., *A. resiliens* Kunze, *A. serra* Langsd., *A. triphyllum* Presl and *A. tucumanense* Hieron. (Sota, 1977; Ponce, 1996).

Species of sections *Asplenium* and *Sphenopteris* from North-West Argentina, have free venation and are differentiated by their blades texture, sorus location and rachis indument (Morton & Lellinger, 1966; Sota, 1977). The spore characteristics were not mentioned in these studies.

According to Murakami & Moran (1993), *Asplenium laetum* Sw. (section *Hymenasplenium*) is widely distributed and the only represented of that section which reaches the North- West of Argentina. The authors characterised the spore of section *Hymenasplenium* as echinate, papilose or cristate as in *A. laetum*.

The spore morphology of *Asplenium* was quoted in floristic contributions like those of Tryon & Tryon (1982) and Murakami & Moran (1993) and, in studies where morphology and ultrastructure were studied in detail, such as those of Nayar & Devi (1964) and

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Nayar *et al.* (1964), Viane & Van Cotthem (1977), Puttock & Quinn (1980), Perez Raya *et al.* (1986), Pangua & Prada (1988), Prada *et al.* (1989), Braggins & Large (1990), Tryon (1990), Tryon & Lugardon (1991) and Johns (2000).

With respect to the species that grow in the area of study, *A. formosum*, *A. praemorsum*, *A. auritum*, *A. monanthes* and *A. serra* were illustrated and described by Prada *et al.* (1989) based on material from Colombia and Perú, those of *A. monanthes*, *A. resiliens*, *A. serra* and *A. auritum* were illustrated with SEM photographs in Tryon & Tryon (1982) and Tryon & Lugardon (1991) and, those of *A. laetum* and *A. pumilum* in Tryon & Lugardon (1991).

Michelena (1993) studied the species of *Asplenium* that grow in Buenos Aires Province and Morbelli (1980) studied those of Patagonia.

In the works of Puttock & Quinn (1980) and Braggins & Large (1990), the perispore features were analysed and taken into account in order to establish the spore types in species of Australia and New Zealand.

Tryon (1990) found a correlation between the spore ornamentation and the ecology in species of *Asplenium*. She found that epiphytic species have more complexity in the spore sculpture than those of terrestrial and epilithic species.

In Love *et al.* (1977) and Tryon & Tryon (1982) references to cytological levels in *Asplenium* were found. It was observed that polyploidy is frequent in species that grow in America as well as in Europe. This characteristic was related with an increase in spore size (Perez Raya *et al.*, 1986; Tryon & Tryon, *l.c.*; Tryon, 1990).

Our aim in this study is to analyse the spores of the seventeen species present in North-West Argentina under light microscopy and scanning electron microscopy in order to provide more information about the species that form part of Flora projects in our country. With that information we will try to find out the characteristics shared by all the species as well as those that could be useful for systematic purposes.

## MATERIAL AND METHODS

The work was based on herbarium material from the following institutions: BA, CT, LP, LIL and SI. The spores were studied using light microscope (LM) and scanning electron microscopy (SEM). For LM, the material was acetolyzed according to Erdtman

(1960) after treatment with hot 3% sodium carbonate for 2 minutes. For SEM, the material was treated with hot 3% sodium carbonate, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates and coated with gold. In order to study the ultrastructure using the SEM wall fractures were made with ultrasound.

The observations were made with a JEOL JSMT-100 scanning electron microscope at Museo de Ciencias Naturales de La Plata.

The spores measures were estimated on 25 spores in each sample quoted in Material and Methods, and given in  $\mu\text{m}$ . The figures correspond to minimum, average (in brackets) and maximum values respectively. The values of the main diameters include the perispore thickness.

The terms and concepts proposed by Viane & Van Cotthem (1977), Puttock & Quinn (1980) and Tryon & Lugardon (1991) were used to describe the perispore features and structure.

### *Specimens Investigated*

The letters **MP** in this list and the figure legends indicate the reference number of each palynological sample which is filed at in the Laboratorio de Palinología, Facultad de Ciencias Naturales and Museo de La Plata.

#### *Asplenium achalense*

*Prov. Tucumán: Dpto. Monteros, Qda. Pueblo Viejo, De la Sota 4060, I-1965(LP), MP 3733. Prov. Salta: Dpto. La Caldera, Alto La Sierra, camino de cornisa, alt. 1400 m., Palací 1036 (MCNS), MP 3612.*

#### *Asplenium argentinum*

*Prov. Jujuy: Dpto. Capital, C° Labrado, De la Sota 4368, 11-III-1966 (LP); MP 3741.; Idem, Yala.*

*Sotelo s/n, 9-VI-1948 (LP); MP 3751., Dpto. Ledesma, camino a Valle Grande; Cabrera & Fabris 22620, 13-V-1972 (LP); MP 3752. Prov. Salta: Dpto. Capital, Loc. Qda. San Lorenzo, alt. 1600 m.s.m., (MCNS 167); MP 3611.; Dpto. Capital, Loc. Qda. de San Lorenzo, alt. 1600 m.s.m., MCNS, Nro. 168; MP 3610.; Sta. Victoria, 1400 m.s.m., Marmol *et al.*, 9205c, 21-IX-1972; MP 3743.; Dpto. Sta. Victoria, camino a Baritú, 1530 m.s.m. Marmol, Cuezco & Cuezco (h), 18-IX-1972 (LP); MP 3754; Prov. Tucumán: Dpto. Monteros, Qda. Pueblo Viejo, De la Sota 4075, I-1965 (LP); MP 3742; Dpto. Taquí del Valle, Yerba Buena, Venturi 184, 15-1919 (LP); MP 3753.*

*Asplenium auritum*

Prov. Jujuy: Dpto. Capital, El Cucho, De la Sota 4311, 9-III-1966 (LP); **MP 3744**; Dpto. Ledesma, Valle Grande, Cabrera & Kiesling 20319, 11-XII-1969 (LP). Prov. Salta: Dpto. Orán, Loc. Aguas Blancas, finca El Arrazayal, alt. 850 m, MCNS Nro. 91; **MP 3613**; Dpto. La Caldera, Loc. camino de cornisa, Alto La Sierra, 1400 m, MCNS Nro. 1037; **MP 3614**; *Idem*, Loc. Ruta 9, camino de cornisa a Jujuy, 8km arriba de Santa Laura, alt. 1500 m.s.m., Novara, MCNS Nro. 3276; **MP 3615**; Prov. Tucumán: Dpto. Tañi del Valle, Yerba Buena, Venturi 201, 21-II-1919 (LP); **MP 3745**; Dpto. Monteros, Eda. Pueblo Viejo, De la Sota 4073, I-1965 (LP)

*Asplenium depauperatum*

Prov. Catamarca: Dpto. Belén, El Rodeo, Castillón 2018, 15-I-1911 (LIL); **MP 3765**; Prov. Salta: Dpto. Capital, Loc. Cerro San José (Río Wierna), alt. 1300 m.s.m., MCNS 604; **MP 3616**; Prov. Salta: Dpto. Rosario de la Frontera, Candelaria, Venturi 3837, 14-IV-1925 (LIL); **MP 3767**.

*Asplenium formosum*

Prov. Salta: Dpto. Orán, Río Pescado, Abra Arasayal, Vervoorst & Cuezco 7599c, 26-X-1970; **MP 3762**; *Idem*, Puesto Rivero, Hiuck 127, V-1950 (LIL), **MP 3769**; *idem*, Río Pescado, Vervoorst & Cuezco 7808c, 25-X-1970 (LIL), **MP 3770**. Prov. Tucumán: Quebrada de Lules, Castillón 11668, VIII-1911 (LIL); **MP 3761**.

*Asplenium gilliesii*

Prov. Jujuy: Dpto. Valle Grande, C° Hermoso, Agarde 477, 21-I-95 (LIL), **MP 3766**. Prov. Salta: Dpto. Cachi, Loc. Valle Encantado, alt. 3000 m.s.m., MCNS Nro. 966; **MP 3617**. Prov. Tucumán: Dpto. Tañi del Valle, Castillón 644, 26-XII-1907 (LIL), **MP 3764**.

*Asplenium laetum*

Prov. Salta: Dpto. Orán, Río Pescado, Abra Arasayal, Vervoorst & Cuezco 7598c, 26-X-1970 (LIL), **MP 3763**.

*Asplenium lilloanum*

Prov. Salta: Dpto. Anta, P.N. El Rey, Brown 865 (1), 9-VII-1979; **MP 3783**

*Asplenium lorentzii*

Prov. Catamarca: Dpto. Belén, Potrero Ambato, Qda. del Nacimiento, LIL 41534; **MP 3621**. Prov. Jujuy: Dpto. Capital, Loc. Lagunas de Yala, LIL 187938, 10-01-47; **MP 3620**. Salta: Dpto. Capital, Loc. Qda. de San Lorenzo, alt. 1500 m.s.m., MCNS Nro. 130, 11-03-94; **MP 3626**; Qda. de San Lorenzo, alt. 1500-2000 m.s.m., MCNS Nro. 165, 25-08-85; **MP 3627**; Dpto. Rosario de Lerma, Campo Quijano, alt. 1550 m.s.m., MCNS Nro. 330, 1-04-96; **MP 3625**.

*Asplenium monanthes*

Jujuy: Dpto. Valle Grande, serranía de Calilegua, Fabris, Crisci & Petriella 5933, 15-10/II/1965 (LP); **MP 3746**; Dpto. Capital, Lagunas de Yala, Cabrera 16365, 28-X-1964 (LP). Salta: Dpto. Capital, Qda. de San Lorenzo, alt. 1600 m.s.m., MCNS, Nro. 158; **MP 3624**; Dpto. Rosario de Lerma, Loc. 2 km pasando Corralito, camino a El Manzano, alt. 1750 m.s.m., MCNS, Nro. 3537, **MP 3623**. Tucumán: La Cueva, Rodríguez 483, 3-IV-1912 (LP); **MP 3747**.

*Asplenium palmeri*

Salta: Dpto. Anta, P.N. El Rey, Ayo. Los Puestos, Brown 525 (LP), **MP 3860**.

*Asplenium praemorsum*

Jujuy: Dpto. Capital, El Cucho, De la Sota 4335, 10-III-1966 (LP); **MP 3748**. Salta: Dpto. La Caldera, Loc. Ruta 9, camino de cornisa a Jujuy, 8 km antes de Abra Santa Laura, 1500 m.s.m.; Nro. 3278; **MP 3622**; Dpto. Anta, "El Rey", camino a Pozo Verde, Brown 437, 19-I-1979 (LP); **MP 3750**; Dpto. Orán, Aguas Blancas, Palací 102, 24-VII-85 (LP); **MP 3755**. Tucumán: Dpto. Tañi del Valle, Venturi 277, III-1919 (LP); **MP 3749**.

*Asplenium pumilum*

Tucumán: Dpto. Capital, Barranca Colorada, Venturi 817 (LP), 5-V-1920; **MP 3734 - MP 3760**; Dpto. Cruz Alta, Río Salí, Schreiter 8, 2-V-1923 (LIL); **MP 3768**.

*Asplenium resiliens*

Salta: Dpto. Anta, P.N. El Rey, Brown 985(1) (LP), 19-7-1979; **MP 3735**. Jujuy: Dpto. Valle Grande, Valle Colorado, Kiesling et al. 541 (LP), 21-I-1974; **MP 3736**; Dpto. Capital, El Cucho, C° Labrado, De la Sota 4379 (LP), 11-III-1966; **MP 3737**.

*Asplenium serra*

Jujuy: Dpto. Ledesma, camino a Abra de Cañas, 1000-1700 m.s.m., De la Sota 4410 (LP), 17-III-1966; **MP 3738**. Salta: Dpto. Orán, Aguas Blancas, 1200 m.s.m., Palací 111 (LP), 24-VIII-85; **MP 3739**; Dpto. Santa Victoria, camino de Baritú a Porengal, Cuezco, Cuezco (h) & Mármol 9277c (LP), 18-IX-1972; **MP 3740**.

*Asplenium triphyllum*

Jujuy: Dpto. Rinconada, Mina Piriquitas, I-III-1964, Schwabe 905 (LP); **MP 458**

*Asplenium tucumanense*

Jujuy: Dpto. Capital, S.S. de Jujuy, Parque Botánico, Vázquez 82 (Fac. Cs. Agrarias), 15-IV-1987; **MP 3585**; Dpto. Ledesma, camino a Valle Grande, Abra de Cañas, Cabrera et al. 25679 (LP), 31-X-1974; **MP 3581**; Dpto. Valle Grande, Vervoorst & Cuezco 7829c (LP); **MP 3582**. Salta: Dpto. Anta, P.N. El Rey,

Brown 102(1) (LP), 18-VII-1979; **MP 3584**; *Dpto. Chuquisaca*, Monte Grande, Saravia Toledo 11909 (CTES), 20-VI-1993; **MP 3583**; *Tucumán: Dpto. Famaillá*, Villa Nogués, Venturi s/n° (GH), 23-V-1922; **MP 3669**; Bajo de Anfama, Lillo 5036 (GH), 8-VI-1906; **MP 3668**.

**RESULTS**

The spores of all the taxa analysed are monolete, ellipsoidal to spheroidal in polar view and plane to concave-convex in equatorial view. The shape is only evident using the LM since it is modified by the features of the outer layer of the sporoderm (perispore).

By the comparative analysis of the polar and equatorial diameters (Table 1, Fig. 1 and Fig. 2) it is possible to differentiate three groups: **1.** Spores up to 40 µm (*A. argentinum*, *A. depauperatum* and *A. lorentzii*); **2.** Spores from 40 to 50 µm (*A. achalense*, *A. formosum*, *A. gilliesii*, *A. laetum*, *A. lilloanum*, *A. palmeri*, *A. praemorsum*, *A. pumilum*, *A. serra*, *A. triphyllum* and *A. tucumanense*). **3.** Spores with diameters higher than 50 µm (*A. auritum*, *A. monanthes* and *A. resiliens*).

The laesura is monolete, 16 - 30 µm long, straight and tenuimarginate at the exospore level. It is always marked on the perispore by a continuous fold.

The exospore is light-brown and darker than the perispore in acetolyzed material when observed with LM. It is 1.2- 2.5 µm thick, single-layered and has a smooth surface.

The perispore generally appears as one-layered with three strata in section and is camerate. The inner stratum (Pi) is thin and attached to the exospore. The middle stratum (Pm) is basically a continuous space filled with radial pillars (rodlets) that can also be single, digitate or concrescent. The pillars are more evident at LM in *Asplenium achalense*. The outer stratum (Po) is thin and generally perforate; it bears the elements of the sculpture and rises forming folds.

The perispore thickness ranges from 1 to 10 µm due to its camerate and folded conditions. Then these values vary according to the part of the spore where they are considered.

No evidence of a middle stratum with pillars was found in the perispore of *Asplenium praemorsum*. The perispore seems to be compact at this level. The typical spaces of this stratum are apparently confined to the inside of the folds in this species.

In all the spores analysed a supralaesural fold is present.

According to the terminology proposed by Puttock & Quinn (1980), the folds in the studied species are:

- 1. winged (with wings):** they are present in *A. argentinum* (Fig. 3 D-F), *A. auritum* (Fig. 3 G-I), *A. gilliesii* (Fig. 4F,H,I), *A. laetum* (Fig. 5A-C), *A. lilloanum* (Fig. 5D, E, G), *A. lorentzii* (Fig. 5 F,H), *A. monanthes* (Fig. 5 I, J), *A. palmeri* (Fig. 6 A-C), *A. pumilum* (Fig. 6 F-H), *A. resiliens* (Fig. 7 A-C), *A. serra* (Fig. 7 D-F) and *A. tucumanense* (Fig. 7 J-L);
- 2. ridged (with ridges):** they are present in *A. achalense* (Fig. 3 A-B), *A. depauperatum* (Fig. 4 A-B), *A. formosum* (Fig. 4 C-E, G), *A. praemorsum* (Fig. 6 D, E) and *A. triphyllum* (Fig. 7 G-I).

**Table 1.** Quantitative data of spores of *Asplenium* L. from North-West Argentina. Dimensions (in µm) are presented in the form: minimum (mean) maximum.

SPECIES	EQUATORIAL DIAMETER (MAJOR)	EQUATORIAL DIAMETER (MINOR)	POLAR DIAMETER	LAESURA LENGHT	EXOSPORE THICKNES	PERISPORE	
						thickness	folds
<i>A. achalense</i>	31.5 (46.5) 51.3	31.5 (39.0) 52.2	40.5 (47.9) 51.3	18.0 (30.3) 36.0	1.1 (1.5) 1.9	0.4 (0.6) 0.7	3.6 - 6.0
<i>A. argentinum</i>	23.4 (27.9) 32.6	22.5 (21.5) 26.9	24.8 (27.4) 31.9	14.2 (16.3) 22.7	0.4 (0.7) 1.0	0.3 (0.4) 0.6	3.2 - 5.2
<i>A. auritum</i>	43.2 (50.4) 58.5	31.5 (34.2) 36.9	42.3 (51.3) 58.5	26.1 (29.9) 32.4	0.5 (0.7) 1.0	0.4 (0.5) 0.6	2.0 - 5.3
<i>A. depauperatum</i>	35.4 (38.6) 41.6	27.6 (31.7) 35.4	32.5 (37.2) 41.2	17.5 (19.5) 22.5	1.2 (1.8) 2.1	0.4 (0.6) 0.7	4.0 - 8.0
<i>A. formosum</i>	37.5 (47.1) 55.0	27.5 (36.4) 46.2	40.0 (47.3) 52.5	18.7 (23.1) 34.5	1.2 (1.9) 2.5	0.4 (0.5) 0.6	2.5 - 5.0
<i>A. gilliesii</i>	43.7 (47.7) 50.6	35.0 (40.8) 44.4	43.1 (47.1) 50.6	21.9 (27.8) 31.2	1.5 (1.9) 2.5	0.4 (0.6) 0.8	3.7 - 6.2
<i>A. laetum</i>	40.0 (47.8) 51.2	32.5 (36.5) 38.7	41.2 (45.1) 50.0	25.0 (26.4) 27.5	1.8 (1.9) 2.5	0.4 (0.5) 0.6	5.0 - 7.5
<i>A. lilloanum</i>	45.0 (48.0) 54.0	36.0 (41.6) 45.0	43.2 (46.5) 47.7	18.0 (22.4) 27.0	0.9 (1.2) 1.8	0.4 (0.8) 1.3	3.6 - 7.2
<i>A. lorentzii</i>	30.8 (33.4) 36.6	27.1 (29.2) 33.3	27.9 (31.9) 35.4	15.0 (17.8) 22.1	1.2 (1.5) 1.8	0.3 (0.5) 0.7	3.7 - 6.2
<i>A. monanthes</i>	43.7 (47.1) 51.9	35.0 (41.0) 46.9	43.1 (50.1) 53.1	21.9 (30.6) 37.5	1.2 (1.5) 1.8	0.3 (0.4) 0.6	2.5 - 7.5
<i>A. palmeri</i>	36.9 (43.9) 50.4	29.7 (33.5) 36.0	40.5 (42.0) 42.3	18.0 (19.8) 22.1	0.7 (0.9) 1.5	0.3 (0.4) 0.6	3.6 - 5.4
<i>A. praemorsum</i>	42.5 (45.5) 49.7	31.5 (34.8) 38.8	43.4 (46.5) 51.5	21.6 (26.1) 31.5	1.1 (1.0) 1.5	0.6 (0.7) 1.0	1.0 - 1.5
<i>A. pumilum</i>	34.0 (45.9) 55.0	37.5 (35.8) 47.5	33.7 (42.6) 51.2	18.0 (25.9) 27.5	1.8 (2.2) 2.5	0.4 (0.5) 0.8	6.2 - 10.0
<i>A. resiliens</i>	52.5 (56.6) 60.3	40.8 (45.9) 52.9	50.4 (56.1) 60.8	26.3 (29.0) 31.6	1.8 (2.2) 2.5	0.4 (0.6) 0.8	5.0 - 10.0
<i>A. serra</i>	41.2 (44.5) 48.1	27.5 (32.1) 36.9	38.7 (44.4) 49.4	19.4 (21.4) 23.1	1.5 (1.8) 2.1	0.9 (1.5) 1.8	6.2 - 8.7
<i>A. triphyllum</i>	43.7 (50.4) 51.2	31.2 (37.7) 43.7	42.5 (47.3) 52.5	21.2 (25.3) 30.0	1.8 (2.1) 2.5	0.3 (0.5) 0.8	2.5 - 5.0
<i>A. tucumanense</i>	40.8 (47.5) 53.7	33.3 (38.9) 43.3	43.3 (48.9) 52.5	21.2 (26.9) 30.4	1.2 (1.4) 1.8	0.3 (0.6) 0.8	5.0 - 11.2

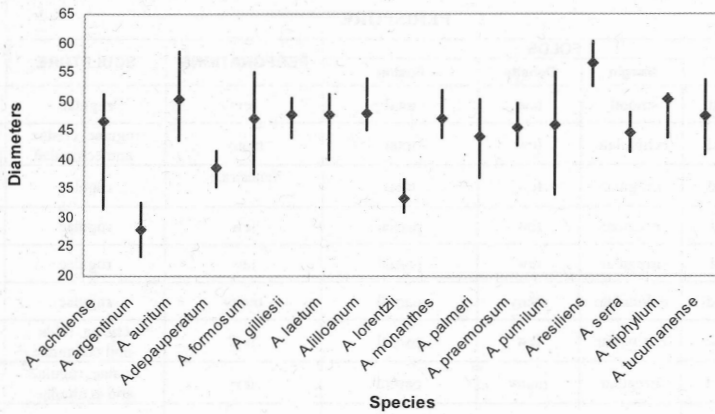


Fig. 1. Comparative mayoral equatorial diameters ( $\mu\text{m}$ ) in spores of *Asplenium* species from North - West Argentina

Variations were observed with respect to the contour in spores with ridged folds. They can be narrow like in *A. achalense*, *A. depauperatum* and *A. formosum* or broad like in *A. praemorsum* and *A. triphyllum*.

The folds can be isolated, partially or totally fused determining a pattern which is unique in each species (Table 2).

The fold margin is echinulate, irregular or smooth. Most of the studied species have echinulated folds whereas the folds in *A. depauperatum*, *A. formosum*, *A. auritum* and *A. lilloanum* are irregular. The folds with a smooth margin are present in *Asplenium achalense* (Fig. 3 A, B) and *A. praemorsum* (Fig. 6 D, E).

According to the degree of perforation of the outer stratum of the perispore (Po), three types can

be recognized: **1. Reticulate**, with large and numerous perforations resulting thus in an outer stratum totally discontinuous (*A. serra*, Fig. 7 D- F). **2. Fenestrate**, with middle-sized perforations, located between folds (*A. auritum*, Fig. 3 G-H and *A. monanthes*, Fig. 5 I, J) resulting in an outer stratum partially discontinuous. **3. Scarcely perforate** (in most of the species), with a few perforations of variable size, on the fold margin or between folds and associated with the elements of the sculpture (*A. gilliesii*, Fig. 4 I; *A. lorentzii*, Fig. 5 F).

The elements of the sculpture are echinulae, cristae, rugulae and ridges which show variation in density and location even within the same specimen. These elements can be distributed uniformly in the whole surface or restricted to certain areas such as

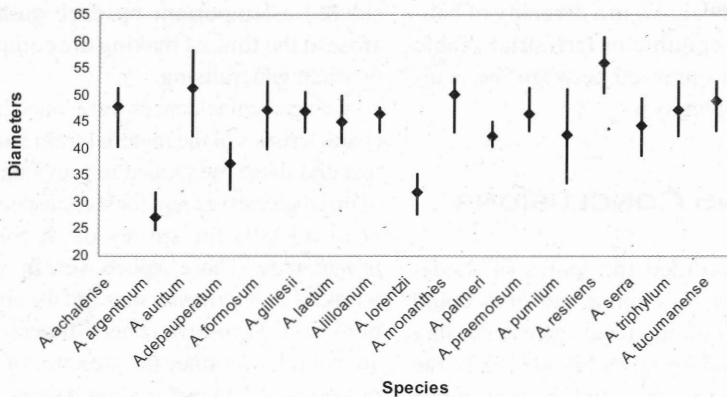


Fig. 2. Comparative polar diameters ( $\mu\text{m}$ ) in spores of *Asplenium* species from North - West Argentina.

Table 2. Character state of perispore and plant habit of *Asplenium* species from North-West Argentina

SPECIES	PERISPORE						PLANT HABIT
	FOLDS				PERFORATIONS	SCULPTURE	
	Type	Margin	Density	Fusion			
<i>A. achalense</i>	Ridged	smooth	few	total	few	rugulae	epiphyte, epilithic
<i>A. argentinum</i>	winged	echinulate	few	total	many	rugulae, cristae and echinulae	terrestrial
<i>A. auritum</i>	winged	irregular	few	total	fenestrate	rugulae	epiphyte, epilithic
<i>A. depauperatum</i>	ridged	irregular	few	partial	few	rugulae	terrestrial, epilithic
<i>A. formosum</i>	ridged	irregular	few	partial	few	rugulae	epiphyte, epilithic
<i>A. gilliesii</i>	winged	echinulate	many	partial	many	rugulae	epilithic
<i>A. laetum</i>	winged	echinulate	few	partial	few	cristae, folds and echinulae	terrestrial
<i>A. lilloanum</i>	winged	irregular	many	partial	few	cristae, rugulae and echinulae	epilithic
<i>A. lorentzii</i>	winged	echinulate	many	partial	many	rugulae	epilithic, terrestrial
<i>A. monanthes</i>	winged	echinulate	many	partial	fenestrate	Rugulae and echinulae	epilithic
<i>A. palmeri</i>	winged	irregular	few	total	few	echinulae	epilithic
<i>A. praemorsum</i>	ridged	smooth	many	total	few	rugulae	epiphyte, epilithic
<i>A. pumilum</i>	winged	echinulate	many	total	many	Echinulae and cristae	terrestrial
<i>A. resiliens</i>	winged	echinulate	many	partial	few	rugulae	epilithic
<i>A. serra</i>	winged	echinulate	few	partial	reticulate	rugulae	epiphyte, epilithic
<i>A. triphyllum</i>	ridged	echinulate	many	partial	few	Echinulae and baculae	epilithic
<i>A. tucumanense</i>	winged	echinulate	many	total	few	rugulae and echinulae	terrestrial

fold margins or between folds. In some cases the echinulae fuse at their bases or are situated on short ridges (Fig. 5 B-C; Fig. 6 G-H). The sculptural elements are clearly observed in *A. argentinum* (Fig. 3 D-E), *A. gilliesii* (Fig. 4 F, H), *A. laetum* (Fig. 5 A-C), *A. palmeri* (Fig. 6 A-C), *A. pumilum* (Fig. 6 F-H), *A. triphyllum* (Fig. 7 I) and *A. serra* (Fig. 7 D-F).

The perispore character states and the ecology of the analysed taxa are summarised in Table 2. The taxa analysed in this work grow in a diversity of habits, such as epiphytic, epilithic or terrestrial (Table 2). No correlation was observed between the ecology and perispore complexity.

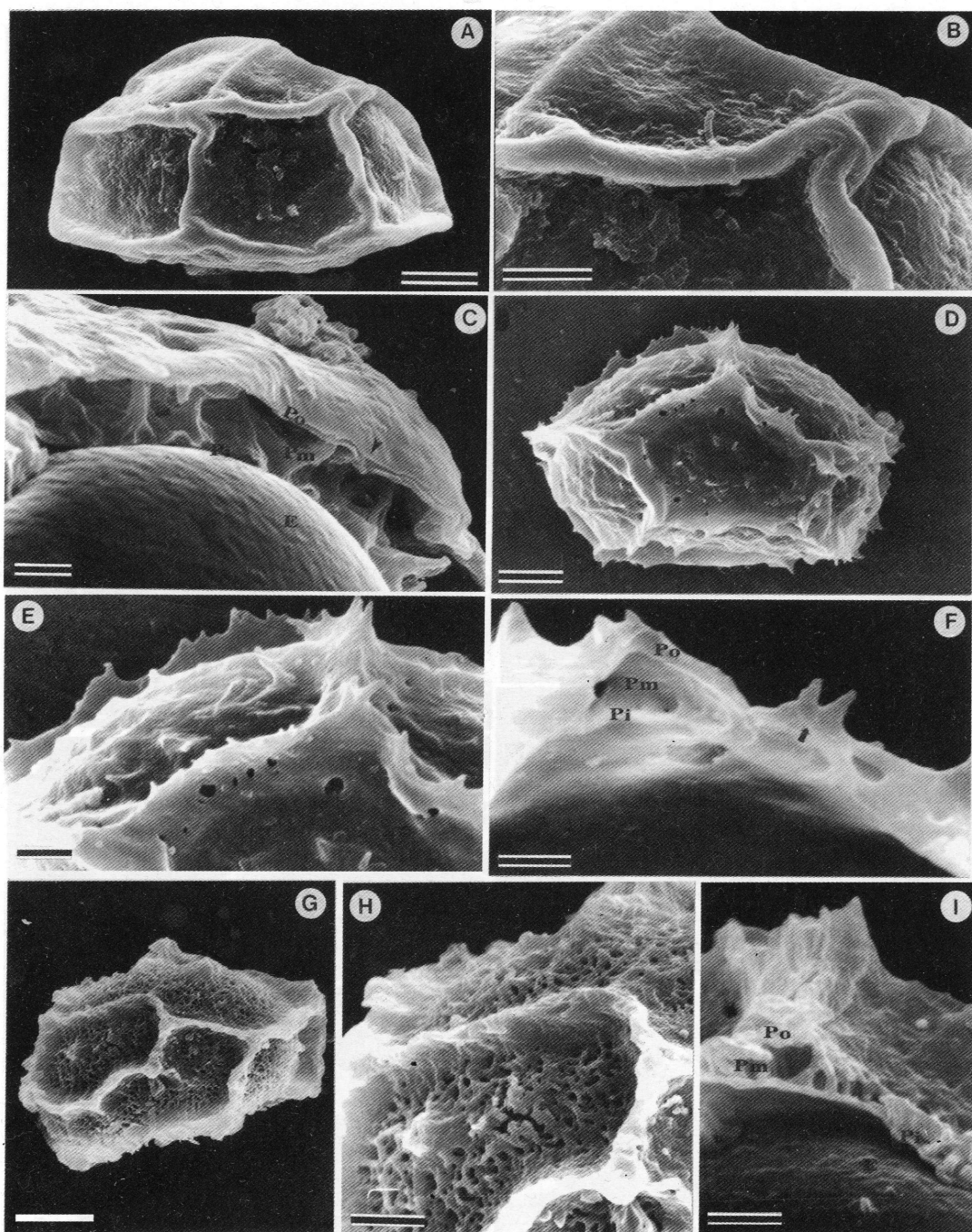
## DISCUSSION AND CONCLUSIONS

The largest size recorded for spores of *Asplenium auritum*, *A. monanthes* and *A. resiliens* could be related with a higher ploidy level since according to Löve *et al.* (1977) and Tryon & Tryon (1982), the two first species are tetraploids and the last one is an hexaploid. Spores of large size related with an increase in the ploidy level and with the epilithic habit

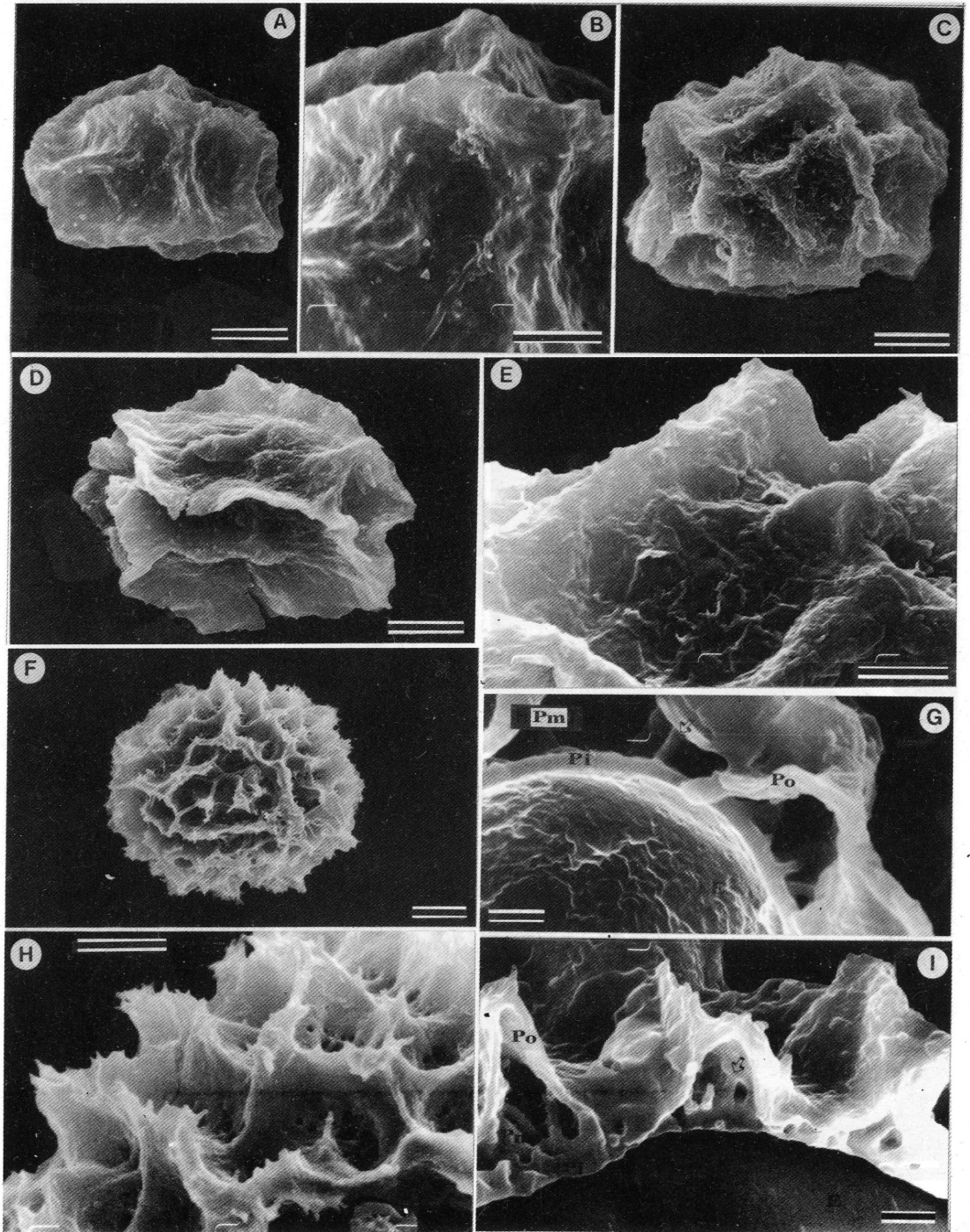
were quoted by Perez Raya *et al.* (1986) in species of *Asplenium* of Andalucía.

Palynological studies done in *Asplenium* like those of Viane & Van Cotthem (1977), Puttock & Quinn (1980), Pangua & Prada (1988), Prada *et al.* (1989) and Johns (2000), considered groups based on different characteristics always related with the spore surface. In this way the groups depended on the characteristics or groups of characteristics considered as important by each author. Difficulties arose at the time of making the comparative analysis or when generalising.

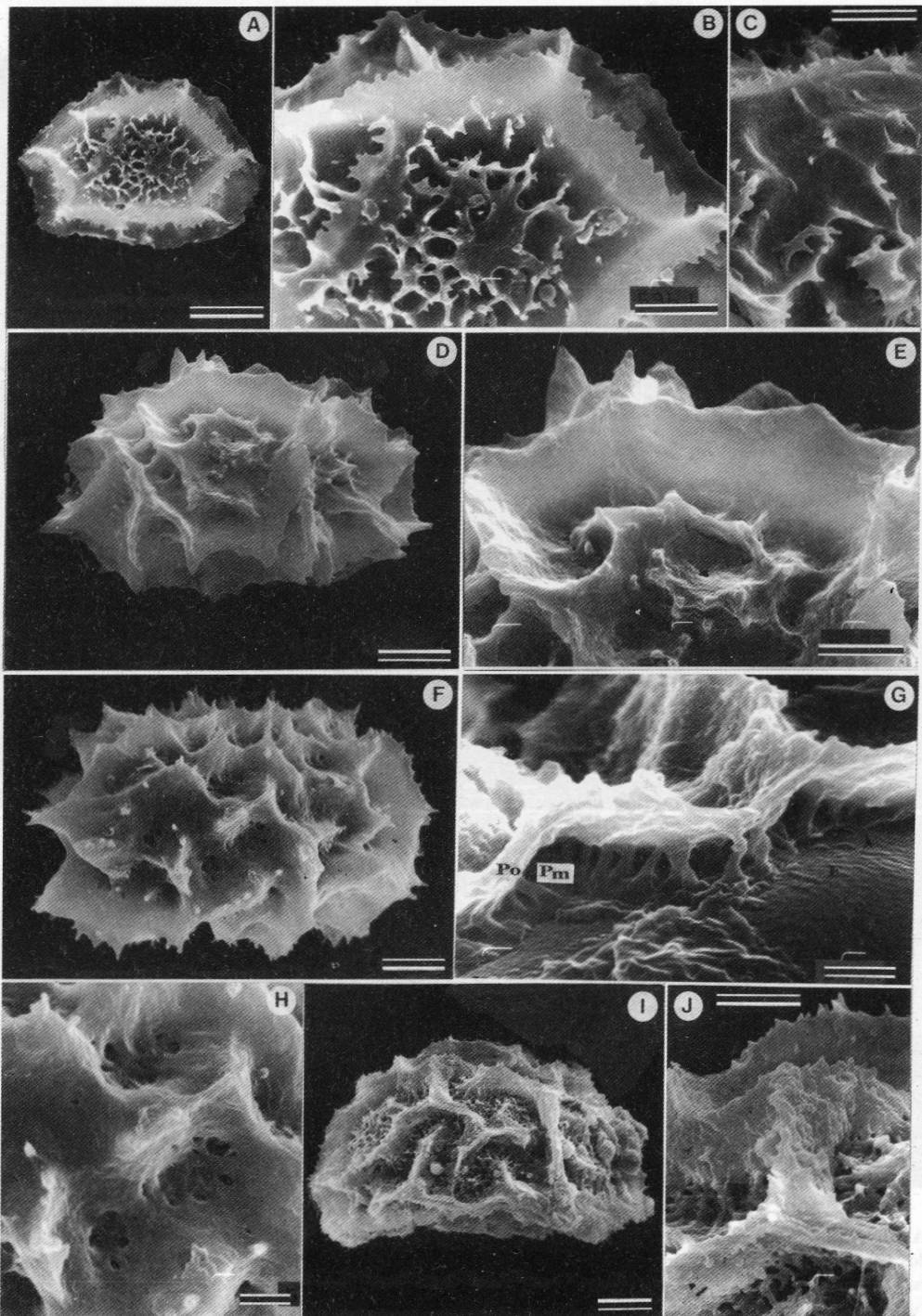
General coincidences were found between the spore characteristics of the material from North-West Argentina and those mentioned in previous studies on some of these species, except for some aspects given by Prada *et al.* (1989) for spores of *A. formosum* and *A. praemorsum*. Those aspects were the presence of small wrinkles in *A. praemorsum* and the absence of perforations in *A. formosum*. This differences in our opinion are not relevant since the presence of such characteristics do not change the main pattern of the spore and could be due, at least in part, to modifications and limitations determined by dehydration.



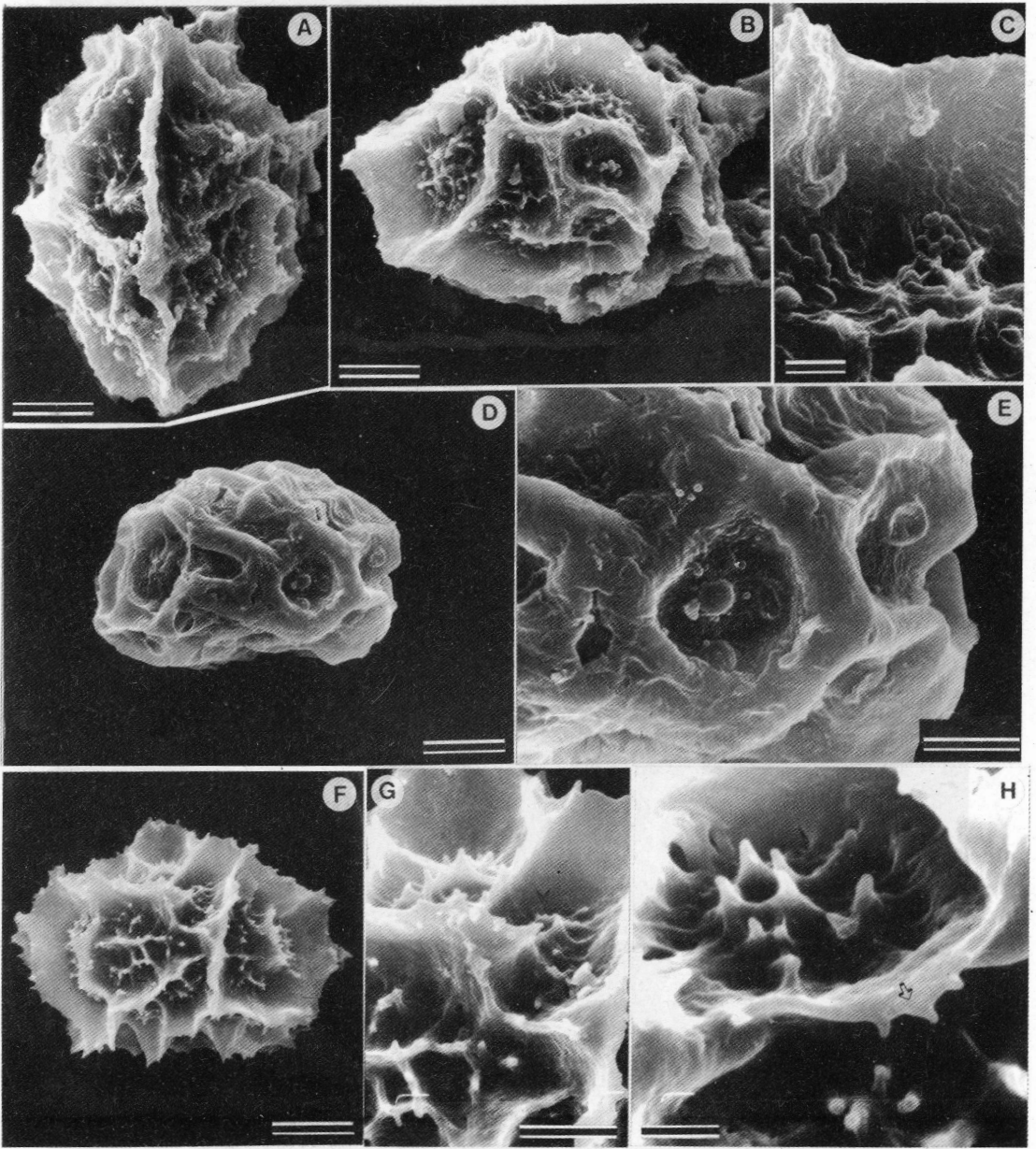
**Fig. 3.** A-C: *A. achalense* (MP 3733) A: Spore in equatorial view with fused ridged folds with a smooth margin. B: detail of the surface that shows ridged folds with a smooth margin and rugulate area between folds. C: a perispore fracture that exposes its three strata: inner stratum (Pi), middle stratum (Pm) with concentric rodlets (arrow) and outer stratum (Po) apparently composed by two substrata (arrowhead). D-F: *A. argentinum* (MP 3743), D: spore in equatorial view with a few fused winged folds with an echinulated margin determining polygonal areas, E: detail of the surface showing baculiform or spiniform processes and perforations on and between folds; margin of folds is echinulate. F: a perispore fracture that shows its three strata; the outer stratum (Po) is echinulate and two echinulae are fused at their bases (arrow); the camerae are evident in the middle stratum (Pm) and the inner stratum (Pi) is thin and continuous. G-I: *A. auritum* (MP 3613) G: a spore in equatorial view with winged folds with scarcely echinulate margins. The area between folds is fenestrate. H: detail of the surface showing folds with irregular margins and area between folds is fenestrate. I: a perispore fracture that shows its three strata; the inner stratum (Pi) is thin and continuous; the middle stratum (Pm) has radial rodlets, some of them are fused at different levels and the outer stratum (Po) is perforated between folds. There are also some cones and echinulae on the folds margins. E: exospore, P: perispore. Bars: 10  $\mu$ m.



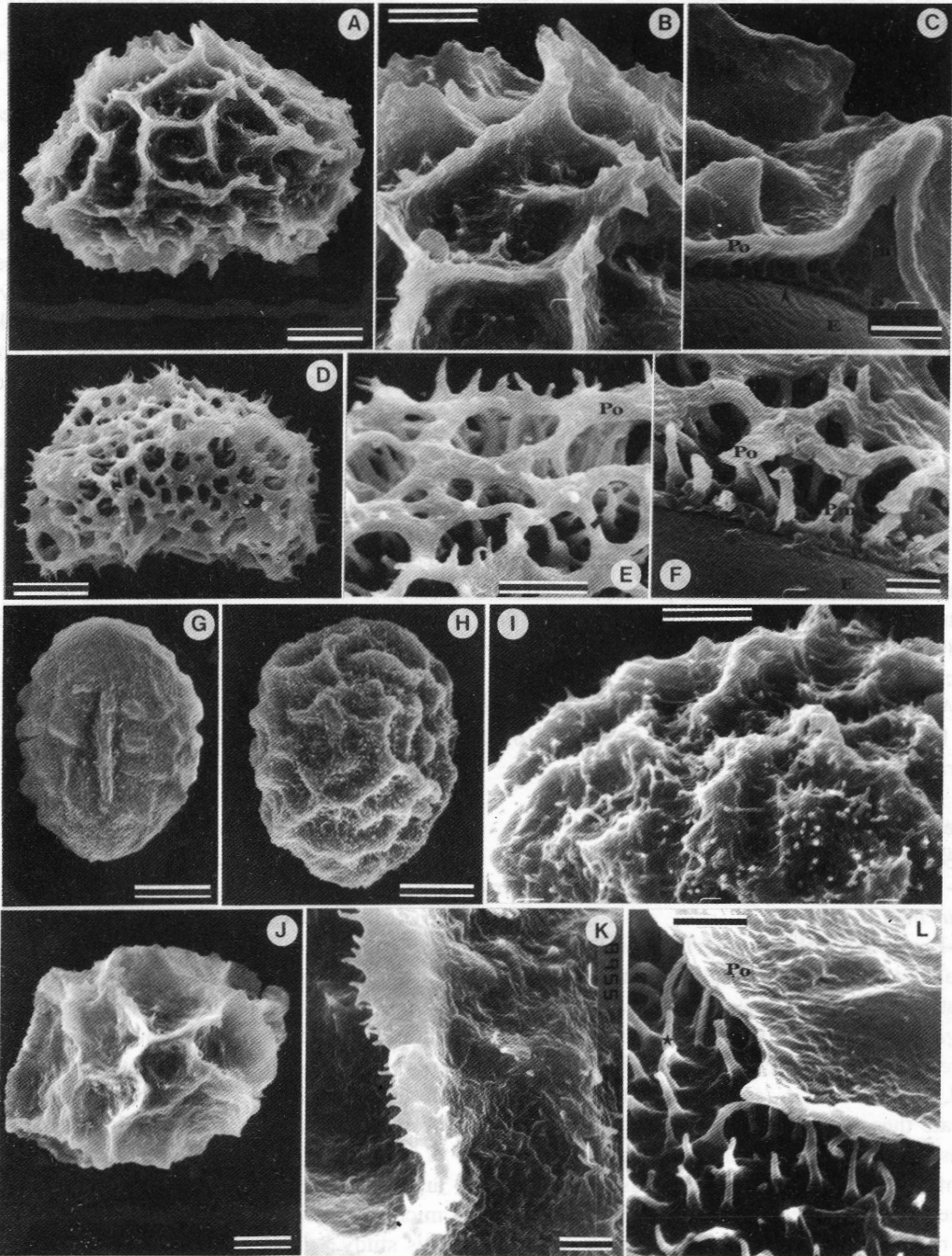
**Fig. 4. A-B:** *A. depauperatum* (MP 3616). **A:** spore in equatorial view with ridged folds with an irregular margin and partially fused. **B:** detail of the surface showing folds with an irregular margin and area between folds is rugulate. **C, D, E, G:** *A. formosum* (MP 3770) **C:** spore in equatorial view with ridged folds partially fused. **D:** spore in proximal view. An evident supralaesural fold is present. **E:** detail of the surface with folds with an irregular margin and with rugulae, few echinulae and cones. **G:** a perispore fracture that exposes its three strata: inner stratum (Pi), middle stratum (Pm) with single rodlets (arrow) and outer stratum (Po) which rises forming folds. **F, H, I:** *A. gilliesii* (MP 3764) **F:** distal view of a spore with winged folds partially fused. Perforations are also present. **H:** detail of the surface that shows folds with an echinulate margin and perforations on the whole surface. **I:** a perispore fracture that shows a perforate outer stratum (Po) which rises forming folds, a middle stratum (Pm) with single rodlets (arrow) and an inner stratum (Pi) which is thin and continuous. **E:** exospore. Bars: 10  $\mu$ m.



**Fig. 5. A-C:** *A. laetum* (MP 3763) **A:** spore in equatorial view with winged folds which are fused. The area between folds is sculptured. **B:** detail of the surface that shows folds with an echinulate margin. The folds are fused forming a reticulum. Echinulae and cristae are evident in the area between folds. **C:** detail of cristae and echinulae in the area between folds. **D, E, G:** *A. lilloanum*. (MP 3783) **D:** spore in equatorial view with winged folds, partially fused. **E:** detail of the surface showing folds with an irregular margin and surface between folds with rugulae, echinulae and cristae **G:** a perispore fracture that shows the perispore with its three strata (\*Po, Pm, Pi) and the exospore (E). Pi is detached from the exospore and is pointed by an arrow. **F, H:** *A. lorentzii* (MP 3627). **F:** distal view of a spore with abundant, winged folds with echinulate margin. **H:** detail of the surface between folds with rugulae and perforations. **I, J:** *A. monanthes* (MP 3746). **I:** equatorial view of a spore with abundant partially fused winged folds and with an area between folds which is fenestrate. **J:** detail of the surface with fold with an echinulate margin and surface between folds with rugulae and echinulae. Bars: 10 µm.



**Fig.6. A-C:** *A. palmeri* (MP 3860). **A:** Spore in proximal view with winged folds. A supralesural fold is evident. **B:** distal view. The folds have an irregular margin and are fused determining irregular areas. The surface between folds has echinulae fused at their bases. **C:** detail of the surface between folds with echinulae and rugulae. **D-E:** *A. praemorsum* (MP 3622). **D:** spore in equatorial view with ridged, fused folds. **E:** detail of the surface showing folds with a smooth margin and rugulae between folds. In some cases a verruca is evident in the centre of the areola. **F-H:** *A. pumilum* (MP 3760). **F:** spore in distal view with winged folds partially fused. **G** and **H:** detail of the surface that shows folds with echinulate margins (arrow) and area between folds with echinulae, cristae (arrowhead) and perforations. Bars: 10 µm.



**Fig. 7. A-C:** *A. resiliens* (MP 3736). **A:** spore in equatorial view with winged narrow folds. **B:** detail of the surface showing folds with an irregular margin and surface between folds. **C:** a perispore fracture at the place where a fold rises that shows its three strata. The continuous outer stratum (Po) is risen forming folds; the middle stratum (Pm) is camerate and the inner stratum (marked by an arrowhead) is thin, continuous and attached to the exospore (E). **D-F:** *A. serra* (MP 3740). **D:** spore in equatorial view with winged folds and reticulate surface. **E:** detail of the surface. The outer stratum (Po) is discontinuous, completely perforated and folded. The fold margin is echinulated. The middle stratum has evident digitate rodlets (arrow). **F:** a perispore fracture that exposes its strata: outer stratum (Po) with large perforations, middle stratum (Pm) with rodlets, baculae and echinulae. The bases of which are on the inner stratum (Pi). **E:** exospore. **G-I:** *A. triphyllum* (MP 458). **G:** spore in proximal view with ridged folds. A supralesural fold is evident. **H:** distal view with many short folds partially fused. **I:** detail of the surface with folds with echinulate margins and echinulae and baculae in the area between folds. **J-L:** *A. tucumanense* (MP 3585). **J:** spore in distal view with winged, narrow and fused folds. **K:** detail of the surface with folds with an echinulated margin and rugulae and echinulae in the area between folds. **L:** a perispore fracture that exposes its three strata: outer stratum (Po), middle stratum with rodlets (star) whose bases are on the inner stratum (Pi). Bars: 10  $\mu$ m.

There are also a few studies done on sporoderm ultrastructure with TEM and SEM on this genus (Pettitt, 1966; Lugardon, 1972, 1974; Tryon & Lugardon, 1991).

Most of the *Asplenium* species studied and observed in section with LM and SEM have a complex perispore which is characterised by having spaces with radial rodlets (pillars) in its middle part. In *A. praemorsum* the spaces are located and confined to the inside of the folds and no rodlets (or pillars) were observed neither in the middle stratum nor in the inside of folds. It means that spaces in the spores of this species are restricted to areas; however in the rest of the studied species a continuous space exists between the two strata (Pi and Po) of the perispore.

Observations made by Tigerschiöld (1981) on spores of *Asplenium trichomanes* in different stages of development indicate that the features of perispore outer stratum are formed during late steps. We consider that studies on sporoderm development and ultrastructure would provide useful data about morphology related to function and that they would allow us to search for similarities with other Filicopsids.

As a result of this analysis the studied species can be classified into two groups according to the characters states of the perispore surface:

### 1-Perispore with winged folds

-Perispore reticulate: *A. serra*

-Perispore fenestrate: *A. monanthes* and *A. auritum*

-Perispore with a few perforations: with varied ornamentation, like: echinulae, cristae and rugulae located in the area between folds: *A. argentinum*, *A. gilliesii*, *A. lilloanum*, *A. lorentzii*, *A. palmeri*, *A. resiliens* and *A. tucumanense*. It is more evident in *A. pumilum* and *A. laetum*. It is difficult to differentiate these species by their spores due to variations in ornamentation, density of the sculptural elements and characteristics of the folds margins. Nevertheless, *A. resiliens* could be distinguished from the rest by their large spores. In the same way, *A. laetum* and *A. pumilum* can be distinguished from the rest by their folds with markedly echinulated margins and the surface between folds with cristae together with fused echinulae forming a reticulum.

### 2. Perispore with ridged folds

- folds with a smooth margin: present in *A. achalense* and *A. praemorsum*

- folds with an irregular margin : present in *A. depauperatum* and *A. formosum*

- folds with an echinulate margin: present in *A. triphyllum*

Thus seven out of seventeen species studied could be determined by their spores, they are *A. achalense*, *A. auritum*, *A. monanthes*, *A. praemorsum*, *A. resiliens*, *A. serra* and *A. triphyllum*.

The groups obtained based on spore characteristics, do not match with the systematic groups based on vegetative characteristics established by De la Sota (1977), Tryon & Tryon (1982) and Murakami & Moran (1993). Nevertheless, it should be taken into account that these classifications were made without using palynological characteristics.

We would like to highlight that in the case of *A. serra* there is a congruence between exo-morphological characteristics (blade architecture, size and pinnae margins) and spore characteristics which makes these species easily determined in the area of study.

As we have previously pointed out not correlation was found between the perispore surface complexity and the ecology in the studied species, since we observed in epiphytic species slightly ornamented-perforate species (*A. achalense*) to rugulate-slightly perforate (*A. formosum*, *A. praemorsum*) to fenestrate (*A. auritum*) to the extreme case of those with completely perforate (reticulate) surface in *A. serra*. A direct correlation between the complexity of the perispore surface and the ecology was quoted by Tryon (1990) based on the analysis of 111 species of *Asplenium*.

According to the concepts given in previous paragraphs, we consider that spore characteristics should be taken into account together with other morphological features and that they could help in the determination of most taxa of *Asplenium* of North-West Argentina. In the next contribution (Morbelli *et al.*, in prep.), the sporoderm ultrastructure will be analysed under scanning and transmission electron microscopy in the structural patterns recognised in this study.

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