



ECOSYSTEMS

Asplenium sylvaticum (Aspleniaceae), a new fern species from Yungas and Paraná forests, Neotropical region

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Abstract: A new species of *Asplenium* L. (Aspleniaceae) is described and illustrated. *Asplenium sylvaticum* is endemic of the Yungas and Paraná biogeographic provinces in the Southern Cone. The main diagnostic characters for this taxon are: apical pinnae with one to four caudate lobes at the base, similar in size to lateral pinnae, smooth pinnate blades with 18-36 pairs of caudate pinnae and spores with reticulate perispore, winged folds with scarce equinulae on the margins and the laesurae wide with smooth margin. The new species belongs to the *A. serra* species complex group, characterised by xeromorphic habit, creeping rhizome and coriaceous fronds with branched scales on both surfaces, and resembles the other species inhabiting in Argentina, *A. achalense* and *A. serra*, in overall leaf architecture and shape but differs of the aforementioned species (and the other species of the *A. serra* complex) by the unique combination of characters of its rhizomatic scales, fronds and spores. A key with the morphological and palynological characters that differentiate *A. sylvaticum* from the other taxa belonging to the *A. serra* complex in the South American Cone and Bolivia is given.

Key words: Argentina, *Asplenium*, Bolivia, Brazil, ferns, Polypodiopsida.

INTRODUCTION

The asplenioid ferns, including the genus *Asplenium* L. and its putative segregates, constitute one of the most species-rich groups among leptosporangiate ferns, mainly tropical in distribution (Schneider et al. 2004, Smith et al. 2006, Xu et al. 2020).

Asplenium is a genus with about 700 species worldwide (Kramer & Viane 1990, Bellefroid et al. 2010, PPG I 2016). Although widely accepted as monophyletic, the subdivision of the genus *Asplenium* is complicated due to the scarcity of suitable distinguishing characters at the intrageneric level. This genus is characterized by usually having erect rhizomes with clathrate scales and dictyostelic or radial steles, leaves that are simple to 4-pinnate, laminae with

non-articulate petioles and distinctly sulcate rachises with a raised ridge in the centre and two grooves on each side, rachis-costa architecture characterized by a terete and alate rachis with wings that are confluent with the basiscopic pinna margin, elongate sori with a narrow indusium borne on a vein, and a base chromosome number $x = 36$ (Murakami & Moran 1993, Cheng & Murakami 1998, Lin & Viane 2013, Sundue & Rothfels 2014, Xu et al. 2020).

In Argentina, *Asplenium* constitutes the most species-rich fern genus, with 38 taxa assigned to the native flora distributed mainly in the Neotropical area of the country and few species in the Andean region (Ganem et al. 2016, Arana et al. 2020). Among the Neotropical species, those surrounding *A. serra* complex,

mainly characterised by xeromorphic habit, creeping rhizome and coriaceous fronds with branched scales on both surfaces, as delimited by Tryon & Tryon (1982), Tryon & Stolze (1993) and Moran (1995) are complicated taxonomically due to the high variability in characters such as rhizome scale colour and size; pinna size, number and margin, and degree of pinna dissection (Kessler & Smith 2006). Two species within the *A. serra* complex have been cited for the flora of Argentina: *A. achalense* and *A. serra* (Ganem et al. 2016, Arana et al. 2022).

As part of a project dealing with the revision of the lycophytes and ferns Flora of Argentina, we are updating and clarifying the nomenclature, taxonomy and distribution of the taxa, including Aspleniaceae (Ganem et al. 2016, Arana et al. 2022). Through the exhaustive analysis of herbarium specimens and living specimens observed in field trips, a taxon belonging to the *A. serra* complex with different characteristics from those mentioned above was detected.

The aim of this work was to describe a new species of *Asplenium* endemics of Yungas and Paraná biogeographic provinces, previously identified as *A. achalense* Hieron. in Bolivia and with *A. serra* Langsd. & Fisch. in Argentina and Brazil.

MATERIALS AND METHODS

Fresh and herbarium samples were used to compare the characteristics of the new species with those included in the *Asplenium serra* complex growing in Argentina, Brazil and Bolivia. For this, specimens from B, CTES, JUA, LE, LIL, LP, MA, MNHN, MO, RB, RCVC and SI herbaria were consulted, including types. Also, a bibliographical analysis of the protologues of all species belonging to the *A. serra* species complex from Argentina, Bolivia and Brazil was carried out. To morphological observations,

rhizome and leaf scales were mounted in 20% glycerin and examined under a Nikon SMZ1000 stereoscopic microscope and a Nikon E200 light microscope (LM) (D'Ambroggio de Argüeso 1986). The spores were analysed under both, LM and scanning electron microscope (SEM). For LM, the spores were mounted in 20% glycerin without prior treatment. For SEM, the spores were mounted without pre-treatment and covered with gold for 10 minutes. Observations were made in a microscope FEI Quanta 200 model (Servicio de Microscopía Electrónica de Barrido y Microanálisis del LIMF, Facultad de Ingeniería, Universidad Nacional de La Plata) with a 15 kV voltage. The spore characteristics analysed were: size, colour, shape in polar and equatorial view, laesura type and wall ornamentation. The quantitative data refers to the major and minor equatorial diameters, polar diameter and laesura length. The measures of spores were randomly estimated on 60 spores taken from the type and paratypes of the new species. Terminology follows Tryon & Lugardon (1991) and Lellinger (2002).

The lectotype of *A. serra*, selected by Ganem et al. (2016): Brazil, Santa Catarina, *G. H. von Langsdorff* s. n., LE 0000008!, and available at Virtual herbarium of Komarov Botanical Institute RAS — <http://re.herbariumle.ru/00000008>, as well as the isolectotype (Brazil, Santa Catarina, *G. H. von Langsdorff* s. n., B -W 19880 -01 0!, available at <https://herbarium.bgbm.org/object/BW19880010>), and also other four original specimens of *A. serra* at LE (available at <https://en.herbariumle.ru/?t=occ&s=Asplenium%20serra&f=%5Ball%5D>) consist only in upper blade fragments and therefore the rhizome characters are missing. Thus, for the present study, we analysed the high-quality digital images of the types and other specimens from south eastern Brazil, collected in the same area from which comes the type material. We also observed living

plants of *A. serra* inhabiting Northeast Argentina (Misiones) and North-western Argentina (Jujuy).

RESULTS

Taxonomic treatment

Asplenium sylvaticum Arana, Luna & Ganem, *sp. nov.* TYPE: Argentina, Jujuy province: Department Libertador General San Martín, Calilegua National Park, Aguada del Tigre, 23° 41' 03" S, 64° 53' 40" W, 1630 m a.s.l., 24 may 2011, A. Ganem, L. Luna & M. Arana 286 (Holotype JUA!, Isotype RCVC!) (Figures 1a-d, 2a,b, 3a-m).

Diagnosis

Asplenium sylvaticum is recognised and characterized by the rhizome scales margins with

conspicuous, capitate glandular trichomes (vs. rhizome scales margin without such trichomes in *A. achalense* and *A. serra*). The pinnate fronds with pinnae margin slightly serrate and long caudate apex; apical pinnae conform, similar in size to the lateral ones, with one to four caudate lobes at the base (vs. erect or patent fronds, laminae pinnate, pinnae margin serrate, apex obtuse to acute, apical pinnae entire and half length of the lateral ones in *A. serra*, and patent to deflexed fronds, laminae pinnate, pinnae margin strongly biserrate, apical pinna pinnatifid in *A. achalense*). The spores of *A. sylvaticum* are $48 \times 45 \mu\text{m}$ in equatorial diameter, with reticulated perispore and winged folds with equinulated margins and large and numerous perforations, laesura wide with smooth margin



Figure 1. Habitats and habit of *Asplenium sylvaticum*. a) Paraná biogeographic province at Iguazú National Park; b) Yungas biogeographic province at Calilegua National Park; c) living plant of *A. sylvaticum* growing in Yungas; d) detail of the frond apical and lateral pinnae.

(vs. winged folds with equinate margin, laesura thin with equinaes in *A. serra*, and spores $39 \times 36 \mu\text{m}$, perispore folded- ridged, fused ridged folds with a smooth margin and scarcely perforated in *A. achalense*).

Terrestrial or growing in profound rock crevices; *rhizome* creeping, 5-12 mm in diameter; *rhizome scales* lanceolate to linear-lanceolate, clathrate, 2-3.5 mm long, bright dark brown to blackish, auriculate at the bases, margin with 1-2 capitate glandular trichomes at the margins; apices long attenuate to filiform, minutely remotely denticulate; *fronds* arching, up to 160 cm long; *petioles* 13,5-50 cm ca. 1/3 of the laminae length, dull brown, sulcate adaxially.

Petiole bases scaly, scales similar to those of the rhizome but without glandular trichomes at the margins, glabrescent distally, sparsely scaly, the scales linear to subulate; *blades*: one-pinnate, strongly reduced at the bases, herbaceous, lanceolate to lanceolate-acuminate, widest at the middle, up to 16 cm width, the apices acuminate, conform, non-proliferous, the bases truncate. Rachises adaxially grooved, with scattered brown scales 0.7-1.2 mm long., subulate to linear lanceolate with filiform apex and several processes at the base; *pinnae*: 18-36 pairs, subalternate, short-petiollulate (1-3 mm), pinnae midveins dark brown coloured to the costal bases or green; herbaceous,

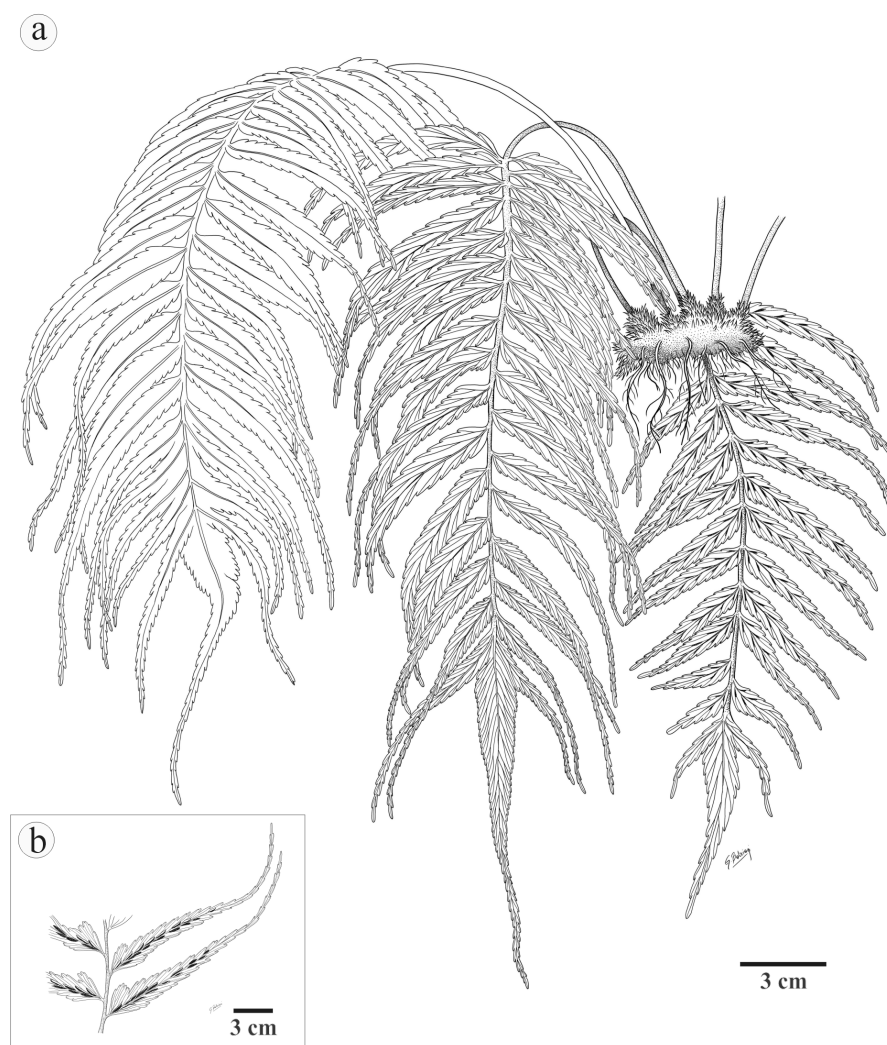


Figure 2. *Asplenium sylvaticum*. a) plant habit; b) detail of lateral pinnae with slightly serrate margins, venation, caudate apices and sori position.

almost glabrous on both sides, abaxially with scattered hyaline filiform, tortuous scales, with several processes at the base on the veins and costae; pinnae lanceolate to linear lanceolate, falcate, ascendent, caudate, the bases cuneate

to truncate, apices long-acuminate to almost linear, up to 123 mm x 15 mm, the basal ones sometimes obsolete, margin finely and regularly serrulate, veins 1-2 forked, each secondary vein ending in a marginal teeth without hydathodes;

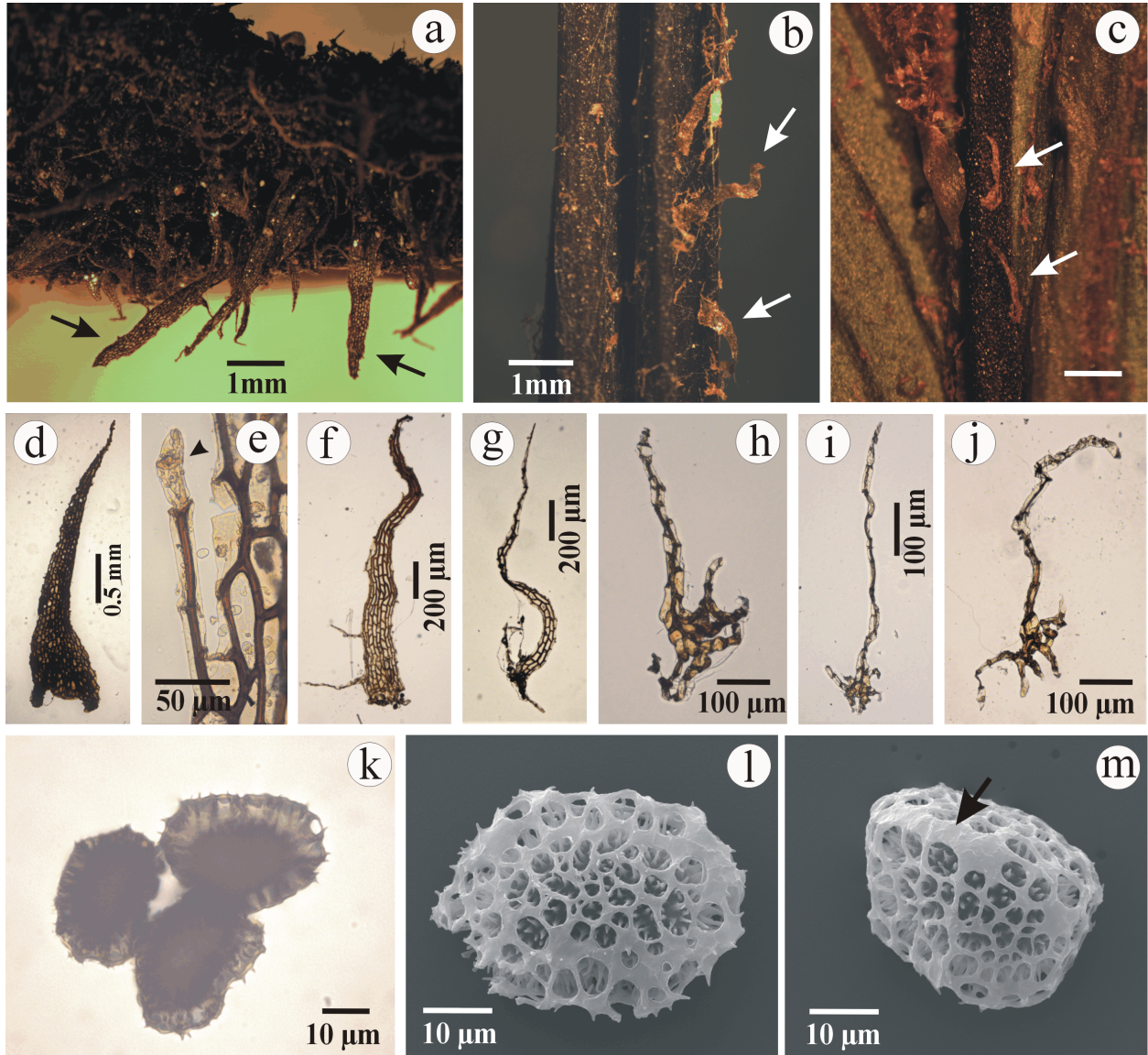


Figure 3. Indument and spores of *Asplenium sylvaticum*. a) rhizome scales (arrows); b) leaf rachis scales (arrows); c) scales on pinna midvein (arrows); d) lanceolate rhizome scale auriculate at the base; e) detail of a marginal glandular hair in a rhizomatic scale, with an apical secretory cell (arrowhead); f) scale from basal rachis, with some processes towards the base; g) scale from apical part of the rachis, narrowly lanceolate with a filiform apex and processes at the base; h) scale from the laminar tissue between veins, linear lanceolate with filiform apex and several processes at the base; i) scale from pinna costae, filiform with processes at the base; j) scale from lateral veins, filiform, tortuous, with several processes at the base; k) LM micrograph of spores; l) SEM micrograph of a spore showing perispore reticulated, folded winged and scarce echinulae on the margins; m) SEM micrograph of a spore, the arrow points the wide laesura with smooth margin.

apical pinnae conform, with one to four falcate, largely caudate lobes near the bases; *sori* linear, 3–32 per pinna, placed on lowermost acroscopic vein branch on both sides, close to the midvein and nearly parallel to it; indusia ca. 5–12 × 0.8–1 mm, membranous, whitish, glabrous, entire; sporangia light brown. Spores dark-brown, monolete, 48 × 45 µm in equatorial diameter, perispore reticulated, winged folds with echinulated margin and large and numerous perforations, laesura wide with smooth margin.

Etymology

The specific epithet “*sylvaticum*” is proposed due to the species habitat preference for Yungas and Paraná rainforests.

Additional specimens examined (Paratypes)

Argentina, Jujuy province: Department Libertador General San Martín, Abra de Cañas, en quebraditas húmedas, 1700 m sm., 17 march 1966, *E. de la Sota* 4465 (LP). Parque Nacional Calilegua, february 2009, *J. P. Ramos Giacosa et al.*, s.n. (JUA). Aguada de tigre, 7 june 2023, *A. Ganem, L. Luna & G. Armella* 299 (JUA, RCVC). Misiones province: Department San Ignacio, Teyú Cuaré, february 1910, *E. Wagner* s.n. (SI 18962). Salta province: Department Oran, Aguas Blancas, Serranía de las Pavas, 1200 m sm., 24 july 1985, *C. Palací* 3 (LP). Department Santa Victoria, camino de Baritú a Porongal, 1600 m sm., 18 september 1972, *L. Marmol et al.* 9277 (LP). Camino de Toldos a Lipeo, a 20 km de Toldos, 1600 m sm., 7 september 1971, *P. Legname & A. Cuezio* 8502 (LP). 8 km antes de llegar a Porongal, 1400 m s.m., 21 september 1972, *L. Marmol et al.*, 9201 (LP). Baritú, 1800 m sm., 13 july 1999, *M. A. Ganem et al.* 137 (JUA, LP). Bolivia: Department Cochabamba, Yacaba, Incachaca, despeñadero sombrío, 2500 m s.m, 5 september 1921, *J. Steinbach* 5772 (SI). Brazil: State of Rio Grande do Sul, Porto Alegre, *J. Flach* s.n. (SI 18958).

Distribution and habitat

Asplenium sylvaticum grows in Yungas and Paraná biogeographic provinces (Arana et al. 2021, Morrone et al. 2022) of Southern Bolivia, Southern Brazil and North-east (Paraná) and North-western (Yungas) Argentina. These biogeographic provinces are considered high priority areas for conservation at global and regional scales, due the elevated levels of species richness and endemism (Arana et al. 2021). The authors pointed out that these hotspots have a complex biogeographical history and contain numerous species of lineages with different patterns of diversification. The Yungas province comprises the eastern slopes of the Andes, between 500 to 3,500 m a.s.l., from Northern Peru to North-western Argentina (Morrone et al. 2022). The mountain forests are dominated by *Ocotea porphyria* (Griseb.) van der Werff (Lauraceae) accompanied by *Juglans australis* Griseb. (Juglandaceae), *Zantoxylum coco* Gillies ex Hook. & Arn (Rutaceae), *Duranta serratifolia* (Griseb.) Kuntze (Verbenaceae) and several Myrtaceae tree species, the most common of which is *Blepharocalyx salicifolius* (Kunth) O. Berg. The precipitations average is 2000 mm per year, with a dry period from May to August and frequent drizzle and fog during the lower rain period. In this environment, *A. sylvaticum* grows near watercourses and very humid places at elevations from 800 to 1600 m asl. The Paraná biogeographic province comprises south-eastern Brazil west of the Serra do Mar to central Rio Grande do Sul, north-eastern Argentina, and eastern Paraguay (Morrone et al. 2022). The precipitations average is 2000 mm per year. The topography ranges from relatively plain areas to a relatively flat plateau with elevations ranging from 550 to 800 m a.s.l., where tropical humid forests with trees of 20–30 meters in height develop, as well as savannas and isolated trees (Cabrera & Willink 1980,

Morrone 2017, Arana et al. 2021). Dominant plant species include *Aspidosperma polyneuron* Müll. Arg. (Apocynaceae), *Myracrodruon balansae* (Engl.) Santin (Anacardiaceae), *Balfourodendron riedelianum* (Engl.) Engl. (Rutaceae), *Nectandra angustifolia* (Schrad.) Nees & Mart. (Lauraceae), *Cedrela fissilis* Vell. (Meliaceae), *Chusquea tenella* Nees (Poaceae), *Euterpe edulis* Mart. and *Syagrus romanzoffiana* (Cham.) Glassman (Arecaceae). In these environments, as in Yungas, *A. sylvaticum* grows as terrestrial in shadow places near watercourses.

DISCUSSION

In classical Argentinean floras, *Asplenium sylvaticum* was referred previously to “*Asplenium serra*” (de la Sota 1977, Ganem et al. 2007, 2016), whereas the same taxon was named *A. achalense* by Kessler & Smith (2006, 2018) and Jonas (2011), when they revised the genus *Asplenium* for the Bolivian flora. Arana et al. (2022) noticed that some specimens identified as *A. achalense* in Bolivia and as *A. serra* in Argentina could belong to an unnamed species, proposed here as *Asplenium sylvaticum*. The three species belong to the so called “*Asplenium serra* complex” (Tryon & Stolze 1993), a group highly variable in several characteristics (Kessler & Smith 2006). Particularly, the distinction between *A. serra* and related species (specially *A. incurvatum*, *A. mosetenense* and *A. tunquiniense*) remain poorly understood (Kessler & Smith 2006, 2018).

The disjunct distributional pattern of *A. sylvaticum*, connecting Paraná and Yungas forests, is shared by other species of *Asplenium* (Ganem et al. 2016), as well as taxa of lycophytes (Arana et al. 2012), other ferns (Yañez et al. 2014, Arana & Ponce 2015), angiosperms (Pennington et al. 2000, Garcia et al. 2014) and animal taxa as birds (Nores 1992). Yungas and Paraná forests could constitute relicts of a unique large forest

existing during the Pleistocene and Holocene interglacial periods (Van der Hammen 1974, Bigarella & Andrade Lima 1982, Pennington et al. 2000). This large forest was gradually fragmented into two parts by progressive aridification and the advance of the xerophilous forests of the Chacoan domain, influenced by the uplifting of the Andes (Arana et al. 2021 and references herein), remaining one part in the west (Yungas) and another in the east (Paraná), in two distinct areas between 23°S and 29°S (Morrone & Coscarón 1996, Pennington et al. 2000, Katinas & Crisci 2008, Romo & Morrone 2011, Simões et al. 2012).

The relevance of spore morphology and wall ornamentation to the taxonomy of ferns is well documented (Tryon & Lugardon 1991). It has been demonstrated in many cases that the analyses under scanning electron microscope provides valuable information for elucidating systematic issues in complex fern taxa (e.g. Braggins & Large 1990, Giudice et al. 2002, Regalado & Sánchez 2002, Ganem et al. 2013, Vaganov et al. 2017, Adeonipekun et al. 2021, Gorrer et al. 2021, Morajkar et al. 2021, Arana et al. 2022, Irfan et al. 2022). Concerning spore morphology and wall sculpture in *A. serra* complex species, Viane (1992) mentioned that they share the same reticulate perispore pattern. In the present work, we found differences of spore wall sculpture pattern in the Argentinian, Bolivian and Brazilian species of *A. serra* complex, which allow differentiating them. Although the spores of *A. sylvaticum* may resemble those of *A. serra* (more than to those of *A. achalense*) they are larger in size (48 × 45 µm in equatorial diameter), and the perispore with scarce echinulae, and laesura wide with smooth margin, without spines.

After the analyses of diagnostic characters in herbaria specimens belonging to the *A. serra* complex from Argentina, Bolivia and Brazil, along with the observation of high-quality digital

images of specimens, we found that most of the Bolivian specimens identified as *A. achalense*, as well as most of the Argentinian specimens assigned to *A. serra* that inhabit the Yungas and Paraná biogeographic provinces, correspond to a new species designed here as *A. sylvaticum*. Even the illustration referring to *A. serra* in the classic work Pteridophyta of Jujuy (De la Sota 1977) corresponds to *A. sylvaticum*.

Key to differentiate the species of the *Asplenium serra* complex inhabiting Paraná and Yungas provinces:

1. Blades 1-pinnate-pinnatisect to 2-pinnate-pinnatifid, at least proximal pinnae incised fully to the costae *A. tunquiniense*

1. Blades 1-pinnate, if proximal pinnae incised, then not fully to the costae..... 2

2(1). Rhizome scales up to 2 mm long
..... *A. micropaleatum*

2. Rhizome scales 3-18 mm long..... 3

3(2). Rhizome scales 6–18 mm long 4

3. Rhizome scales 3-5 mm long.....5

4(3). Rhizome scales dark brown to blackish..... *A. incurvatum*

4. Rhizome scales orange to castaneous..... *A. mosetenense*

5(4). Petioles and rachises scaly and glandular-pubescent, hairs bicellular with apical rounded cell. Pinnae margin strongly biserrate. Apical pinnae pinnatifid. Spores brownish, 39 x 36 µm, perispore folded- ridged, fused ridged folds with a smooth margin and scarcely perforated*A. achalense*

5. Petioles and rachises only scaly, without such hairs. Pinnae margin only slightly serrate. Apical pinnae conform. Spores dark-brown, 48 x 45 µm, perispore folded- winged, winged folds with echinulated or echinated margins and large and numerous perforations.....6

6(5) Rhizome scales with entire margins; blade widest at the bases, pinnae 4–16 pairs per blade; pinnae 23–45 mm wide, straight or slightly

ascendent, usually little reduced towards blade apices; pinnae apices acute to short acuminate. Apical pinnae entire, as long as half the length of the medial lateral pinnae. Spores with perispore with echinated surface, laesura thin with surficial echinae..... *A. serra*

6. Rhizome scales with 1-2 glandular trichomes at margins; blade widest at the middle, pinnae 18-36 pairs per blade; pinnae 12–20 mm wide, ascendant, caudate, often gradually reduced towards blade apices; pinnae apices long attenuate, strongly curved upwards. Apical pinnae as long as the medial lateral pinnae, with one to four acute and caudate lobes at the base. Spores with perispore with scarce echinulae, laesura wide with smooth margin without spines.....*A. sylvaticum*

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