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Author(s): João Paulo S. Condack Monique A. McHenry Rita E. Morero Lana S. Sylvestre David S. Barrington

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***Polystichum montevidense* Demystified: Molecular and Morphological Data Reveal a Cohesive, Widespread South American Species**

JOÃO PAULO S. CONDACK

Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s.n., São Cristóvão, Rio de Janeiro, Brazil 20940-040, e-mail: jpcondack@gmail.com

MONIQUE A. MCHENRY

University of Vermont, Pringle Herbarium, Torrey Hall, 27 Colchester Ave, Burlington, VT 05405, USA, e-mail: mmchenry@uvm.edu

RITA E. MORERO

Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET) y Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Córdoba, Argentina, e-mail: ritamorero@hotmail.com

LANA S. SYLVESTRE

Universidade Federal do Rio de Janeiro, Instituto de Biologia, Av. Carlos Chagas Filho, 373, Cidade Universitária, Rio de Janeiro, Brazil 21941-902, e-mail: lana@biologia.ufrj.br

DAVID S. BARRINGTON

University of Vermont, Pringle Herbarium, Torrey Hall, 27 Colchester Ave, Burlington, VT 05405, USA, e-mail: dbarrington@uvm.edu

ABSTRACT.—The genus *Polystichum* presents striking variation in morphology and habitat preference in the Central Andes, the Serra do Mar, and adjacent regions. Among these taxa, *Polystichum montevidense* is a name long applied to an array of twice-pinnate species with dark petiole scales, broad leaves, and no vegetative propagules. Using a classical morphological approach combined with inferences gleaned from molecular data, we analyzed *P. montevidense* and its widespread and ecologically prominent array of allies. Results from our combined molecular and morphological analysis suggest the name *P. montevidense* should be applied to collections from the Central Andes south to Argentina, and east to Uruguay and the southernmost portion of Brazil. Most Brazilian plants determined as *P. montevidense* in herbaria are *P. platylepis*.

KEY WORDS.—Dryopteridaceae, South America, Andes, Brazil, biogeography, phylogeny

Polystichum (Dryopteridaceae) is a monophyletic genus with the inclusion of a few satellite genera and part of *Cyrtomium* (Little and Barrington, 2003; Li *et al.*, 2007). One of the largest fern genera (between 200 and 300 species; Little and Barrington, 2003), it is found worldwide mainly in tropical and warm temperate areas. Its two centers of greatest diversity are in montane forests of warm temperate eastern Asia (Zhang and Barrington, in press) and tropical America (Barrington, 2011). Studies on *Polystichum* in the New World have been focused outside of South America; the main contributions address the genus in Western North America (Wagner, 1979), the West Indies (Mickel 1997, Morejón and Sánchez, 2012), and Mesoamerica (Barrington 1985a, 1985b, 1989, 1990, 1992, 1995, 2003, 2005, 2011).

Though *Polystichum* itself is morphologically distinct, species delimitation has been a problem for a very long time (e.g., Christensen, 1905–1906; Domin, 1929). This taxonomic problem can be found across all of the centers of diversity for the genus. For instance, Fraser-Jenkins (1997) accepted only one of the 11 *Polystichum* species described in the previous 15 years for the Indo-Himalayan region; he considered the remainder to be synonyms.

In South America the level of confusion is similar, and the name *Polystichum montevidense* emerges as perhaps the most misapplied on the continent (Kessler *et al.* 2005). The taxonomic confusion surrounding this species begins with its brief and vague description by Sprengel (1827). Baker (1870) in *Flora Brasiliensis*, placed it in synonymy with *Polystichum aculeatum* (L.) Roth var. β *phegopteroideum* Baker, a name that included a broad array of Andean exindusiate polystichums. Fée (1869, 1872–1873) in his major work on the Brazilian ferns, did not even mention *P. montevidense*, though he recognized 14 species in *Polystichum* s.l. including ten newly described by him.

Almost a century after its original description, Rosenstock (1906) combined Sprengel's *Polypodium montevidense* into *Polystichum*. Rosenstock's conception of the species was very broad, including as synonyms six of the species described by Fée from the Serra do Mar region of tropical Brazil.

Sehnem (1979), in the most recent flora addressing *Polystichum* diversity in Brazil, rejected Rosenstock's (1906) species circumscriptions and returned to Fée's concept of the genus. In his treatment, following Fée, Sehnem excluded *P. montevidense* entirely. Nevertheless, the name *Polystichum montevidense* has remained in widespread use in Brazil; it is the most common name on Brazilian *Polystichum* specimens, being used extensively for mid-sized 2-pinnate plants with dark, matte-textured petiole-base scales.

Further complicating the situation was the broad application, by Tryon and Stolze (1991), of *P. montevidense* (with two varieties, var. *montevidense* and var. *nudicaule* (Rosenst.) Tryon), to plants distributed from Venezuela to Bolivia and Argentina as well as Brazil and Uruguay. Kessler *et al.* (2005) also recognized a broadly circumscribed *P. montevidense*. They alluded to morphological differences between Bolivian and Southeastern Brazilian plants historically placed in *P. montevidense*, but they did not distinguish them taxonomically. Neither Tryon and Stolze (1991) nor Kessler *et al.* (2005) located Sprengel's type of *P. montevidense*; thus critical study of authentic material for *Polystichum montevidense*, using modern morphological analysis, has not been possible.

In this paper we seek resolution of the long-standing problems surrounding the name *Polystichum montevidense*. Our approach has been to relocate the collections on which the name was based, to collect material for molecular and morphological study from the presumptive type locality north of Montevideo, Uruguay, to perform cytological analysis of the species, to do a molecular phylogenetic analysis of the relevant related *Polystichum* species, and to review the morphological characters of *P. montevidense* and related taxa. This combined morphological, cytological, and molecular analysis yields clear

insights into the species biology and taxonomy of this long-misunderstood taxon.

METHODS

Taxon sampling.—Our sample consists of 17 *Polystichum* accessions from across the northern and central Andean region, Argentina, Uruguay, and Brazil. We sampled all taxa shown to have close evolutionary relationships to *P. montevidense* in the phylogeny of McHenry (2012). Also included are three accessions from the senior author's dense sampling of the Serra do Mar region, and one accession of *P. montevidense* from the tropical Andes, one from Argentina, and one from Uruguay, to adequately represent the morphological diversity of the group across its range. We excluded accessions that showed signs of hybridity (intermediate morphology and misshapen spores). Voucher data for all of our collections are listed in the Appendix.

DNA extraction, amplification and sequencing.—Total genomic DNA was extracted from fresh (0.1g) or silica-dried (0.02g) material. Leaf material collected in the field was preserved fresh at 4°C or in silica desiccant gel and stored at –80°C until extraction. Total genomic DNA was extracted from pinnules following a modified CTAB protocol (Doyle and Doyle, 1987). Four plastid DNA regions were amplified using by PCR: two genes (*rps4* and *rbcl*) and two intergenic spacers (the region between *trnL* and *trnF* (*trnLF*) and the region between *trnS* and *rps4* (*trnS-rps4*)). Primers for amplification and sequencing were taken from the literature: *rbcl* (Little and Barrington, 2003), *rps4* (Shaw *et al.*, 2005), *trnLF* (Taberlet *et al.*, 1991), and *trnS-rps4* (Souza-Chies *et al.*, 1997). PCR amplification was performed in a TC-312 or TC-3000 thermal cycler (Techne, Burlington, New Jersey, USA) following protocols in McKeown *et al.* (2012). PCR products were cleaned using ExoSAP-IT (USB Corporation, Cleveland, OH, USA). Sequencing of the cleaned PCR products employed a cycle-sequence reaction using the BigDye Terminator Cycle Sequence Ready Reaction Kit v. 3.1 (Perkin–Elmer/Applied Biosystems, Foster City, CA, USA). Sequences were resolved on an ABI Prism 3100-Avant Genetic Analyzer (Vermont Cancer Center DNA Analysis Facility, Burlington, VT, USA). Consensus sequences from the raw chromatographs (using both the forward and reverse reads) were assembled for each gene using Sequencher 4.5 (Genes Code Corporation, Ann Arbor, MI, USA) or Geneious Pro v.5.0.3 (Drummond *et al.*, 2007).

Sequence alignment and phylogenetic analysis.—Consensus sequences were aligned with MUSCLE (Edgar, 2004) as implemented in Geneious Pro v.5.0.3 (Drummond *et al.*, 2007). All phylogenetically informative indels were coded following the simple gap coding of Simmons and Ochoterena (2000) and added as additional binary characters at the end of the NEXUS file. The concatenated sequences were analyzed by Bayesian inference (BI) using MrBayes v. 3.2 (Ronquist *et al.*, 2012). The data were partitioned by plastid region, and optimal models (Table 1) were applied to each of the molecular partitions. The model selection was done using jModelTest v. 2.1 under the Akaike

TABLE 1. Characteristics of the markers used in the phylogenetic analysis.

Marker	Model	Length (bp)	Variable sites	Sampled taxa
<i>rbcL</i>	K80+G	1165	33 (2.8%)	17
<i>trnLF</i>	TPM2uf+G	331	29 (8.8%)	17
<i>rps4</i>	TPM3uf+G	450	25 (5.6%)	17
<i>trnS-rps4</i>	TrN+G	460	50 (10.9%)	17

Information Criterion (AIC; Darriba *et al.* 2012). We employed a mixed-model Bayesian analysis with different models allowed for each partition. The parameters for each data partition were able to vary freely. Indels were treated as single, independent, and binary characters. BI was preformed in Mr. Bayes by running two independent analyses for 5 million generations with trees sampled every 1,000 generations. Stationarity was calculated by plotting the log-likelihood scores for each run against generation in the program Tracer v1.5 (Rambault and Drummond, 2007). All trees prior to this point (the first 500,000) were discarded as the burn-in phase and a 50% majority rule consensus tree was calculated for the remaining trees.

Chromosome analysis.—Sporophytes collected from wild populations from Córdoba province, Argentina, were cultivated at the Universidad de Córdoba; voucher specimens were deposited at CORD. For the analysis of mitotic chromosomes, croziers were excised into small fragments approximately 2 mm wide. The fragments were treated in a solution of 2mM 8-hydroxyquinoline for 1 h at room temperature, followed by 8 h at 14°C, then fixed in ethanol-acetic acid (3:1) and stored at -20°C. For chromosome analysis, the crozier fragments were washed in distilled water 4 to 5 times, then hydrolyzed in 3 ml of cellulase 2%-pectinase 20%, for 2 h at 37°C. The hydrolyzed cells were stained in alcoholic hydrochloric acid-carminine and squashed following established protocols (Manton, 1950).

RESULTS

Molecular analysis.—The analysis of South American accessions relevant to the disposition of the name *P. montevidense* yielded a highly resolved phylogeny (Fig. 1). In this phylogeny, our accession from the area of the type collection near Montevideo, Uruguay, resolves in a clade with Argentine and Bolivian accessions of *P. montevidense* (Clade 2; posterior probability [PP]=1.0). This trio of accessions is sister to a clade of Andean plants including *P. solomonii* and *P. albomarginatum*. Together they form a well-supported clade (PP=0.96) with *P. opacum*, the Brazilian representative of the lineage that includes the widespread *P. platyphyllum*. In contrast, our accessions of specimens matching plants commonly determined as *P. montevidense* from the northern Serra do Mar, Brazil, resolve in a well-supported clade (Clade 1, PP=1.0) with the tropical Southeast Brazilian endemic species *P. platylepis* and *P. pallidum* (Condack, 2012).

Morphological analysis.—We found that the Sello collections of *Polypodium montevidense* from near Montevideo, Uruguay (Brazil in Sello's time), which

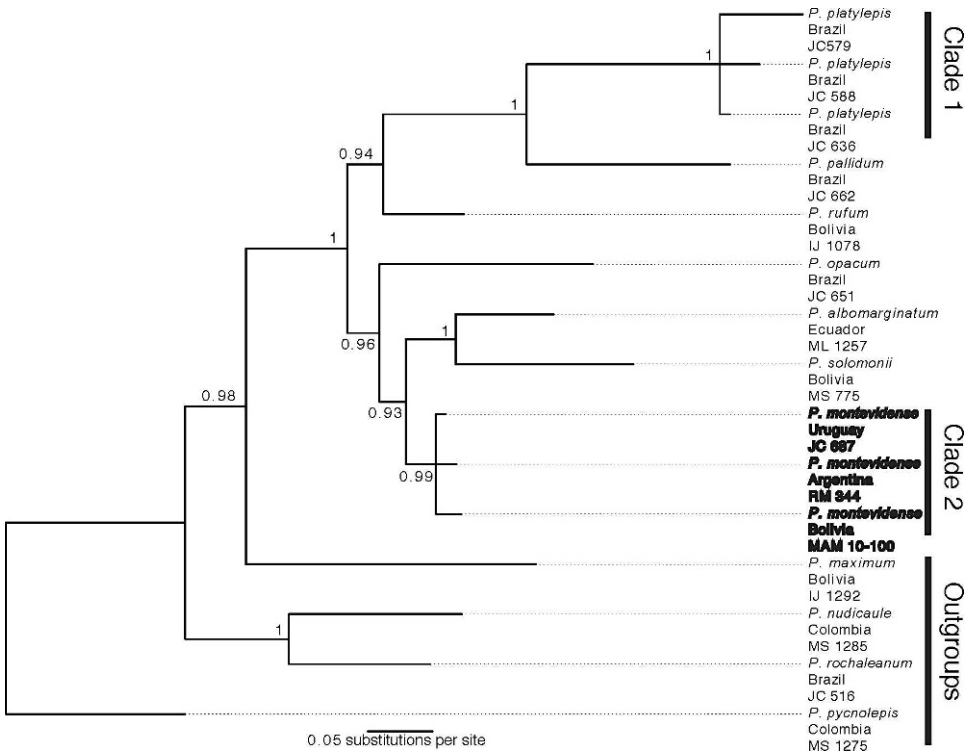


FIG. 1. Phylogeny of *Polystichum montevidense* and its nearest allies. The tree is the 50% majority rule phylogram from the Bayesian inference analysis. Posterior probabilities are given at each node. *Polystichum montevidense* accessions are in bold. Country of each accession, collector initials, and collection number are below each accession (see Appendix).

survive at Berlin, include six sheets numbered *d. 654*. One of these (B 20 0156392) is determined as *Polypodium montevidense* in a hand annotated as Sprengel's. These specimens, which we take to be syntypes of *Polystichum montevidense*, are morphologically cohesive, allowing a clear morphological circumscription of the taxon. Additionally, a specimen deposited in the Gaudichaud herbarium at Paris (P 01456828) has a field label attached at the petiole with the same "d 654" found on the original material at B. From these materials, we diagnose the species as having ferruginous stem scales that contrast with the large basal petiole-scales, which have atropurpureous centers and rufous edges; long, delicate petiole-scale cilia; 2-pinnate laminae that lack a bulbil; lanceolate rachis scales that are either entirely rufous or bicolorous with dark centers; long-lanceolate, rufous pinna-rachis scales; serrate pinnules with strongly developed spinules; glandular trichomes on both sides of the pinnules, and no true indusium (Figs. 2, 3). The glandular trichomes are especially significant as they are extraordinarily rare in *Polystichum*, so far known only from juvenile plants of Andean *Polystichum* species (McHenry, 2012).

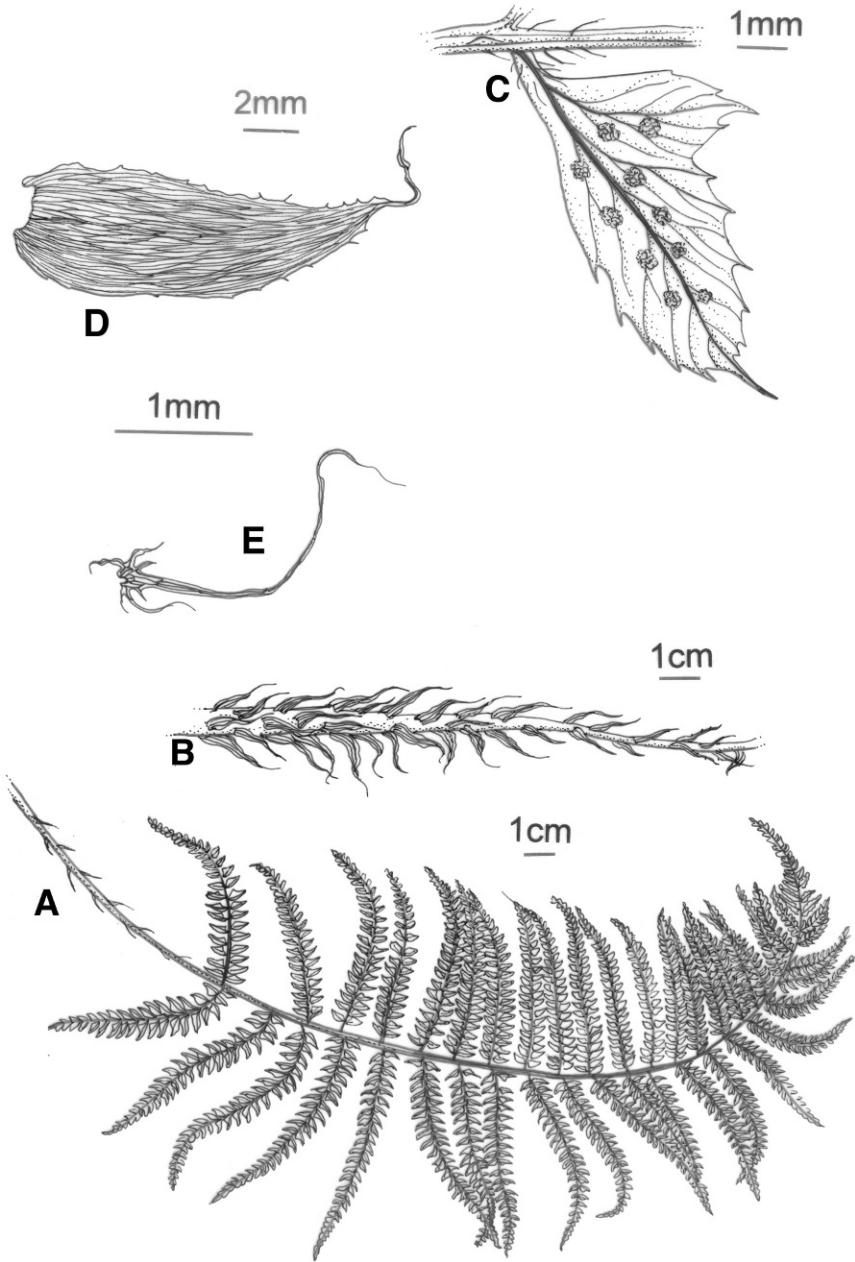


FIG. 2. *Polystichum montevidense*. A. Lamina. B. Petiole base. C. Pinnule with portion of pinna-rachis. D. Basal petiole scale. E. Scale from abaxial surface of pinna-rachis between pinnules. Original material: Uruguay. Pr. Maldonado, J.P.S. Condack 694 (R).

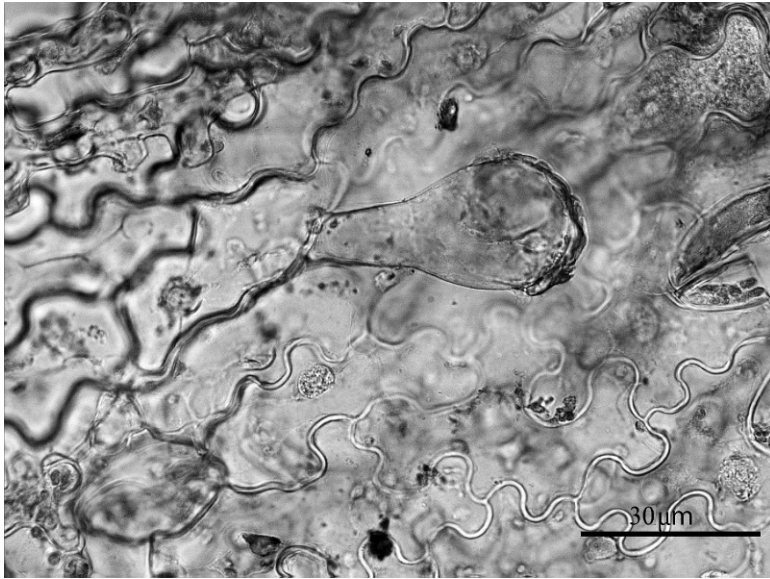


FIG. 3. Epidermal gland from the abaxial lamina of *Polystichum montevidense* (from Morero 344 [CORD]).

Geographic distribution.—As circumscribed here, *Polystichum montevidense* is found in the Central Andes including the eastern slopes in north and central Argentina, to the southernmost part of Brazil, and Uruguay. We did not locate specimens of *P. montevidense* from Paraguay, though they are to be expected. It is diploid (Fig. 4) based on three counts from northern Argentina: two of the plants are from the Sierras Chicas, the eastern range of the Sierras Pampeanas (Pr. Córdoba, *R. Morero 342, 348*), the third is from the Sierra El Aconquija, Pr. Tucumán, (*R. Morero 352*).

In Brazil the species has been found only south of 30° S latitude in the southernmost state of Rio Grande do Sul. Its range lies between the 4° and 8° minimum-annual-temperature isotherms in regions where the aridity index is near 1.0 (Santibañez, 2004). Habitat is either on soil or among rocks in forested regions or on hilltops in both protected areas under shrubs, and exposed areas.

TAXONOMIC TREATMENT

Polystichum montevidense (Spreng.) Rosenst.

Polypodium montevidense Spreng., Linnaeus, Syst. Veget. ed. 16. 4(1): 59. LECTOTYPE (here designated).—Uruguay, near Montevideo [orig. label: “Brasilia, prope Monte Video”], *Sello d 654* (B 20 0156392; duplicates: B [B 20 0156391, B 20 0156394, B 20 0156395, B 20 0148065], P 01456828). *Phegopteris montevidensis* (Spreng.) Salomon, Nomencl. Gefässkrypt. 276.

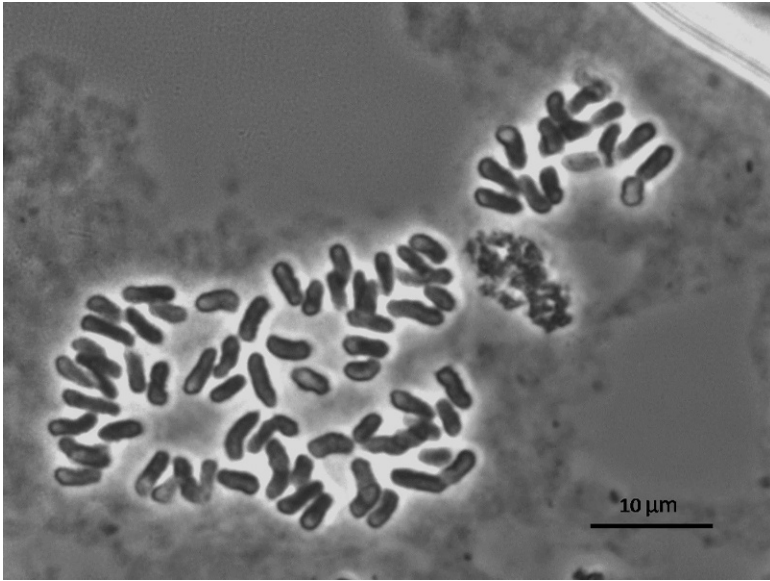


FIG. 4. Mitotic prophase ($2n=82$) in *P. montevidense* (from a crozier of *Morero 342* [CORD]).

1883. *Aspidium montevidense* (Spreng.) Hieron., Engl. Jahrb. 22:364. 1896. *Aspidium montevidense* (Spreng.) Hieron. in Rosenst., Hedwigia 43:223. 1904. *Polystichum aculeatum* (L.) Roth var. 9, C.Chr. Index Filic. 576. 1905. *Polystichum montevidense* (Spreng.) Rosenst., Hedwigia 46: 111. 1906. *Polystichum montevidense* (Spreng.) Hieron., Hedwigia 46: 356. 1907.

Polystichum sellowianum C. Presl nom. nud., Tent. Pterid. 83.1836. AUTHENTIC MATERIAL, Monte Video, *Sello d 654* (B 20 0156396, with Herb. Mettenius label bearing determination “*Phegopteris montevidensis* (Spreng.) Mett.” and a second label with determination “*Polystich. sellowianum* Pr.” in Mettenius’s hand.).

Excluded name:

Polystichum montevidense (Spreng.) Rosenst. var. ***squamulosa***, Hieron 1907, Hedwigia 46:356. *Aspidium montevidense* (Spreng.) forma *squamulosa* Hieron., Englers Botan. Jhrb. 22:366.1896.

Based on specimens from near Córdoba, Argentina. This name pertains to *Polystichum pycnolepis* (Kze. ex Kl.) T.Moore (see McHenry, 2012).

DISCUSSION

The correct identity of *Polystichum montevidense*, a name commonly applied to plants across central South America, has been confused for over one hundred years. Even in recent floristic treatments the circumscription of the

species was not informed by the study of authentic material such as the type. Our study is the first to combine extensive fieldwork with analysis of the type collections using modern morphological and molecular methods to clarify the identity of this species.

With the resolution of the major taxonomic problems in Brazilian *Polystichum* (Condack, 2012), comes the realization that *P. montevidense* has been confused most often with a single species, *P. platylepis* (or one of its many synonyms). Plants pertaining to *P. platylepis* were prominent in the collections that Auguste François Marie Glaziou made in the Serra do Mar during the late 1860s. These collections, as they so often served as types for Fée in his *Cryptogames vasculaires du Brésil* (1869; 1872–3), have apparently left botanists confused about the limits of *P. montevidense* and similar species in the Serra do Mar for the last 140 years.

However, the question remains, why is *P. montevidense* so morphologically similar to *P. platylepis*? They share an array of characters including lamina dissection, pinna shape and size, pinnule attachment, and pinnule shape, and petiole scales are similar in shape and hue. They differ in a few key but less salient features, such as petiole-scale cilia development, lamina-indument shape and hue, and spinule development on the pinnules. Two possible explanations merit consideration. First, given the strong tendency to morphological convergence observed in the genus, they may look alike in spite of independent recent histories. Second, however, it is possible that *P. platylepis* is the result of hybridization between *P. montevidense* and another Brazilian species followed by restoration of fertility.

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APPENDIX

Selected specimens examined including all those used in the molecular analysis, which have GenBank accession numbers (marker sequence is *rbcl*, *rps4* gene, *trnLF*, *trnS-rps4*). Herbarium abbreviations throughout follow Index Herbariorum (Theirs, 2012). GAN = GenBank accession number.

Polystichum albomarginatum M. Kessler & A.R. Smith. ECUADOR. **Zamora-Chinchipe**: Bombuscaro, Podocarpus National Park, *M. Lehnert 1257* (VT) (GANs: KC819964; KC819994; KC819949; KC819979).

Polystichum maximum M. Kessler & A.R. Smith. BOLIVIA. **Cochabamba**: Prov. Ayopaya, San Cristobal, 16°39'S, 66°43'W, 2550, *I. Jiménez 1292* (LPB) (GANs: KC819965; KC819995; KC819950; KC819980).

Polystichum montevidense (Spreng.) Rosenst. BOLIVIA. **Santa Cruz**: Caballero, *M. Sundue 844* (VT, NY). **Cochabamba**: Prov. Carrasco, 3.3 km NW of Kayarani, *M. Sundue 621* (VT, NY). **La Paz**: Calle Sorata a Laripata, *M. McHenry 10-95* (VT); Sorata, *M. McHenry 10-100* (VT) (GANs: KC819967; KC819997; KC819952; KC819982). **Tarija**: Arce, Padcaya, *H. Huaylla 1514* (NY). ARGENTINA. **Córdoba**: Dpto. Punilla, Los Gigantes, *R. Morero 342*

(CORD), 344 (CORD) (GANs: KC819968; KC819983; KC819953; KC819998).
Tucumán: Dpto. Chichilgasta. Cuesta del Clavillo, *R. Morero* 352 (CORD).

URUGUAY. **Montevideo:** without precise locality, *F. Sello* d 654 (B, P);
Maldonado: Cerro Pan de Azúcar, *Gibert* 857 (K); Idem, *A. Lombardo* (BM ex MVM10907); Idem, *A. Lombardo* 2568 (MVJB); Idem, *A. Lombardo* 4732 (MVJB); Idem, *A. Lombardo* 5436 (MVJB); Idem, *J. Arechavaleta* 784 (MVFA); Idem, *O. del Puerto et al.* 9702 (MVFA); Idem, *E. Marchesi* 1379 (MVFA); Idem, *M. Berro* 1261 (MVFA); Idem, *J. Condack & F. Muñoz* 687 (R) (GANs: KC819966; KC819996; KC819951; KC819981); Idem, *J. Condack* 694 (R). **Tacuarembó:** Montes de Tacuarembó, *J. Arechavaleta* 4187 (MVM). URUGUAY/BRASIL.
Artigas: Cuarein (border of Brazil and Uruguay), *M. Berro* 5617 (MVFA).

Polystichum nudicaule Rosenst. COLOMBIA. **Boyacá:** Mun. Chisacá-San Pedro de Iquaque, *M. Sundue* 1285 (VT) (GANs: KC819969; KC819999; KC819954; KC819984).

Polystichum opacum Rosenst. BRASIL. **Rio Grande do Sul:** Santa Cruz do Sul, *J. Condack & M. Brito* 651 (R, VT) (GANs: KC819970; KC820000; KC819955; KC819985).

Polystichum pallidum Gardn. BRASIL. **Rio de Janeiro:** Parque Nacional da Tijuca, *J. Condack* 662 (R, VT) (GANs: KC819971; KC820001; KC819956; KC819986).

Polystichum platylepis Fée BRASIL. **Bahia:** Amargosa, *J. Paixão & M. Nascimento* 1377 (UEFS). **Espírito Santo:** Castelo, Parque Estadual do Forno Grande, *P. Labiak et al.* 4820 (MBML, RB, UPCB). **Mato Grosso:** Vila Bela da Santíssima Trindade, Serra Ricardo Franco, *P. Windisch* 1350 (GH, HB, ICN); **Minas Gerais:** Bocaina de Minas, Parque Nacional do Itatiaia, Alto dos Brejos, *J. Condack et al.* 328 (RB, NY); **Paraná:** Clevelândia, *G. Hatschbach* 22695 (MBM, NY, PACA); **Rio de Janeiro:** Itatiaia, *J. Condack & A. Vasco* 636 (R, RBR, VT) (GANs: KC819974; KC820004; KC819959; KC819989). **Rio Grande do Sul:** São José dos Ausentes, *J. Condack & P. Schwartsburd* 588 (R, RBR, VT) (GANs: KC819973; KC820003; KC819958; KC819988). **Santa Catarina:** Urubici, Morro da Igreja, *J. Condack & P. Schwartsburd* 579 (R, RBR, VT) (GANs: KC819972; KC820002; KC819957; KC819987). **São Paulo:** Bananal, Serra da Bocaina, *A. Brade* 15203 (RB).

Polystichum platyphyllum (Willd.) C. Presl. BRASIL. **Goiás:** Caiapônia, Serra do Caiapó, *H. Irwin et al.* 17873 (GH, NY, RB, UB, US).

Polystichum pycnolepis (Kunze ex Klotzsch) T. Moore. COLOMBIA. **Boyacá:** Mun. Villa de Leyva, *M. Sundue* 1275 (VT) (GANs: KC819975; KC820005; KC819960; KC819990).

Polystichum rochaleanum Fée. BRASIL. **Rio de Janeiro:** Itatiaia, Pico das Agulhas Negras, *J. Condack & C. Ramos 516* (RB, VT) (GANs: KC819976; KC820006; KC819961; KC819991).

Polystichum rufum M. Kessler & A. R. Smith. BOLIVIA. **F. Tamaya:** PN-ANMI Madidi, *I. Jiménez 1078* (UC) (GANs: KC819977; KC820007; KC819962; KC819992).

Polystichum solomonii M. Kessler & A. R. Smith. BOLIVIA. **Caballero:** 1.5 km down from Empalme, *M. Sundue 775* (VT) (GANs: KC819978; KC820008; KC819963; KC819993).