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: jpccondack@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: dharring@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.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Model</th>
<th>Length (bp)</th>
<th>Variable sites</th>
<th>Sampled taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>rbcl</td>
<td>K80+G</td>
<td>1155</td>
<td>33 (2.8%)</td>
<td>17</td>
</tr>
<tr>
<td>trnlF</td>
<td>TPM2uf+G</td>
<td>331</td>
<td>29 (8.8%)</td>
<td>17</td>
</tr>
<tr>
<td>rps4</td>
<td>TPM3uf+G</td>
<td>450</td>
<td>25 (5.6%)</td>
<td>17</td>
</tr>
<tr>
<td>trnS-rps4</td>
<td>TrN+G</td>
<td>460</td>
<td>50 (10.9%)</td>
<td>17</td>
</tr>
</tbody>
</table>

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-carmine 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. alhomarginatum. 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
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 bicolored 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. 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, 346), 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.

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’ hand.).

Excluded name:

Polystichum montevidense (Spreng.) Rosenst. var. squamulosa, Hieron 1907,
Hedwigia 46:356. Aspidium montevidense (Spreng.) forma squamulosa

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.

ACKNOWLEDGMENTS

The authors thank CAPES for providing a doctoral scholarship to Joao Condack. This work was supported by CNPq (Proc. n. 309415/2008-0 to Lana Sylvestre). We thank the curators of the following herbaria AAU, B, BHCB, BM, CEPEC, CESJ, FURB, GH, GUA, HAS, HB, HBR, HUEFS, HUCS, ICN, K, LPB, MA, MBM, MBML, MO, MVFA, MVFQ, MVM, MVJ, NY, OUPR, P, PAG, QCA, R, RB, RBR, RFA, SJRP, SP, SPF, UB, UPCB, UC, US, VIES and VT (herbarium abbreviations follow Thiers 2012) for providing material for this study. Maria Alice Rezende prepared Figure 2. Work on the Andean accessions was in part supported by an American Society of Plant Taxonomists Graduate Student Research Grant to Monique McHenry.

LITERATURE CITED


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


Polystichum solomonii M. Kessler & A. R. Smith. BOLIVIA. Caballero: 1.5 km down from Empalme, M. Sundue 775 (VT) (GANs: KC819978; KC820008; KC819963; KC819993).
https://doi.org/10.1640/0002-8444-103.2.118.

View This Item Online: https://www.biodiversitylibrary.org/item/200536
DOI: https://doi.org/10.1640/0002-8444-103.2.118
Permalink: https://www.biodiversitylibrary.org/partpdf/231065

Holding Institution
Missouri Botanical Garden, Peter H. Raven Library

Sponsored by
Missouri Botanical Garden

Copyright & Reuse
Copyright Status: Permission to digitize granted by rights holder
Rights: https://www.biodiversitylibrary.org/permissions

This document was created from content at the Biodiversity Heritage Library, the world’s largest open access digital library for biodiversity literature and archives. Visit BHL at https://www.biodiversitylibrary.org.

This file was generated 16 April 2022 at 03:28 UTC