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cpDNA supports the identification of the major lineages of American *Blechnum* (Blechnaceae, Polypodiopsida) established by morphology

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Abstract: Blechnaceae is an important leptosporangiate family (9–10 genera, about 250 species). It is monophyletic and distributed mainly in tropical America and Australasia. Among the species 80% belong to *Blechnum*, a genus with a very complex taxonomy and uncertain internal relationships. In terms of American diversity, the results of morphological studies have arrived at 8 informal groups. Molecular works on this genus are scarce, and there is no information for the majority of American species. The main objective of this work was to evaluate whether the groups proposed to organise the diversity of American of *Blechnum* are consistent with a molecular analysis. We sequenced 2 chloroplastic regions from species representing all of the groups. In our molecular analysis most of the informal groups were maintained as well supported clades. Only 2 species, *B. brasiliense* and *B. spicant*, appear to be isolated from their alleged relatives. Combining our molecular results with previous morphological knowledge, we propose the recognition of 4 lineages: 1) *B. serrulatum*, 2) *B. spicant*, and 3) core *Blechnum*, which represents a large clade that can be divided into core *Blechnum* I (arborescent species, cordatoids, and *B. brasiliense*) and core *Blechnum* II (epiphytic species and herbaceous terrestrials, both monomorphic and dimorphic groups).

Key words: America, *Blechnum*, chloroplast DNA, morphology

1. Introduction

Blechnaceae is a medium-sized leptosporangiate fern family that comprises 220–250 species in an uncertain number of genera, sometimes reaching 10 (Kramer et al., 1990; Smith et al., 2006; Rothfels et al., 2012b). Around 80% of the species fall within *Blechnum* L. The family is sub-cosmopolitan with 2 notable areas of diversity, one in Central and South America, and the other in Southeast Asia and Oceania. Blechnaceae seems to be monophyletic (Smith et al., 2006; Schuettpelz & Pryer, 2007; Kuo et al., 2011; Rothfels et al., 2012a); however, genera and species relationships are still unclear. *Blechnum* is undoubtedly paraphyletic because *Sadleria* Kaulf. and *Doodia* R.Br. are embedded within it (Cranfill & Kato, 2003; Shepherd et al., 2007). A new conception of *Blechnum* will probably result in the rearrangement of almost all of the genera (Cranfill et al., 2001).

Much effort has been made to establish order in *Blechnum*, including its segregation into several smaller genera (e.g., *Lomaria* Willd., *Lomaridium* C.Presl, *Struthiopteris* Scop., *Blechnopsis* C.Presl, *Distaxia* C.Presl,

and *Spicanta* C.Presl). In a modern morphological conception, all of these genera are considered *Blechnum* subgenera or sections (Kramer et al., 1990; Smith et al., 2006); however, even moved into infrageneric positions they have rarely been used (Murillo, 1968; Ramos Giacosa, 2008). Thus, knowledge of the genus *Blechnum* remains obscure and is complicated by the continuous publication of new local species, lack of a general review, and an exorbitant nomenclature.

In order to avoid a taxonomically uncertain scenario, specialists have proposed informal groups within *Blechnum* using morphological criteria. For the American diversity, where around 80 species are recognised (Rolleri & Prada, 2006a), a first approach involving 8 groups has been made, mostly according to characters of rhizome, frond architecture, dimorphism, and habit (Figure 1): *Blechnum loxense* (Kunth) Hieron. group; *B. cordatum* (Desv.) Hieron. group; *B. tabulare* (Thunb.) Kuhn group, originally named *B. buchtienii* Rosenst. group (Rolleri et al., 2013); *B. fragile* (Liebm.) C.V.Morton & Lellinger group; *B. penna-marina* (Poir.) Kuhn group; *B. occidentale* L. group;

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Figure 1. *Blechnum* species silhouettes representing some of the morphological groups assessed in the current work (from herbaria material). A- *B. loxense* (gr. *loxense*); B- *B. stipitellatum* (gr. *cordatum*), sterile frond; C- *B. tabulare* (gr. *tabulare*), fertile and sterile fronds; D- *B. fragile* (gr. *fragile*), sterile fronds; E- *B. penna-marina* (gr. *penna-marina*), individual with sterile and fertile fronds; F- *B. laevigatum* (gr. *occidentale*). Scale bar: A, B = 11 cm; C = 12.5 cm; D = 8 cm; E, F = 5 cm.

B. divergens (Kunze) Mett. group; and *B. serrulatum* Rich. group (Tryon & Tryon, 1982; Durán, 1997; Dittrich, 2005; Rolleri & Prada, 2006b; Passarelli, 2007; Prada et al., 2007; Gabriel y Galán et al., 2008; Ramos Giacosa, 2008; Rolleri et al., 2008; Passarelli et al., 2010; Rolleri et al., 2010;

Gabriel y Galán et al., 2011; Rolleri et al., 2012a; Rolleri et al., 2012b; Rolleri et al., 2013).

Molecular studies focussing on *Blechnum* are very scarce and only partial in their taxonomic and geographical scope. The first verification that Blechnaceae

is monophyletic but *Blechnum* is not was assessed using morphology and plastid markers (Cranfill, 2001; Cranfill et al., 2001) and later confirmed in several works (e.g., Cranfill & Kato, 2003; Schuettpelz & Pryer, 2007). Very few American taxa were included in these studies. More recently, a phylogeny of 18 New Zealand *Blechnum* species (plus some other indigenous Blechnaceae) was completed using trnL-trnF sequences (Shepherd et al., 2007).

The main objective of this work was to evaluate whether the 8 informal morphological groups proposed to order the American diversity of *Blechnum* are coherent evolutionary lineages, by evaluating whether the groups are consistent with molecular analysis. Therefore, this study is not intended to be a phylogeny of the genus; instead, it will help to expand taxonomical criteria beyond morphological data, in the search for a more comprehensive infrageneric ordering of the genus *Blechnum*.

2. Materials and methods

2.1. Plant materials

Biological materials representing all morphological groups of *Blechnum* (Tryon & Tryon, 1982) were taken either from field samples, preserved in silica gel until extraction, or from herbarium material. The selected species are listed in Table 1. All samples correspond to American collections, except *B. spicant* (L.) Sm., which is

from a European locality. Among the samples, 2 identified as *B. schomburgkii* (Klotzsch) C.Chr. appeared in different positions within the tree, and we decided to maintain the labels as *B. schomburgkii* 1 (Costa Rica) and *B. schomburgkii* 2 (Peru). *Woodwardia radicans* (L.) Sm., from a European collection, was used as outgroup due to the known position of the genus (Cranfill & Kato, 2003). We also included the American *Salpichlaena volubilis* (Kaulf.) J.Sm. in order to assess its position in a broader framework. Vouchers of all samples have been deposited in the herbarium of the Botanical Garden of Madrid (MA).

2.2. DNA extraction, PCR, and sequencing

Total DNA was extracted with a DNeasy Plant Mini Kit (Quiagen). PCR was used to amplify 2 chloroplast regions, trnL-trnF and trnG-trnR, both successfully employed in the evaluation of fern species relationships including *Blechnum* (Shepherd et al., 2007). The primers used are as follows (Table 2): universal primers D, E, and F for trnL-trnF (Taberlet et al., 1991; Coskunlebi et al., 2012); FERN1 (Shepherd et al., 2007) and 2 new primers designed for this work (LF-BLEC1 and LF-BLEC2R); 1F and 22R for trnL-trnG (Nagalingum et al., 2007) and 4 new primers developed for this work (GR-BLEC1, GR-BLECR1, GR-BLEC2, and GR-BLECR2).

PCR was performed according to protocols specified for Quiagen Taq polymerase and previously published

Table 1. Biological materials ordered by species with indication of country, collector, herbarium, and GenBank accession number (the first stands for trnL-trnF and the second for trnG-trnR). All cited accessions correspond to new sequences.

Taxa	Voucher information: country, collector, and herbarium		trnL-trnF GenBank accession number	trnG-trnR GenBank accession number
<i>Blechnum australe</i>	Argentina	Prada s/n (MA)	JQ907366	JQ907347
<i>Blechnum brasiliense</i>	Argentina	Prada s/n (MA)	JQ907369	JQ907348
<i>Blechnum fragile</i>	Costa Rica	Gabriel y Galán s/n (MA)	JQ907372	JQ907349
<i>Blechnum laevigatum</i>	Argentina	Prada s/n (MA)	JQ907374	JQ907350
<i>Blechnum loxense</i>	Peru	Gabriel y Galán s/n (MA)	JQ907375	JQ907351
<i>Blechnum magellanicum</i>	Argentina	Prada s/n (MA)	JQ907376	JQ907352
<i>Blechnum microphyllum</i>	Argentina	Prada s/n (MA)	JQ907377	JQ907353
<i>Blechnum mochaenum</i>	Argentina	Prada s/n (MA)	JQ907378	JQ907354
<i>Blechnum moritzianum</i>	Argentina	Prada s/n (MA)	JQ907379	JQ907355
<i>Blechnum penna-marina</i>	Argentina	Prada s/n (MA)	JQ907380	JQ907356
<i>Blechnum sampaoianum</i>	Brazil	Silva 1651 et al. (UC)	JQ907381	JQ907357
<i>Blechnum schomburgkii</i> 1	Costa Rica	Gabriel y Galán s/n (MA)	JQ907383	JQ907358
<i>Blechnum schomburgkii</i> 2	Peru	Gabriel y Galán s/n (MA)	JQ907384	JQ907359
<i>Blechnum serrulatum</i>	Argentina	Prada s/n (MA)	JQ907385	JQ907360
<i>Blechnum spicant</i>	France	Gabriel y Galán s/n (MA)	JQ907386	JQ907361
<i>Blechnum stipitellatum</i>	Ecuador	Neill & Manzanares 13118 (UC)	JQ907388	JQ907362
<i>Blechnum tabulare</i>	Argentina	Prada s/n (MA)	JQ907389	JQ907363
<i>Salpichlaena volubilis</i>	Costa Rica	Gabriel y Galán s/n (MA)	JQ907390	JQ907364
<i>Woodwardia radicans</i>	Spain	Gabriel y Galán s/n (MA)	JQ907391	JQ907365

Table 2. Primers used in current work to amplify 2 chloroplastic regions, trnL-trnF and trnG-trnR. Some of these primers were used previously with ferns; proper references are given. Other primers were developed ad-hoc in the current study for *Blechnum*.

Region	Name	Sequence (5'→3')	Source
trnL-F	FERN1	GGCAGCCCCCARATTCAGGGRAACC	Previous works (Shepherd et al., 2007)
	F	ATTTGAACTGGTGACACGAG	Previous works (Taberlet et al., 1991; Coşkunçelebi et al., 2012)
	E	GGTTCAAGTCCCTCTATCCC	Previous works (Taberlet et al., 1991; Coşkunçelebi et al., 2012)
	D	GGGGATAGAGGGACTTGAAC	Previous works (Taberlet et al., 1991; Coşkunçelebi et al., 2012)
	LF-BLEC1	ATAGGTACAGAGACTCGACG	Current work
	LF-BLEC2R	ACGGATAAAACTCAACTAAA	Current work
	1F	GCGGGTATAGTTTAGTGGTAA	Previous works (Nagalingum et al., 2007)
	22R	CTATCCATTAGACGATGGACG	Previous works (Nagalingum et al., 2007)
trnG-R	GR-BLEC1	CTCAACTAAATCAAAAAGCTTTA	Current work
	GR-BLECR1	CAAATCGTGAACAAACGCTAAC	Current work
	GR-BLEC2	AGTCGTACCGGTTAGTGACG	Current work
	GR-BLECR2	GCGGGAATCGAACCCGCAT	Current work

procedures (Aydın et al., 2013), with the following reaction conditions: 1 U of Taq polymerase (5 U/μL), 5 μL at 10X buffer (which included 15 mM of MgCl₂), 2 μL of each primer at 5 μM, 1 μL of dNTPs at 10 mM, and dH₂O to a final volume of 50 μL with 1–3 μL of DNA template. Cycling conditions were as follows; for trnL-trnF: 35 cycles of 1 min at 94 °C, 1 min at 51–56 °C (depending on the primer pairs), 1 min 30 s at 72 °C plus an extension period of 6 min at 72 °C; for trnG-trnR: 35 cycles of 1 min at 94 °C, 1 min at 50–58 °C (depending on the primer pairs), 2 min 30 s at 72 °C plus an extension period of 10 min at 72 °C. Cycling programmes were initiated with 5 min at 95 °C for enzyme activation. PCR products were checked by 1% agarose gel electrophoresis. Sizes were about 800 pb for trnL-trnF and 900 pb for trnG-trnR. Amplification products were purified using semi-automated kits (Quiagen PCR purification kit), following manufacturer instructions; binding DNA to a column, double washing, and eluting to obtain a final clean volume of 40–60 μL.

Sequencing was conducted at the Genomic Service, Universidad Complutense de Madrid (ABI Prism 3730, Applied Biosystems) and an external service (Macrogen, ABI Prism 3730XL, Applied Biosystems).

2.3. Data analysis

We obtained 38 useful sequences, 19 from each cpDNA region, and they have been published in GenBank (Table 2). Geneious 5.5 software (Drummond et al., 2011) was used to edit the sequences and create an alignment. All ambiguous sites were removed from the sequences. Finally, datasets from both trnL-trnF and trnG-trnR regions were concatenated into a single, 2-locus dataset that reached a total of 1671 sites.

The software jModeltest 2.1 (Darriba et al., 2012) was used for estimating evolutionary models and MEGA5

(Tamura et al., 2011) for conducting molecular analyses. Indels were coded as binary characters (Simmons & Ochoterena, 2000). Trees were constructed using the maximum likelihood method based on the GTR+G model. Bootstrap test values were inferred from 1000 replicates. A heuristic search was done by automatically obtaining the initial trees and applying the nearest neighbour interchange (NNI) algorithm.

3. Results

The maximum likelihood phylogram for cpDNA trnG-trnR + trnL-trnF combined regions (Figure 2) for the 19 species shows that most of the morphological groups slated for evaluation are well maintained in the tree. *Blechnum brasiliense* Desv. (usually considered part of *B. tabulare* group) and *B. spicant* (related to *B. penna-marina* group) are the only 2 species that appear separate from their respective morphological groups.

Blechnum serrulatum appears in the base of the tree and forms a group with *S. volubilis*. This clade is very well supported with high bootstrap values (99%) and occupies a position sister to the rest of taxa. *Blechnum spicant*, very distant from its supposed relatives (*Blechnum penna-marina* group), occupies a position sister to the rest of the taxa and is also highly supported (100%). The rest of species form a large, well-supported (93%) clade, sister to *B. spicant*.

Within this clade there are 2 branches; one comprises *Blechnum cordatum*, *B. loxense*, and *B. tabulare* groups and the other forms the rest of the groups (i.e. *Blechnum fragile*, *B. occidentale*, *B. divergens*, and *B. penna-marina* groups). Segregation between the branches is not well defined as

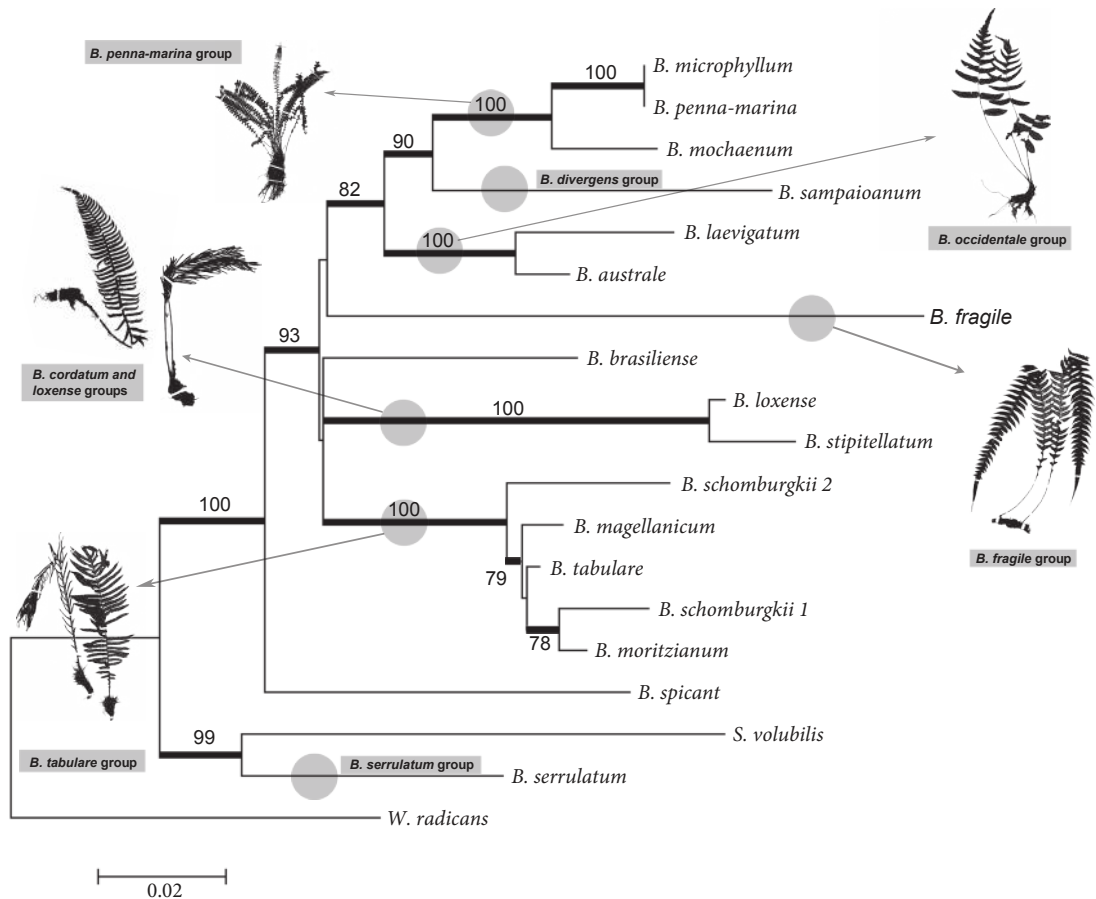


Figure 2. Maximum likelihood consensus phylogram of American groups of *Blechnum*, based on 2 cpDNA regions (trnG-trnR + trnL-trnF). Branches with bootstrap support values >70% have been thickened. Circles and silhouettes plotted over the tree indicate situation of the morphological groups. Tree is drawn to scale, with branch lengths measured in number of substitutions per site.

these 2 clades are not supported by the bootstrap test.

The first clade presents an unresolved polytomy with 3 branches: cordatoids (*B. cordatum* group) + *B. loxense* group, arborescents (*B. tabulare* group), and *B. brasiliense* alone. The studied species representing the *B. cordatum* and *B. loxense* groups form a highly supported (100%) clade. The arborescent species group, without *B. brasiliense*, also forms a clear, well-supported (100%) monophyletic clade. Sequences of 2 individuals of *B. schomburgkii* from different geographical locations appeared at different positions within this group. *Blechnum brasiliense* appeared alone in its own phylogenetic lineage but quite near the arborescent group in which it was traditionally placed.

The second large clade includes small-to-medium-sized herbaceous plants and epiphytes. The group of *Blechnum* epiphytes, represented in the tree by *B. fragile*, is sister to the rest of the groups. The last 3 groups form a well-supported clade with an 82% bootstrap value. The *B. occidentale* group forms a monophyletic lineage

with bootstrap value support of 100% and is sister to a clade that comprises the *B. divergens* (represented by *B. sampaioanum* Brade) and *B. penna-marina* groups, which are also well supported (90%).

4. Discussion

Several previous molecular works stated that *Blechnum* is not a monophyletic genus (Cranfill et al., 2001), a fact also derived from our analyses, as *Salpichlaena volubilis* appeared nested within *Blechnum*. Other known genera that are also embedded in *Blechnum* are *Doodia* (Schuettpelz & Pryer, 2007; Shepherd et al., 2007) and *Sadleria* (Shepherd et al., 2007).

The morphological groups proposed to arrange American *Blechnum* (Tryon & Tryon, 1982) are mostly maintained under our molecular analyses, with few notable exceptions. Main phylogenetic lineages supported by high bootstrap values seem to have diverged in correlation with basic sets of morphological characters.

Blechnum serrulatum was included alone in the *B. serrulatum* group (Tryon & Tryon, 1982) due to distinctive morphology characterised, for example, by articulate pinnae (Rolleri et al., 2010). In our molecular study *B. serrulatum* is strongly related to American *Salpichlaena volubilis* and, in previous molecular reports, to American *S. thalassica* Grayun & R.C.Moran and Philippine *Stenochlaena milnei* Underw. (Cranfill & Kato, 2003). All of these species share interesting morphological features. *Salpichlaena* J.Sm. and *Stenochlaena* J.Sm. are high-climbing hemi-epiphytic ferns with 1- or 2-pinnate fronds. *Blechnum serrulatum* is a terrestrial, 1-pinnate species, but it has also been considered a climber in some localities (Rolleri et al., 2010). Some species of *Stenochlaena* such as *S. palustris* live in swamps, like *B. serrulatum* (known as swamp fern). Finally, *B. serrulatum* and *Salpichlaena* species have spores with orbiculate perispores carrying irregularly arranged rugose orbicules (Tryon & Lugardon, 1991; Giudice et al., 2008; Passarelli et al., 2010). Although it has been stated that these orbicules or spherules are common in *Blechnum* (Tryon & Lugardon, 1991), we have only seen them in *B. serrulatum* and *B. fernandezianum*, which belong to the *B. penna-marina* group (Passarelli, 2007; Passarelli et al., 2010). The major differences between these taxa are the absence of indusia in *Stenochlaena*, and the fact that *B. serrulatum* is monomorphic while all *Salpichlaena* and *Stenochlaena* species are strongly dimorphic. In conclusion, *Blechnum serrulatum* is quite far from the rest of *Blechnum* and may deserve a distinct taxonomic treatment related to *Salpichlaena*.

The case of *Blechnum spicant* is striking. In all of the analyses performed it always appears in an isolate branch at the base of the tree as sister to the rest of the groups. According to its morphological traits, the species was placed within the *Blechnum penna-marina* group (Rolleri & Prada, 2006b); habit, rhizome scales, frond architecture, spores, and foliar dimorphism clearly informs this relationship. The reason why genetic data segregate *B. spicant* and place it so far from its presumed group could be related to its distribution. Effectively, this is the only *Blechnum* species with a temperate northern distribution (North Africa, Europe to the Caucasus, and North America). Further analyses are required to precisely determine the nature of the morphological set shared between *B. spicant* and *B. penna-marina* group, particularly if it is due to convergence with independent origins. In addition, analyses should be performed to investigate relationships with *Woodwardia*, the only other *Blechnaceae* with a temperate northern distribution (Kramer et al., 1990). We propose the creation of a new group, the monotypic *B. spicant* group, to include herbaceous, stoloniferous species with dimorphic fronds and adnate pinnae coming from temperate, northern continents.

Concerning the rest of the morphological groups, which could be called core *Blechnum*, 5 main lineages appear as well-defined clades. These tend to form 2 groups, core *Blechnum* I and core *Blechnum* II: I) arborescents (*B. tabulare* group) + *B. brasiliense* + cordatoids (*B. cordatum* + *B. loxense* groups); II) epiphytes (*B. fragile* group) + herbaceous, small or medium-sized species (*B. occidentale* + *B. divergens* + *B. penna-marina* groups).

The group comprising the arborescent and sub-arborescent species of *Blechnum*, the so-called *Blechnum tabulare* group (Tryon & Tryon, 1982), forms a monophyletic clade if *B. brasiliense* is extracted. This arborescent clade is supported by many synapomorphic characters. Some morphological ones include the stout, massive vertical rhizomes, forming a more or less tall caudex; the long, acicular scales; an epidermal type of more-or-less rectangular cells; and densely folded spores (Ramos Giacosa, 2008; Rolleri et al., 2008; Passarelli et al., 2010; Rolleri et al., 2013). They also present some shared nucleotide indels in the trnL-trnF region.

In our molecular tree and previous trees (Shepherd et al., 2007) *Blechnum brasiliense* is segregated from this arborescent group, in which it has been traditionally included (Rolleri et al., 2013). Some morphological features beyond the arborescent habit could support this position; *B. brasiliense* is the only arborescent species with monomorphic fronds and adnate pinnae that become reduced towards the base of the lamina. Apart from its evident molecular connection to the rest of the arborescent species, *B. brasiliense* is known for its strong relationship to the Asian-Austro-Pacific *Doodia* (Shepherd et al., 2007). There is no morphological evidence to support this relationship, as *Doodia* is very different from *Blechnum* in its anastomosing veins and discrete sori. Regardless, we think the available molecular data suggest that *B. brasiliense* merits an isolated taxonomic position within *Blechnum*, with the same rank as the rest of the groups.

Blechnum cordatum and *B. loxense* groups come together to form a highly supported group that is related to the arborescent clade and also to *B. brasiliense*. The phylogenetic position of *B. loxense*, the only representative of its group (Tryon & Tryon, 1982), has been debated for a long time. Its isolated situation is sustained by a number of unique and interesting morphological features such as the strongly recurved pinnae with papillate-digitate marginal cells, papillate indusium, mucilaginous rhizomes, and slight dimorphism (Rolleri et al., 2012a). Similarities between *B. stipitellatum* (a cordatoid element) and *B. loxense* have been reported to such an extent that both have been considered mere shadow forms (Tryon & Stolze, 1993). With the addition of the results from the current study, we propose joining both morphological groups into one evolutionary lineage, as the basic cordatoid

morphologies (habit and general frond architecture) are also present in *B. loxense*.

The second large lineage core *Blechnum* II concerns terrestrial or epiphytic herbaceous plants that have slender, horizontal or vertical rhizomes and are never massive or arborescent. We include here 2 different morphological clades that are well supported by molecular data. The first is the group of epiphytic species (*B. fragile* group), which is strongly supported by both morphology (habit, foliar dimorphism, and adnate pinnae) and molecular information (one large nucleotide indel in the trnL-trnF region). There is a bibliographic consensus for treating this group as a natural entity, an idea reinforced by our results. The second is a large clade comprising 3 different morphological groups: *B. divergens*, *B. occidentale*, and *B. penna-marina*. These species are either monomorphic (*B. occidentale* group) or dimorphic (*B. penna-marina* and *B. divergens* groups).

We included *Blechnum sampaioanum*, which was previously part of the *B. lineatum* (Sw.) C.Chr. group (Tryon & Tryon, 1982), as representative of the *B. divergens* group. Morphological characters (Dittrich, 2005; Prada et al., 2007) and our molecular results unquestionably segregate both groups. Chloroplast data relate the *B. divergens* group to *B. penna-marina* and *B. occidentale* groups, far from the cordatoids. In addition, despite the gross resemblance of species of this group and elements of the *B. fragile* group, our results indicate a molecularly evident difference between them.

The *Blechnum occidentale* group is quite distinctive because it contains monomorphic or subdimorphic herbaceous plants with pinnatisect or mostly 1-pinnate fronds (Rolleri et al., 2012b), and it has been treated as *Blechnum* subgen. *Blechnum* (Murillo, 1968). This group deserves more attention, due to the high incidence of polyploidy and hybridisation processes (Murillo, 1968; Rolleri & Prada, 2006a).

The *Blechnum penna-marina* group is an evidently monophyletic clade, from both a morphological (Rolleri & Prada, 2006b; Passarelli, 2007) and molecular perspective, with the exception of *B. spicant*. From our analyses we can derive a virtually complete molecular similarity between *B. penna-marina* and *B. microphyllum*. This could explain the difficulty in distinguishing the 2 species morphologically (Rolleri & Prada, 2006b). Perhaps the combination of both species into a single entity is appropriate. In addition, the *B. penna-marina* group presents another outstanding feature; a large geographical disjunction. Several of its members, such as *B. penna-marina* and *B. blechnoides*, are distributed in South America and Oceania.

In conclusion, cpDNA demonstrated its value for deciphering some of the taxonomical problems in *Blechnum*. Most of the groups traditionally recognised with morphological characters are maintained in a molecular framework, so they can be seen as main natural evolutionary lineages within American *Blechnum*. By combining all the morphological and molecular information available, we propose the following 4 evolutionary lineages with their sub-clades:

- *Blechnum serrulatum* [+ *Salpichlaena*]
- *B. spicant*
- Core *Blechnum*
 - o Core *Blechnum* I
 - Arborescents (*B. tabulare* group without *B. brasiliense*)
 - Cordatoids (*B. cordatum* + *B. loxense* groups)
 - *B. brasiliense* [+ *Doodia*]
 - o Core *Blechnum* II
 - Epiphytes (*B. fragile* group)
 - Herbaceous terrestrials
 - Monomorphic (*B. occidentale* group)
 - Dimorphic (groups *B. divergens* + *B. penna-marina* without *B. spicant*)

It is obvious that more molecular accessions are needed to complete a comprehensive taxonomical ordering of American *Blechnum*. Morphological knowledge has already included all of the 8 informal groups (after reviewing most of the American diversity), and we feel with some confidence that new species will fit into the lineages we propose here. In the future we will be able to present a global phylogeny (the inclusion of more nucleotides will aid in statistically resolving some problematic branches) with proposals for formal taxonomic entities.

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References

- Aydın O, Coşkunçelebi K, Gültepe M & Güzel ME (2013). A contribution to taxonomy of *Centaurea* including *Psephellus* (Asteraceae) based on anatomical and molecular data. *Turkish Journal of Botany* 37: 419–427.
- Coşkunçelebi K, Terzioğlu S, Karaköşe M & Güzel ME (2012). Contributions to the description and molecular properties of *Erodium hendrikii* Alpinar (Geraniaceae), endemic to Turkey. *Turkish Journal of Botany* 36: 455–461.
- Cranfill R (2001). Phylogenetic Studies in the Polypodiales (Pteridophyta) with an Emphasis on the Family *Blechnaceae*. Berkeley, CA: University of California.
- Cranfill R, Nakahira Y & Kato M (2001). A molecular phylogeny of *Blechnaceae* inferred from three plastid gene sequences. *Fifth International Flora Malesiana Symposium Abstract*.
- Cranfill R & Kato M (2003). Phylogenetics, biogeography, and classification of the woodwardioid ferns (*Blechnaceae*). In: Chandra S & Srivastava M (eds.) *Pteridology in the New Millennium*. Netherlands: Kluwer Academic Publishers.
- Darriba D, Taboada G, Doallo R & Posada D (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772–772.
- Dittrich V (2005). Estudos taxonômicos no gênero *Blechnum* L. (Pteridophyta-Blechnaceae) para as regiões Sudeste e Sul do Brasil. Universidad Estadual Paulista Julio de Mesquita Filho, Brasil.
- Drummond A, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T & Wilson A (2011). Geneious 5.5. Website <http://www.geneious.com> [accessed 30 November 2010].
- Durán M (1997). Estudios morfológicos, taxonómicos y biosistemáticos en el género *Blechnum* (Blechnaceae-Pteridophyta). Universidad Nacional de Córdoba, Córdoba, Argentina.
- Gabriel y Galán JM, Passarelli LM, Prada C & Rolleri CH (2008). Sporophyte morphology and gametophyte development of the fern *Blechnum sprucei* (Pteridophyta: Blechnaceae). *Revista de Biología Tropical* 56: 2027–2040.
- Gabriel y Galán JM, Prada C, Rolleri CH, Lahoz-Beltrá R & Martínez-Calvo C (2011). A biometrical study of stomata in *Blechnum* species (Blechnaceae) with some taxonomic and ecological implications for the ferns. *Revista de Biología Tropical* 59: 403–415.
- Giudice GE, Luna ML, Carrion C & de La Sota ER (2008). Revision of the genus *Salpichlaena* J. Sm. *American Fern Journal* 98: 49–60.
- Kramer K, Chambers T & Hennipman E (1990). *Blechnaceae*. In: Kramer K & Green P (eds.) *The Families and Genera of Vascular Plants: I. Pteridophytes and Gymnosperms*. Berlin: Springer.
- Kuo L-Y, Li F-W, Chiou W-L & Wang C-N (2011). First insights into fern matK phylogeny. *Molecular Phylogenetics and Evolution* 59: 556–566.
- Murillo M (1968). *Blechnum* subgénero *Blechnum* en Sur América, con especial referencia a las especies de Colombia. *Nova Hedwigia* 16: 329–366.
- Nagalingum N, Schneider H & Pryer K (2007). Molecular phylogenetic relationships and morphological evolution in the heterosporous fern genus *Marsilea*. *Systematic Botany* 32: 16–25.
- Passarelli L (2007). Estudios esporales en especies del grupo *Blechnum penna-marina* (Blechnaceae-Pteridophyta). *Acta Botanica Malacitana* 32: 1–19.
- Passarelli L, Gabriel y Galán JM, Prada C & Rolleri CH (2010). Spore morphology and ornamentation in the genus *Blechnum* (Blechnaceae, Pteridophyta). *Grana* 49: 243–262.
- Prada C, Rolleri C & Passarelli L (2007). Morfología, caracterización y distribución geográfica de *Blechnum cordatum* (Blechnaceae-Pteridophyta). *Acta Botanica Malacitana* 33: 29–46.
- Ramos Giacosa JP (2008) Revisión sistemática, análisis cladístico y biogeográfico de la sección *Lomariocycas* (J. Sm.) C. V. Morton del género *Blechnum* L. (Blechnaceae, Pteridophyta) en América. Universidad Nacional de La Plata, La Plata, Argentina.
- Rolleri C & Prada C (2006a). Catálogo comentado de las especies de *Blechnum* (Blechnaceae-Pteridophyta) de Mesoamérica y Sudamérica. *Anales del Jardín Botánico de Madrid* 63: 67–106.
- Rolleri C & Prada C (2006b). Revisión de los grupos de especies del género *Blechnum* (Blechnaceae-Pteridophyta): el grupo *B. penna-marina*. *Acta Botanica Malacitana* 31: 7–50.
- Rolleri C, Prada C & Passarelli L (2008). Estudios morfológicos y taxonómicos en *Blechnum* (Blechnaceae-Pteridophyta): *Blechnum tabulare* y *B. magellanicum*. *Anales del Jardín Botánico de Madrid* 65: 179–195.
- Rolleri C, Prada C, Gabriel y Galán JM, Passarelli LM & Ciciarelli MM (2010). Morphology of the sporophyte and gametophyte of the swamp fern, *Blechnum serrulatum* (Blechnaceae, Pteridophyta). *Australian Journal of Botany* 58: 508–518.
- Rolleri C, Prada C, Passarelli L & Gabriel y Galán JM (2012a). Revisión de dos especies de *Blechnum* de las regiones montañosas tropicales e intertropicales de Centroamérica y Sudamérica. *Actes du Colloque Les Fougères d'Alsace, d'Europe et du Monde* 1: 149–161.
- Rolleri C, Prada C, Passarelli L, Gabriel y Galán JM & Ciciarelli MM (2012b). Revisión de especies monomórficas y subdimórficas del género *Blechnum* (Blechnaceae-Polypodiophyta). *Botanica Complutensis* 36: 51–77.
- Rolleri C, Prada C, Gabriel y Galán JM & Passarelli L (2013). Caracterización y revisión de ocho especies arborescentes del género *Blechnum* (Blechnaceae-Polypodiophyta). *Revista de Biología Tropical* 61: 377–408.
- Rothfels CJ, Larsson A, Kuo L-Y, Korall P, Chiou W-L & Pryer KM (2012a). Overcoming deep roots, fast rates, and short internodes to resolve the ancient rapid radiation of eupolypod II ferns. *Systematic Biology* 61: 490–509.

- Rothfels CJ, Sundue MA, Kuo L-Y, Larsson A, Kato M, Schuettpelz E & Pryer KM (2012b). A revised family-level classification for eupolypod II ferns (Polypodiidae: Polypodiales). *Taxon* 61: 515–533.
- Schuettpelz E & Pryer KM (2007). Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56: 1037–1050.
- Shepherd LD, Perrie LR, Parris BS & Brownsey PJ (2007). A molecular phylogeny for the New Zealand *Blechnaceae* ferns from analyses of chloroplast trnL-trnF DNA sequences. *New Zealand Journal of Botany* 45: 67–80.
- Simmons MP & Ochoterena H (2000). Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–81.
- Smith AR, Pryer KM, Schuettpelz E, Korall P, Schneider H & Wolf PG (2006). A classification for extant ferns. *Taxon* 55: 705–731.
- Taberlet P, Gielly L, Patou G & Bouvet J (1991). Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.
- Tryon AF & Lugardon B (1991). *Spores of the Pteridophyta*. New York: Springer.
- Tryon RM & Tryon AF (1982). *Ferns and Allied Plants with Special Reference to Tropical America*. New York: Springer.
- Tryon RM & Stolze RG (1993). Pteridophyta of Peru. Part. V: Aspleniaceae-Polypodiaceae. *Fieldiana Botany* 32: 54–70.