





SYSTEMATICS AND PHYLOGENY

A multi-character analysis of *Struthiopteris* leads to the rescue of *Spicantopsis* (Blechnaceae, Polypodiopsida)

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Abstract The family Blechnaceae is a moderately sized leptosporangiate fern lineage, with 24 genera and around 250 species. *Struthiopteris* accommodates small to medium-sized, dimorphic, pinnate species. It is composed of six northern species: *S. spicant* is distributed in western parts of Europe and North America; *S. fallax* is endemic to Iceland; *S. niponica*, *S. amabilis* and *S. castanea* are endemic to Japan, and *S. hancockii* occurs in Japan and Taiwan. Due to the lack of a global review and to its highly interesting geographical distribution, this genus merits further study to clarify its taxonomy, species relationships, and distributional pattern. The present study aims to achieve the following goals: (a) identify and describe morphological characters supporting the taxonomy of *Struthiopteris*; (b) reconstruct a complete phylogeny for the genus; (c) study the biogeographical history of *Struthiopteris* at a global scale. The morphological study involved the observation of characters ranging from rhizome scales to spores over 164 individuals. Phylogenies were constructed applying ML and BI techniques over 51 newly produced sequences of three chloroplast markers (*rbcL*, *trnL-trnF*, *psbA-trnH*), using the species *Blechnidium melanopus* and *Brainea insignis* as closest relatives. For the molecular dating and historical biogeography analyses, we estimated and compared ancestral ranges under several models. Most of the morphological characters led us to discern two groups of species: the *S. spicant* group (*S. spicant*, *S. fallax*, and *S. castanea*) and the *S. niponica* group (the remaining species). In our molecular phylogeny, the supposed sister genus *Blechnidium* always appeared as nested within *Struthiopteris*, rendering this genus non-monophyletic. The two groups identified by the morphology appeared as monophyletic clades within *Struthiopteris*, with the clade *S. spicant* more closely related to *Blechnidium* than to the clade *S. niponica*. For all these reasons, we propose to rescue the now-disused genus *Spicantopsis* for the species belonging to the *S. niponica* group: indeed, this genus was created c. 100 years ago to reunite the same species *S. amabilis*, *S. niponica*, and *S. hancockii*. Our results suggest that all members of this group of genera (*Blechnidium*, *Struthiopteris*, *Spicantopsis*) emerged in East Asia about 85 mya, at a time when Japan was still part of the mainland. It appears that, for most of their history, the members of these genera have been confined to East Asia, with one dispersal to the Americas by an ancestor within *Struthiopteris* s.str., and additional dispersals to India and the Philippines by *Blechnidium melanopus*.

Keywords *Blechnidium*; historical biogeography; morphology; phylogeny

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

The family Blechnaceae is a moderately sized leptosporangiate fern lineage (Polypodiopsida), with around 250 species (Smith & al., 2006; PPG1, 2016). It has a subcosmopolitan distribution, with two important southern centers of diversity in South America and the Austropacific area, but with a few genera also occurring in temperate-cold zones of the Northern Hemisphere (Kramer & al., 1990). Historically, 9–10 genera were recognized within the family, with *Blechnum* L. being the most diverse and including

around 80% of the species in Blechnaceae (Kramer & al., 1990). Due to its high diversity, the genus' taxonomy was extremely complicated (Rolleri & Prada, 2006; Passarelli & al., 2010; Rolleri & al., 2012; Dittrich & al., 2015). Based on recent phylogenetic studies (Shepherd & al., 2007; Gabriel y Galán & al., 2013; Perrie & al., 2014; Gasper & al., 2017) a new classification has been published in which the family has been split into 24 genera, which greatly affected *Blechnum* in particular, drastically reducing its diversity (Gasper & al., 2016; PPG1, 2016). In general (and excluding the monotypic genera in the new classification),

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our knowledge about the taxonomy and phylogeny within each of the new, and also some of the old, genera of Blechnaceae is still far from being resolved, mainly due to the restricted distribution of many of the species, which as a result occur infrequently in herbaria. Therefore, it is necessary to continue detailed molecular and morphological studies on the terminal lineages of the family.

One of the cases that needs further investigation is the genus *Struthiopteris* Scop., the subject of the present study. Gasper & al. (2016) recovered this disused genus (Ching, 1940; Tagawa, 1952) in their recent classification in order to accommodate the small to medium-sized, dimorphic, pinnate species of *Blechnum* (Fig. 1A). In its current circumscription, *Struthiopteris* is composed of six species distributed in the Northern Hemisphere. The most widely distributed is *Struthiopteris spicant* (L.) Weiss, which has a wide holarctic distribution with two disjunct centers: (a) Europe, where it is very common in territories from Sweden to Romania and westwards to Iceland and the Iberian Peninsula; it is present but extremely rare in eastern Europe, North Africa and the Macaronesian archipelagos (Canary Islands, Madeira, and the Azores); and (b) the Pacific coast area of North America (both the United States (California, Oregon, Washington, Alaska) and Canada (Fig. 1B). *Struthiopteris fallax* (Lange) S.Molino & al., which has only recently been recognized at the species level (Molino & al., 2019), is endemic to Iceland

(Fig. 1B). The rest of the species in *Struthiopteris* are restricted to East Asia, with an obvious center of diversity in Japan: *S. niponica* (Kunze) Nakai, *S. amabilis* (Makino) Ching, and *S. castanea* (Makino & Nemoto) Nakai are all endemic to the Japanese archipelago, and *S. hancockii* (Hance) Tagawa occurs there as well as in Taiwan (Fig. 1C) (GBIF, 2018).

The taxonomy of the species of *Struthiopteris* has been discussed previously by several other authors. *Struthiopteris amabilis*, *S. niponica*, and *S. hancockii* were considered as a different genus, *Spicantopsis* Nakai (Nakai, 1933), until Ching (1940) (see also Tagawa, 1952) decided to recombine them into *Struthiopteris* just a few years later. Regarding *S. spicant*, some authors considered that the American populations show more affinity to *S. niponica* than to the European populations of *S. spicant* (Löve & Löve, 1966), resulting in the description of a new subspecies (*Blechnum spicant* subsp. *nipponicum* (Kunze) Á.Löve & D.Löve), based largely on cytotoxic data (i.e., chromosome counts) (though information from additional Asian species was not considered according to Nakato, 1987). Furthermore, some of the species of *Struthiopteris* (for example, *S. spicant*, *S. niponica*, and *S. castanea*) show a high level of phenotypic plasticity (Tagawa, 1936; Löve & Löve, 1966, 1968; Iwatsuki, 1992), which has resulted in the description of numerous ecological forms and varieties (up to 22 in the case of *S. spicant*; see Löve & Löve, 1968).

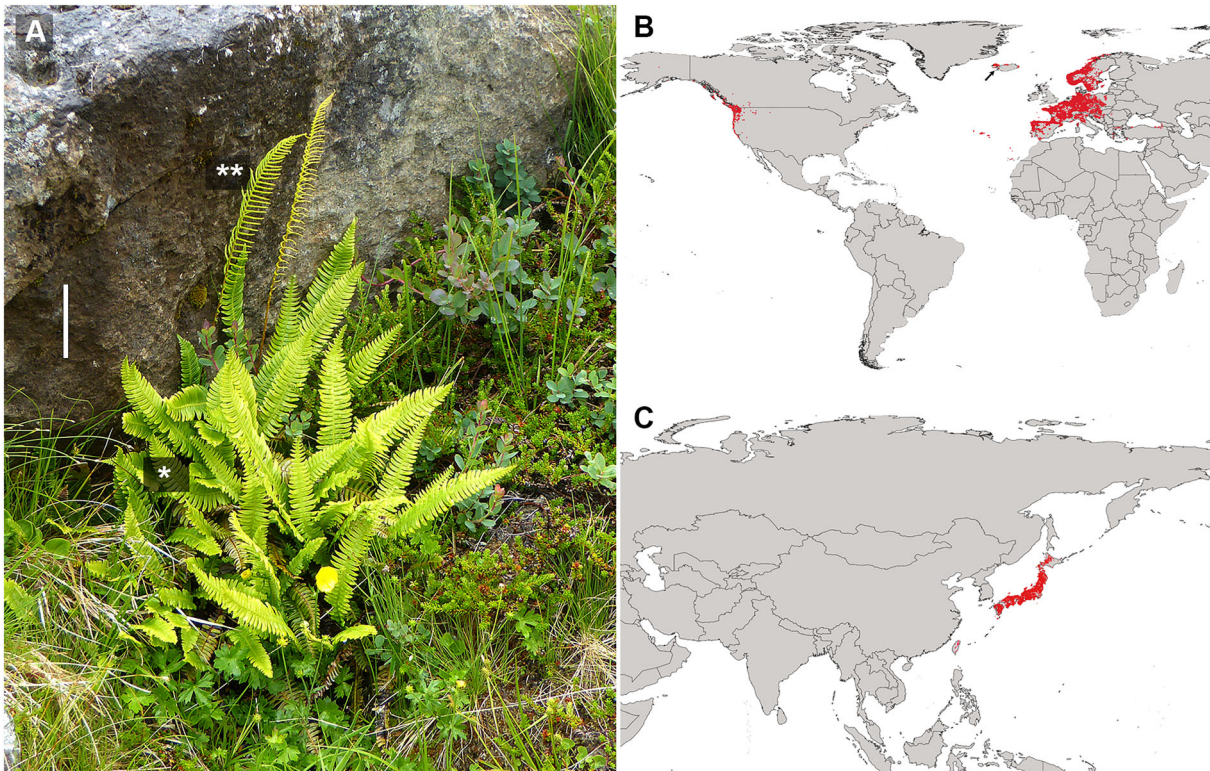


Fig. 1. **A**, Main features of a representative *Struthiopteris* species (*S. spicant*): medium-sized plants, dimorphic, with pinnate sterile fronds (*) and longer fertile fronds (**), with strongly contracted pinnae. Bar = 10 cm. Photograph by J.M. Gabriel y Galán (2016). **B**, Distribution map of *S. spicant* and *S. fallax* (black arrow pointing to Iceland). **C**, Combined distribution map of the rest of the species in *Struthiopteris*. Chorological data extracted from GBIF (2018).

Up to now, no completely sampled phylogenetic analysis for *Struthiopteris* has been conducted and only some species have been included in more general phylogenetic studies of the Blechnaceae. For example, Shepherd & al. (2007) used a cultivated sample of unknown origin of *S. spicant* (sub *Blechnum spicant* (L.) Roth) in their phylogeny of the New Zealand Blechnaceae. In a study that combined morphology and molecular phylogenetics of the American functional groups of Blechnaceae, Gabriel y Galán & al. (2013) again included *S. spicant* (sub *B. spicant*). The same species was evaluated by Perrie & al. (2014) in an approach to a global phylogeny of the family, along with *S. amabilis* (sub *B. amabile* Makino) and *S. hancockii* (sub *B. hancockii* Hance). Finally, Gasper & al. (2017) reconstructed the largest phylogeny known to date for the Blechnaceae and included two *Struthiopteris* species: *S. spicant* and *S. castanea*. In these studies, *Struthiopteris* was recovered as related in various ways, and with different phylogenetic support (usually low), to other genera such as *Brainea* J.Sm., *Blechnidium* T.Moore, *Blechnopsis* C.Presl, and *Sadleria* Kaulf. (or their equivalents as species of *Blechnum* s.l.).

The genus *Struthiopteris* merits further study to clarify its taxonomy, species relationships, and distributional pattern. These are the three main points of interest. First, there is a need to search for alternative morphological characters and evaluate them over a larger sample of individuals, as some interesting characters of known utility have not been described for every *Struthiopteris* species (e.g., rhizome scales and the anatomy of the pinnae) (Rolleri & Prada, 2006; Rothfels & al., 2012; Prada & al., 2016; Vicent, 2017). Second, and in part for the same reason, it is necessary to propose a comprehensive and well-supported hypothesis of species relationships in this group. Finally, there is a clearly understudied aspect of the family Blechnaceae in general, which is its geographical distribution pattern and the historical events that have led to current distributions (Moran & Smith, 2001; Vicent & al., 2017). In this sense, *Struthiopteris* emerges as a paradigmatic example of a complex distribution, with a set of species confined to a geographically reduced area (Japan) and another species with an intriguing extreme disjunction (i.e., western parts of the European and American continents).

In this context, the present study aims to achieve the following goals: (a) Identify and describe morphological characters supporting the taxonomy of *Struthiopteris*; (b) Reconstruct a complete phylogeny for the genus; (c) Study the biogeographical history of *Struthiopteris* at a global scale.

■ MATERIALS AND METHODS

Plant material. — This study involved the use of 164 specimens belonging to the following taxa: *Struthiopteris spicant* var. *spicant*, *S. spicant* var. *homophyllum* (Merino) Gabriel y Galán & R.Pino, *S. spicant* var. *pradae* S.Molino

& Gabriel y Galán, *S. fallax*, *S. castanea*, *S. niponica* var. *niponica*, *S. niponica* var. *minima* (Tagawa) Masam., *S. amabilis*, and *S. hancockii*. Part of the material of *S. spicant* and *S. fallax*, and a few specimens of *S. niponica* came from new field collections throughout the Iberian Peninsula, Iceland, and Japan; a voucher of each collection was prepared and stored at the Herbarium of the Faculty of Biology, Universidad Complutense, Madrid (MACB). The remaining specimens were loaned from the following herbaria: National Museum of Nature and Science, Tokyo (TNS), Naturhistoriska Riksmuseet, Stockholm (S) and Muséum National d'Histoire Naturelle, Paris (P). Also, some digital, high-resolution images of *S. castanea* and *S. hancockii* were reviewed through web-platforms of the herbaria TNS (National Museum of Nature and Science, 2008) and the National Taiwan University TAI (Herbarium of National Taiwan University, 2012), and of *S. spicant* from the Real Jardín Botánico de Madrid MA (Real Jardín Botánico de Madrid, 2018). All the material used in this work is cited in Appendix 1.

Morphological and anatomical study. — All characters were observed in no fewer than nine individuals per taxon, except the following: for *S. niponica* var. *minima*, the herbarium sheet we could examine (with three different individuals on it) was an isotype, so we lacked available material for the anatomical study; and *S. fallax*, because it is a rare species with reduced and endangered populations, of which we were able to examine only three individuals.

The following characters were observed: rhizome scales, general macro-morphology of the fronds, and anatomy of both fertile and sterile pinnae, epidermis, and spores.

Macromorphological characters were observed by eye and with the use of a stereoscopic microscope (Leica EZ4D). For the anatomical observations, common protocols in plant microscopy (Ruzin, 1999) were applied over hand-made sections. Anatomical sections were stained with TBO (toluidine O Blue) 1%, which has been used previously in several anatomical studies of ferns (Prada & al., 2016; Vicent, 2017), and studied under a light compound microscope (Nikon Labophot 2 with a camera Coolpix MDC). In addition, we checked scales, fronds, and spores in three individuals of *Blechnidium melanopus*, the only species of that genus, which was previously found to be the sister group to *Struthiopteris* (Gasper & al., 2017).

PCR and sequencing. — Specimens used for the molecular study are listed in Appendix 2. Total DNA was extracted from dried material (≈ 20 mg) with a DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.) following the manufacturer's protocols. PCR was used to amplify three plastid regions that have been widely used in the evaluation of fern phylogenetic relationships: *rbcL*, *trnL-trnF*, and *psbA-trnH*. The polymerase chain reaction (PCR) protocol was as follows: 1 U Taq polymerase (5 U/mL), 5 mL buffer (containing 15 mM MgCl₂), 3 mL of each primer (5 mM), 1 mL dNTPs (10 mM), 5 mL BSA (2.5 g/L), 10 mL sol Q (Qiagen, Hilden, Germany), and dH₂O to a final volume of

50 mL with 1–3 mL of DNA template. Primers and reaction conditions followed those of previous studies; for *rbcL*: 1F/1361R, 35× [45 s 94°C, 1 min 55°C, 2 min 72°C] + 10 min 72°C (Vicent & al., 2017; Sessa & al., 2018); for *trnL-trnF*: Fern1/F, 35× [1 min 94°C, 1 min 55°C, 1 min 30 s 72°C] + 16 min 72°C (Gabriel y Galán & al., 2013; Vicent & al., 2017; Sessa & al., 2018); for *psbA-trnH*: psbA39f/trnHf, 28× [1 min 94°C, 1 min 48°C, 1 min 72°C] + 7 min 72°C (Sessa & al., 2018). PCR products were checked using 1% agarose gel electrophoresis. After purification (QIAquick PCR Purification kit, Qiagen), samples were sequenced on an ABI3730XL sequencer (Macrogen, Amsterdam, the Netherlands). We generated 51 new sequences (Appendix 2).

Phylogenetic analyses. — We used Geneious R11 (<http://www.geneious.com>, Kearse & al., 2012) to edit sequences and assemble contigs. Alignments (suppl. Appendix S1) were constructed using the ClustalW v.2.1 algorithm (Larkin & al., 2007), with the following conditions: gap open cost 15 and gap extend cost 6. As chloroplast markers are linked and behave as a single non-recombining marker (Naumann & al., 2011), our three individual markers were concatenated in a single matrix that was analyzed as a whole but partitioned by marker for model assignment. We carried out maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses. MP analyses were conducted with the software MEGA7 (Kumar & al., 2016), ML analyses with PhyML v.2.2.3 (Guindon & Gascuel, 2003), and BI analyses with MrBayes v.2.0.9 (Ronquist & Huelsenbeck, 2003), using the best models of nucleotide evolution identified for the data partitions by jModelTest v.2 (Darriba & al., 2012). The following conditions were set in each case. For MP: subtree-pruning-regrafting (SPR) heuristic for tree inference and a bootstrap analysis with 500 replicates to determine branch support. For ML: nearest-neighbor-interchange (NNI) heuristic search method and a bootstrap analysis with 500 replicates to determine branch support. For BI: Multiple chains (4), chain length 5,100,000 generations with burn-in of 100,000 generations and unconstrained branch lengths. We checked for stationarity in the Bayesian analysis by evaluating the posterior probability distribution in Tracer 1.6 (Rambaut & al., 2014). We included in the analyses the following species, due to their suggested phylogenetic position as close relatives to *Struthiopteris* (Gasper & al., 2017): *Blechnidium melanopus* (Hook.) T.Moore, *Blechnopsis orientalis* (L.) C.Presl, *Brainea insignis* (Hook.) J.Sm., *Cleistoblechnum eburneum* (Christ) Gasper & Salino, *Sadleria cyatheoides* Kaulf., *Telmatoblechnum indicum* (Burm.f.) Perrie, D.J.Ohlsen & Brownsey, and *Woodwardia prolifera* Hook. & Arn. The latter was set as outgroup.

Molecular dating and historical biogeography. — We conducted molecular dating analyses using BEAST v.2.4.2 (Bouckaert & al., 2014). The fossil record of Blechnaceae is sparse, with only a handful of reliably identified fossils available for calibration of divergence time analyses (see

Vicent & al., 2017 for discussion). Phylogenetically, the closest unambiguously assigned fossil belongs to *Onoclea sensibilis* (Rothwell & Stockey, 1991), a member of the Onocleaceae, the sister family to the Blechnaceae. Our sampling did not allow us to include this fossil as a calibration point, and so instead we used a secondary age estimate derived from an earlier study by members of our research group on divergence times in another group of Blechnaceae (Vicent & al., 2017). In that analysis, we found the crown age of the clade that is the focus of the present paper, and which includes all members of *Struthiopteris*, *Blechnidium*, and *Brainea*, to be 72.2 million years old, with the 95% highest posterior density (HPD) interval for this node encompassing the time period 112–36 million years. We therefore modeled the age of this node in our analyses using a gamma prior distribution with the age set to 72.2 and alpha and beta set to 3.0 and 5.0, respectively, in order to capture the bulk of the HPD interval from the prior distribution in the present analysis.

The BEAST analysis used one representative of each taxon and an uncorrelated, lognormal relaxed clock model with a birth-death process tree prior and the best nucleotide substitution model identified for each locus as described above. The analysis ran for 20 million generations, with trees saved every 4000 generations and all other parameters every 200 generations. We examined the posterior distribution and estimated sample size (ESS) of all parameters with Tracer v.1.6 (Drummond & Rambaut, 2007), and determined that the analysis had run for sufficiently long when all ESS values were above 200. We used TreeAnnotator v.2.4.2 (Bouckaert & al., 2014) to combine and summarize a post-burn-in set of trees, compute the 95% HPD intervals for all node ages, and generate a maximum clade credibility chronogram for use in ancestral range estimation analyses.

We used BioGeoBEARS (Matzke, 2014) to estimate and compare ancestral ranges under several models: DEC (dispersal-extinction-cladogenesis; Ree & Smith, 2008), DIVA-like (dispersal-vicariance analysis; Yu & al., 2010), and BayArea-like (Landis & al., 2013). Each of these models was tested with and without the “jump dispersal” (j) parameter available in BioGeoBEARS. Likelihood ratio tests were used to identify the model that produced the most likely set of ancestral ranges. The analysis was time-stratified to accommodate changes in proximity of geographic areas over the last >65 million years, with six time “slices” in the analysis: >65 mya, 65–40 mya, 40–18 mya, 18–2.5 mya, 2.5–0.01 mya, and 0.01–0 mya. The latter three periods were constructed in order to account for glaciation in the Northern Hemisphere and the appearance of several island groups (e.g., Taiwan) over the last roughly 18 million years.

Dispersal parameters (Appendix 3) between geographic areas in each time slice were based on a survey of relevant studies that have performed similar analyses over similar temporal and spatial scales (Sessa & al., 2012; Spalink & al., 2016; Vicent & al., 2017). Ranges of extant taxa were determined from a survey of the literature.

RESULTS

Comparative morphology of *Struthiopteris*. — All the measured morphological traits, except the rhizome scales, were variable among species in a way that resulted principally in the differentiation of two distinct species group (Table 1). One of these, the *S. niponica* group includes the species *S. niponica*, *S. hancockii*, and *S. amabilis*, which share the following features: pale petioles (Fig. 2A), no epidermal hairs (Fig. 2C), stomata independent of the veins, sori with straight base (Fig. 2E) and long, thin, enrolled indusium (Fig. 2G), and spores with compact perispore (Fig. 2I). The other group, the *S. spicant* group, includes the species *S. spicant*, *S. fallax*, and *S. castanea*, which share the following features: dark petioles (Fig. 2B), epidermal hairs (Fig. 2D), stomata located along the veins, sori decurrent (Fig. 2F), indusium thick, complex, shorter and not or only slightly enrolled (Fig. 2H), and spores with alveolate perispore (Fig. 2J).

Phylogeny of *Struthiopteris*. — The combined dataset of *rbcL+trnL-trnF+psbA-trnH* was 2538 nucleotides in length and jModelTest identified HKY+ Γ as the best model of evolution for each marker. MP, ML and BI analyses produced trees sharing the same general topology (Fig. 3), including three main clades each supported by high statistical values (bootstraps and posterior probabilities). First is a clade formed by *Blechnidium melanopus* and the *S. spicant* group of species (*S. spicant* and its varieties plus *S. fallax* and *S. castanea*); this clade is supported by a posterior

probability of 1.00 and bootstrap support of 96.4% (ML) and 98.5% (MP). Second, within this clade, the *S. spicant* group is maximally supported as monophyletic and sister to *Blechnidium melanopus*. Within the *S. spicant* group, *S. castanea* was further maximally supported as sister to the remaining species. Third, a clade including the species of the *S. niponica* group also received maximal or nearly maximal support (BI PP = 1.0, ML BS = 100%, MP BS = 99%). The species *S. amabilis* is maximally supported as sister to the other taxa in this clade, while *S. niponica* (excluding var. *minima*) and *S. hancockii* were resolved as separate subclades but without support.

Biogeographical history of *Struthiopteris*. — The molecular dating analysis produced a topology identical to the MP, ML and BI analyses, with an estimated divergence between *Brainea insignis* and the remaining species about 85 mya (Fig. 4). The next split occurred roughly 81 mya, separating the *S. niponica* group from the *S. spicant* group plus *Blechnidium*. Divergences within those two groups began at 44 and ~60 mya, respectively. The earliest history of *Struthiopteris* and *Blechnidium* is inferred to have been in Asia, with the area that is now the Japanese archipelago having played a critical role as the ancestral range for much of the group's history. The *S. niponica* group has remained in Japan until the present, with one inferred dispersal to Taiwan (Fig. 4), while part of the *S. spicant* group eventually migrated to North America, Iceland, and Europe, where two extant taxa occur today.

Table 1. Comparative morphological features of plants belonging to *Struthiopteris* and *Blechnidium*. Two groups of taxa can be identified in *Struthiopteris*, the *S. niponica* group and the *S. spicant* group

	Rhizome scales	Colour of petiole	Epidermal hairs	Stomata position	Base of sori	Mature indusium	Perispore
<i>Struthiopteris niponica</i> group							
<i>S. amabilis</i>	Ovate, no sclerosed center	Pale	No	Not following veins	Straight, not decurrent	Thin, long, enrolled	Compact
<i>S. niponica</i>	Linear, sclerosed center	Pale	No	Not following veins	Straight, not decurrent	Thin, long, enrolled	Compact
<i>S. hancockii</i>	Linear, sclerosed center	Pale	No	Not following veins	Straight, not decurrent	Thin, long, enrolled	Compact
<i>Struthiopteris spicant</i> group							
<i>S. castanea</i>	Linear-lanceolate, sclerosed center	Dark	Yes	Following veins	Decurrent	Thick, short, not enrolled	Alveolate
<i>S. spicant</i> var. <i>spicant</i>	Linear-lanceolate, sclerosed center	Dark	Yes	Following veins	Decurrent	Thick, short, not enrolled	Alveolate
<i>S. fallax</i>	Lanceolate, no sclerosed center	Dark	Yes	Following veins	Decurrent	Thick, short, not enrolled	Alveolate
<i>S. spicant</i> var. <i>homophyllum</i>	Triangular, no sclerosed center	Dark	Yes	Following veins	Decurrent	Thick, short, not enrolled	Alveolate
<i>S. spicant</i> var. <i>pradae</i>	Linear-lanceolate, sclerosed center	Dark	Yes	Following veins	Decurrent	Thick, short, not enrolled	Alveolate
<i>Blechnidium</i>							
<i>B. melanopus</i>	Lanceolate, no sclerosed center	Dark	Yes	Following veins	Does not apply	Thin, short, not enrolled	Alveolate

DISCUSSION

We have conducted an integrated study using a multi-character approach to clarify the taxonomy of *Struthiopteris*, a genus recently resurrected by Gasper & al. (2016) to accommodate dimorphic, medium-sized blechnoid ferns. We have taken into consideration analyses of both morphological features (including macro- and micro-characters) as well as molecular sequence data.

The phylogeny shown here (Fig. 3), which includes all the taxa currently accepted in *Struthiopteris* (including all the varieties of *S. spicant* and *S. niponica*), clearly demonstrates that this genus is not a monophyletic entity in its

current circumscription. The main reason is that *Blechnidium melanopus*, a monomorphic fern with anastomosing veins, appears squarely nested within *Struthiopteris*, a phylogenetic position supported by high statistical values. Consequently, *Struthiopteris* appears to include members of two distinct clades: the *S. spicant* group (*S. spicant*, *S. fallax*, and *S. castanea*) and the *S. niponica* group (*S. niponica*, *S. hancockii*, and *S. amabilis*). The former group is more closely related to *Blechnidium melanopus* than to the *S. niponica* group.

We have also detected a considerable number of morphological features that support the differentiation of these two groups of species, ranging from the color of the petioles to the structure of the perispore (Table 1, Fig. 2). These data clearly support the natural grouping of these species into two distinct sets and can easily be used to separate the *S. niponica* clade from the clade of *Blechnidium melanopus*+the *S. spicant* group by, for example, the color of petioles, the stomatal pattern, the existence of epidermal hairs, and the structure of the perispore.

Faced with this information, and in order to propose a natural classification for the group that better reflects species' relationships, one immediate choice is to transfer *Blechnidium melanopus* to *Struthiopteris*. In this way, we could deal with a single, larger genus which would comprise two subclasses: the *S. niponica* group sister to a possible subgenus formed by “*B. melanopus*”+the *S. spicant* group. However, four facts prevent us from adopting this proposal: first, the monomorphic condition of *B. melanopus*, which strongly contrasts with the rest of the species of the supposed genus *Struthiopteris*, all of which have dimorphic (separate fertile and sterile) leaves; second, *Blechnidium* has veins partially anastomosing, while *Struthiopteris* has free veins. These morphological facts would require revising the taxonomic description of *Struthiopteris* to be a genus including both dimorphic and monomorphic plants, and whose species have both free and anastomosing veins. This is a considerable though not insurmountable obstacle, but two additional facts moved us toward a different taxonomic solution: the strong phylogenetic support for the *S. niponica* group and the *B. melanopus* + *S. spicant* group as distinct clades separated by substantial genetic divergence (Fig. 3); and finally, the fact that all of the species that belong to the *S. niponica* group (*S. niponica*, *S. hancockii*, and *S. amabilis*) have already been considered previously to be an independent genus: *Spicantopsis* Nakai (Nakai, 1933).

Spicantopsis was originally separated from *Struthiopteris* because the stomata follow the veins in *S. spicant* and *S. castanea*, but not in the rest of *Struthiopteris* (Nakai, 1933). In addition, it had been previously observed that the developmental pattern of the stomata was also different (Kondo, 1929), further supporting Nakai's recognition of *Spicantopsis*. *Spicantopsis* was in use for a few years, until it was considered that these stomatal characters were not enough to support *Spicantopsis* as a different genus, and *Struthiopteris* was recovered for those species (Ching, 1940; Tagawa, 1952). Later, *Struthiopteris* was recombined in *Blechnum* (Kramer & al.,

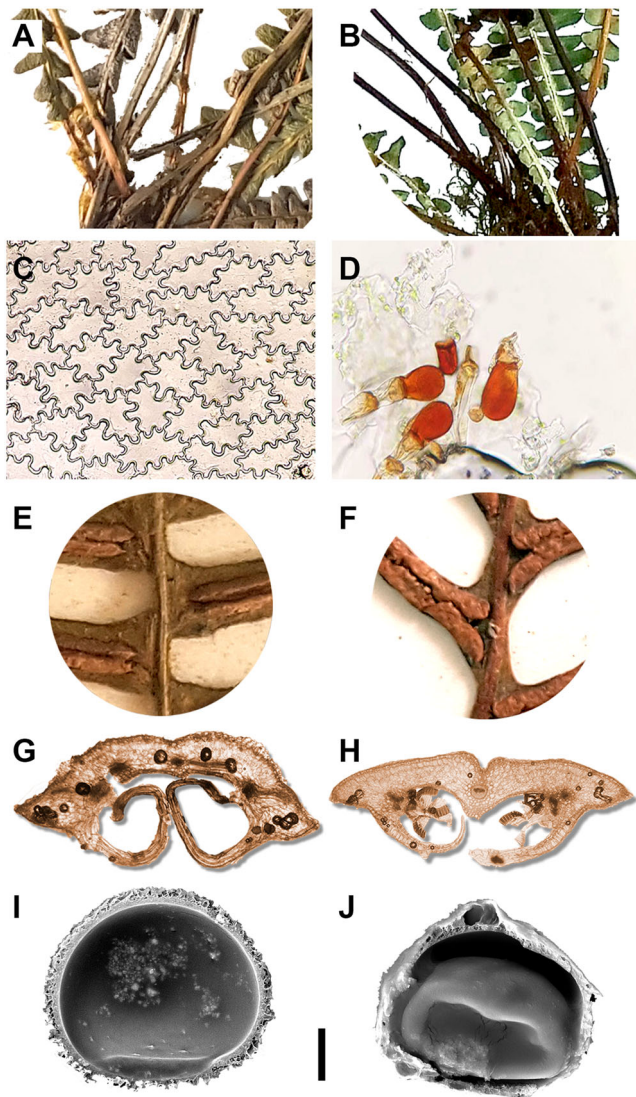


Fig. 2. Morphological characters that differ between the *Struthiopteris niponica* group (left) and the *S. spicant* group (right): color of petioles pale (A) or dark (B); epidermal hairs absent (C) or present (D); base of sori straight (E) or decurrent (F); indusia thin and enrolled (G) or thick and not enrolled (H); perispore compact (I) or alveolate (J). — Scale bar: A & B, 1.3 cm; C, 70 μ m; D, 50 μ m; E & F, 2.7 mm; G & H, 0.4 mm; I & J, 10 μ m.

1990), where it has been included until its recent separation as *Struthiopteris* again (Gasper & al., 2016, 2017).

In the present study, we have observed the original stomatic character along with many more traits that support the recognition of two groups, such as the color of the petioles (Ohwi, 1965; Chiou & al., 1994; Faguo & al., 2013), the presence of epidermal hairs (which has been previously described for *S. spicant* and *S. fallax* [Rolleri & Prada, 2006; Molino & al., 2019] but not for *S. castanea*), the base of the sori (decurent or not), differences in the anatomy of the pinnae, which is an important character for classifying ferns, especially within the family Blechnaceae (Prada & al., 2016; Vicent, 2017), and also differences in the perispore, which is another extremely important character for fern classification (Tryon & Lugardon, 1991), particularly in Blechnaceae (Passarelli, 2007; Passarelli

& al., 2010). Cytogenetic data also exist that support this segregation. For example, Nakato (1987), after counting chromosomes for the four Asian species of *Struthiopteris*, divided them into two groups, with *S. amabilis*, *S. niponica*, and *S. hancockii* being diploid with $x = 31$, and *S. castanea* being decaploid with $x = 34$. He pointed out that *S. castanea* was therefore likely more closely related to *S. spicant* and *S. fallax*, which are diploids but also have $x = 34$ (Löve & Löve, 1968).

Finally, our phylogenetic analyses undoubtedly support the idea that *S. amabilis*, *S. hancockii*, and *S. niponica* form a clade apart from the rest of the species currently included in *Struthiopteris*. We, therefore, conclude that we should recover the genus *Spicantopsis* for those taxa.

Our biogeographic results suggest that all members of this group of genera (*Blechnidium*, *Struthiopteris*,

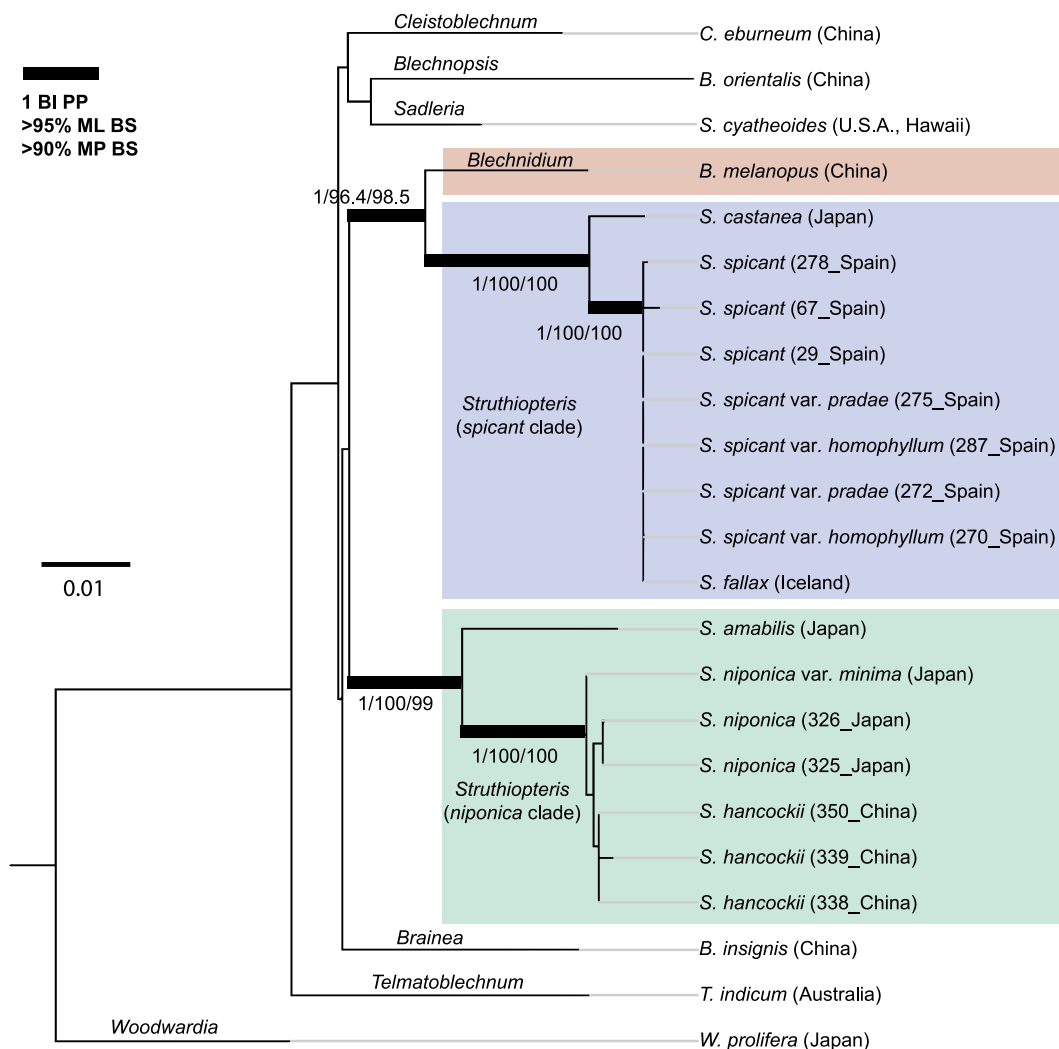


Fig. 3. Phylogeny of *Struthiopteris* and outgroups based on combined *rbcl*, *trnL-trnF*, and *psbA-trnH* sequences of 66 accessions (51 newly generated plus 15 obtained from GenBank). Branches with high statistical support (1.0 BI PP, >95% ML BS, and >90% MP BS) have been highlighted with a bold line. Numbers near the branches indicate, in this order, posterior probabilities, maximum likelihood bootstrap support, and maximum parsimony bootstrap support. Three major lineages are supported by high BS and PP values: *Blechnidium* (red), and two clades corresponding to the *Struthiopteris spicant* (blue) and *Struthiopteris niponica* (green, = *Spicantopsis*) groups. Length of branches is proportional to the number of substitutions per site.

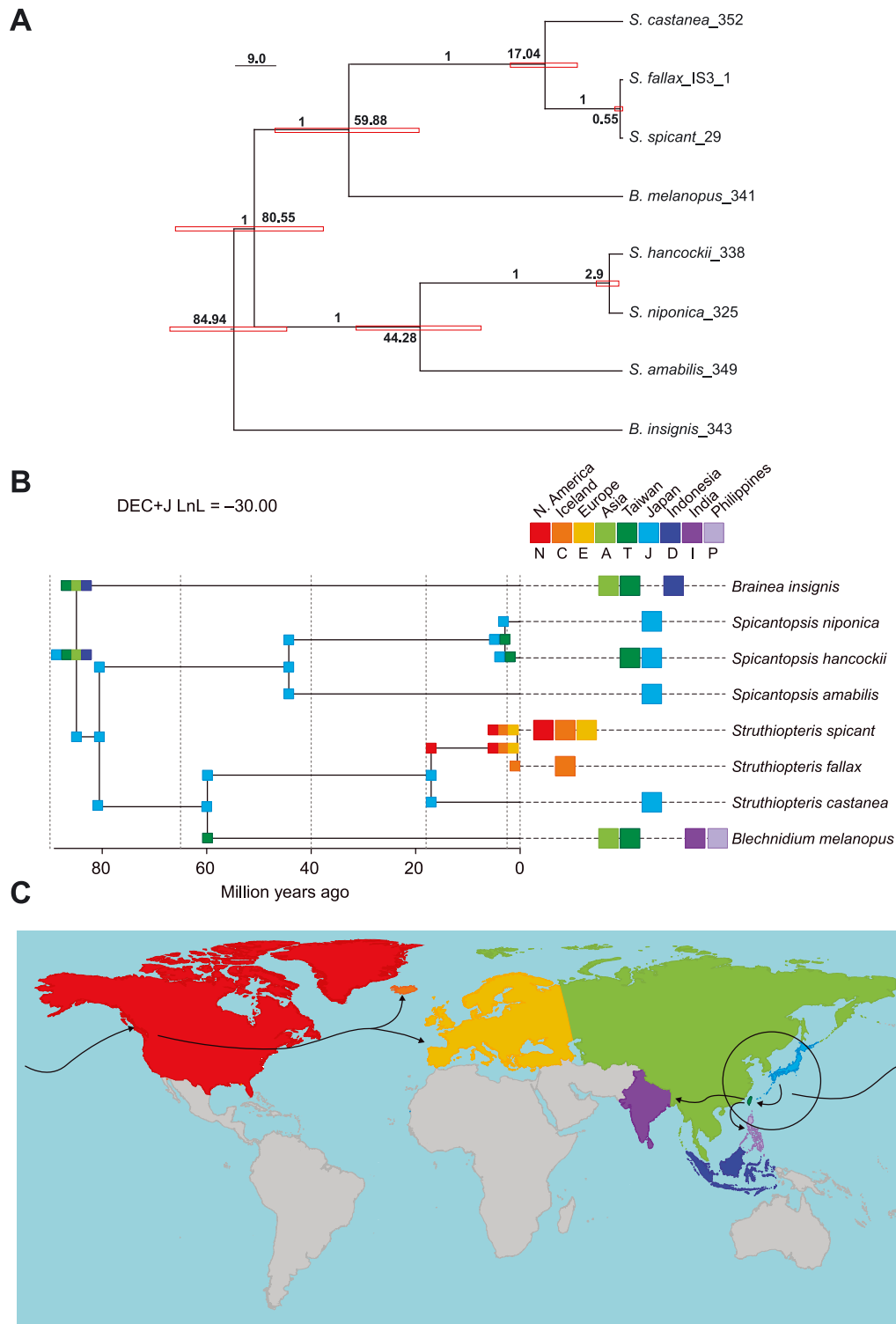


Fig. 4. Molecular dating and ancestral range reconstruction for the group *Spicantopsis*+*Struthiopteris*+*Blechnidium*. **A**, Maximum clade credibility chronogram from the BEAST analysis, showing statistical support (as PP) over the branches, and estimation of the age of the nodes. **B**, The same chronogram with ranges of extant taxa indicated at the tips of the tree, and reconstructed ancestral ranges calculated in BioGeoBEARS under the best-performing model (DEC+J) shown at internal nodes. When several colored boxes appear at nodes, that ancestor was assumed to have inhabited a range encompassing multiple areas prior to cladogenesis. Boxes at the corners show ranges immediately following cladogenesis. The vertical dotted lines in the phylogeny indicate the time slices used in the BioGeoBEARS analysis. **C**, Map with the inferred historical events for the group. The circle rounds the approximate estimated area of emergence of the ancestor of the three genera, and the lines indicate the probable dispersal routes of the ancestors for each clade of *Struthiopteris* and *Spicantopsis*. The colors of the map coincide with those of the ranges used for the analysis.

Spicantopsis) emerged in East Asia at a time when Japan was still part of the mainland. Based on our analyses of extant taxa it appears that, for most of their history, the members of these genera have been confined to East Asia. The most probable set of dispersal events includes one dispersal to the Americas by an ancestor within *Struthiopteris* s.str. in the last 20 million years, and additional dispersals to India and the Philippines by *Blechnidium melanopus*. These latter movements could have taken place at any time since the divergence of *B. melanopus* from *Struthiopteris*, which occurred ~60 million years ago. An interesting case is that of *S. spicant* and *S. fallax*, which diverged from one another very recently, after an inferred dispersal to North America by their shared ancestor (Fig. 4). *Struthiopteris spicant* is now widespread in North America, Iceland, and Western Europe, while *S. fallax* is a narrow endemic in Iceland only. It may be that *S. fallax* is a product of local adaptation to this extreme, volcanically active and geothermal environment. Although the taxon is known just from one locality in western Iceland, we cannot rule out the possibility that it was more widespread in the past. For now, it is also unclear whether the striking morphological differences between *S. spicant* and *S. fallax* evolved due to adaptation to the local, geothermal conditions in Iceland or whether geothermal sites in Iceland merely created a refuge for a now-rare species that evolved and subsequently went extinct in other regions. The latter option is quite probable due to the very young age of the Icelandic flora (Wasowicz & al., 2018) and to the fact that there are well-documented examples of fairly recent (i.e., younger than 4 kya) natural, long distance plant dispersals to Iceland (Wasowicz & al., 2018). It seems that a comprehensive explanation of the current distributions of these extant taxa will require further investigation and additional evidence, perhaps from fossil plant remains.

■ TAXONOMIC TREATMENT

We present a key to identify the three genera *Blechnidium*, *Spicantopsis*, and *Struthiopteris*. Full taxonomic descriptions for *Spicantopsis* and *Struthiopteris* are included, along with the names of their infrageneric taxa. Taxonomic terms have been adjusted to match Lellinger (2002).

1. Black, dark brown, or purplish petiole; glandular hairs on the lamina present..... 2
1. Pale or straw-colored petiole; glandular hairs on the lamina absent..... *Spicantopsis*
2. Dimorphic plants, infrequently sub- or monomorphic; veins free..... *Struthiopteris*
2. Monomorphic plants; veins anastomosing, forming areolae..... *Blechnidium*

Spicantopsis Nakai in Bot. Mag. (Tokyo) 47: 180–181. 1933 – Type: *S. niponica* (Kunze) Nakai (≡ *Lomaria niponica* Kunze).

Terrestrial plants; thin and creeping or thick and slender rhizomes, non-stoloniferous, dark, covered by concolorous or discolorous scales, pale brown to dark brown, linear-lanceolate to ovate-lanceolate, membranaceous or papyraceous, with entire margins; dimorphic fronds; sterile fronds with slender, short, pale brown or green petioles, with scales in the proximal zone, sterile laminae lanceolate, rosulated, erect or arched, from pinnate to pinnatisect, with reduced pinnae towards the basal zone, conform apex, adnate pinnae, linear-oblong to linear-falcate, entire margins; free veins, bifurcate, ended in submarginal, adaxial and obvious hydathodes; series of intramarginal stomata, parallel between the costa and the margin, not following the secondary veins; erect and pinnate fertile fronds, with heavily contracted pinnae, and rachis with few filiform scales, or without (*S. amabilis*); linear and continuous cenosori on both sides of the costa, occupying all the length of the pinnae, not decurrent towards the rachis; linear indusium, continuous, long and enrolled, opened towards the costa; sporangia with 19–20 cells in the annulus; spores monoete, with brown, irregularly reticulate or verrucate granulose perispore. $x = 31$.

Spicantopsis amabilis (Makino) Nakai in Bot. Mag. (Tokyo) 47: 184. 1933 ≡ *Blechnum amabile* Makino in Bot. Mag. (Tokyo) 11: 83. 1897.

Spicantopsis hancockii (Hance) Masam., Short Fl. Formosa: 29. 1936 ≡ *Blechnum hancockii* Hance in J. Bot. 21: 267. 1883.

Spicantopsis niponica (Kunze) Nakai in Bot. Mag. (Tokyo) 47: 181. 1933 ≡ *Lomaria niponica* Kunze in Bot. Zeitung (Berlin) 6: 508. 1848.

Spicantopsis niponica var. *minima* Tagawa in J. Jap. Bot. 14(11): 706. 1936.

Struthiopteris Scop., Meth. Pl.: 25. 1754 – Type: *S. spicant* (L.) Weiss (≡ *Osmunda spicant* L.).

Terrestrial plants; slender rhizomes, non-stoloniferous, dark, covered with scales with entire margins, linear-lanceolate, triangular-lanceolate or ovate-lanceolate, concolorous or discolorous, pale to dark brown, with central cells occluded or not; dimorphic, subdimorphic (*S. spicant* var. *homophyllum*) or monomorphic (*S. fallax*) fronds; sterile fronds with slender petioles, short, dark brown or black, sometimes purplish, with scales in the proximal zone and glabrous in the distal zone, lanceolate laminae, pinnate or pinnatifid, very gradually reduced towards the base, with glandular hairs, pinnatifid apexes, more rarely conform; adnate pinnae, linear-falcate to oblong, with entire margins; free furcate veins, inconspicuous, ending in submarginal adaxial hydathodes; aligned stomata following the veins in the abaxial side of the pinnae; fertile fronds (when dimorphic) with longer petioles, slender and heavily contracted pinnae; linear cenosori on both sides of the costa, sometimes interrupted; indusium linear, continuous or not, entire, usually

wrapping the sporangia at maturity; sporangia with 12–20 cells in the annulus; spores monolete, with brown, irregularly plicate perispore. $x = 34$.

Struthiopteris castanea (Makino & Nemoto) Nakai in Bot. Mag. (Tokyo) 47: 186. 1933 \equiv *Blechnum castaneum* Makino & Nemoto, Fl. Japan, ed. 1: 1591. 1925.

The designation “*Lomaria castanea*” Makino, used to be cited as the basionym of *S. castanea*, is a name not validly published (nomen nudum) (Makino, 1892). Therefore, it can’t be used as the basionym of any combination. The correct name for this taxon was *Blechnum castaneum* Makino & Nemoto (Makino & Nemoto, 1925: 1591), which was transferred to *Struthiopteris* by Nakai (1933). In consequence, the proper name under *Struthiopteris* is *S. castanea* (Makino & Nemoto) Nakai.

Struthiopteris fallax (Lange) S.Molino, Gabriel y Galán & Wasowicz in Pl. Syst. Evol. 305: 266. 2019 \equiv *Blechnum spicant* var. *fallax* Lange in Oeder, Fl. Dan. 17(50): 11, t. 1988. 1880.

Struthiopteris spicant (L.) Weiss., Pl. Crypt. Fl. Gott.: 287. 1770 \equiv *Osmunda spicant* L., Sp. Pl.: 1066. 1753.

Struthiopteris spicant var. *homophyllum* (Merino) Gabriel y Galán & R.Pino in Phytotaxa 302(2): 198. 2017 \equiv *Blechnum spicant* var. *homophyllum* Merino ex H.Christ in Bull. Acad. Int. Géogr. Bot., sér. 3, 13: 79. 1904.

Struthiopteris spicant var. *pradae* S.Molino & Gabriel y Galán in Pl. Syst. Evol. 305: 266. 2019.

■ AUTHOR CONTRIBUTIONS

SM: material collection, laboratory observations, molecular procedures, manuscript writing, and final approval. JMgYg: conception of the initial idea, material collection, laboratory observations, manuscript writing, and final approval. EBS: conceptual framework, molecular procedures, manuscript writing, English review, and final approval. PW: material collection, laboratory observations, molecular procedures manuscript writing, and final approval. — SM, <https://orcid.org/0000-0003-2396-4649>; JMgYg, <https://orcid.org/0000-0003-2786-0062>; EBS, <https://orcid.org/0000-0002-6496-5536>; PW, <https://orcid.org/0000-0002-6864-6786>

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Appendix 1. Material reviewed for this study.

For each taxon we indicate the locality, collector and collection number, and date (if available), and the herbarium voucher. When more than one, the number of individuals is indicated in brackets after the voucher number.

Blechnidium melanopus (Hook.) T.Moore. **TAIWAN:** Taitung: Lidao, *Knapp 3355*, 1/03/2014 (P02439093); *Ibidem* (P02439094). Yilan: Taipingshan, Cuifenghu, *Knapp & Huang 262*, 6/02/2005 (P02436518). **Brainea insignis** (Hook.) J.Sm. **TAIWAN:** Nantou: Forest Station Huison, *Knapp 1849*, 29/05/2009 (P02437798); Forestry Station Huison, *Knapp 3409*, 10/05/2014 (P02439156). Taichung: Dongmaoshan, *Knapp 4022*, 1/02/2016 (P02435287). **Spicantopsis amabilis** (Makino) Nakai. **JAPAN:** *Christ 94*, 9/02/1910 (P01406586); *Coreé s.n.*, 08/1913 (P01575934); *Faurié 1566*, 20/08/1898 (P01406590); no collector or date (P01406642); *Faurié 5612*, 07/1904 (P01406648); *Ibidem* (P01406647). Honshū: Echigo, Takidani, *Weigel s.n.*, 24/08/1903 (P01406649); Echigo, Tsugawa, *Togashi 1576*, 16/09/1957 (P01406637); *Ibidem* (S1952); Gunma, Kusatsu, *Dickins s.n.*, 1877 (P01406525); Kii, Owase-machi, Kita-muro-gun, *M. Furuse s.n.*, 9/10/1956 (S1951); Kyoto, Tadasu-no-mori, *Faurié 614*, 10/07/1888 (P01567610); Mie, Utobi, *Seto 6672*, 23/11/1956 (P01608624); Musasi, Ryōgami, *Tagawa 341*, 16/09/1957 (P01406650); Nagano, Togakushi, *Weigel s.n.*, 20/08/1906 (P01571952); *Ibidem* (P01406644); Nara, Shōfuku-dake, *Tawaga & Iwatsuki 5129*, 4/08/1962 (P01575933); Niigata, Itoigawa, *Matsumoto s.n.*, 3/08/2009 (TNS9544305); Saitama, Chichibu-gun, Ohtaki-mura, *Iwata s.n.*, 9/07/2002 (TNS001147002); *Ibidem* (TNS01147001); Shizuoka, Hamamatsu-shi, *Nakamura s.n.*, 08/08/2000 (TNS01019335); Tochigi, *Tagawa & Iwatsuki 1913*, 13/09/1958 (P01608623); Tomaya, Tateyama, *Faurié s.n.*, 08/1913 (S1953); *Ibidem* (P01406643); *Ibidem* (P01406645); *Ibidem* (P01406646); Yamanashi, Fusiya, *Tschonoski s.n.*, 1864 (P01406532). Kyūshū: Kagoshima, Satsuma, *Faurié s.n.*, 07/1888 (P01567609). Yakushima: Kagoshima, Kumage-gun, Yaku-chō, *Ebihara & Ito s.n.*, 10/12/2006 (TNS763339). **Spicantopsis hancockii** (Hance) Masam. **TAIWAN:** Chiayi: Alishan, Arisan, *Iwasaki s.n.*, 22/07/1970 (TNS832514); Alishan, Tashan, *Ebihara & Kuo s.n.*, 2/07/2014 (TNS01219667). Hualien: Muh-Kwa, *Kao 4141*, 24/07/1961 (TAI004259). Kaphsiung: Chienchin-Kuaishan, Miharasi-Hinokiyama, *Yamamoto 615*, 9/12/1937 (TAI004265). Nantou: Dayuling, *Knapp 1413*, 14/07/2007 (P02437438). Taipei: Beitou, *Knapp 3338*, 18/01/2014 (P02439073); Sitsei, Chihsingshan, *Suzuki s.n.*, 2/09/1926 (TAI004269); Taiping, *Feung & Kao 336*, 20/02/1963 (TAI004261). Taichung: Pahsinshan Logging Station, Chuang, *Kou & Kao 2751*, 7/08/1959 (TAI004262); Pahsinshan, *Huang 1321*, 7/08/1959 (TAI004272). Pahsinshan, Taitung: Taiwu, *Knapp 3430*, 2/06/2014 (P02439164); *Ibidem* (P02439165). Yilan: Nan'ao Township, Taipingshan, *Knapp 4311*, 3/09/2016 (P02435597); *Ibidem*, *Knapp & Huang 239*, 5/12/2005 (P02436496); Nan'ao Township, Taipingshan, National Forest Recreation Area, Cueifong Lake Circle Trail, *Ebihara, Tsutsumi, Kokubugata & Huang s.n.*, 20/06/2008 (TNS776516). **Without geographical information:** *Shakurai s.n.*, 1/08/1906 (P01406577). **Spicantopsis niponica** (Kunze) Nakai var. *niponica*. **JAPAN:** *Christ s.n.*, 9/02/1910 (P01406588); *Nerville s.n.*, 12/08/1908 (P01571953); *Matsumura 48* (P01619262); no collector, 20/08/1913 (P01406574); no collector or date (P01406582). Hokkaidō: Nopporo, *Dorsett & Morse 1038*, 09/1929 (P01575938). Honshū: *Kramer 2280*, 1866–1871 (P01406581); *Tschonoski s.n.*, 1866 (P01406591); *Ibidem* (P01406593); Aomori, *Faurié 146*, 1866 (P01406584); Aomori, Agamushi, *Rosenstock 27*, 12/11/1913 (P01406533); Aomori, Towada, *Drake & Faurié 13280*, 26/06/1894 (P01406585); Fukushima, Hanami Yama, *Savatier s.n.*, 1866–1871 (P01406583); Hakone, *Drake 1558*, 07/1869 (P01406579); Hyōgo, Myōkō-san, Hikami-gun, *Tagawa & Iwatsuki 3014*, 23/09/1960 (P01575937); Kamagawa, Hakone, Komagatake, *Sakurai s.n.*, 1/08/1906 (P01406576); Kanawaga, Hakone, Sagami, *Weigel s.n.*, 21/10/1902 (P01406578); Kanawaga, Yokosuka, *Henschel 1558*, 1866–1871 (P01557335); Kanawaga, Kantō, Yokohama, *Maximowicz s.n.*, 1862 (P01406592); Kyoto, Shishigatani, *Tagawa 7150*, 30/10/1955 (P01608625); Tottori, Tottori-shi, *B. Estèbanez s.n.*, 17/07/2017 (MACB110656); Yamagata, Mikawa, Okazaki, *Umemura 37*, 5/10/1911 (P01406521); Yamamoto, Sottsu, *Togashi 710*, 10/02/1953 (S2061); Wakayama, Tanabe-shi, Nakahechi-cho, Mizukami, *B. Estèbanez s.n.*, 21/08/2017 (MACB110657). Kyūshū: Amagi, no collector, 28/12/1890 (P01406575); Amagi, *Owi & Okamoto 1402*, 9/10/1956 (P01406594); Kōchi, Tosa, *Christ s.n.*, 07/1889 (P01406589); Nagasaki, *Maximowicz s.n.*, 1863 (S2144). **Spicantopsis niponica** var. *minima* Tagawa. **JAPAN:** Kyūshū: Kagoshima, *Tagawa 853*, 18/08/1933 (P00748579) (three individuals). **Struthiopteris castanea** (Makino) Nakai. **JAPAN:** Hokkaidō: Aomori, Hakkōda, *Maximowicz s.n.*, 1861 (S2145); Aomori, Hakkōda, Iwakura, *Iwatsuki 5049*, 5/09/1959 (S1971); Aomori, Shichinohe-machi, *Nakaïke 8422*, 31/08/1980 (TNS732190); *Ibidem*, no collector (TNS739308). Honshū: Akita, *Makino 32953*, 1962 (S1980); Fukui, Imadate-gun, Ikeda-cho, Hekosan, *Kawahara s.n.*, 14/08/1992 (TNS792167); Fukui, Imadate-gun, Ikeda-cho, Kanmuri-yama, *Saito s.n.*, no date (TNS792168); Fukushima, Hinoemata-mura, *Okuyama 410*, 22/07/1934 (TNS45261); Iwate Isawa-cho, no collector, 1/08/1968 (TNS426905); Iwate, Shizukuishi-cho, no collector, 28/09/1980 (TNS739310); Iwate, Wakayanagi-mura, *Karizumi s.n.*, 29/08/1951 (TNS115371); Gifu, Ohno-gun, Shirakawa-mura, Sanpoiw adake, *Murase s.n.*, 15/08/1992 (TNS792169); Gunma, Minakami-mura, *Watanabe 310*, 23/08/1894 (TNS63209); Gunma, Tone-gun, Minakami-chō, Tanigawa Mt., Tenjindaira, Kuma-ana sawa hinangoya, *Ebihara, Okuyama & Saito 2782*, 6/06/2011 (TNS108194); Hyōgo, Mikana-gun, Onsen-cho, Ueyama-kogen, *Tobayashi s.n.*, 25/07/1999 (TNS700642); Nagano, Otari-mura, *Futoshi 81107*, 14/09/1985 (TNS832504); Nagano, Otari-mura, *Haginiwa s.n.*, 27/07/1979 (TNS979503); Niigata, Koide-machi, *Konta 6405*, 26/08/1967 (TNS924267); *Ibidem* (TNS924268); Niigata, Shibata-shi, *Konta 3340*, 24/19/1963 (TNS924263); *Ibidem*, *Konta 3350* (TNS924266); Niigata, Yamato-machi, *Ikegami s.n.*, 8/08/1964 (TNS184551); Niigata, Yamato-mura, *Iwano s.n.*, 17/07/1954 (TNS110853); Shinano, Shumominochi-gun, Minochi-mura, *Mizushima 13853*, 28/06/1956 (S1982); Toyama, Yatsuo-machi, *Kirino s.n.*, 15/08/1955 (TNS280676); Yamagata, Iide-machi, *Shimizu s.n.*, 18/08/1966 (TNS172556); *Ibidem* (TNS172559). **Struthiopteris fallax** (Lange) S.Molino, Gabriel y Galán & Wasowicz. **ICELAND:** Deildartunguhver, *Wasowicz & Gabriel y Galán s.n.* (MACB109359) (three individuals). **Struthiopteris spicant** (L.) Weiss var. *spicant*. **AUSTRIA:** Salzburg, Hohe Tauern, *Eberwein & Vitek 64-193*, 20/7/1994 (MA767022). **BELGIUM:** Turnhout, Engels Kamp park, *W. van Cotthem 1123*, 18/6/1973 (MA809713). **FRANCE:** Sources Forêt de l'Estérel, *G. Gavelle s.n.*, 01/08/1958 (MA186653); Serans (Oise), *P. Bosserdet s.n.*, 22/07/1921 (P01001059). **ICELAND:** Vestfirðir, *Gabriel y Galán & Wasowicz s.n.*, 8/07/2016 (MACB110659). **IRELAND:** Iveragh, Caherdaniel, *Molino & Pachón s.n.*, 17/07/2016 (MACB109619). **NORWAY:** Telemark, Nottoden, *P. Sunding s.n.*, 15/08/2004 (MA747921). **SPAIN:** Asturias: Valdés, Paladepierre, *Gabriel y Galán s.n.*, 22/03/2016 (MACB109612) (eight individuals); Valdés, Lueca, *Gabriel y Galán s.n.*, 12/04/2017 (MACB109615) (seven individuals). Canary Islands: Anaga, Tenerife, *Santos s.n.*, 25/10/1989 (MACB36152); Anaga, Tenerife, *Gabriel y Galán s.n.*, 14/09/2017 (MACB110658) (two individuals). Cantabria: Camaleño, Cosgaya, *Gabriel y Galán s.n.*, 8/10/2016 (MACB109622) (four individuals). Madrid: Dehesa de Somosierra, *Gabriel y Galán & Molino s.n.*, 31/05/2017 (MACB109611). Pontevedra: Monte Aloya, Tuy, *Pajarón & Pangua s.n.*, 15/07/1993 (MACB59142). Zamora: Aciberos, *Molino & al. s.n.*, 23/09/2017 (MACB110655). **UNITED KINGDOM:** South Somerset, Seven Wells Wood, *J.A. Crabbe 11822*, 30/07/1968 (MA195093). Mid Ebudes, Mull, Tobermory, *Crabbe & Jermy 11837*, 06/07/1970 (MA195091). **Struthiopteris spicant** var. *homophyllum* (Merino) Gabriel y Galán & R.Pino. **PORTUGAL:** Braga, Vieira do Minho, *Prada s.n.*, 1/10/2004 (MACB109621). **SPAIN:** La Coruña: Santiago, Canteleta, *Barrera s.n.*, 29/07/1967 (MACB32367). Pontevedra: between Tabagón and Tomiño, *Gabriel y Galán s.n.*, 19/03/2016 (MACB109617); Mondariz, *Gabriel y Galán s.n.*, 20/03/2016 (MACB109618). Salamanca: Batuecas, *Gabriel y Galán s.n.*, 15/05/2016 (MACB109626) (five individuals). **Struthiopteris spicant** var. *pradae* S.Molino & Gabriel y Galán. **SPAIN:** Asturias: Valdés, Paladepierre, *Gabriel y Galán*, 22/03/2016 (MACB109613) (three individuals); *Ibidem*, 3/08/2016 (MACB109615) (three individuals); Lueca, *Gabriel y Galán s.n.*, 26/07/2017 (MACB110660); Otur, *Gabriel y Galán s.n.*, 17/08/17 (MACB110661). Burgos: Sierra de San Millán, *Fuentes s.n.*, 27/09/1975 (MACB5994). Zamora: Aciberos, *Molino & al. s.n.*, 23/09/2017 (MACB110654).

Appendix 2. Sequences used in this study.

Information is ordered alphabetically by taxon and includes: sample_id, country of origin: herbarium voucher, the collector and collection number (for the newly generated sequences); GenBank accessions for *rbcL*, *trnL-trnF*, and *psbA-trnH* (asterisk indicates new sequences).

Blechnidium melanopus (Hook) T.Moore Sample_341, China: *Ralf Knapp 3355* (P02439093); MH644117*, MH644127*, MH644151*. *Blechnopsis orientalis* C.Presl Sample_GB, China (cultivated): KC254350, KC254424, GU592475. *Brainea insignis* (Hook.) J.Sm. Sample_343, China: *Ralf Knapp 4022* (P02435287); MH644110*, MH644137*, MH644144*. *Cleistoblechnum eburneum* (Christ) Gasper & Salino Sample_GB, China: MG183277, MG183577, -. *Sadleria cyatheoides* Kaulf. Sample_GB, U.S.A. (Hawaii): EF463161, DQ683431, -. *Spicantopsis amabilis* (Makino) Nakai Sample_349, Japan: *A. Ebihara, J.H. Nitta & M. Ito s.n.* (TNS763339); MH644111*, MH644138*, MH644145*. *Spicantopsis hancockii* (Hance) Masam. Sample_338, China: *Ralf Knapp 4311* (P02435597); MH644114*, MH644141*, MH644149*. Sample_339, China: *Ralf Knapp 3338* (P02439073); MH644113*, MH644143*, MH644146*. Sample_350, China: *A. Ebihara, C. Tsutsumi, G. Kokubugata & C.-I. Huang s.n.* (TNS776516); MH644115*, MH644142*, MH644148*. *Spicantopsis niponica* var. *minima* Tagawa Sample_GB, Japan: (TNS763250); AB575054, -, AB575684. *Spicantopsis niponica* var. *niponica* (Kunze) Nakai Sample_325, Japan: *B. Estébanez s.n.* (MACB110657); MH644116*, MH644139*, MH644150*. Sample_326, Japan: *B. Estébanez s.n.* (MACB110656); MH644112*, MH644140*, MH644147*. *Struthiopteris castanea* (Makino & Nemoto) Nakai Sample_352, Japan: *A. Ebihara, Y. Okuyama & Y. Saito 2782* (TNS108194); MH644118*, MH644128*, MH644152*. *Struthiopteris fallax* (Lange) S.Molino, Gabriel y Galán & Wasowicz Sample_IS3-1, Iceland: *Wasowicz & Gabriel y Galán s.n.* (MACB109359); MH644122*, MH644132*, MH644159*. *Struthiopteris spicant* var. *homophyllum* (Merino) Gabriel y Galán & R.Pino Sample_287, ind.01, Spain: *Gabriel y Galán s.n.* (MACB109626); MH644119*, MH644133*, MH644158*. Sample_270, ind.02, Spain: *Gabriel y Galán s.n.* (MACB109626); MH644120*, MH644136*, MH644160*. *Struthiopteris spicant* var. *pradae* S.Molino & Gabriel y Galán Sample_272, Spain: *Gabriel y Galán s.n.* (MACB109613); MH644123*, MH644134*, MH644157*. Sample_275, Spain: *Fuentes s.n.* (MACB5994); MH644125*, MH644131*, MH644156*. *Struthiopteris spicant* var. *spicant* (L.) Weiss Sample_29, Spain: *Gabriel y Galán s.n.* (MACB109612); MH644121*, MH644135*, MH644155*. Sample_67, Spain: *Gabriel y Galán s.n.* (MACB109615); MH644124*, MH644129*, MH644154*. Sample_278, Spain: *Pajarón & Pangua s.n.* (MACB59142); MH644126*, MH644130*, MH644153*. *Telmatoblechnum indicum* (Burm.f.) Perrie, D.J.Ohlsen & Brownsey Sample_GB: Australia: KJ170830, KJ170857, KC572549. *Woodwardia prolifera* Hook. & Arn. Sample_GB, Japan: AY137666, DQ683433, GU592476.

Appendix 3. Dispersal multipliers used in the BioGeoBears analysis for each of six time slices. C = Iceland, E = Europe, N = North America, J = Japan, T = Taiwan, A = Asian mainland, I = India, D = Indonesia, P = the Philippines.

0.01 mya–present									
C	E	N	J	T	A	I	D	P	
1	0	0	0	0	0	0	0	0	0
0	1	0.25	0.25	0.25	0.75	0.75	0.25	0.25	0.25
0	0.25	1	0.75	0.75	0.75	0.70	0.70	0.75	0.75
0	0.25	0.75	1	1	1	0.90	1	1	1
0	0.25	0.75	1	1	1	1	1	1	1
0	0.75	0.75	1	1	1	1	1	1	1
0	0.75	0.70	0.90	1	1	1	1	1	1
0	0.25	0.70	1	1	1	1	1	1	1
0	0.25	0.75	1	1	1	1	1	1	1
2.5–0.01 mya									
C	E	N	J	T	A	I	D	P	
1	1	1	0.25	0.25	0.75	0.50	0.25	0.25	0.25
1	1	0.90	0.75	0.75	1	0.90	0.75	0.75	0.75
1	0.90	1	0.80	0.80	0.80	0.75	0.75	0.80	0.80
0.25	0.75	0.80	1	1	1	0.90	1	1	1
0.25	0.75	0.80	1	1	1	1	1	1	1
0.75	1	0.80	1	1	1	1	1	1	1
0.5	0.90	0.75	0.90	1	1	1	1	1	1
0.25	0.75	0.75	1	1	1	1	1	1	1
0.25	0.75	0.80	1	1	1	1	1	1	1
18–2.5 mya									
C	E	N	J	T	A	I	D	P	
1	1	1	0.25	0.25	0.75	0.50	0	0	0
1	1	0.90	0.75	0.75	1	0.90	0	0	0

Appendix 3. Continued.

18–2.5 mya								
C	E	N	J	T	A	I	D	P
0.25	0.75	0.80	1	1	1	0.90	0	0
0.25	0.75	0.80	1	1	1	1	0	0
0.75	1	0.80	1	1	1	1	0	0
0.5	0.90	0.75	0.90	1	1	1	0	0
0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	1
40–18 mya								
C	E	N	J	T	A	I	D	P
1	0	0	0	0	0	0	0	0
0	1	1	0.75	0.75	1	0.90	0	0
0	1	1	0.80	0.80	0.85	0.75	0	0
0	0.75	0.80	1	1	1	0.90	0	0
0	0.75	0.80	1	1	1	1	0	0
0	1	0.85	1	1	1	1	0	0
0	0.90	0.75	0.90	1	1	1	0	0
0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	1
65–40 mya								
C	E	N	J	T	A	I	D	P
1	0	0	0	0	0	0	0	0
0	1	1	0.75	0.75	1	0.75	0	0
0	1	1	0.70	0.70	0.85	0.70	0	0
0	0.75	0.70	1	1	1	0.90	0	0
0	0.75	0.70	1	1	1	1	0	0
0	1	0.85	1	1	1	1	0	0
0	0.75	0.70	0.90	1	1	1	0	0
0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	1
90–65 mya								
C	E	N	J	T	A	I	D	P
1	0	0	0	0	0	0	0	0
0	1	1	0.75	0.75	1	0.60	0	0
0	1	1	0.70	0.70	0.85	0.60	0	0
0	0.75	0.70	1	1	1	0.70	0	0
0	0.75	0.70	1	1	1	1	0	0
0	1	0.85	1	1	1	1	0	0
0	0.60	0.60	0.70	1	1	1	0	0
0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	1