

## A Reassessment of the Little-Known Amazonian Fern *Diplazium praestans* Based on Molecular and Morphological Evidence

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**Abstract**—Family- and genus-level circumscription of ferns in the suborder Aspleniineae (eupolypods II) has long been controversial, due in part to confusion about the relationship among the families Aspleniaceae and Athyriaceae. Recent studies have demonstrated that character states traditionally used to infer a close relationship between these two families are either symplesiomorphic or homoplastic, and re-examination of numerous taxa has led to the recircumscription of several clades, and the description of several new families and genera. In light of these findings, we re-evaluated the taxonomic affinities of *Diplazium praestans*, a little-known fern from western Amazonia that is morphologically disparate to the remainder of Neotropical *Diplazium*. Using sequence data from three chloroplast markers and analysis of eight morphological characters, we demonstrate that *Diplazium praestans* was erroneously placed in that genus and instead is a *Hymenasplenium*. We place it in a phylogenetic context, reassess its morphology in light of our findings, evaluate its conservation status under IUCN criteria, and provide a new combination: *Hymenasplenium praestans*. We also provide an updated key to the Neotropical species of *Hymenasplenium* and discuss unresolved taxonomic problems in the genus.

**Resumen**—Durante mucho tiempo, la circunscripción de familias y géneros en el suborden de helechos Aspleniineae ha sido controversial, debido en parte a la incertidumbre sobre las afinidades de las familias Aspleniaceae y Athyriaceae. Estudios recientes han demostrado que los caracteres que se han utilizado para inferir la estrecha relación entre estas familias son simplesiomorfías u homoplasias. Por lo tanto, la reconsideración de varios taxones ha llevado a la recircunscripción de varios clados y a la descripción de varias familias y géneros nuevos. A la luz de estos descubrimientos, reevaluamos las afinidades taxonómicas de *Diplazium praestans*, un helecho poco conocido de la Amazonia occidental que es morfológicamente dispar a las demás especies neotropicales de *Diplazium*. Utilizamos secuencias de tres regiones del cloroplasto y analizamos ocho caracteres morfológicos para demostrar que *D. praestans* se consideró erróneamente en ese género y que, en su lugar, es un *Hymenasplenium*. Por primera vez, ponemos a *D. praestans* en un contexto filogenético, reevaluamos su morfología, evaluamos su estado de conservación bajo criterios de la UICN y proveemos una nueva combinación: *Hymenasplenium praestans*. También proporcionamos una clave para las especies neotropicales de *Hymenasplenium* y discutimos problemas taxonómicos no resueltos en el género.

**Keywords**—Aspleniaceae, asplenoid, Athyriaceae, classification, IUCN, taxonomy.

The relationships of ‘asplenoid’ and ‘athyroid’ ferns, both traditionally characterized by the presence of elongate sori and two vascular bundles in the petiole, have long been a source of uncertainty for fern taxonomists. The morphological similarity between these groups led taxonomists to transfer numerous species between their constituent genera, especially *Asplenium* L., *Athyrium* Roth, and *Diplazium* Sw. (Makino 1899; Copeland 1948; Ching 1964; Kato 1975a, 1975b, 1977; Smith 1976; Tryon and Tryon 1982; Kato and Darnaedi 1988; Kramer and Kato 1990; Wang et al. 2004). Molecular evidence later demonstrated that the Aspleniaceae and Athyriaceae are only distantly related, and found that several taxa long-treated in these families belong to neither (Sano et al. 2000; Tzeng 2002; Wang et al. 2003); these have since been transferred to their own families: Desmophlebiaceae (Mynssen et al. 2016), Diplaziopsidaceae (Wei et al. 2010), Hemidictyaceae (Rothfels et al. 2012b), and Rhachidosoraceae (Kuo et al. 2011). A re-evaluation of the morphology of these lineages by Sundue and Rothfels (2014) revealed that the traits by which they were traditionally united are in fact a mix of symplesiomorphic and homoplastic character states, highlighting the challenge of clade circumscription in the Aspleniineae.

At the center of this complicated taxonomic history is the difficulty in separating some Aspleniaceae from *Diplazium*. Both groups are species-rich and morphologically diverse, and the characters that have been commonly used to circumscribe them (single-sided vs. double-sided sori, clathrate vs. non-clathrate rhizome scales, chromosome base number) are sometimes difficult to interpret or unavailable for some species.

Particularly confusing have been species of *Hymenasplenium* Hayata (previously *Asplenium* sect. *Hymenasplenium* (Hayata) K.Iwats.), which were being treated in *Diplazium*, because of their often terrestrial habit and similar leaf division. Insights into differentiating between these groups were presented by Smith (1976), who provided a detailed study of characters in *Asplenium* and *Diplazium* and demonstrated that widespread *Diplazium delitescens* Maxon in fact belonged to *Asplenium* and appeared to be allied to species in *Asplenium* sect. *Hymenasplenium*. Characters supporting the placement of that species in *Asplenium* sensu lato included the number of annular cells in the sporangia (~15 in *Diplazium*, 20–23 in *Asplenium*), sporangial stalk width (two or three cells wide in *Diplazium*, one cell wide in *Asplenium*), spore perispore morphology (loosely folded in *Diplazium*, sharp folds in *Asplenium*), rhizome habit (generally erect in *Diplazium*, creeping in *Asplenium* sect. *Hymenasplenium*), the presence of swollen petiole bases (absent in *Diplazium*, present in *Asplenium* sect. *Hymenasplenium*), and petiole vasculature (bundles fusing distally to form a “U” in *Diplazium*, forming an “X” in *Asplenium*). Guided by Smith’s findings, Murakami and Moran (1993) included this species in their treatment of Neotropical *Asplenium* sect. *Hymenasplenium*, and it was later transferred to *Hymenasplenium* by Regalado and Prada (2011). Although the diagnostic power of the characters examined by Smith (1976) has been confirmed by later studies (Murakami and Moran 1993; Sundue and Rothfels 2014), there is little evidence of their subsequent use to re-evaluate species circumscriptions in the Neotropics.

As part of a recent study on hybrids in *Diplazium* (Testo et al. 2017), two of us (WT and MS) examined specimens of all three Neotropical *Diplazium* species with simple leaves: *Diplazium aberrans* Maxon & C.V.Morton, *Diplazium plantaginifolium* (L.) Urb., and *Diplazium praestans* (Copel.) C.V.Morton. Based on our observations of its morphology, we concluded that *D. praestans* was not closely related to any other Neotropical *Diplazium* species and differed from other members of the genus by a lack of double-sided sori, sporangia with uniseriate stalks, and annuli of > 20 cells. These differences suggested it might instead belong to the Aspleniaceae and prompted the present study.

*Diplazium praestans* is a medium-sized terrestrial fern that occurs in floodplain forests of the western Amazonian basin of Brazil, Bolivia, and Peru (Tryon and Stolze 1991; Mynssen and Sylvestre 2019) (Fig. 1). Its distinctiveness was apparently first noted by Maxon, who provided the determination "*Diplazium* (?) *praestans* Maxon sp. nov." for Klug 4002 (CAS, F, MO, NY, US); herbarium codes follow Thiers (2020). Maxon apparently never published the name, however. Copeland, who lumped *Diplazium* in *Athyrium* (Copeland 1948), first described it as an *Athyrium* but remarked that it likely warranted recognition as a distinct genus, principally based on its anastomosing venation (Copeland 1948). After being transferred to *Diplazium* by Morton (1967), this taxon has received little attention beyond regional treatments (Tryon and Conant 1975; Tryon and Stolze 1991; Prado et al. 2017; Mynssen and

Sylvestre 2019). Given its unusual morphology (Fig. 2) and the fact that it has been largely overlooked during the last seven decades, we ask the question: is *Diplazium praestans* really a *Diplazium*?

#### MATERIALS AND METHODS

**Taxonomic Sampling and Sequence Data**—To place *D. praestans* in a phylogenetic context, we generated sequence data for three chloroplast markers commonly used in studies of ferns: one coding region (*rbcL*) and two intergenic spacers (*trnL-trnF*, *rps4-trnS*). Total genomic DNA was extracted from an herbarium specimen of *D. praestans* (Woytkowski 5118, GH) using the Qiagen DNeasy plant mini kit (Valencia, California, US.) following the manufacturer's protocol. Previous attempts to extract DNA from five other specimens had failed, likely due to their old age and the poor quality of the specimens. Amplification was performed using the polymerase chain reaction (PCR) using 3  $\mu$ L of genomic DNA, 2.5  $\mu$ L of 10  $\times$  PCR buffer (MgCl<sub>2</sub>), 0.75  $\mu$ L of 50 mM MgCl<sub>2</sub>, 0.5  $\mu$ L of 10  $\mu$ M deoxyribonucleotide triphosphates, 0.5  $\mu$ L of each primer at 10  $\mu$ M concentration, 0.2  $\mu$ L of Platinum Taq DNA polymerase, and 17.05  $\mu$ L of purified water. For the *rbcL* gene, we used the primers ESRBCL1F and ESRBCL1361R (Schuettpezel and Pryer 2007) for amplification; the primers ES645F and ES663R (Schuettpezel and Pryer 2007) were also sent with the amplification primers for sequencing. For the intergenic spacer *trnL-trnF* we used the primers Fern-1 (Trewick et al. 2002) and f (Taberlet et al. 1991), and for the spacer *rps4-trnS* we used the primers *rps4F2* (Hennequin et al. 2003) and *trnS* (Souza-Chies et al. 1997); the same primers used for amplification of the intergenic spacers were sent for sequencing. Amplification of all three markers was performed on an Eppendorf Mastercycler pro S thermocycler. The PCR conditions for *rbcL* consisted of an initial denaturation cycle of 4 min at 94°C and then 35 cycles of 1 min at 94°C, 1 min at 52°C, 2 min at 72°C, followed by a final extension period of 10 min at 72°C. PCR conditions for *trnL-trnF* consisted of an initial

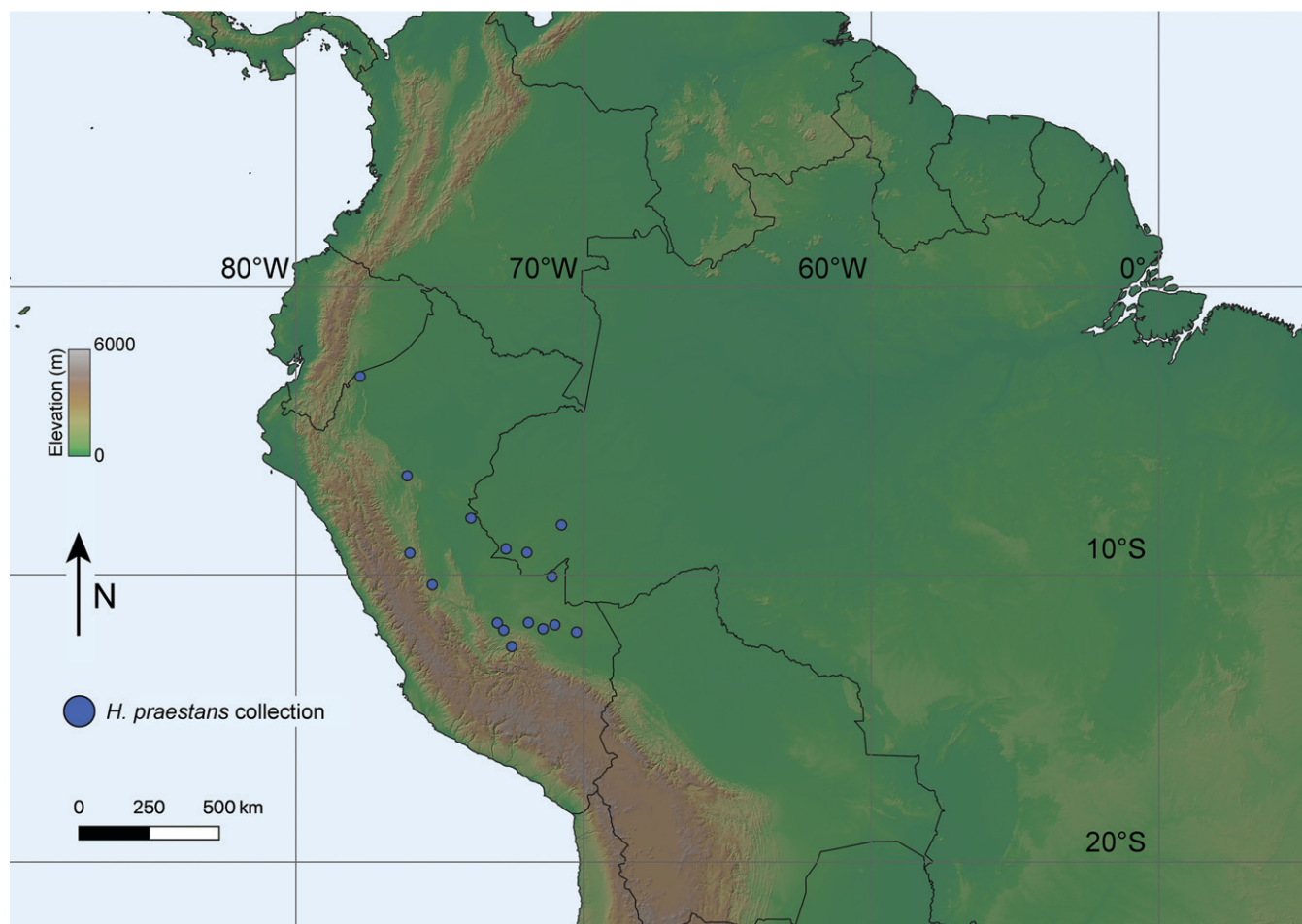


FIG. 1. Distribution map of *Hymenasplenium praestans*. Blue circles represent vouchered occurrence records from GBIF.org (as of May 2020).

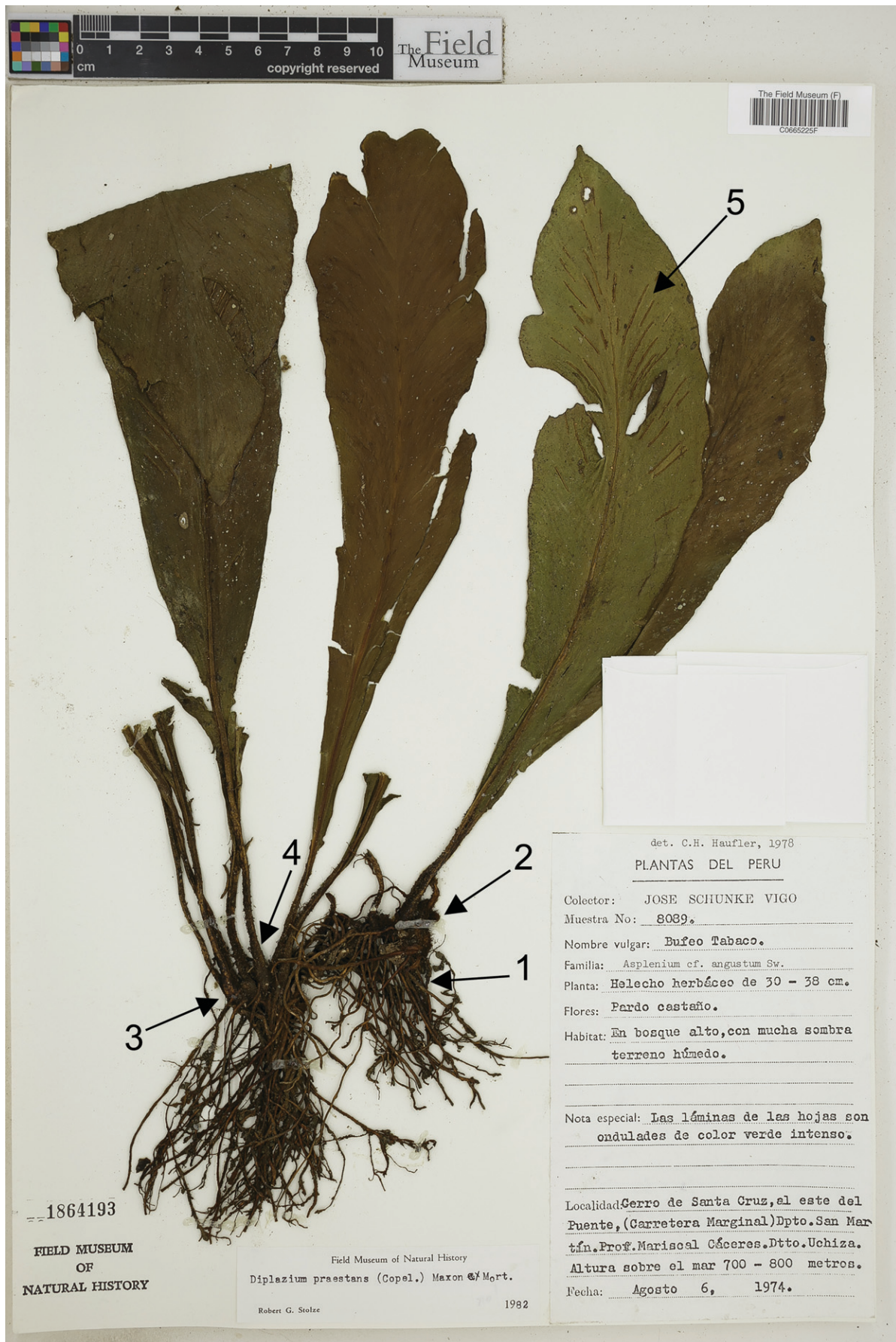


FIG. 2. *Hymenasplenium praestans* specimen (Schunke Vigo 8089, F) showing diagnostic characters. Numbered arrows indicate 1) ventrally inserted roots, 2) creeping rhizome, 3) distichous leaf arrangement, 4) swollen petiole bases, and 5) elongate, single-sided sori.

denaturation cycle of 2 min at 94°C and then 35 cycles of 15 s at 94°C, 30 s at 48°C, 1.5 min at 72°C, followed by a final extension period of 3 min at 72°C. PCR conditions for *rps4-trnS* consisted of an initial denaturation cycle of 4 min at 94°C and then 35 cycles of 1 min at 94°C, 1 min at 55°C, 1.5 min at 72°C, followed by a final extension period of 10 min at 72°C. The PCR products were electrophoresed and visualized on 1% agarose gel with ethidium bromide and purified using ExoSAP-IT (USB, Cleveland, Ohio) following manufacturer protocols. Purified PCR products and primers for each marker were sent to Eurofins Genomics (Louisville, Kentucky) for Sanger sequencing. Sequence data for an additional 50 taxa were obtained from GenBank, including representatives from 11 families: Aspleniaceae, Athyriaceae, Blechnaceae, Cystopteridaceae, Desmophlebiaceae, Diplaziopsidaceae, Hemidictyaceae, Onocleaceae, Rhachidosoraceae, Thelypteridaceae, and Woodsiaceae (Wolf et al. 1994; Murakami et al. 1999; Pinter et al. 2002; Smith and Cranfill 2002; Cranfill and Kato 2003; Wang et al. 2003; Pryer et al. 2004; Schneider et al. 2004a, 2004b, 2005; Tsutsumi and Kato 2006; Ebihara et al. 2010; Chunxiang et al. 2011; Rothfels et al. 2012a; Vicent et al. 2013; Wei et al. 2013; Ohlsen et al. 2015; Shao et al. 2015; Mynssen et al. 2016; Xu et al. 2018d). The newly generated sequences for *D. praestans* were uploaded to GenBank and accession numbers for all sequences included in our study are available in Appendix 1.

**Phylogenetic Analyses**—Sequence alignment was performed for each marker separately using the MAFFT (Katoh and Standley 2013) plugin for Geneious Prime 2019.2.1 (Biomatters Ltd.). Sequences were concatenated for the phylogenetic analyses with model selection performed using AICc model selection in Partition Finder 2.1.1 (Lanfear et al. 2017) under a greedy search algorithm (Lanfear et al. 2012) and PhyML (Guindon et al. 2010). The resulting substitution models and partition scheme were used in a maximum likelihood (ML) phylogenetic analysis in RAxML 8.2.12 (Stamatakis 2014) and a Bayesian inference (BI) phylogenetic analysis in MrBayes 3.2 (Ronquist et al. 2012) on the CIPRES portal (Miller et al. 2010). The ML analyses were run using the GTRGAMMA + I model and 25 rate categories; 1000 bootstrap replicates were performed. Our MrBayes analysis consisted of an MCMC run of 10 million generations with a sampling frequency of 2500 generations, resulting in 4000 trees. We used Tracer v. 1.6 (Rambaut and Drummond 2007) to assess convergence and ensure that ESS values for all parameters exceeded 200; the initial 500 trees were excluded as burn-in.

**Reconstruction of Ancestral Character States**—To evaluate the morphology of *Diplazium praestans* in a phylogenetic context, we performed ancestral character state reconstructions for eight discrete traits: sporangium stalk width, sorus shape, annulus cell number, phyllotaxy, rhizome habit, root insertion position, presence of swollen petiole bases, and rhizome stele orientation. Characters were scored based on study of herbarium specimens (see Representative Specimens), high resolution specimen images, and descriptions provided in previous studies (Murakami and Moran 1993; Sundue and Rothfels 2014). We studied these characters because they have been demonstrated to be informative for distinguishing genera of Aspleniaceae and especially for separating *Diplazium* and Aspleniaceae (Smith 1976; Sundue and Rothfels 2014). In particular, the first four characters listed above were used to differentiate between Athyriaceae and Aspleniaceae, whereas the last four characters were used to distinguish between the genera *Asplenium* and *Hymenasplenium*. A complete character matrix, including character states and scoring, is provided in Appendix 2. Ancestral character state reconstructions were carried out in a maximum likelihood framework with an equal rate character state transition model using the ‘ace’ function in the R package ‘ape’ (Paradis et al. 2004). Node character state estimates were visualized using the ‘lik.anc’ function in the R package ‘phytools’ (Revell 2012). These characters were reconstructed on the 50% majority-rule consensus tree; for visualization purposes, the tree was ultrametricized using the ‘compute.brln’ function in ‘ape’.

**Preliminary Conservation Assessment**—We used the R package ‘speciesgeocodeR’ (Töpel et al. 2017) and all georeferenced occurrence records of *Diplazium praestans* on GBIF (GBIF 2020; DOI: <https://doi.org/10.15468/dl.xxfxnv>) to assess its preliminary conservation status following the IUCN Red List (IUCN 2020) guidelines. Because detailed information on population sizes is not available for *D. praestans*, we based our assessment on the IUCN Criterion B (Geographic Range) by calculating extent of occurrence (EOO, criterion B1), area of occupancy (AOO, criterion B2), the number of occurrence locations (criterion B(a)), and the inferred change in habitat extent (criterion Bb(iii)). We estimated EOO and AOO using the ‘CalcRangeSize’ function. We used the ‘pseudospherical’ method of convex hull area estimation and 10 replicates to estimate EOO and employed a grid size of 4 km<sup>2</sup> for our AOO estimation. To avoid overestimation of the number of distinct locations, we used the ‘gBuffer’ function in the R package ‘rgeos’ (Bivand et al. 2017) to create a buffer with a 10 km radius around

each occurrence point and then counted all non-overlapping occurrences as distinct locations.

## RESULTS

**Phylogeny**—The final concatenated alignment was 2959 base pairs in length. The partitioning scheme returned by PartitionFinder included seven subsets with GTR + G selected as the best model for all but the second and third codon positions of *rbcl* for which GTR + I + G was selected as the best model (Table 1). The concatenated DNA alignment and tree files from both the ML and BI analyses are freely available on TreeBASE (<http://purl.org/phylo/treebase/phylogs/study/TB2:S26935>), and all data are available from the Dryad Digital Repository (Testo et al. 2021).

The ML and BI analyses recovered similar topologies with relatively strong support for all major clades. Relationships among the Aspleniaceae presented here are generally concordant with previously published phylogenies (Sano et al. 2000; Schneider et al. 2004a; Wei et al. 2013, 2017; Rothfels et al. 2015; Mynssen et al. 2016) with *Diplazium* (*D. plantaginifolium* and *D. cristatum* (Desr.) Alston) forming a fully-supported clade within a monophyletic Athyriaceae. *Hymenasplenium* and *Asplenium* each form a well-supported clade which together form a monophyletic Aspleniaceae (Fig. 3). *Diplazium praestans* is nested within *Hymenasplenium* with strong support (Fig. 3) and is sister to the Old World *Hymenasplenium* clade, though support for that relationship is low.

**Morphological Analyses**—SPORANGIUM STALK WIDTH—Sporangium stalks of *D. praestans* are uniformly uniseriate. Our character state reconstruction supports the finding that occurrence of uniseriate sporangial stalks is a synapomorphy for the Aspleniaceae (Fig. 4A).

SORUS SHAPE—All *D. praestans* specimens examined for this study displayed elongate sori along a single vein (i.e. asplenioid sori) (Fig. 2). In some cases, sori appeared to occur on both sides of a vein (i.e. diplazioid sori), but closer inspection revealed them to actually be asplenioid sori on adjacent veins. Our character state reconstruction supports single-sided sori as a synapomorphy for the clade comprising Aspleniaceae and Rhachidosoraceae, Diplaziopsidaceae, Desmophlebiaceae, and Hemidictyaceae (Fig. 4B).

ANNULUS CELL NUMBER—The mean number of annulus cells in examined sporangia of *D. praestans* is 20 (range: 19–22), similar to other members of Aspleniaceae, which range from 18–28 (mean = 19). The average number of annular cells for Athyriaceae is 14 (Sundue and Rothfels 2014); the *Diplazium* species studied here on average have 13 (*D. cristatum*) or 14 (*D. plantaginifolium*) annulus cells (Fig. 4C).

TABLE 1. Characteristics of the sequence data used in ML and BI phylogenetic analyses.

cpDNA region	Aligned length (bp)	Percent variable sites	Optimal model (AICc criterion)
<i>rbcl</i>	1269	35.6%	GTR+G (first codon position), GTR+I+G (second and third codon positions)
<i>rps4-trnS</i>	1132	71.4%	GTR+G
<i>trnL-trnF</i>	545	85.5%	GTR+G

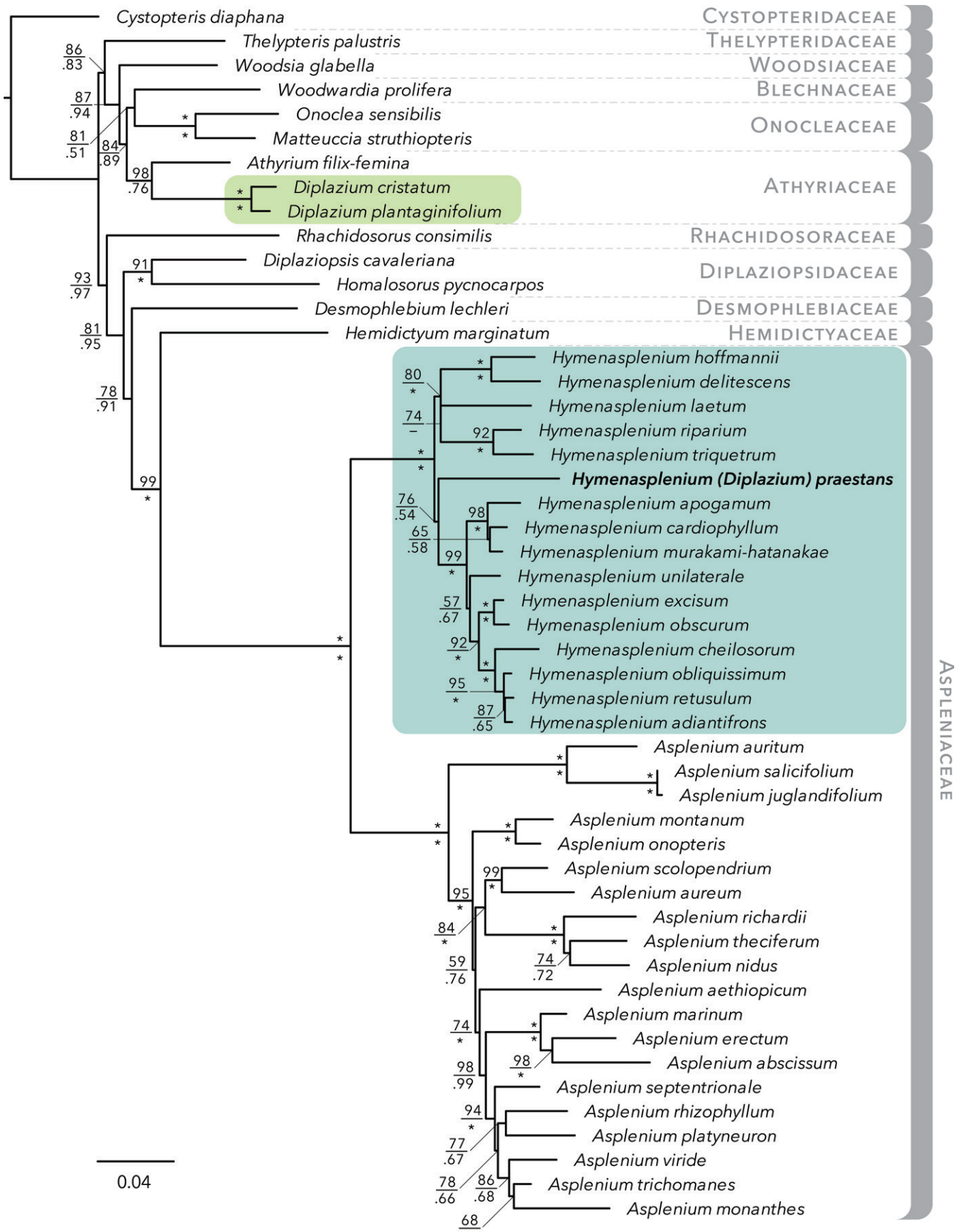


FIG. 3. Maximum likelihood phylogeny showing major relationships among the Aspleniaceae (eupolypods II). The clades representing *Diplazium* and *Hymenasplenium* are highlighted in green and turquoise boxes, respectively; family names are in gray. Support values above branches are ML bootstrap values and values below the branches are BI posterior probabilities; full support (100/1) is shown as an asterisk (\*), and support below 50/0.5 is represented by an em dash (—).

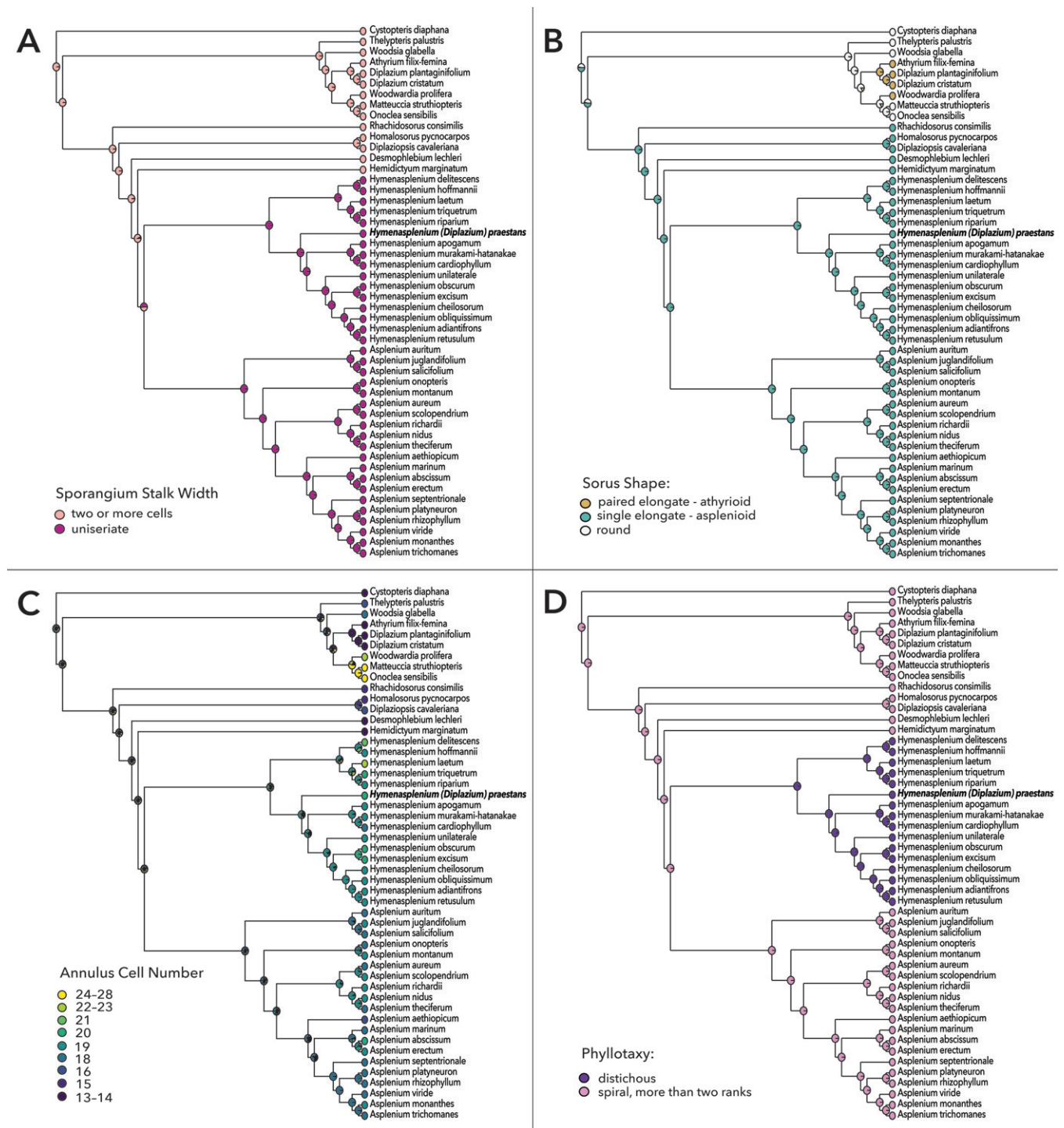


FIG. 4. Maximum likelihood ancestral character state reconstructions for four traits across Aspleniineae (eupolypods II). *Hymenasplenium praestans* is highlighted with bold font. A. Width of the sporangium stalk: two or more cells wide (light pink), uniseriate (magenta). B. Sorus shape: athyroid (gold), asplenioid (turquoise), round (white). C. Number of annulus cells: 13–16 (purple and blue), 18–20 (turquoise), 21–23 (greens), 24–28 (yellow). D. Phyllotaxy: distichous (purple), spiral and more than two ranks (pink).

**PHYLLOTAXY**—Although phyllotaxy is difficult to observe in *D. praestans* due to its relatively compact rhizome and short internodes, its leaves are distichous (Fig. 2). Distichous leaf arrangement was also found in all members of *Hymenasplenium* examined and is a synapomorphy for that genus. The species of *Asplenium* and *Diplazium* included in our analysis had spiral phyllotaxy with > 2 ranks (Fig. 4D).

**RHIZOME HABIT**—Rhizomes of *D. praestans* are short-creeping (Fig. 2), although many specimens do not clearly show this character due to the short length of internodes or because the collections are fragmentary. Some specimens (e.g. *Schunke Vigo 8089*, F; *Schunke Vigo 4250*, NY) are more intact and demonstrate that the species has creeping, albeit short, rhizomes. Creeping rhizomes are also present in *Hymenasplenium*, and

although rhizomes in that clade are generally long-creeping, having a creeping rhizome (regardless of length) is a synapomorphy for *Hymenasplenium*. The species of *Asplenium* and *Diplazium* evaluated here have erect rhizomes; however, creeping rhizomes occur (rarely) in both genera (Fig. 5A).

**ROOT INSERTION**—Roots of *D. praestans* are ventrally inserted (Fig. 2); this character state is shared by all *Hymenasplenium* species and is a synapomorphy for the genus. Both *Asplenium* and *Diplazium* have radially inserted roots (Fig. 5B).

**RHIZOME STELE ORIENTATION**—The rhizome stele of *D. praestans* is dorsiventrally oriented, as are those of *Hymenasplenium* species. Rhizomes of *Asplenium* and *Diplazium* species studied here are radially symmetrical (Fig. 5C).

**PRESENCE OF SWOLLEN PETIOLE BASES**—The leaves of *D. praestans* have swollen petiole bases (Fig. 2). Swollen petiole bases are present in numerous Aspleniineae genera, including *Hymenasplenium*. Swollen petiole bases were absent in the *Asplenium* and *Diplazium* species examined (Fig. 5D).

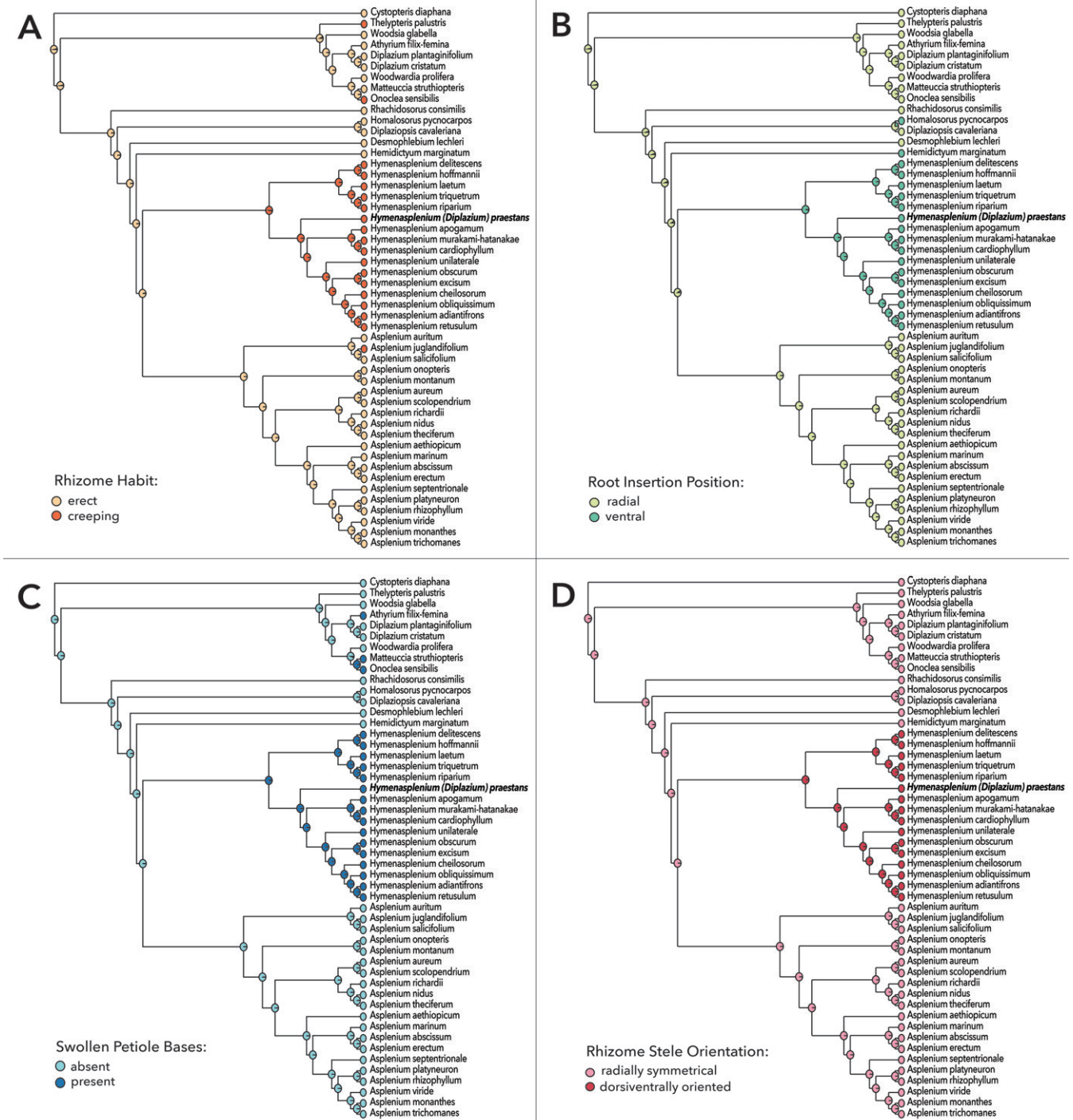


FIG. 5. Maximum likelihood ancestral character state reconstructions for four traits across Aspleniineae (eupolypods II). *Hymenasplenium praestans* is highlighted with bold font. A. Rhizome habit: erect (yellow), creeping (orange). B. Root insertion position: radial (chartreuse), ventral (turquoise). C. Presence of swollen petiole bases: absent (light blue), present (dark blue). D. Rhizome stele orientation: radially symmetrical (pink), dorsiventrally oriented (red).

Given its phylogenetic placement and morphology, we recognize *Diplazium praestans* as a member of *Hymenasplenium*; we provide a combination for it in that genus and a taxonomic treatment for the species here. We also provide a key to the Neotropical species of *Hymenasplenium*, which is derived principally from that of Murakami and Moran (1993); a key in Spanish is available at <http://www.westontesto.com/helpful-stuff.html>.

#### TAXONOMIC TREATMENT

*Hymenasplenium praestans* (Copel.) Testo, Riibe & Sundue, comb. nov. *Athyrium praestans* Copel., Amer. Fern J. 38: 132 (1948). *Diplazium praestans* (Copel.) Maxon ex Morton, Contr. U. S. Natl. Herb. 38: 4 (1967). TYPE: PERU. San Martín, Chazuta, Río Huagalla, March 1935, G. Klug 4002 (holotype: US!, barcode: 1592381; isotypes: CAS!, barcode: 1051; NY!, barcode: 149348; F image!, barcode: V0075630F; K image!, barcode: K000632800; MO!, barcode: 1827268).

Plants terrestrial; **rhizome** short-creeping, at times appearing erect or decumbent, scales 1–3 mm long, brown, concolorous, with entire margins; **leaves** to 35 cm long, erect, simple, margins entire to undulate, arranged in two ranks; **petiole** 0–5 cm long, brown, with linear to lanceolate scales, like scales of the rhizome but longer and broader; **lamina** 20–33 cm long and 5–9 cm broad, glabrous, ovate to oblanceolate, brilliant green in live plants and olive-green in pressed specimens, the base attenuate and asymmetrically decurrent, apex obtuse to sub-acute; **rachis** slightly grooved adaxially; **veins** irregularly anastomosing, without included veinlets; **sori** linear, slightly arcuate, up to 5 cm long, single along vein, rarely appearing paired when veins are closely spaced; **indusia** narrow, membranaceous, persistent; **spores** monolete, reniform.

**Distribution and Habitat**—*Hymenasplenium praestans* is primarily distributed in Peru, with populations in northern Bolivia (Departamento Beni) and Amazonian Brazil (Estado Acre) (Fig. 1). It occurs in dense, humid forests in deep shade; often along streams and riverbanks in moist soils; sometimes on rocky limestone substrate.

**Preliminary Conservation Status**—*Hymenasplenium praestans* is known from a total of 23 collections and 16 distinct locations in western Amazonia of Peru, Bolivia, and Brazil. By our estimates, its EOO is 392,405 km<sup>2</sup> and its AOO is 256 km<sup>2</sup>. Rates of deforestation in the region of western Amazonia where *H. praestans* occurs are increasing, largely due to gold mining practices (Asner and Tupayachi 2017; Caballero Espejo et al. 2018), indicating a decline in the extent and quality of habitat throughout its range. Because the number of locations where *H. praestans* is thought to occur (based on data from herbarium specimens) exceeds 10, and its EOO exceeds 20,000 km<sup>2</sup>, it may not qualify for IUCN listing under a threatened category at this time (IUCN 2020). However, because of the continued

and accelerating deforestation observed in the western Amazon, its geographic range is likely to become severely fragmented in the foreseeable future. Therefore, criteria B2b(iii) of the IUCN guidelines could be applied, and due to its low AOO (< 500 km<sup>2</sup> threshold for ‘Endangered’ threat category) and the threat of fragmentation and loss of native forest where it occurs, *H. praestans* could be preliminarily assigned as Near Threatened (IUCN 2020).

**Notes**—*Hymenasplenium praestans* can be confused with *Diplazium aberrans*, a member of *Diplazium* subgenus *Callipteris* (Bory) R. Wei & X.C. Zhang (Wei and Zhang 2020). *Diplazium aberrans* is another Neotropical fern with simple leaves and anastomosing veins, and it differs from *H. praestans* in its larger leaves (up to 60 cm vs. 35 cm in *H. praestans*), double-sided sori (vs. single-sided in *H. praestans*), vascular bundles in the petiole uniting distally to form a “U” (vs. vascular bundles uniting distally to form an “X” in *H. praestans*), and the presence of dark-margined petiole scales with bifid teeth on the leaf (vs. concolorous and entire-margined scales restricted to the rhizome and petiole in *H. praestans*).

The range of *Hymenasplenium praestans* overlaps with those of at least four other species: *H. delitescens* (Maxon) L. Regalado & Prada, *H. laetum* (Sw.) L. Regalado & Prada, *H. ortegae* (N. Murak. & R.C. Moran) L. Regalado & Prada, and *H. repandulum* (Kunze) L. Regalado & Prada. Fieldwork is needed to reveal if they co-occur.

**Representative Specimens**—**Brazil**. —ACRE: Jordão, Ao longo do Rio Jordão, [-9.2244°, -71.9739°], R. Goldenberg 1325 (RB image!, NY!); Marechal Thaumaturgo, Rio Juruá, Reserva Extrativista do Alto Juruá, N of Sao Joao do Breu, [-9.1°, -73.7°], D. C. Daly 7721 (NY!); Tarauacá, Rio Muru, 12 km above confluence with Rio Tarauacá, G. T. Prance 7400 (F image!, NY!, L image!). **Bolivia**. —BENI: Gral. Ballivian, ca. 3 km S of Rurrenabaque, J. Weigelt 90373 (LPB, TUR, UC, Z). **Peru**. —CUSCO: La Convención, Distr. Echarati, Armihuari-Río Camisea, [-11.932°, -72.7775°], P. Nuñez V. 24216 (US!); La Convención, Distr. Echarati, Armihuari-Río Camisea, [-11.932°, -72.7775°], P. Nuñez 24109 (US!); Pagoreni well site [-11.68°, -73°], P. Nuñez 21609 (US!). —HUÁNUCO: Tingo María, R. C. Moran 3680 (NY!). —JUNÍN: Cahuapapas, on Río Pichis, E. P. Killip 26798 (NY!); Río Pinedo, north of La Merced, E. P. Killip 23621 (NY!). —LORETO: Puerto Arturo, Lower Río Huallaga below Yurimaguas, E. P. Killip 27800 (NY!); Prov. Coronel Portillo, Dto. Iparia, Bosque Nacional de Iparia, A 1 km. abajo de Iparia, al nor oeste del Río Ucayali, J. M. Schunke Vigo 2752 (NY!); San Alejandro river bank, F. Woytkowski 5118 (GH!). —MADRE DE DIOS: Manu National Park, Across river from Cocha Cashu camp, [-12°, -70.25°], A. Gentry 27205 (F image!); Prov. Manu, Manu Park, Cocha Cashu uplands, [-11.75°, -71°], P. Nuñez 5725 (F image!); Prov. Manu, Cucha cashu, Nuñez 1839 (F image!); Prov. Manu, Parque Nacional Manu, Río Manu: Cocha Cashu Station [-12°, -70.25°] R. B. Foster 7193 (F image!); Prov. Manu, Parque Nacional Manu, Río Manu: Río Sotileja, [-11.67°, -71.92°], R. B. Foster 11722 (US image!); Prov. Manu, Parque Nacional Manu, Río Manu: Río Sotileja, [-11.67°, -71.92°], R. B. Foster 11723 (F image!); Prov. Manu, Shintuya a Pilcapata, C. Vargas 23754 (L image!). —PASCO: Oxapampa, Dist. Palcazú, Parque Nacional Yanachanga-Chemillén, sector Paujil-Trocha Venado, I. Huamantupa 10651 (NY!). —SAN MARTÍN: Prov. Mariscal Cáceres, Dto. Uchiza, Cerro de Santa Cruz, al este del Puente, (Carretera Marginal), J. M. Schunke Vigo 8089 (F image!, NY!); Prov. Mariscal Cáceres, Dto. Campanilla, Quebrada de Mashuyacu, (margen izquierda del Río Huallaga), J. M. Schunke Vigo 4250 (F image!, NY!). —UCAYALI: Prov. de Purús, Distrito Purús, Río Curanja, cerca la comunidad nativa de Colombiana, [-10.07°, -71.1°], J. G. Graham 1133 (NY!).

#### KEY TO THE NEOTROPICAL SPECIES OF HYMENASPLENIUM

1. Leaves of mature plants simple; veins anastomosing. . . . . *H. praestans*
1. Leaves of mature plants pinnate to pinnate-pinnatifid; veins free. . . . . 2
2. Plants epiphytic or hemiepiphytic; space between leaves in the same rank 1–4 cm; petioles 1/7–1/3 the length of the leaf. . . . . 3
3. Rhizomes growing linearly (not twining) on large tree trunks; rachises strongly carinate (keel may be pushed to one side in pressed specimens), alate, the wings adaxial and in the same plane as the lamina (wings may shrivel upon drying obscuring this character); plants from eastern Ecuador and Peru. . . . . *Hymenasplenium repandulum* (Kunze) L. Regalado & C. Prada
3. Rhizomes twining spirally around saplings and small tree trunks; rachises terete to sub-terete, alate, the wings adaxial and perpendicular to the lamina (wings may shrivel upon drying or appear to be in the same plane as the lamina when pressed flat; best to observe wing orientation at



- pinnae junctures); plants from Costa Rica, Panamá, Colombia, and western Ecuador. . . . . *Hymenasplenium volubile* (N.Murak. & R.C.Moran) L.Regalado & C.Prada
2. Plants terrestrial or epilithic (or rheophytic); space between leaves in the same rank < 1 cm (up to 2 cm in *H. triquetrum*); petioles 1/3–1/2 the length of the leaf. . . . . 4
4. Rachises not alate . . . . . 5
5. Petioles and rachises atropurpureous to black; pinna pairs 10–25. . . . . *Hymenasplenium laetum* (Sw.) L.Regalado & C.Prada
5. Petioles and rachises stramineous to greenish-brown; pinna pairs 3–10(–11). . . . . 6
6. Plants epilithic, often growing near or under waterfalls; rhizomes of living plants green; lamina < 20 cm long, fleshy; basal pinnae deeply lobed, basal lobes sometimes free; spores papillose. . . . . *Hymenasplenium obtusifolium* (L.) L.Regalado & C.Prada
6. Plants terrestrial, sometimes growing on rocks; rhizomes of living plants reddish-brown to black; lamina > 20 cm long, not fleshy; basal pinnae not deeply lobed, acroscopic auricles present but not free; spores cristate. *Hymenasplenium delitescens* (Maxon) L.Regalado & C.Prada
4. Rachises alate, wings green and herbaceous in living plants. . . . . 7
7. Pinnae regularly pinnatifid, with a basal acroscopic lobe or auricle that is deeply incised and sometimes free. . . . . 8
8. Pinna pairs (10–)14–18, basisopic pinnules of basal pinnae elongate; vein forks 4–8 per segment lobe; plants from the eastern slopes of the Andes in Bolivia. . . . . *Hymenasplenium basisopicum* (R.C.Moran & M.A.Sundue) L.Regalado & C.Prada
8. Pinna pairs 8–11, basisopic pinnules of basal pinnae excavate or not elongate; vein forks 3–4 per segment lobe; plants from the western side of the Andes in Ecuador. . . . . *Hymenasplenium purpurascens* (Mett. ex Kuhn) L.Regalado & C.Prada
7. Pinnae with doubly-serrate to nearly entire margins but not pinnatifid, basal acroscopic auricle sometimes present but < 4 mm and never free. . . . . 9
9. Rachis strongly carinate, adaxial rachis wings in the same plane as the lamina; pinna pairs 12–17. . . . . *Hymenasplenium triquetrum* (N.Murak. & R.C.Moran) L.Regalado & C.Prada
9. Rachis terete to sub-terete, adaxial rachis wings perpendicular to the lamina (wings may shrivel upon drying or appear to be in the same plane as the lamina when pressed flat; best to observe wing orientation at pinnae junctures); pinna pairs 2–12(–14). . . . . 10
10. Plants terrestrial; petioles atropurpureous; lamina deltate to oblong. . . . . *Hymenasplenium ortegae* (N.Murak. & R.C.Moran) L. Regalado & C.Prada
10. Plants epilithic; petioles greenish or brown; lamina lanceolate to oblong. . . . . 11
11. Pinna pairs 8–14, auricles 1–4 mm; plantlets produced from sori of senescent leaves. . . . . *Hymenasplenium riparium* (Liebm.) L.Regalado & C.Prada
11. Pinna pairs 2–6(–8), lacking auricles; plantlets lacking on senescent leaves. . . . . *Hymenasplenium hoffmannii* (Hieron.) L.Regalado & C.Prada

#### DISCUSSION

Morphological similarity of athyrioid and asplenioid ferns has long been a source of uncertainty for fern taxonomists. Relationships among these taxa and the Aspleniaceae clade as a whole have largely been clarified in recent years as a result of numerous molecular phylogenetic studies (Sano et al. 2000; Schuettpelz and Pryer 2007; Wei et al. 2010; Kuo et al. 2011; Rothfels et al. 2012a; Mynssen et al. 2016). These studies coupled with detailed morphological analysis (Sundue and Rothfels 2014) have demonstrated that the apparent similarity of these clades is due to a combination of plesiomorphic and homoplastic character states, including elongate sori and sulcate rachises. One recurring finding from such studies has been that several taxa formerly treated in the genus *Diplazium* are in fact more closely allied to Aspleniaceae. In the modern era, this was first demonstrated by Smith (1976), who used morphological, anatomical, and cytological data to transfer *Diplazium delitescens* to *Hymenasplenium*, a move supported by later molecular studies. More recently, a small number of Asian, Malesian, and eastern North American species that had been treated within various athyrioid genera were found to be only distantly related to Athyriaceae and are now placed in their own family, Diplaziopsidaceae (Sano et al. 2000; Wei et al. 2010; Kuo et al. 2011). Similarly, molecular and morphological evidence led to the transfer of members of the genus *Rhachidosorus* Ching to the monogeneric family Rhachidosoraceae (Sano et al. 2000; Tzeng 2002; Wang et al. 2003; Kuo et al. 2011; Rothfels et al. 2012b). In the most recent such case, two Neotropical species of *Diplazium* with once-pinnate leaves and a unique venation type were found to be sister to Hemidictyaceae + Aspleniaceae, and are now placed in the genus *Desmophlebium* Mynssen, A.Vasco, Sylvestre, R.C.Moran & Rouhan (Desmophlebiaceae) (Mynssen et al. 2016). *Hymenasplenium praestans* is clearly another such example of pteridological mistaken identity.

The fact that the identity of *H. praestans* has not been re-evaluated since it was transferred to *Diplazium* from *Athyrium* by Morton in 1967 is likely due to a combination of its rarity, occurrence in under-collected and understudied regions, and its unusual morphology. Moreover, the taxon is poorly represented in herbaria (just 23 distinct records on GBIF and 27 total images on pteridoportal.org, the data portal for the Pteridophyte Collections Consortium, as of May, 2020), which undoubtedly limited the attention it has received from taxonomists. Further, *H. praestans* has been included in only a few floristic and taxonomic treatments (Tryon and Stolze 1991; Prado et al. 2017; Mynssen and Sylvestre 2019) and few specimens were examined in those works. In addition to problems posed by the paucity of collections and field observations, the gross morphology of *H. praestans* does not immediately suggest a close affinity to Aspleniaceae, and the arrangement of its sori does closely resemble that of some *Diplazium* species. It was only as part of an earlier study of *Diplazium* hybrids (Testo et al. 2017) that we examined the morphology of *H. praestans* more closely and brought into question its identity.

Detailed study of the morphology of *H. praestans* clearly shows its affinity with Aspleniaceae rather than Athyriaceae. Like all members of Aspleniaceae, *H. praestans* possesses a uniseriate sporangial stalk, whereas Athyriaceae have sporangial stalks that are two or three cells wide (Smith 1976; Sundue and Rothfels 2014). The number of annulus cells per sporangium is also diagnostic; *H. praestans* (19–22) falls within the observed range in Aspleniaceae (18–28, mean = 19) and is well above the number known from Athyriaceae (mean = 14) (Sundue and Rothfels 2014). We were unable to find convincing evidence of true diplazioid sori in *H. praestans*, despite the fact that this character state has been reported for this species previously. In some cases, sori of *H. praestans* appear to be diplazioid, but closer examination shows that these sori are in fact on adjacent veins. We suspect that this ‘pseudo-diplazioid’ arrangement, which appears to be caused by dense,

anastomosing venation, contributed to the placement of this taxon in *Diplazium*. Interestingly, the two known species of *Hymenasplenium* with simple leaves (*H. cardiophyllum* (Hance) Nakaike and *H. ikenoi* (Makino) Viane) also have anastomosing veins; Holttum (1954) hypothesized that these character states co-evolve as a product of laminar fusion or reduction. Given the numerous cases of anastomosing venation in simple-leaved ferns, especially in Aspleniaceae, Holttum's hypothesis warrants further attention.

There are several characters which also unite *H. praestans* with *Hymenasplenium* and distinguish it from both *Diplazium* and *Asplenium*. These include distichous phyllotaxy (members of *Diplazium* and *Asplenium* display phyllotaxy of greater than two ranks), creeping rhizome habit (members of *Diplazium* and *Asplenium* generally have erect rhizomes), ventral root insertion position (members of *Diplazium* and *Asplenium* have radially inserted roots), dorsiventral rhizome stele orientation (members of *Diplazium* and *Asplenium* have radially symmetrical rhizome steles), and presence of swollen petiole bases (absent in members of *Diplazium* and *Asplenium*). Some of these characters were initially difficult to recognize in *D. praestans* because of the poor quality of many specimens and the fact that the rhizome, although creeping, is much more compact than that of many other *Hymenasplenium* species. This short rhizome may represent an adaptation to floodplain forests in which it grows, where a long-creeping rhizome may not be as beneficial as it would be in habitats where other *Hymenasplenium* species often occur, such as on rocks or on tree trunks (Murakami and Moran 1993; Watts et al. 2019). More collections of *H. praestans* and more detailed observations of its ecology are needed to better understand its growth form.

Our findings highlight the need for additional systematic study of *Hymenasplenium* to appropriately document the genus' true diversity. A recent study by Xu et al. (2018d) uncovered extensive cryptic speciation within the Old-World members of the genus, with four of the widespread "species" in their analysis being non-monophyletic. They concluded these four species represented a total of 17 taxa, most of which are narrowly distributed. Following this phylogenetic work, a large number of new species of *Hymenasplenium* have recently been described, particularly from China and Vietnam (Xu et al. 2018a, 2018b, 2018c, 2018e, 2019). In total, 25 new species of *Hymenasplenium* have been described in the last four years (PPG I 2016), raising the total number of species recognized worldwide to approximately 65.

In contrast to the Old-World members of the genus, Neotropical *Hymenasplenium* remain poorly sampled in phylogenetic studies with few molecular analyses which include both Old-World and Neotropical taxa (Murakami and Schaal 1994; Murakami 1995; Iwatsuki et al. 1998; Murakami et al. 1999; Xu et al. 2018d). Of these, Xu et al. (2018d) had the most complete sampling of both regions (5 Neotropical and 19 Old-World species) and recovered a Neotropical clade sister to the Old-World clade. In our analyses *H. praestans* resolved sister to the Old-World members of *Hymenasplenium* rather than to the other five Neotropical species. While our results suggest that Neotropical *Hymenasplenium* may not be monophyletic, support for this relationship in our study is weak (Fig. 3). Increased sampling of the Neotropical taxa may yield better resolution of these relationships, and would likely uncover additional species diversity as several taxonomic studies have detected the apparent occurrence of numerous cryptic taxa. Murakami and Moran (1993) segregated *Hymenasplenium*

*ortegae* from *Hymenasplenium purpurascens* on the basis of subtle morphological differences and geography: *H. purpurascens* is restricted to the western side of the Andes, whereas *H. ortegae* is mostly restricted to the eastern side. Later, Moran and Sundue (2004) split another geographically distinct species from *H. purpurascens*, showing that specimens formerly treated as *H. purpurascens* from the eastern side of the Andes in Bolivia actually represent a novel taxon, which they named *Hymenasplenium basiscopium*. In addition, cytological data and spore counts indicate that hybridization and polyploidy are widespread in Neotropical *Hymenasplenium*. Murakami and Moran (1993) reported the existence of two geographically distinct 'races' of *Hymenasplenium obtusifolium*, with a 32-spored race (likely an apomictic lineage) present in Central America, Jamaica, and northwestern South America, and a 64-spored race (sexual lineage) occurring in northern Venezuela, throughout the Lesser Antilles, and Puerto Rico. The widespread *Hymenasplenium laetum* also appears to comprise a polyploid complex involving frequent hybridization, and at least four other hybrids are known (Murakami and Moran 1993; Regalado and Prada 2011). Detailed work integrating morphology, cytology, geography, and nuclear and plastid DNA sequence data is badly needed both to resolve these reticulate complexes and identify other *Hymenasplenium* species that, like *H. praestans*, are "hiding in plain sight."

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#### AUTHOR CONTRIBUTIONS

LR and WT performed lab work and carried out the phylogenetic analyses. WT and MS scored morphological characters and compiled the morphological matrix. LR wrote the taxonomic treatment and species key. LR and WT were the primary authors of the manuscript, with contributions by ES and MS.

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APPENDIX 1. GenBank accession numbers of samples used in this study, listed in alphabetical order by species name. The data for existing sequences are ordered: Taxon name, *rbcL* accession number, *rps4-trnS* accession number, *trnL-trnF* accession number. New sequence data generated for this study are in bold, and these data are ordered: Taxon name, voucher, herbarium code, *rbcL* accession number, *rps4-trnS* accession number, *trnL-trnF* accession number.

*Asplenium abscissum*, AY300102, AY549768, AY300049; *Asplenium aethiopicum*, AF240654, AY549823, AF525233; *Asplenium aureum*, AF240642, AY549767, AF525258; *Asplenium auritum*, —, AY549759, AF240667; *Asplenium erectum*, AY300113, AY549770, AY300060; *Asplenium juglandifolium*, AF525269, AY459168, AF525245; *Asplenium marinum*, AF240647, AY549773, AF240662; *Asplenium monanthes*, AY300125, AY549797, AY300072; *Asplenium montanum*, AY300126, JX068776, AY300073; *Asplenium nidus*, AF525270, AY549807, AF525246; *Asplenium (Hymenasplenium) obliquissimum*, MH065413, MH065356, MH065569; *Asplenium onopteris*, AY300131, KF923965, AY300078; *Asplenium platyneuron*, AF525272, —, AF525240; *Asplenium rhizophyllum*, AY300136, AY549781, AY300083; *Asplenium richardii*, AY300138, AY549810, AY300085; *Asplenium salicifolium*, AY300139, —, AY300086; *Asplenium scolopendrium*, AF240645, AY612650, AF525262; *Asplenium septentrionale*, AF525275, AY549777, AF525248; *Asplenium theciferum*, AY300123, AY549821, AY300070; *Asplenium trichomanes*, —, —, AF525237; *Asplenium viride*, AF240649, AY549782, —; *Athyrium filix-femina*, —, AF425152, —; *Cystopteris diaphana*, KT329389, KT329399, KT329412; *Desmophlebium lechleri*, KT329390, KT329403, KT329416; *Diplaziopsis cavaleriana*, JN168016, JN168089, AF515251; *Diplazium cristatum*, KC254362, KC254515, KC254434; *Diplazium plantaginifolium*, KC254361, KC254514, KC254433; ***Hymenasplenium praestans***, **Woytkowski 5118, GH, MT936350, MT936351, MT936352**; *Hemidictyum marginatum*, KT329397, KT329410, KT329423; *Homalosorus pycnocarpus*, AF425101, AF425154, AF425124; *Hymenasplenium adiantifrons*, —, MH065322, MH065559; *Hymenasplenium apogamum*, MH065437, MH065376, MH065604; *Hymenasplenium cardiophyllum*, MH065387, MH065306, MH065534; *Hymenasplenium cheilosorum*, MH065421, MH065362, MH065575; *Hymenasplenium delitescens*, MH065443, MH065338, MH065609; *Hymenasplenium excisum*, KP774884, KP851882, KP851914; *Hymenasplenium hoffmannii*, MH065442, MH065337, MH065608; *Hymenasplenium laetum*, AB014707, —, —; *Hymenasplenium murakami-hatanakae*, AB574891, —, —; *Hymenasplenium obscurum*, MH065380, MH065342, MH065530; *Hymenasplenium retusulum*, MH065379, MH065304, MH065529; *Hymenasplenium riparium*, AB014708, —, —; *Hymenasplenium triquetrum*, MH065444, MH065339, MH065610; *Hymenasplenium unilaterale*, AF240652, —, AF525232; *Matteuccia struthiopteris*, AB232415, AF425158, KC254425; *Onoclea sensibilis*, JF832076, AF425159, —; *Rhachidosorus consimilis*, NC\_035862, NC\_035862, NC\_035862; *Thelypteris palustris*, U05947, AF425189, AF425145; *Woodsia glabella*, KP226763, —, KP226788; *Woodwardia prolifera*, AB040603, AF533864, —.

APPENDIX 2. Matrix of morphological characters scored for this study. The data are ordered: Taxon name, rhizome habit (0 = creeping, 1 = erect), rhizome stele orientation (0 = dorsiventral, 1 = radial), root insertion position (0 = ventral, 1 = radial), number of annulus cells, sorus shape (0 = round, 1 = elongate-diplazioid, 2 = elongate-asplenioid), width of the sporangium stalk (1 = uniseriate, 2 = 2–3 cells), presence of swollen petiole bases (absent = 0, present = 1), phyllotaxy (0 = distichous, 1 = > 2 ranks).

*Asplenium abscissum*, 1, 1, 1, 20, 2, 1, 0, 1; *Asplenium aethiopicum*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium aureum*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium auritum*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium erectum*, 1, 1, 1, 19, 2, 1, 0, 1; *Asplenium juglandifolium*, 0, 1, 1, 19, 2, 1, 0, 1; *Asplenium marinum*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium monanthes*, 1, 1, 1, 19, 2, 1, 0, 1; *Asplenium montanum*, 1, 1, 1, 19, 2, 1,

0, 1; *Asplenium nidus*, 1, 1, 1, 19, 2, 1, 0, 1; *Asplenium onopteris*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium platyneuron*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium rhizophyllum*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium richardii*, 1, 1, 1, 19, 2, 1, 0, 1; *Asplenium salicifolium*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium scolopendrium*, 1, 1, 1, 19, 2, 1, 0, 1; *Asplenium septentrionale*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium theciferum*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium trichomanes*, 1, 1, 1, 18, 2, 1, 0, 1; *Asplenium viride*, 1, 1, 1, 18, 2, 1, 0, 1; *Cystopteris diaphana*, 1, 1, 1, 13, 0, 2, 0, 1; *Athyrium filix-femina*, 1, 1, 1, 13, 1, 2, 1, 1; *Desmophlebium lechleri*, 1, 1, 1, 14, 2, 2, 0, 1; *Diplaziopsis cavaleriana*, 1, 1, 1, 16, 2, 2, 0, 1; *Diplazium cristatum*, 1, 1, 1, 13, 1, 2, 0, 1; *Diplazium plantaginifolium*, 1, 1, 1, 14, 1, 2, 0, 1; *Diplazium (Hymenasplenium) praestans*, 0, 0, 0, 20, 2, 1, 1, 0; *Hemidictyum marginatum*, 1, 1, 0, 14, 2, 2, 0, 1; *Homalosorus pycnocarpus*, 1, 1, 0, 15, 2, 2, 0, 1; *Hymenasplenium adiantifrons*, 0, 0, 0, 19, 2, 1, 1, 0; *Hymenasplenium apogamum*, 0,

0, 0, 19, 2, 1, 1, 0; *Hymenasplenium cardiophyllum*, 0, 0, 0, 18, 2, 1, 1, 0; *Hymenasplenium cheilosorum*, 0, 0, 0, 19, 2, 1, 1, 0; *Hymenasplenium delitescens*, 0, 0, 0, 21, 2, 1, 1, 0; *Hymenasplenium excisum*, 0, 0, 0, 20, 2, 1, 1, 0; *Hymenasplenium hoffmannii*, 0, 0, 0, 19, 2, 1, 1, 0; *Hymenasplenium laetum*, 0, 0, 0, 23, 2, 1, 1, 0; *Hymenasplenium murakami-hatanakae*, 0, 0, 0, 19, 2, 1, 1, 0; *Hymenasplenium obliquissimum*, 0, 0, 0, 19, 2, 1, 1, 0; *Hymenasplenium obscurum*, 0, 0, 0, 20, 2, 1, 1, 0; *Hymenasplenium retusulum*, 0, 0, 0, 19, 2, 1, 1, 0; *Hymenasplenium riparium*, 0, 0, 0, 19, 2, 1, 1, 0; *Hymenasplenium triquetrum*, 0, 0, 0, 20, 2, 1, 1, 0; *Hymenasplenium unilaterale*, 0, 0, 0, 19, 2, 1, 1, 0; *Macrothelypteris torresiana*, 1, 1, 1, 16, 0, 2, 1, 1; *Matteuccia struthiopteris*, 1, 1, 1, 24, 0, 2, 1, 1; *Onoclea sensibilis*, 0, 1, 1, 28, 0, 2, 1, 1; *Rhachidosorus consimilis*, 1, 1, 1, 15, 2, 2, 0, 1; *Thelypteris palustris*, 0, 1, 1, 16, 0, 2, 0, 1; *Woodsia glabella*, 1, 1, 1, 18, 0, 2, 0, 1; *Woodwardia prolifera*, 1, 1, 1, 22, 1, 2, 0, 1.