Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften
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# EVOLUTION AND CLASSIFICATION OF *ELAPHOGLOSSUM* AND *ASPLENIUM* FERNS ON CUBA, AND DISCOVERY OF A MIOCENE *ELAPHOGLOSSUM* IN DOMINICAN AMBER

Josmaily Lóriga Piñeiro

München, 15. Januar 2018

**A mi familia y mis amigos, donde quiera que estén**

*To my family and my friends, wherever they are*

# PREFACE

## Statutory declaration

## Erklärung

Diese Dissertation wurde im Sinne von §12 der Promotionsordnung von Prof. Dr. Jochen Heinrichs betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

## Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Josmaily Lóriga, 15. Januar 2018
(Unterschrift)

1. Gutachter: Prof. Dr. Susanne S. Renner
2. Gutachter: Prof. Dr. Reinhard Agerer

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**Declaration of contribution**

In this thesis, I present the results from my doctoral research, carried out in Munich from March 2013 to January 2018 (maternity leave between February and December 2017) under the guidance of Prof. Dr. Jochen Heinrichs. My thesis resulted in three published manuscripts presented in Chapters 2 to 4. I generated all data and conducted all analyses myself with some exceptions: Chapter 2, Alejandra Vasco produced Fig. 4; Chapter 3, Ledis Regalado conducted the study of gametophytes and generated the drawings of Fig. 1; and Chapter 4, Kathrin Feldberg contributed to the divergence time estimates and Alexander R. Schmidt produced Fig. 1. Writing and discussion involved collaboration with J. Heinrichs, with input from the co-authors.

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| Josmaily Lóriga | Prof. Susanne S. Renner |
| (Signature) | (Signature) |

**List of publications**

***Peer-reviewed journal articles***

LÓRIGA, J., VASCO, A., REGALADO, L., HEINRICHS, J., MORAN, R.C. 2014. Phylogeny and classification of the Cuban species of *Elaphoglossum* (Dryopteridaceae), with description of *Elaphoglossum* sect. *Wrightiana* sect. nov. *Plant Systematics and Evolution* 300, 937–951.

LÓRIGA, J., SCHMIDT, A.R., MORAN, R.C., FELDBERG, K., SCHNEIDER, H., HEINRICHS, J. 2014. The first fossil of a bolbitidoid fern belongs to the early-divergent lineages of *Elaphoglossum* (Dryopteridaceae). *American Journal of Botany* 101, 1466–1475.

LÓRIGA, J., REGALADO, L., PRADA, C., SCHNEIDER, H., HEINRICHS, J. 2016. Phylogenetic relationships of two Cuban spleenworts with unusual morphology: *Asplenium* (*Schaffneria*) *nigripes* and *Asplenium pumilum* (Aspleniaceae, leptosporangiate ferns). *Plant Systematics and Evolution* 303, 165–176.

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# CONTENTS

## SUMMARY

This dissertation deals with the systematics and evolution of Neotropical ferns of the genera *Elaphoglossum* and *Asplenium*, with particular focus on the species of Cuba and the West Indies. It also includes an analysis and description of an *Elaphoglossum* frond fragment preserved in Miocene Dominican amber. The worldwide genera *Elaphoglossum* with 600 species and *Asplenium* with 685 species are the most species-rich groups of leptosporaniate ferns. On Cuba, *Elaphoglossum* has 34 species and *Asplenium* 32. I performed phylogenetic analyses of plastid DNA sequence matrices that included almost 300 sequences of *Elaphoglossum* and its closest outgroups, with especially dense sampling of the Cuban *Elaphoglossum*, mostly newly sequenced during my research. The Cuban endemic *E. wrightii* was found to be an early-diverging lineage of *Elaphoglossum*, not a member of section *Squamipedia* in which it had previously been classified; I therefore created a separate section for this species. This species climbs upwards on the lower portions of tree trunks but never loses its connection with the soil while most remaining species of *Elaphoglossum* retain no connection to the soil and are holo-epiphytes. The plastid DNA phylogeny in combination with an in-depth analysis of the morphology of West Indian *Elaphoglossum* allowed me to confidently assign a fern inclusion from Miocene Dominican amber to the genus by reconstructing the evolution of relevant morphological characters (preserved in the fossil) on the molecular phylogeny of extant taxa.

The infrageneric classification of *Asplenium* is notoriously difficult as a result of extensive morphological homoplasy and plasticity. Molecular-phylogenetic studies have shed light on major lineages within *Asplenium* including some morphologically highly distinct species. Among these is *Asplenium nigripes*, a species occurring in Costa Rica, Guatemala, and Cuba where it grows on rocks in mountain forests between 900 and 1500 m. The species is unusual in having entire suborbicular to rhomboid fleshy blades that do not look like typical fern fronds. My molecular phylogenetic analysis revealed that it is the sister species to *A. pumilum*, also occurring on Cuba but with 'normal' fern leaves except for unusual whitish hairs. Using micro-morphological leaf and spore traits, I tried to find additional support for a close relationship of these two species, but was unable to detect any synapomorphies, which highlights both the importance of molecular characters for investigating species relationships in *Asplenium* and our still incomplete knowledge of the phenotypic traits of Cuban ferns.

# Chapter 1

# GENERAL INTRODUCTION

Islands have been of disproportionate importance to the study of evolution since the voyages of Charles Darwin to the Galapagos Archipelago and of Alfred Wallace to the Malaysian Archipelago in the 19th century. The distinct boundaries of islands, their geographic isolation, and the fact that groups of islands can function as replicates suit them for analyses of the interplay of ecological and evolutionary processes in the generation of biological diversity (see reviews by Losos and Ricklefs, 2009; Santos, Field, and Ricklefs, 2016). The West Indies archipelago in the Caribbean Sea is one such 'natural laboratory' for biogeographic and evolutionary studies since it comprises three groups of islands that differ in age, size and geological origin. The intermediate degree of geographic isolation of the West Indies from the nearest continental plates, as well as their age and habitat heterogeneity, have allowed both *in situ* speciation and dynamic interaction of populations on the islands and the continent (Ricklefs and Bermingham, 2008). The region constitutes one of the world's biodiversity hotspots with around 7780 species of endemic plants and vertebrates (Myers et al., 2000). The origin of this biodiversity has long been a subject of study (Rosen, 1975; Hedges, Hass, and Maxson, 1992; Hedges, 1996; Iturralde-Vinent and MacPhee, 1999; Iturralde-Vinent, 2006). For its animal diversity, phylogenetic and biogeographic patterns of West Indian butterflies (Matos-Maraví et al., 2014; Lewis et al., 2015), cobweb spiders (Dzik et al., 2015), sloths, rodents, and primates (Dávalos, 2004) imply both island-to-island and overland colonization during times of low sea levels (Fabre et al., 2014; Moonlight et al., 2015; Uit de Weerd, Robinson, and Rosenberg, 2016). While fewer biogeographic studies have focused on the plants of the West Indies, the basic patterns of overland colonization and much in situ diversification are the same (Acevedo and Strong, 2008; Filipowicz and Renner, 2012).

When I started my doctoral research, few West Indian ferns had been included in published molecular phylogenetic analyses. This was surprising because there are excellent taxonomic treatments of the ferns of Jamaica (Proctor, 1985), Puerto Rico, the Virgin Islands (Proctor, 1989), the Lesser Antilles (Proctor, 1977), and Cuba (Hymenophyllaceae, Sánchez, 2000; Cyatheaceae, Caluff and Shelton, 2003; Aspleniaceae, Sánchez and Regalado, 2003; Ophioglossaceae, Caluff and Palacios-Rios, 2006; Isoetaceae, Palacios-Rios, Caluff, and Oviedo, 2006c; Salviniaceae, Palacios-Rios, Caluff, and Oviedo, 2006b; Azollaceae, Palacios-Rios, Caluff, and Oviedo, 2006a; Marsileaceae, Palacios-Rios et al., 2006; Psilotaceae, Palacios-Rios, Caluff, and Shelton, 2006f; Plagiogyriaceae, Palacios-Rios, Caluff, and Shelton, 2006e; Osmundaceae,

Palacios-Rios, Caluff, and Shelton, 2006d; Lophosoriaceae, Palacios-Rios, Caluff, and Shelton, 2006c; Dicksoniaceae, Palacios-Rios, Caluff, and Shelton, 2006b; Oleandraceae, Palacios-Rios, Caluff, and Shelton, 2006a; Thelypteridaceae, Sánchez, Caluff, and Regalado, 2006). However, researchers specializing on the ferns of Cuba until recently did not have access to freshly collected material for DNA sequencing and probably did not succeed in extracting and amplifying DNA from old herbarium collections.

Worldwide, there are about 10,578 species of ferns of which some 10,323 belong to the Leptosporangiate lineage (PPG I, 2016). *Elaphoglossum* with 600 species and *Asplenium* with 685 species are the most species rich genera of leptosporaniate ferns (Smith et al., 2006; PPG I, 2016; Schneider et al., 2017). With more than 100,000 $km^2$ in area (for comparison, Germany covers 375,021 $km^2$) and elevations of up to 1,942 m, Cuba is the largest of the West Indian Islands and exhibits a mosaic of almost every ecosystem also occurring elsewhere in the West Indies (Gebelein, 2012). In my thesis, I selected *Elaphoglossum*, which has 34 species on Cuba (Lóriga, 2012) and *Asplenium*, which has 32 (Sánchez and Regalado, 2003; with updates in Regalado, 2009), as study systems to deepen our knowledge of the evolution and classification of ferns in my home country Cuba. I visited 59 localities that in combination allowed me to sample much of the diversity of *Asplenium* and *Elaphoglossum* (Fig. 1). During these field trips, I made 528 collections, always including fresh tissue dried in silica powder. I recorded aspects of the ferns' natural habitats and took photographs of the microhabitats. Specimens and duplicates are stored in the herbarium of the Academia de Ciencias, La Habana, acronym HAC (373) and the herbarium of Munich, Botanische Staatssammlung, acronym M (155). The following two sections outline relevant aspects of the morphology and phylogeny of the two genera.

### 1.1 *Elaphoglossum*: Phylogeny and morphology

*Elaphoglossum* belongs the bolbitidoid clade (Moran, Labiak, and Sundue, 2010) within Dryopteridaceae (Smith et al., 2006; Christenhusz, Zhang, and Schneider, 2011; PPG I, 2016). Other genera belonging to this clade and their corresponding species riches are as follow (global species richness *sensu* Moran, Labiak, and Sundue (2010), Cuban species richness *sensu* Sánchez (2017)): *Arthrobotrya* (3, 0), *Bolbitis* (55, 2), *Lomagramma* (22, 0), *Mickelia* (10, 2), and *Teratophyllum* (11, 0). Synapomorphies of bolbitidoids ferns are the dorsiventral rhizomes with an elongated ventral meristele (resembling a smiling mouth in cross section) bearing the roots, lack of hairs on the

leaves, sterile–fertile leaf dimorphy, and acrostichoid sporangial arrangement, meaning that the sporangia are distributed over the lower surface of the blade (Moran, Labiak, and Sundue, 2010). Figure 2 shows a typical such fern, with taxonomically important features highlighted.

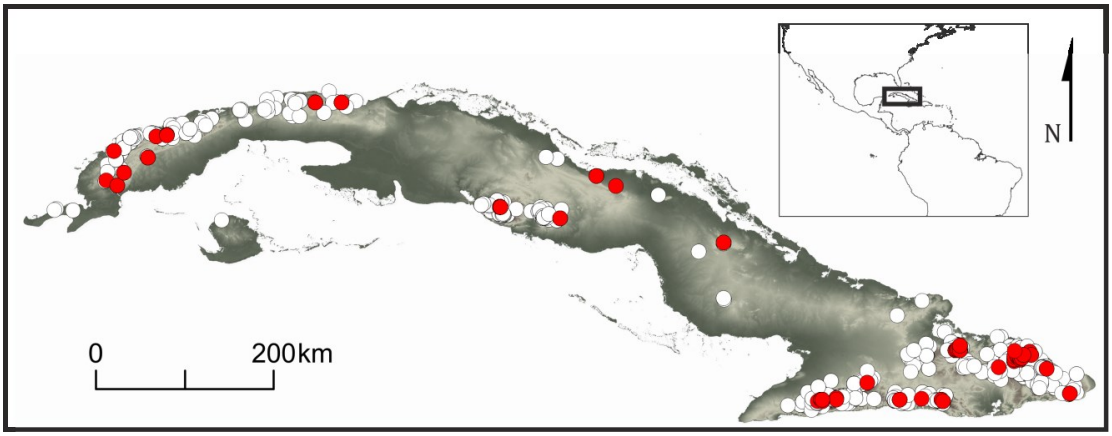


**Figure 1.** Locations on Cuban from which material of *Asplenium* and *Elaphoglossum* was analysed for this study, with herbarium specimens indicated by white circles and the author's personal collecting sites by red circles.

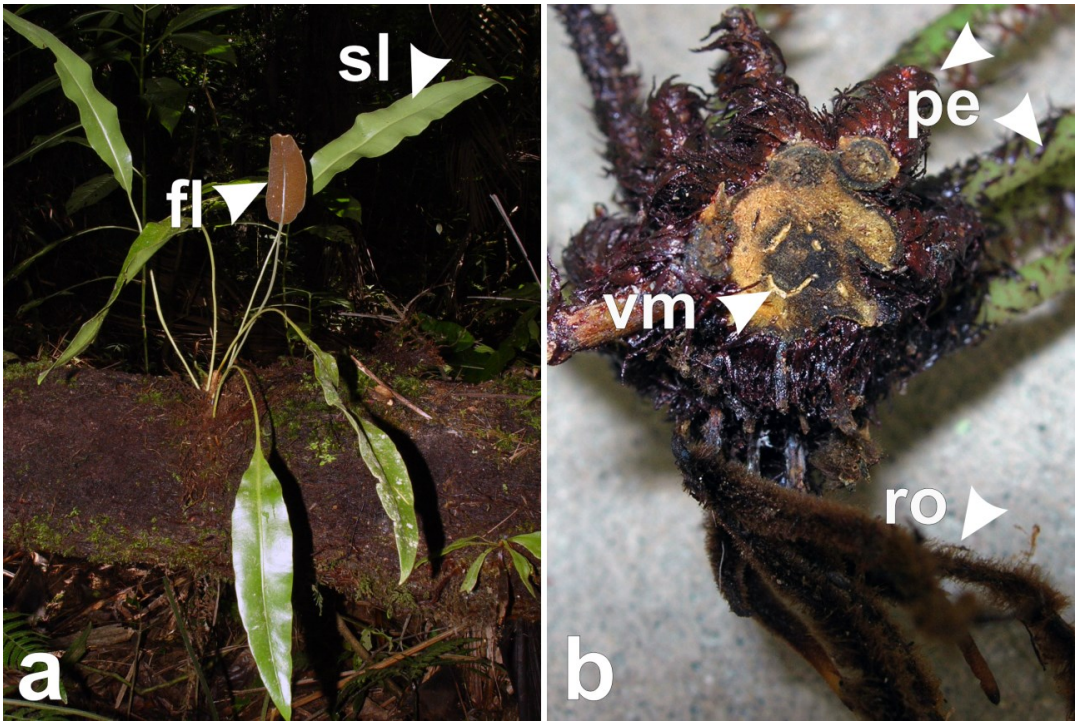


**Figure 2**. Synapomorphies of bolbitidoids ferns. (a) *Elaphoglossum maxoni* in his natural habitat showing the sterile–fertile frond dimorphy, and the acrostichoid sporangial arrangement. (b) Rhizome with dorsiventral symmetry showing in cross section an elongated ventral meristele. *fl* fertile leaf, *pe* leaf petiole, *sl* sterile leaf, *ro* roots, *vm* ventral meristele.

The vast majority of the 600 species of *Elaphoglossum* has simple and entire laminae, phyllopodia, and free veins (Moran, Labiak, and Sundue, 2010). Early molecular phylogenetic studies of *Elaphoglossum* identified six major clades (Rouhan et al., 2004:123 species included; Skog et al., 2004: 48 species included), which were ranked at the sectional level, following Mickel and Atehortúa (1980). My own phylogenetic analyses (described in Chapter 2) recovered a seventh Cuban lineage, which I described as a new section, *Wrightiana* J. Lóriga, A. Vasco, L. Regalado, Heinrichs & R. C. Moran. Details on the morphological identity of each the seven clades can be found in Chapters 2 and 4. Figure 3 show representative species of each of the six sections present in Cuba.


**Figure 3**. Representative species of the *Elaphoglossum* sections present in Cuba. (a) *E. maxonii*, Section *Elaphoglossum*; (b) *E. crinitum*, section *Polytrichia*, (c) *E. pusillum*, section *Setosa*; (d) *E. peltatum*, section *Squamipedia*; (e) *E. eggersii*, section *Lepidoglossa*; (f) *E. wrightii*, section *Wrightiana*.

The genus *Elaphoglossum* comprises mostly root-climbing ferns, a growth form already described by Darwin (1865: page 105) as plants climbing up from the soil towards tree trunks without losing the connection to the soil. Some of the species, however, germinate on trees, grow as pure epiphytes, and only later make secondary contact with the soil, by growing roots from the tree trunk downwards (Lagomarsino, Grusz, and Moran, 2012).

### 1.2 Systematics and Evolution of *Asplenium*

*Asplenium* comprises an estimated 685 species worldwide, 32 of which are known from Cuba (Sánchez and Regalado, 2003; Regalado, 2009; Schneider et al., 2017). Character states typical of this genus are x-shaped vascular bundles in the distal portion of the petiole, clathrate scales attached to the rhizome and basal portion of the petiole, sporangia arranged in linear sori along the veins and covered by laterally attached indusia, 1-rowed sporangial stalks, and monolete spores (Morton and Lellinger, 1966; Murakami et al., 1999; Schneider et al., 2004c; Sundue and Rothfels, 2014). The taxonomy of *Asplenium* is far from resolved due to the high frequency of hybridization coupled with a great morphological disparity among the species. This makes it difficult for researchers to find morphological traits that might be useful for grouping species together. At present, only *Hymenasplenium* (ca. 35 species, 16 of them included in molecular phylogenies; Schneider et al., 2017) and *Asplenium* (ca. 685 species, 276 of them included in molecular phylogenies; Schneider et al., 2017) have been recovered as monophyletic by molecular phylogenetic analyses (Murakami, 1995: 11 species of *Hymenasplenium* or 21 species of *Asplenium* included; Murakami et al., 1999; 6 species of *Hymenasplenium* or 21 species of *Asplenium* included; Schneider et al., 2004c: 1 species of *Hymenasplenium* or 70 species of *Asplenium* included; Schneider et al., 2017: 16 species of *Hymenasplenium* or 276 species of *Asplenium*). Cytological and morphological characters can also distinguish these two genera. The species of *Asplenium* are mostly epiphytic or saxicolous, have erect rhizomes with radial vascular system, non-swollen petiole bases, and a diploid chromosome number of 36; whereas of *Hymenasplenium* have long creeping rhizomes with dorsi-ventral vascular system, swollen petiole bases, and diploid chromosome numbers of 39 or 38 (Mitui, Murakami, and Iwatsuki, 1989; Murakami, 1992; Murakami and Moran, 1993; Regalado and Prada, 2011).

*Asplenium* and *Hymenasplenium* have 35 species on Cuba (Sánchez and Regalado, 2003; Regalado, Sánchez, and Prada, 2006; Regalado, 2011; Regalado and

Prada, 2011; Chapter 3). Regalado (2009, a partially unpublished Ph.D. thesis) proposed seven species groups (*Hymenasplenium* and six groups within *Asplenium*) of related species based on morphological data. Figure 4 shows representative species of each of the seven groups. Five species could not be assigned to any of the seven groups. Particularly, *Asplenium nigripes* (Fée ex T. Moore) Hook., a species known from few localities in Mexico, Guatemala, Costa Rica and Cuba (Moran and Riba, 1995; Sánchez and Regalado, 2003; Mickel and Smith, 2004) is morphologically unusual in having entire suborbicular to rhomboid fleshy leaves. This species has sometimes been ranked as a separate genus (*Schaffneria nigripes* Fée ex T.Moore). Another distinctive Cuban spleenwort (the common name for *Asplenium* and *Hymenasplenium*) is *Asplenium pumilum* Sw. This species is also present in other West Indian islands but also occurs in tropical South and Central America as well as Africa and Madagascar (Moran and Smith, 2001). It is among the few Aspleniaceae species with whitish hairs on the leaves. Prior to my work, no material of *A. nigripes* or *A. pumilum* had been sequenced. In Chapter 3, I investigate the phylogenetic position of these two species and evaluate their morphological affinities to related taxa.

### 1.3 Dominican amber as a source of fern fossils

Amber fossils from the Dominican Republic are an important source of microfossils of small vertebrates (Poinar Jr. and Cannatella, 1987), invertebrates (Iturralde-Vinent and MacPhee, 1996), fungi (Poinar and Singer, 1990) as well as liverworts and mosses (Frahm and Newton, 2005; Lee et al., 2017; Heinrichs et al., 2018). This amber is thought to date to the Miocene, 15 to 20 Ma (Iturralde-Vinent and MacPhee, 1996) and was probably produced by Fabaceae trees from the genus *Hymenaea* (Langenheim, 1990; Poinar, 1991). For ferns, amber inclusions are of particular value because of their preservation of micro-structures, such as fern sporangia, which are usually poorly preserved in sedimentary fossils. At the beginning of my doctoral research, a fern specimen in Dominican amber became available for study, and using extensive analysis of traits, such as leaf and spore morphology, and petiolar scales, I assigned it to *Elaphoglossum* (Chapter 4). Previously described ferns from Dominican amber are a species of *Grammitis* (Gómez, 1982), recently transferred to *Polymniopteris* (Sundue and Poinar, 2016), and a specimen assigned to *Pleopeltis* (Schneider et al., 2015). The extraordinary preservation of the *Elaphoglossum* amber inclusion that I was able to study

allowed the reconstruction of morphological characters of the fossil in a light of a new phylogenetic tree of *Elaphoglossum*. Details about this are presented in Chapter 4.

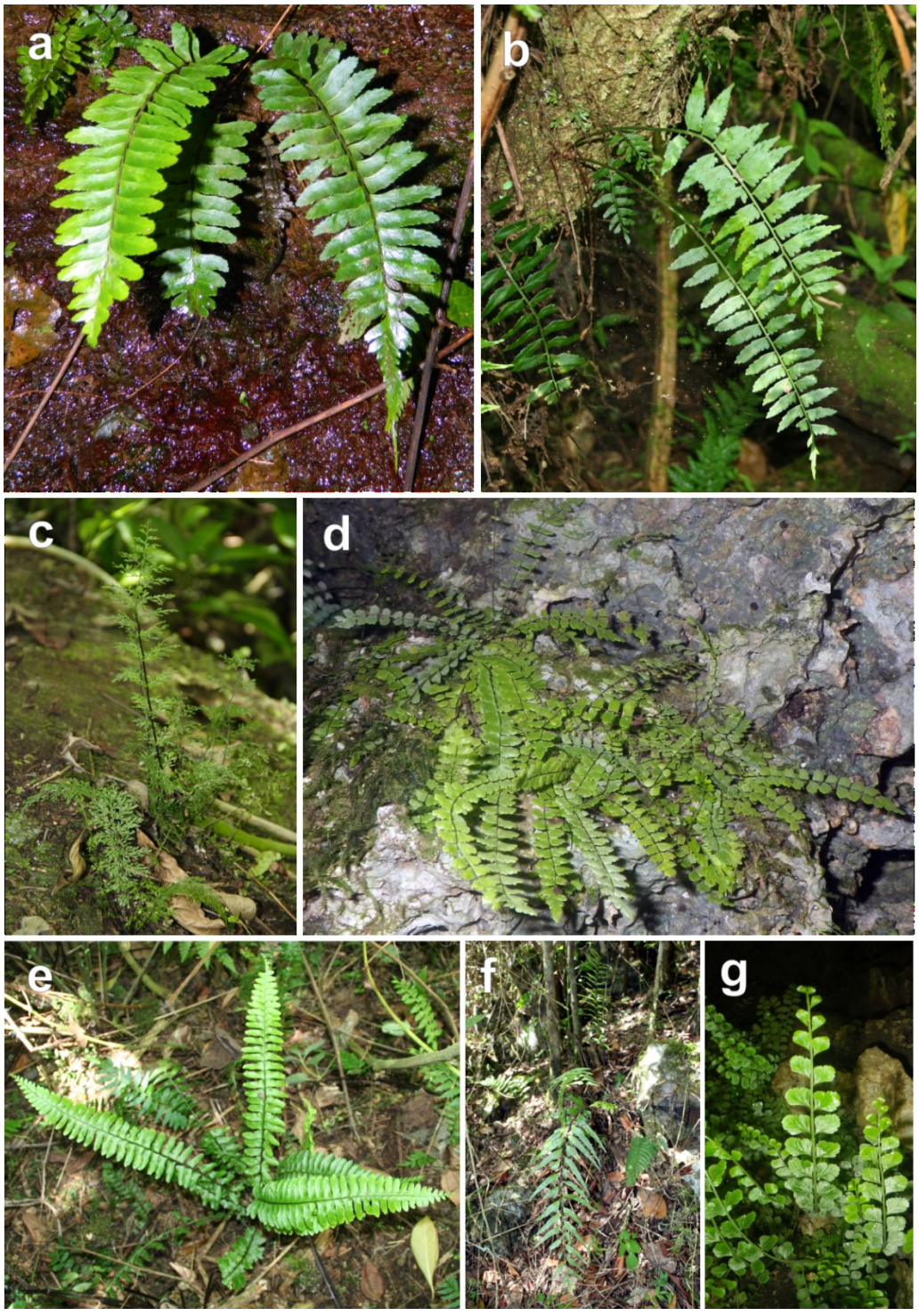


**Figure 4.** Diversity of Cuban Aspleniaceae illustrated with field photographs of species in the seven morphologic groups recognized by Regalado (2009) (a) *Hymenasplenium laetum*, (b) *Asplenium auriculatum*, (c) *A. mortonii*, (d) *A. heterochroum*, (e) *A. formosum*, (f) *A. erosum*, and (g) *A. dentatum*.

## 1.4 Research questions

When I started my doctoral research, the systematics of the West Indian ferns was based entirely on morphological traits (e.g. Proctor, 1977, 1985, 1989; Guala et al., 2002). Unquestionably, morphologic studies are the basis for much of today's classification of the genera and families of ferns, but in groups lacking suitably discrete distributions of phenotypic characters, molecular data matrices outperform morphological features for inferring evolutionary relationships. I took advantage of my knowledge on the fern morphospecies occurring on Cuba and my experience with collecting and observing ferns in their natural habitats to test the morphology-based classification of West Indian ferns and to improve the taxonomy of *Elaphoglossum* and *Asplenium*. My research was not driven by specific expectations (other than that molecular data would more confidently resolve species and genus relationships), but addressed the morphological evolution, species relationship, and taxonomy of ferns on Cuba and Hispaniola.

Chapter 2

# PHYLOGENY AND CLASSIFICATION OF THE CUBAN SPECIES OF *ELAPHOGLOSSUM* (DRYOPTERIDACEAE), WITH DESCRIPTION OF *ELAPHOGLOSSUM* SECT. *WRIGHTIANA* SECT. NOV.

Lóriga, J., A. Vasco, L. Regalado, J. Heinrichs, and R.C. Moran

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ORIGINAL ARTICLE

# Phylogeny and classification of the Cuban species of *Elaphoglossum* (Dryopteridaceae), with description of *Elaphoglossum* sect. *Wrightiana* sect. nov.

**Josmaily Lóriga · Alejandra Vasco · Ledis Regalado · Jochen Heinrichs · Robbin C. Moran**

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**Abstract** Although a worldwide phylogeny of the bolbitidoid fern genus *Elaphoglossum* is now available, little is known about the phylogenetic position of the 34 Cuban species. We performed a phylogenetic analysis of a chloroplast DNA dataset for *atp*ß-*rbc*L (including a fragment of the gene *atp*ß), *rps*4-*trn*S, and *trn*L-*trn*F. The dataset included 79 new sequences of *Elaphoglossum* (67 from Cuba) and 299 GenBank sequences of *Elaphoglossum* and its most closely related outgroups, the bolbitidoid genera *Arthrobotrya*, *Bolbitis*, *Lomagramma*, *Mickelia*, and *Teratophyllum*. We obtained a well-resolved phylogeny including the seven main lineages recovered in previous phylogenetic studies of *Elaphoglossum*. The Cuban endemic *E. wrightii* was found to be an early diverging lineage of *Elaphoglossum,* not a member of *E.* sect. *Squamipedia* where it was previously classified. We propose a new section for this species: *E.* sect. *Wrightiana*. The early diverging position of *E. wrightii* is of particular interest because the species is a root climber (i.e., climbing from the soil on the lower portions of tree trunks and not losing its connection with the soil), a growth habit it shares with its closest bolbitidoid outgroup genera. This suggests that holoepiphytism evolved later in *Elaphoglossum*, and the primary hemiepiphytism of *E. amygdalifolium,* which is sister to the rest of the genus, was derived independently from ancestors that were root climbers. Based on our phylogenetic analysis and morphological investigations, the species of Cuban *Elaphoglossum* were found to occur in *E.* sects. *Elaphoglossum*, *Lepidoglossa*, *Polytrichia, Setosa*, and *Squamipedia*.

**Keywords** Bolbitidoid fern · Chloroplast DNA sequences · Growth habit · Holoepiphytism · Primary hemiepiphytism · Root climber · Taxonomy

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## Introduction

With some 600 species, *Elaphoglossum* Schott ex J. Sm. is among the largest and taxonomically most complex genera of ferns. It has a pantropical distribution with a center of diversity in the Neotropics, where more than 450 species have been recognized (Mickel and Smith 2004; Kessler and Mickel 2006). Most *Elaphoglossum* species are holoepiphytes, a few are terrestrial, and one was recently found to be a primary hemiephiphyte (Lagomarsino et al. 2012). *Elaphoglossum* is a member of the bolbitidoid clade of Dryopteridaceae (Moran et al. 2010a). Morphologically, the bolbitidoids are characterized by dorsiventral rhizomes, lack of hairs (with a few exceptions in *Elaphoglossum*), dimorphic sterile and fertile leaves, and acrostichoid sori. Within the bolbitidoids, *Elaphoglossum* is characterized by phyllopodia, simple and entire (rarely divided) leaves, and free veins (Moran et al. 2010a).

Several molecular phylogenetic studies have provided insights into the evolution and biogeography of *Elaphoglossum* and related bolbitidoid genera. These identified and described the Neotropical genus *Mickelia* R.C. Moran,

Labiak & Sundue as distinct from *Bolbitis* Schott and showed that *Elaphoglossum* is sister to this new genus (Moran et al. 2010a, b). Molecular phylogenetic studies also improved the classification of *Elaphoglossum* and shed light on its morphological evolution, especially in relation to the main clades of the genus (Eastwood et al. 2004; Rouhan et al. 2004, 2007; Skog et al. 2004; Vasco et al. 2009a). These molecular phylogenetic studies have also recovered clades that have been the basis for several monographic studies (Vasco et al. 2009b, 2013; Roux 2011; Vasco 2011).

Previous molecular phylogenetic studies focused on the global phylogeny of *Elaphoglossum* (Rouhan et al. 2004) or clades within the genus (Rouhan et al. 2007; Vasco et al. 2009a). These studies included several species from oceanic islands but few from the West Indies. This region has a complex geological history and has been considered a laboratory for studying evolutionary processes such as colonization, diversification, and extinction (Ricklefs and Bermingham 2008). Studies of *Elaphoglossum* in the region have been greatly aided by taxonomic treatments published for Jamaica (Anderson and Anderson 1985), the Lesser Antilles (Proctor 1977), and Puerto Rico and the Virgin Islands (Proctor 1989). These studies recognized 43 species of *Elaphoglossum* in the region, about half of which are endemic.

With more than 100,000 sq km and elevations up to 1,942 m, Cuba is the largest of the West Indian Islands. Its diversity of topographic relief, soil types, wind exposures, and rainfall patterns result in a mosaic of almost every ecosystem also occurring elsewhere in the West Indies (Gebelein 2012). The wide range of habitats harbors about 715 species of ferns and lycophytes in Cuba (Caluff et al. 2008), which is about 60 % of the known diversity of those groups in the Antilles (Moran 2008).

As part of a revision of *Elaphoglossum* for the *Flora de Cuba*, we conducted extensive fieldwork, herbarium work, and obtained chloroplast DNA sequences from 18 Cuban species. Here, we present the results of phylogenetic analyses of the newly generated Cuban sequences integrated with previously published sequences from GenBank. Based on these analyses and accompanying morphological studies, the Cuban species of *Elaphoglossum* are assigned to five previously recognized sections in the genus (Mickel and Atehortúa 1980; Rouhan et al. 2004) and a new section is created for the Cuban endemic *E. wrightii* (Mett ex D. C. Eaton) T. Moore, which is shown to be a fern climbing from the soil onto the lower portions of tree trunks without losing its connection with the soil (root climber sensu Darwin 1865).

## Materials and methods

### Taxon sampling

Thirty-four species of *Elaphoglossum* occur in Cuba (Lóriga et al., in preparation). Fresh tissue from field-collected material of 17 of these species were collected and stored in silica. Voucher specimens are deposited in HAC. We also used unpublished sequences from an old collection of *E. minutum* (Pohl ex Fée) T. Moore from Cuba. Additional material for DNA extraction was obtained from four herbarium specimens from Dominican Republic deposited at NY (Table 1). These specimens resemble the Cuban species *E. ocoense* C. Chr. and *E. piloselloides* (C. Presl) T. Moore that were not sampled in the field, and *E. pusillum* (Mett. ex Kuhn) C. Chr. and *E. minutum* that were sampled in Cuba. To complete our three-marker dataset, we also used unpublished sequences from *E. decoratum* (Kunze) T. Moore, *E. luridum* (Fée) Christ and *E. succisaefolium* (Thouars) T.Moore. All unpublished sequences were obtained at the molecular laboratory of the New York Botanical Garden following the methodology described below.

### DNA extraction, amplification and sequencing

Total genomic DNA was extracted from leaf samples using the DNeasy Plant Mini Kit (Qiagen). DNA extraction from herbarium specimens was carried out with the addition of a proteinase K digestion in the lysis step (Vasco et al. 2009a). We amplified the noncoding intergenic chloroplast DNA spacers *atp*ß-*rbc*L (including a fragment of the gene *atp*ß), *rps*4-*trn*S and *trn*L-*trn*F using the primer sets of Rouhan et al. (2007) and Vasco et al. (2009a). The successfully amplified products were sent to the High-Throughput Genomics Unit, Department of Genome Sciences, University of Washington, for purification and bidirectional sequencing. ExoSap-IT (USB Corporation) was used for eliminating unincorporated primers and dNTPs and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) for the sequencing reaction running on an ABI Prism 3130 DNA Analyzer (Applied Biosystems). Chromatograms were checked and, when necessary, sequences were corrected by hand using CodonCode Aligner (v. 3.5.6, Codon Code Corporation). The newly identified sequences were submitted to GenBank (KF212374–KF212448) and EMBL (HG428762, HG425357– HG425359) (Table 1).

### Outgroup selection and sequence alignment

We added the new sequences to a large set of published bolbitidoid fern sequences (Rouhan et al. 2004, 2007; Skog et al. 2004; Vasco et al. 2009a) from GenBank. Species belonging to the bolbitidoid genera *Arthrobotrya* J. Sm.,

**Table 1** Voucher information for sequences used in this study and their GenBank accession numbers (in bold are the new sequences generated for this study)

| Species | Voucher and herbaria | Country | *atpß- rbc*L | *rps*4-*trn*S | *trn*L-*trn*F |
|---|---|---|---|---|---|
| *Arthrobotrya wilkesiana* (Brack.) Copel. | Ranker 1937 (UC) | French Polynesia | – | GU376719 | GU376569 |
| *Bolbitis auriculata* (Lam.) Alston | Rouhan 101 (NY) | Mauritius | – | GU376649 | GU376505 |
| *Bolbitis auriculata* (Lam.) Alston | Fay 1110 (NY) | Sierra Leone | EF040664 | – | – |
| *Elaphoglossum achroalepis* (Baker) C. Chr. | Rakotondrainibe 6485 (P) | Madagascar | EF040636 | AY540225 | AY536288 |
| *Elaphoglossum acrostichoides* (Hook. & Grev.) Schelpe | Rouhan 229 (P) | La Réunion | EF040654 | EF040628 | EF040614 |
| *Elaphoglossum aemulum* (Kaulf.) Brack | Lorence 8514 (PTBG) | Hawaii | – | AY540227 | AY536290 |
| *Elaphoglossum aff. ciliatum* (C. Presl) T. Moore | Moran 6711 (NY) | Ecuador | EU907673 | EU907748 | EU907813 |
| *Elaphoglossum affine* (M. Martens & Galeotti) T. Moore | Mickel 9694 (NY) | Mexico | – | AY536169 | AY534841 |
| *Elaphoglossum albescens* (Sodiro) Christ | Vasco 739 (HUA, NY) | Colombia | – | GU376678 | GU376532 |
| *Elaphoglossum alismaefolium* (Fée) T. Moore | Lóriga and Rodríguez 159 (HAC) | Cuba | **KF212374** | **KF212425** | **KF212399** |
| *Elaphoglossum amygdalifolium* (Mett. ex Kuhn) Christ | Herrera 2063 (CR, INB, NY, USJ) | Costa Rica | – | AY536173 | AY534845 |
| *Elaphoglossum angulatum* (Blume) T. Moore | Rouhan 220 (NY, P) | La Réunion | EF040655 | AY540230 | AY536293 |
| *Elaphoglossum apodum* (Kaulf.) Schott ex J. Sm. | Lóriga and Rodríguez 68 (HAC) | Cuba | – | – | **KF212400** |
| *Elaphoglossum apodum* (Kaulf.) Schott ex J. Sm. | Trusty 120 (NY) | Costa Rica | EF040651 | EF040625 | EF040611 |
| *Elaphoglossum asterolepis* (Baker) C. Chr. | Kessler 12751 (P) | Madagascar | EF040642 | AY540231 | AY536294 |
| *Elaphoglossum aubertii* (Desv.) T.Moore | Rouhan 110 (P) | Comoros | EF040647 | EF040622 | EF040608 |
| *Elaphoglossum auricomum* (Kunze) T. Moore | Hammer 3 (NY) | Mexico | – | AY536145 | AY534817 |
| *Elaphoglossum auripilum* Christ | Moran 6377 (NY) | Costa Rica | EF040652 | EF040626 | EF040612 |
| *Elaphoglossum avaratraense* Rakotondr. | Rakotondrainibe 1456 (P) | Madagascar | EU907660 | AY540233 | AY536296 |
| *Elaphoglossum backhousianum* T. Moore | Moran 6321 (CR, INB, NY, UCR) | Costa Rica | – | AY540234 | AY536297 |
| *Elaphoglossum bifurcatum* (Jacq.) Mickel | Eastwood 215 (–) | St. Helena | EU907661 | EU907737 | AY194070 |
| *Elaphoglossum biolleyi* Christ | Boyle 6397 (CR, INB, NY, UCR) | Costa Rica | – | AY540235 | AY536298 |
| *Elaphoglossum boryanum* (Fée) T. Moore | Meier et al. 6768 (NY, VEN) | Venezuela | – | AY536133 | AY534804 |
| *Elaphoglossum burchellii* (Baker) C. Chr. | Jimenez 2460 (NY) | Bolivia | EU907663 | EU907738 | EU907803 |
| *Elaphoglossum cardiophyllum* (Hook.) T. Moore | Holm-Nielsen 17480 (AAU, NY) | Ecuador | – | AY536171 | AY534842 |
| *Elaphoglossum cf. erinaceum* (Fée) T. Moore | NYBG living collection 554/79A | Mexico | – | AY536135 | AY534806 |
| *Elaphoglossum cf. longifolium* (Jacq.) J. Sm. | Lóriga and Rodríguez 151 (HAC) | Cuba | **KF212375** | **KF212426** | **KF212401** |
| *Elaphoglossum cf. longifolium* (Jacq.) J. Sm. | Lóriga and Regalado 2 (HAC) | Cuba | **KF212376** | **KF212427** | **KF212402** |
| *Elaphoglossum cf. petiolatum* (Sw.) Urb. | Moran 7573 (NY) | Ecuador | EU907714 | EU907785 | EU907848 |
| *Elaphoglossum ciliatum* (C. Presl) T. Moore | Vasco 468 (HUA, MO, NY) | Colombia | EU907670 | EU907745 | EU907810 |
| *Elaphoglossum ciliatum* (C. Presl) T. Moore | Vasco 504 (HUA, MO, NY) | Colombia | EU907671 | EU907746 | EU907811 |
| *Elaphoglossum cismense* Rosenst. | Van Ee 327 (CR, INB, NY, UCR) | Costa Rica | – | AY540237 | AY536300 |
| *Elaphoglossum coriaceum* Bonap. | Rouhan 145 (P) | Seychelles | EF040653 | EF040627 | EF040613 |
| *Elaphoglossum coursii* Tardieu | Rouhan 127 (NY, P) | Comoros | – | AY540240 | AY536303 |
| *Elaphoglossum crinitum* (L.) Christ. | Lóriga and Rodríguez 258 (HAC) | Cuba | **KF212377** | **KF212428** | **KF212403** |
| *Elaphoglossum crinitum* (L.) Christ | NYBG living collection 233/94 | Dominican Republic | – | AY536134 | AY534805 |

**Table 1** continued

| Species | Voucher and herbaria | Country | *atpß- rbc*L | *rps*4-*trn*S | *trn*L-*trn*F |
|---|---|---|---|---|---|
| *Elaphoglossum croatii* Mickel | Moran 6378 (CR, INB, NY, UCR) | Costa Rica | – | AY540241 | AY536304 |
| *Elaphoglossum cubense* (Mett. ex Kuhn) C. Chr. | Lóriga and Rodríguez 155 (HAC) | Cuba | **KF212378** | **KF212429** | **KF212404** |
| *Elaphoglossum cubense* (Mett. ex Kuhn) C. Chr. | Regalado s.n. (HAC) | Cuba | **KF212379** | **KF212430** | **KF212405** |
| *Elaphoglossum cuspidatum* (Willd.) T. Moore | Jiménez 754 (LPB) | Bolivia | EU907675 | EU907750 | EU907815 |
| *Elaphoglossum davidsei* Mickel | Moran 6366 (CR, INB, NY, UCR) | Costa Rica | – | AY540242 | AY536305 |
| *Elaphoglossum decaryanum* Tardieu | Rakotondrainibe 6326 (P) | Madagascar | EF040658 | AY540243 | AY536306 |
| *Elaphoglossum deckenii* (Kuhn) C. Chr. | Rouhan 105 (CNDRS, NY, P, PTBG) | Comoros | – | AY540244 | AY536307 |
| *Elaphoglossum decoratum* (Kunze) T. Moore | Labiak 4074 (UPCB) | Brazil | **KF212380** | GU376681 | GU376534 |
| *Elaphoglossum dendricola* (Baker) C. Chr. | Moran 6853 (NY, QCA, QCNE) | Ecuador | EU907676 | EU907751 | EU907816 |
| *Elaphoglossum dimorphum* (Hook. & Grev.) T. | Eastwood 302 (–) | St. Helena | EU907677 | EU907752 | AY194068 |
| *Elaphoglossum dussii* Underw. ex Maxon | Sanchez 138 (NY) | Puerto Rico | EU907681 | EU907755 | EU907819 |
| *Elaphoglossum dussii* Underw. ex Maxon | Christenhusz 4011 (NY) | Guadeloupe | EU907679 | EU907754 | EU907818 |
| *Elaphoglossum edwallii* Rosenstock | Prado et al. 1123 (NY) | Brazil | – | AY536144 | AY534816 |
| *Elaphoglossum eggersii* (Baker) Christ | Lóriga and Rodríguez 157 (HAC) | Cuba | **KF212381** | **KF212431** | **KF212406** |
| *Elaphoglossum erinaceum* (Fée) T. Moore | Lóriga and Rodríguez 162 (HAC) | Cuba | – | **KF212432** | **KF212407** |
| *Elaphoglossum eximium* (Mett.) Christ | Moraga 485 (NY) | Costa Rica | – | AY536132 | AY534803 |
| *Elaphoglossum firmum* (Mett. ex. Kuhn) Urb. | Lóriga and Rodríguez 74 (HAC) | Cuba | **KF212382** | **KF212433** | **KF212408** |
| *Elaphoglossum flaccidum* (Fée) T. Moore | Mori 25578 (NY) | French Guiana | EF040657 | AY540246 | AY536309 |
| *Elaphoglossum forsythii-majoris* Christ | Kessler 12678 (P) | Madagascar | EF040644 | EF040620 | EF040606 |
| *Elaphoglossum fournierianum* L. D. Gómez | Moran 6336 (CR, INB, NY, UCR) | Costa Rica | – | AY540248 | AY536311 |
| *Elaphoglossum gayanum* (Fée) T. Moore | Mickel 9695 (NY) | Mexico | – | AY536166 | AY534838 |
| *Elaphoglossum glabellum* J. Sm. | Prado et al. 1129 (NY) | Brazil | – | AY536167 | AY534839 |
| *Elaphoglossum gramineum* (Jenman) Urb. | Lóriga and Rodríguez 331 (HAC) | Cuba | **KF212383** | **KF212434** | **KF212409** |
| *Elaphoglossum gramineum* (Jenman) Urb. | Anderson 3223 (US) | Jamaica | EU907682 | EU907756 | EU907820 |
| *Elaphoglossum gramineum* (Jenman) Urb. | Proctor 3907 (US) | Jamaica | – | EU907757 | EU907821 |
| *Elaphoglossum grayumii* Mickel | Moran 6329 (CR, INB, NY, UCR) | Costa Rica | – | AY540250 | AY536313 |
| *Elaphoglossum guatemalense* (Klotzsch) T. Moore | Mickel 9701 (NY) | Mexico | – | AY536164 | AY534836 |
| *Elaphoglossum guentheri* Rosenst. | Lehnert 1306 (NY) | Ecuador | – | GU376682 | GU376535 |
| *Elaphoglossum herminieri* (Bory & Fée) T. Moore | Lóriga and Rodríguez 77 (HAC) | Cuba | **KF212384** | **KF212435** | **KF212410** |
| *Elaphoglossum herminieri* (Bory & Fée) T. Moore | Blanco 1559 (F, USJ) | Costa Rica | – | AY536163 | AY534835 |
| *Elaphoglossum heterolepis* (Fée) T. Moore | Rouhan 177 (P) | Mauritius | EU907683 | AY540251 | AY536314 |
| *Elaphoglossum hoffmannii* (Mett. ex Kuhn) Christ | Moran 6365 (CR, INB, NY, UCR) | Costa Rica | – | AY540252 | AY536315 |
| *Elaphoglossum huacsaro* (Ruíz) Christ | Vasco 568 (HUA, NY) | Colombia | EU907694 | EU907769 | EU907832 |
| *Elaphoglossum humbertii* C. Chr. | Rouhan 466 (P) | Madagascar | EU907696 | EU907771 | EU907834 |
| *Elaphoglossum hybridum* (Bory) Brack. | Rouhan 250 (P) | La Réunion | EU907697 | EU907772 | EU907835 |
| *Elaphoglossum ipshookense* Mickel | Mickel 4748 (NY) | Mexico | EU907698 | EU907773 | EU907836 |
| *Elaphoglossum lanatum* (Bojer ex Baker) Lorence | Rouhan 194 (MAU, NY, P, PTBG) | Mauritius | – | AY540258 | AY536321 |

**Table 1** continued

| Species | Voucher and herbaria | Country | *atpß- rbc*L | *rps*4-*trn*S | *trn*L-*trn*F |
|---|---|---|---|---|---|
| *Elaphoglossum lancifolium* (Desv.) C.V. Morton | Rouhan 201 (NY, P) | La Réunion | EU907699 | AY540259 | AY536322 |
| *Elaphoglossum langsdorffii* (Hook. & Grev.) T. Moore | Labiak 4113 (UPCB) | Brazil | – | GU376683 | GU376536 |
| *Elaphoglossum leucolepis* (Baker) Krajina ex Tardieu | Rakotondrainibe 6339 (P) | Madagascar | EF040638 | AY540261 | AY536324 |
| *Elaphoglossum lindenii* (Bory ex Fée) T. Moore | Mickel 9652 (NY) | Mexico | – | AY536130 | AY534801 |
| *Elaphoglossum lingua* (C. Presl) Brack. | Moran 6380 (NY) | Costa Rica | – | AY540262 | AY536325 |
| *Elaphoglossum lloense* (Hook.) T. Moore | Vasco 539 (HUA, NY) | Colombia | – | GU376684 | GU376537 |
| *Elaphoglossum lonchophyllum* (Fée) T. Moore | Hammer 9 (NY) | Mexico | – | AY536136 | AY534807 |
| *Elaphoglossum luridum* (Fée) Christ | NYBG living collections 2001-0052 | Peru | **KF212385** | AY540263 | AY536326 |
| *Elaphoglossum macropodium* (Fée) T. Moore | Rouhan 209 (NY, P) | La Réunion | – | AY540264 | AY536327 |
| *Elaphoglossum malgassicum* C. Chr. | Kessler 12725 (NY) | Madagascar | EF040659 | AY540265 | AY536328 |
| *Elaphoglossum marojejyense* Tardieu | Rakotondrainibe 6429 (P) | Madagascar | EF040630 | AY540266 | AY536329 |
| *Elaphoglossum martinicense* (Desv.) T. Moore | Lóriga and Rodríguez 255 (NY) | Cuba | **KF212386** | **KF212436** | **KF212411** |
| *Elaphoglossum martinicense* (Desv.) T. Moore | Lóriga and Rodríguez 366 (HAC) | Cuba | **KF212387** | **KF212437** | **KF212412** |
| *Elaphoglossum maxonii* Underw. ex C.V. Morton | Lóriga and Rodríguez 341 (HAC) | Cuba | **KF212388** | **KF212438** | **KF212413** |
| *Elaphoglossum micropogon* Mickel | Moran 6353 (NY) | Costa Rica | EF040643 | AY540268 | AY536331 |
| *Elaphoglossum minutum* (Pohl ex Fée) T. Moore | Ekman 14764 (NY) | Cuba | **KF212389** | **KF212439** | – |
| *Elaphoglossum minutum* (Pohl ex Fée) T. Moore | Zanoni et al. 30916 (NY) | Dominican Republic | **KF212390** | **KF212440** | **HG425359** |
| *Elaphoglossum mitorrhizum* Mickel | Boyle 6410 (CR, INB, NY, USJ) | Costa Rica | EF040656 | AY540269 | AY536332 |
| *Elaphoglossum nervosum* C. Chr. | Eastwood 367 (–) | St. Helena | EU907701 | EU907775 | EU907837 |
| *Elaphoglossum nidiforme* Mickel | Lehnert 1316 (NY) | Bolivia | EF040662 | EF040629 | EF040616 |
| *Elaphoglossum nidusoides* Rouhan & Rakotondr | Rouhan 387 (P) | Madagascar | EF040634 | EF040618 | EF040604 |
| *Elaphoglossum nigrescens* (Hook.) T. Moore ex Diels | Moran 7491 (NY) | Ecuador | EU907708 | EU907781 | EU907843 |
| *Elaphoglossum nigrocostatum* Mickel | Luteyn 11051 (NY) | Venezuela | – | AY536152 | AY534824 |
| *Elaphoglossum oblanceolatum* C. Chr. | Gomez 21000 (NY) | Costa Rica | – | AY540271 | AY536334 |
| *Elaphoglossum ocoense* C. Chr. | Jones and Norris 1120 (NY) | Dominican Republic | – | **KF212441** | **KF212414** |
| *Elaphoglossum orbignyanum* (Fée) T. Moore | Bach 1773 (NY) | Bolivia | EU907710 | EU907783 | EU907845 |
| *Elaphoglossum ovalilimbatum* Bonap. | Humbert 24895 (P) | Madagascar | – | AY540272 | AY536335 |
| *Elaphoglossum ovatum* (Hook. & Grev.) T. Moore | Smith 2872 (UC) | Ecuador | EF040641 | AY540273 | AY536336 |
| *Elaphoglossum paleaceum* (Hook. & Grev.) Sledge | Mickel 9710 (NY) | Hawaii | EU907711 | EU907784 | EU907846 |
| *Elaphoglossum palmeri* Underw. & Maxon | Lóriga and Rodríguez 67 (HAC) | Cuba | **KF212391** | **KF212442** | **KF212415** |
| *Elaphoglossum palmeri* Underw. & Maxon | Lóriga and Rodríguez 92 (HAC) | Cuba | **KF212392** | **KF212443** | **KF212416** |
| *Elaphoglossum papillosum* (Baker) Christ | Boyle 5816 (CR, INB, NY, USJ) | Costa Rica | – | AY536129 | AY534800 |
| *Elaphoglossum peltatum* (Sw.) Urb., | Lóriga and Rodríguez 355 (HAC) | Cuba | **KF212393** | **KF212444** | **KF212417** |
| *Elaphoglossum peltatum* (Sw.) Urb. | Mickel 9703 (NY) | Mexico | EF040631 | AY536159 | AY534831 |
| *Elaphoglossum petiolatum* (Sw.) Urb. | Nicholson and 782-01-A (NY) | Mexico | EU907712 | AY540275 | AY536338 |
| *Elaphoglossum phanerophlebium* C. Chr. | Rakotondrainibe 6430 (P) | Madagascar | EF040646 | AY540276 | AY536339 |

**Table 1** continued

| Species | Voucher and herbaria | Country | *atpß- rbc*L | *rps*4-*trn*S | *trn*L-*trn*F |
|---|---|---|---|---|---|
| *Elaphoglossum piloselloides* (C. Presl) T. Moore | Howard and Howard 9038 (NY) | Dominican Republic | – | **KF212445** | **KF212418** |
| *Elaphoglossum piloselloides* (C. Presl) T. Moore | Mickel 9708 (NY) | Mexico | – | AY536141 | AY534812 |
| *Elaphoglossum pilosius* Mickel | Moran 6338 (CR, INB, NY, UCR) | Costa Rica | – | AY540277 | AY536340 |
| *Elaphoglossum poolii* (Baker) Christ | Kessler 12702 (NY) | Madagascar | EF040639 | AY540278 | AY536341 |
| *Elaphoglossum potosianum* Christ | Hinton 22679 (NY) | Mexico | EU907715 | EU907786 | EU907849 |
| *Elaphoglossum prestonii* (Baker) J. Sm. | Prado et al. 1117 (NY) | Brazil | – | AY536139 | AY534810 |
| *Elaphoglossum pringlei* (Davenp.) C. Chr. | Campos 2650 (NY) | Mexico | EU907716 | EU907787 | EU907850 |
| *Elaphoglossum productum* Rosenst. | Moran s.n. (CR, INB, NY, UCR) | Costa Rica | EU907733 | AY540279 | EU907861 |
| *Elaphoglossum pusillum* (Mett. ex Kuhn) C. Chr. | Lóriga and Rodríguez 325 (HAC) | Cuba | **KF212394** | **HG428762** | **KF212420** |
| *Elaphoglossum pusillum* (Mett. ex Kuhn) C. Chr. | Valeur 568 (US) | Dominican Republic | – | **HG425357** | **KF212419** |
| *Elaphoglossum pygmaeum* (Mett. ex Kuhn) Christ | Smith 2826 (UC) | Ecuador | – | AY540281 | AY536344 |
| *Elaphoglossum rapense* Copel. | Motley 2677 (NY) | French Polynesia | – | AY540283 | AY536365 |
| *Elaphoglossum richardii* (Bory ex Fée) H. Christ | Rouhan 205 (P) | La Réunion | EF040645 | EF040621 | EF040607 |
| *Elaphoglossum rufidulum* C. Chr. | Rakotondrainibe 6396 (P) | Madagascar | – | AY540285 | AY536348 |
| *Elaphoglossum russelliae* Mickel | Moran 6360 (CR, INB, NY, UCR) | Costa Rica | – | AY540286 | AY536349 |
| *Elaphoglossum rzedowskii* Mickel | Bartholomeus 2691 (NY) | Mexico | EU907718 | EU907788 | EU907851 |
| *Elaphoglossum samoense* Brack. | Motley 2875 (NY) | Rapa | – | AY540287 | AY536350 |
| *Elaphoglossum sartorii* (Liebm.) Mickel | Mickel 9700 (NY) | Mexico | – | AY536161 | AY534833 |
| *Elaphoglossum scolopendriforme* Tardieu | Rakotondrainibe 6426 (P) | Madagascar | EU907719 | AY540288 | AY536351 |
| *Elaphoglossum setigerum* (Sodiro) Diels | Van Ee 328 (CR, INB, NY, UCR) | Costa Rica | – | AY540289 | AY536352 |
| *Elaphoglossum sieberi* (Hook. & Grev.) T. Moore | Rouhan 169 (MAU, NY, P, PTBG) | Mauritius | EU907720 | AY540290 | AY536353 |
| *Elaphoglossum siliquoides* (Jenman) C. Chr. | Lóriga and Rodríguez 220 (HAC) | Cuba | – | **HG425358** | **KF212421** |
| *Elaphoglossum siliquoides* (Jenman) C. Chr. | Smith 2631 (UC) | Costa Rica | – | AY536127 | AY534798 |
| *Elaphoglossum smithii* (Baker) Christ | Boyle 6409 (CR, INB, NY, UCR) | Costa Rica | – | AY540291 | AY536354 |
| *Elaphoglossum spatulatum* (Bory) T. Moore | Rakotondrainibe 6125 (NY, P, PTBG) | Madagascar | EF040649 | EF040623 | EF040609 |
| *Elaphoglossum splendens* (Bory ex Willd.) Brack. | Rouhan 247 (P) | La Réunion | EU907721 | AY540296 | AY536359 |
| *Elaphoglossum squamipes* (Hook.) T. Moore | Moran 6308 (CR, INB, NY, USJ) | Costa Rica | EF040635 | AY536157 | AY534829 |
| *Elaphoglossum squamipes* (Hook.) T. Moore | Labiak et al. 1253 (NY, P) | Brazil | – | AY536158 | AY534830 |
| *Elaphoglossum succisaefolium* (Thouars) T.Moore | Marthel-Thoumian 1A (P) | Amsterdam Island | **KF212395** | AY540299 | AY536362 |
| *Elaphoglossum tectum* (Humb. & Bonpl. ex Willd.) T. Moore | Prado et al. 1126 (NY) | Brazil | – | AY536142 | AY534813 |
| *Elaphoglossum tenuiculum* (Fée) T. Moore ex Baker | Vasco 558 (NY) | Colombia | EU907722 | – | EU907852 |
| *Elaphoglossum tomentosum* (Bory ex Willd.) Christ | Rouhan 174 (P) | Mauritius | EU907723 | AY540300 | AY536363 |
| *Elaphoglossum tripartitum* (Hook. & Grev.) Mickel | Fay and Fay 3344 (MO) | Ecuador | – | AY536156 | AY534828 |
| *Elaphoglossum vestitum* (Sw.) T. Moore | Mickel 9699 (NY) | Costa Rica | – | AY536146 | AY534818 |

**Table 1** continued

| Species | Voucher and herbaria | Country | *atpß- rbc*L | *rps*4-*trn*S | *trn*L-*trn*F |
|---|---|---|---|---|---|
| *Elaphoglossum vieillardii* (Mett.) T. Moore | Munzinger 1361 (P) | New Caledonia | – | AY540301 | AY536364 |
| *Elaphoglossum wawrae* (Luerss.) C. Chr. | Lorence 8511 (PTBG) | Hawaii | – | AY540302 | AY536365 |
| *Elaphoglossum welwitschii* (Baker) C. Chr. | Taylor 9099 (P) | Tanzania | – | AY540303 | AY536366 |
| *Elaphoglossum wrightii* (Mett. ex D.C. Eaton) T. Moore | Lóriga and Rodríguez 254 (HAC) | Cuba | **KF212397** | **KF212447** | **KF212423** |
| *Elaphoglossum wrightii* (Mett. ex D.C. Eaton) T. Moore | Lóriga and Rodríguez 348 (HAC) | Cuba | **KF212398** | **KF212448** | **KF212424** |
| *Elaphoglossum wrightii* (Mett. ex D.C. Eaton) T. Moore | Lóriga and Rodríguez 242 (HAC) | Cuba | **KF212396** | **KF212446** | **KF212422** |
| *Elaphoglossum yungense* de la Sota | Jimenez 2487 (NY) | Bolivia | EU907731 | EU907796 | EU907859 |
| *Lomagramma sinuata* C. Chr. | Grether 4056 (US) | Papua New Guinea | – | GU376706 | GU376557 |
| *Mickelia guianensis* (Aubl.) R.C. Moran, Labiak & Sundue | Secco 288 (NY) | Brazil | – | GU376698 | GU376549 |
| *Mickelia nicotianifolia* (Sw.) R.C. Moran, Labiak & Sundue | Christenhusz 4062 (TUR) | Guadeloupe | EF463382 | – | – |
| *Mickelia nicotianifolia* (Sw.) R.C. Moran, Labiak & Sundue | Sanchez 124 (NY) | Puerto Rico | – | GU376669 | GU376522 |
| *Mickelia oligarchica* (Baker) R.C.Moran, Labiak & Sundue | Moran 6244 (NY) | Ecuador | – | GU376668 | GU376521 |
| *Teratophyllum ludens* (Fée) Holttum | Molesworth-Allen 3196 (US) | Malaysia | – | GU376717 | GU376568 |

A '–' indicates no information or data available

*Bolbitis*, *Lomagramma* J. Sm., *Mickelia*, and *Teratophyllum* Mett. ex Kuhn. were used as outgroup. The three marker sets of *Bolbitis auriculata* (Lam.) Alston, and *Mickelia nicotianifolia* (Sw.) R.C. Moran, Labiak & Sundue derived from different specimens of these species. For this study, 450 sequences were used from a total of 156 specimens (Table 1). All sequences were aligned using Muscle 3.6 (Edgar 2004) under default parameters implemented in MEGA 5.1 (Tamura et al. 2011). The resulting alignment was manually edited in BioEdit 5.0.9 (Hall 1999). Ambiguous positions were excluded from the alignment. The alignment is available at http://treebase.org (S14716).

## Phylogenetic analyses

We used 79 new sequences of *Elaphoglossum* (67 of which were from Cuba) and 299 GenBank sequences of *Elaphoglossum* and other bolbitidoids. Missing nucleotides and indels in the aligned sequences were coded as missing data. Phylogenetic trees were inferred using maximum parsimony (MP) criteria implemented in PAUP* 4.0b10 (Swofford 2000) and maximum likelihood criteria implemented in RaxML 7.4.2 (Stamatakis 2006). Bayesian inference (BI) of phylogeny was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). MP analyses were performed with the following options implemented: heuristic search mode with 1,000 random-addition-sequence replicates, tree bisection-reconnection branch swapping (TBR), MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Bootstrap support (BS) values were estimated by calculating 1,000 bootstrap replicates (Felsenstein 1985), each with ten random-addition-sequence replicates, TBR branch swapping, and MULTrees on. Rearrangements were limited to one million per replicate. For BI and ML analyses, the partitioning schemes and the best-fitting nucleotide substitution models were determined under the Akaike Information Criterion (AIC) implemented in PartitionFinder (Lanfear et al. 2012). This resulted in the following four partitions and corresponding substitution models: *atpß*, first and second nucleotide position (GTR + I); *atpß*, third position and *atpß-rbc*L (GTR + Γ); *rps*4-*trn*S (GTR + Γ) and *trn*L-*trn*F (GTR + Γ). For ML analyses, we conducted a rapid Bootstrap (BS) analysis and search for the best-scoring tree with 1,000 bootstrap replicates using the GTR + Γ model for all five partitions. Bayesian inference was implemented using the pertinent GTR substitution models. A Bayesian search was carried out with four simultaneous Markov chains for ten million generations, sampling every 1,000th generation. The first 25 % of the sampled trees were discarded prior to summarizing the remaining trees in a 50 % majority rule consensus tree and generating Bayesian posterior probability (PP) confidence values. We considered nodes to be well supported when PP $\geq$ 0.95 (Larget and Simon 1999) and BS $\geq$ 70 (Hillis and Bull 1993).

Phylogenetic trees were edited in FigTree 1.4 and CorelDRAW 14.

## Results

### Molecular investigation

Of the 1,580 character sites in the concatenated matrix, 793 were constant, 280 autapomorphic, and 507 parsimony informative (see Table 2 for character state distributions within the single markers). The three phylogenetic analyses led to similar topologies. The MP analysis resulted in more than 500,000 equally parsimonious trees with a length of 1,739 steps, a consistency index (CI) of 0.6 and a retention index (RI) of 0.86. The MP strict consensus tree is depicted in Fig. 1. The ML phylogram is not depicted, but ML-BS is shown on the Bayesian tree (Fig. 2). A sister relationship of *Elaphoglossum amygdalifolium* (Mett. ex Kuhn.) Christ and the rest of the genus is strongly supported. A clade with three specimens of the Cuban endemic *E. wrightii* and a clade with a specimen of the Hawaiian *E. aemulum* (Kaulf.) Brack. are separated from the rest of *Elaphoglossum* with strong support. The relationship of these two species is unresolved in the MP analysis (Fig. 1) and lacks statistical support in the BI and the ML analyses (Fig. 2). A well-supported clade assigned to *E.* sect. *Elaphoglossum* is placed sister to a clade consisting of representatives of *E.* sects. *Lepidoglossa* Christ, *Polytrichia* Christ, *Setosa* (Christ) Mickel & Atehortúa and *Squamipedia* Mickel & Atehortúa. The sister relationship of *E.* sect. *Lepidoglossa* with *E.* sects. *Polytrichia* and *Setosa* is also strongly supported. *Elaphoglossum* sects. *Polytrichia* and *Setosa* are the only sections not supported in our analysis, however together form a well-supported clade (Figs. 1, 2). Multiple accessions of *Elaphoglossum* species usually form monophyletic lineages. However, two specimens of *E. palmeri* Underw. & Maxon are placed in a polytomy with *E. dussii* Underw. ex Maxon specimens from Guadeloupe and Puerto Rico; and specimens of *E. erinaceum* (Fée) T. Moore from Cuba and Mexico form separate lineages.

**Table 2** Distribution of constant and phylogenetically informative sites for aligned positions of the three chloroplast DNA regions used in this study

| Matrix | *atp*B-*rbc*L | *rps*4-*trn*S | *trn*L-*trn*F | Total |
|---|---|---|---|---|
| Number of sites in matrix | 874 | 371 | 335 | 1,580 |
| Constant | 585 | 110 | 98 | 793 |
| Autapomorphic | 127 | 81 | 72 | 280 |
| Parsimony informative (% of the total matrix) | 162 (10.3) | 180 (11.4) | 165 (10.4) | 507 (32.1) |

Cuban specimens of *E. martinicense* (Desv.) T. Moore are placed sister to a clade with *E. coriaceum* Bonap. from the Seychelles and *E. coursii* Tardieu from the Comoros. In general, Cuban species of *Elaphoglossum* are resolved in *E.* sects. *Elaphoglossum*, *Lepidoglossa*, *Polytrichia*, *Setosa*, and *Squamipedia*; however, *E. wrightii* is placed in its own lineage sister to all other species in the genus except *E. amygdalifolium* and *E. aemulum.*

### Infrageneric classification

Based on our phylogenetic analysis and morphological observations, we classify the Cuban species of *Elaphoglossum* in five previously recognized sections: *E.* sect. *Elaphoglossum*, *E.* sect. *Lepidoglossa*, *E.* sect. *Polytrichia*, *E.* sect. *Setosa*, and *E.* sect. *Squamipedia* (Table 3). The Cuban endemic *E. wrightii* is classified in a new section, as follows:

*Elaphoglossum* sect. *Wrightiana* J. Lóriga, A. Vasco, L. Regalado, Heinrichs & R.C. Moran, sect. nov.

Type: *Acrostichum wrightii* Mett. ex D.C. Eaton, Mem. Amer. Acad. Arts, n.s. 8: 194. 1860. [=*Elaphoglossum wrightii* (Mett. ex D.C. Eaton) T. Moore].

Diagnosis: Root climbers, with long-creeping rhizomes that begin growth on the ground and eventually climb trunks to heights of 1–2 m, maintaining the connection to the ground by the rhizome and by roots emitted from the lower portions of the climbing rhizome; phyllopodia present, hydathodes absent, laminar scales flat (not subulate), with marginal processes or teeth ending in a slightly swollen cell (i.e., scales never with acicular marginal cells as in *E.* sect. *Lepidoglossa*) (Fig. 3).

## Discussion

### Infrageneric classification and evolution of *Elaphoglossum*

Collectively, the Cuban species of *Elaphoglossum* included in the phylogenetic analyses were resolved in the following sections of the genus: *E.* sects. *Elaphoglossum*, *Lepidoglossa*, *Polytrichia*, *Setosa* and *Squamipedia*. These sections can be identified using combinations of morphological character states such as growth habit, scales, hydathodes, rhizome habit, and presence or absence of phyllopodia (Mickel and Atehortúa 1980; Rouhan et al. 2004). Using such characters (Table 3), we assigned to sectional rank the Cuban species not included in our molecular studies [*E. decursivum* Mickel, *E. denudatum* (Jenman) Maxon ex Morton, *E. inaequalifolium* (Jenman) C. Chr., *E. muscosum* (Sw.) T. Moore, *E. procurrens* (Mett. ex D. C. Eaton) T. Moore, *E. simplex* (Sw.) Schott. ex J.

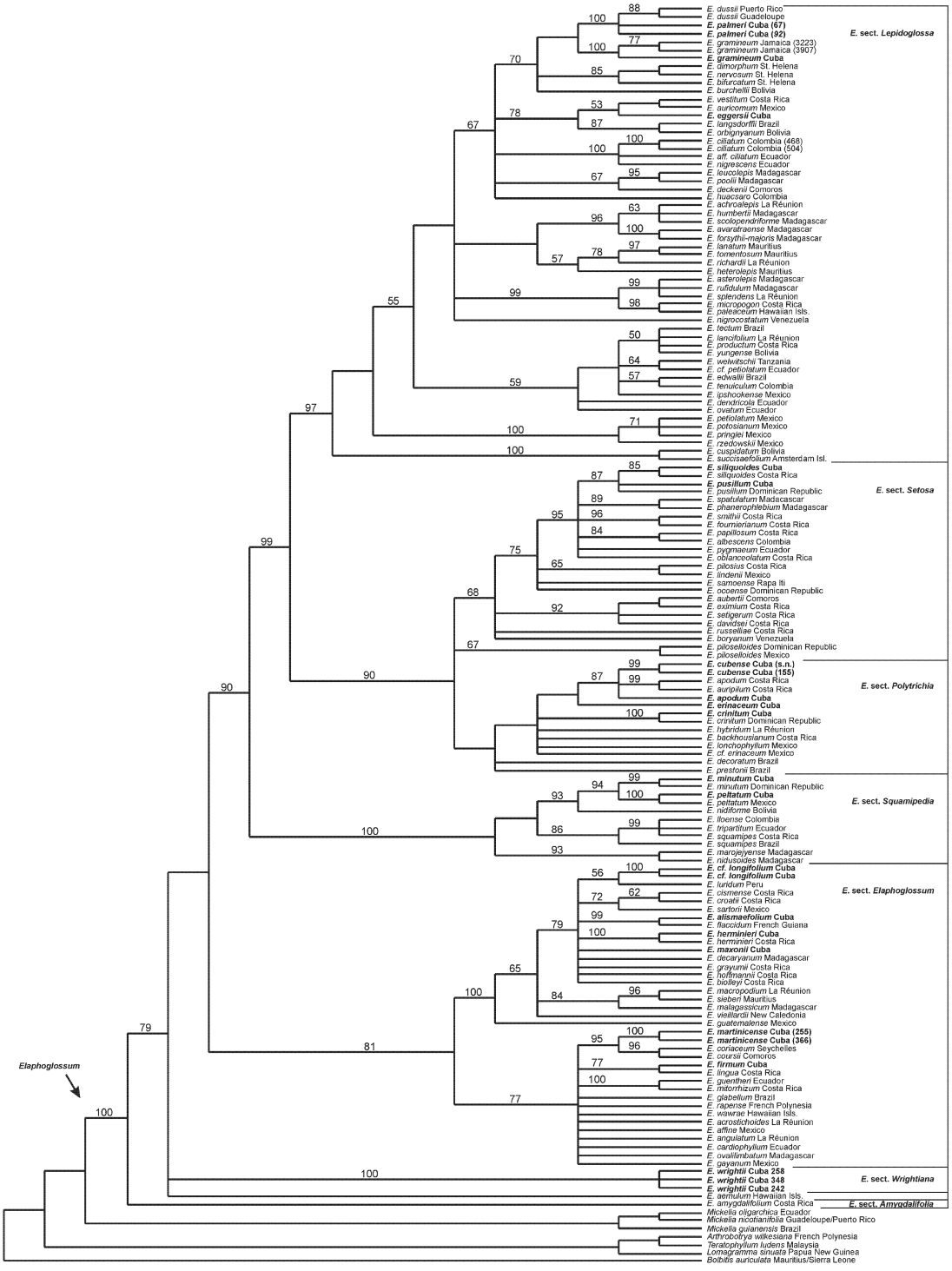


**Fig. 1** Rooted strict consensus of more than 500,000 trees recovered during MP heuristic searches of the chloroplast DNA dataset. Bootstrap percentage values ≥50 are indicated at branches, as well as a refined infrageneric classification of *Elaphoglossum*

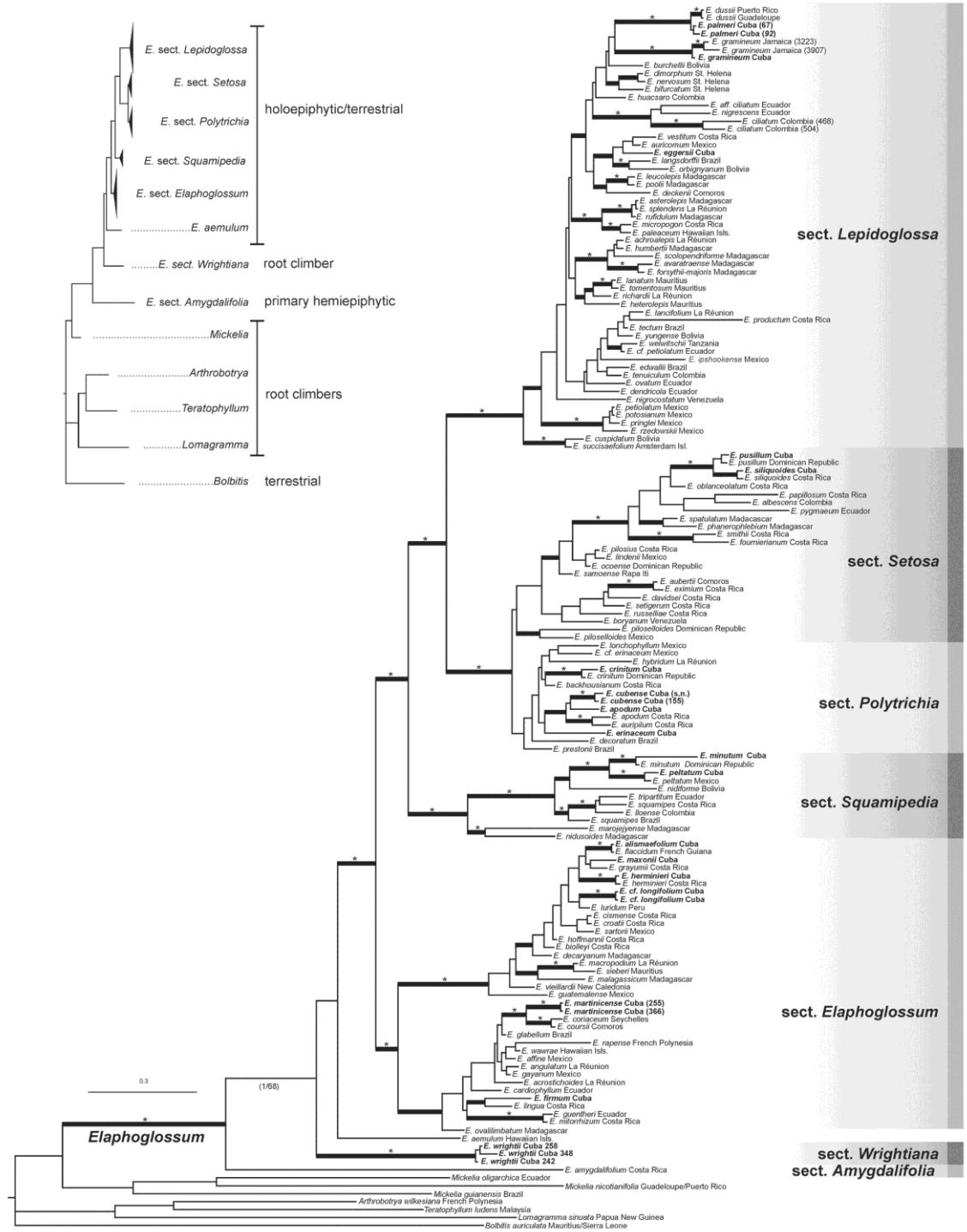


**Fig. 2** Majority rule consensus tree recovered in Bayesian inference analysis. *Thick branches* indicate Bayesian posterior probabilities (PP) $\geq$ 0.95 and maximum likelihood (ML) bootstrap percentage values (BS) $\geq$ 70 %. A *star* indicates Bayesian PP of 1.0 and ML-BS $\geq$ 90 %. The growth habit of the sections and outgroup clades is provided in a schematic topology in the *upper left* of the *panel*

**Table 3** Distinctive characters of *Elaphoglossum* sections and the assignment of the Cuban species based on either the phylogenetic reconstruction or morphological characters

| Character | *E.* sect. *Amygdalifolia* | *E.* sect. *Elaphoglossum* | *E.* sect. *Lepidoglossa* | *E.* sect. *Setosa* | *E.* sect. *Polytrichia* | *E.* sect. *Squamipedia* | *E.* sect. *Wrightiana* |
|---|---|---|---|---|---|---|---|
| Subulate scales on the leaves | Absent | Absent | Absent | Present | Present | Absent | Absent |
| Rhizome scales with acicular marginal cells | Absent | Absent | Present | Absent | Absent | Absent | Absent |
| Rhizome habit | Long creeping | Erect | Erect/short creeping | Erect/short creeping | Erect/short creeping/ long creeping | Long creeping | Long creeping |
| Phyllopodia | Present | Present | Present | Present | Present | Absent | Present |
| Hydathodes | Present | Absent | Absent | Present | Absent | Absent | Absent |
| Young fronds color | Reddish | Green | Green | Green | Green | Green | Green |
| Growth habit | Primary hemiepiphytic | Holoepiphytic/ terrestrial | Holoepiphytic/ terrestrial | Holoepiphytic/ terrestrial | Holoepiphytic/ terrestrial | Holoepiphytic | Root climbers |
| **Cuban species** | | ***E. alismaefolium*** | ***E. eggersii*** | ***E. ocoense*** | ***E. apodum*** | ***E. peltatum*** | ***E. wrightii*****|
| | | *E. decursivum** | ***E. gramineum*** | ***E. piloselloides*** | ***E. crinitum*** | ***E. minutum*** | |
| | | ***E. firmum*** | *E. muscosum* | ***E. pusillum*** | ***E. cubense*** | | |
| | | ***E. flaccidum*** | ***E. paleaceum*** | ***E. siliquoides*** | ***E. decoratum*** | | |
| | | ***E. glabellum*** | ***E. palmeri**** | | *E. denudatum** | | |
| | | ***E. herminieri*** | ***E. tectum*** | | ***E. erinaceum*** | | |
| | | *E. inaequalifolium* | | | *E. procurrens* | | |
| | | ***E. longifolium*** | | | | | |
| | | ***E. martinicense**** | | | | | |
| | | ***E. maxonii*** | | | | | |
| | | *E. simplex* | | | | | |

Names in bold represent the species included in the phylogenetic analyses; species with one asterisk are reported for the first time for Cuba, species with two asterisks are Cuban endemics. "Primary hemiepiphytic" refers to plants that start growing on a support tree and secondary contact with the soil, "epiphytic" refers to plants that never contact the soil; "terrestrial" to plants growing exclusively in the soil; "root climbers" are plants that start growing on the ground and eventually climb trunks. Three species are still unidentified; two belong to *E.* sect. *Elaphoglossum* and one to *E.* sect. *Lepidoglossa*

Sm. and *E.* spp. indet. 1–3]. *Elaphoglossum amygdalifolium,* the sole member of *E.* sect. *Amygdalifolia* (Christ) Mickel & Atehortúa, has been cited for Cuba (Mickel 1995; Lagomarsino et al. 2012), but we cannot find a voucher specimen and suspect that the record is based on a misidentification.

A new finding reported here is that *Elaphoglossum wrightii* merits its own section, as described above. Formerly, this species was considered to belong to *E.* sect. *Squamipedia*, which is characterized by long-creeping rhizomes, absence of phyllopodia, and echinulate spores (Mickel and Atehortúa 1980; Moran et al. 2007). *Elaphoglossum wrightii* differs, however, from other representatives of *E.* sect. *Squamipedia* by non-echinulate spores (Fig. 4), presence of phyllopodia, and most importantly its growth habit. To our knowledge, *E. wrightii* is the only species of *Elaphoglossum* that starts growth on the soil and climbs from there to the lower portions of tree trunks. This lends support to its early diverging phylogenetic position. This growth habit is typical for the bolbitidoid outgroup genera of *Arthrobotrya*, *Lomagramma*, *Mickelia*, and *Teratophyllum* (Moran et al. 2010a). This suggests that the growth habit of *E. wrightii* is plesiomorphic, and that the primary hemiepiphytism of *E. amygdalifolium* (Lagomarsino et al. 2012) and the holoepiphytism found elsewhere in the genus might be derived from it. *Elaphoglossum amygdalifolium*, the sister species of all other *Elaphoglossum* species investigated so far (Rouhan et al. 2004), is the only primary hemiepiphyte within the genus, initiating sporophyte growth on a support tree and later developing contact with the soil by downward growing roots (Lagomarsino et al. 2012). Its rhizomes are long creeping. In contrast, most *Elaphoglossum* species have short, compact rhizomes. It can be hypothesized that the development of

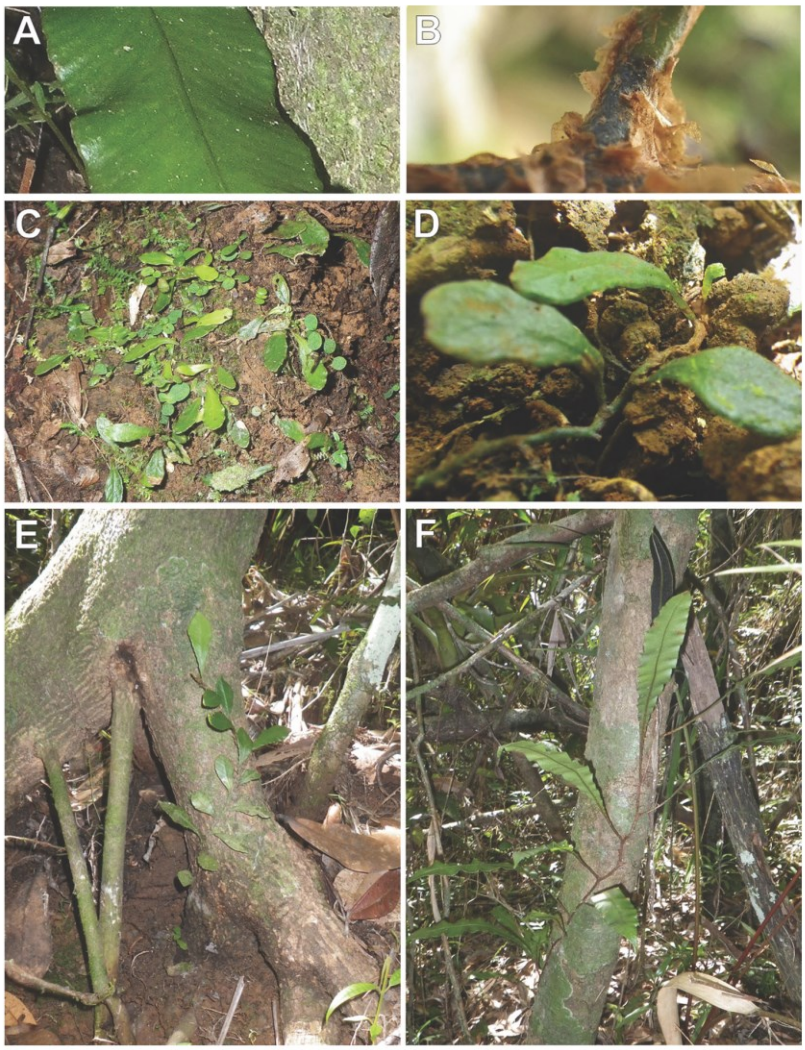


**Fig. 3** The Cuban endemic *Elaphoglossum wrightii* in its natural habitat. Spores germinate on soil; the sporophyte begins growth on the ground and later climbs a support tree. **a** Sterile blade lacking hydathodes. **b** Phyllopodium covered by scales. **c** Juvenile sporophyte growing on soil. **d** Creeping rhizome of young sporophyte. **e** Juvenile sporophyte climbing support tree. **f** Mature sporophyte on support tree

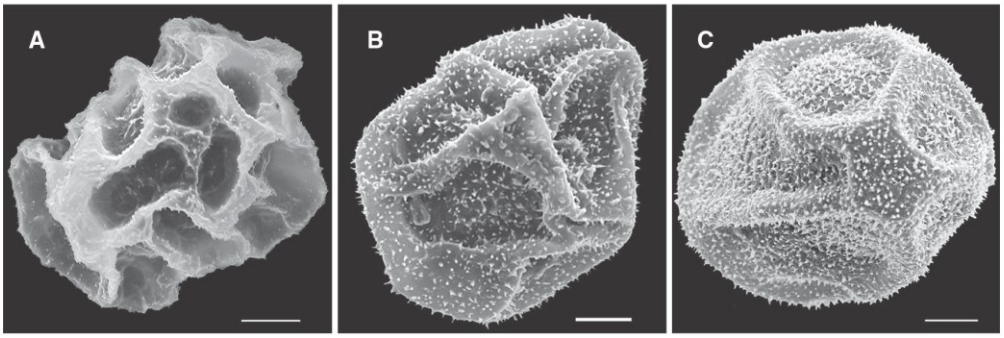


**Fig. 4** Comparison of perispores in *Elaphoglossum* sect. *Wrightiana* (**a**) and sect. *Squamipedia* (**b**, **c**). **a** *E. wrightii* (Cuba, *Ekman 3882*, NY). **b** *E. minutum* (Guiana, *Clarke 4963*, NY). **c** *E. peltatum* f. *peltatum* (Mexico, *Mendez 7931*, NY). *Scale bars* 10 μm

short, compact rhizomes allowed *Elaphoglossum* to colonize epiphytic habitats, and to leave the terrestrial environment which is otherwise typical for bolbitidoid ferns. Compact rhizomes could be a key innovation (Schneider et al. 2010; Yoder et al. 2010) that could explain the evolutionary success of *Elaphoglossum,* but additional physiological and ecological studies are necessary to evaluate this hypothesis.

Rouhan et al. (2004) recovered another monotypic lineage for the Neotropical *E. glaucum* T. Moore but questioned the result. Our initial analyses of the related *trn*L-*trn*F and *rps*4-*trn*S sequences (GenBank accessions AY534844 and AY536172) provided evidence for a conflicting phylogenetic signal and low statistical support for the related node. We later excluded these sequences and found that it improved the robustness of our topologies. Further samples of *E. glaucum* should be included in future studies to clarify its position within the genus. Its thick, sparsely scaly laminae suggest that it is a typical member of *E.* sect. *Elaphoglossum*. Unfortunately, spore morphology is not distinctive enough to assign it to this section or any others in the genus (Moran et al. 2007).

## Species concepts

*Elaphoglossum* is notorious for its relatively uniform leaf shapes (nearly all simple and entire) and subtle morphological differences. Given this, it is helpful to test morphological species concepts using molecular evidence (e.g., Vasco et al. 2009a). The monophyly of several specimens thought to represent the same morphological species from Cuba, other islands in the West Indies, and Central America points to congruence of morphological and molecular species concepts. This congruence is seen in the monophyly exhibited by the following species in our analyses that had multiple samples: *E. crinitum*, *E. gramineum*, *E. herminieri*, *E. peltatum*, *E. pusillum*, *E. minutum*, and *E. siliquoides*. In contrast, the samples of *E. erinaceum* were resolved polyphyletic. This species is highly variable (pers. obs.) and probably consists of several species.

The phylogenetic position of the Cuban endemic *Elaphoglossum palmeri*, in a clade with the West Indian endemic *E. dussii*, suggests that this species belongs to the *E. ciliatum* group sensu Vasco et al. (2009a). The two species are atypical in the *E. ciliatum* group because they lack echinate perispores and resinous rhizomes, characteristics typical of the other species in this clade (Vasco et al. 2009a, b). Both species are similar in the DNA sequences, yet differ in morphology: *E. palmeri* lacks resinous dots (present in *E. dussii*) on the blades, and its rhizome scales have only half the length of those of *E. dussii*.

## Biogeography

*Elaphoglossum* is likely of Neotropical origin. This is suggested by its sister relationship with *Mickelia,* an entirely Neotropical genus (Moran et al. 2010a), and its many early diverging species being Neotropical. Several Afro-Malagasy species are nested within Neotropical lineages, indicating a Neotropical origin by long-distance dispersal of these species or their ancestors. Examples include *E. lancifolium* (Desv.) C.V. Morton and *E. welwitschii* (Baker) C. Chr. (both from *E.* sect. *Lepidoglossa*), and *E. aubertii* (Desv.) T.Moore, *E. phanerophlebium* C. Chr., and *E. spatulatum* (*E.* sect. *Setosa*) (Fig. 2). Dispersal from the Neotropics to Africa seems to be common and has been inferred for several other lineages of ferns (Janssen et al. 2007; Moran and Smith, 2001), angiosperms (Renner 2004), and bryophytes (Feldberg et al. 2010).

Remarkably, two of the early diverging lineages within *Elaphoglossum* (*E.* sects. *Amygdalifolia* and *Wrightiana*) are monotypic, a pattern that suggests widespread extinctions in the early history of this genus. This idea requires testing with more comprehensive sampling, with emphasis in the less well-sampled Eastern Asian and Indonesian species. Within the Neotropics, the biogeographic pattern of *Elaphoglossum* shows evidence of both long-distance dispersal and local speciation events along its evolutionary history. The long-distance dispersal capability of *Elaphoglossum* is shown by its numerous occurrences on oceanic islands (Rouhan et al. 2004, 2008; Eastwood et al. 2004; Vasco et al. 2009a). In our results, it is shown by the close relationship between the West Indian *E. martinicense* and Old World *E. coriaceum* (Seychelles) and *E. coursii* (Comoros). In general, however, Cuban species of *Elaphoglossum* are most closely related to congeners in the West Indies and Central America. This suggests local speciation.

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Chapter 3

# PHYLOGENETIC RELATIONSHIPS OF TWO CUBAN SPLEENWORTS WITH UNUSUAL MORPHOLOGY: *ASPLENIUM* (*SCHAFFNERIA*) *NIGRIPES* AND *ASPLENIUM PUMILUM* (ASPLENIACEAE, LEPTOSPORANGIATE FERNS)

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ORIGINAL ARTICLE

# Phylogenetic relationships of two Cuban spleenworts with unusual morphology: *Asplenium* (*Schaffneria*) *nigripes* and *Asplenium pumilum* (Aspleniaceae, leptosporangiate ferns)

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**Abstract** The infrageneric classification of *Asplenium*, the most species-rich genus of ferns, is notoriously difficult as a result of extensive morphological homoplasy combined with exceptional morphological disparity. Besides a core *Asplenium*, 29 satellite genera have been described, but most of them have not been widely accepted. In recent years, molecular phylogenetic studies found most of these satellite genera to be nested in *Asplenium*, but several morphologically distinct taxa have not yet been included in such studies. One of these elements is the monospecific neotropical genus *Schaffneria* which is characterized by undivided suborbicular blades, lack of a costa, black stipes, netted veins and single or paired sori. Maximum likelihood and Bayesian phylogenetic inference based on the chloroplast DNA markers *rbc*L, *rps*4, *rps*4-*trn*S and *trn*L-*trn*F indicated a position of *Schaffneria nigripes* within *Asplenium*. We thus propose to treat *Schaffneria* as a synonym of *Asplenium* and adopt the name *Asplenium nigripes*. With the current sampling, *Asplenium* (*Schaffneria*) *nigripes* is placed sister to *A. pumilum*, the only species of *Asplenium* with whitish catenate hairs on its leaves. Despite considerable morphological differences, both species resemble each other in several features including filiform-lanceolate, mostly entire, brown-blackish rhizome scales with a dark-sclerotic center and some marginal projections, a striate, hairy epidermis, echinolophate spore ornamentation with slim microechinate folds forming small lacunae, and *Aspidium*-type gametophytes.

**Keywords** Chloroplast DNA · Greater Antilles · Mesoamerica · Molecular phylogeny · Polypodiales · Satellite genera

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## Introduction

Aspleniaceae as defined by Smith et al. (2006) and Christenhusz et al. (2011) is a globally distributed family of the eupolypod II clade of leptosporangiate ferns including more than 700 species. Members of Aspleniaceae occur in a wide array of terrestrial, epiphytic and rock habitats in tropical and temperate zones (e.g., Murakami et al. 1999a; Pinter et al. 2002; Schneider et al. 2004, 2005; Perrie and Brownsey 2005; Schuettpelz and Pryer 2007; Rothfels et al. 2012). Diagnostic features of the family are *x*-shaped vascular bundles in the distal portion of the petiole, clathrate scales attached to the rhizome and basal portion of the petiole, sporangia arranged in linear sori along the veins that are covered by laterally attached indusia, 1-rowed sporangial stalks, and monolete spores (Morton and Lellinger 1966; Murakami et al. 1999a; Schneider et al. 2004; Sundue and Rothfels 2014).

More than 90% of the species of Aspleniaceae have been placed within *Asplenium* L. (Copeland 1947; Pichi-Sermolli 1977; Tryon and Tryon 1982). The rest has variously been assigned to 29 species poor satellite genera (see Lovis 1973; Schneider et al. 2004) of which only *Hymenasplenium* Hayata is currently accepted (Murakami and Schaal 1994; Murakami 1995; Murakami et al. 1999a; Schneider et al. 2004; Smith et al. 2006; Christenhusz et al. 2011). Many of the putative satellite genera have been confirmed to be nested in *Asplenium* or *Hymenasplenium* in molecular phylogenetic studies and are therefore treated as synonyms. Examples include *Boniniella* Hayata, *Camptosorus* Link, *Ceterach* Willd., *Ceterachopsis* (J.Sm.) Ching, *Lepichroa* T.Moore, *Loxoscaphe* T.Moore, *Neottopteris* J.Sm., *Phyllitis* Hill, *Pleurosorus* Fée, *Tarachia* C.Presl and *Thamnopteris* (C.Presl) C.Presl (Murakami et al. 1999a, b; Gastony and Johnson 2001; Pinter et al. 2002; Schneider et al. 2004, 2005). Other putative *Asplenium* elements have not yet been included in molecular phylogenies, e.g., *Holodictyum* Maxon and *Schaffneria* Fée ex T.Moore.

*Schaffneria* is known from a few localities in Mexico, Guatemala, Costa Rica and Cuba and comprises only a single species, *S. nigripes* Fée (Fig. 1a, b). This species grows on rock in mountain forests between 900 and 1500 m (Moran and Riba 1995; Sánchez and Regalado 2003; Mickel and Smith 2004) and stands out by its entire suborbicular to rhomboid fleshy blade, lack of a costa, black stipes, netted veins, and single or "scolopendroid" sori, i.e., paired sori on adjacent veins that open toward each other (Gómez 1973; Riba et al. 1992; Sánchez and Regalado 2003; Mickel and Smith 2004). It has alternatively been placed in the genera *Antigramma* C. Presl. (Smith 1875), *Asplenium* (Hooker 1857), *Phyllitis* (Kuntze 1891) and *Scolopendrium* (Hooker 1862). Another distinctive species with unclear taxonomic position is the Afro-American *Asplenium pumilum* Sw. This species occurs in tropical South America, Central America, the Caribbean islands, tropical Africa and Madagascar (Moran and Smith 2001) and grows on limestone outcrops. It is characterized by whitish catenate hairs on the leaves and a basal pair of pinnae of which the basiscopic (proximal) side is more strongly developed than the acroscopic (distal) side (Fig. 1d, e). Given the unusual indument, it is surprising that this widespread species has not been recognized at generic rank in the past. The Mexican *Asplenium arcanum* A.R.Sm. and *A. minimum* M.Martens & Galeotti resemble *A. pumilum* in leaf dissection but lack the whitish catenate hairs (Mickel and Smith 2004). The unusual morphology of *A. arcanum*, *A. minimum* and *A. pumilum* led Mickel and

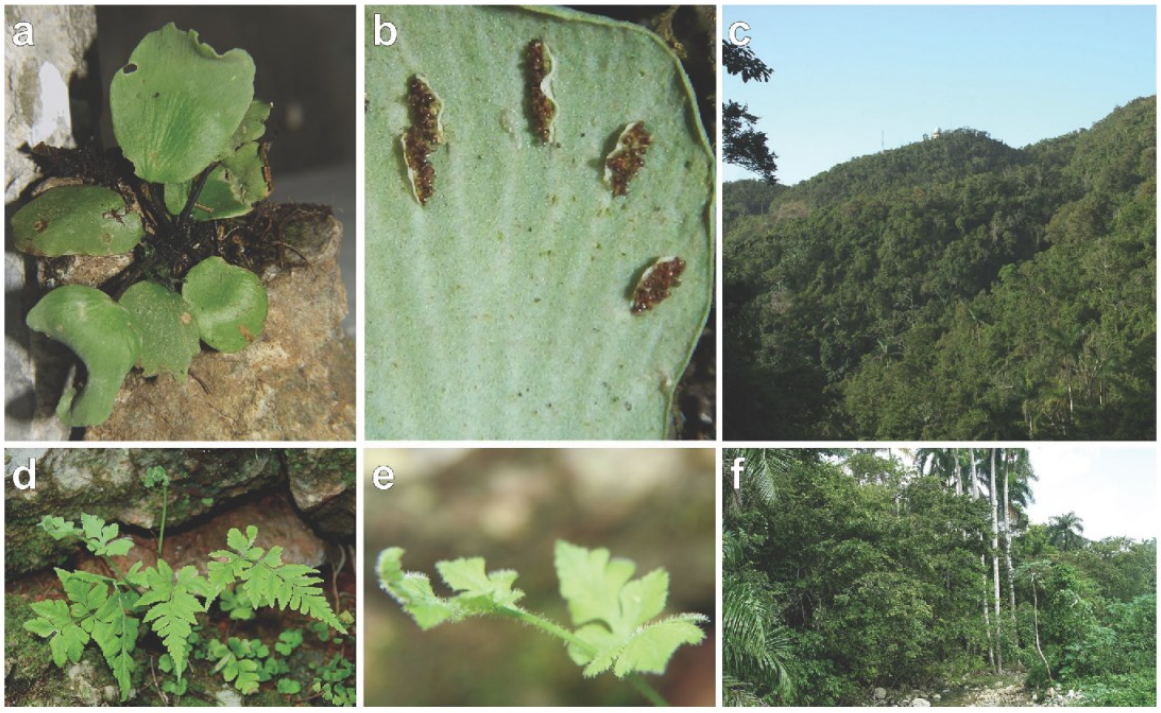


**Fig. 1** *Schaffneria nigripes* and *Asplenium pumilum* in their natural habitat. *Schaffneria nigripes*, **a** habit, **b** detail of the abaxial surface of a fertile leaf showing single and paired ("scolopendrioid") sori, **c** habitat. *A. pumilum*, **d** habit, **e** leaf detail showing catenate white hairs, **f** habitat

Smith (2004) to conclude that the relationships of these species within *Asplenium* are uncertain.

Here we present the first molecular phylogeny of Aspleniaceae that includes *Schaffneria* and *A. pumilum*. We investigate the phylogenetic position of both species and evaluate their morphological affinities to related species.

## Materials and methods

Two samples of *Schaffneria* and four of *A. pumilum* were gathered during collecting trips in Cuba in 2014 and 2015. Samples of *Schaffneria* originated from a limestone hill northwest of Pico San Juan (80.147822°W, 21.990544°N; 1107 m.s.m.) in Cumanayagua, Central Cuba (Fig. 1a–c). Samples of *A. pumilum* were gathered on limestone outcrops of riverine forests near Canimar River mouth, Matanzas, West Cuba (81.494761°W, 23.036753°N), at sea level (Fig. 1d–f) and in Poza del Cura, Cumanayagua, Central Cuba (80.207941°W, 21.992629°N; 483 m.s.m.). From each individual, sterile leaf tissue fragments were preserved in 96% ethanol for DNA extraction. Fertile leaves for spore examination were preserved in silica. Vouchers were deposited in the Herbario de la Academia de Ciencias, La Habana (HAC) and the Botanische Staatssammlung München (M).

Total genomic DNA was extracted from leaf tissue using the Invisorb Spin Plant Mini Kit (STRATEC). We amplified four chloroplast DNA markers commonly used in published Aspleniaceae phylogenies, namely the genes *rbc*L and *rps*4, and the intergenic spacers *rps*4-*trn*S and *trn*L-*trn*F (including partial *trn*L intron) (see Table 1 for primers used). Polymerase chain reactions were performed in a final volume of 12 µl, using 0.3 µl each of 10 µM forward and reverse primers, 0.25 µl of total dNTP 10 mM, 0.08 µl of 5 u/µl GoTaq and 2.5 µl 5 × GoTaq Reaction Buffer. Conditions for *rbc*L comprised initial denaturation at 94 °C (3 min) and then 30 cycles of denaturation at 94 °C (45 s), primer annealing at 57 °C (30 s) and elongation at 72 °C (90 s), followed by a final extension step at 72 °C (5 min). For the other three markers, primer annealing was set to 49 °C (30 s) and the final extension step was reduced to 1 min.

The successfully amplified products were purified using exonuclease I and shrimp alkaline phosphatase (SAP) or antarctic phosphatase (AP) according to the manufacturer's instructions (New England Biolabs Inc.). Bidirectional sequences were generated using dye-labeled didesoxy terminator cycle sequencing on an ABI 3130 DNA sequencer (Applied Biosystems). Sequences were assembled using CodonCode Aligner (v. 3.5.6, Codon Code Corporation) and submitted to GenBank (Table 2).

The newly generated sequences of *Schaffneria* and *A. pumilum* were compared with GenBank sequences using the BLASTN program (Altschul et al. 1990). The BLAST searches suggested an affiliation of both species to *Asplenium* clade VII of Schneider et al. (2004). Therefore, we downloaded all available *rbc*L, *rps*4, *rps*4-*trn*S and *trn*L-*trn*F sequences of members of *Asplenium* clade VII (Murakami et al. 1999a; Schneider et al. 2004, 2005; Dyer et al. 2012; Chang et al. 2013; Ohlsen et al. 2015). This initial dataset was reduced to one individual per species prioritizing vouchers with the most complete sequence stretches. We also downloaded sequences from two to three species of the remaining *Asplenium* clades retrieved by Schneider et al. (2004). Three species of *Hymenasplenium* where chosen as outgroup based on phylogenetic hypotheses of Murakami and Schaal (1994), Murakami (1995), Murakami et al. (1999a) and Schneider et al. (2004, 2005). The final dataset included 51 species represented by 148 sequences (Table 2). We used Muscle 3.6 (Edgar 2004) under default parameters implemented in the MEGA 6 package (Tamura et al. 2011) to align the sequences and manually adjusted the resulting alignment. Ambiguously aligned sections of noncoding regions were removed using Gblocks 0.91b (Castresana 2000) under relaxed selection of blocks (Talavera and Castresana 2007). The final alignment included 2948 characters. The alignment is available at http://treebase.org, study 19965.

**Table 1** Amplification and sequencing primers used in this study

| DNA marker | Primer | Sequences (5′–3′) | References |
|---|---|---|---|
| *rbc*L | ESRBCL1F | ATGTCACCACAAACGGAGACTAAAGC | Schuettpelz and Pryer (2007) |
| | ESRBCL1361R | TCAGGACTCCACTTACTAGCTTCACG | Schuettpelz and Pryer (2007) |
| | ESRBCL628F* | CCATTYATGCGTTGGAGAGATCG | Schuettpelz and Pryer (2007) |
| | ESRBCL654R* | GAARCGATCTCTCCAACGCAT | Schuettpelz and Pryer (2007) |
| *rps*4 + *rps*4-*trn*S | – | ATGTCMCGTTAYCGAGGRCCTCGT | Schneider et al. (2005) |
| | R | TACCGAGGGTTCGAATC | Smith and Cranfill (2002) |
| *trn*L + *trn*L-*trn*F | Fern-1 | GGCAGCCCCCARATTCAGGGRAACC | Trewick et al. (2002) |
| | f | ATTTGAACTGGTGACACGAG | Taberlet et al. (1991) |

An asterisk indicates primers used only for sequencing

**Table 2** Taxa used in the present study, including information about the origin of the studied material, voucher information, as well as GenBank accession numbers

| Species | Voucher (herbarium) | Country | *rbc*L | *rps*4 to *rps*4-*trn*S | *trn*L-*trn*F |
|---|---|---|---|---|---|
| *Asplenium adiantum-nigrum* L. | ADI 46 (—) | UK | JX068689 | JX068764 | JX068722 |
| *Asplenium affine* Sw. | *Schneider 954* (SAR) | Borneo | AY300104 | AY549826 | AY300051 |
| *Asplenium anceps* Lowe ex Hook. & Grev. | *Vogel 1111* (BM) | Azores | AY300105 | AY549795 | AY300052 |
| *Asplenium angustum* Sw. | *Boudrie 3254* (BM) | French Guiana | AY300106 | AY549822 | AY300053 |
| *Asplenium aureum* Cav. | JCV Cet-116 (BM) | Canary Islands | AF240642 | AY549767 | AF525258 |
| *Asplenium ceterach* L. | CV225 (—) | Cyprus | AF538313 | – | AY162334 |
| *Asplenium cristatum* Lam. | *Cranfill s.n.* (UC) | Costa Rica | AY549731 | AF425146 | AY549834 |
| *Asplenium cuspidatum* Lam. | *Grantham* and *Parsons 0233090* (UC) | Costa Rica | AY300111 | AY549760 | AY300058 |
| *Asplenium dielerectum* Viane | *Wood 7775* (PTBG) | Hawaii | AY549737 | AY549786 | AY549840 |
| *Asplenium dielfalcatum* Viane | *Wood 7826* (PTBG) | Hawaii | AY549738 | AY549787 | AY549841 |
| *Asplenium dielmannii* Viane | *Perlman SP18502* (PTBG) | Hawaii | AY549739 | AY549788 | AY549842 |
| *Asplenium dielpallidum* N.Snow | SP18502 (PTBG) | Hawaii | AY549740 | AY549789 | AY549843 |
| *Asplenium erectum* Bory ex Willd. | *Hemp 14* (BM) | Kenya | AY300113 | AY549770 | AY300060 |
| *Asplenium fontanum* (L.) Bernh. | *Vogel F-3-92* (BM) | Germany | AF525268 | AY549806 | AF525239 |
| *Asplenium formosum* Willd. | *Vogel AZO34* (BM) | Belize | AY300116 | AY549796 | AY300063 |
| *Asplenium friesiorum* C.Chr. | *Hemp 21* (BM) | Kenya | AY549756 | AY549828 | AY549860 |
| *Asplenium gulingense* Ching & S.H.Wu | 102303 (HITBC) | China | JX152738 | JQ724309 | JQ724224 |
| *Asplenium hallbergii* Mickel & Beitel | *Vogel 350* (BM) | cult | AY300118 | AY549798 | AY300065 |
| *Asplenium hemionitis* L. | *Vogel HEM-9* (BM) | Azores | AF240648 | AY549776 | AF240663 |
| *Asplenium heterochroum* Kunze | *Hughes 42* (BM) | Belize | AY549745 | AY549799 | AY549849 |
| *Asplenium hobdyi* W.H.Wagner | *Ranker 1806* (COLO) | Hawaii | AY549736 | AY549785 | AY549839 |
| *Asplenium* x *joellaui* N.Snow | *Wood 7797* (PTBG) | Hawaii | AY549742 | AY549791 | AY549845 |
| *Asplenium juglandifolium* Lam. | *Boudrie M 3249* (BM) | French Guiana | AF525269 | AY459168 | AF525245 |
| *Asplenium laciniatum* D.Don | *Cheng s.n.* (BM) | China | AY549747 | AY549801 | AY549851 |
| *Asplenium lushanense* C.Chr. | *Lu SG/D21* (PYU) | China | AY545481 | AY725042 | AY725033 |
| *Asplenium marinum* L. | *Vogel MAR-5* (BM) | UK | AF240647 | – | AF240662 |
| *Asplenium nidus* L. | *Kessler 13726* (UZH) | Papua New Guinea | KP774889 | KP835428 | KP835367 |
| *Asplenium nigripes* (Fée) Hook. | JLTS971 = JL709 (M) | Cuba | **KX856359** | **KX856365** | **KX856354** |
| *Asplenium nigripes* (Fée) Hook. | JLTS972 (no voucher) | Cuba | **KX856360** | **KX856366** | **KX856355** |
| *Asplenium normale* D.Don | HITBC 102003 | Hawaii | JX152759 | JQ724306 | JQ724222 |
| *Asplenium oligophlebium* Baker | 102404 (HITBC) | Japan | JX152751 | JQ724310 | JQ724225 |
| *Asplenium papaverifolium* (Kunze) Viane | PLE CHI (—) | Chile | JX068707 | JX068790 | JX068750 |
| *Asplenium pekinense* Hance | *Lu SG/C67* (PYU) | China | AY545479 | AY725040 | AY725037 |
| *Asplenium petrarchae* subsp. *bivalens* Lovis & Reichst. | *Vogel PET-4* (BM) | Majorca | AF525271 | AY549804 | AF525249 |
| *Asplenium protensum* Schrad. | *Hemp 2* (BM) | Kenya | AY300135 | AY549825 | AY300081 |
| *Asplenium pumilum* Sw. | JLTS1004 = JL719 (M) | Cuba | **KX856361** | **KX856367** | **KX856356** |
| *Asplenium pumilum* Sw. | JLTS1005 (no voucher) | Cuba | **KX856362** | **KX856368** | **KX856357** |
| *Asplenium pumilum* Sw. | JLTS658 (no voucher) | Cuba | **KX856363** | **KX856369** | **KX856358** |
| *Asplenium pumilum* Sw. | JLTS659 (no voucher) | Cuba | **KX856364** | – | – |
| *Asplenium resiliens* Kunze | *Shaw 19* (ISC) | USA | AY549746 | AY549800 | AY549850 |
| *Asplenium scolopendrium* L. | *Vogel SCOL-73* (BM) | France | AF240645 | – | AF525262 |
| *Asplenium septentrionale* subsp. *caucasicum* Fraser-Jenk. & Lovis | *Vogel SEPT-17* (BM) | Turkey | AF525275 | AY549777 | AF525248 |

**Table 2** continued

| Species | Voucher (herbarium) | Country | *rbc*L | *rps*4 to *rps*4-*trn*S | *trn*L-*trn*F |
|---|---|---|---|---|---|
| *Asplenium tricholepis* Rosenst. | *Kessler 12603* (—) | Bolivia | AY549729 | AY549761 | AY549832 |
| *Asplenium trichomanes* subsp. *quadrivalens* D.E.Mey. | *Vogel Q-272* (BM) | Romania | AY549744 | AY549794 | AY549847 |
| *Asplenium unisorum* (W.H.Wagner) Viane | *Wood 7706* (PTBG) | Hawaii | AY549741 | AY549790 | AY549844 |
| *Asplenium varians* Wall. ex Hook. & Grev. | *Fraser-Jenkins 10046* (BM) | China | AY300147 | AY549802 | AY300094 |
| *Asplenium viride* Wall. ex Hook. & Grev. | *Vogel 1334* (BM) | Austria | AY549734 | AY549782 | AF525238 |
| *Hymenasplenium cheilosorum* (Kunze ex Mett.) Tagawa | *Cranfill TW013* (UC) | Taiwan | – | AY549757 | AY549830 |
| *Hymenasplenium excisum* (C.Presl) S.Lindsay | *Ranker 1786* (COLO) | Hawaii | AY549728 | AY549758 | AY549831 |
| *Hymenasplenium unilaterale* (Lam.) Hayata | *Hemp 18* (BM) | Kenya | AF240652 | – | AF525232 |

New sequences in bold face

We reconstructed the phylogeny using a concatenated DNA matrix with partitions. The partitioning schemes and the best-fitting nucleotide substitution models of the dataset were estimated under the Bayesian information criterion (BIC) implemented in PartitionFinder (Lanfear et al. 2012). This resulted in the following four partitions and corresponding substitution models: *rbc*L, first nucleotide position (HKY + I + Γ); *rbc*L, second nucleotide position (JC + I); *rps*4, first and second nucleotide position (K80 + Γ); *rbc*L and *rps*4, third nucleotide position (GTR + Γ); and *rps*4-*trn*S and *trn*L-*trn*F (GTR + Γ). We conducted a maximum likelihood (ML) and Bayesian inference (BI) search using RaxML 7 (Stamatakis 2006) and MrBayes 3.2 (Ronquist and Huelsenbeck 2003), respectively. For ML analyses, we conducted a rapid bootstrap (BS) analysis and searched for the best-scoring tree with 1000 bootstrap replicates using the GTR + Γ model for the five partitions. Bayesian searches were carried out with four simultaneous Markov chains for ten million generations and sampling every 1000th generation. The first 25% of the sampled trees were discarded; the remaining trees were summarized in a 50% majority rule consensus tree with Bayesian posterior probabilities (PP) indicated at branches.

Spores of *Schaffneria nigripes* and *A. pumilum* were studied using scanning electron microscopy. Air-dried spores of 3–5 sporangia selected from two individuals of each species were mounted on stubs with double-sided tape, coated with gold palladium (Au/Pd, c. 20 nm) and examined using a SEM Jeol JSM 25 S-11. Morphological features of the epidermis were studied following Peña and Saralegui (1982). Stipes were distally cut, about 2 mm below the basal pair of pinnae and fixed in formalin–acetic acid–alcohol solution. Cross sections were made with a razor blade. Petioles were cleared in 3% NaOCl solution for 3 min and washed in distilled water for 2–3 min. Sections were stained with toluidine blue and mounted on permanent slides. Spores were cultured on mineral agar (Dyer 1979) in Petri dishes for the study of first stages of prothallial development. The cultures were kept in a growth chamber at 20 °C and 12 h of illumination with fluorescent tubes (28mEm 22 s 21)/12 h darkness. Gametophytes were stained with chloral hydrate acetocarmine (Edwards and Miller 1972) and mounted in water for the morphological study.

Six morphological characters of *S. nigripes* commonly used to distinguish *S. nigripes* from other aspleniod ferns and two used to distinguish *A. pumilum* were scored based on the literature (e.g., Wagner 1953a, b, 1979; Khare and Shankar 1989; Tryon and Stolze 1993; Moran and Riba 1995; Pinter et al. 2002; Mickel and Smith 2004; Bercu 2005; Lin and Viane 2013) and inspection of specimens from the herbaria BM, HAC, HAJB, M and MABC as well as living plants. Mesquite 3.04 (Maddison and Maddison 2011) was used to build a morphological character matrix and display the character states at the terminals of the reconstructed Bayesian phylogenetic tree. Diagnostic characters of *S. nigripes* were coded as (1) blade shape (orbicular, non-orbicular); (2) blade dissection (simple, divided); (3) sori (scolopendrioid, non-scolopendrioid); (4) venation (netted, free); (5) midrib blade (absent, present); (6) petiole color (dark brown to blackish, green). The diagnostic characters of *A. pumilum* were coded as (7) basiscopic side of the basal pinnae in relation to the acroscopic side (markedly more developed, equal or less developed) and (8) catenate leaf hairs (absent, present). During the scoring of these characters, we encountered several challenges. For example, *S. nigripes* specimens show both scolopendrioid and non-scolopendrioid sori. Reticulate venation occurs in various forms in spleenworts ranging from regular netted veins (as in *Asplenium rhizophyllum* L.) to nettings retracted to a submarginal commissure (as in *Asplenium nidus* L.). A high degree of continuity is found in the petiole color which makes it

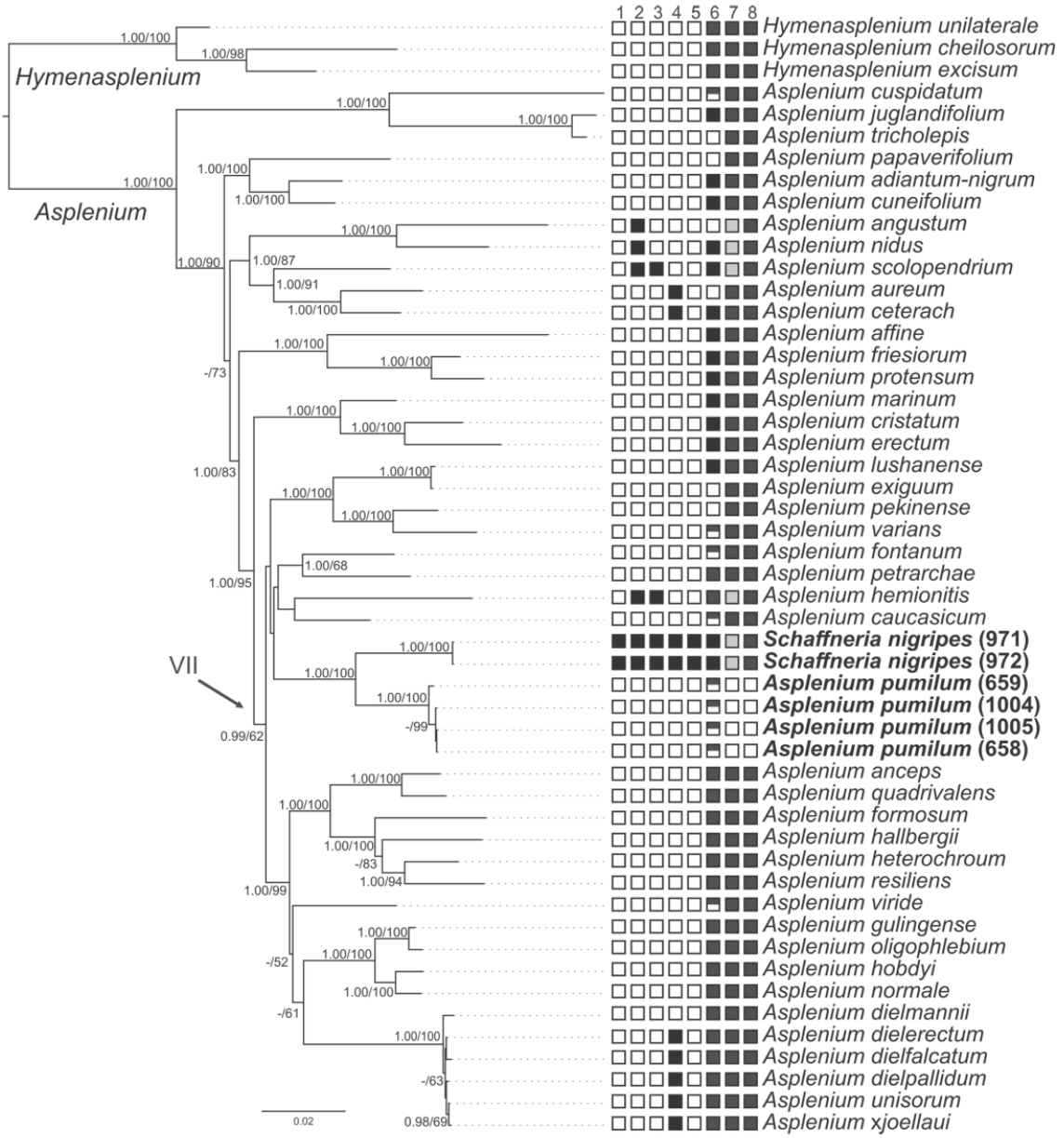


**Fig. 2** Majority rule consensus tree of trees recovered in stationary phase of Bayesian search showing the distribution of diagnostic characters of *Schaffneria nigripes* and *Asplenium pumilum*. Bayesian posterior probabilities (PP) ≥0.95 and maximum likelihood (ML) bootstrap percentage values (BS) ≥50% depicted at branches. The arrow indicates clade VII of Schneider et al. (2004). Morphological characters are displayed in the terminals of the tree as follows: *1* blade shape (*filled square* orbicular, *open square* non-orbicular); *2* blade dissection (*filled square* simple, *open square* divided); *3* sori (*filled square* scolopendrioid, *open square* non-scolopendrioid); *4* venation (*filled square* netted, *open square* free); *5* midrib blade (*filled square* absent, *open square* present); *6* petiole color (*filled square* dark brown to blackish, *open square* green); *7* basiscopic side of the basal pinnae in relation to the acroscopic side (*filled square* equally developed, *open square* conspicuously more developed); *8* catenate leaf hairs (*filled square* absent, *open square* present). *Shaded square* non-applicable character, *filled* and *open square* intermediate states

difficult to define informative discrete character states. In all these cases, we focused on the character states used in diagnostic keys.

## Results

The concatenated DNA matrix contained 2948 characters; 435 (14.8%) of which were variable and 882 (29.9%) were parsimony informative. The topologies obtained in BI and ML analyses were largely similar; hence, only the Bayesian tree is depicted, with PP (≥0.95) and BS values (≥50%) shown at branches (Fig. 2). A clade with *Asplenium cuspidatum* Lam., *A. juglandifolium* Lam. and *A. tricholepis* Rosenst. (PP 1.00, BS 100%) was found sister to the rest of the genus. The remaining species were clustered in five main clades: *A. papaverifolium* (Kunze) Viane to *A. cuneifolium* Viv. (PP 1.00, BS 100%), *A. angustatum* C.Presl to *A. ceterach* L. (PP 1.00, BS 87%), *A. affine* Sw. to *A. protensum* Schrad. (PP 1.00, BS 100%), *A. marinum* L. to *A. erectum* Bory ex Willd. (PP 1.00, BS 100%) and *A. lushanense* C.Chr. to *A.* x *joelliaui* N.Snow (PP 99, BS 62%). The latter includes a clade with the two accessions of *S. nigripes* (PP 1.00, BS 100%) and four accessions of *A. pumilum* (PP 1.00, BS 100%). The sister relationship of *S. nigripes* and *A. pumilum* achieved a PP of 1.00 and a BS of 100%. The backbone of clade VII was largely unresolved.

Most of the diagnostic character states of *S. nigripes*, e.g., simple blade, scolopendrioid sori, black petiole and netted venation, were also found in a few other in-group species. The orbicular blade and the absence of a blade midrib were scored only for *S. nigripes*. The basiscopic side of the basal pinna markedly more developed than the acroscopic side, and catenate leaf hairs were found only in *A. pumilum* (Fig. 2). *Schaffneria nigripes* and *A. pumilum* share filiform-lanceolate, mostly entire, brown-blackish rhizome scales with a dark-sclerotic center and some marginal projections, a striate, hairy epidermis, echinolophate spore ornamentation with slim microechinate folds forming small lacunae, and *Aspidium*-type gametophytes with cylindrical hairs (Figs. 3, 4).

## Discussion

Based on our phylogenetic reconstructions, we propose to treat *Schaffneria* as a synonym of *Asplenium* and adopt the name *Asplenium nigripes* (Fée) Hook. The newly recovered position of *Schaffneria* is consistent with the trend to recognize a broadly defined *Asplenium* that includes all satellite genera except *Hymenasplenium* (Murakami et al. 1999a, b; Gastony and Johnson 2001; Pinter et al. 2002; Schneider et al. 2004, 2005; Smith et al. 2006). The results add to growing evidence that a monophyletic supraspecific classification of *Asplenium* is hampered by extensive morphological homoplasy combined with an unusual range of morphological disparity and frequent reticulate evolution (e.g., Dyer et al. 2012; Chang et al. 2013; Schneider et al. 2013). Indeed, most of the diagnostic characters of *A. nigripes* have evolved several times in the history of the genus *Asplenium* (Fig. 2) yet molecular data unequivocally identify *A. nigripes* as a member of *Asplenium* clade VII of Schneider et al. (2004). This clade is a morphologically and geographically heterogeneous assemblage of species with representatives in nearly the entire range of the genus.

Species with paired sori on adjacent veins opening toward each other such as *Asplenium nigripes* were placed in the genus *Scolopendrium* in early fern classifications (e.g., Hooker and Baker 1868). Later, scolopendrioid ferns were segregated into different genera based on different venation patterns and leaf shape, yet these concepts were not supported by molecular phylogenetic evidence (Murakami et al. 1999a, b; Pinter et al. 2002; Schneider et al. 2004, 2005). The phylogenetic position of *A. nigripes* corroborates that the presence of scolopendroid sori is not suited to define genera of Aspleniaceae and that these occur in different main clades of *Asplenium* (Fig. 2). The character is probably linked with the establishment of undivided laminae although it is not found in all spleenworts with undivided leaves.

The robust sister relationship of *Asplenium nigripes* and *A. pumilum* is somewhat unexpected considering the morphological differences between both species; however, sister pairs of morphologically distinct taxa have also been found in other lineages of spleenworts (Murakami et al. 1999a, b; Gastony and Johnson 2001; Pinter et al. 2002; Schneider et al. 2004, 2005). Furthermore, characters restricted to one or a few spleenwort species may have evolved independently in spleenworts and other closely related ferns. For example, catenate leaf hairs are found in several distantly related genera such as *Deparia* Hook. and Grev. and *Acystopteris* Nakai (Sundue and Rothfels 2014). The occasional establishment of unique characters or character combinations is found in several putative satellite genera of *Asplenium* that are currently treated as synonyms, e.g., *Ceterach* or *Loxoscaphe*, and these expansions of the morphological disparity of spleenworts are arguably one of the main challenges in the taxonomy of these ferns.

In an attempt to discover features that may be consistent with the sister relationship of *Asplenium nigripes* and *A. pumilum*, we explored micromorphological characters of their sporophytes, namely petiole cross sections, rhizome scales, epidermis (Fig. 3a–f) and spores (Fig. 4), and morphology and development of young gametophytes (Fig. 3g, h). The lower and upper epidermis of both species

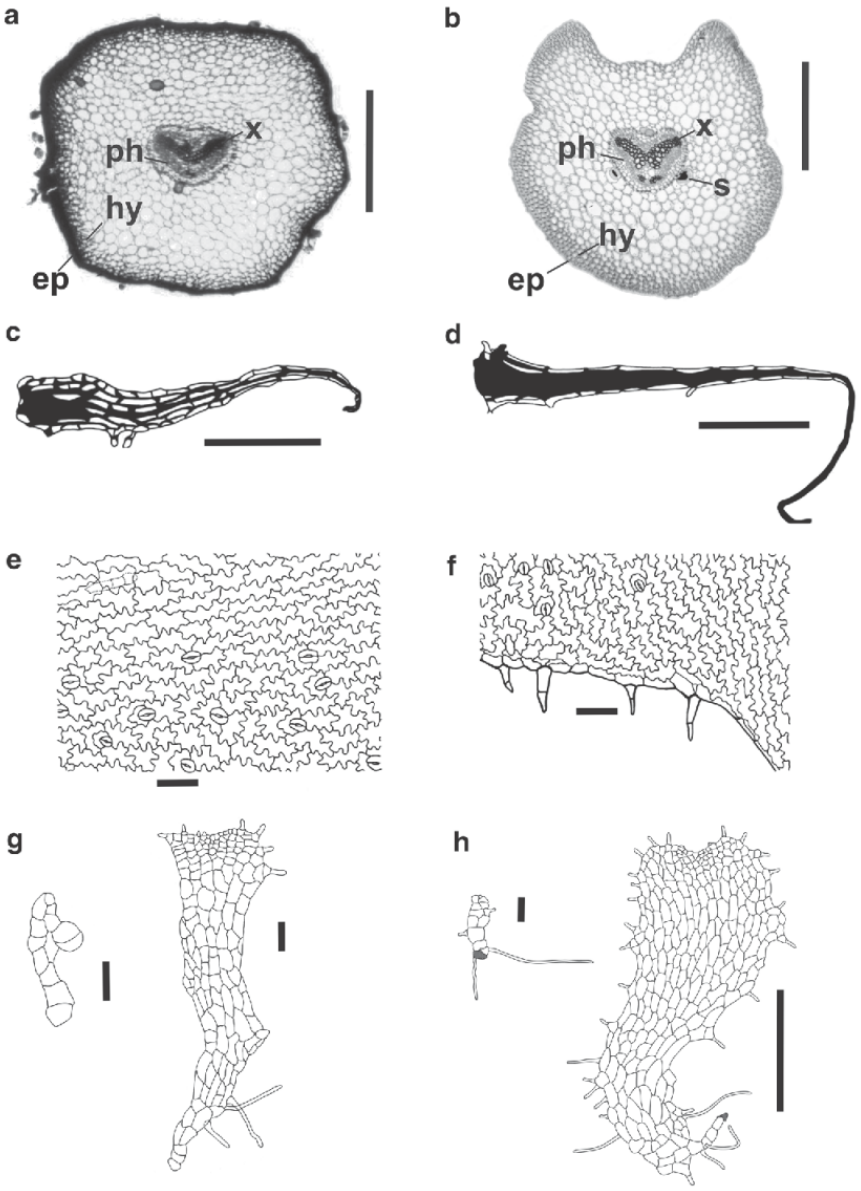


**Fig. 3** Sporophytes and young gametophytes of *Schaffneria nigripes* (**a**, **c**, **e**, **g**) and *A. pumilum* (**b**, **d**, **f**, **h**). Cross sections through middle portion of adult petiole **a** with sclerotized epidermis and hypodermis, **b** with non-sclerotized epidermis and hypodermis, and discontinuous bands of sclerenchyma. **c**, **d** Clathrate scales of stem with some cell lumen obliterated. **e**, **f** Abaxial epidermis in surface view. **g**, **h** Young gametophyte and bidimensional phase [**a**, **c**, **g** from *Sánchez* and *Regalado 42379* (HAC), **b** from *Lóriga* and *Regalado JL624* (M), **d**, **f** from *Béquer s.n.* (HAC), **e** from *Sánchez* and *Regalado 42380* (HAC), **h** from Regalado et al. *90169* (MABC); *scale bars* 0,1 mm **a**, **b**, **e**–**h**, **i**, **j**; 0.5 mm **c**, **d**, **j**; *ep* epidermis, *hy* hypodermis, *ph* phloem, *s* sclerenchyma, *x* xylem]

is striate and bears hairs (2–4 celled in *Asplenium nigripes* and 3–6 celled in *A. pumilum*). The epidermal cells of both species have undulate anticlinal walls, and the stomata are mainly basipolocytic (in addition, the anomocytic type can be found in *A. nigripes*) (Fig. 3e, f). The spore ornamentation of Aspleniaceae is very variable, usually with prominent folds forming long wings or crests, but can also be echinate or reticulate (Tryon and Lugardon 1991). Nevertheless, some closely related species have similar spore ornamentation (Nayar and Devi 1964; Viane and Van Cotthem 1977; Puttock and Quinn 1980; Pangua and Prada 1988; Prada et al. 1989; Braggins and Large 1990; Regalado and Sánchez 2002). Indeed, *A. nigripes* and *A. pumilum* share echinolophate spore ornamentation, with slim microechinate folds forming small regular lacunae (Fig. 4). However, other closely related species show highly distinct spore ornamentations despite being rather similar in their sporophyte morphology (Johns 2000; Wei and Dong 2012). Unfortunately, the current knowledge on spleenwort spore ornamentation is insufficient to allow the

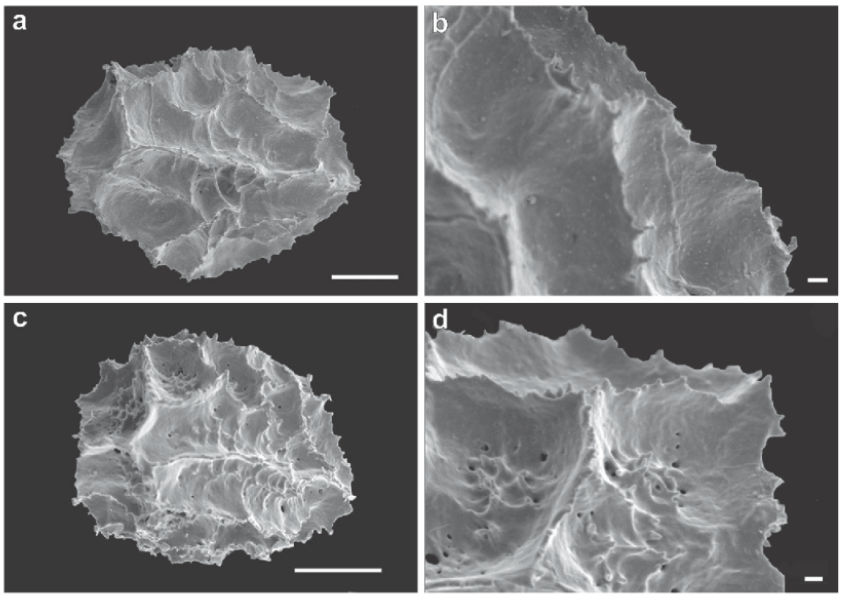


**Fig. 4** Spore ornamentation of *Schaffneria nigripes* **a**, **b**, (*Álvarez 17783* SV) and *A. pumilum* **c**–**f**, **c**–**e** (*Regalado s.n.* HAC), **f** (*Jack 3150* AJBC). *Scale bars* 1 μm **b**, **d**; 10 μm **a**, **c**, **e**–**f**

reconstruction of the evolution of these character states as required to evaluate their informativeness.

Some studies have considered gametophytic characters as a valuable source for detecting phylogenetic relationships within Aspleniaceae. Wagner (1953b) pointed out that the species of the *Diellia* group sensu Schneider et al. (2005) share gametophytes with unique glandular hairs, and Herrero et al. (2002) and Prada et al. (1995, 1996) showed that closely related taxa share the same gametophyte development type. Similarities in the gametophytic generation likewise support the phylogenetic relationship of *Asplenium nigripes* and *A. pumilum*. Both taxa exhibit the *Aspidium*-type gametophyte development described by Nayar and Kaur (1969, 1971). Besides *Aspidium* type, *Adiantum* type (Nayar and Kaur 1969, 1971) has been reported for Aspleniaceae yet the distribution of both types within *Asplenium* is poorly known. The gametophytes of *A. nigripes* and *A. pumilum* also resemble each other by having cylindrical hairs (Fig. 3g, h) appearing in the filamentous phase. Nevertheless, the bidimensional phase of *A. nigripes* initiates from a subapical cell, and the mature gametophytes are strap shaped, whereas in *A. pumilum* the bidimensional phase starts from a terminal cell, and the gametophytes are heart shaped. Currently, the taxonomic importance of *Asplenium* gametophytes cannot be evaluated conclusively given the lack of reliable reports on the gametophytic generation of the majority of *Asplenium* species; however, the available data identify *Asplenium* gametophytes as a promising source for exploring relationships within this genus.

The rhizome scales of *Asplenium nigripes* and *A. pumilum* are brown-blackish with a dark-sclerotic center, filiform-lanceolate, mostly entire, and provided with some marginal projections (Fig. 2). Similar scales have been reported for many species of the "black-stemmed" spleenwort lineages *A. monanthes* L. complex, *A. normale* D. Don complex, *A. trichomanes* L. complex, the *Diellia* Brackenridge group and *A. viride* Hudson (Wagner 1953a, b; Bercu 2007). Members of these lineages and *Asplenium nigripes* share a conspicuously sclerotized epidermis and hypodermis in the petioles. In contrast, the epidermis and hypodermis of *A. pumilum* are only slightly sclerotized. In addition, *A. pumilum* has discrete bands of sclerenchyma surrounding the bundles. Such bands are missing in *A. nigripes* (Fig. 3a, b). In summary, it can be stated that there is only a limited amount of morphological support for the sister relationship of *A. nigripes* and *A. pumilum* and that molecular data are of prime importance for the reconstruction of relationships within the species-rich genus *Asplenium*. However, the lack of carefully assembled micromorphological evidence hampered our ability to explore the phylogenetic informativeness and biological importance of these characters.

## Perspectives

Extension of the taxon sampling led to further improvements of the classification of asplenioid ferns and provided convincing evidence that *Schaffneria*, despite its unusual morphology, belongs to the genus *Asplenium*. However, an extended sampling is desirable to arrive at a more reliable

hypothesis on the relationship of *Asplenium nigripes* and *A. pumilum* since we were not able to include several putative allies in our molecular study. Tardieu-Blot (1957) and Copeland (1947) suggested a close relationship of the neotropical *A. nigripes* and the Chinese *A. delavayi* based on their small size, black petioles, entire, rounded blades, reticulate venation and scolopendrioid sori. Despite its shared character states, Mickel (1976) transferred *A. delavayi* to a monospecific genus *Sinephropteris* Mickel, arguing that, besides the geographic disjunction, the latter has less regular netted venation and most of its sori are scolopendrioid, whereas in *A. nigripes* simple sori are more common. We do not rule out that *A. nigripes* is closely related to *A. delavayi* yet a more definite statement should be based on molecular data. *Asplenium minimum* and *A. arcanum* are species with a basal pair of pinnae whose basiscopic side is more developed than the acroscopic side and thus could also belong to the *A. nigripes–A. pumilum* lineage. *Antigramma purdieana* also has this type of pinnae. As defined by Sylvestre and Windisch (2002), *Antigramma* comprises four species from tropical America and one from Africa and has netted venation, scolopendrioid sori, and usually entire, simple laminas. This combination of character states resembles that of *Asplenium nigripes* and *A. delavayi*. On the other hand, *Antigramma purdieana*, the only *Antigramma* with a pinnate lamina, has the basiscopic side of the basal pinnae more developed than the acroscopic side. The phylogenetic relationships of this group are currently being studied independently (Sylvestre pers. comm.; see also http://www.botanica.org.br/trabalhos-cientificos/64CNBot/resumo-ins20467-id4651.pdf).

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**Compliance with ethical standards**

**Conflict of interest** The authors declare they have no conflict of interest.

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Chapter 4

# THE FIRST FOSSIL OF A BOLBITIDOID FERN BELONGS TO THE EARLY-DIVERGENT LINEAGES OF *ELAPHOGLOSSUM* (DRYOPTERIDACEAE)

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# The first fossil of a bolbitidoid fern belongs to the early-divergent lineages of *Elaphoglossum* (Dryopteridaceae)[1]

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- *Premise of the study:* Closing gaps in the fossil record and elucidating phylogenetic relationships of mostly incomplete fossils are major challenges in the reconstruction of the diversification of fern lineages through time. The cosmopolitan family Dryopteridaceae represents one of the most species-rich families of leptosporangiate ferns, yet its fossil record is sparse and poorly understood. Here, we describe a fern inclusion in Miocene Dominican amber and investigate its relationships to extant Dryopteridaceae.
- *Methods:* The morphology of the fossil was compared with descriptions of extant ferns, resulting in it being tentatively assigned to the bolbitidoid fern genus *Elaphoglossum*. This assignment was confirmed by reconstructing the evolution of the morphological characters preserved in the inclusion on a molecular phylogeny of 158 extant bolbitidoid ferns. To assess the morphology-based assignment of the fossil to *Elaphoglossum*, we examined DNA-calibrated divergence time estimates against the age of the amber deposits from which it came.
- *Key results:* The fossil belongs to *Elaphoglossum* and is the first of a bolbitidoid fern. Its assignment to a particular section of *Elaphoglossum* could not be determined; however, sects. *Lepidoglossa*, *Polytrichia*, and *Setosa* can be discounted because the fossil lacks subulate scales or scales with acicular marginal hairs. Thus, the fossil might belong to either sects. *Amygdalifolia*, *Wrightiana*, *Elaphoglossum*, or *Squamipedia* or to an extinct lineage.
- *Conclusions:* The discovery of a Miocene *Elaphoglossum* fossil provides remarkable support to current molecular clock-based estimates of the diversification of these ferns.

**Key words:** ancestral state reconstruction; bolbitidoid fern; Dominican amber; *Elaphoglossum*; eupolypods I; fossil fern; Miocene; Polypodiales.

Molecular clock-based studies have been increasingly employed to explore macroevolution and macroecology of ferns including aspects of their diversification in the past 120 Myr (e.g., Schneider et al., 2004, 2010; Schuettpelz and Pryer, 2009; Sessa et al., 2012; Liu et al., 2014). These studies challenged the fossil record as the main source of information about fern diversification by using molecular-based estimates of diversification times of extant lineages using DNA sequences. Although most of these studies incorporate one or more fossils as time constraints, little attention has been given to the consistency of the obtained hypotheses and the known fossil record. Recent reviews of the fern fossil record document a limited availability of reliably determined fossils especially for derived ferns (Collinson, 2001; Skog, 2001). In fact, some authors consider the fern fossil record inadequate for comprehensive time calibrations of molecular topologies (Lehtonen et al., 2012). This view, however, has not been backed up by exploring the information from published fossils, which have not yet been used for calibration purposes, or by newly discovered fossils using an integrative approach as suggested in Schneider et al. (2009).

The Dryopteridaceae provide an outstanding example to explore the impact of newly discovered fossils on our understanding of fern diversification as outlined in molecular clock-based studies (Schneider et al., 2004; Schuettpelz and Pryer, 2009; Sessa et al., 2012; Liu et al., 2014). With about 1700 species in some 36 genera, the family is one of the most species-rich among derived ferns (Smith et al., 2006; Liu et al., 2007; Moran et al., 2010a, b; Christenhusz et al., 2011; McHenry et al., 2013). Phylogenetic studies reported two core lineages of Dryopteridaceae (Schuettpelz and Pryer, 2007; Lehtonen, 2011; Liu et al., 2014). The first lineage corresponds to the Dryopteridoideae and contains genera such as *Arachniodes*, *Ctenitis*, *Dryopteris*, and *Polystichum*. The second lineage corresponds to the subfamily Elaphoglossoideae (Christenhusz et al., 2011) and contains genera such as *Polybotrya*, *Megalastrum*, and *Stigmatopteris.* It also includes the well-supported, species-rich, and almost entirely tropical clade known as the bolbitidoid ferns (Schuettpelz and Pryer, 2007; Moran et al., 2010a; Liu et al., 2014). This clade is characterized morphologically by the synapomorphies of dorsiventral rhizomes with an elongated

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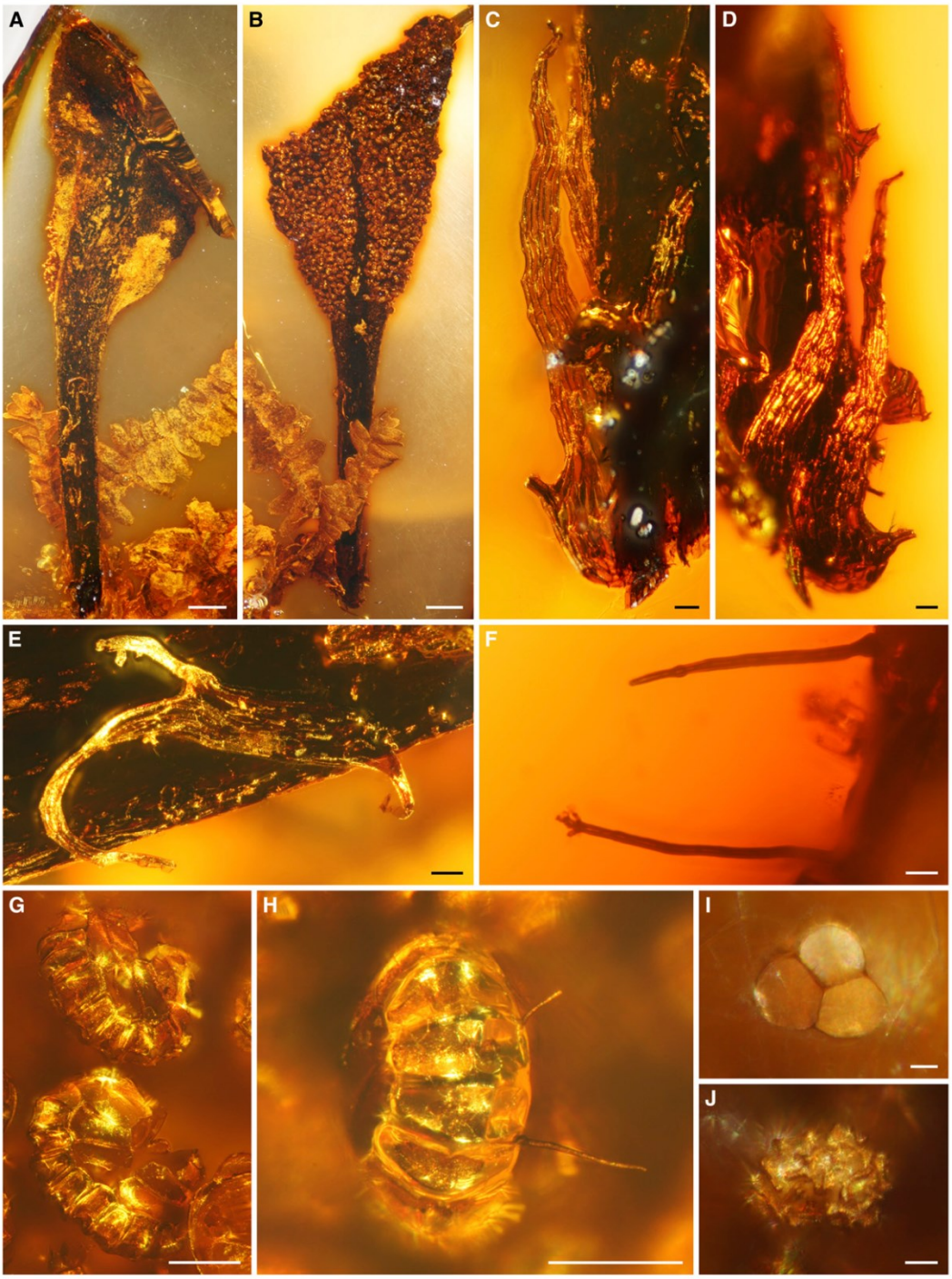



Fig. 1. Holotype of *Elaphoglossum miocenicum* sp. nov. in Miocene Dominican amber (USNM 414283). (A) Upper surface of the leaf. (B) Lower surface of the leaf with sporangia covering the blade. (C, D) Basal petiolar scales. (E) Middle petiolar scale. (F) Syninclusion of fungal conidiophores at the margin of the leaf. (G) Sporangia in oblique-lateral view showing the vertical annulus and the transversal stomium. (H) Sporangium in dorsal view showing fungal conidiophores emerging between the annulus cells. (I) Cross section of the 3-seriate sporangium stalk. (J) Spore with continuous broadly folded perine. Scale bars = 1 mm (A, B), 100 μm (C–E, G, H), and 10 μm (F, I, J).

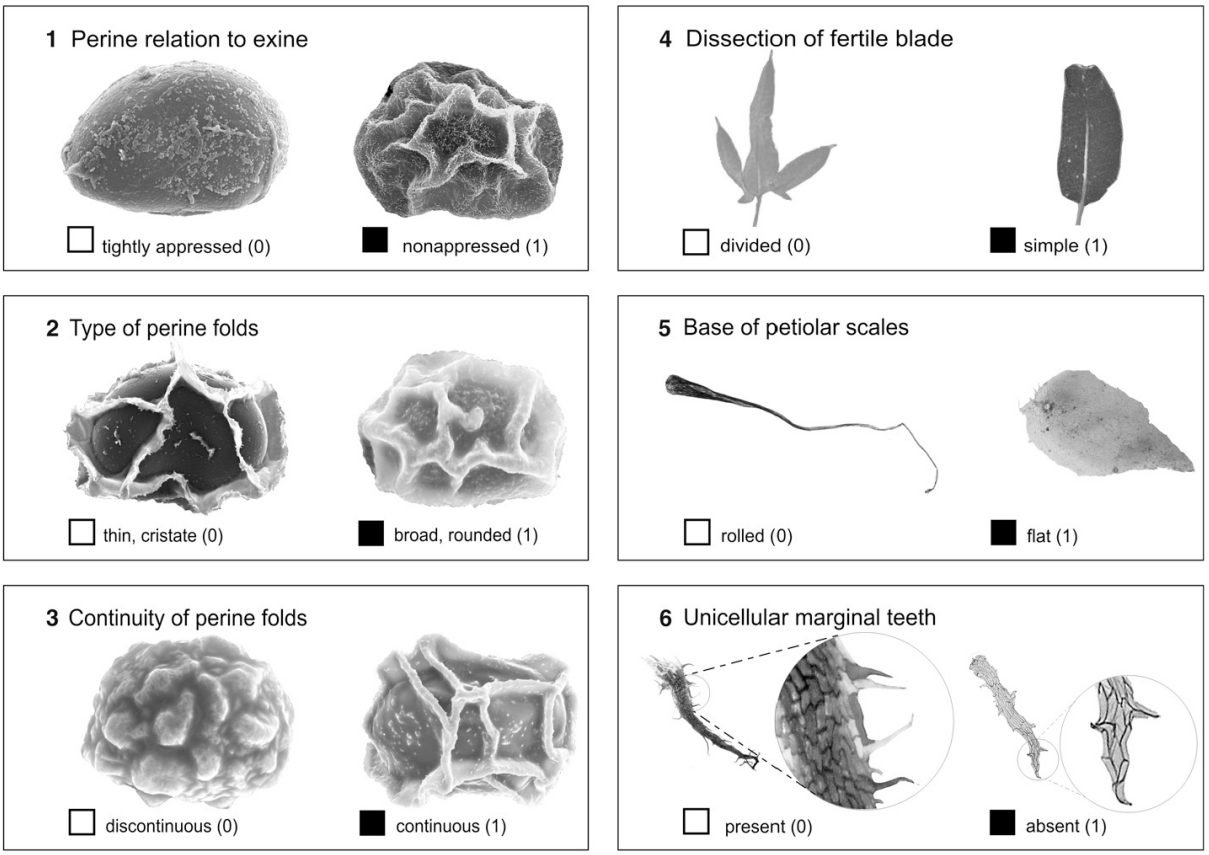



Fig. 2. Morphological characters observed in the amber fossil of *Elaphoglossum miocenicum* sp. nov. Black squares represent the character states present in the fossil as used in the ancestral character state reconstruction (see Fig. 4).

(in transverse section) ventral meristele, roots borne only from this ventral meristele, lack of hairs on the leaves, sterile–fertile leaf dimorphy, and acrostichoid sporangial arrangement, i.e., the sporangia are distributed over the lower surface of the blade (Moran et al., 2010a). Within the bolbitidoid ferns, the largest genus is *Elaphoglossum,* a largely epiphytic, pantropical genus. The other bolbitidoid genera are typically either terrestrial (*Bolbitis*) or climbing from the soil up tree trunks (*Arthrobotrya*, *Lomagramma*, *Mickelia*, and *Teratophyllum*) (Moran et al., 2010a).

So far, few fossils have been attributed to the Dryopteridaceae, and no fossils of bolbitidoid ferns have been documented (Collinson, 2001). Some fossils previously assigned to the family (see discussion of these in Collinson, 2001) are unlikely to belong to the Dryopteridaceae as defined by Smith et al. (2006). This is largely because earlier authors used the wider definition of Dryopteridaceae provided by Kramer (1990), a definition that includes genera now considered to belong to families in eupolypods I and II, such as Athyriacae, Onocleaceae, Tectariaceae, Thelypteridaceae, and Woodsiaceae (Smith et al., 2006; Schuettpelz and Pryer, 2007; Lehtonen, 2011). Late Miocene *Dryopteris* fossils (Sessa et al., 2012) and Eocene fossils assigned to the extant genus *Rumohra* (Collinson, 2001) appear to be the most reliable fossils of the Dryopteridaceae. The family placement of these fossils, however, has not been determined by reconstructing the evolution of the fossils' characters on a phylogenetic tree. This approach is now widely considered crucial to achieve reliable assignments of fossil taxa and to overcome shortcomings of the previously used similarity assignments (Parham et al., 2012).

In the present study, we describe an inclusion of a fertile fern in amber from the Dominican Republic. The amber has been dated as early Miocene, 20 to 15 Myr old (Iturralde-Vinent and MacPhee, 1996), and was exuded by resin-bearing species of *Hymenaea* in the Fabaceae (Poinar, 1991; Langenheim, 1995). We identify the fossil as *Elaphoglossum*, a member of the bolbitidoid lineage of the Dryopteridaceae. We use a molecular phylogeny of bolbitidoid ferns to reconstruct the ancestral states of characters preserved in the fossil. Finally, we examine whether the fossil's age is consistent with estimated divergence times of bolbitidoid ferns based on calibrations from other fossils used previously in other large-scale phylogenetic analyses of ferns.

## MATERIALS AND METHODS

The fossil is from the Dominican Republic and preserved in the amber collection of the U. S. National Museum of Natural History at the Smithsonian

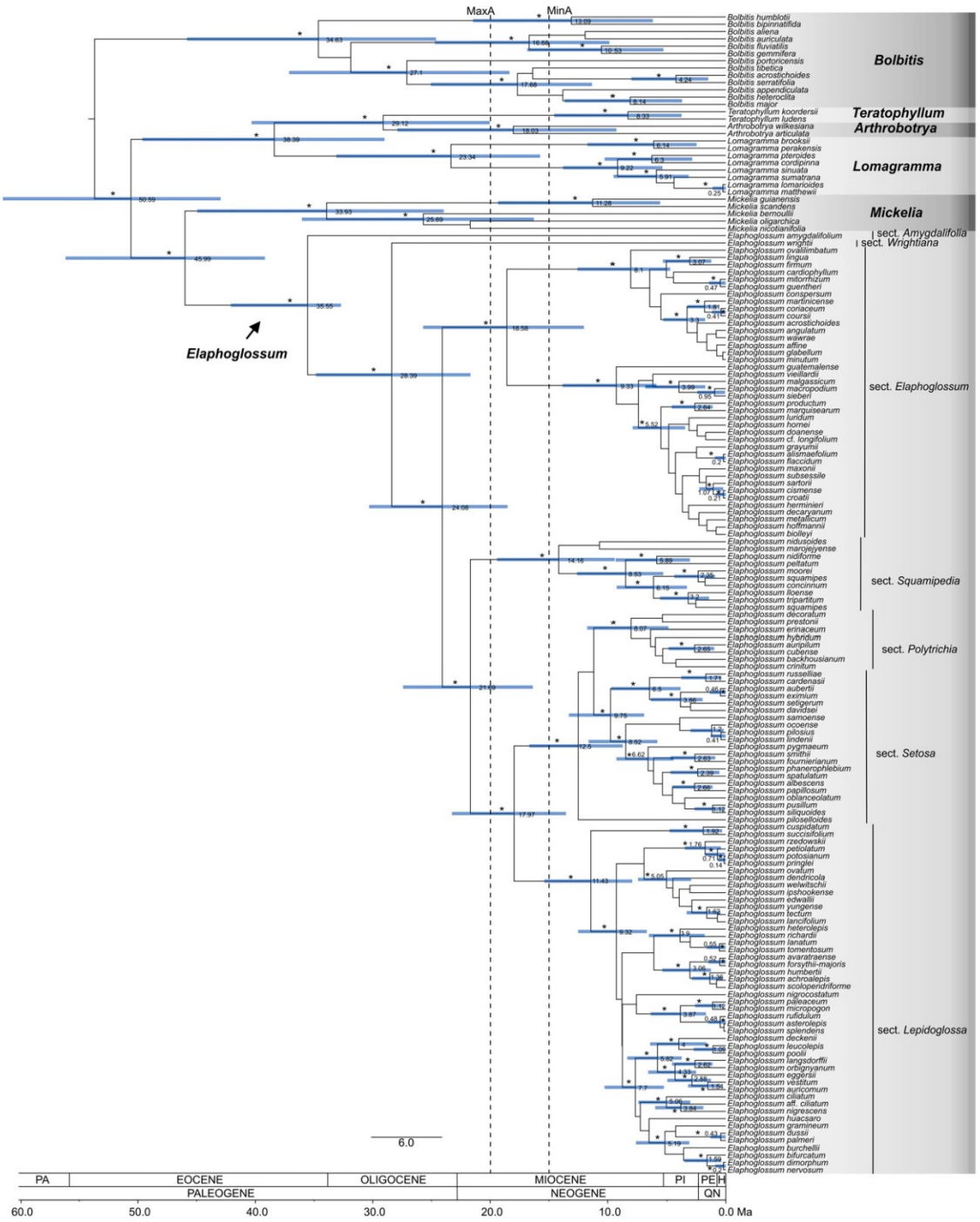
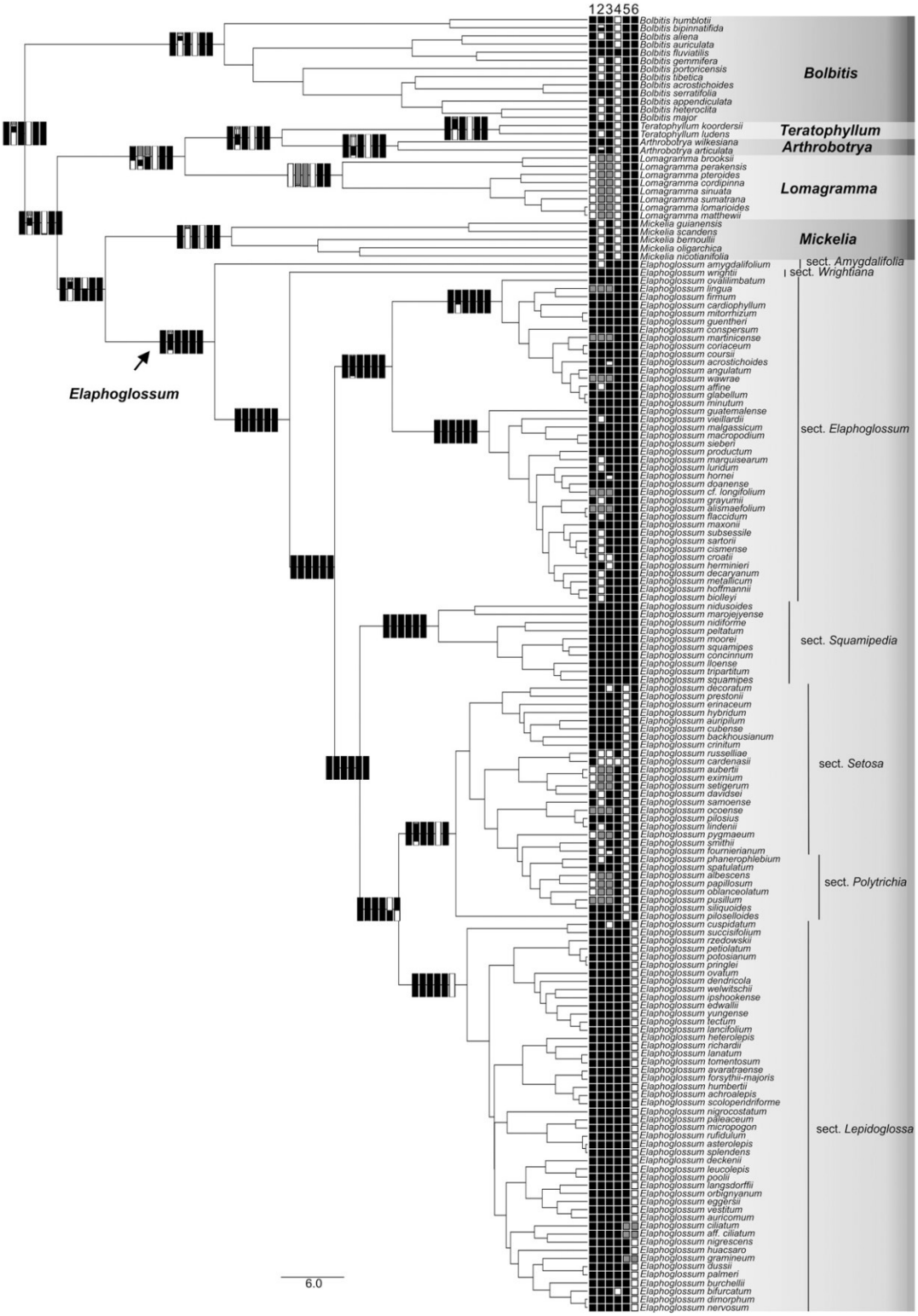



Fig. 3. Time-calibrated phylogeny of bolbitidoid ferns. Nodes with a posterior probability ≥0.95 are marked by asterisks. The mean age (million years from present) of these nodes is indicated; bars represent the 95% highest posterior density (HPD) credibility intervals. Dashed vertical lines represent the age range estimated for Dominican amber. The geologic timescale follows Gradstein et al. (2012): PA, Paleocene; PI, Pliocene; PE, Pleistocene; H, Holocene; QN, Quaternary. Mean ages and 95% HPD credibility intervals of every node are provided in Appendix S2.


1 2 3 4 5 6
Elaphoglossum
Bolbitis humblotii
Bolbitis bipinnatifida
Bolbitis alsena
Bolbitis auriculata
Bolbitis fluviatilis
Bolbitis gemmifera
Bolbitis portoricensis
Bolbitis tibetica
Bolbitis acrostichoides
Bolbitis serratifolia
Bolbitis appendiculata
Bolbitis heteroclita
Bolbitis major
Bolbitis
Teratophyllum koordersii
Teratophyllum ludens
Teratophyllum
Arthrobotrya wilkesiana
Arthrobotrya articulata
Arthrobotrya
Lomagramma brooksii
Lomagramma perakensis
Lomagramma pteroides
Lomagramma cordipinna
Lomagramma sinuata
Lomagramma sumatrana
Lomagramma lomarioides
Lomagramma matthewii
Lomagramma
Mickelia guianensis
Mickelia scandens
Mickelia bernoullii
Mickelia oligarchica
Mickelia nicotianifolia
Mickelia
Elaphoglossum amygdalifolium
sect. Amygdalifolia
Elaphoglossum wrightii
Elaphoglossum ovalilimbatum
sect. Wrightiana
Elaphoglossum lingua
Elaphoglossum firmum
Elaphoglossum cardiophyllum
Elaphoglossum mitorrhizum
Elaphoglossum guentheri
Elaphoglossum conspersum
Elaphoglossum martinicense
Elaphoglossum coriaceum
Elaphoglossum coursii
Elaphoglossum acrostichoides
Elaphoglossum angulatum
Elaphoglossum wawrae
Elaphoglossum affine
Elaphoglossum glabellum
Elaphoglossum minutum
Elaphoglossum guatemalense
Elaphoglossum vieillardii
Elaphoglossum malgassicum
Elaphoglossum macropodium
Elaphoglossum sieberi
Elaphoglossum productum
Elaphoglossum marquisearum
Elaphoglossum luridum
Elaphoglossum hornei
Elaphoglossum doanense
Elaphoglossum cf. longifolium
Elaphoglossum grayumii
Elaphoglossum alismaefolium
Elaphoglossum flaccidum
Elaphoglossum maxonii
Elaphoglossum subsessile
Elaphoglossum sartorii
Elaphoglossum cismense
Elaphoglossum croatii
Elaphoglossum herminieri
Elaphoglossum decaryanum
Elaphoglossum metallicum
Elaphoglossum hoffmannii
Elaphoglossum bioleyi
sect. Elaphoglossum
Elaphoglossum nidusoides
Elaphoglossum marojejyense
Elaphoglossum nidiforme
Elaphoglossum peltatum
Elaphoglossum moorei
Elaphoglossum squamipes
Elaphoglossum concinnum
Elaphoglossum lloense
Elaphoglossum tripartitum
Elaphoglossum squamipes
sect. Squamipedia
Elaphoglossum decoratum
Elaphoglossum prestonii
Elaphoglossum ernaceum
Elaphoglossum hybridum
Elaphoglossum auripilum
Elaphoglossum cubense
Elaphoglossum backhousianum
Elaphoglossum crinitum
Elaphoglossum russelliae
Elaphoglossum cardenasii
Elaphoglossum aubertii
Elaphoglossum eximium
Elaphoglossum setigerum
Elaphoglossum davidsei
Elaphoglossum samoense
Elaphoglossum oceense
Elaphoglossum pilosius
Elaphoglossum lindenii
Elaphoglossum pygmaeum
Elaphoglossum smithii
Elaphoglossum fournierianum
sect. Setosa
Elaphoglossum phanerophlebium
Elaphoglossum spatulatum
Elaphoglossum albescens
Elaphoglossum papillosum
Elaphoglossum oblanceolatum
Elaphoglossum pusillum
Elaphoglossum siliquoides
Elaphoglossum piloselloides
sect. Polytrichia
Elaphoglossum cuspidatum
Elaphoglossum succisifolium
Elaphoglossum czedowskii
Elaphoglossum petiolatum
Elaphoglossum potosianum
Elaphoglossum pringlei
Elaphoglossum ovatum
Elaphoglossum dendricola
Elaphoglossum wellwitschii
Elaphoglossum ipshookense
Elaphoglossum edwallii
Elaphoglossum yungense
Elaphoglossum tectum
Elaphoglossum lancifolium
Elaphoglossum heterolepis
Elaphoglossum richardii
Elaphoglossum lanatum
Elaphoglossum tomentosum
Elaphoglossum avaratraense
Elaphoglossum forsythii-majoris
Elaphoglossum humbertii
Elaphoglossum achroalepis
Elaphoglossum scoloperidriforme
Elaphoglossum nigrocostatum
Elaphoglossum paleaceum
Elaphoglossum micropogon
Elaphoglossum rufidulum
Elaphoglossum asterolepis
Elaphoglossum splendens
Elaphoglossum deckenii
Elaphoglossum leucolepis
Elaphoglossum poolii
Elaphoglossum langsdorffii
Elaphoglossum orbignyanum
Elaphoglossum eggersii
Elaphoglossum vestitum
Elaphoglossum auricomum
Elaphoglossum ciliatum
Elaphoglossum aff. ciliatum
Elaphoglossum nigrescens
Elaphoglossum huacsaro
Elaphoglossum gramineum
Elaphoglossum flussii
Elaphoglossum palmeri
Elaphoglossum burchellii
Elaphoglossum bifurcatum
Elaphoglossum dimorphum
Elaphoglossum nervosum
sect. Lepidoglossa
6.0

Institution (coll. no. USNM 414283). The amber inclusion was investigated using a Zeiss Stemi 2000 dissection microscope and a Zeiss AxioScope A1 compound microscope, each equipped with a Canon 60D digital camera. In most instances, incident and transmitted light were used simultaneously. The images of Fig. 1 are digitally stacked photomicrographic composites of up to 40 individual focal planes obtained using the software package HeliconFocus 5.0 (HeliconSoft, http://www.heliconsoft.com) for a better illustration of the three-dimensional inclusions.

The fossil was compared with published morphological descriptions of extant ferns (e.g., Rouhan et al., 2004, 2008; Moran et al., 2007; Vasco et al., 2009a, 2013; Vasco, 2011; Lóriga et al., 2014) and putatively assigned to *Elaphoglossum*. This assignment was investigated with two independent approaches. First, divergence times of bolbitidoid ferns were estimated without incorporating the fossil as a time constraint. Second, the evolution of the fossil's morphological characters was reconstructed on a phylogeny of the bolbitidoid ferns. These approaches also tested the morphology-based assignment of the fossil to certain clades (sections) within *Elaphoglossum*, and the consistency of molecular clock-based time estimates with the age of the amber as determined by geologists (Iturralde-Vinent and MacPhee, 1996).

The taxonomic samping of bolbitidoid ferns was based on those species included in published phylogenies (Rouhan et al., 2004, 2007; Skog et al., 2004; Vasco et al., 2009b, in press; Moran et al., 2010a; Lóriga et al., 2014). The genera included were *Arthrobotrya* (2 species), *Bolbitis* (13 species), *Lomagramma* (8 species), *Mickelia* (5 species), *Elaphoglossum* (127 species), and *Teratopyllum* (2 species). The sampling of *Elaphoglossum* included all sections recognized by Rouhan et al. (2004) (i.e., sects. *Amygdalifolia*, *Elaphoglossum*, *Lepidoglossa*, *Polytrichia*, *Setosa*, *Squamipedia*), with the addition of sect. *Wrightiana* recognized by Lóriga et al. (2014). Noncoding intergenic plastid DNA sequences of the *rps4-trnS* and *trnL-trnF* regions of all investigated 158 bolbitidoid species were downloaded from GenBank (Appendix 1) and aligned with the program Muscle 3.6 (Edgar, 2004) under default parameters implemented in the program MEGA 5.1 (Tamura et al., 2011). The resulting alignment was manually edited in BioEdit 7.0.5.3 (Hall, 1999), and ambiguous positions were excluded. The final alignment with 712 bp (*rps4-trnS*, 371 bp; *trnL-trnF*, 341 bp) is available at TreeBase (http://treebase.org, study 16183).

Divergence time estimates were performed with the BEAST v1.8.0 package (Drummond et al., 2006; Drummond and Rambaut, 2007) by assigning node-age information from Schuettpelz and Pryer (2009) for the split of *Bolbitis* and the rest of the bolbitidoid ferns at 46.3 Ma, and the split of *Elaphoglossum* and *Mickelia* at 32.7 Ma. Because the results of Liu et al. (2014) indicated somewhat older ages for this split than those estimated by Schuettpelz and Pryer (2009), a minimum-age approach was adopted by modeling the age constraint for the root as a truncated normal prior distribution with a mean of 46.3 Ma, a standard deviation of 10, and a truncation from 46.3–1000 Ma (Knoop and Müller, 2009). The age constraint for *Elaphoglossum* had a truncated normal prior distribution with a mean of 32.7 Ma, a standard deviation of 10 and a truncation from 32.7–1000. The TVM+G model of evolution was chosen using the Bayesian information criterion of the program jModeltest v2.1.4 (Darriba et al., 2012), with PhyML implemented (Guindon & Gascuel, 2003). The analysis setup was done with the program BEAUTi 1.8.0, employing the above constraints, a lognormal relaxed clock and a birth–death model for incomplete sampling (Stadler, 2009). The analysis was run for 200 million generations and a sampling of every 20000th tree. After a burnin of 25%, a maximum credibility tree was assembled with the program TreeAnnotator v1.8.0. The performance of the analysis was examined with the program TRACER 1.5 (Rambaut and Drummond, 2007). ESS values > 200 were regarded as good support. FigTree (http://tree/bio.ed.ac.uk/software/figtree) was used to depict the maximum credibility tree.

The ancestral state of six discrete morphological characters preserved in the fossil (Fig. 2) was reconstructed to identify the relationships of the fossil. Three of these characters related to the ornamentation of the perine and were coded following Moran et al. (2007, 2010c). Information on the characters of most species is available online at http://www.plantsystematics.org/index.html or in online databases of the herbaria B, NY, and MNHN. The morphological character matrix is provided in Appendix S1 (see Supplemental Data with the online version of this article). Ancestral character state reconstructions (ASR) were carried out using the ace function of the ape package in R (Paradis et al., 2004). The maximum likelihood method for ASR (Pagel, 1994, 1999) was used over the time-calibrated consensus tree obtained from the Bayesian divergence time analysis. We implemented a model with equal rates of transition between states. Intermediate character states were treated as a new state. Missing data and not applicable characters were coded as lacking.

## RESULTS

Of the 712 character sites in the concatenated DNA matrix, 140 were constant and 450 parsimony informative. All six bolbitidoid genera were resolved monophyletic (Figs. 3, 4), with *Mickelia* in a sister relationship to *Elaphoglossum*. Two monospecific sections of *Elaphoglossum,* sects. *Amygdalifolia* and *Wrightiana*, were placed in serial sister relationships to the rest of the genus. Section *Elaphoglossum* was placed sister to a clade with sects. *Squamipedia*, *Setosa*, *Polytrichia*, and *Lepidoglossa*. Section *Squamipedia* was recovered as sister to a clade comprising sect. *Lepidoglossa* and the sister sects. *Setosa* and *Polytrichia*. Divergence time estimates (Fig. 3) indicated an Eocene origin of *Elaphoglossum*, an Oligocene age of its core group (all sections with the exception of the monospecific sect. *Amygdalifolia*), and the presence of all sectional lineages in the middle Miocene. Node mean ages and 95% highest posterior density (HPD) credibility intervals are provided in Appendix S2 (see online Supplemental Data).

Reconstruction of ancestral character states (Fig. 4) suggested that the most recent common ancestor of all bolbitidoid ferns had divided fertile blades (PL = 1.00) (proportional likelihood values [PL] are provided in Appendix S2, node identification numbers in online Appendix S3). All bolbitidoid genera retained this ancestral character state except *Elaphoglossum*. The divided blades of *E. bifurcatum* and *E. cardenasii* were secondarily derived. It is ambiguous whether the most recent common ancestor of *Elaphoglossum* and *Mickelia* had either entire or divided fertile blades (PL = 0.53 vs. PL = 0.47). The perine folds of the most recent common ancestor of *Mickelia* were reconstructed as thin cristate with a probability of PL = 0.85. It is equivocal whether the perine folds of the most recent common ancestor of *Elaphoglossum* and *Mickelia* were thin and cristate or broad and rounded (PL = 0.51 vs. PL = 0.31).

The ancestors of several early-diverging lineages of *Elaphoglossum* most likely exhibited the same set of characters observed in the fossil; namely, perine nonappressed and with continuous, broad, rounded folds (characters of all Eupolypod ferns), fertile blades simple (characters of nearly all species of *Elaphoglossum*), and petiolar scales not rolled at the base and lacking unicellular marginal teeth (Fig. 1). Within *Elaphoglosssum*, the sections that exhibit these characters include sects. *Amygdalifo-*

←

Fig. 4. Time-calibrated phylogeny of bolbitidoid ferns presented in Fig. 3 showing the ancestral state reconstruction of morphological characters observed in *Elaphoglossum miocenicum* sp. nov. Morphological characters are displayed in the terminals of the tree in the same order as described in Fig. 2 (squares with two colors indicate intermediate states; gray squares indicate not applicable characters or lack of data). Rectangles at main internal nodes of the tree represent the proportional likelihoods of character presence for characters 1–6 as inferred by the ancestral state reconstructions. Morphological states scored for every species are provided in Appendix S1, and proportional likelihood values of character presence in every node is provided in Appendix S2.

*lia*, *Lepidoglossa*, *Squamipedia*, and *Wrightiana*. The most recent common ancestor of sects. *Setosa* and *Polytrichia* had basally enrolled scales (PL = 1.00), whereas the scales were flat in the fossil and the rest of bolbitidoids. The most recent common ancestor of *Elaphoglossum* sect. *Lepidoglossa* probably had scales with acicular marginal appendages consisting of a single cell (PL = 1.00), whereas in the fossil and the rest of bolbitidoids marginal teeth were formed by the upturned ends of two adjacent cells.

## DISCUSSION AND TAXONOMIC TREATMENT

The fossil has simple and entire leaves and an acrostichoid arrangement of sporangia (Fig. 1), suggesting it is an *Elaphoglossum*, a bolbitidoid fern genus in the Dryopteridaceae. The most recent common ancestor of *Elaphoglossum* and *Mickelia* was reconstructed to have had either entire or divided fertile blades (PL = 0.53 vs. PL = 0.47), but the perine folds of the ancestor of *Mickelia* were reconstructed as thin and cristate (PL = 0.85). The fossil had broadly rounded perine folds (Fig. 1J), which are frequent in *Elaphoglossum* (Moran et al., 2007). *Elaphoglossum* is the only fern genus characterized by the combination of simple and entire leaves, the presence of phyllopodia, an acrostichoid arrangement of sporangia, and free veins. Unfortunately, petiole bases, which would allow determination of the presence or absence of phyllopodia, were not present in the fossil, and venation was not visible on the fertile lamina preserved in the inclusion. Simple and entire leaves with acrostichoid sori occur also in several genera belonging to distinct lineages such as Dipteridaceae, Polypodiaceae, and Tectariaceae. The Dipteridaceae can be discounted because the fossil is not a simple and entire-leaved *Cheiropleuria*. That genus has 4-seriate sporangial stalks, and slightly oblique, complete annuli, and tetrahedral, trilete spores (Smith et al., 2006). It also lacks foliar scales. In contrast, the fossil has three-seriate sporangium stalks, vertical annuli interrupted at the stalks (i.e., not bypassing the stalk and completely encircling the sporangial capsules), and bean-shaped monolete spores (Fig. 1G–J). In the Polypodiaceae, some species of *Leptochilus* (including *Colysis*) have simple blades with an acrostichoid arrangement of sporangia, but unlike the fossil (Fig. 1A–E), these ferns lack scales on the petioles of fertile leaves (R. C. Moran and H. Schneider, personal observations). Moreover, *Leptochilus*, like most Polypodiaceae, has a thin perine tightly appressed to the exine (Tryon and Lugardon, 1991), not a broadly folded perine as found in the fossil. Finally, it seems unlikely that the fossil belongs to the Tectariaceae. Laminar scales, such as are common on the fossil, are rare or absent in that family, as are also sporangia with an acrostichoid arrangement (R. C. Moran, personal observations).

Given the evidence, the fossil most likely belongs to *Elaphoglossum*. It could not be assigned to a section within *Elaphoglossum* because important characters were not preserved or visible, such as rhizome habit and presence/absence of hydathodes (Rouhan et al., 2004; Moran et al., 2010a; Lóriga et al., 2014). Assuming consistency of sectional character states through time, however, the fossil can be excluded from three sections. *Elaphoglossum* sect. *Lepidoglossa* can be eliminated because the scales of the fossil lack unicellular marginal teeth, which occur in all extant species of this section (Vasco et al., 2009b), and were estimated to be present in the most recent ancestor of the section with a probability of PL = 1.00 (Fig. 4). Similarly, sects. *Polytrichia* or *Setosa* can be excluded because they have subulate (longitudinally enrolled) scales on the leaves (Mickel and Atehortúa, 1980). Some species in these two sections bear flat scales on parts of the lamina, especially the margins. These flat scales, however, are always accompanied by subulate scales elsewhere on the same leaf, in contrast to the consistently flat scales of the fossil. Given the elimination of these three sections, the fossil belongs either to one of the remaining sections of *Elaphoglossum* (i.e., sects. *Amygdalifolia*, *Elaphoglossum*, *Squamipedia*, or *Wrightiana*), or to an extinct lineage not part of any extant section.

Previous divergence time analyses (Schuettpelz and Pryer, 2009; Liu et al., 2014) provided evidence for a Paleogene origin of *Elaphoglossum*. This is consistent with our newly obtained divergence-time analysis (Fig. 3) and the interpretation of the fossil as a member of *Elaphoglossum*. The morphology of the fossil does not exclude the possibility that it belongs to an extant species of *Elaphoglossum*. Miocene amber inclusions of bryophytes from the Dominican Republic have frequently been assigned to extant species (Gradstein, 1993; Frahm and Newton, 2005), although uncertainty remains since these inclusions show only a subset of the features visible in living plant material (Heinrichs et al., 2013). To assess whether the fossil belongs to an extant species, we estimated the ages of the sectional crown groups of *Elaphoglossum*. Our divergence-time analysis allowed slightly older ages than those presented in other studies (Schuettpelz and Pryer, 2009); however, despite this conservative approach, we found the extant species of the sectional crown groups to be younger than the fossil. Hence, the fossil is considered to represent a stem lineage element of one of the above sections, or an extinct member of the early-diverging sects. *Amygdalifolia* and *Wrightiana*. These two sections are monospecific and may or may not represent survivors of the early divergence of the genus (Lóriga et al., 2014). Finally, the possibility cannot be ruled out that the fossil belongs to an extinct lineage of *Elaphoglossum* that is not part of an extant section.

Given the above analyses that provide evidence the amber inclusion is an extinct crown group representative of *Elaphoglossum*, we describe it here as a new species.

***New species*—*Elaphoglossum miocenicum*** Lóriga, A. R. Schmidt, R. C. Moran, K. Feldberg, H. Schneid. & Heinrichs, sp. nov.

***Holotype*—**National Museum of Natural History of the Smithsonian Institution, amber inclusion no. USNM 414283. Fragment of fertile leaf with acrostichoid arrangement of sporangia (Fig. 1). Type locality: Dominican Republic, Santiago area. Age and stratigraphic position: Early Miocene, about 15 to 20 Myr ago. Syninclusions: Conidiophores of a fungus and the leafy liverworts *Bazzania* sp. (Lepidoziaceae) and *Cheilolejeunea antiqua* (Lejeuneaceae).

***Diagnosis*—**Bolbitidoid fern with simple, entire, fertile leaves and sporangia covering the lower surface of the blade; leaf scales flat, with toothed margins, unicellular marginal teeth lacking; perines with broad, continuous folds.

***Description*—**The fossil consists of a fragment of a fertile leaf including the petiole and the basal half of the blade. Fertile leaf simple, entire; petiole 0.9 cm long, 1.5 mm broad; scales

scattered on the petiole and blade, lanceolate to irregularly shaped, becoming larger towards the petiole base, up to 3.6 mm long, basifixed, narrowly lanceolate, brown, margin entire to dentate; blade wedge-shaped, base long-decurrent. Leptosporangia densely covering the abaxial surface of the blade (acrostichoid sporangial arrangement), stalks 3-celled; annulus vertical, interrupted at the stalk, stomium transverse; spores monolete, reniform, equatorial diameter 36.0 (25.0–45.0) × 21.7 (20.0–25.0) µm, perine with continuous, broad folds.

*Perspectives*—Dominican amber is a well-known source of plant microinclusions and especially famous for its numerous liverwort and moss fossils that indicate a conserved generic composition of epiphytic bryophyte communities during the Miocene of the Caribbean (Frahm and Newton, 2005; Heinrichs et al., 2014). In contrast, only a few fern inclusions have been recognized so far (Grimaldi, 1996), of which only one has been treated taxonomically, as *Grammitis succinea* (Gómez, 1982). The present study documents the second fern genus in Dominican amber and the first fossil of a bolbitidoid fern. The extraordinary preservation of the amber inclusion revealed morphological details, such as the cross section of the sporangium stalk, and allowed for a reliable classification of the fern as a crown group member of *Elaphoglossum*. Today, *Elaphoglossum* is a common element of the epiphyte flora of the Caribbean and elsewhere in tropical America. The fossil provides evidence that it was also present in the epiphytic communities of the local Miocene amber forests. Most important, the age of this fossil is consistent with molecular clock-based estimates.

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APPENDIX 1. Species and GenBank accession numbers of the DNA sequences used in this study.

***Species***; GenBank accessions: *rps4-trnS*; *trnL-trnF*.

***Arthrobotrya articulata*** (Fée) J. Sm.; GU376714; GU376565. ***A. wilkesiana*** (Brack.) Copel.; GU376719; GU376569.

***Bolbitis acrostichoides*** (Afzel. ex Sw.) Ching; GU376644; GU376500. ***B. aliena*** (Sw.) Alston; GU376646; GU376502. ***B. appendiculata*** (Willd.) K. Iwats.; GU376648; GU376504. ***B. auriculata*** (Sw.) Alston; GU376649; GU376505. ***B. bipinnatifida*** (J. Sm.) K. Iwats.; GU376676; GU376530. ***B. fluviatilis*** (Hook.) Ching; GU376656; GU376510. ***B. gemmifera*** (Hieron.) C. Chr.; GU376657; GU376511. ***B. heteroclita*** (C. Presl) Ching; GU376660; GU376514. ***B. humblotii*** (Baker) Ching; GU376663; GU376516. ***B. major*** (Bedd.) Hennipman; GU376665; GU376518. ***B. portoricensis*** (Spreng.) Hennipman; GU376670; GU376523. ***B. serratifolia*** (Mert. ex Kaulf.) Schott; GU376673; GU376527. ***B. tibetica*** Ching & S.K. Wu; GU376677; GU376531.

***Elaphoglossum achroalepis*** (Baker) C. Chr.; AY540225; AY536288. ***E. acrostichoides*** (Hook. & Grev.) Schelpe; EF040628; EF040614. ***E.* aff. *ciliatum*** (C. Presl) T. Moore; EU907748; EU907813. ***E. affine*** (M. Martens & Galeotti) T. Moore; AY536169; AY534841. ***E. albescens*** (Sodiro) Christ; GU376678; GU376532. ***E. alismaefolium*** (Feé) T. Moore; KF212425; KF212399. ***E. amygdalifolium*** (Mett. ex Kuhn) Christ; AY536173; AY534845. ***E. angulatum*** (Blume) T. Moore; AY540230; AY536293. ***E. asterolepis*** (Baker) C. Chr.; AY540231; AY536294. ***E. aubertii*** (Desv.) T. Moore; EF040622; EF040608. ***E. auricomum*** (Kunze) T. Moore; AY536145; AY534817. ***E. auripilum*** Christ; EF040626; EF040612. ***E. avaratraense*** Rakotondr.; AY540233; AY536296. ***E. backhouseanum*** T.Moore; AY540234; AY536297. ***E. bifurcatum*** (Jacq.) Mickel; EU907737; AY194070. ***E. bolleyi*** Christ; AY540235; AY536298. ***E. burchellii*** (Baker) C. Chr.; EU907738; EU90780. ***E. cardenasii*** W.H. Wagner; AY536131; AY534802. ***E. cardiophyllum*** (Hook.) T. Moore; AY53617; AY534842. ***E. cf. longifolium*** (Jacq.) J. Sm.; KF212426; KF212402. ***E. ciliatum*** (Hook.) T. Moore ex Diels; EU907745; EU907810. ***E. cismense*** Rosenst.; AY540237; AY536300. ***E. concinnum*** Mickel; KJ528151; KJ528179. ***E. conspersum*** Crhist; AY540238; AY536301. ***E. coriaceum*** Bonap.; EF040627; EF040613. ***E. coursii*** Tardieu; AY540240; AY536303. ***E. crinitum*** (L.) Christ; AY536134; AY534805. ***E. croatii*** Mickel; AY540241; AY536304. ***E. cubense*** (Mett. ex Kuhn) C. Chr.; KF212429; KF212404. ***E. cuspidatum*** (Willd.) T. Moore; EU907750; EU907815. ***E. davidsei*** Mickel; AY540242; AY536305. ***E. decaryanum*** Tardieu; AY540243; AY536306. ***E. deckenii*** (Kuhn) C. Chr.; AY540244; AY536307. ***E. decoratum*** (Kunze) T. Moore; GU376681; GU376534. ***E. dendricola*** (Baker) Christ; EU907751; EU907816. ***E. dimorphum*** (Hook. & Grev.) T. Moore; EU907753; EU907817. ***E. doanense*** L.D. Gómez; AY540245; AY536308. ***E. dussii*** Underw. & Maxon; EU907755; EU907819. ***E. edwallii*** Rosenst.; AY536144; AY534816. ***E. eggersii*** (Baker) Christ; KF212431; KF212406. ***E. erinaceum*** (Fée) T. Moore; KF212432; KF212407. ***E. eximium*** (Mett.) Christ; AY536132; AY534803. ***E. firmum*** (Mett. ex Kuhn) Urb.; KF212382; KF212408. ***E. flaccidum*** (Fée) T. Moore; AY540246; AY536309. ***E. forsythii-majoris*** Christ; EF040620; EF040606. ***E. fournierianum*** L.D. Gómez; AY540248; AY536311. ***E. gayanum*** Mickel; AY534838; AY536166. ***E. glabellum*** J. Sm.; AY536167; AY534839. ***E. gramineum*** (Jenman) Urb.; KF212383; KF212409. ***E. grayumii*** Mickel; AY540250; AY536313. ***E. guatemalense*** (Klotzsch) T. Moore; AY536164; AY534836. ***E. guentheri*** Rosenst.; GU376682; GU376535. ***E. herminieri*** (Bory & Fée) T. Moore; KF212435; KF212410. ***E. heterolepis*** T.Moore; AY540251; AY53631. ***E. hoffmannii*** (Mett. ex Kuhn) Christ; AY540252; AY536315. ***E. hornei*** C.Chr.; AY540253; AY536316. ***E. huacsaro*** (Ruiz) Christ; HG425357; KF212419. ***E. humbertii*** C. Chr.; EU907771; EU907834. ***E. hybridum*** (Bory) Brack.; EU907772; EU907835. ***E. ipshookense*** Mickel; EU907773; EU907836. ***E. lanatum*** Lorence; AY540258; AY536321. ***E. lancifolium*** (Desv.) C.V. Morton; AY540259; AY536322. ***E. langsdorffii*** (Hook. & Grev.) T. Moore; GU376536; GU376683. ***E. leucolepis*** (Baker) Krajina ex Tardieu; AY540261; AY536324. ***E. lindenii*** (Bory ex Fée) T. Moore; AY536130; AY534801. ***E. lingua*** (C. Presl) Brack.; AY540262; AY536325. ***E. lloense*** (Hook.) T. Moore; GU376684; GU376537. ***E. luridum*** (Fée) Christ; AY540263; AY536326. ***E. macropodium*** (Fée) T. Moore; AY54026; AY536327. ***E. malgassicum*** C. Chr.; AY540265; AY536328. ***E. marojejyense*** Tardieu; AY540266; AY536329. ***E. marquisearum*** Bonap.; AY540267; AY536330. ***E. martinicense*** (Desv.) T. Moore; KF212386; KF212411. ***E. maxonii*** Underw. ex Maxon; KF212438; KF212413. ***E. metallicum*** Mickel; AY536160; AY534832. ***E. micropogon*** Mickel; AY540268; AY536331. ***E. mitorrhizum*** Mickel; AY540269; AY536332. ***E. moorei*** (E. Britton) Christ; KJ528150; KJ528208. ***E. nervosum*** (Bory) Christ; EU907775; EU907837. ***E. nidiformis*** Mickel; EF040629; EF040616. ***E. nidusoides*** Rouhan & Rakotondr; EF040618; EF040604. ***E. nigrescens*** (Hook.) T. Moore ex Diels; EU907781; EU907843. ***E. nigrocostatum*** Mickel; AY536152; AY534824. ***E. oblanceolatum*** C. Chr.; AY540271; AY536334. ***E. ocoense*** C. Chr.; KF212414; KF212441. ***E. orbignyanum*** (Fée) T. Moore; EU907783; EU907845. ***E. ovalilimbatum*** Bonap.; AY540272; AY536335. ***E. ovatum*** (Hook. & Grev.) T. Moore; AY540273; AY536336. ***E. paleaceum*** (Hook. & Grev.) Sledge; EU907784; EU907846. ***E. palmeri*** Underw. & Maxon; KF212442; KF212415. ***E. papillosum*** (Baker) Christ; AY536129; AY534800. ***E. peltatum*** (Sw.) Urb.; KF212444; KF212417. ***E. petiolatum*** (Sw.) Urb.; AY540275; AY536338. ***E. phanerophlebium*** C. Chr.; AY540276; AY536339. ***E. piloselloides*** (C. Presl) T. Moore; KF212445; KF212418. ***E. pilosius*** Mickel; AY540277; AY536340. ***E. poolii*** Christ; AY540278; AY536341. ***E. potosianum*** Christ; EU907786; EU907849. ***E. prestonii*** (Baker) J. Sm.; AY534810; AY53481. ***E. pringlei*** (Davenp.) C. Chr.; EU907716; EU907850. ***E. productum*** Rosenst.; AY540279; AY536342. ***E. pusillum*** (Mett. ex Kuhn) C. Chr.; HG428762; KF212420. ***E. pygmaeum*** (Mett. ex Kuhn) Christ; AY540281; AY536344. ***E. richardii*** (Bory) Christ; EF040621; EF040607. ***E. rufidulum*** C. Chr.; AY540285; AY536348. ***E. russelliae*** Mickel; AY540286; AY536349. ***E. rzedowskii*** Mickel; EU907788; EU907851. ***E. samoense*** Brack.; AY540287; AY536350. ***E. sartorii*** (Liebm.) Mickel; AY536161; AY534833. ***E. scolopendriforme*** Tardieu; AY540288; AY536351. ***E. setigerum*** (Sodiro) Diels; AY540289; AY536352. ***E. sieberi*** (Hook. & Grev.) T. Moore; AY540290; AY536353. ***E. siliquoides*** (Jenman) C. Chr.; AY536127; AY534798. ***E. smithii*** (Baker) Christ; AY540291; AY536354. ***E. spatulatum*** (Bory) T. Moore; EF040623; EF040609. ***E. splendens*** Brack.; AY540296; AY536359. ***E. squamipes*** (Hook.) T. Moore; AY536157; AY534829. ***E. squamipes*** (Hook.) T. Moore; AY536158; AY534830. ***E. subsessile*** (Baker) C. Chr.; AY540298; AY536361. ***E. succisifolium*** (Willd.) T. Moore; AY540299; AY536362. ***E. tectum*** (Humb. & Bonpl. ex Willd.) T. Moore; AY536142; AY534813. ***E. tomentosum*** (Bory ex Willd.) Christ; AY540300; AY53636. ***E. tripartitum*** (Hook. & Grev.) Mickel; AY536156; AY534828. ***E. vestitum*** (Schltdl. & Cham.) T. Moore; AY536146; AY534818. ***E. vieillardii*** (Mett.) T. Moore; AY54030; AY536364. ***E. wawrae*** C. Chr.; AY540302; AY536365. ***E. welwitschii*** (Baker) C. Chr.; AY540303; AY536366. ***E. wrightii*** (Mett. ex D.C. Eaton) T. Moore; KF212447; KF212423. ***E. yungense*** de la Sota; EU907796; EU907859.

***Lomagramma brooksii*** Copel.; GU376691; GU376542. ***L. cordipinna*** Holttum; GU376695; GU376546. ***L. lomarioides*** (Blume) J. Sm.; GU376699; GU376550. ***L. matthewii*** (Ching) Holttum; GU376700; GU376551. ***L. perakensis*** Bedd.; GU376703; GU376554. ***L. pteroides*** J. Sm.; GU376704; GU376555. ***L. sinuata*** C. Chr.; GU376706; GU376557. ***L. sumatrana*** Alderw.; GU376708; GU376559.

***Mickelia bernoullii*** (Kuhn ex Christ) R.C. Moran, Labiak & Sundue; GU376651; GU376506. ***M. guianensis*** (Aubl.) R.C. Moran, Sundue & Labiak; GU376698; GU376549. ***M. nicotianifolia*** (Sw.) R.C. Moran, Labiak & Sundue; GU376669; GU376522. ***M. oligarchica*** (Baker) R.C. Moran, Labiak & Sundue; GU376668; GU376521. ***M. scandens*** (Raddi) R.C. Moran, Labiak & Sundue; GU376696; GU376547.

***Teratophyllum koordersii*** Holttum; GU376715; GU376566. ***T. ludens*** (Fée) Holttum; GU376717; GU376568.

**Appendix S1.** List of morphological characters and states scored for the bolbitidoid ferns used in the ancestral state reconstructions.

Six-digit numbers indicate states for traits 1–6, from left to right. Traits and states are as follows: (1) Perine relation to exine: tightly appressed = 0, nonappressed = 1. (2) Type of perine folds: thin, cristate = 0, broad, rounded = 1. (3) Continuity of perine folds: discontinuous = 0, continuous = 1. (4) Dissection of the fertile blade: divided = 0, simple = 1. (5) Base of petiolar scales: rolled = 0; flat = 1. (6) Unicellular marginal teeth: present = 0; absent = 1. A dash in place of a number indicates a nonapplicable character state, a question mark indicates no data, and a slash indicates an intermediate state.

| Species | Character states |
|---|---|
| *Arthrobotrya articulata* | 111011 |
| *Arthrobotrya wilkesiana* | 1/1011 |
| *Bolbitis acrostichoides* | 111011 |
| *Bolbitis aliena* | 101011 |
| *Bolbitis appendiculata* | 101011 |
| *Bolbitis auriculata* | 111011 |
| *Bolbitis bipinnatifida* | 111011 |
| *Bolbitis fluviatilis* | 111111 |
| *Bolbitis gemmifera* | 101011 |
| *Bolbitis heteroclita* | 101011 |
| *Bolbitis humblotii* | 1/1011 |
| *Bolbitis major* | 101011 |
| *Bolbitis portoricensis* | 101011 |
| *Bolbitis serratifolia* | 111011 |
| *Bolbitis tibetica* | 101011 |
| *Elaphoglossum achroalepis* | 111110 |
| *Elaphoglossum acrostichoides* | 11/111 |
| *Elaphoglossum* aff. *ciliatum* | 1111?? |
| *Elaphoglossum affine* | 101111 |
| *Elaphoglossum albescens* | 0--101 |
| *Elaphoglossum alismaefolium* | ???111 |
| *Elaphoglossum amygdalifolium* | 101111 |
| *Elaphoglossum angulatum* | 111111 |
| *Elaphoglossum asterolepis* | 111110 |
| *Elaphoglossum aubertii* | 0--101 |
| *Elaphoglossum auricomum* | 111110 |
| *Elaphoglossum auripilum* | 111101 |
| *Elaphoglossum avaratraense* | 111110 |
| *Elaphoglossum backhousianum* | 111101 |
| *Elaphoglossum bifurcatum* | 111010 |
| *Elaphoglossum biolleyi* | 101111 |
| *Elaphoglossum burchellii* | 111110 |
| *Elaphoglossum cardenasii* | 100001 |
| *Elaphoglossum cardiophyllum* | 111111 |
| *Elaphoglossum* cf. *longifolium* | ???111 |

| Species | Character states |
|---|---|
| *Elaphoglossum ciliatum* | 1111?? |
| *Elaphoglossum cismense* | 101111 |
| *Elaphoglossum concinnum* | 111111 |
| *Elaphoglossum conspersum* | 111111 |
| *Elaphoglossum coriaceum* | 111111 |
| *Elaphoglossum coursii* | 111111 |
| *Elaphoglossum crinitum* | 111101 |
| *Elaphoglossum croatii* | 100111 |
| *Elaphoglossum cubense* | 111101 |
| *Elaphoglossum cuspidatum* | 110110 |
| *Elaphoglossum davidsei* | 101101 |
| *Elaphoglossum decaryanum* | 101111 |
| *Elaphoglossum deckenii* | 111110 |
| *Elaphoglossum decoratum* | 111101 |
| *Elaphoglossum dendricola* | 111110 |
| *Elaphoglossum dimorphum* | 111110 |
| *Elaphoglossum doanense* | 111111 |
| *Elaphoglossum dussii* | 111110 |
| *Elaphoglossum edwallii* | 111110 |
| *Elaphoglossum eggersii* | 111110 |
| *Elaphoglossum erinaceum* | 111101 |
| *Elaphoglossum eximium* | 0--101 |
| *Elaphoglossum firmum* | ???111 |
| *Elaphoglossum flaccidum* | 101111 |
| *Elaphoglossum forsythia-majoris* | 111110 |
| *Elaphoglossum fournierianum* | 10/101 |
| *Elaphoglossum glabellum* | 111111 |
| *Elaphoglossum gramineum* | 1111?? |
| *Elaphoglossum grayumii* | 101111 |
| *Elaphoglossum guatemalense* | 111111 |
| *Elaphoglossum guentheri* | 111111 |
| *Elaphoglossum herminieri* | 110111 |
| *Elaphoglossum heterolepis* | 111110 |
| *Elaphoglossum hoffmannii* | 101111 |
| *Elaphoglossum hornei* | 11/111 |
| *Elaphoglossum huacsaro* | 111110 |
| *Elaphoglossum humbertii* | 111110 |
| *Elaphoglossum hybridum* | 111101 |
| *Elaphoglossum ipshookense* | 111110 |
| *Elaphoglossum lanatum* | 111110 |
| *Elaphoglossum lancifolium* | 111110 |
| *Elaphoglossum langsdorffii* | 111110 |
| *Elaphoglossum leucolepis* | 111110 |
| *Elaphoglossum lindenii* | 101101 |
| *Elaphoglossum lingua* | 111111 |

| Species | Character states |
|---|---|
| *Elaphoglossum lloense* | 111111 |
| *Elaphoglossum luridum* | 101111 |
| *Elaphoglossum macropodium* | 111111 |
| *Elaphoglossum malgassicum* | 111111 |
| *Elaphoglossum marojejyense* | 111111 |
| *Elaphoglossum marquisearum* | 101111 |
| *Elaphoglossum martinicense* | ???111 |
| *Elaphoglossum maxonii* | 111111 |
| *Elaphoglossum metallicum* | 101111 |
| *Elaphoglossum micropogon* | 111110 |
| *Elaphoglossum minutum* | 111111 |
| *Elaphoglossum mitorrhizum* | 111111 |
| *Elaphoglossum moorei* | 111111 |
| *Elaphoglossum nervosum* | 111110 |
| *Elaphoglossum nidiforme* | 111111 |
| *Elaphoglossum nidusoides* | 111111 |
| *Elaphoglossum nigrescens* | 111110 |
| *Elaphoglossum nigrocostatum* | 111110 |
| *Elaphoglossum oblanceolatum* | 0--101 |
| *Elaphoglossum ocoense* | ???101 |
| *Elaphoglossum orbignyanum* | 111110 |
| *Elaphoglossum ovalilimbatum* | 111111 |
| *Elaphoglossum ovatum* | 111110 |
| *Elaphoglossum paleaceum* | 111110 |
| *Elaphoglossum palmeri* | 111110 |
| *Elaphoglossum papillosum* | 0--101 |
| *Elaphoglossum peltatum* | 111111 |
| *Elaphoglossum petiolatum* | 111110 |
| *Elaphoglossum phanerophlebium* | 101101 |
| *Elaphoglossum piloselloides* | 111101 |
| *Elaphoglossum pilosius* | 111101 |
| *Elaphoglossum poolii* | 111110 |
| *Elaphoglossum potosianum* | 111110 |
| *Elaphoglossum prestonii* | 110101 |
| *Elaphoglossum pringlei* | 111110 |
| *Elaphoglossum productum* | 111111 |
| *Elaphoglossum pusillum* | ???101 |
| *Elaphoglossum pygmaeum* | 0--101 |
| *Elaphoglossum richardii* | 111110 |
| *Elaphoglossum rufidulum* | 111110 |
| *Elaphoglossum russelliae* | 100101 |
| *Elaphoglossum rzedowskii* | 111110 |
| *Elaphoglossum samoense* | 101101 |
| *Elaphoglossum sartorii* | 101111 |
| *Elaphoglossum scolopendriforme* | 111110 |

| Species | Character states |
|---|---|
| *Elaphoglossum setigerum* | 0--101 |
| *Elaphoglossum sieberi* | 111111 |
| *Elaphoglossum siliquoides* | 111101 |
| *Elaphoglossum smithii* | 101101 |
| *Elaphoglossum spatulatum* | 111101 |
| *Elaphoglossum splendens* | 111110 |
| *Elaphoglossum squamipes* | 111111 |
| *Elaphoglossum squamipes* | 111111 |
| *Elaphoglossum subsessile* | 101111 |
| *Elaphoglossum succisifolium* | 111110 |
| *Elaphoglossum tectum* | 111110 |
| *Elaphoglossum tomentosum* | 111110 |
| *Elaphoglossum tripartitum* | 111111 |
| *Elaphoglossum vestitum* | 111110 |
| *Elaphoglossum vieillardii* | 101111 |
| *Elaphoglossum wawrae* | ???111 |
| *Elaphoglossum welwitschii* | 111110 |
| *Elaphoglossum wrightii* | 111111 |
| *Elaphoglossum yungense* | 111110 |
| *Lomagramma brooksii* | 0--011 |
| *Lomagramma cordipinna* | 0--011 |
| *Lomagramma lomarioides* | 0--011 |
| *Lomagramma matthewii* | 0--011 |
| *Lomagramma perakensis* | 0--011 |
| *Lomagramma pteroides* | 0--011 |
| *Lomagramma sinuata* | 0--011 |
| *Lomagramma sumatrana* | 0--011 |
| *Mickelia bernoullii* | 101011 |
| *Mickelia guianensis* | 101011 |
| *Mickelia nicotianifolia* | 101011 |
| *Mickelia oligarchica* | 101011 |
| *Mickelia scandens* | 101011 |
| *Teratophyllum koordersii* | 101011 |
| *Teratophyllum ludens* | 111011 |

**Appendix S2.** Divergence time and proportional likelihood of character states for all the nodes in the newly obtained phylogeny of bolbitidoid ferns (see Figs. 3 and 4). Node age estimates were obtained from a Bayesian phylogenetic inference analysis with divergence time dating in BEAST, and are presented as mean and 95% HPD credibility intervals. Proportional likelihood values of each possible state of six morphological characters were inferred using the ape package in R. Node numbers correspond to those in Appendix S3. States 0 and 1 for each character as described in Fig. 2; state 2 represents intermediate character states; ND/NA state means No Data or Not Applicable.

| Node | Age (95% HPD) | Traits | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Perine relation to exine | | | Type of perine folds | | | | Continuity of the perine folds | | | | Dissection of the fertile blade | | Base of petiolar scales | | | Unicellular marginal teeth | | |
| | | 0 | 1 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 0 | 1 | ND/NA | 0 | 1 | ND/NA |
| 1 | 53.69 (46.32–63.96) | 0.005 | 0.993 | 0.003 | 0.545 | 0.260 | 0.082 | 0.114 | 0.988 | 0.003 | 0.003 | 0.005 | 0.004 | 0.996 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 2 | 34.63 (24.62–45.83) | 0.003 | 0.995 | 0.003 | 0.650 | 0.197 | 0.097 | 0.057 | 0.991 | 0.003 | 0.003 | 0.003 | 0.001 | 0.999 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 3 | 13.09 (6.21–21.46) | 0.006 | 0.989 | 0.006 | 0.074 | 0.426 | 0.426 | 0.074 | 0.980 | 0.007 | 0.007 | 0.007 | 0.002 | 0.998 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 4 | 31.88 (23.17–42.32) | 0.003 | 0.994 | 0.003 | 0.806 | 0.116 | 0.039 | 0.039 | 0.988 | 0.004 | 0.004 | 0.004 | 0.001 | 0.999 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 5 | 16.68 (9.85–24.72) | 0.001 | 0.998 | 0.001 | 0.467 | 0.467 | 0.033 | 0.033 | 0.996 | 0.001 | 0.001 | 0.001 | 0.017 | 0.983 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 6 | 11.92 (5.67–19.1) | 0.005 | 0.991 | 0.005 | 0.432 | 0.432 | 0.068 | 0.068 | 0.983 | 0.006 | 0.006 | 0.006 | 0.002 | 0.998 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 7 | 10.53 (5.31–16.85) | 0.004 | 0.993 | 0.004 | 0.440 | 0.440 | 0.060 | 0.060 | 0.987 | 0.004 | 0.004 | 0.004 | 0.500 | 0.500 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 8 | 27.1 (18.36–37.13) | 0.009 | 0.983 | 0.009 | 0.838 | 0.067 | 0.048 | 0.048 | 0.969 | 0.010 | 0.010 | 0.010 | 0.003 | 0.997 | 0.999 | 0.001 | 0.001 | 0.001 | 0.999 | 0.001 |
| 9 | 17.68 (11.33–25.05) | 0.000 | 0.999 | 0.000 | 0.895 | 0.076 | 0.014 | 0.014 | 0.999 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 10 | 16.38 (10.23–23.8) | 0.007 | 0.987 | 0.007 | 0.356 | 0.486 | 0.079 | 0.079 | 0.976 | 0.008 | 0.008 | 0.008 | 0.002 | 0.998 | 0.999 | 0.001 | 0.001 | 0.001 | 0.999 | 0.001 |
| 11 | 4.24 (1.5–8.04) | 0.001 | 0.999 | 0.001 | 0.003 | 0.992 | 0.003 | 0.003 | 0.998 | 0.001 | 0.001 | 0.001 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 12 | 13.82 (8.16–20.52) | 0.003 | 0.994 | 0.003 | 0.957 | 0.014 | 0.014 | 0.014 | 0.990 | 0.003 | 0.003 | 0.003 | 0.001 | 0.999 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 13 | 8.14 (3.72–13.68) | 0.002 | 0.996 | 0.002 | 0.969 | 0.010 | 0.010 | 0.010 | 0.992 | 0.003 | 0.003 | 0.003 | 0.001 | 0.999 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 14 | 50.59 (42.96–61.5) | 0.021 | 0.973 | 0.005 | 0.387 | 0.331 | 0.104 | 0.178 | 0.964 | 0.006 | 0.006 | 0.024 | 0.046 | 0.954 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 15 | 38.39 (29–49.63) | 0.381 | 0.582 | 0.037 | 0.153 | 0.259 | 0.165 | 0.422 | 0.557 | 0.039 | 0.039 | 0.364 | 0.002 | 0.998 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 16 | 29.12 (20.04–40.33) | 0.009 | 0.981 | 0.009 | 0.209 | 0.445 | 0.235 | 0.111 | 0.966 | 0.011 | 0.011 | 0.011 | 0.003 | 0.997 | 0.999 | 0.001 | 0.001 | 0.001 | 0.999 | 0.001 |
| 17 | 8.33 (3.77–14.55) | 0.002 | 0.995 | 0.002 | 0.452 | 0.452 | 0.048 | 0.048 | 0.992 | 0.003 | 0.003 | 0.003 | 0.001 | 0.999 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 18 | 18.03 (9.32–27.91) | 0.011 | 0.978 | 0.011 | 0.099 | 0.401 | 0.401 | 0.099 | 0.961 | 0.013 | 0.013 | 0.013 | 0.004 | 0.996 | 0.998 | 0.001 | 0.001 | 0.001 | 0.998 | 0.001 |
| 19 | 23.34 (15.75–33.11) | 0.983 | 0.008 | 0.008 | 0.040 | 0.040 | 0.040 | 0.879 | 0.010 | 0.010 | 0.010 | 0.970 | 0.003 | 0.997 | 0.999 | 0.001 | 0.001 | 0.001 | 0.999 | 0.001 |
| 20 | 6.14 (2.48–11.73) | 0.998 | 0.001 | 0.001 | 0.006 | 0.006 | 0.006 | 0.983 | 0.001 | 0.001 | 0.001 | 0.996 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 21 | 9.22 (5.4–13.8) | 0.999 | 0.000 | 0.000 | 0.002 | 0.002 | 0.002 | 0.995 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 22 | 6.3 (2.86–10.25) | 0.997 | 0.001 | 0.001 | 0.006 | 0.006 | 0.006 | 0.982 | 0.001 | 0.001 | 0.001 | 0.996 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 23 | 5.91 (3.16–9.56) | 0.999 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.996 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 24 | 4.41 (1.86–7.47) | 0.999 | 0.001 | 0.001 | 0.003 | 0.003 | 0.003 | 0.992 | 0.001 | 0.001 | 0.001 | 0.998 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 25 | 0.25 (0–1.13) | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 26 | 45.99 (39.19–56.17) | 0.005 | 0.989 | 0.005 | 0.511 | 0.313 | 0.088 | 0.088 | 0.980 | 0.007 | 0.007 | 0.007 | 0.529 | 0.471 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 27 | 33.93 (23.96–44.95) | 0.007 | 0.985 | 0.007 | 0.852 | 0.049 | 0.049 | 0.049 | 0.973 | 0.009 | 0.009 | 0.009 | 0.002 | 0.998 | 0.999 | 0.001 | 0.001 | 0.001 | 0.999 | 0.001 |

| Node | Age (95% HPD) | Traits | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Perine relation to exine | | | Type of perine folds | | | | Continuity of the perine folds | | | | Dissection of the fertile blade | | Base of petiolar scales | | | Unicellular marginal teeth | | |
| | | 0 | 1 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 0 | 1 | ND/NA | 0 | 1 | ND/NA |
| 28 | 11.28 (5.59–19.33) | 0.004 | 0.992 | 0.004 | 0.939 | 0.020 | 0.020 | 0.020 | 0.985 | 0.005 | 0.005 | 0.005 | 0.001 | 0.999 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 29 | 25.69 (16.28–36.04) | 0.006 | 0.988 | 0.006 | 0.861 | 0.046 | 0.046 | 0.046 | 0.977 | 0.008 | 0.008 | 0.008 | 0.002 | 0.998 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 30 | 21.7 (13.25–32.4) | 0.016 | 0.968 | 0.016 | 0.784 | 0.072 | 0.072 | 0.072 | 0.942 | 0.019 | 0.019 | 0.019 | 0.005 | 0.995 | 0.997 | 0.001 | 0.001 | 0.001 | 0.998 | 0.001 |
| 31 | 35.55 (32.7–42.1) | 0.010 | 0.981 | 0.010 | 0.156 | 0.685 | 0.080 | 0.080 | 0.965 | 0.012 | 0.012 | 0.012 | 0.997 | 0.003 | 0.999 | 0.001 | 0.001 | 0.001 | 0.999 | 0.001 |
| 32 | 28.39 (21.68–34.84) | 0.004 | 0.992 | 0.004 | 0.022 | 0.935 | 0.021 | 0.021 | 0.985 | 0.005 | 0.005 | 0.005 | 0.999 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 33 | 24.08 (18.52–30.31) | 0.001 | 0.999 | 0.001 | 0.006 | 0.987 | 0.003 | 0.004 | 0.998 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 34 | 18.58 (12.02–25.72) | 0.003 | 0.993 | 0.003 | 0.084 | 0.868 | 0.024 | 0.024 | 0.989 | 0.004 | 0.004 | 0.004 | 0.999 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 35 | 8.1 (4.74–12.57) | 0.000 | 0.999 | 0.000 | 0.002 | 0.994 | 0.002 | 0.002 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 36 | 6.48 (3.92–9.64) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 37 | 5.07 (2.92–7.69) | 0.000 | 0.996 | 0.004 | 0.001 | 0.989 | 0.001 | 0.010 | 0.995 | 0.000 | 0.000 | 0.004 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 38 | 3.07 (1.24–5.36) | 0.009 | 0.496 | 0.496 | 0.018 | 0.482 | 0.018 | 0.482 | 0.491 | 0.009 | 0.009 | 0.491 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 39 | 4.45 (2.45–6.92) | 0.001 | 0.999 | 0.001 | 0.003 | 0.992 | 0.003 | 0.003 | 0.998 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 40 | 0.47 (0.02–1.43) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 41 | 5.53 (3.26–8.48) | 0.000 | 0.999 | 0.001 | 0.002 | 0.994 | 0.002 | 0.002 | 0.998 | 0.000 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 42 | 3.3 (1.75–5.33) | 0.000 | 0.996 | 0.004 | 0.001 | 0.990 | 0.000 | 0.009 | 0.979 | 0.000 | 0.008 | 0.013 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 43 | 1.81 (0.67–3.27) | 0.004 | 0.561 | 0.434 | 0.009 | 0.554 | 0.009 | 0.428 | 0.559 | 0.005 | 0.005 | 0.432 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 44 | 0.41 (0.01–1.18) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 45 | 2.44 (0.96–4.17) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.000 | 0.001 | 0.739 | 0.004 | 0.250 | 0.007 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 46 | 1.62 (0.51–3.13) | 0.000 | 0.995 | 0.005 | 0.018 | 0.960 | 0.001 | 0.022 | 0.995 | 0.000 | 0.000 | 0.005 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 47 | 0.81 (0.1–2.07) | 0.002 | 0.499 | 0.499 | 0.005 | 0.495 | 0.005 | 0.495 | 0.498 | 0.002 | 0.002 | 0.498 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 48 | 0.77 (0.13–1.94) | 0.000 | 1.000 | 0.000 | 0.397 | 0.596 | 0.004 | 0.004 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 49 | 0.26 (0–1.16) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 50 | 9.33 (5.9–13.84) | 0.001 | 0.999 | 0.001 | 0.381 | 0.597 | 0.011 | 0.011 | 0.998 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 51 | 7.44 (4.82–10.78) | 0.000 | 1.000 | 0.000 | 0.853 | 0.141 | 0.003 | 0.003 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 52 | 6.21 (3.42–9.47) | 0.000 | 0.999 | 0.000 | 0.258 | 0.703 | 0.020 | 0.020 | 0.998 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 53 | 3.99 (1.75–6.86) | 0.000 | 0.999 | 0.000 | 0.002 | 0.995 | 0.002 | 0.002 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 54 | 0.95 (0.07–2.43) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 55 | 5.52 (3.47–7.93) | 0.000 | 1.000 | 0.000 | 0.960 | 0.038 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 56 | 2.64 (1.12–4.6) | 0.000 | 1.000 | 0.000 | 0.485 | 0.485 | 0.015 | 0.015 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 57 | 4.87 (3.1–7.06) | 0.000 | 1.000 | 0.000 | 0.966 | 0.031 | 0.001 | 0.002 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |

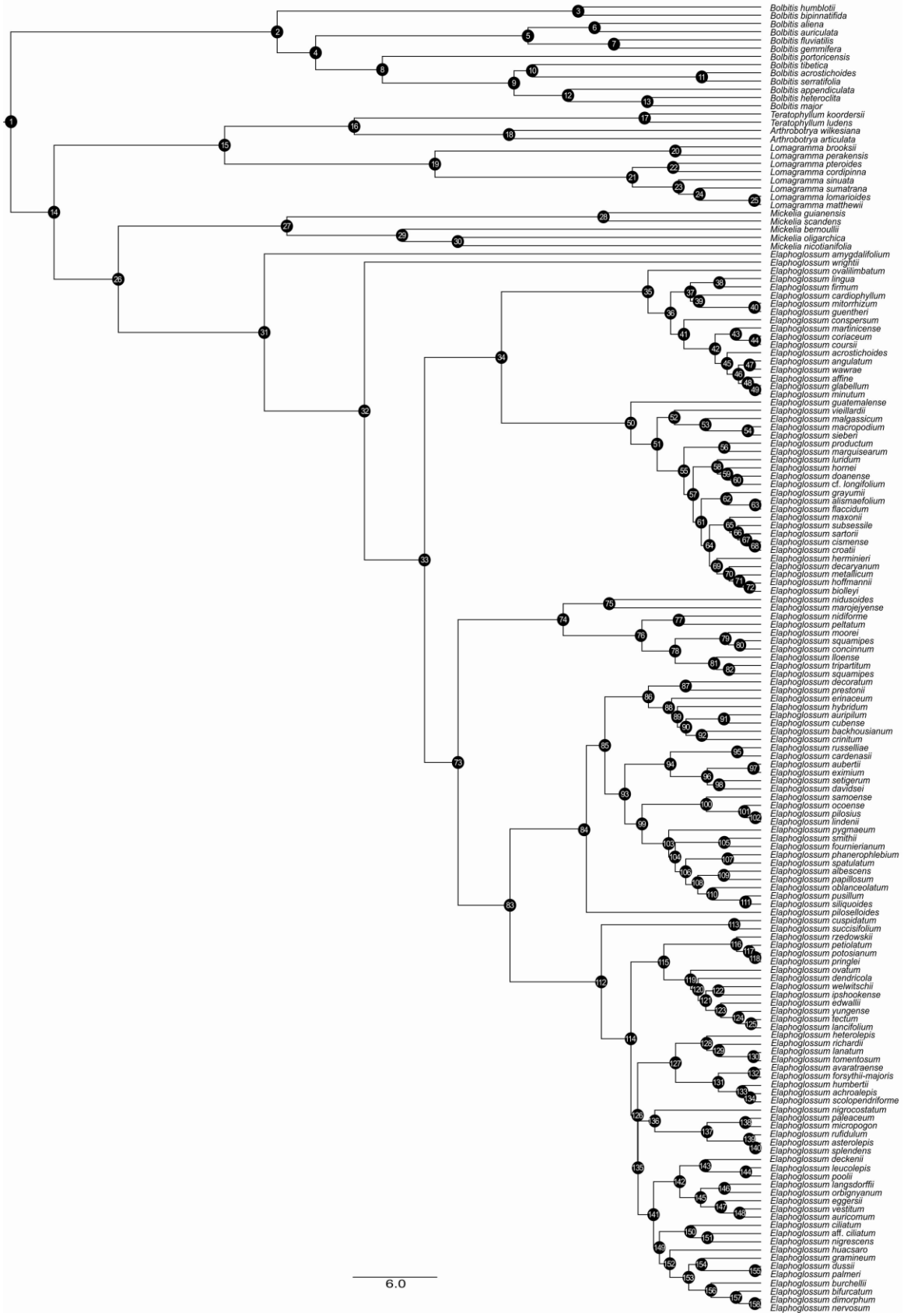
| Node | Age (95% HPD) | Traits | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Perine relation to exine | | | Type of perine folds | | | | Continuity of the perine folds | | | | Dissection of the fertile blade | | Base of petiolar scales | | | Unicellular marginal teeth | | |
| | | 0 | 1 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 0 | 1 | ND/NA | 0 | 1 | ND/NA |
| 58 | 3.14 (1.51–5.13) | 0.000 | 1.000 | 0.000 | 0.194 | 0.770 | 0.007 | 0.029 | 0.958 | 0.001 | 0.023 | 0.018 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 59 | 2.44 (1.1–4.28) | 0.000 | 0.986 | 0.014 | 0.001 | 0.970 | 0.001 | 0.028 | 0.303 | 0.006 | 0.389 | 0.303 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 60 | 1.71 (0.57–3.18) | 0.005 | 0.498 | 0.498 | 0.010 | 0.490 | 0.010 | 0.490 | 0.495 | 0.005 | 0.005 | 0.495 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 61 | 4.29 (2.78–6.52) | 0.000 | 1.000 | 0.000 | 0.997 | 0.002 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 62 | 2.49 (0.9–4.47) | 0.000 | 0.986 | 0.014 | 0.968 | 0.002 | 0.002 | 0.029 | 0.985 | 0.000 | 0.000 | 0.015 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 63 | 0.2 (0–0.94) | 0.001 | 0.500 | 0.500 | 0.499 | 0.001 | 0.001 | 0.499 | 0.499 | 0.001 | 0.001 | 0.499 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 64 | 3.7 (2.06–5.56) | 0.000 | 1.000 | 0.000 | 0.927 | 0.072 | 0.000 | 0.000 | 0.997 | 0.002 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 65 | 2.25 (0.86–4) | 0.000 | 1.000 | 0.000 | 0.786 | 0.204 | 0.005 | 0.005 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 66 | 1.67 (0.59–3.3) | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 67 | 1.07 (0.21–2.26) | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.994 | 0.006 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 68 | 0.21 (0–0.82) | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.499 | 0.499 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 69 | 3.17 (1.52–4.92) | 0.000 | 1.000 | 0.000 | 0.771 | 0.213 | 0.008 | 0.008 | 0.777 | 0.215 | 0.004 | 0.004 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 70 | 2.29 (0.77–4.23) | 0.000 | 1.000 | 0.000 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 71 | 1.61 (0.31–3.58) | 0.000 | 1.000 | 0.000 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 72 | 0.82 (0.04–2.39) | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 73 | 21.69 (16.37–27.42) | 0.001 | 0.998 | 0.001 | 0.007 | 0.982 | 0.005 | 0.006 | 0.997 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 0.985 | 0.014 | 0.000 | 0.010 | 0.990 | 0.000 |
| 74 | 14.16 (9.46–19.43) | 0.001 | 0.998 | 0.001 | 0.004 | 0.987 | 0.004 | 0.004 | 0.997 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 75 | 10.7 (5.71–16.17) | 0.004 | 0.992 | 0.004 | 0.018 | 0.946 | 0.018 | 0.018 | 0.987 | 0.004 | 0.004 | 0.004 | 0.999 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 |
| 76 | 8.53 (5.33–12.61) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 77 | 5.89 (3.05–9.39) | 0.001 | 0.998 | 0.001 | 0.005 | 0.984 | 0.005 | 0.005 | 0.996 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 78 | 6.15 (3.31–9.29) | 0.000 | 0.999 | 0.000 | 0.002 | 0.995 | 0.002 | 0.002 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 79 | 2.35 (0.92–4.38) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 80 | 1.77 (0.57–3.49) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 81 | 3.2 (1.43–5.6) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 82 | 2.55 (1.07–4.81) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 83 | 17.97 (13.54–23.26) | 0.001 | 0.998 | 0.001 | 0.023 | 0.953 | 0.008 | 0.015 | 0.995 | 0.002 | 0.001 | 0.002 | 1.000 | 0.000 | 0.453 | 0.542 | 0.005 | 0.453 | 0.542 | 0.005 |
| 84 | 12.5 (8.78–16.67) | 0.001 | 0.999 | 0.001 | 0.163 | 0.746 | 0.010 | 0.081 | 0.993 | 0.001 | 0.001 | 0.005 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 85 | 11.18 (8.12–15.08) | 0.001 | 0.999 | 0.000 | 0.444 | 0.330 | 0.012 | 0.213 | 0.941 | 0.003 | 0.001 | 0.055 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 86 | 8.07 (4.89–11.75) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 0.989 | 0.010 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 87 | 5.4 (1.72–9.67) | 0.001 | 0.998 | 0.001 | 0.004 | 0.987 | 0.004 | 0.004 | 0.484 | 0.484 | 0.016 | 0.016 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |

| Node | Age (95% HPD) | Traits | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Perine relation to exine | | | Type of perine folds | | | | Continuity of the perine folds | | | | Dissection of the fertile blade | | Base of petiolar scales | | | Unicellular marginal teeth | | |
| | | 0 | 1 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 0 | 1 | ND/NA | 0 | 1 | ND/NA |
| 88 | 6.41 (3.83–9.52) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 89 | 5.99 (3.51–8.8) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 90 | 5.38 (3.23–8.38) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 91 | 2.65 (0.99–4.88) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 92 | 4.25 (1.51–7.31) | 0.001 | 0.999 | 0.001 | 0.003 | 0.992 | 0.003 | 0.003 | 0.998 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 93 | 9.75 (6.95–13.3) | 0.024 | 0.976 | 0.000 | 0.678 | 0.003 | 0.002 | 0.317 | 0.239 | 0.023 | 0.002 | 0.736 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 94 | 6.5 (3.85–9.7) | 0.441 | 0.552 | 0.007 | 0.535 | 0.015 | 0.015 | 0.435 | 0.022 | 0.352 | 0.010 | 0.615 | 0.991 | 0.009 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 95 | 1.71 (0.37–3.79) | 0.000 | 1.000 | 0.000 | 0.999 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.500 | 0.500 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 96 | 3.86 (1.98–6.38) | 0.981 | 0.019 | 0.000 | 0.039 | 0.002 | 0.002 | 0.956 | 0.020 | 0.001 | 0.001 | 0.979 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 97 | 0.46 (0–1.37) | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 98 | 3 (1.22–5.31) | 0.496 | 0.496 | 0.008 | 0.483 | 0.017 | 0.017 | 0.483 | 0.491 | 0.009 | 0.009 | 0.491 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 99 | 8.52 (5.83–11.63) | 0.023 | 0.976 | 0.001 | 0.632 | 0.011 | 0.006 | 0.351 | 0.832 | 0.002 | 0.002 | 0.164 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 100 | 3.92 (1.07–8.13) | 0.001 | 0.985 | 0.015 | 0.886 | 0.042 | 0.005 | 0.066 | 0.983 | 0.001 | 0.001 | 0.015 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 101 | 1.2 (0.17–3.01) | 0.003 | 0.602 | 0.396 | 0.273 | 0.273 | 0.006 | 0.447 | 0.600 | 0.003 | 0.003 | 0.394 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 102 | 0.41 (0.02–1.29) | 0.000 | 1.000 | 0.000 | 0.498 | 0.498 | 0.002 | 0.002 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 103 | 6.62 (4.45–9.32) | 0.464 | 0.535 | 0.002 | 0.177 | 0.010 | 0.003 | 0.811 | 0.172 | 0.001 | 0.007 | 0.820 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 104 | 6.15 (4.03–8.67) | 0.029 | 0.970 | 0.001 | 0.700 | 0.033 | 0.006 | 0.262 | 0.814 | 0.002 | 0.030 | 0.155 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 105 | 2.63 (0.9–4.7) | 0.000 | 1.000 | 0.000 | 0.997 | 0.001 | 0.001 | 0.001 | 0.492 | 0.008 | 0.492 | 0.008 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 106 | 5.4 (3.37–7.81) | 0.596 | 0.397 | 0.007 | 0.087 | 0.094 | 0.008 | 0.811 | 0.227 | 0.004 | 0.004 | 0.765 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 107 | 2.39 (0.55–4.71) | 0.000 | 1.000 | 0.000 | 0.486 | 0.486 | 0.014 | 0.014 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 108 | 4.54 (2.63–6.68) | 0.987 | 0.007 | 0.007 | 0.000 | 0.001 | 0.000 | 0.998 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 109 | 2.66 (1.12–4.57) | 1.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.997 | 0.000 | 0.000 | 0.000 | 0.999 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 110 | 3.49 (1.61–5.89) | 0.452 | 0.274 | 0.274 | 0.003 | 0.040 | 0.003 | 0.954 | 0.021 | 0.001 | 0.001 | 0.978 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 111 | 1.12 (0.11–2.67) | 0.003 | 0.498 | 0.498 | 0.007 | 0.493 | 0.007 | 0.493 | 0.497 | 0.003 | 0.003 | 0.497 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| 112 | 11.43 (7.97–15.39) | 0.001 | 0.999 | 0.001 | 0.003 | 0.991 | 0.003 | 0.003 | 0.984 | 0.012 | 0.002 | 0.002 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 113 | 1.92 (0.32–4.79) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 0.494 | 0.494 | 0.006 | 0.006 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 114 | 9.32 (6.73–12.51) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 115 | 6.96 (4.45–9.91) | 0.000 | 0.999 | 0.000 | 0.001 | 0.996 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 116 | 1.76 (0.42–3.49) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 117 | 0.71 (0.08–1.77) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |

| Node | Age (95% HPD) | Traits | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Perine relation to exine | | | Type of perine folds | | | | Continuity of the perine folds | | | | Dissection of the fertile blade | | Base of petiolar scales | | | Unicellular marginal teeth | | |
| | | 0 | 1 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 0 | 1 | ND/NA | 0 | 1 | ND/NA |
| 118 | 0.14 (0–0.65) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 119 | 5.05 (2.96–7.47) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 120 | 4.5 (2.79–6.96) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 121 | 3.96 (2.18–6.09) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 122 | 3.06 (1.59–5.07) | 0.000 | 0.999 | 0.000 | 0.001 | 0.996 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 123 | 2.89 (1.23–4.95) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 124 | 1.62 (0.46–3.33) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 125 | 0.72 (0.01–1.94) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 126 | 8.84 (6.47–11.69) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 127 | 6.12 (3.58–9.18) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 128 | 3.9 (1.8–6.56) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 129 | 3.03 (1.18–5.46) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 130 | 0.55 (0.01–1.63) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 131 | 3.06 (1.27–5.41) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 132 | 0.52 (0.01–1.49) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 133 | 1.36 (0.3–2.9) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 134 | 0.79 (0.06–1.92) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 135 | 8.81 (6.36–11.35) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 136 | 7.61 (5.08–10.6) | 0.001 | 0.998 | 0.001 | 0.004 | 0.987 | 0.004 | 0.004 | 0.997 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 137 | 3.87 (1.69–6.39) | 0.000 | 0.999 | 0.000 | 0.001 | 0.996 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 138 | 1.12 (0.2–2.62) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 139 | 0.48 (0.01–1.51) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 140 | 0.16 (0–0.71) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 141 | 7.7 (5.36–10.35) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 142 | 5.82 (3.76–8.39) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 143 | 4 (1.66–6.44) | 0.000 | 0.999 | 0.000 | 0.002 | 0.995 | 0.002 | 0.002 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 144 | 1.09 (0.1–2.74) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 145 | 4.33 (2.53–6.61) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 146 | 2.62 (1.13–4.58) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 147 | 2.88 (1.27–4.97) | 0.000 | 1.000 | 0.000 | 0.001 | 0.998 | 0.001 | 0.001 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |

| Node | Age (95% HPD) | Traits | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Perine relation to exine | | | Type of perine folds | | | | Continuity of the perine folds | | | | Dissection of the fertile blade | | Base of petiolar scales | | | Unicellular marginal teeth | | |
| | | 0 | 1 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 2 | ND/NA | 0 | 1 | 0 | 1 | ND/NA | 0 | 1 | ND/NA |
| 148 | 1.54 (0.4–3.22) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 149 | 7.28 (5–9.76) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.904 | 0.000 | 0.096 | 0.904 | 0.000 | 0.096 |
| 150 | 5.06 (3.03–7.45) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.008 | 0.000 | 0.992 | 0.008 | 0.000 | 0.992 |
| 151 | 3.84 (1.91–6.02) | 0.000 | 0.999 | 0.000 | 0.002 | 0.994 | 0.002 | 0.002 | 0.998 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 0.498 | 0.003 | 0.498 | 0.498 | 0.003 | 0.498 |
| 152 | 6.53 (4.32–9.26) | 0.000 | 0.999 | 0.000 | 0.001 | 0.996 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 153 | 5.19 (3.11–7.65) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.998 | 0.000 | 0.002 | 0.998 | 0.000 | 0.002 |
| 154 | 4.27 (2.21–6.57) | 0.001 | 0.999 | 0.001 | 0.002 | 0.993 | 0.002 | 0.002 | 0.998 | 0.001 | 0.001 | 0.001 | 1.000 | 0.000 | 0.525 | 0.003 | 0.472 | 0.525 | 0.003 | 0.472 |
| 155 | 0.43 (0.02–1.3) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 156 | 3.59 (1.55–6.28) | 0.000 | 1.000 | 0.000 | 0.001 | 0.997 | 0.001 | 0.001 | 0.999 | 0.000 | 0.000 | 0.000 | 0.990 | 0.010 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 157 | 1.59 (0.38–3.52) | 0.000 | 1.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.533 | 0.467 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |
| 158 | 0.2 (0–0.89) | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 |

Appendix S3: Bolbitidoid phylogeny showing node numbers.



# Chapter 5

# General Discussion

## 5.1 Evolution and classification of Cuban *Elaphoglossum* and *Asplenium*

### 5.1.1 *Elaphoglossum*

To test the morphology-based classification of the Cuban *Elaphoglossum* and shed light on the evolution of the genus in the West Indies, I conducted phylogenetic analyses of a chloroplast DNA dataset that included 79 new sequences representing 20 species of *Elaphoglossum* (18 from Cuba, and two from Dominican Republic) and 299 other GenBank-downloaded sequences of *Elaphoglossum* and its most closely related outgroups, the genera *Arthrobotrya*, *Bolbitis*, *Lomagramma*, *Mickelia*, and *Teratophyllum*. The results (Chapter 2) confirm the existence of the seven main lineages of *Elaphoglossum* recovered in previous phylogenetic studies, but revealed an eighth lineage, the Cuban endemic *E. wrightii*, which diverged early in the evolution of the genus. I therefore proposed a new section for this species, section *Wrightiana*. My data for the first time reveal the precise relationships of various Cuban species of *Elaphoglossum* (Chapter 2). The phylogenetic position of *E. wrightii* was unexpected; the species had previously been classified with species in section *Squamipedia* that have long-creeping rhizomes and echinulate spores and that lack phyllopodia. My discovery that *E. wrightii* is not closely related to those species implies that those morphological traits were uninformative about species relationships or had been misinterpreted. The study of living specimens and more herbarium material of *E. wrightii* led me to conclude that this species differs from other representatives of section *Squamipedia* by lacking echinulate spores (Chapter 2: Fig. 4) and by possessing phyllopodia (Chapter 2: Fig. 3b). Most importantly, *E. wrightii* starts growing on the soil and climbs from there to the lower portions of tree trunks (Chapter 2: Fig. 3c-f). It is the only species of *Elaphoglossum* that is a root climber, a growth form otherwise typical of the closely related genera *Arthrobotrya*, *Lomagramma*, *Mickelia*, and *Teratophyllum* (Moran, Labiak, and Sundue, 2010). The distribution of this character on the phylogeny suggested that the root-climbing growth habit may be plesiomorphic, and that the predominant holo-epiphytism (growth without connection to the ground) in the remaining species of *Elaphoglossum* might be derived.

The contrast between the huge species diversity of *Elaphoglossum* (600 species) and that of its closest related lineages *Arthrobotrya* (3 species), *Bolbitis* (55), *Lomagramma* (22), *Mickelia* (10), and *Teratophyllum* (11) suggests that the epiphytic habit that evolved in *Elaphoglossum* might have fuelled species diversification in this lineage. The rhizomes of the early-diverging species *E. wrightii* and also *E.*

*amygdalifolium* are elongate and creeping, while most other *Elaphoglossum* species have short, compact rhizomes. Compact rhizomes could have been a precondition for the evolution of holo-epiphytism (Schneider et al., 2010), but additional physiological and ecological studies are necessary to evaluate this hypothesis. Any attribution of increased speciation or lowered extinction rates (over millions of years and vast regions of the Earth) to single morphological traits in my view may be naïve. This, based on limited species sampling, Schuettpelz and Pryer (2009) claimed that high Cenozoic diversification rates of ferns were associated with the evolution of epiphytism, but statistically it is not possible to distinguish between increased speciation and decreased extinction rates. More recent studies found no differences between the diversification rates of epiphytic and non-epiphytic ferns (Sundue, Testo, and Ranker, 2015; Testo and Sundue, 2016). Perhaps chlorophyllous spores, which have more rapid germination than achlorophyllous spores, facilitated the radiation of grammitid ferns in epiphytic habitats (Schneider et al., 2004b), and such spores also occur in my target genus *Elaphoglossum* (Sundue, Vasco, and Moran, 2011). It would be interesting to study the evolution of this feature in *Elaphoglossum*, using field and lab experiments on germination rates under controlled conditions.

From a biogeographic perspective, the phylogenetic placement of the Cuban endemic *E. wrightii* as an early diverging species of *Elaphoglossum* could be explained by colonization from the continent in the relatively recent past, followed by extinction of the ancestral populations on the continent. It is also possible that my sampling of species from Florida, Central America, Colombia, and Venezuela is too sparse for me to have detected the ancestral or most closely related species

### 5.1.2 *Asplenium*

To elucidate the phylogenetic position of two species of Aspleniaceae with unique morphology, *Schaffneria nigripes*, and *Asplenium pumilum*, I conducted phylogenetic analyses of a plastid DNA dataset that included 16 new sequences representatives of the distribution of the two species in Cuba. The results (Chapter 3) recovered *S. nigripes* within *Asplenium*, and I am therefore treating *Schaffneria* as a synonym of *Asplenium* and retaining Hooker's placement of the species in *Asplenium*. Based on my current sampling, *Asplenium* (*Schaffneria*) *nigripes* is sister to *A. pumilum*, but I could not detect morphological characters that support the sister relationship recovered by the DNA phylogeny. *Asplenium nigripes* and *A. pumilum* share features of

the rhizome scales, spore ornamentation, and gametophyte development (Chapter 3: Figs. 3, 4), but those can also be found elsewhere in the phylogeny. An important limitation for the definition of morphological synapomorphies between these two species is that many potentially informative characters have not been studied for all species.

My phylogenetic results also show that *Asplenium nigripes* and *A. pumilum* are clustered within the clade VII of Schneider et al. (2004c), which is constituted mostly by four lineages of the so-called "black-stemmed" rock spleenworts (Schneider et al., 2005; Dyer, Savolainen, and Schneider, 2012; Chang et al., 2013). Inferring the relationship of the *A. nigripes/A. pumilum* clade and other lineages within this group with higher confidence will require further species sampling. A species that could be a key is the Chinese *A. delavayi*, which resembles the neotropical *A. nigripes* in its small size, black leaf petioles, entire, rounded leaf blades, reticulate venation, and scolopendrioid sori (see Copeland, 1947; Tardieu-Blot, 1957; Chapter 3: Fig. 1a–b). Other important species missing in my sampling are the Mexican *A. minimum* and *A. arcanum* (see Mickel and Smith, 2004) whose leaves resemble *A. pumilum* in the basal pair of pinnae with the basiscopic side more developed than the acroscopic side (Chapter 3: Fig. 1d–e).

Some 69 species of *Asplenium* have been reported for the Antilles, with 39% of them (27 species) endemic to the region. The highest species richness is distributed in the Greater Antilles, 50 in Hispaniola (72% of the Antillean species richness), 41 in Jamaica (59%), 32 in Cuba (46%) and 23 in Puerto Rico (33%) (Lóriga, unpublished data). The knowledge of this diversity is almost entirely based on morphological data. In addition to the results presented in Chapter 3, exploratory molecular phylogenetic and morphologic analyses of Cuban samples suggest the existence of an important level of cryptic diversity. For example, I found two lineages with overlapping distributions and variable spore sizes within what is currently known as *A. dentatum* (Fig. 5a). This species is the most widely distributed *Asplenium* in Cuba and is also present in other West Indian islands, Florida, Central America and northern South America. Another example is *A. heterochroum*, a species known from few localities in western and central Cuba and also present on Central America, Mexico and Florida. I found that the samples from western Cuba (very close to the type locality), are genetically divergent from conspecific samples from Central America (Fig. 5b). Upon close inspection, I found the Cuban specimens have 64 spores per sporangium but the Central American specimens are known to have 32 (Dyer, Savolainen, and Schneider, 2012). Under this scenario, the Central American specimens may then represent a different taxon. These preliminary results highlight the

importance of additional studies on the diversity of Antillean *Asplenium* with an integrative approach.

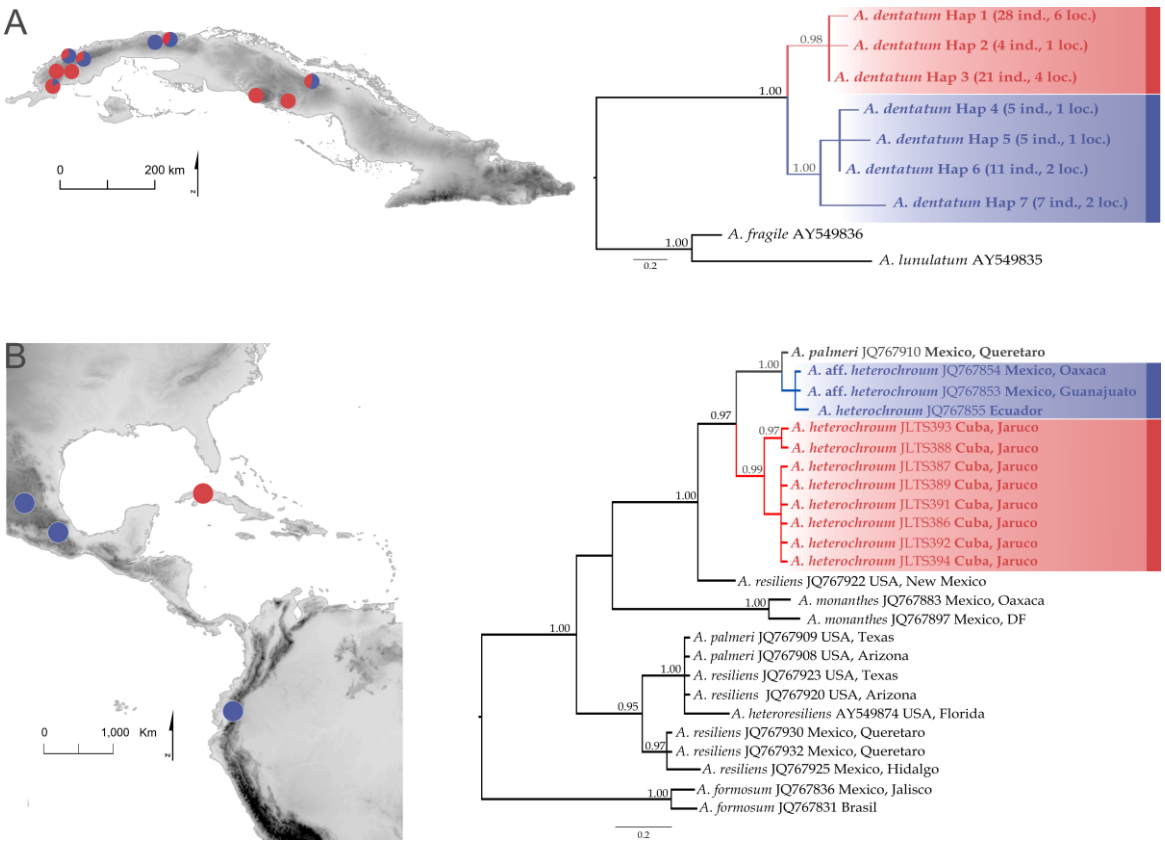



**Figure 5.** Underestimation of genetic diversity in Antillean / Neotropical *Asplenium* ferns. Preliminary results on two species complexes are shown in a topographic map with the distribution of localities sampled (left) and a phylogenetic tree obtained from a Bayesian analysis of aligned DNA sequences of the chloroplast trnL-trnF region (right). A: Phylogenetic analysis of 78 individuals of *A. dentatum* from 10 localities reveals two reciprocally-monophyletic clades with overlapping geographic distributions in Cuba (haplotype frequencies per locality are indicated by pie charts). B: Phylogenetic analysis of eight samples of *A. heterochrum* from a single locality in Western Cuba and homologous GenBank sequences renders the species paraphyletic and stresses the need for a taxonomic revision.

## 5.2 The inclusion of *Elaphoglossum* in Miocene Dominican amber

Well-preserved fossils of epiphytic ferns are scarce (Schneider et al., 2004a; Schuettpelz and Pryer, 2009). Yet it is precisely among the epiphytic lineages where most of the species-rich radiations occur, and the gaps in the fossil record thus hinder our understanding of the evolutionary history of key lineages. In recent years, Dominican amber is becoming an important source of epiphytic fern fossils (cf. section 1.3 in the Introduction of this thesis). During my doctoral research, a fern inclusion in Dominican

amber became available for study, and the comparison of the morphological characters preserved in the fossil with those of extant ferns allowed me to assign it to the genus *Elaphoglossum*. To reduce the uncertainty in the placement of this fossil in the phylogeny, I generated a molecular phylogeny of 158 extant species of *Elaphoglossum* and its relatives and reconstructed the evolution of the morphological characters observable in the fossil, assuming that the morphology of the genus has remained stable through time. This approach supported the placement of the amber fossil within *Elaphoglossum*, and the age of the amber, estimated as 15–20 Myr (Iturralde- Vinent and MacPhee, 1996), matches molecular-clock based age estimates of the group obtained without including the fossil as an age constraint.

The seven clades of *Elaphoglossum* currently ranked at sectional level are well supported by molecular and morphological characters (see Chapter 2). The fossil, however, could not be assigned to any section because important characters, such as rhizome habit and presence or absence of hydathodes, are not preserved (Rouhan et al., 2004; Moran, Labiak, and Sundue, 2010; Chapter 2). Nevertheless, I exclude a possibly placement in section *Lepidoglossa* because the scales of the fossil lack the unicellular marginal teeth characteristic in the extant species of this section (Vasco, 2010; Chapter 2: Fig. 2, Character 6). Sections, *Polytrichia* and *Setosa* were also excluded because they have subulate (longitudinally enrolled) scales on the leaves (Mickel and Atehortúa, 1980; Chapter 2: Fig. 2, character 5). Thus, I propose that the fossil probably belong to one of the remaining sections, namely *Amygdalifolia*, *Elaphoglossum*, *Squamipedia*, or *Wrightiana*. Other studies of organisms preserved in Dominican amber suggest a relative stability of the structure of morphologic diversity in plants and vertebrate communities inhabiting the Miocene forests of Hispaniola (Sherratt et al., 2015; Kaasalainen et al., 2017; Lee et al., 2017), and the *Elaphoglossum* fossil that I studied, together with other fern inclusions, point to the existence, during the mid- to late Miocene, of an epiphytic fern community composed of representatives of the lineages that still inhabit the region today, such as *Pleopeltis* (Schneider et al., 2015) and grammitid ferns (Gómez, 1982; Sundue and Poinar, 2016).

### 5.3 General conclusion and perspective

My studies on *Elaphoglossum* resulted in a phylogeny that includes 25 of the Cuban species (Chapter 2). The most important finding is that the endemic *E. wrightii* is an early-diverging lineage of the genus, which caused me to rank it as a new section,

*Wrightiana*. The Cuban species so far sequenced belong to six of the (now) seven sections of *Elaphoglossum*, but some eight Cuban species still need to be investigated using DNA sequencing. My other research focus, on the Cuban Aspleniaceae (Chapter 3), revealed that *Schaffneria nigripes* is nested within *Asplenium* and sister to *A. pumilum*, both being species with an unusual morphology, which is why morphological studies were unable to detect their true relationships. More species sampling is needed to understand the relationship between these two species and other species in "clade VII" of Schneider et al. (2004c). Most importantly, the results of my doctoral research show the importance of Cuba in the evolution of the *Elaphoglossum* and *Asplenium*, two of the most species-rich genera of ferns today.

My third research focus, the study of a frond fragment included in Dominican amber, led to the discovery of the first known fossil of *Elaphoglossum* and one of the few well-preserved fossils for the family Dryopteridaceae, making this amber inclusion a useful calibration point for future molecular divergence-time estimates in ferns.

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# CURRICULUM VITAE

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## EDUCATION

| | |
|---|---|
| 03/2013 – 01/2018 | Ph.D.: "Evolution and classification of *Elaphoglossum* and *Asplenium* ferns on Cuba, and discovery of a Miocene *Elaphoglossum* in Dominican amber"<br>Advisor: Prof. Dr. J. Heinrichs |
| 2012 | Master in Biological Sciences. Mention: Systematic of Vascular Plants, University of Havana.<br>Thesis: "Diversity, geographical distribution and priority areas for the conservation of the genus *Elaphoglossum* (Dryopteridaceae) in Cuba"<br>Advisors: Dr. J. Perez, Dr. L. Regalado |
| 10/1999 – 07/2004 | Bachelor in Biological Sciences, University of Havana.<br>Thesis: "Taxonomical study of subulate scaled-species of the genus *Elaphoglossum* (Lomariopsidaceae: Pteridophyta) in Cuba"<br>Advisors: Prof. Dr. C. Sánchez, Dr. L. Regalado |

## PEER-REVIEWED PUBLICATIONS

Regalado, L., **Lóriga, J.**, Bechteler, J., Beck, A., Schneider, H., Heinrichs, J. 2018. Phylogenetic biogeography reveals the timing and source areas of the *Adiantum* species (Pteridaceae) in the West Indies, with a special focus on Cuba. *Journal of Biogeography*.

Schneider, H., Liu, H.-M., Chang, Y.-F., Ohlsen, D., Perrie, L.R., Shepherd, L., Kessler, M., Karger, D.N., Hennequin, S., Marquardt, J., Russell, S., Ansell, S., Lu, N.T., Kamau, P., **Lóriga, J.**, Regalado, L., Heinrichs, J., Ebihara, A., Smith, A.R., Gibby, M. 2017. Neo- and Paleopolyploidy contribute to the species diversity of Asplenium—the most species-rich genus of ferns. *Journal of Systematics and Evolution*, 55, 353–364.

**Lóriga, J.**, Regalado, L., Prada, C., Schneider, H., Heinrichs, J. 2016. Phylogenetic relationships of two Cuban spleenworts with unusual morphology: *Asplenium*

(*Schaffneria*) *nigripes* and *Asplenium pumilum* (Aspleniaceae, leptosporangiate ferns). *Plant Systematics and Evolution* 303, 165–176.

Vasco, A., **Lóriga, J.**, Rouhan, G., Ambrose, B.A., Moran, R.C. 2015. Divided leaves in the genus *Elaphoglossum* (Dryopteridaceae): a phylogeny of *Elaphoglossum* section *Squamipedia*. *Systematic Botany* 40, 46–55.

**Lóriga, J.**, Schmidt, A.R., Moran, R.C., Feldberg, K., Schneider, H., Heinrichs, J. 2014. The first fossil of a bolbitidoid fern belongs to the early-divergent lineages of *Elaphoglossum* (Dryopteridaceae). *American Journal of Botany* 101, 1466–1475.

**Lóriga, J**., Vasco, A., Regalado, L., Heinrichs, J., Moran, R.C. 2014. Phylogeny and classification of the Cuban species of *Elaphoglossum* (Dryopteridaceae), with description of *Elaphoglossum* sect. *Wrightiana* sect. nov. *Plant Systematics and Evolution* 300, 937–951.

Regalado, L., **Lóriga, J.** 2009-2010. Los helechos y licófitos de la Sierra de la Güira y sus alrededores, Pinar del Río, Cuba. *Revista del Jardín Botánico Nacional* 30–31, 131–140.

**RESEARCH PRESENTATIONS (*Speaker)**

Vasco, A., **Lóriga, J.**, Moran, R.C. 2013. "Systematics and Phylogeny of *Elaphoglossum* section *Squamipedia*", Botanical Society of America annual Conference (New Orleans, USA).

**Lóriga, J.** *. 2012. "Los helechos del género *Elaphoglossum* (Dryopteridaceae) en Cuba", XIII National Workshop Flora of the Republic of Cuba (Havana, Cuba).

**Lóriga, J.** *. 2011. "Exploring biodiversity of the fern genus *Elaphoglossum* in Cuba", Institute seminar, The New York Botanical Garden seminar (New York, USA)

**Lóriga, J**.* 2010. "Avances en la taxonomía del género *Elaphoglossum* (Dryopteridaceae) en Cuba", XI National Workshop Flora of the Republic of Cuba (Havana, Cuba).

**Lóriga, J**.* 2008. "Distribución geográfica del género *Elaphoglossum* (Dryopteridaceae) en Cuba", VIII Cuban Symposium of Botany (Havana, Cuba).

Regalado L., **Lóriga, J.** 2005. "Redescubrimento de *Asplenium delitescens*, Aspleniaceae: Pteridophyta", X Botanical Meeting Johannes Bisse in memorian (Camagüey, Cuba).

Regalado L., **Lóriga, J.** 2005. "Helechos y plantas afines de la Sierra la Güira", V Biodiversity Workshop BIOECO (Santiago de Cuba, Cuba).

## RESEARCH EXPERIENCE

10/2004 – 07/2012: Researcher at the Division of Vascular Plants, Department of Botany and National Herbarium, Institute of Ecology and Systematics (IES), Ciudad de la Habana, Cuba. Involved in projects of: database and Herbarium collection management, floristic inventories of ferns, Systematic and Taxonomy of ferns, Biodiversity conservation, and Invasive species in Cuba.

## RELEVANT SKILLS

Laboratory skills (ferns): genomic DNA extraction, PCR amplification, sanger-sequencing.

Data analysis: Phylogenetic, biogeographic, Divergence time estimates, and ancestral state reconstruction analyses (RAxML, MrBayes, BEAST, R, PartitionFinder, jModelTest).

Geographic Information Systems (GIS): Mapping and species distributions analyses.

Fern morphology, anatomy and taxonomy (mainly from West Indies).

## TEACHING

2014: Practices assistant in "Evolution der Farne und Bärlappe" taught by Heinrichs and Feldberg

## AWARDS AND SCHOLARSHIPS

International Association for Plant Taxonomy (IAPT) research grant for the studies on Taxonomic treatment of the fern genus *Elaphoglossum* (Dryopteridaceae) in Cuba (August, 2009).

DAAD Research Grant for Doctoral Candidates for the project "Exploring the origin and diversification of spleenwort fern flora of the Caribbean with focus on Greater Antilles" (September 2013 – August 2016)

International Association for Plant Taxonomy (IAPT) research grant for the study Integrative taxonomy of the spleenwort fern flora of Cuba (March, 2013).