

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

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Received: 23 May 2013 / Accepted: 12 October 2013  
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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 *atpβ-rbcL* (including a fragment of the gene *atpβ*), *rps4-trnS*, and *trnL-trnF*. 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*, *Lomagamma*, *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

## 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 hemiepiphyte (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,

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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 *atpB-rbcL* (including a fragment of the gene *atpB*), *rps4-trnS* and *trnL-trnF* 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 bolbitoid fern sequences (Rouhan et al. 2004, 2007; Skog et al. 2004; Vasco et al. 2009a) from GenBank. Species belonging to the bolbitoid 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	<i>atpβ- rbcL</i>	<i>rps4-trnS</i>	<i>trnL-trnF</i>
<i>Arthrobotrya wilkesiana</i> (Brack.) Copel.	Ranker 1937 (UC)	French Polynesia	–	GU376719	GU376569
<i>Bolbitis auriculata</i> (Lam.) Alston	Rouhan 101 (NY)	Mauritius	–	GU376649	GU376505
<i>Bolbitis auriculata</i> (Lam.) Alston	Fay 1110 (NY)	Sierra Leone	EF040664	–	–
<i>Elaphoglossum achroalepis</i> (Baker) C. Chr.	Rakotondrainibe 6485 (P)	Madagascar	EF040636	AY540225	AY536288
<i>Elaphoglossum acrostichoides</i> (Hook. & Grev.) Schelpe	Rouhan 229 (P)	La Réunion	EF040654	EF040628	EF040614
<i>Elaphoglossum aemulum</i> (Kaulf.) Brack	Lorence 8514 (PTBG)	Hawaii	–	AY540227	AY536290
<i>Elaphoglossum aff. ciliatum</i> (C. Presl) T. Moore	Moran 6711 (NY)	Ecuador	EU907673	EU907748	EU907813
<i>Elaphoglossum affine</i> (M. Martens & Galeotti) T. Moore	Mickel 9694 (NY)	Mexico	–	AY536169	AY534841
<i>Elaphoglossum albescens</i> (Sodiolo) Christ	Vasco 739 (HUA, NY)	Colombia	–	GU376678	GU376532
<i>Elaphoglossum alismaefolium</i> (Fée) T. Moore	Lóriga and Rodríguez 159 (HAC)	Cuba	<b>KF212374</b>	<b>KF212425</b>	<b>KF212399</b>
<i>Elaphoglossum amygdalifolium</i> (Mett. ex Kuhn) Christ	Herrera 2063 (CR, INB, NY, USJ)	Costa Rica	–	AY536173	AY534845
<i>Elaphoglossum angulatum</i> (Blume) T. Moore	Rouhan 220 (NY, P)	La Réunion	EF040655	AY540230	AY536293
<i>Elaphoglossum apodum</i> (Kaulf.) Schott ex J. Sm.	Lóriga and Rodríguez 68 (HAC)	Cuba	–	–	<b>KF212400</b>
<i>Elaphoglossum apodum</i> (Kaulf.) Schott ex J. Sm.	Trusty 120 (NY)	Costa Rica	EF040651	EF040625	EF040611
<i>Elaphoglossum asterolepis</i> (Baker) C. Chr.	Kessler 12751 (P)	Madagascar	EF040642	AY540231	AY536294
<i>Elaphoglossum aubertii</i> (Desv.) T. Moore	Rouhan 110 (P)	Comoros	EF040647	EF040622	EF040608
<i>Elaphoglossum auricomum</i> (Kunze) T. Moore	Hammer 3 (NY)	Mexico	–	AY536145	AY534817
<i>Elaphoglossum auripilum</i> Christ	Moran 6377 (NY)	Costa Rica	EF040652	EF040626	EF040612
<i>Elaphoglossum avaratraense</i> Rakotondr.	Rakotondrainibe 1456 (P)	Madagascar	EU907660	AY540233	AY536296
<i>Elaphoglossum backhousianum</i> T. Moore	Moran 6321 (CR, INB, NY, UCR)	Costa Rica	–	AY540234	AY536297
<i>Elaphoglossum bifurcatum</i> (Jacq.) Mickel	Eastwood 215 (–)	St. Helena	EU907661	EU907737	AY194070
<i>Elaphoglossum biolleyi</i> Christ	Boyle 6397 (CR, INB, NY, UCR)	Costa Rica	–	AY540235	AY536298
<i>Elaphoglossum boryanum</i> (Fée) T. Moore	Meier et al. 6768 (NY, VEN)	Venezuela	–	AY536133	AY534804
<i>Elaphoglossum burchellii</i> (Baker) C. Chr.	Jimenez 2460 (NY)	Bolivia	EU907663	EU907738	EU907803
<i>Elaphoglossum cardiophyllum</i> (Hook.) T. Moore	Holm-Nielsen 17480 (AAU, NY)	Ecuador	–	AY536171	AY534842
<i>Elaphoglossum cf. erinaceum</i> (Fée) T. Moore	NYBG living collection 554/79A	Mexico	–	AY536135	AY534806
<i>Elaphoglossum cf. longifolium</i> (Jacq.) J. Sm.	Lóriga and Rodríguez 151 (HAC)	Cuba	<b>KF212375</b>	<b>KF212426</b>	<b>KF212401</b>
<i>Elaphoglossum cf. longifolium</i> (Jacq.) J. Sm.	Lóriga and Regalado 2 (HAC)	Cuba	<b>KF212376</b>	<b>KF212427</b>	<b>KF212402</b>
<i>Elaphoglossum cf. petiolatum</i> (Sw.) Urb.	Moran 7573 (NY)	Ecuador	EU907714	EU907785	EU907848
<i>Elaphoglossum ciliatum</i> (C. Presl) T. Moore	Vasco 468 (HUA, MO, NY)	Colombia	EU907670	EU907745	EU907810
<i>Elaphoglossum ciliatum</i> (C. Presl) T. Moore	Vasco 504 (HUA, MO, NY)	Colombia	EU907671	EU907746	EU907811
<i>Elaphoglossum cismense</i> Rosenst.	Van Ee 327 (CR, INB, NY, UCR)	Costa Rica	–	AY540237	AY536300
<i>Elaphoglossum coriaceum</i> Bonap.	Rouhan 145 (P)	Seychelles	EF040653	EF040627	EF040613
<i>Elaphoglossum coursii</i> Tardieu	Rouhan 127 (NY, P)	Comoros	–	AY540240	AY536303
<i>Elaphoglossum crinitum</i> (L.) Christ.	Lóriga and Rodríguez 258 (HAC)	Cuba	<b>KF212377</b>	<b>KF212428</b>	<b>KF212403</b>
<i>Elaphoglossum crinitum</i> (L.) Christ	NYBG living collection 233/94	Dominican Republic	–	AY536134	AY534805

Table 1 continued

Species	Voucher and herbaria	Country	<i>atpβ- rbcL</i>	<i>rps4-trnS</i>	<i>trnL-trnF</i>
<i>Elaphoglossum croatii</i> Mickel	Moran 6378 (CR, INB, NY, UCR)	Costa Rica	–	AY540241	AY536304
<i>Elaphoglossum cubense</i> (Mett. ex Kuhn) C. Chr.	Lóriga and Rodríguez 155 (HAC)	Cuba	<b>KF212378</b>	<b>KF212429</b>	<b>KF212404</b>
<i>Elaphoglossum cubense</i> (Mett. ex Kuhn) C. Chr.	Regalado s.n. (HAC)	Cuba	<b>KF212379</b>	<b>KF212430</b>	<b>KF212405</b>
<i>Elaphoglossum cuspidatum</i> (Willd.) T. Moore	Jiménez 754 (LPB)	Bolivia	EU907675	EU907750	EU907815
<i>Elaphoglossum davidsei</i> Mickel	Moran 6366 (CR, INB, NY, UCR)	Costa Rica	–	AY540242	AY536305
<i>Elaphoglossum decaryanum</i> Tardieu	Rakotondrainibe 6326 (P)	Madagascar	EF040658	AY540243	AY536306
<i>Elaphoglossum deckenii</i> (Kuhn) C. Chr.	Rouhan 105 (CNDRS, NY, P, PTBG)	Comoros	–	AY540244	AY536307
<i>Elaphoglossum decoratum</i> (Kunze) T. Moore	Labiak 4074 (UPCB)	Brazil	<b>KF212380</b>	GU376681	GU376534
<i>Elaphoglossum dendricola</i> (Baker) C. Chr.	Moran 6853 (NY, QCA, QCNE)	Ecuador	EU907676	EU907751	EU907816
<i>Elaphoglossum dimorphum</i> (Hook. & Grev.) T.	Eastwood 302 (–)	St. Helena	EU907677	EU907752	AY194068
<i>Elaphoglossum dussii</i> Underw. ex Maxon	Sanchez 138 (NY)	Puerto Rico	EU907681	EU907755	EU907819
<i>Elaphoglossum dussii</i> Underw. ex Maxon	Christenhusz 4011 (NY)	Guadeloupe	EU907679	EU907754	EU907818
<i>Elaphoglossum edwallii</i> Rosenstock	Prado et al. 1123 (NY)	Brazil	–	AY536144	AY534816
<i>Elaphoglossum eggersii</i> (Baker) Christ	Lóriga and Rodríguez 157 (HAC)	Cuba	<b>KF212381</b>	<b>KF212431</b>	<b>KF212406</b>
<i>Elaphoglossum erinaceum</i> (Fée) T. Moore	Lóriga and Rodríguez 162 (HAC)	Cuba	–	<b>KF212432</b>	<b>KF212407</b>
<i>Elaphoglossum eximium</i> (Mett.) Christ	Moraga 485 (NY)	Costa Rica	–	AY536132	AY534803
<i>Elaphoglossum firmum</i> (Mett. ex. Kuhn) Urb.	Lóriga and Rodríguez 74 (HAC)	Cuba	<b>KF212382</b>	<b>KF212433</b>	<b>KF212408</b>
<i>Elaphoglossum flaccidum</i> (Fée) T. Moore	Mori 25578 (NY)	French Guiana	EF040657	AY540246	AY536309
<i>Elaphoglossum forsythii-majoris</i> Christ	Kessler 12678 (P)	Madagascar	EF040644	EF040620	EF040606
<i>Elaphoglossum fournierianum</i> L. D. Gómez	Moran 6336 (CR, INB, NY, UCR)	Costa Rica	–	AY540248	AY536311
<i>Elaphoglossum gayanum</i> (Fée) T. Moore	Mickel 9695 (NY)	Mexico	–	AY536166	AY534838
<i>Elaphoglossum glabellum</i> J. Sm.	Prado et al. 1129 (NY)	Brazil	–	AY536167	AY534839
<i>Elaphoglossum gramineum</i> (Jenman) Urb.	Lóriga and Rodríguez 331 (HAC)	Cuba	<b>KF212383</b>	<b>KF212434</b>	<b>KF212409</b>
<i>Elaphoglossum gramineum</i> (Jenman) Urb.	Anderson 3223 (US)	Jamaica	EU907682	EU907756	EU907820
<i>Elaphoglossum gramineum</i> (Jenman) Urb.	Proctor 3907 (US)	Jamaica	–	EU907757	EU907821
<i>Elaphoglossum grayumii</i> Mickel	Moran 6329 (CR, INB, NY, UCR)	Costa Rica	–	AY540250	AY536313
<i>Elaphoglossum guatemalense</i> (Klotzsch) T. Moore	Mickel 9701 (NY)	Mexico	–	AY536164	AY534836
<i>Elaphoglossum guentheri</i> Rosenst.	Lehnert 1306 (NY)	Ecuador	–	GU376682	GU376535
<i>Elaphoglossum herminieri</i> (Bory & Fée) T. Moore	Lóriga and Rodríguez 77 (HAC)	Cuba	<b>KF212384</b>	<b>KF212435</b>	<b>KF212410</b>
<i>Elaphoglossum herminieri</i> (Bory & Fée) T. Moore	Blanco 1559 (F, USJ)	Costa Rica	–	AY536163	AY534835
<i>Elaphoglossum heterolepis</i> (Fée) T. Moore	Rouhan 177 (P)	Mauritius	EU907683	AY540251	AY536314
<i>Elaphoglossum hoffmannii</i> (Mett. ex Kuhn) Christ	Moran 6365 (CR, INB, NY, UCR)	Costa Rica	–	AY540252	AY536315
<i>Elaphoglossum huacsaro</i> (Ruíz) Christ	Vasco 568 (HUA, NY)	Colombia	EU907694	EU907769	EU907832
<i>Elaphoglossum humbertii</i> C. Chr.	Rouhan 466 (P)	Madagascar	EU907696	EU907771	EU907834
<i>Elaphoglossum hybridum</i> (Bory) Brack.	Rouhan 250 (P)	La Réunion	EU907697	EU907772	EU907835
<i>Elaphoglossum ipshookense</i> Mickel	Mickel 4748 (NY)	Mexico	EU907698	EU907773	EU907836
<i>Elaphoglossum lanatum</i> (Bojer ex Baker) Lorence	Rouhan 194 (MAU, NY, P, PTBG)	Mauritius	–	AY540258	AY536321

Table 1 continued

Species	Voucher and herbaria	Country	<i>atpβ- rbcL</i>	<i>rps4-trnS</i>	<i>trnL-trnF</i>
<i>Elaphoglossum lancifolium</i> (Desv.) C.V. Morton	Rouhan 201 (NY, P)	La Réunion	EU907699	AY540259	AY536322
<i>Elaphoglossum langsdorffii</i> (Hook. & Grev.) T. Moore	Labiak 4113 (UPCB)	Brazil	–	GU376683	GU376536
<i>Elaphoglossum leucolepis</i> (Baker) Krajina ex Tardieu	Rakotondrainibe 6339 (P)	Madagascar	EF040638	AY540261	AY536324
<i>Elaphoglossum lindenii</i> (Bory ex Fée) T. Moore	Mickel 9652 (NY)	Mexico	–	AY536130	AY534801
<i>Elaphoglossum lingua</i> (C. Presl) Brack.	Moran 6380 (NY)	Costa Rica	–	AY540262	AY536325
<i>Elaphoglossum lloense</i> (Hook.) T. Moore	Vasco 539 (HUA, NY)	Colombia	–	GU376684	GU376537
<i>Elaphoglossum lonchophyllum</i> (Fée) T. Moore	Hammer 9 (NY)	Mexico	–	AY536136	AY534807
<i>Elaphoglossum luridum</i> (Fée) Christ	NYBG living collections 2001-0052	Peru	<b>KF212385</b>	AY540263	AY536326
<i>Elaphoglossum macropodium</i> (Fée) T. Moore	Rouhan 209 (NY, P)	La Réunion	–	AY540264	AY536327
<i>Elaphoglossum malgassicum</i> C. Chr.	Kessler 12725 (NY)	Madagascar	EF040659	AY540265	AY536328
<i>Elaphoglossum marojejense</i> Tardieu	Rakotondrainibe 6429 (P)	Madagascar	EF040630	AY540266	AY536329
<i>Elaphoglossum martinicense</i> (Desv.) T. Moore	Lóriga and Rodríguez 255 (NY)	Cuba	<b>KF212386</b>	<b>KF212436</b>	<b>KF212411</b>
<i>Elaphoglossum martinicense</i> (Desv.) T. Moore	Lóriga and Rodríguez 366 (HAC)	Cuba	<b>KF212387</b>	<b>KF212437</b>	<b>KF212412</b>
<i>Elaphoglossum maxonii</i> Underw. ex C.V. Morton	Lóriga and Rodríguez 341 (HAC)	Cuba	<b>KF212388</b>	<b>KF212438</b>	<b>KF212413</b>
<i>Elaphoglossum micropogon</i> Mickel	Moran 6353 (NY)	Costa Rica	EF040643	AY540268	AY536331
<i>Elaphoglossum minutum</i> (Pohl ex Fée) T. Moore	Ekman 14764 (NY)	Cuba	<b>KF212389</b>	<b>KF212439</b>	–
<i>Elaphoglossum minutum</i> (Pohl ex Fée) T. Moore	Zanoni et al. 30916 (NY)	Dominican Republic	<b>KF212390</b>	<b>KF212440</b>	<b>HG425359</b>
<i>Elaphoglossum mitorrhizum</i> Mickel	Boyle 6410 (CR, INB, NY, USJ)	Costa Rica	EF040656	AY540269	AY536332
<i>Elaphoglossum nervosum</i> C. Chr.	Eastwood 367 (–)	St. Helena	EU907701	EU907775	EU907837
<i>Elaphoglossum nidiforme</i> Mickel	Lehnert 1316 (NY)	Bolivia	EF040662	EF040629	EF040616
<i>Elaphoglossum nidusoides</i> Rouhan & Rakotondr	Rouhan 387 (P)	Madagascar	EF040634	EF040618	EF040604
<i>Elaphoglossum nigrescens</i> (Hook.) T. Moore ex Diels	Moran 7491 (NY)	Ecuador	EU907708	EU907781	EU907843
<i>Elaphoglossum nigrocostatum</i> Mickel	Luteyn 11051 (NY)	Venezuela	–	AY536152	AY534824
<i>Elaphoglossum oblanceolatum</i> C. Chr.	Gomez 21000 (NY)	Costa Rica	–	AY540271	AY536334
<i>Elaphoglossum ocoense</i> C. Chr.	Jones and Norris 1120 (NY)	Dominican Republic	–	<b>KF212441</b>	<b>KF212414</b>
<i>Elaphoglossum orbignyanum</i> (Fée) T. Moore	Bach 1773 (NY)	Bolivia	EU907710	EU907783	EU907845
<i>Elaphoglossum ovalilimbatum</i> Bonap.	Humbert 24895 (P)	Madagascar	–	AY540272	AY536335
<i>Elaphoglossum ovatum</i> (Hook. & Grev.) T. Moore	Smith 2872 (UC)	Ecuador	EF040641	AY540273	AY536336
<i>Elaphoglossum paleaceum</i> (Hook. & Grev.) Sledge	Mickel 9710 (NY)	Hawaii	EU907711	EU907784	EU907846
<i>Elaphoglossum palmeri</i> Underw. & Maxon	Lóriga and Rodríguez 67 (HAC)	Cuba	<b>KF212391</b>	<b>KF212442</b>	<b>KF212415</b>
<i>Elaphoglossum palmeri</i> Underw. & Maxon	Lóriga and Rodríguez 92 (HAC)	Cuba	<b>KF212392</b>	<b>KF212443</b>	<b>KF212416</b>
<i>Elaphoglossum papillosum</i> (Baker) Christ	Boyle 5816 (CR, INB, NY, USJ)	Costa Rica	–	AY536129	AY534800
<i>Elaphoglossum peltatum</i> (Sw.) Urb.,	Lóriga and Rodríguez 355 (HAC)	Cuba	<b>KF212393</b>	<b>KF212444</b>	<b>KF212417</b>
<i>Elaphoglossum peltatum</i> (Sw.) Urb.	Mickel 9703 (NY)	Mexico	EF040631	AY536159	AY534831
<i>Elaphoglossum petiolatum</i> (Sw.) Urb.	Nicholson and 782-01-A (NY)	Mexico	EU907712	AY540275	AY536338
<i>Elaphoglossum phanerophlebium</i> C. Chr.	Rakotondrainibe 6430 (P)	Madagascar	EF040646	AY540276	AY536339

Table 1 continued

Species	Voucher and herbaria	Country	<i>atpβ- rbcL</i>	<i>rps4-trnS</i>	<i>trnL-trnF</i>
<i>Elaphoglossum piloselloides</i> (C. Presl) T. Moore	Howard and Howard 9038 (NY)	Dominican Republic	–	<b>KF212445</b>	<b>KF212418</b>
<i>Elaphoglossum piloselloides</i> (C. Presl) T. Moore	Mickel 9708 (NY)	Mexico	–	AY536141	AY534812
<i>Elaphoglossum pilosius</i> Mickel	Moran 6338 (CR, INB, NY, UCR)	Costa Rica	–	AY540277	AY536340
<i>Elaphoglossum poolii</i> (Baker) Christ	Kessler 12702 (NY)	Madagascar	EF040639	AY540278	AY536341
<i>Elaphoglossum potosianum</i> Christ	Hinton 22679 (NY)	Mexico	EU907715	EU907786	EU907849
<i>Elaphoglossum prestonii</i> (Baker) J. Sm.	Prado et al. 1117 (NY)	Brazil	–	AY536139	AY534810
<i>Elaphoglossum pringlei</i> (Davenp.) C. Chr.	Campos 2650 (NY)	Mexico	EU907716	EU907787	EU907850
<i>Elaphoglossum productum</i> Rosenst.	Moran s.n. (CR, INB, NY, UCR)	Costa Rica	EU907733	AY540279	EU907861
<i>Elaphoglossum pusillum</i> (Mett. ex Kuhn) C. Chr.	Lóriga and Rodríguez 325 (HAC)	Cuba	<b>KF212394</b>	<b>HG428762</b>	<b>KF212420</b>
<i>Elaphoglossum pusillum</i> (Mett. ex Kuhn) C. Chr.	Valeur 568 (US)	Dominican Republic	–	<b>HG425357</b>	<b>KF212419</b>
<i>Elaphoglossum pygmaeum</i> (Mett. ex Kuhn) Christ	Smith 2826 (UC)	Ecuador	–	AY540281	AY536344
<i>Elaphoglossum rapense</i> Copel.	Motley 2677 (NY)	French Polynesia	–	AY540283	AY536365
<i>Elaphoglossum richardii</i> (Bory ex Fée) H. Christ	Rouhan 205 (P)	La Réunion	EF040645	EF040621	EF040607
<i>Elaphoglossum rufidulum</i> C. Chr.	Rakotondrainibe 6396 (P)	Madagascar	–	AY540285	AY536348
<i>Elaphoglossum russelliae</i> Mickel	Moran 6360 (CR, INB, NY, UCR)	Costa Rica	–	AY540286	AY536349
<i>Elaphoglossum rzedowskii</i> Mickel	Bartholomeus 2691 (NY)	Mexico	EU907718	EU907788	EU907851
<i>Elaphoglossum samoense</i> Brack.	Motley 2875 (NY)	Rapa	–	AY540287	AY536350
<i>Elaphoglossum sartorii</i> (Liebm.) Mickel	Mickel 9700 (NY)	Mexico	–	AY536161	AY534833
<i>Elaphoglossum scolopendriforme</i> Tardieu	Rakotondrainibe 6426 (P)	Madagascar	EU907719	AY540288	AY536351
<i>Elaphoglossum setigerum</i> (Sodirol) Diels	Van Ee 328 (CR, INB, NY, UCR)	Costa Rica	–	AY540289	AY536352
<i>Elaphoglossum sieberi</i> (Hook. & Grev.) T. Moore	Rouhan 169 (MAU, NY, P, PTBG)	Mauritius	EU907720	AY540290	AY536353
<i>Elaphoglossum siliquoides</i> (Jenman) C. Chr.	Lóriga and Rodríguez 220 (HAC)	Cuba	–	<b>HG425358</b>	<b>KF212421</b>
<i>Elaphoglossum siliquoides</i> (Jenman) C. Chr.	Smith 2631 (UC)	Costa Rica	–	AY536127	AY534798
<i>Elaphoglossum smithii</i> (Baker) Christ	Boyle 6409 (CR, INB, NY, UCR)	Costa Rica	–	AY540291	AY536354
<i>Elaphoglossum spatulatum</i> (Bory) T. Moore	Rakotondrainibe 6125 (NY, P, PTBG)	Madagascar	EF040649	EF040623	EF040609
<i>Elaphoglossum splendens</i> (Bory ex Willd.) Brack.	Rouhan 247 (P)	La Réunion	EU907721	AY540296	AY536359
<i>Elaphoglossum squamipes</i> (Hook.) T. Moore	Moran 6308 (CR, INB, NY, USJ)	Costa Rica	EF040635	AY536157	AY534829
<i>Elaphoglossum squamipes</i> (Hook.) T. Moore	Labiak et al. 1253 (NY, P)	Brazil	–	AY536158	AY534830
<i>Elaphoglossum succisaefolium</i> (Thouars) T. Moore	Marthel-Thoumian 1A (P)	Amsterdam Island	<b>KF212395</b>	AY540299	AY536362
<i>Elaphoglossum tectum</i> (Humb. & Bonpl. ex Willd.) T. Moore	Prado et al. 1126 (NY)	Brazil	–	AY536142	AY534813
<i>Elaphoglossum tenuiculum</i> (Fée) T. Moore ex Baker	Vasco 558 (NY)	Colombia	EU907722	–	EU907852
<i>Elaphoglossum tomentosum</i> (Bory ex Willd.) Christ	Rouhan 174 (P)	Mauritius	EU907723	AY540300	AY536363
<i>Elaphoglossum tripartitum</i> (Hook. & Grev.) Mickel	Fay and Fay 3344 (MO)	Ecuador	–	AY536156	AY534828
<i>Elaphoglossum vestitum</i> (Sw.) T. Moore	Mickel 9699 (NY)	Costa Rica	–	AY536146	AY534818

**Table 1** continued

Species	Voucher and herbaria	Country	<i>atpβ- rbcL</i>	<i>rps4-trnS</i>	<i>trnL-trnF</i>
<i>Elaphoglossum vieillardii</i> (Mett.) T. Moore	Munzinger 1361 (P)	New Caledonia	–	AY540301	AY536364
<i>Elaphoglossum wawrae</i> (Luerss.) C. Chr.	Lorence 8511 (PTBG)	Hawaii	–	AY540302	AY536365
<i>Elaphoglossum welwitschii</i> (Baker) C. Chr.	Taylor 9099 (P)	Tanzania	–	AY540303	AY536366
<i>Elaphoglossum wrightii</i> (Mett. ex D.C. Eaton) T. Moore	Lóriga and Rodríguez 254 (HAC)	Cuba	<b>KF212397</b>	<b>KF212447</b>	<b>KF212423</b>
<i>Elaphoglossum wrightii</i> (Mett. ex D.C. Eaton) T. Moore	Lóriga and Rodríguez 348 (HAC)	Cuba	<b>KF212398</b>	<b>KF212448</b>	<b>KF212424</b>
<i>Elaphoglossum wrightii</i> (Mett. ex D.C. Eaton) T. Moore	Lóriga and Rodríguez 242 (HAC)	Cuba	<b>KF212396</b>	<b>KF212446</b>	<b>KF212422</b>
<i>Elaphoglossum yungense</i> de la Sota	Jimenez 2487 (NY)	Bolivia	EU907731	EU907796	EU907859
<i>Lomagramma sinuata</i> C. Chr.	Grether 4056 (US)	Papua New Guinea	–	GU376706	GU376557
<i>Mickelia guianensis</i> (Aubl.) R.C. Moran, Labiak & Sundue	Secco 288 (NY)	Brazil	–	GU376698	GU376549
<i>Mickelia nicotianifolia</i> (Sw.) R.C. Moran, Labiak & Sundue	Christenhusz 4062 (TUR)	Guadeloupe	EF463382	–	–
<i>Mickelia nicotianifolia</i> (Sw.) R.C. Moran, Labiak & Sundue	Sanchez 124 (NY)	Puerto Rico	–	GU376669	GU376522
<i>Mickelia oligarchica</i> (Baker) R.C. Moran, Labiak & Sundue	Moran 6244 (NY)	Ecuador	–	GU376668	GU376521
<i>Teratophyllum ludens</i> (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: *atpβ*, first and second nucleotide position (GTR + I); *atpβ*, third position and *atpβ-rbcL* (GTR +  $\Gamma$ ); *rps4-trnS* (GTR +  $\Gamma$ ) and *trnL-trnF* (GTR +  $\Gamma$ ). 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 +  $\Gamma$  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	<i>atpB</i> - <i>rbcL</i>	<i>rps4</i> - <i>trnS</i>	<i>trnL</i> - <i>trnF</i>	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).

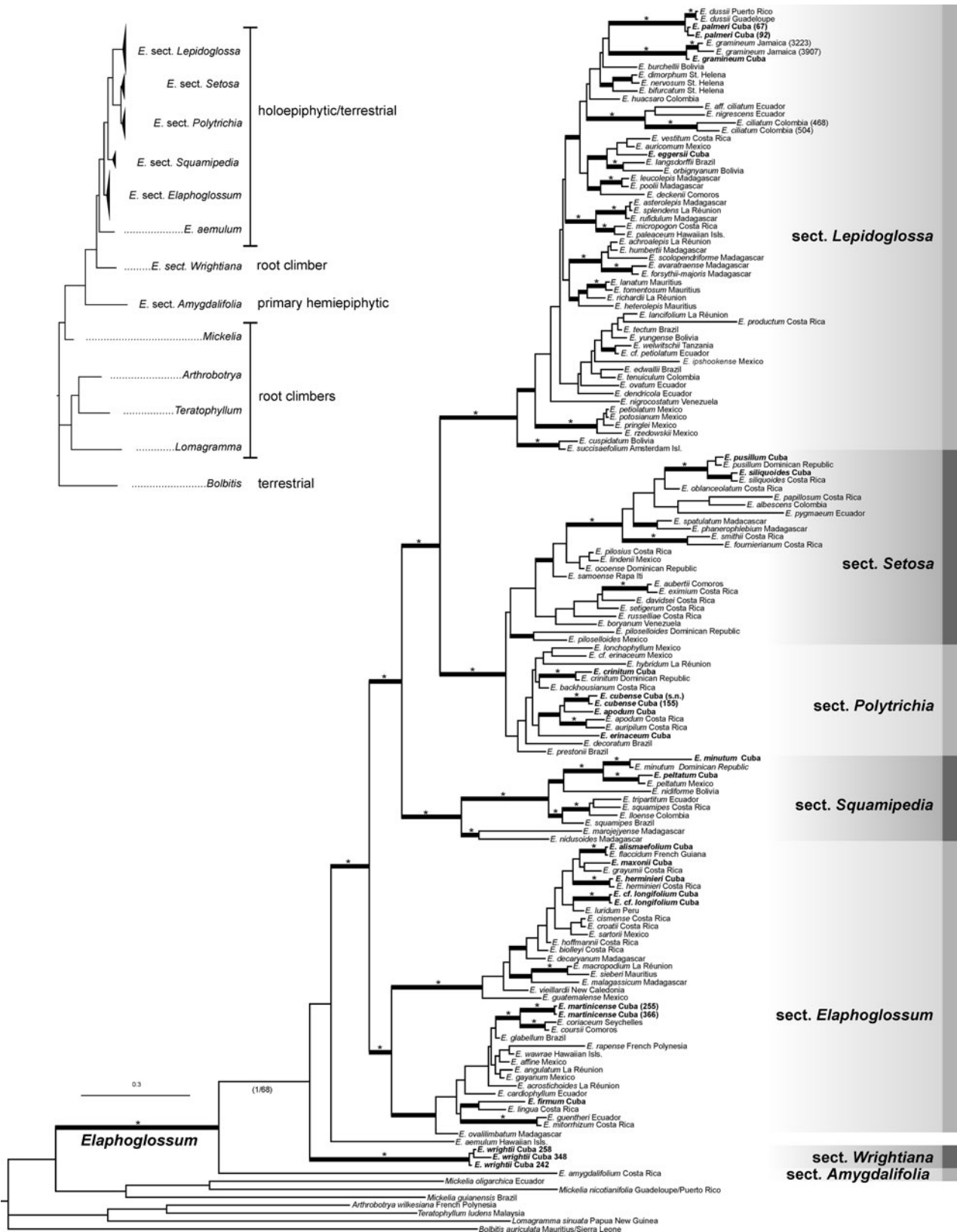
## Discussion

### 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. procurrans* (Mett. ex D. C. Eaton) T. Moore, *E. simplex* (Sw.) Schott. ex J.







**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	<i>E. sect. Amygdalifolia</i>	<i>E. sect. Elaphoglossum</i>	<i>E. sect. Lepidoglossa</i>	<i>E. sect. Setosa</i>	<i>E. sect. Polytrichia</i>	<i>E. sect. Squamipedia</i>	<i>E. sect. Wrightiana</i>
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
<b>Cuban species</b>		<b><i>E. alismaefolium</i></b> <i>E. decursivum</i> * <b><i>E. firmum</i></b> <b><i>E. flaccidum</i></b> <b><i>E. glabellum</i></b>  <b><i>E. herminieri</i></b> <i>E. inaequalifolium</i> <b><i>E. longifolium</i></b> <b><i>E. martinicense</i></b> * <b><i>E. maxonii</i></b> <i>E. simplex</i>	<b><i>E. eggersii</i></b> <b><i>E. gramineum</i></b> <i>E. muscosum</i> <b><i>E. paleaceum</i></b> <b><i>E. palmeri</i>**</b>  <b><i>E. tectum</i></b>	<b><i>E. ocoense</i></b> <b><i>E. piloselloides</i></b> <b><i>E. pusillum</i></b> <b><i>E. siliquoides</i></b>	<b><i>E. apodum</i></b> <b><i>E. crinitum</i></b> <b><i>E. cubense</i></b> <b><i>E. decoratum</i></b> <i>E. denudatum</i> * <b><i>E. erinaceum</i></b> <i>E. procurrens</i>	<b><i>E. peltatum</i></b> <b><i>E. minutum</i></b>	<b><i>E. wrightii</i>**</b>

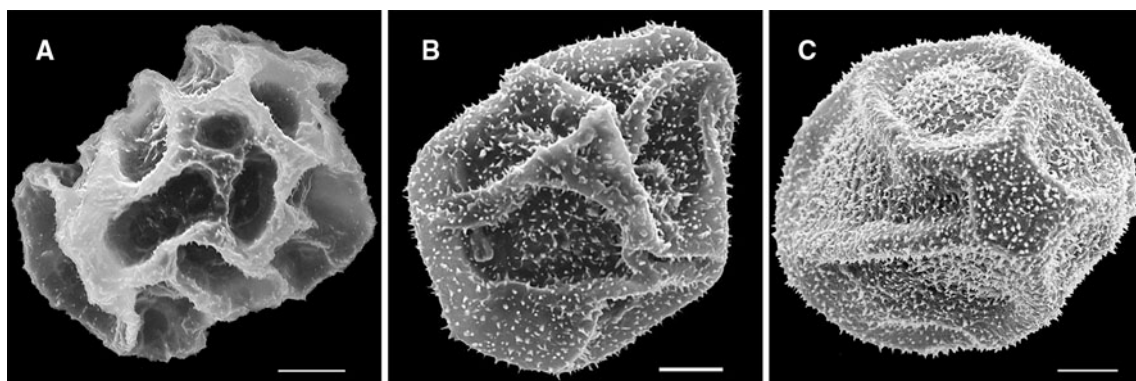
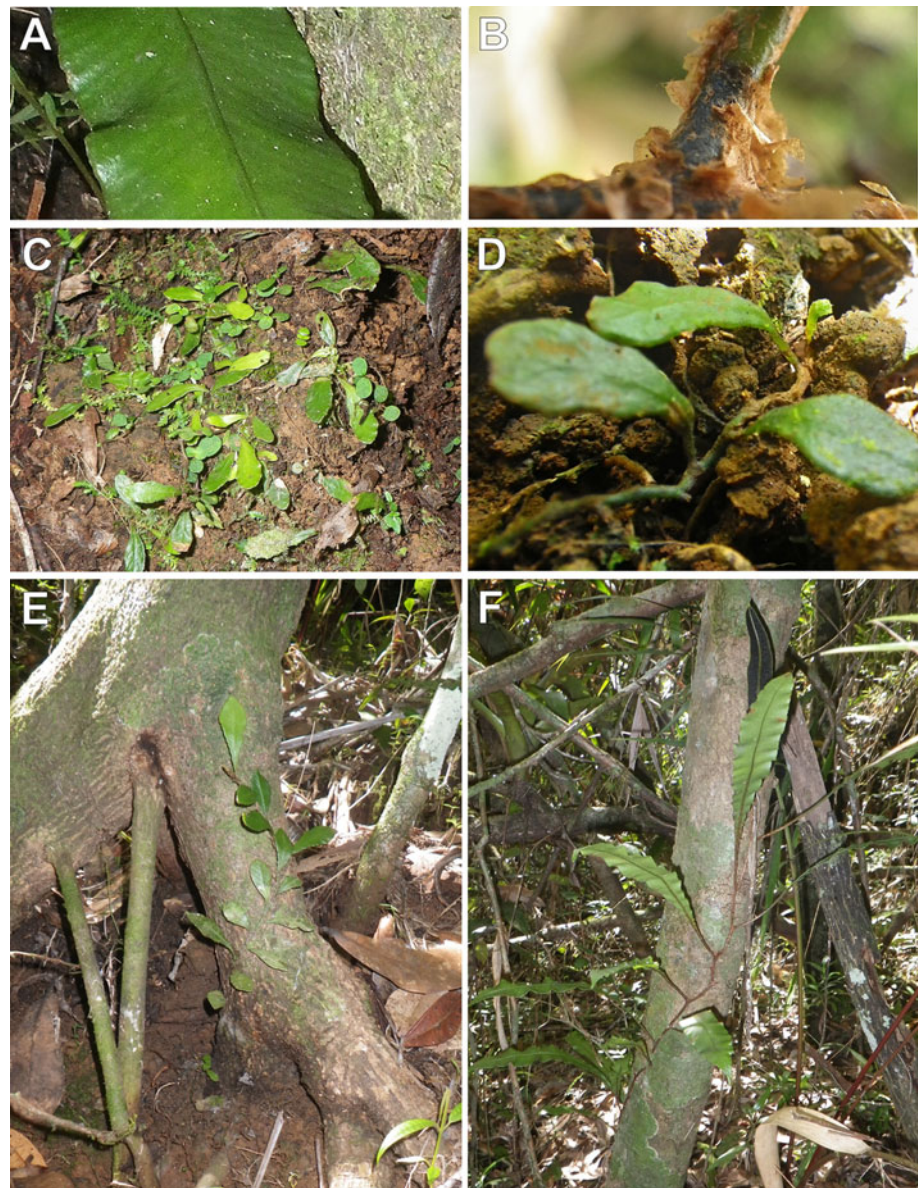
Names in bold represent the species included in the phylogenetic analyses; species with one asterisk are reported for the first time for Cuba, species with two asterisks are Cuban endemics. “Primary hemiepiphytic” refers to plants that start growing on a support tree and secondary contact with the soil, “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  $\mu$ m

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 *trnL-trnF* and *rps4-trnS* 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.

**Acknowledgments** We thank the curators and directors of the herbaria B, BSC, BM, BR, G, GH, HAJB, K, M, MBG, NY, P, S, US, W and YU for access to collections and the loan of specimens. The first author's visit to NY for herbarium and molecular phylogenetic research was supported by the Christopher Reynold Foundation, and we thank Brian Boom for his assistance in obtaining that grant. We also acknowledge a grant from the United States National Science Foundation (DEB 1020443) to R. C. Moran and B. A. Ambrose. The Swedish Institute granted funds for revising type specimens and additional material to L. Regalado. Financial support from the International Association of Plant Taxonomists to J. Lóriga, and further support from the Cuban Agency of Environment (CITMA, projects AMA204 and DB02) to J. Lóriga and L. Regalado, enabled field work in Cuba. We are grateful to Judith Garrison-Hanks for taking photomicrographs of the spores, and to Tynisha Smalls for helping with the molecular work at NY. Special thanks to Miguel Vences and Ariel Rodríguez for kindly providing advice on phylogenetic methods and access to the computer pool of the Zoological Institute of the Technical University of Braunschweig, and to John T. Mickel and Carlos Sánchez for discussions about *Elaphoglossum*. We

are very grateful to two anonymous reviewers for their helpful comments on the manuscript.

## References

- Anderson WR, Anderson C (1985) *Elaphoglossum*. In: Proctor GR (ed) Ferns of Jamaica. British Museum of Natural History, London, pp 486–518
- Caluff MG, Sánchez C, Shelton G (2008) Helechos y plantas afines (Pteridophyta) de Cuba. I. Fitogeografía. Rev Jard Bot Nac 29:21–49
- Darwin C (1865) On the movements and habits of climbing plants. J Linn Soc Bot 9:1–118
- Eastwood A, Cronk QCB, Vogel JC, Hemp A, Gibby M (2004) Comparison of molecular and morphological data on *St. Helena: Elaphoglossum*. Pl Syst Evol 245:93–106
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl Acids Res 32:1792–1797
- Feldberg K, Vaña J, Long DG, Shaw AJ, Hentschel J, Heinrichs J (2010) A phylogeny of Adelanthaceae (Jungermanniales, Marchantiophyta) based on nuclear and chloroplast DNA markers, with comments on classification, cryptic speciation and biogeography. Molec Phylogen Evol 55:293–304
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
- Gebelein J (2012) A geographic perspective of Cuban landscapes. Landscape. Springer, New York
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing the confidence in phylogenetic analysis. Syst Biol 42:182–192
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinforma 17:754–755
- Janssen T, Kreier H-P, Schneider H (2007) Origin and diversification of African ferns with special emphasis on Polypodiaceae. Brittonia 59:159–181
- Kessler M, Mickel J (2006) Nineteen new species of *Elaphoglossum* (Elaphoglossaceae, Pteridophyta) from Bolivia. Brittonia 58:93–118
- Lagamarsino L, Grusz A, Moran RC (2012) Primary hemiepiphytism and gametophyte morphology in *Elaphoglossum amygdalifolium* (Dryopteridaceae). Brittonia 64:226–235
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol 29:1695–1701
- Larget B, Simon DL (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol Biol Evol 16:750–759
- Mickel JT (1995) *Elaphoglossum*. In: Moran RC, Riba R (eds) Flora Mesoamericana, vol I. Psilotaceae a Salviniaceae. Universidad Nacional Autónoma de México, México, D. F., pp 250–283
- Mickel JT, Atehortúa LG (1980) Subdivision of the genus *Elaphoglossum*. Am Fern J 70:47–68
- Mickel JT, Smith AR (2004) *Elaphoglossum*. In: Mickel JT, Smith AR (eds) The Pteridophytes of Mexico. Memoris of the New York Botanical Garden, vol 88. New York Botanical Garden Press, New York, pp 282–315
- Moran RC (2008) Diversity, biogeography, and floristics. In: Ranker TA, Haufler CH (eds) Biology and evolution of ferns and lycophytes. Cambridge University Press, New York, pp 367–394
- Moran RC, Smith AR (2001) Phytogeographic relationships between neotropical and African-Madagascan pteridophytes. Brittonia 53:304–351
- Moran RC, Hanks JG, Rouhan G (2007) Spore morphology in relation to phylogeny in the fern genus *Elaphoglossum* (Dryopteridaceae). Int J Pl Sci 168:905–929
- Moran RC, Labiak PH, Sundue M (2010a) Phylogeny and character evolution of the bolbitidoid ferns (Dryopteridaceae). Int J Pl Sci 171:547–559
- Moran RC, Labiak PH, Sundue M (2010b) Synopsis of *Mickelia*, a newly recognized genus of bolbitidoid ferns (Dryopteridaceae). Brittonia 62:337–356
- Proctor GR (1977) *Elaphoglossum*. In: Proctor GR (ed) Flora of the Lesser Antilles: Leeward and Windward Islands, vol 2. Pteridophyta. Arnold Arboretum, Harvard University, Massachusetts, pp 197–217
- Proctor GR (1989) *Elaphoglossum*. In: Proctor GR (ed) Ferns of Puerto Rico and the Virgin Islands. Memoris of the New York Botanical Garden, vol 53. New York Botanical Garden Press, New York, pp 289–301
- Renner S (2004) Tropical trans-Atlantic disjunctions, sea surface currents, and wind patterns. Int J Pl Sci 165:S23–S33
- Ricklefs RE, Bermingham E (2008) The West Indies as a laboratory of biogeography and evolution. Phil Trans R Soc B Biol Sci 363:2393–2413
- Rouhan G, Dubuisson J-Y, Rakotondrainibe F, Motley TJ, Mickel JT, Labat J-N, Moran RC (2004) Molecular phylogeny of the fern genus *Elaphoglossum* (Elaphoglossaceae) based on chloroplast non-coding DNA sequences: contributions of species from the Indian Ocean area. Molec Phylogen Evol 33:745–763
- Rouhan G, Rakotondrainibe F, Moran RC (2007) *Elaphoglossum nidusoides* (Dryopteridaceae), a new species of fern from Madagascar with an unusual phylogenetic position in the *Squamipedia* group. Syst Bot 32:227–235
- Rouhan G, Lorence DH, Motley TJ, Hanks JG, Moran RC (2008) Systematic revision of *Elaphoglossum* (Dryopteridaceae) in French Polynesia, with the description of three new species. Bot J Linn Soc 158:309–331
- Roux JP (2011) The fern genus *Elaphoglossum* section *Lepidoglossa* (Dryopteridaceae) in Africa, Macaronesia, the mid-Atlantic and southern Indian Ocean Islands. Bot J Linn Soc 165:20–63
- Schneider H, Kreier H-P, Janssen T, Otto E, Muth H, Heinrichs J (2010) Key innovations versus key opportunities: identifying causes of rapid radiations in derived ferns. In: Glaubrecht M (ed) Evolution in action. Springer, Berlin, Heidelberg, pp 61–75
- Skog JE, Mickel JT, Moran RC, Volovsek M, Zimmer EA (2004) Molecular studies of representative species in the fern genus *Elaphoglossum* (Dryopteridaceae) based on cpDNA sequences *rbcl*, *trnL-F*, and *rps4-trnS*. Int J Pl Sci 165:1063–1075
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690
- Swofford DL (2000) PAUP\*. Phylogenetic analysis using parsimony (\*and other methods). Sinauer Associates, Sunderland
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
- Vasco A (2011) Taxonomic revision of *Elaphoglossum* section *Muscosa* (Dryopteridaceae). Blumea 56:165–202
- Vasco A, Moran RC, Rouhan G (2009a) Circumscription and phylogeny of the *Elaphoglossum ciliatum* group (*E. sect. Lepidoglossa*, Dryopteridaceae) based on cpDNA sequences. Taxon 58:825–834
- Vasco A, Moran RC, Rouhan G (2009b) Monograph of the *Elaphoglossum ciliatum* group (Dryopteridaceae). Brittonia 61: 241–272
- Vasco A, Mickel JT, Moran RC (2013) Monograph of the neotropical species of *Elaphoglossum* sect. *Squamipedia* (Dryopteridaceae). Ann Missouri Bot Gard (in press; accepted April 2013)

Yoder JB, Clancey E, Des Roches S, Eastman JM, Gentry L, Godsoe W, Hagey TJ, Jochimsen D, Oswald BP, Robertson J, Sarver BAJ, Schenk JJ, Spear SF, Harmon LJ (2010) Ecological

opportunity and the origin of adaptive radiations. *J Evol Biol* 23:1581–1596