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Molecular phylogeny of the fern genus *Elaphoglossum* (Elaphoglossaceae) based on chloroplast non-coding DNA sequences: contributions of species from the Indian Ocean area

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Abstract

We performed a phylogenetic analysis of the fern genus *Elaphoglossum* using two non-coding chloroplast spacers: *trnL-trnF* and *rps4-trnS*. The sampling includes 123 species, of which 80 have not been previously sequenced, and for the first time includes species from Africa and the Indian Ocean area. The results of this expanded study largely agree with an earlier molecular study based on a smaller group of neotropical species and with the morphology-based classification of Mickel and Atehortúa. We found, however, that some infrageneric groups such as section *Elaphoglossum* are not monophyletic. Besides section *Elaphoglossum pro parte*, we recognize six sections: two new monospecific, unnamed sections, and the previously established sections *Lepidoglossa*, *Squamipedia*, *Amygdalifolia*, and "*Subulate-scaled clade*." We divide the subulate-scaled clade into subsection *Setosa* (hydathodes present) and *Polytrichia* (hydathodes absent), and section *Elaphoglossum* is divided into subsections *Platyglossa* and *Pachyglossa*, two groups that do not appear to be supported by any single morphological character. In general, however, the main clades are supported by morphology.

Finally, we discuss the species of the Indian Ocean region and their affinities with the neotropical ones. Out of the 11 species pairs postulated by Moran and Smith on the basis of morphology, two are well supported (*E. eximium–E. aubertii*; *E. piloselloides–E. spatulatum*) and three are not supported (*E. ciliatum–E. humbertii*; *E. muscosum–E. poolii*; *E. paleaceum–E. deckenii*), and two remain unresolved (*E. erinaceum–E. hybridum*; *E. glabellum–E. acrostichoides*) because our molecular markers were not variable enough. Four species pairs could not be tested because specimens were lacking. Unsupported species pairs are best interpreted as morphological convergences. Two additional species pairs are proposed: *E. cuspidatum–E. succisaefolium*; *E. doanense–E. hornei*. Placement of the species from the Indian Ocean suggests that at least 13 long-distance dispersal events occurred between the Neotropics and the Indian Ocean-Africa.

Keywords: *Elaphoglossum*; Elaphoglossaceae; Ferns; Lomariopsidaceae; *trnL-trnF*; *rps4-trnS*; Phylogeny; Systematics; Indian Ocean; Biogeography; Islands

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1. Introduction

Elaphoglossum (Elaphoglossaceae) is one of the most diversified fern genera, with over 600 species. These grow primarily in wet montane forests and evergreen cloud forests, either terrestrially or as epiphytes. The genus is distributed worldwide in the tropics with some species represented in temperate regions to 39° of north latitude (Azores Archipelago) and to 50° south latitude (Marion Islands and South Sandwich Islands). About 75% of the described species occur in the American tropics. Nearly all the species are characterized by a transversely elongated ventral meristele in the rhizome, simple fronds, free veins, dimorphic sterile and fertile fronds, and acrostichoid sori. The main characters used to distinguish species are the rhizome and frond scales.

Distinctive and morphologically uniform, *Elaphoglossum* appears to have no clear relatives among other ferns. Pichi-Sermolli (1968) excluded it from Lomariopsidaceae and created the new monogeneric family Elaphoglossaceae. In contrast, other authors considered the genus related to *Bolbitis*, *Lomariopsis*, *Thysanosoria*, *Teratophyllum*, and *Lomagramma* in the Lomariopsidaceae, but with uncertain affinities (Holttum, 1947; Kaur, 1974; Kramer and Green, 1990). The present study, however, focuses on evolutionary relationships within *Elaphoglossum* instead of its generic relationships.

Because of the acrostichoid sori, many species of *Elaphoglossum* were originally described in *Acrostichum*. Fée (1845) first proposed an infrageneric classification based on morphological characters such as frond shape and texture, and scale variations. He subdivided the genus into two primary groups: *Oligolepideae* and *Polylepideae*. Later, Fée (1850–1852) recognized two additional groups: *Pilosellae* and *Chromatolepideae*. In his *Monographie des Genus Elaphoglossum*, Christ (1899) subdivided the genus into two primary groups: "Ordo" *Stenoneura*, based on secondary veins without thickened vein ends, and "Ordo" *Condyloneura*, based on secondary veins ending near the margin and thickened at the tip (i.e., non-hydathodous and hydathodous species, respectively). Although Christ's treatment is the most detailed for the genus, Mickel and Atehortúa (1980) found some morphological inconsistencies in his classification, the most important being the separation of species with subulate scales into several unrelated groups. Mickel and Atehortúa (1980) provided a useful subgeneric treatment of the genus with a key for nine sections and 21 subsections, taking into account morphological characters from the rhizome, frond shape, frond venation, scales, and spores.

Given the morphological uniformity of the genus, molecular data were expected to be helpful in depicting phylogenetic relationships. This expectation was born out by the first phylogenetic study of the genus, a study done on 52 neotropical species and based on *rbcL*, *trnL-trnF*, and *rps4-trnS* (Skog et al., 2004). This work concluded that *Elaphoglossum* was monophyletic and, among the outgroups they used, sister to *Bolbitis*. Within *Elaphoglossum*, six clades were supported: (1) one informally named "*Subulate scales*," including all the species with subulate scales (enrolled at the base); (2) the *Lepidoglossa* clade, sister group of the "*Subulate scales*" clade, characterized by phyllopodia and conspicuously scaly laminae; (3) the *Squamipedia* clade grouping species with a long-creeping rhizome, distichously arranged fronds, 1–2 mm long peg-like aerophores, small fronds (less than 15 cm long), and echinulate spores; (4) the *Amygdalifolia* clade consisting of the single species *E. amygdalifolium* (Mett. ex Kuhn) H. Christ, characterized by long-creeping rhizomes, hydathodes, phyllopodia, and reddish young fronds; (5) *Platyglossa*; and (6) *Pachyglossa*. The latter clades are sister groups and contain species with phyllopodia and inconspicuously scaly or glabrous laminae.

Based on morphology, Moran and Smith (2001) proposed 11 species pairs between *Elaphoglossum* species from the Neotropics and Indian Ocean area (i.e., Madagascar, the Comoros, Mascarenes, Seychelles, and Africa). The species pair concept designates two species that appear more similar to each other on the basis of morphology than to any other species in their genus (Moran and Smith, 2001). This should not be confused with "sister species," two species with a common ancestor shared by no other descendant. The 11 proposed pairs suggest that species from Africa-Madagascar do not form a monophyletic group.

Since 1993, chloroplast-coding *rbcL* sequences have been commonly used to resolve evolutionary relationships in pteridophytes at the family or higher levels (Hasebe et al., 1993, 1994, 1995; Kato and Setoguchi, 1999; Korall and Kenrick, 2002; Murakami et al., 1999; Pryer et al., 2001; Sano et al., 2000; Wolf et al., 1999). Some authors examined the usefulness of *rbcL* at the genus level (Dubuisson, 1997; Hennequin et al., 2003; Murakami, 1995; Schulze et al., 2001) and the best resolution were found among the heterosporous ferns (Pryer, 1999), the cheilanthoid ferns (Gastony and Rollo, 1995), *Trichomanes* (Hymenophyllaceae; Dubuisson, 1997; Dubuisson et al., 2003; Pryer et al., 2001), and *Polystichum* (Dryopteridaceae; Little and Barrington, 2003). Nevertheless, within *Elaphoglossum*, despite a good support for the monophyly of the genus, *rbcL* sequence data provided little resolution (Skog et al., 2004).

In the present study, two different DNA sequence regions are used: the *trnL-trnF* and *rps4-trnS* chloroplast intergenic spacers. The *trnL-trnF* spacer is the non-coding, intergenic spacer between *trnL* (UAA)3′ and *trnF* (GAA) exons. Because of its fast evolution, this marker is often useful for studies of closely related species (Taberlet et al., 1991). Although *trnL-trnF* spacer region is more conserved than *rbcL* in palms (Asmussen and

Chase, 2001), it resolved relationships of several other plant groups. In Iridaceae, it evolves three times faster than *rbcL* (Soltis et al., 1998). In pteridophytes, it was successfully used for eusporangiate ferns such as Ophioglossaceae (Hauk et al., 1996), and for leptosporangiate ferns such as Schizaeaceae (Skog et al., 2002), *Asplenium* (Aspleniaceae; Van den Heede et al., 2003), *Adenophorus* (Grammitidaceae; Ranker et al., 2003). The second intergenic spacer, *rps4-trnS*, has also proven useful in the phylogenetic analysis of ferns (Thelypteridaceae, Smith and Cranfill, 2002; *Hymenophyllum*, Hennequin et al., 2003; and *Elaphoglossum*, Skog et al., 2004).

This study had three main objectives: (1) to infer a molecular phylogeny for *Elaphoglossum* that included for the first time species outside the Neotropics; (2) to use this extended geographical and taxonomical sampling to confirm the monophyly of the genus and test the robustness of the six clades obtained in the previous study based on neotropical taxa (Skog et al., 2004), and (3) to study the biogeographic relationships of the Indian Ocean species in relation to the neotropical ones, especially evaluating the species pairs suggested by Moran and Smith (2001) on the basis of morphology.

2. Materials and methods

2.1. Taxon sampling

Material for this study was collected in the wild or, in a few cases, from cultivated plants at The New York Botanical Garden. Within *Elaphoglossum*, we sampled 123 species representative of the diversity of sections according to Mickel and Atehortúa (1980), and representative of the pantropical distribution of the genus (Table 1). For outgroups we used *Bolbitis*, *Lomariopsis*, *Rumohra*, and *Athyrium*. The first two genera are often classified in the Lomariopsidaceae (the family into which *Elaphoglossum* is often placed), because of their dimorphic sterile and fertile fronds, acrostichoid sori, and creeping rhizomes with an elongated ventral vascular bundle (Kramer and Green, 1990). *Rumohra* and *Athyrium* were chosen because both are traditionally included into another closely related family, Dryopteridaceae (Kramer and Green, 1990; Tryon and Tryon, 1982). Also, *Rumohra* was found, among a limited sample of fern genera, the one most closely related to *Elaphoglossum* (Hasebe et al., 1995; Pryer et al., 1995). Voucher informations and GenBank accession numbers are reported in Table 1.

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was isolated from either field-collected, silica gel-dried fronds or herbarium specimens when fresh material was unavailable (Table 1). To avoid contamination, we collected young fronds free of epiphyllous bryophytes and fungal or insect parasites. For silica gel-dried samples, total genomic DNA was extracted from approximately 1 cm^2 of leaf tissue using a modified CTAB protocol of Doyle and Doyle (1987; see Struwe et al., 1998). For herbarium material, the DNEasy Plant Mini kit Qiagen (Valencia, CA) was used following the manufacturer protocol but with a proteinase K digestion during the lysis step: 30 μL of proteinase K were added per tube and tubes were incubated on a tipping plate at 42 °C for 24 h.

The polymerase chain reaction (PCR) was used to amplify both chloroplast regions, *trnL-trnF* and *rps4-trnS* spacers. The *trnL-trnF* spacer was amplified and sequenced using universal primers "e" (5′-GGT TCA AGT CCC TCT ATC CC-3′), and "f" (5′-ATT TGA ACT GGT GAC ACG AG-3′) designed by Taberlet et al. (1991). Primers "rps4-3r.f" (5′-AGT TGT TAG TTG TTG AGT AT-3′; Skog et al., 2004) and "trnS-r" (5′-TAC CGA GGG TTC GAA TC-3′; Smith and Cranfill, 2002) were used to amplify and sequence the *rps4-trnS* spacer. PCR amplifications were typically prepared in 25 μL reactions using 0.1–0.75 μL of non-diluted genomic DNA, 2.5μL of 10× *Taq* buffer with 15 mM $MgCl_2$ (1.5 mM $MgCl_2$ final), 2.5 μL of dNTPs (250 μM final of each), 5 μL of 5 M betaine solution (Q-solution), 2.5 μL of 2.5 μg/μL BSA solution, 1 μL of each primer at 10 μM, 1 U Qiagen Inc. *Taq* DNA Polymerase, and purified water to volume. For both regions, a typical amplification program began with one initial denaturation step for 5 min at 94 °C, then 35 cycles of 1 min at 94 °C, 30 s at 50 °C, 1 min at 72 °C, followed by an extension period of 7 min at 72 °C, and was performed on a DNA Engine DYAD Peltier Thermal Cycler. The resulting PCR products were checked on a 1% agarose gel with ethidium bromide and purified using QIAquick PCR purification kit (Qiagen). They were directly sequenced using the amplification primers with a Perkin Elmer ABI 377XL automated sequencer. Forward and reverse sequences obtained were edited and assembled using Sequencher (version 4; Gene Codes Corporation, Ann Arbor, MI). Thus, new complete sequences for *rps4-trnS* and *trnL-trnF* intergenic spacers were obtained for 80 taxa (Table 1).

2.3. Sequence alignment, indel coding, and phylogenetic analyses

Automatic alignments first were generated using ClustalX (Thompson et al., 1997). These were unsatisfactory even after trying different values of gap opening and elongation costs. Therefore, alignments were done manually with the MUST package (Philippe, 1993), followed by preparation of formatted files for analyses. The data matrices obtained were analyzed under the maximum parsimony procedure using PAUP* version

Table 1
Taxa used in this study, with the sections according to Mickel and Atehortúa (1980), localities, collection and voucher information, and GenBank accession numbers

Species	Section	Locality, *collector*, *collection number* (herbarium)	GenBank accession numbers	
			trnL-trnF	*rps4-trnS*
Outgroups				
Bolbitis auriculata (Lam.) Alston		Mauritius, Rouhan 183 (MAU, P)	AY536367	AY540304
Athyrium filix-femina (L.) Roth		U.S.A., Virginia, Kelloff 101 (GMU)	AY540046*	AY540050*
Lomariopsis marginata (Schrad.) Kuhn		Brazil, Amorim 1920 (NY)	AY540045*	AY540049*
Rumohra adiantiformis (Forster) Ching		Country unknown, cultivated GMU, Skog s.n. (NY)	AY540044*	AY540048*
Elaphoglossum				
E. achroalepis (Baker) C. Chr.	sect. *Lepidoglossa*	Madagascar, *Rakotondrainibe 6485* (P)	AY536288	AY540225
E. acrostichoides (Hook. and Grev.) Schelpe	sect. *Elaphoglossum*	Comoros, *Rouhan 113* (P)	AY536289	AY540226
E. aemulum (Kaulf.) Brack.	sect. *Elaphoglossum*	U.S.A., Hawaï, *Lorence 8514* (PTBG)	AY536290	AY540227
E. aff. conforme (Sw.) J. Sm.	sect. *Elaphoglossum*	Madagascar, *Rakotondrainibe 6359* (P)	AY536291	AY540228
E. aff. latifolium (Sw.) J. Sm.	sect. *Elaphoglossum*	Guatemala, *collector unknown*, *Selby Botanical Garden 94.063*	AY542627	AY542628
E. aff. petiolatum (Sw.) Urban	sect. *Lepidoglossa*	Guadeloupe (Lesser Antilles), *Jérémie s.n.* (P)	AY536292	AY540229
E. affine (M. Martens & Galeotii) T. Moore	sect. *Elaphoglossum*	Mexico, *Mickel 9694* (NY)	AY534841*	AY536169*
E. amygdalifolium (Mett. ex Kuhn) H. Christ	sect. *Amygdalifolia*	Costa Rica, *Herrera 2063* (CR, INB, NY, USJ)	AY534845*	AY536173*
E. angulatum (Blume) T.Moore	sect. *Elaphoglossum*	La Réunion, *Rouhan 220* (NY, P)	AY536293	AY540230
E. asterolepis (Baker) C.Chr.	sect. *Lepidoglossa*	Madagascar, *Kessler 12751* (NY)	AY536294	AY540231
E. apodum (Kaulf.) Schott ex J. Sm.	sect. *Polytrichia*	NYBG *living plant collection 1368/76A*	AY534808*	AY536137*
E. aubertii (Desv.) T.Moore	sect. *Eximia*	La Réunion, *Rouhan 241* (NY, P)	AY536295	AY540232
E. auricomum (Kunze) T. Moore	sect. *Lepidoglossa*	Mexico, *Hammer 3* (NY)	AY534817*	AY536145*
E. auripilum H. Christ var. *auripilum*	sect. *Polytrichia*	Costa Rica, *NYBG living plant collection no. 1368/76A*	AY534809*	AY536138*
E. avaratraense Rakotondr.	sect. *Lepidoglossa*	Madagascar, *Rakotondrainibe 1456* (P)	AY536296	AY540233
E. backhousianum T. Moore	sect. *Setosa*	Costa Rica, *Moran 6321* (CR, INB, NY, UCR)	AY536297	AY540234
E. biolleyi H. Christ	sect. *Elaphoglossum*	Costa Rica, *Boyle 6397* (CR, INB, NY, UCR)	AY536298	AY540235
E. boryanum (Fée) T. Moore var. *eutecnum* Mickel	sect. *Undulata*	Venezuela, *Meier et al. 6768* (NY, VEN)	AY534804*	AY536133*
E. burchellii (Baker) C. Chr.	sect. *Lepidoglossa*	Brazil, *Prado et al. 1109* (NY)	AY534822*	AY536150*
E. cardenasii W. H. Wagner	sect. *Eximia*	Bolivia, *Beck 21894* (NY)	AY534802*	AY536131*
E. cardiophyllum (Hook.) T. Moore	sect. *Squamipedia*	Ecuador, *Holm-Nielsen 17480* (AAU, NY)	AY534842*	AY536171*
E. caricifolium Mickel	sect. *Lepidoglossa*	Costa Rica, *Halley 7* (NY)	AY534814*	AY536143*
E. cf. *proximum* (J. Bommer) H. Christ	sect. *Elaphoglossum*	Costa Rica, *Zamora 3340* (CR, INB, UCR)	AY536299	AY540236
E. ciliatum (C. Presl) T. Moore	sect. *Lepidoglossa*	Dominican Republic, *NYBG living plant collection no. 234/94A*	AY534820*	AY536148*
E. cismense Mickel	sect. *Elaphoglossum*	Costa Rica, *Van Ee 327* (CR, INB, NY, UCR)	AY536300	AY540237
E. conspersum H. Christ	sect. *Elaphoglossum*	Costa Rica, *Moran 6343* (CR, INB, NY, UCR)	AY536301	AY540238
E. coriaceum Bonap.	sect. *Elaphoglossum*	Madagascar, *Rakotondrainibe 6353* (P)	AY536302	AY540239
E. costaricense H. Christ	sect. *Setosa*	Costa Rica, *Williams s.n.* (NY)	AY534799*	AY536128*
E. coursii Tardieu	sect. *Elaphoglossum*	Comoros, *Rouhan 127* (NY, P)	AY536303	AY540240
E. craspedariiforme (Fée) Brade ex Alston	sect. *Squamipedia*	Brazil, *Labiak et al. 1253* (NY)	AY534830*	AY536158*
E. crinitum (L.) H. Christ	sect. *Polytrichia*	Dominican Republic, *NYBG living plant collection no. 233/94*	AY534805*	AY536134*
E. croatii Mickel	sect. *Elaphoglossum*	Costa Rica, *Moran 6378* (CR, INB, NY, UCR)	AY536304	AY540241
E. cuspidatum (Willd.) T. Moore	sect. *Lepidoglossa*	Venezuela, *Fernández 16424* (VEN)	AY534827*	AY536155*
E. davidsei Mickel	sect. *Eximia*	Costa Rica, *Moran 6366* (CR, INB, NY, UCR)	AY536305	AY540242
E. decaryanum Tardieu	sect. *Elaphoglossum*	Madagascar, *Rakotondrainibe 6326* (P)	AY536306	AY540243
E. deckenii (Kuhn) C. Chr.	sect. *Lepidoglossa*	Comoros, *Rouhan 105* (CNDRS[a] , NY, P, PTBG)	AY536307	AY540244
E. decoratum (Kunze) T. Moore	sect. *Decorata*	Colombia, *NYBG living plant collection no. 391/77B*	AY534811*	AY536140*
E. doanense L. D. Gómez	sect. *Elaphoglossum*	Costa Rica, *Moran 6324* (CR, INB, NY, UCR)	AY536308	AY540245
E. edwallii Rosenstock	sect. *Lepidoglossa*	Brazil, *Prado et al. 1123* (NY)	AY534816*	AY536144*

E. erinaceum (Fée) T. Moore	sect. *Polytrichia*	Mexico, *NYBG living plant collection no. 554/79A*	AY534806*	AY536135*
E. eximium (Mett.) H. Christ	sect. *Eximia*	Costa Rica, *Moraga 485* (NY)	AY534803*	AY536132*
E. flaccidum (Jenm.) Alston	sect. *Elaphoglossum*	French Guiana, *Mori 25578* (NY)	AY536309	AY540246
E. forsythii majoris H. Christ	sect. *Lepidoglossa*	Madagascar, *Rakotondrainibe 6340* (P)	AY536310	AY540247
E. fournierianum L. D. Gómez	sect. *Setosa*	Costa Rica, *Moran 6336* (CR, INB, NY, UCR)	AY536311	AY540248
E. furfuraceum (Mett. ex Kuhn) H. Christ	sect. *Lepidoglossa*	Costa Rica, *Moran 6367* (CR, INB, NY, UCR)	AY536312	AY540249
E. glabellum J. Sm.	sect. *Elaphoglossum*	Brazil, *Prado et al. 1129* (NY)	AY534839*	AY536167*
E. glaucum T. Moore	sect. *Elaphoglossum*	Mexico, *Mickel 9696* (NY)	AY534844*	AY536172*
E. grayumii Mickel	sect. *Elaphoglossum*	Costa Rica, *Moran 6329* (CR, INB, NY, UCR)	AY536313	AY540250
E. guatemalense (Klotzsch) T. Moore	sect. *Elaphoglossum*	Mexico, *Mickel 9701* (NY)	AY534836*	AY536164*
E. herminieri (Bory ex Fée) T. Moore	sect. *Elaphoglossum*	Costa Rica, *Blanco 1559* (F, USJ)	AY534835*	AY536163*
E. heterolepis. (Fée) T. Moore	sect. *Lepidoglossa*	Mauritius, *Rouhan 177* (MAU, NY, P)	AY536314	AY540251
E. hoffmannii (Mett. ex Kuhn) H. Christ	sect. *Elaphoglossum*	Costa Rica, *Moran 6365* (CR, INB, NY, UCR)	AY536315	AY540252
E. hornei C. Chr.	sect. *Elaphoglossum*	Seychelles, *Rouhan 156* (NY, P, PTBG, SEY)	AY536316	AY540253
E. huacsaro (Rúiz) H. Christ	sect. *Lepidoglossa*	Brazil, *Prado et al. 1099* (NY)	AY534823*	AY536151*
E. humbertii C. Chr.	sect. *Lepidoglossa*	Madagascar, *Humbert 22448bis* (P)**	AY536317	AY540254
E. hybridum (Bory) Brack.	sect. *Polytrichia*	Tristan Da Cunha, *Mejland 690* (P)	AY536318	AY540255
E. hybridum (Bory) Brack. var. *vulcanii* (Lepervanche ex Fée) H. Christ	sect. *Polytrichia*	La Réunion, *Rouhan 222* (NY, P)	AY536319	AY540256
E. killipii Mickel	sect. *Lepidoglossa*	Bolivia, *Sundue 581* (LPB, NY, USZ)	AY536320	AY540257
E. lanatum (Bojer ex Baker) Lorence	sect. *Lepidoglossa*	Mauritius, *Rouhan 194* (MAU, NY, P, PTBG)	AY536321	AY540258
E. lancifolium (Desv.) C.V.Morton	sect. *Lepidoglossa*	La Réunion, *Rouhan 201* (NY, P)	AY536322	AY540259
E. latifolium (Sw.) J. Sm.	sect. *Elaphoglossum*	Country unknown, *NYBG living plant collection no. 1462/76A*	AY534837*	AY536165*
E. lepervanchii (Bory ex Fée) T. Moore	sect. *Elaphoglossum*	Seychelles, *Rouhan 162* (P)	AY536323	AY540260
E. leucolepis (Baker) Krajina ex Tardieu	sect. *Lepidoglossa*	Madagascar, *Rakotondrainibe 6339* (P)	AY536324	AY540261
E. lindenii (Bory ex Fée) T. Moore	sect. *Setosa*	Mexico, *Mickel 9652* (NY)	AY534801*	AY536130*
E. lingua (C. Presl) Brack.	sect. *Elaphoglossum*	Costa Rica, *Moran 6380* (NY)	AY536325	AY540262
E. luridum (Fée) H. Christ	sect. *Elaphoglossum*	Peru, *NYBG living collections no. 2001-0052*	AY536326	AY540263
E. macropodium (Fée) T. Moore	sect. *Elaphoglossum*	La Réunion, *Rouhan 209* (NY, P)	AY536327	AY540264
E. malgassicum C. Chr.	sect. *Elaphoglossum*	Madagascar, *Kessler 12725* (NY)	AY536328	AY540265
E. marojejyense Tardieu	sect. *Squamipedia*	Madagascar, *Rakotondrainibe 6429* (P)	AY536329	AY540266
E. marquisearum Bonap.	sect. *Lepidoglossa*	Marquisas, *Lorence 8937* (PTBG)	AY536330	AY540267
E. metallicum Mickel	sect. *Elaphoglossum*	Peru, *NYBG living plant collection no. 2790/95*	AY534832*	AY536160*
E. micropogon Mickel	sect. *Lepidoglossa*	Costa Rica, *Moran 6353* (CR, INB, NY, UCR)	AY536331	AY540268
E. minutum (Pohl ex Fée) T. Moore	sect. *Elaphoglossum*	Mexico, *Mickel 9695* (NY)	AY534838*	AY536166*
E. mitorrhizum Mickel	sect. *Elaphoglossum*	Costa Rica, *Boyle 6410* (CR, INB, NY, UCR)	AY536332	AY540269
E. muscosum (Sw.) T. Moore	sect. *Lepidoglossa*	Costa Rica, *Moran 6342* (CR, INB, NY, UCR)	AY536333	AY540270
E. nigrocostatum Mickel	sect. *Lepidoglossa*	Venezuela, *Luteyn 11051* (NY)	AY534824*	AY536152*
E. oblanceolatum C. Chr.	sect. *Setosa*	Costa Rica, *Gomez 21000* (NY)	AY536334	AY540271
E. orbignyanum (Fée) T. Moore	sect. *Lepidoglossa*	Bolivia, *NYBG living plant collection no. 386/94A*	AY534819*	AY536147*
E. ovalilimbatum Bonap.	sect. *Elaphoglossum*	Madagascar, *Humbert 24895*(P)**	AY536335	AY540272
E. ovatum (Hook. and Grev.) T. Moore	sect. *Lepidoglossa*	Ecuador, *Smith 2872* (UC)	AY536336	AY540273
E. paleaceum (Hook. and Grev.) Sledge	sect. *Lepidoglossa*	Hawaii, *Annable 3792* (NY)	AY534825*	AY536153*
E. palmense H. Christ	sect. *Lepidoglossa*	Costa Rica, *Moran 6332* (CR, INB, NY, UCR)	AY536337	AY540274
E. papillosum (Baker) H. Christ	sect. *Undulata*	Costa Rica, *Boyle 5816* (CR, INB, NY, USJ)	AY534800*	AY536129*
E. peltatum (Sw.) Urb.	sect. *Squamipedia*	Mexico, *Mickel 9703.* (NY)	AY534831 *	AY536159*
E. petiolatum (Sw.) Urb.	sect. *Lepidoglossa*	Mexico, *Nicholson 782-01-A* (NY)	AY536338	AY540275
E. phanerophlebium C. Chr.	sect. *Setosa*	Madagascar, *Rakotondrainibe 6430* (P)	AY536339	AY540276
E. piloselloides (C. Presl) T. Moore	sect. *Setosa*	Mexico, *Mickel 9708* (NY)	AY534812*	AY536141*

(*continued on next page*)

Table 1 (*continued*)

Species	Section	Locality, *collector*, *collection number* (herbarium)	GenBank accession numbers	
			trnL-trnF	*rps4-trnS*
E. pilosius Mickel	sect. *Setosa*	Costa Rica, *Moran 6338* (CR, INB, NY, UCR)	AY536340	AY540277
E. poolii (Baker) H. Christ	sect. *Lepidoglossa*	Madagascar, *Kessler 12702* (NY)	AY536341	AY540278
E. prestonii J. Sm.	sect. *Polytrichia*	Brazil, *Prado et al. 1117* (NY)	AY534810*	AY536139*
E. pringlei (Davenp.) C. Chr.	sect. *Lepidoglossa*	Mexico, *Mickel 9693.* (NY)	AY534826*	AY536154*
E. productum Rosenst.	sect. *Elaphoglossum*	Costa Rica, *Moran s.n.* (CR, INB, NY, UCR)	AY536342	AY540279
E. pteropus C. Chr.	sect. *Elaphoglossum*	French Guiana, *Mori 25579* (NY)	AY536343	AY540280
E. pygmaeum (Mett. ex Kuhn) H. Christ	sect. *Setosa*	Ecuador, *Smith 2826* (UC)	AY536344	AY540281
E. randii Alston and Schelpe	sect. *Lepidoglossa*	Marion Island, *Huntley 2072* (P)**	AY536345	AY540282
E. rapense Copel.	sect. *Setosa*	French Polynesia, *Motley 2677* (NY)	AY536346	AY540283
E. richardii (Bory ex Fée) H. Christ	sect. *Lepidoglossa*	La Réunion, *Rouhan 231* (NY, P, PTBG)	AY536347	AY540284
E. rufidulum (Willd. ex Kuhn) C. Chr.	sect. *Lepidoglossa*	Madagascar, *Rakotondrainibe 6396* (P)	AY536348	AY540285
E. russelliae Mickel	sect. *Undulata*	Costa Rica, *Moran 6360* (CR, INB, NY, UCR)	AY536349	AY540286
E. samoense Brack.	sect. *Setosa*	Rapa, *Motley 2875* (NY)	AY536350	AY540287
E. sartorii (Liebm.) Mickel	sect. *Elaphoglossum*	Mexico, *Mickel 9700* (NY)	AY534833*	AY536161*
E. scolopendriforme Tardieu	sect. *Lepidoglossa*	Madagascar, *Rakotondrainibe 6426* (P)	AY536351	AY540288
E. setigerum (Sodiro) Diels	sect. *Setosa*	Costa Rica, *Van Ee 328* (CR, INB, NY, UCR)	AY536352	AY540289
E. sieberi (Hook. and Grev.) T.Moore	sect. *Elaphoglossum*	Mauritius, *Rouhan 169* (MAU, NY, P, PTBG)	AY536353	AY540290
E. siliquoides (Jenm.) C. Chr.	sect. *Setosa*	Costa Rica, *Smith 2631* (UC)	AY534798*	AY536127*
E. smithii (Baker) H. Christ	sect. *Setosa*	Costa Rica, *Boyle 6409* (CR, INB, NY, UCR)	AY536354	AY540291
E. sp.	sect. *Lepidoglossa*	Marquisas, *Lorence 9011* (PTBG)	AY536355	AY540292
E. sp. *nov.* cf. *hayesii* (Mett. ex Kuhn) Maxon	sect. *Setosa*	Costa Rica, *Moran 6344* (CR, INB, NY, UCR)	AY536356	AY540293
E. sp. *nov.* cf. *terrestre* A. Rojas	sect. *Elaphoglossum*	Costa Rica, *Moran 6368* (CR, INB, NY, UCR)	AY536357	AY540294
E. spatulatum (Bory) T. Moore	sect. *Setosa*	La Réunion, *Rouhan 246* (NY, P, PTBG)	AY536358	AY540295
E. splendens (Bory ex Willd.) Brack.	sect. *Lepidoglossa*	La Réunion, *Rouhan 247* (NY, P, PTBG)	AY536359	AY540296
E. squamipes (Hook.) T. Moore	sect. *Squamipedia*	Costa Rica, *Moran 6308* (INB, NY, USJ)	AY534829*	AY536157*
E. stipitatum (Bory ex Fée) T. Moore	sect. *Lepidoglossa*	La Réunion, *Rouhan 212* (NY, P, PTBG)	AY536360	AY540297
E. subsessile (Baker) C. Chr.	sect. *Elaphoglossum*	Madagascar, *Rakotondrainibe 6332* (P)	AY536361	AY540298
E. succisaefolium (Thouars) T. Moore	sect. *Lepidoglossa*	Amsterdam Island, *Marthel-Thoumian 1A* (P)	AY536362	AY540299
E. tectum (Humb. & Bonpl. ex Willd.) T. Moore	sect. *Lepidoglossa*	Brazil, *Prado et al. 1126* (NY)	AY534813*	AY536142*
E. tomentosum (Bory ex Willd.) H. Christ	sect. *Lepidoglossa*	Mauritius, *Rouhan 174* (MAU, NY, P)	AY536363	AY540300
E. tripartitum (Hook. & Grev.) Mickel	sect. *Squamipedia*	Ecuador, *Fay & Fay 3344* (MO)	AY534828*	AY536156*
E. vestitum (Schltdl. & Cham.) T. Moore	sect. *Lepidoglossa*	Mexico, *Mickel 9699* (NY)	AY534818*	AY536146*
E. vieillardii (Mett.) T. Moore	sect. *Elaphoglossum*	New Caledonia, *Munzinger 1740* (P)	AY536364	AY540301
E. wawrae (Luerss.) C. Chr.	sect. *Elaphoglossum*	U.S.A., Hawaï, *Lorence 8511* (PTBG)	AY536365	AY540302
E. wackettii Rosenst.	sect. *Lepidoglossa*	Bolivia, *Janet Kuhn s.n.*, *NYBG living collections 1638/76A* (NY)	AY534821*	AY536149*
E. welwitschii (Baker) C. Chr.	sect. *Lepidoglossa*	Tanzania, Taylor 9099 (P)	AY536366	AY540303

[a] CNDRS: Centre National de Documentation et de Recherche Scientifique, Moroni, Comoros.

* Previously published sequences (Skog et al., 2004).

** DNA was extracted from herbarium laminae for specimens marked with **; otherwise, DNA was extracted from silica-dried lamina material.

4.0b10 (Swofford, 2002) on a PC Athlon 1.2 GHz. We chose to overweight empirically the less frequent character-state changes in order to decrease the probable influence of the likely saturated most frequent ones. This method was already applied on Hymenophyllaceae and showed slightly better support for some branches (Pryer et al., 2001). Thus, character-state changes were unequally weighted according to a procedure that takes into account the relative frequencies from the matrix of each possible reciprocal substitution event (A ↔ C, A ↔ G, A ↔ T, C ↔ G, C ↔ T, G ↔ T) for each position. The probabilities of reciprocal change are converted to costs of change using the negative neperian logarithm of the observed frequencies (Felsenstein, 1981; Maddison and Maddison, 1992; Wheeler, 1990). Each of these costs was rounded off to the second decimal point and used to construct a step matrix. Two different step matrices corresponding to each marker (*trnL-trnF* and *rps4-trnS*) were constructed separately and implemented simultaneously (for combined matrix analysis) in the Assumptions block of the nexus file. PAUP* automatically tested each step matrix for internal consistency and checked that the triangle inequality was not violated.

The introduction of many indels was necessary in both *trnL-trnF* and *rps4-trnS* alignments. Given our wide sampling comprising 127 sequences in each matrix, this is not unexpected. As pointed out by Lutzoni et al. (2000), gaps contain historical information suitable for phylogenetic analysis, and coded indels integrated into sequences improved resolution, while in many other studies gaps are most often excluded from phylogenetic analyses. The effects of gaps as a source of phylogenetic data were explored applying the ID coding method (Barriel, 1994). An alternative approach is to treat indels as a fifth character state, but such treatment could result in biased phylogenies because of a potential long-branch attraction (Felsenstein, 1978) concerning species sharing a long indel. Indels were thus treated in both matrices (*trnL-trnF* and *rps4-trnS*) according to the ID coding method (ID; Barriel, 1994) using the program Barcod (kindly provided by Cyril Gallut, Université Paris 6). With this treatment, each gap string that has different 5′ and/or 3′ termini is coded as separate binary (as present/absent) character. According to the hierarchy of internested states of characters (sites), this strategy introduces question marks in the data matrix: whenever a gap is being coded and the region it spans is completely included within the span of another gap, the sequences having the longer gap are scored as inapplicable (question marks). They are not scored as missing data, but rather as methodological codes, neutral to any a priori phylogenetic hypotheses (Barriel, 1994).

The most parsimony (MP) heuristic searches analyses were conducted using 100 random-addition sequence replicates, tree bisection-reconnection (TBR) branch swapping and MulTrees option on. Non parametric bootstrap analysis (Felsenstein, 1985) was used to evaluate the robustness of each node, using 1000 replicates of similar heuristic searches (but with one random addition sequence per bootstrap replicate). Because all characters are equally sampled, uninformative characters can have a significant effect on robustness, that is neither logical nor desirable. Therefore, uninformative characters were removed before the bootstrap procedure (DeSalle et al., 2002). Because unequal weighting results in non-integral numbers of steps, decay indices (Bremer, 1988; Donoghue et al., 1992) were not calculated. In order to test clades for phylogenetic stability to treatment choice, we performed different phylogenetic analyses, applying ID coding or treating indels as missing data.

2.4. Test of data partition incongruence

We employed the incongruence length difference test (ILD; Farris et al., 1994, 1995), as implemented in PAUP* (called partition homogeneity test) to test the null hypothesis that our two data sets were homogeneous with respect to phylogenetic information. Invariant sites were removed for the test (Cunningham, 1997), and 10,000 replications were performed.

3. Results

3.1. Sequence variation

The length of the *trnL-trnF* intergenic spacer-sequences ranged among the ingroup from 296 bp (*E. poolii*) to 370 bp (*E. auricomum*), and in the outgroups from 343 bp (*Lomariopsis marginata*) to 380 bp (*Bolbitis auriculata*. Total length of aligned *trnL-trnF* sequences resulted in 488 bp due to insertion of indels. Thus, out of the 488 aligned sites, 210 were variable across ingroup species, and 123 were phylogenetically informative.

For the *rps4-trnS* intergenic spacer, sequence lengths varied considerably, ranging in the ingroup species from 121 bp (*E. siliquoides*, *E. costaricense*, *E. papillosum*, *E. fournierianum*, *E. sp.nov.* cf *hayesii*, *E. oblanceolatum*, *E. phanerophlebium*, *E. pygmaeum*, and *E. smithii*) to 408 bp (*E. peltatum*), and in the outgroup species ranging from 419 bp (*Bolbitis auriculata*) to 425 bp (*Lomariopsis marginata*. Consequently, many indels were necessary to achieve an aligned matrix of 530 bp. Out of the 530 aligned sites, 201 were variable across ingroup species and 124 were phylogenetically informative.

For the *trnL-trnF* and *rps4-trnS* alignments, the percentage of variable sites (43.0 and 37.9%, respectively) and the percentage of informative sites (58.6% for *trnL-trnF* and 61.7% for *rps4-trnS*) was not considerably different between the data sets. When outgroup taxa

were included, the percentage of variable sites of each total fragment length was slightly higher for *rps4-trnS* (58.7% vs. 54.7% for *trnL-trnF*). In contrast, the percentage of informative sites out of the number of variable sites was only slightly higher for *trnL-trnF* (65.5% vs 63.0% for *rps4-trnS*).

3.2. Phylogenetic results

For each analysis, the statistical results (number of sites, number and percentages of variable and informative sites, number of MP trees, tree length, CI, RI; Farris, 1989) are summarized in Table 2. In the text bootstrap scores (BS) are specified most often into brackets as the two values obtained, respectively, without indel coding, and with indel coding (ID).

3.2.1. rps4-trnS data

Regardless of the treatment, parsimony analyses of *rps4-trnS* sequences produced many equally parsimonious trees, not less than 16,501 (Table 2). This result reflects that there is little resolution in the terminal nodes. In the strict consensus trees (Fig. 1A–B) the internal clades are more or less strongly supported, and the taxa are indicated by the corresponding sectional name *sensu* Skog et al. (2004) at the terminal branches where resolution was low.

Equally and unequally weighted parsimony analyses resulted in the same topology with similar branch support, but two different topologies were obtained depending on the indel treatment used: one treating the indel data as missing data (Fig. 1A), and the other treating the indels as additional characters (Fig. 1B). In both analyses, the monophyly of *Elaphoglossum* is strongly supported (100% BS) and the sister relationship between *Elaphoglossum* and *Bolbitis* (99% and 100% BS) were retrieved.

Within *Elaphoglossum*, two species, *E. aemulum* and *E. amygdalifolium*, were retrieved as basal branches in the trees. The latter species is sister to the rest of the genus, although this relationship is weakly supported (< 5 and 51% BS). The remaining species relationships of the consensus trees (Figs. 1A–B) were poorly resolved, but three major clades were distinguished. The largest clade was formed by 80 species and was only weakly supported (BS < 50%). It comprises a large polytomy with species belonging to sections *Lepidoglossa*, *Squamipedia*, and "*Subulate scales*", along with *E. latifolium*. The latter species belongs to the section *Elaphoglossum* subsection *Pachyglossa sensu* Mickel and Atehortúa (1980) or to the section *Pachyglossa sensu* Skog et al. (2004). The second major clade, receiving better support (79 and 76% BS), corresponds to all species belonging to the section *Platyglossa sensu* Skog et al. (2004). The third major clade, the best supported (92 and 97% BS), corresponds to section *Pachyglossa sensu* Skog et al. (2004) but excludes *E. latifolium*, *E. glaucum*, and *E. aemulum*.

3.2.2. trnL-trnF data

Compared to the *rps4-trnS* data, *trnL-trnF* produced better resolution and stronger branch support (Figs. 1C–D). Phylogenetic analyses of this region retrieved the same strongly supported basal relationships as shown by *rps4-trnS*: the sister-taxon relationships of *Bolbitis* and *Elaphoglossum* (96 and 97% BS) and the monophyly of the genus *Elaphoglossum* (88 and 93% BS). Regardless of the treatment and with a strong support, *E. amygdalifolium* appeared sister to the rest of the genus (99 and 100% BS). This species defines its own section corresponding to *Amygdalifolia*.

Several relationships unresolved with *rps4-trnS* were resolved with *trnL-trnF*. Besides *Amygdalifolia*, five other sections *sensu* Skog et al. (2004) were retrieved: *Lepidoglossa*, "*Subulate scales*", *Squamipedia*, *Platyglossa*, and *Pachyglossa*. Both *E. glaucum* and *E. aemulum* are exceptions, each being isolated on a branch and not included in the previously defined sections. Only three sections were well supported, *Lepidoglossa* (94 and 98% BS), "*Subulate scales*" (99% BS) and *Pachy-*

Table 2
Statistical results of the phylogenetic analysis using different treatments to separate or combined data matrix

Statistics	Data set:	*rps4-trnS*		*trnL-trnF*		*Rps4-trnS + trnL-trnF*	
	Treatment:	ID		ID		ID	
Number of sites		530	595	488	623	1018	1218
Number of variable sites		311	372	267	390	578	762
(% of total)		(58.7)	(62.5)	(54.7)	(62.6)	(56.8)	(74.8)
Number of informative sites		196	217	175	231	371	448
(% of variable sites)		(63.0)	(58.3)	(65.5)	(59.2)	(64.2)	(58.8)
Number of MP trees		16501	66868	104819	165	74745	1008
Number of steps		1079.47	1231.61	1031.00	1397.25	2141.90	2674.15
CI[a]		0.5698	0.5633	0.5279	0.5051	0.5391	0.5204
RI		0.8263	0.8344	0.8709	0.8644	0.8469	0.8460

ID is for Indel coding (see Section 2).

[a] CI is calculated excluding uninformative characters.

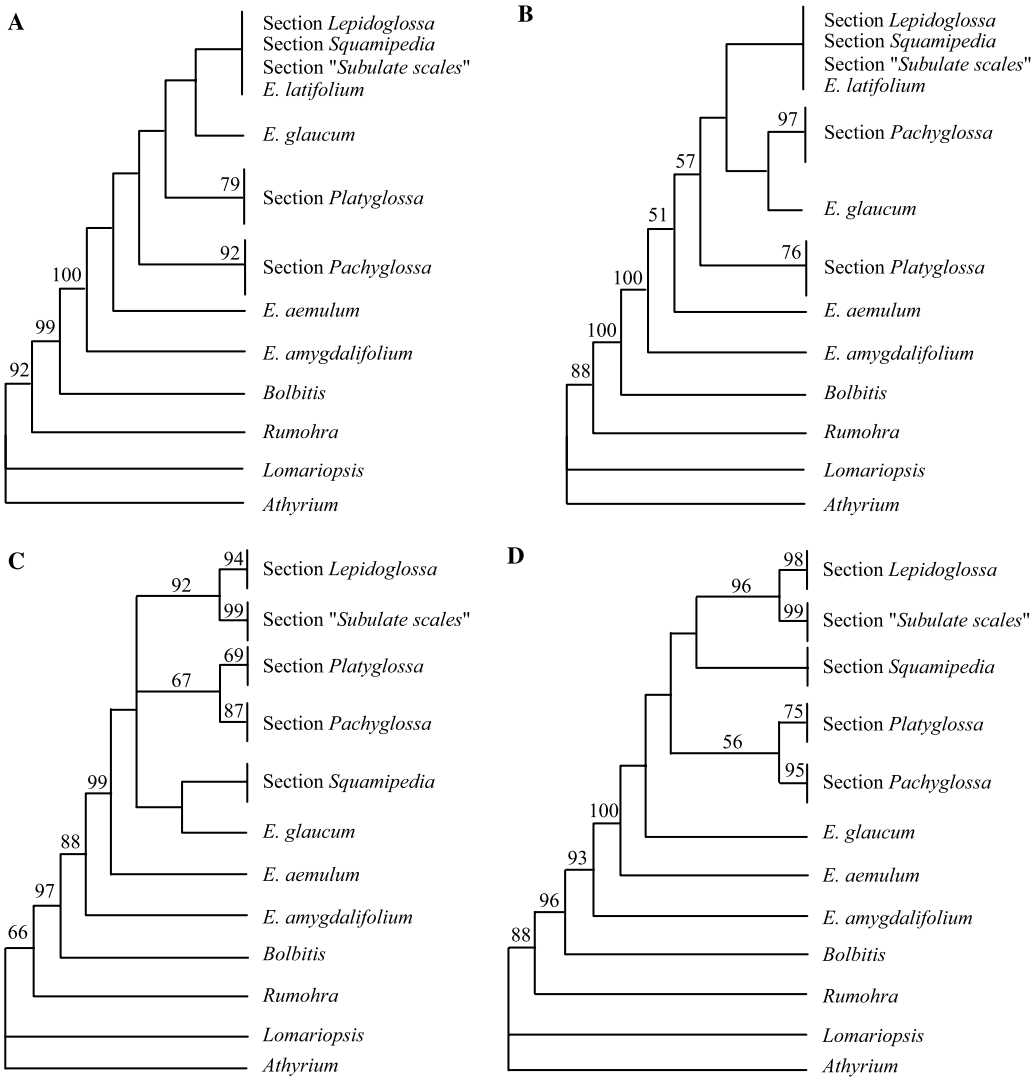

Fig. 1. Strict consensus trees resulting from separate MP analyses of the *rps4-trnS* (A–B) and *trnL-trnS* (C–D) datasets. Only broad major relationships are reported. Trees were rooted with *Athyrium*. Numbers above branches are percent bootstrap support >50%. All names of sections are employed *sensu* Skog et al. (2004). (A) Tree provided by analysis of *rps4-trnS* dataset without indel coding. (B) Tree provided by analysis of *rps4-trnS* dataset, using ID coding. (C) Tree provided by analysis of *trnL-trnS* dataset, without indel coding. (D) Tree provided by analysis of *trnL-trnS* dataset, using ID coding.

glossa (87 and 95% BS). In contrast, *Platyglossa* was less supported (69% and 75% BS), and *Squamipedia* received low support (BS < 50%). The species *E. latifolium* that was included as a separate lineage in the *rps4-trnS* data analyses (Figs. 1A–B), belongs to the section *Pachyglossa* in the *trnL-trnF* data analyses (Fig. 1C–D).

At a higher taxonomic level, two sister relationships between sections were retrieved with the different treatments. The first, between *Lepidoglossa* and "*Subulate scales*", was strongly supported (92 and 96% BS), but the second, between *Platyglossa* and *Pachyglossa*, was weakly supported (67–56% BS).

Concerning the variation observed in results provided by the two treatments, analyses using indels improved resolution, but without better support (Fig. 1D). Independently of the indel treatment, branch support was comparable in trees obtained with analyses conducted with equally or unequally weighted parsimony analyses, but equally weighted parsimony analyses did not resolve relationships between sections *Squamipedia*, *Platyglossa* + *Pachyglossa*, *Lepidoglossa* + "*Subulate scales*", *E. aemulum*, and *E. glaucum*.

3.2.3. Combined analysis

The ILD test indicated that combinations of most of the data partitions resulted in non-significantly incongruent trees. The combined analyses (*rps4-trnS* + *trnL-trnF*) yielded finer phylogenetic resolution than previous analyses conducted with separated matrices (Fig. 2). Bootstrap support for clades retrieved by the combined approaches (Table 3) allows comparison with results of separate analyses. With indel coding we compared the

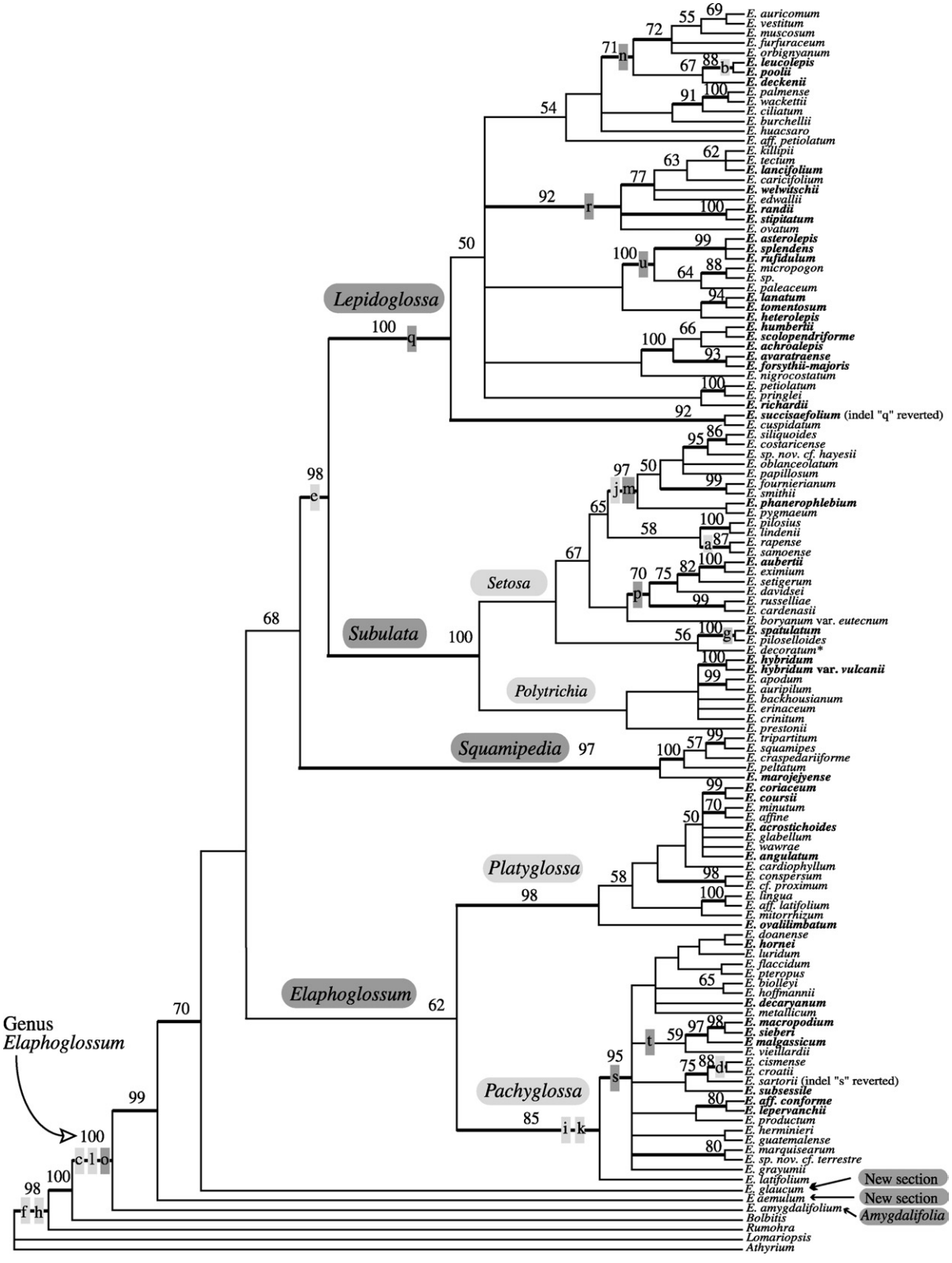

Fig. 2. Strict consensus of 1008 most parsimonious combined trees (*trnL-trnF* and *rps4-trnS*) resulting from an heuristic search using the indel coding ID (tree length = 2674.15). Proposed names for sections are in dark grey frames, those for subsections are in bright grey frames. Numbers above branches are bootstrap percentage values >50%. Branches with thick lines are the most robustly supported (BS > 70%). Single letters in dark and bright grey squares on branches refer respectively to synapomorphic indels in *rps4-trnS* and *trnL-trnS* alignments. The tree was rooted with *Athyrium*. * *Elaphoglossum decoratum*, although included in the subsection *Setosa*, is a species without hydathodes. Taxa names in bold letters identify species from the Indian Ocean area.

Table 3
Node supports estimated by bootstrap percentages for various clades in each of the separate or combined analyses

Clades	Data set:	*rps4-trnS*		*trnL-trnF*		*rps4-trnS* + *trnL-trnF*	
Nomenclatural sections sensu Skog et al., 2004)	Treatment:	ID		ID		ID	
Elaphoglosum + *Rumohra* + *Bolbitis*		92	88	66	88	93	98
Elaphoglossum + *Bolbitis*		99	100	97	96	100	100
Elaphoglossum		100	100	88	93	100	100
Elaphoglossum excluding *Amygdalifolia*; (1)		<5	51	99	100	95	99
(1) excluding *E. aemulum*; (2)		46	57	49	48	75	70
(2) excluding *E. glaucum*		—	—	—	<5	31	<5
Lepidoglossa + "*Subulate scales*" + *Squamipedia*		—	—	—	46	50	68
Lepidoglossa + "*Subulate scales*"		—	—	92	96	96	98
Lepidoglossa		—	—	94	98	100	100
"*Subulate scales*"		—	—	99	99	99	100
Squamipedia		—	—	45	<5	97	97
Platyglossa + *Pachyglossa* (excluding *E. aemulum*, *E. glaucum*)		—	—	67	56	48	62
Platyglossa		79	76	69	75	97	98
Pachyglossa		—	97	87	95	68	85
Pachyglossa (excluding *E. latifolium*)		92	97	—	—	90	95

ID is for Indel coding (see Section 2).

results of the equally and unequally weighted parsimony analyses. With unequal weighting, branch support was equal or slightly higher for each section and for the relationships between sections. Also, unequal weighting improved resolution, especially concerning the placement of *E. glaucum* (although poorly supported).

Regardless of the method, high bootstrap support was obtained for the sister relationships between *Bolbitis* and *Elaphoglossum* (100% BS) and between *Rumohra* and *Elaphoglossum* + *Bolbitis* (100% BS). Within *Elaphoglossum* (100% BS), section *Amygdalifolia* comprising the single species *E. amygdalifolium*, was strongly supported (95 and 99% BS) as sister to the rest of the genus. Of the remaining species, *E. aemulum* and *E. glaucum* form monotypic clades, *E. aemulum* placed as sister-taxon to all the other taxa (75 and 70% BS), and *E. glaucum* isolated on a basal, unsupported branch (BS < 50%). Strong support was obtained for other major clades including *Lepidoglossa* (100% BS), "*Subulate scales*" (99 and 100% BS) named *Subulata* as discussed later, *Squamipedia* (97% BS), *Platyglossa* (97 and 98% BS), and *Pachyglossa* (68 and 85% BS). The latter two clades were placed as sister-group constituting a larger clade, named here *Elaphoglossum* (48 and 62% BS). Sections *Squamipedia*, *Lepidoglossa*, and *Subulata* were grouped in a weakly supported clade (50 and 68% BS), but within this clade the sister-taxon relationship between *Subulata* and *Lepidoglossa* was robustly supported (96 and 98% BS). Within the section *Subulata* two unsupported clades (BS < 50%) were produced corresponding to *Polytrichia* and *Setosa* as discussed later.

We identified several indels that might represent synapomorphies for some clades. Skog et al. (2004) retrieved 12 synapomorphic indels for *Elaphoglossum* in *trnL-trnF* and *rps4-trnS* alignments. Our study identified nine others (Fig. 2; Table 4). The *trnL-trnF* alignment revealed that indels "f" and "h" support the close relationship between the three genera *Rumohra*, *Bolbitis* and *Elaphoglossum*. Indels "c," "l" in *trnL-trnF* and "o" in *rps4-trnS* are synapomorphies for *Elaphoglossum*. In contrast, among the four major clades, *Lepidoglossa*, *Subulata*, "*Squamipedia* " and *Elaphoglossum*, only *Lepidoglossa* received support from an indel ("q" from *rps4-trnS*). Within section *Elaphoglossum*, all species belonging to *Pachyglossa* have indels "i" and "k" in their *trnL-trnF* sequence. The hypothesized close relationship between *Lepidoglossa* and *Subulata* clades is reinforced by the synapomorphic indel "e."

Table 4
Synapomorphic indels revealed by the alignment of both intergenic spacers *trnL-trnF* and *rps4-trnS* sequences

trnL-trnF alignment		*rps4-trnS* alignment	
Indel designation	Position	Indel designation	Position
a	16–20	m (rt 2)	73–449
b	55–125	n (rt 6)	223–226
c	82–83	o (rt 7)	233–265
d	104–110	p (rt 8)	267–270
e	129–130	q (rt 13)	386–390
f	196–197	r (rt 16)	483–493
g	219–223	s (rt 17)	524–530
h (tn 5)	66–71	t	38–54
i (tn 7)	150–156	u	287–290
j (tn 9)	225–228		
k (tn 11)	250–306		
l (tn 12)	311–320		

Correspondences with indels designed by Skog et al. (2004) are in brackets.

The 38 species from the Indian Ocean islands (plus *E. welwitschii* from Africa) were scattered throughout the major clades. Therefore, species from this geographical area do not represent a monophyletic group. Some of the species, however, did form small clades of three or

more species. Within *Lepidoglossa*, three clades were supported: *E. leucolepis* + *E. poolii* + *E. deckenii* (60 and 67% BS), *E. asterolepis* + *E. splendens* + *E. rufidulum* (98 and 99% BS) as sister to a neotropical clade of three taxa (99% and 100% BS), and *E. humbertii* + *E. scolopendriforme* + *E. achroalepis* + *E. avaratraense* + *E. forsythii-majoris* (100% BS). Within *Pachyglossa*, only *E. macropodium* + *E. sieberi* + *E. malgassicum* were grouped together with a high support (91 and 97% BS).

4. Discussion

4.1. Usefulness of indel-coding for phylogenetic work in Elaphoglossum

The insertion of numerous gaps in nucleotide sequences was required during the alignment procedure for both *trnL-trnF* and *rps4-trnS*. This is common in non-coding sequences putatively less affected in their function by insertion and deletion events than coding sequences. Gaps have sometimes been suggested to be unreliable for use as phylogenetic characters (Ford et al., 1995; Golenberg et al., 1993), and most studies disregard them as a source of phylogenetic information, arguing that gaps introduce noise in the data. Yet other studies have shown gaps to be reliable characters (Delarbre et al., 2000; Freudenstein and Chase, 2001; Giribet and Wheeler, 1999; Hennequin et al., 2003; Lloyd and Calder, 1991; Van Dijk et al., 1999). Several methods have been thus developed that integrate indels in the phylogenetic analyses. The simplest way for coding indels may be to treat each different gap string (defined by both size and position) as separate binary character, as proposed by Bruns et al. (1992) and Udovicic et al. (1995). This method did not address the issue of overlapping gap strings, raising the possibility of successive insertions or deletions. ID coding (Barriel, 1994) addresses this problem by introducing missing data. This avoids coding possibly dependent successive indels as independent characters. This method has proven to be useful in several studies (Delarbre et al., 2000; Raymundez et al., 2002). In a comparative study, Hennequin et al. (2003) showed that ID coding appeared the more appropriate indel coding method for *rps4-trnS* data in the Hymenophyllaceae. The results obtained in our study using ID and both markers *rps4-trnS* and *trnL-trnS* demonstrate utility of molecular events such as indels. Indel coding yielded 56 and 21 additional informative sites, respectively in the *trnL-trnF* and *rps4-trnS* data matrix (Table 2) and thus was helpful for increasing potential phylogenetic information and for inferring a phylogeny of *Elaphoglossum*. The gaps and base substitution contained in both matrices was used for the analyses, resulting in a well resolved topology when the data sets were combined.

4.2. Phylogenetic relationships and implication for systematics

The tree from the combined analysis using ID (Fig. 2) exhibits most of the best branch supports. Consequently, the following discussion is based mainly on that tree (Fig. 2).

4.2.1. Monophyly of Elaphoglossum and Lomariopsidaceae

The monophyly of *Elaphoglossum* was supported by a molecular study based only on neotropical species (Skog et al., 2004). Our analyses, which include 46 paleotropical species, also strongly support this conclusion. They also support *Bolbitis* as the sister-group, although our sampling of related genera was limited.

In our analysis, the Lomariopsidaceae *sensu* Alston (1956) was not monophyletic. *Rumohra*, traditionally placed in the Dryopteridaceae (Kramer and Green, 1990), is more closely related to the clade *Elaphoglossum* + *Bolbitis*, whereas *Elaphoglossum*, *Bolbitis* and *Lomariopsis* are traditionally grouped together in the Lomariopsidaceae (Kramer and Green, 1990). Nevertheless, conclusions about generic relationships of *Elaphoglossum* are premature based on our limited sampling of genera. Molecular studies including more genera, especially at least those traditionally placed in the Dryopteridaceae (*Rumohra* and 44 additional genera; Kramer and Green, 1990) and the Lomariopsidaceae (including *Elaphoglossum*, *Bolbitis*, *Lomariopsis*, *Lomagramma*, *Teratophyllum* and *Thysanosoria*; Kramer and Green, 1990; Pichi-Sermolli, 1969) are needed and should be conducted to determine relative phylogenetic placement of each genus.

4.2.2. Phylogenetic status of nomenclatural sections

The sections proposed by Mickel and Atehortúa (1980) on the basis of morphology agree partially with the study conducted by Skog et al. (2004). Our results, based on more species, define additional clades and increase support for many clades recovered in both studies. We confirm the monophyly of the five major clades proposed previously as sections (Skog et al., 2004): *Lepidoglossa*, "*Subulate scales*" (*Subulata* in this study), *Squamipedia*, *Platyglossa*, and *Amygdalifolia*. In contrast, we found that *Pachyglossa sensu* Skog et al. (2004) was not monophyletic. Moreover our results suggest that two new monospecific sections could be recognized, corresponding to *E. aemulum* and *E. glaucum*, respectively (we refrain from naming these clades until current, more inclusive studies are completed).

4.2.3. Section Amygdalifolia and two newly proposed monospecific sections

Elaphoglossum amygdalifolium corresponds to its own monospecific section and is sister to the rest of the

genus. This placement is supported by morphological evidence: *E. amygdalifolium* exhibits a unique combination of characters within the genus, consisting of long-creeping rhizomes, phyllopodia, and hydathodes (Mickel and Atehortúa, 1980). The species can also be distinguished by the reddish young fronds, which appears to be an autapomorphic character within *Elaphoglossum*.

Our analyses suggest two other taxa form monotypic clades: *E. aemulum* and *E. glaucum*. These two species were placed in section *Elaphoglossum* subsection *Pachyglossa* by Mickel and Atehortúa (1980), corresponding to the sections *Pachyglossa* plus *Platyglossa sensu* Skog et al. (2004) as discussed later in the Section 4.2.7. They are similar morphologically to other species placed in the subsection *Pachyglossa*, which forms a large, well supported clade in our phylogeny (Fig. 2, *E. doanense* through *E. latifolium*). *Elaphoglossum aemulum* (Kaulf.) Brack. is endemic in the Hawaiian Islands. It is characterized by well developed rhizomes densely covered with scales more than 1.5 cm long, giving the rhizome the appearance of having an extremely thick diameter of several centimeters. Its leaves form a nest or basket shape that collects fallen leaves and debris, which then decompose in the basket to form humus into which the roots grow. The phylogenetic placement of *E. aemulum* is well supported and might justify a new monospecific section. The similar monotypic placement of *E. glaucum* T. Moore, a species from Central America, might also merit a new section, but position is much less supported and there are few morphological characters to define the clade. More research is needed, both molecular and morphological, to verify the status of *E. aemulum* and *E. glaucum*. For that reason, newly suggested sections are not named. Two reasons suggest that more species might be included in the newly suggested sections or even in new separate lineages. First, our sampling represents only a small proportion of the estimated 600 to 650 species within the genus. Second, morphological characters traditionally used did not seem to support some clades (*e.g.*, *E. glaucum* and *E. aemulum*).

4.2.4. Monophyly of section Lepidoglossa

The clade *Lepidoglossa*, (Fig. 2, *E. auricomum* through *E. cuspidatum*), is very well supported, agreeing with the results of Skog et al. (2004). All species of this section exhibit phyllopodia and blades densely scaly on both surfaces (but never with subulate scales).

Within *Lepidoglossa*, however, are several species without conspicuous scales on the laminae. These species have reddish or yellowish-brown dots (sometimes resinous) that represent the basal cells of minute scales that are soon deciduous (Roux, 1982) or even that might have been lost through evolutionary reduction for some species. This character occurs in both neotropical and African–Madagascan species. In the Old World, it occurs in *E. richardii* from La Réunion in the Indian Ocean (Lorence and Rouhan, 2004), and in *E. scolopendriforme* and *E. humbertii* from Madagascar. Besides resinous dots on otherwise glabrous laminae, these species have creeping, black, resinous rhizomes that lack scales or nearly so. This same combination of characters is found in the neotropical species *E. ciliatum*, *E. burchellii*, *E. palmense*, and *E. wackettii*. Because resinous dots represent the bases of highly reduced scales, these species are placed in section *Lepidoglossa* (Skog et al., 2004; the present study for *E. palmense*), which is typically moderately to densely scaly. Our molecular analysis support this placement. The four neotropical species are nested in one poorly supported subclade, and *E. humbertii* and *E. scolopendriforme* from Madagascar belong to one another subclade. It appears that resinous dots on the blades and long-creeping, resinous, nearly glabrous rhizomes have evolved at least twice in the section—a remarkable example of morphological convergence. Therefore, the species pair proposed on the basis of morphology by Moran and Smith (2001), consisting of the neotropical *E. ciliatum* and the Madagascan *E. humbertii*, is not supported.

4.2.5. Monophyly and relationships within section Subulata

The section *Subulata* is distinguished by subulate scales, which can occur anywhere on the plant. Our study corroborates the monophyly of this section, a monophyly previously proposed by Skog et al. (2004) for what they called the "Subulate scale group" (we use here *Subulata* as a convenient name, but refrain from naming it formally until we complete more extensive studies of the genus). In the Skog et al. (2004) analysis, relationships within this clade were poorly resolved; however, our increased sampling of species and use of indel coding yielded an almost completely dichotomously resolved phylogenetic hypothesis for this section. The basalmost dichotomy separates the hydathodous species (Fig. 2, *E. siliquoides* through *E. decoratum*) from the non-hydathodous ones (Fig. 2, *E. hybridum* through *E. prestonii*). This sister relationship was weakly supported by BS analyses but it was always retrieved. It is well supported morphologically by the presence or absence of hydathodes. The hydathodous clade corresponds to section *Setosa* and the non-hydathodous clade to section *Polytrichia*, both of which were recognized by Mickel and Atehortúa (1980). Because *E. amygdalifolium* (section *Amygdalifolia*) also has hydathodes, hydathodes have apparently evolved twice in the genus.

Within section *Setosa*, the placement of *Elaphoglossum decoratum* is unexpected because it differs morphologically from *E. spatulatum* and *E. piloselloides*, both of which are small species that have hydathodes (*E. decoratum* lacks hydathodes). In the combined analyses without indel coding, *E. decoratum* was also nested within

section *Setosa* but in a clade (although poorly supported) with all other species without hydathodes. Thus, placement of this species is uncertain. Its placement within section *Subulata* was already not expected based on previous morphological studies (Mickel and Atehortúa, 1980) but it is morphologically justified: some scales are indeed slightly enrolled at the base, forming subulate scales (Skog et al., 2004).

Within *Subulata* the sister-taxon to section *Setosa* consists of those species without hydathodes and with long-armed trichomidia on stipes and laminae (Fig. 2, *E. hybridum* through *E. prestonii*). This clade corresponds to section *Polytrichia* defined by Christ (1899) and recognized by Mickel and Atehortúa (1980). The subsection *Apoda sensu* Mickel and Atehortúa (1980) was considered as the single monophyletic subsection by Skog et al. (2004). In this study, three species from subsection *Apoda* were included: *Elaphoglossum apodum*, *E. auripilum* and *E. backhousianum*. The latter species has an inconclusive placement with respect to the two former clustered species. Thus, the monophyly of subsection *Apoda* cannot be assessed with our data.

4.2.6. Monophyly of section Squamipedia

The monophyletic section *Squamipedia*, represented by *Elaphoglossum tripartitum*, *E. squamipes*, *E. craspedariiforme*, and *E. peltatum*, is one of the most distinctive groups, with its small fronds (less than 15 cm long), very long-creeping rhizomes, tan rhizome and stipe scales, and lack of phyllopodia (Mickel and Atehortúa, 1980). Also, all species have a 1–2 mm long, peg-like aerophore on the rhizome near the node, and this probably represents a synapomorphy for the group. In our analysis, these species are sister group to *E. marojejyense*, a species known from Madagascar (Tardieu-Blot, 1955), Zimbabwe (Schelpe, 1969; Burrows, 1990), and Mozambique (Roux, 2001). *Elaphoglossum marojejyense*, however, does not exhibit all the characters of *Squamipedia*: it lacks the characteristic aerophores, and its spores are fenestrate, not echinulate. Moreover, *E. marojejyense* differs by succulent fronds. Roux (2001) included *E. marojejyense* in section *Elaphoglossum* subsection *Pachyglossa*, but *E. marojejyense* lacks phyllopodia whereas these are present in all species in section *Elaphoglossum*. Our molecular results support placement of *E. marojejyense* in section *Squamipedia*, but except for the lack of phyllopodia, morphology does not support its inclusion in this section.

4.2.7. Monophyly of section Elaphoglossum s.s.

All species belonging to the section *Elaphoglossum sensu* Mickel and Atehortúa (1980) (*Elaphoglossum s.l.*) are grouped in one clade except *E. glaucum* and *E. aemulum*, as discussed above. Therefore, section *Elaphoglossum sensu* Mickel and Atehortúa (1980) is polyphyletic. That finding is unexpected because the section is morphologically homogeneous, characterized by the presence of phyllopodia and fronds glabrous or nearly so—characteristics shared by *E. glaucum* and *E. aemulum*. Within the section, however, few characters are available to distinguish the species, as laminar scales or any kind of hair are absent. Whereas other sections are defined mainly by scale characters, too few useful morphological characters could explain that numerous species were placed in section *Elaphoglossum*. Furthermore, this section contains many species. Consequently, it is probably the most difficult group to circumscribe in the whole genus. This section should continue to be recognized, but with the exclusion of *E. aemulum* and *E. glaucum*.

In all analyses, section *Elaphoglossum* consists of two well supported subclades, each of which corresponds to a subsection. Only subsection *Pachyglossa* was recognized by Mickel and Atehortúa (1980), but Skog et al. (2004) pointed out that molecular analyses supported the distinction between two clades: *Platyglossa* and *Pachyglossa*. Both names were first published by Christ (1899), but their morphological definitions must be modified regarding changes of species belonging to each subsection. Subsection *Platyglossa* is represented in our study by 15 species (Fig. 2, *E coriaceum* through *E. ovalilimbatum*) and subsection *Pachyglossa* by 26 species (Fig. 2, *E. doanense* to *E. latifolium*).

4.3. Polyphyly of species with dissected laminae

With few exceptions, all species of *Elaphoglossum* have entire laminae; however, three species with divided laminae were included in this study: *E. peltatum*, *E. tripartitum*, and *E. cardenasii*. All have strong support for their inclusion in *Elaphoglossum* (Skog et al., 2004) and therefore do not merit distinction as separate genera (such as *Peltapteris* Link). Mickel (1980) pointed out that *E. cardenasii*, with pedately divided fronds, is closely related to the group of *E. eximium* (entire fronds) because all these species share thin blades with crenulate margins, hydathodes, and subulate scales. As expected from this morphology, molecular results show *E. cardenasii* in section *Subulata* subsection *Setosa*, placed more precisely in the moderately supported clade (70% BS) defined by indel "p" comprising *E. eximium* and related species *E. aubertii*, *E. setigerum*, *E. davidsei*, and *E. russelliae*. In the same way, *E. peltatum* and *E. tripartitum*, with flabellately divided fronds, were considered related to species with entire laminae in section *Squamipedia* (Christ, 1899; Mickel, 1980). Thus, on the basis of morphology, frond division was thought to have evolved at least twice in the genus. Molecular data support this hypothesis: *E. tripartitum* and *E. peltatum* are indeed not related to *E. cardenasii*, but nested in section *Squamipedia* with the three species *E. squamipes*, *E. craspedariiforme*, and *E. marojejyense* (entire fronds).

4.4. Biogeographic implications for the species from Indian Ocean islands

The 38 species from Indian Ocean islands (in addition to *E. welwitschii* from Africa) are completely dispersed in the phylogeny (Fig. 2) showing the polyphyly of species from this region. Because sections *Lepidoglossa*, *Subulata*, *Squamipedia*, and *Elaphoglossum s.s.* are monophyletic, polyphyly was expected given that species from the region belong to all these sections. Furthermore, the species from the Indian Ocean islands do not form a monophyletic group within each of the sections. Several small clades composed of 2 to 5 species form monophyletic biogeographical units. In contrast, some other species from Indian Ocean islands are not closely related to other species from their region, but instead nested with neotropical taxa. Thus, it is possible to point out some floristic affinities between Neotropics and the Indian Ocean. Molecular results confirm or contradict several species pairs proposed by Moran and Smith (2001) on the basis of morphology. These relationships are summarized in Table 5, and discussed below.

In the section *Subulata* subsection *Setosa*, there are two closely related pairs of species pairs. One pair is *E. aubertii* from Indian Ocean and *E. eximium* from Neotropics. This pair is strongly supported by morphology (Moran and Smith, 2001), and their intergene spacer sequences from *rps4-trnS* and *trnL-trnF* are identical, except for 3 nucleotide positions in the latter. The second pair is *E. spatulatum* from Indian Ocean and *E. piloselloides* from Neotropics. They, too, are also strongly supported by morphology (Moran and Smith, 2001) and their *rps4-trnS* sequences are strictly identical for all nucleotides positions and *trnL-trnF* sequences differ by only two nucleotide bases. These similarities support the suggestion of Schelpe (1969) that *E. spatulatum* and *E. piloselloides* are probably "not... specifically distinct."

Section *Subulata* subsection *Polytrichia*, contains two species proposed by Moran and Smith (2001) as a pair on the basis of morphology: *E. erinaceum* from Neotropics and *E. hybridum* from Indian Ocean. Both belong to an unresolved subclade including *E. apodum*, *E. auripilum*, *E. backhousianum*, and *E. crinitum*. Because our molecular markers were not variable enough for resolving relationships between these six species, we cannot refute or support the species pair *E. hybridum* and *E. erinaceum*. The same limitation of molecular resolution pertains to section *Elaphoglossum* subsection *Platyglossa*, for the proposed species pair *E. glabellum* from Neotropics and *E. acrostichoides* from Indian Ocean.

In section *Elaphoglossum* subsection *Pachyglossa*, *E. doanense* from Neotropics and *E. hornei* from Indian Ocean show a weakly supported sister-species relationship. These species were not listed by Moran and Smith (2001), but their species-pair relation is supported by the following morphological characters: compact to erected rhizome, sterile blade oblanceolate, base attenuate to decurrent, rhizome scales reddish-brown bearing marginal long flexible pluricellular cilia. *Elaphoglossum doanense* differs by its generally short-petiolate sterile frond.

In section *Lepidoglossa*, Moran and Smith (2001) proposed three species pairs. The first was *E. ciliatum* from the Neotropics and *E. humbertii* from Madagascar. Both share long stipes, narrowly oblong blades, resinous dots on the laminae, highly spiculate spores, and long-creeping, resinous, glabrous (or nearly so) rhizomes. Despite these similarities, these two species belong to two different clades within sect. *Lepidoglossa* (Fig. 2). Therefore, their similarities should be interpreted as a remarkable morphological convergence. The second pair proposed was *E. muscosum* from the Neotropics and *E. poolii* from Madagascar. These two species apparently differ only in the slightly longer-ciliate scales on the stipe. Despite their great similarity, their species-pair

Table 5
List of the *Elaphoglossum* species-pairs with regard to their molecular support; each pair involves one species from the Neotropics and one species from the Indian Ocean

Species from Neotropics	Species from Indian Ocean	Support for a sister-species relationship
Species pairs proposed by Moran and Smith (2001), on the basis of morphology		
E. eximium	*E. aubertii*	Supported (100% BS)
E. piloselloides	*E. spatulatum*	Supported (100% BS; indel "g")
E. erinaceum	*E. hybridum*	Inconclusive
E. glabellum	*E. acrostichoides*	Inconclusive
E. ciliatum	*E. humbertii*	Refuted
E. muscosum	*E. poolii*	Refuted
E. paleaceum	*E. deckenii*	Refuted
Species pairs newly proposed in this study, on the basis of molecular results		
E. cuspidatum	*E. succisaefolium*	Supported (92% BS)
E. doanense	*E. hornei*	Supported (BS < 50%)
E. killipii (or *E. tectum*)	*E. lancifolium*	Inconclusive

relationship is not confirmed by our molecular results, *E. poolii* being included in a Malagasy clade with two other species *E. leucolepis* and *E. deckenii*. However, this Malagasy clade is sister to the neotropical clade containing *E. muscosum* and 4 other species—relationship supported by one synapomorphic indel. Therefore, it is justified to consider *E. poolii*, but also *E. leucolepis*, and *E. deckenii* as very close relatives to the neotropical species *E. muscosum* as well to *E. auricomum*, *E. vestitum*, *E. orbignyanum* and *E. furfuraceum*. The third species-pair postulated by Moran and Smith (2001), *E. paleaceum* from the Neotropics and *E. deckenii* from Indian Ocean-Africa, is refuted by molecular data. Although both species are very similar on the basis of their blade shape, caespitose fronds, densely scaly stipes and laminae, and ovate scales with long acicular cilia, *E. paleaceum* differs primarily in the more deeply colored rhizome scales, the acuminate blade apex (*vs*. acute in *E. deckenii*), and the occasional presence of intersporangial scales. Besides the species pairs suggested by Moran and Smith (2001) on the basis of morphology, our analyses reveal the following two species pairs. First, *Elaphoglossum lancifolium* from La Réunion (Indian Ocean) and *E. killipii* and *E. tectum* (Neotropics) form a small polytomy (Fig. 2). These three species bear on the lower lamina surface stellate to substellate scales with long acicular cilia, and they are similar in other respects including rhizome scales, linear-elliptic to linear laminae, and fertile fronds slightly longer than the sterile ones. *Elaphoglossum tectum* has also round and peltate scales on the stipe and upper lamina surface, whereas *E. lancifolium* and *E. killipii* lacks these scales but share lamina and stipe scales with resinous bases. Thus, *E. killipii* seems closest to *E. lancifolium*, and further studies improving the molecular resolution could reveal a sister-species relation between both species. The second species pair involves *E. cuspidatum* from Neotropics and *E. succisaefolium* from Marion Island (southern Indian Ocean). These species grow either terrestrially or as epiphytes and are morphologically similar by their long-creeping rhizomes densely scaly stipes and laminae, similar scales, and presence of intersporangial scales. *Elaphoglossum succisaefolium* differs mainly by its obtuse fronds (never cuspidate) and sometimes by smaller and thicker fronds, what could represent an adaptive character to the exposed habitat and to the temperate to subantarctic climate of the islands where it grows (*E. succisaefolium* is common on all the islands in the Tristan da Cunha and Gough islands groups, and Amsterdam).

Moran and Smith (2001) discussed three hypotheses to explain the origins of species pairs between Neotropical and African-Madagascan ferns, and they concluded that in most cases long-distance dispersal was likely. Long-distance dispersal must be the case for isolated oceanic islands, such as the Indian Ocean islands of the Comoros, La Réunion, Mauritius, and Amsterdam islands, all of which are relatively young and volcanic (Battistini, 1995; Rakotondrainibe et al., 1995), formed long after the continental drift had separated Africa and South America. Given this, it would require at least one initial long-distance dispersal event to give rise to the *Elaphoglossum* flora on the islands of the Indian Ocean. Given our phylogeny, more than one migration is required. *A fortiori*, it is necessary to infer several independent colonizations to explain the occurrence of several species-pairs between Neotropics and Indian Ocean islands. The Neotropics contains about 75% of the species richness in the genus. Taking this into account, we assume *Elaphoglossum* probably migrated from the Neotropics to Africa if the current distribution does not hide numerous extinction events in Africa and Indian Ocean islands. In our phylogeny, 23 monophyletic clades of species or a single species from Indian Ocean were nested among Neotropical species, and this could represent the number of migration events to the area. Considering the lack of resolution between four species from the Indian Ocean (*E. coriaceum*, *E. coursii*, *E. acrostichoides* and *E. angulatum*), at least 21 migration events occurred between the Neotropics and the Indian Ocean. The number of inferred migration events, however, becomes lower if the branch support is taken into account. The strict consensus tree exhibits many branches with low support. If branches with bootstrap values lower than 50% and lower than 70% are collapsed, then the minimum number of migration events becomes 16 and 13, respectively. This many colonizations by long-distance dispersal is conceivable. As pointed out by Tryon (1970), two main complementary processes are involved in the long-distance colonizations: the long-distance dispersal of spores, and the successful establishment of the species in a new area. Ferns spores can disperse easily by wind (Moran and Smith, 2001; Wolf et al., 2001), and are often long-lived (Tryon, 1970; Wolf et al., 2001). Homosporous ferns, such as *Elaphoglossum*, take advantage of bisexual gametophytes allowing self-fertilization and therefore facilitating a successful establishment. Moreover, the growth habit of *Elaphoglossum* gametophytes, like many other epiphytic species (Dassler and Farrar, 2001), is perennial and clone forming (Stokey and Atkinson, 1957; Chiou et al., 1998) increasing the gametophytes space, prolonging their life-span (Chiou and Farrar, 1997), and facilitating successful establishment.

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