

An Indomalesian origin in the Miocene for the diphyletic New World jewel orchids (Goodyerinae, Orchidoideae): molecular dating and biogeographic analyses document non-monophyly of the Neotropical genera

ERIC C. SMIDT^{1,2,*}, GERARDO A. SALAZAR³, ANNA VICTORIA SILVÉRIO RIGHETTO MAUAD¹, MATHIAS ERICH ENGELS¹, JUAN VIRUEL², MARK CLEMENTS⁴, IVÁN JIMÉNEZ PÉREZ⁵ and MARK W. CHASE^{2,6}

¹Universidade Federal do Paraná, Departamento de Botânica, PO Box 19031, 81530-900, Curitiba, Paraná, Brazil

²Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK

³Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-367, 04510 Mexico City, Mexico

⁴Centre for Australian National Biodiversity Research, (CANBR), National Facilities and Collections, CSIRO, GPO Box 1700 Canberra ACT 2601, Australia

⁵Herbario Nacional de Bolivia, Casilla 10077 – Correo Central, La Paz, Bolivia

⁶Department of Environment and Agriculture, Curtin University, Bentley, Western Australia 6102, Australia

Received 2 September 2020; revised 10 December 2020; accepted for publication 20 March 2021

The jewel orchids (Goodyerinae), named after their often colourful leaves, have a pantropical distribution with a clear Asian centre of diversity. However, the Nearctic and Neotropical America together form a second centre of diversity, with one-third of known species of Goodyerinae. Previously, only a few American samples have been included in phylogenetic studies, and their putatively Asian origins and American divergence times were poorly known. To elucidate these topics, we inferred phylogenetic trees, performed molecular dating and reconstructed biogeographic history using nuclear ribosomal ITS and plastid *matK* sequences for 34 species of Goodyerinae from the New World and 76 previously published accessions of Cranichideae. Our well-supported phylogenetic topology suggests two independent dispersal events to the New World from the Indomalesian region during the Miocene. The first inferred dispersal of a Neotropical clade diverged c. 11 Mya from their most recent common ancestor (MRCA), comprising three highly supported subclades that do not match the limits of *Aspidogyne*, *Kreodanthus* and *Microchilus* as previously circumscribed. The second dispersal involved a largely Nearctic clade of *Goodyera* s.l. diverging c. 8.4 Mya from the MRCA and exhibiting a complex biogeographic history with subsequent dispersals between the Nearctic and Indomalesia. The occurrence of these species in gallery forests putatively prevented vicariance events imposed by the expansion of the Chacoan region as previously detected for epiphytic Orchidoideae. Eighty-nine nomenclatural combinations and three new names in *Microchilus* are proposed.

ADDITIONAL KEYWORDS: Atlantic Forest – Chacoan vicariance – *Meliorchis* – Neotropical biogeography – Neotropical flora – *matK* – nrITS – orchid molecular phylogenetics.

INTRODUCTION

Goodyerinae (Orchidoideae) are one of eight subtribes of Cranichideae (Chase *et al.*, 2015), comprising 33 genera and 746 tropical and subtropical species

(Ormerod, 2002, 2005, 2008, 2009, 2013; Pridgeon *et al.*, 2003). The greatest diversity of genera and species occurs in the Palaeotropics and Palaeartic, where 29 genera and 510 species occur, mostly in Asia (Ormerod, 2008; Pridgeon *et al.*, 2003), whereas c. 280 species in four native genera occur in the Neotropics and Nearctic (Meneguzzo, 2012; Ormerod, 2013;

*Corresponding author. E-mail: ecsmidt@yahoo.com.br

Smidt, Engels & Miranda, 2016). One species, *Zeuxine strateumatica* (L.) Schltr., has dispersed widely throughout the tropics but is a native of central and south-eastern Asia and north-eastern Oceania. It has naturalized in North America, Hawaii and the Caribbean and was recently found in south-eastern Brazil (Menini Neto, Miranda & Cruz, 2011; Engels, Barros & Smidt, 2016).

Goodyerinae can be recognised among Orchidoideae by their distinctive terrestrial habit, characterized by a usually prostrate rhizome (except *Pachyplectron* Schltr.) producing roots from their nodes and an upright stem on which leaves are arranged in a spiral (Dressler, 1993; Bona *et al.*, 2020). Leaves may be plain green or variously coloured and often have reticulate venation, giving rise to the common name 'jewel orchids' (Dressler, 1993; Soroka, 2006). Flowers are shortly tubular with the labellum either saccate or projecting into a nectariferous spur, a relatively short, straight or arcuate column and a dorsal, erect anther bearing two bipartite and sectile pollinia with a terminal viscidium (Singer & Szizima, 2001; Ormerod & Cribb in Pridgeon *et al.*, 2003).

Taxonomy of the Neotropical genera, the main focus of this work, has been problematic and requires further study (Chase *et al.*, 2015; Chen *et al.*, 2019), principally because it has been only based on minor differences in floral morphology, which are subject to misinterpretation. Historically, the oldest generic name applied to the Neotropical taxa is *Microchilus* C.Presl, proposed to encompass two Peruvian species (Presl, 1827). These were later subsumed, as were many other subsequently species described, into the illegitimate genus *Physurus* Rich. ex Lindl. or in *Erythrodes* Blume (Ames, 1922). *Erythrodes*, as then circumscribed, occurred in the Neotropics, temperate North America and Asia (Hoehne, 1945; Pridgeon *et al.*, 2003). Garay (1977) published a study of New World Goodyerinae (as Physurinae Lindl. ex Meissn.) based on variation in column morphology, resulting in recognition of six new genera separated from *Erythrodes* (*Aspidogyne* Garay, *Kreodanthus* Garay, *Ligeophila* Garay, *Platythelys* Garay, *Rhamphorhynchus* Garay and *Stephanothelys* Garay). Ormerod (2002) followed this treatment with the proposed resurrection of *Microchilus* to include all New World species previously assigned to *Erythrodes*, distinguishing the former from the latter by the deeply bifid rostellar remnant (vs. divided only on the apical third) and details of the viscidium and pollinia. Thus circumscribed, *Erythrodes* is restricted to tropical and subtropical regions of Asia and the south-western Pacific (POWO, 2020).

Ligeophila, *Platythelys*, *Rhamphorhynchus* and *Stephanothelys* were treated by Meneguzzo (2012)

as synonyms of *Aspidogyne* due to inconsistencies in the morphological characters used by Garay (1977) to define the genera, and this approach has been widely followed (e.g. Ormerod, 2008; Chase *et al.*, 2015). Thus, four Neotropical Goodyerinae genera are currently accepted: *Aspidogyne*, *Kreodanthus* and *Microchilus* (Figs 1, 2) plus *Goodyera* R.Br. (with c. 17 nominal species distributed from Central America to boreal North America in addition to 82 species in the Old World including the Palaearctic; POWO, 2020). *Goodyera repens* (L.) R.Br. is distributed throughout the temperate and boreal Northern Hemisphere (POWO, 2020). *Aspidogyne* has 71 species distributed from south-eastern North America to south-western South America (Ormerod, 2013), *Microchilus* has 135 species found from southern North America to south-western South America (Ormerod, 2009) and *Kreodanthus* comprises 14 species from southern North America (Mexico) to north-western South America (Ormerod, 2008).

From a molecular phylogenetic perspective, New World Goodyerinae have been minimally represented in previous studies aimed at assessing intrafamilial relationships. For instance, Cameron *et al.* (1999, 2004) and Freudenstein *et al.* (2004) included only *Platythelys querceticola* (Lindl.) Garay [now *Aspidogyne querceticola* (Lindl.) Meneguzzo], whereas Górnjak, Paun & Chase (2010) used one accession of *Aspidogyne tuerckheimii* (Schltr.) Garay. Phylogenetic studies focusing on Cranichideae and subtribes also under-represented Goodyerinae (Salazar *et al.*, 2003, 2009; Alvaréz-Molina & Cameron, 2009; Cisternas *et al.*, 2012). Hu *et al.* (2016) conducted the first molecular phylogenetic analysis of Goodyerinae based on plastid (*matK* and *trnL-F*) and nuclear ribosomal (nrITS) DNA sequences for representatives of 25 genera of the subtribe focusing on Asian *Goodyera*. Their sampling included only four North American *Goodyera* spp. and one *Aspidogyne* sp. (as *Platythelys*), which grouped with tropical Asian species of *Hylophila* Lindl., *Lepidogyne* Blume and *Goodyera* section *Otosepalum* Schltr. More recently, Chen *et al.* (2019) assessed phylogenetic relationships in Goodyerinae, analysing two nuclear (rITS and *Xdh*) and four plastid (*matK*, *psaB*, *rbcL*, *trnL-F*) DNA regions and representatives of 27 genera of the subtribe, including four New World genera (*Aspidogyne*, *Kreodanthus*, *Microchilus* and *Goodyera*). The work by Chen *et al.* (2019) was significant because it showed that the molecular results do not support some proposed genera distinguished solely on gynostemium morphology. Moreover, they found a relationship between *Aspidogyne*, *Kreodanthus* and *Microchilus* with some tropical Asian members of *Goodyera* and other Asian genera in a group they

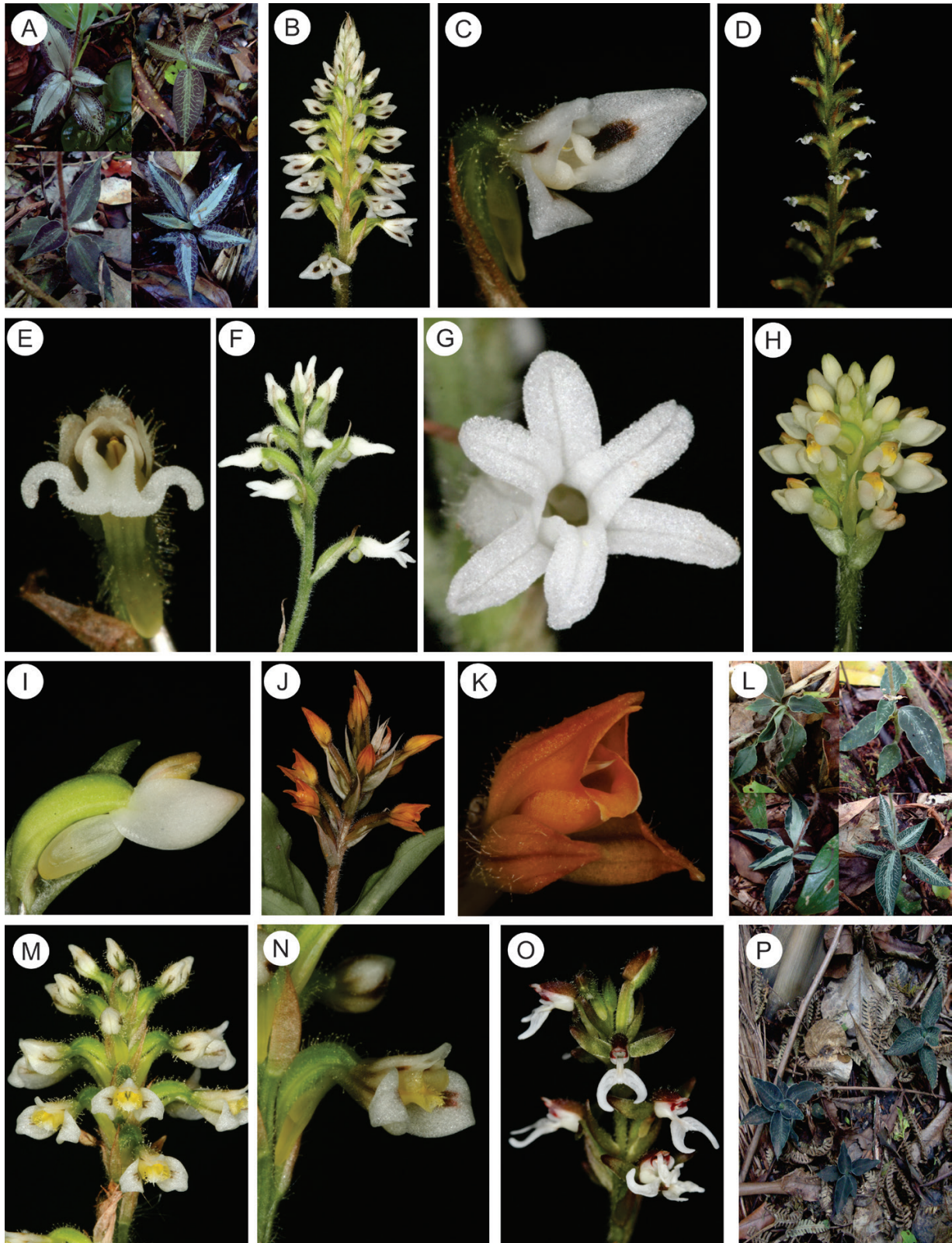


Figure 1. Species of New World Goodyerinae in this study. A, Leaf patterns of *Microchilus argenteus*; B, inflorescence of *M. argenteus*; C, flower of *M. argenteus*; D, inflorescence of *Microchilus arietinus*; E, inflorescence of *Microchilus*

termed the *Microchilus* clade, a name we will use here for continuity, even though this clade comprises many Asian taxa and *Microchilus* is exclusively New World. This large clade was also only weakly supported, making it unwise to consider alternative taxonomic arrangements for the *Goodyera* spp. misplaced in the *Microchilus* clade *sensu* Chen *et al.* (2019). For example, in Hu *et al.* (2016) *G. procera* (Ker Gawl.) Hook. is sister to the remainder of the *Microchilus* clade, but in Chen *et al.* (2019) this species is sister to the main clade of *Goodyera*. Pace (2020) proposed the resurrected genus *Cionisaccus* Breda for *G. procera*, but if the Chen *et al.* (2019) placement is correct, then *G. procera* could be retained in *Goodyera*.

Givnish *et al.* (2015) conducted a phylogenomic analysis of Orchidaceae based on 75 plastid genes and nearly complete tribal/subtribal sampling, providing an estimated age of 32 (± 40 –25) Myr for Goodyerinae, which were represented by eight species, five of them American. Subsequently, Givnish *et al.* (2016) conducted a historical biogeographic analysis of Orchidaceae, but because of the sparse sampling they were unable to specify a particular ancestral area for Goodyerinae, with about the same probabilities of being a combination of the Neotropical/Pacific areas, perhaps connected by an Antarctic route or across the Pacific.

The main objective of our study is to assess the phylogenetic relationships of New World Goodyerinae in much greater detail and investigate their geographical origin and divergence times. We also discuss the taxonomic implications of relationships uncovered as they pertain to the Neotropical genera of Goodyerinae.

MATERIAL AND METHODS

SAMPLING

Voucher information and GenBank accessions for the 110 species included are given in Table 1. These include 34 species sequenced for the first time, including nearly all genera of Goodyerinae and representative species of the other subtribes of Cranichideae *sensu* Chase *et al.* (2015) used as outgroups.

MOLECULAR TECHNIQUES AND ANALYSIS

For the newly sequenced taxa, DNA was extracted from fresh or silica-dried leaf material using the CTAB protocol of Doyle & Doyle (1987) without the addition

of RNase A and scaled to 2 mL tubes. Amplification of nrITS was performed in 20 μ L reactions [1 \times buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 μ M each primer, 10 ng of bovine serum albumin (BSA), 5 M betaine, 2% dimethylsulphoxide (DMSO), 1 unit of Taq DNA polymerase (Invitrogen, Life Technologies Corporation), 20–50 ng of genomic DNA and primers 92 (5' AAG GTT TCC GTA GGT GAA 3') and 75 (5' TAT GCT TAA ACT CAG CGG G 3') Desfeux & Lejeune, 1996]. The partial *matK* exon was amplified with the primers *matK* 2.1f (5' CCTATCCATCTGGAAATCTTAG 3') and *matK* 5R (5' GTTCTAGCACAAGAAAGTGC 3'; Ford *et al.*, 2009). Amplification of *matK* was performed in 20 μ L mixtures containing 1 \times CoralLoad buffer, 0.2 μ M each primer, 1 \times TopTaq Master Mix and 20–50 ng of genomic DNA using the TopTaq Master Mix kit (QIAGEN Biotechnology). The thermocycler was programmed for an initial pre-melt at 94 °C for 1 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C (nrITS)/53 °C (*matK*) for 40 s and extension at 72 °C for 40 s, followed by a final extension for 5 min at 72 °C. PCR products were purified with 10% polyethylene glycol (PEG) and 80% ethanol (Paithankar & Prasad, 1991). Some samples were extracted and amplified with primers and procedures described in Salazar *et al.* (2003). Sequencing reactions used Big Dye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA) manufactured by Macrogen Inc. (Seoul, South Korea; <http://dna.macrogen.com>) or the Federal University of Paraná, Curitiba, Brazil.

The sequences were assembled and edited with Geneious Prime v.2019.1.1 (<https://www.geneious.com>). Multiple sequence alignments were performed using MAFFT 7 (Katoh & Standley, 2013) with default settings in the auto-strategy algorithm (1 PAM, $K = 2$) and visually inspected and manually adjusted in Geneious Prime. Indels (insertions/deletions) were treated as missing data.

Phylogenetic analyses were performed using maximum likelihood, maximum parsimony and Bayesian inference to explore results under different assumptions. Maximum-likelihood phylogenetic trees were calculated using IQ-TREE v.1.6.11 (Nguyen *et al.*, 2015) with 1000 bootstrap replicates in a single run (Chernomor, von Haeseler & Minh, 2016; Kalyaanamoorthy *et al.*, 2017). The best-fit models of substitution also estimated in IQ-TREE using the Akaike information criterion (AIC) were GTR + F + R4 for nrITS and TVM + F + R4 for

austrobrasiliensis; G, flower of *M. austrobrasiliensis*; H, inflorescence of *Microchilus bidentiferus*; I, flower of *M. bidentiferus*; J, inflorescence of *Microchilus commelinoides*; K, Flower of *M. commelinoides*; L, leaf patterns of *Microchilus fimbriolaris*, M, inflorescence of *M. fimbriolaris*; N, flower of *M. fimbriolaris*; O, inflorescence of *Microchilus juruenensis* and P, leaf patterns of *Microchilus kuczynskii* (Photographs by Eric C. Smidt, except A, L, P by Mathias E. Engels).

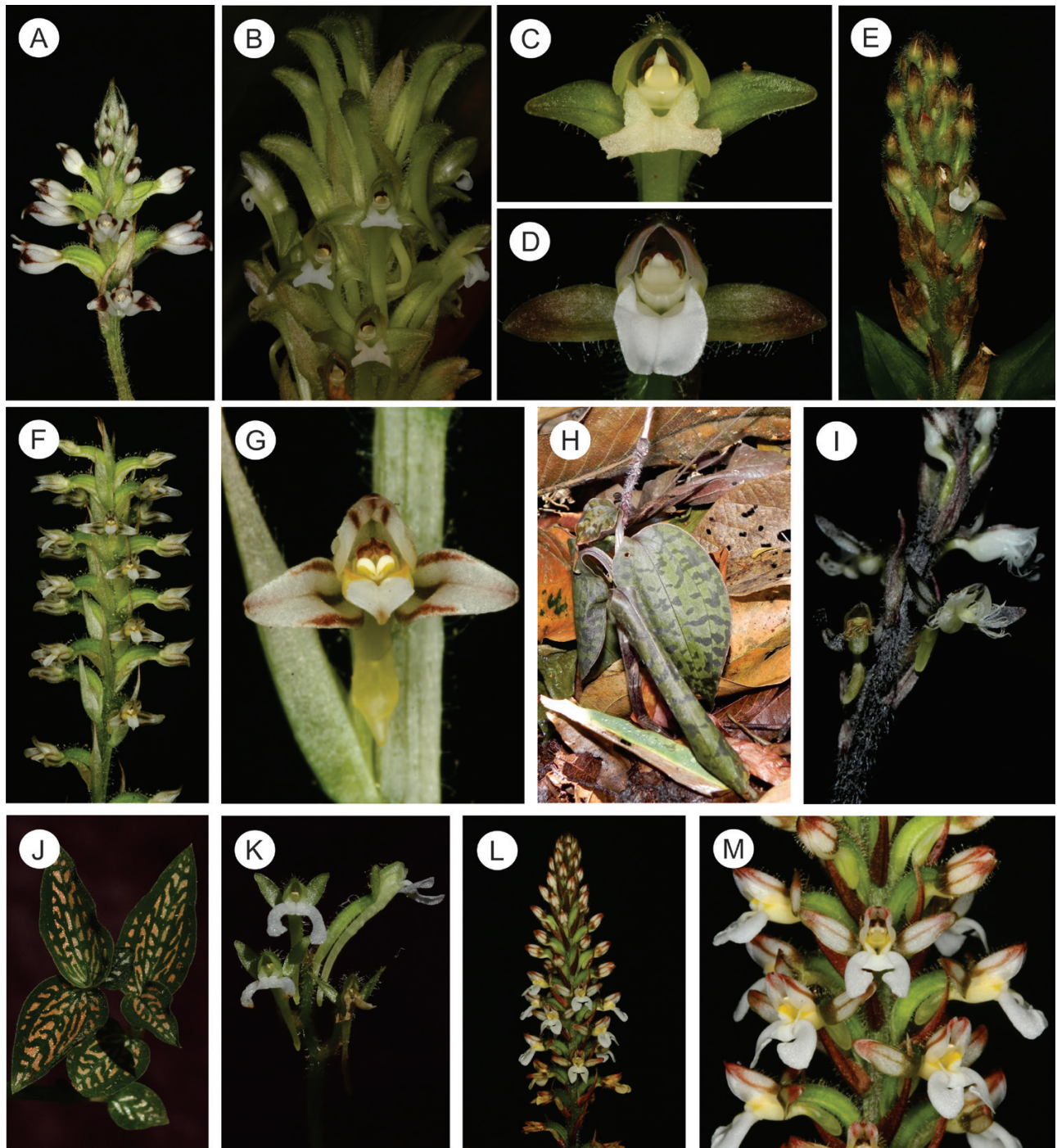


Figure 2. Species of the New World Goodyerinae in this study. A, inflorescence of *M. kuczynskii*; B, inflorescence of *Microchilus longicornu*; C, flower of *M. longicornu*; D, flower of *Microchilus malmei*; E, inflorescence of *M. malmei*; F, inflorescence of *Microchilus metallescens*; G, flower of *M. metallescens*; H, leaf pattern of *Microchilus mystacinus*; I, inflorescence of *M. mystacinus*; J, leaf pattern of *Microchilus pumilus*; K, inflorescence of *M. pumilus*; L, inflorescence of *Microchilus roseus*; M, flower of *M. roseus* (Photographs by Eric C. Smidt, except H, I by Tarcísio M. Rodrigues and J, K by Mathias E. Engels).

matK. Bootstrap support was calculated based on 1000 ultrafast bootstrap approximation (UFBoot) replicates (Minh, Nguyen & von Haeseler, 2013),

with a strategy to reduce the risk of over-estimating support (-bnni). Bayesian inference used MrBayes v.3.2.7a (Ronquist *et al.*, 2012) in the CIPRES

Table 1. Species sampled, GenBank (NCBI) accession numbers and geographical distribution. Geographical regions for Cranichideae^A following Olson *et al.*, (2001) proposal for terrestrial regions: A, Nearctic; B, Neotropic; C, Palearctic; D, Afrotropic; E, Indomalaya; F, Australasia and for the Neotropic^B, following Morrone (2014) B1, Mexican Transition Zone; B2, Mesoamerican; B3, Pacific; B4, northern Brazilian; B5, southern Brazilian; B6, Paraná (Atlantic Forest) and B7, South American Transition Zone; B8, Chacoan; B9, extra-Neotropical. *indicates new sequences generated for this study

Species	ITS	<i>matK</i>	Cranichideae Geography ^A	Neotropical Geography ^B
Chloraeinae				
<i>Chloraea magellanica</i> Hook.f.	AJ539523	GQ917033	B	
Cranichidinae				
<i>Baskervilla colombiana</i> Garay	AM419791	EU214310	B	
<i>Baskervilla paranaensis</i> (Kraenzl.) Schltr.	*MN946471	*MN946485	B	
<i>Cranichis apiculata</i> Lindl.	AM419784	AM900819	B	
<i>Cranichis muscosa</i> Sw.	AM419777	AM900812	B	
<i>Gomphichis adnata</i> (Ridl.) Schltr.	AM419769	AM900804	B	
<i>Ponthieva diptera</i> Linden & Rchb.f.	AM419792	AM900827	B	
<i>Prescottia plantaginea</i> Lindl.	AJ539511	AJ543939	B	
<i>Prescottia stachyodes</i> (Sw.) Lindl.	AM419773	AM900808	B	
<i>Pterichis galeata</i> Lindl.	AM419796	AM900831	B	
<i>Stenoptera ecuadorana</i> Dodson & C.Vargas	AJ539512	AJ543940	B	
Discyphinae				
<i>Discyphus scopulariae</i> (Rchb.f.) Schltr.	LK391732	LK391735	B	
Galeottiellinae				
<i>Galeottiella sarcoglossa</i> (A.Rich. & Galeotti) Schltr.	AJ539518	AJ543945	B	
Goodyerinae				
<i>Anoectochilus albolineatus</i> E.C.Parish & Rchb.f.	JN166058	JN166018	E	
<i>Anoectochilus formosanus</i> Hayata	AY052780	EU797513	ACE	
<i>Anoectochilus koshunensis</i> Hayata	EU700340	EU797512	E	
<i>Aspidogyne argentea</i> (Vell.) Garay	*MN946458	*MN946474	B	B5, B6
<i>Aspidogyne bidentifera</i> (Schltr.) Garay	*MN946459	*MN946475	B	B6
<i>Aspidogyne caraguatatubensis</i> M.R.Miranda, Engels & E.C.Smidt	*MN946460	-	B	B6
<i>Aspidogyne commelinoides</i> (Barb.Rodr.) Garay	*MN946461	-	B	B6
<i>Aspidogyne confusa</i> (C.Schweinf.) Garay	*MT271798	*MT265032	B	B3, B4
<i>Aspidogyne fimbrillaris</i> (B.S.Williams) Garay	*MN946462	*MN946476	B	B6
<i>Aspidogyne juruenensis</i> (Hoehne) Meneguzzo	*MN946464	*MN946478	B	B3, B4, B5, B6, B8
<i>Aspidogyne juruenensis</i> (Hoehne) Meneguzzo1	*MN946463	*MN946477	B	B3, B4, B5, B6, B8
<i>Aspidogyne kuczynskii</i> (Porsch) Garay	*MN946465	*MN946479	B	B6
<i>Aspidogyne longicornu</i> (Cogn.) Garay	*MN946466	*MN946480	B	B6
<i>Aspidogyne malmei</i> (Kraenzl.) Garay	*MN946467	*MN946481	B	B3, B4, B5, B6
<i>Aspidogyne metallescens</i> (Barb.Rodr.) Garay	*MN946468	*MN946482	B	B6
<i>Aspidogyne mystacina</i> (Rchb.f.) Garay	*MN946469	*MN946483	B	B4, B5
<i>Aspidogyne platensis</i> (Hauman) Meneguzzo	*MT271799	*MT265033	B	B8
<i>Aspidogyne pumila</i> (Cogn.) Garay	*MN946470	*MN946484	B	B4, B5, B6
<i>Aspidogyne querceticola</i> (Lindl.) Meneguzzo	FJ473336	AY368386	B	B2, B3, B4
<i>Aspidogyne querceticola</i> (Lindl.) Meneguzzo	*MT271801	*MT265035	B	B2, B3, B4
<i>Aspidogyne rosea</i> (Lindl.) Meneguzzo	*MT271802	*MT265036	B	B4, B5, B6, B8
<i>Aspidogyne utriculata</i> (Dressler) Szlach.	*MT271803	*MT265037	B	B2
<i>Chamaegastrodia shikokiana</i> Makino & F.Maek	JN166061	JN166020	E	

Table 1. Continued

Species	ITS	<i>matK</i>	Cranichideae Geography ^A	Neotropical Geography ^B
<i>Cystorchis aphylla</i> Ridl.	JN166062	JN166062	E	
<i>Cystorchis gracilis</i> (Hook.f.) Holttum	JN166063	JN166033	E	
<i>Dossinia marmorata</i> C.Morren	JN166065	AJ543947	E	
<i>Erythroides blumei</i> (Lindl.) Schltr.	JN166066	JN166024	B	B9
<i>Erythroides latifolia</i> Blume	KT343983	JN166025	B	B9
<i>Gonatostylis vieillardii</i> (Rchb.f.) Schltr.	*MT271804	AJ310034	F	
<i>Goodyera biflora</i> (Lindl.) Hook.f.	KT343989	KT385588	E	
<i>Goodyera bilamellata</i> Hayata	KT343993	KT385592	E	
<i>Goodyera daibuzanensis</i> Yamam.	*MT271807	*MT265039	E	
<i>Goodyera_spp</i>	*MT271806	*MT265038	B	
<i>Goodyera kwangtungensis</i> C.L.Tso	KT344028	KT385620	E	
<i>Goodyera marginata</i> Lindl.	KT344033	KT385623	E	
<i>Goodyera major</i> Ames & Correll	*MT271808	*MT265040	B	
<i>Goodyera oblongifolia</i> Raf.	HM141004	KX676982	ACD	
<i>Goodyera pendula</i> Maxim.	KT344039	KT385628	E	
<i>Goodyera procera</i> (Ker Gawl.) Hook.	HM222488	JN04450	E	
<i>Goodyera procera</i> (Ker Gawl.) Hook.	*MT271809	*MT265041	E	
<i>Goodyera pubescens</i> (Willd.) R.Br.	AJ539519	AJ543954	AB	
<i>Goodyera pusilla</i> Blume	KT344047	JN166026	E	
<i>Goodyera repens</i> (L.) R.Br.	KT338705	KC704633	ACE	
<i>Goodyera robusta</i> Hook.f.	KT344054	KT385637	EF	
<i>Goodyera rubicunda</i> (Blume) Lindl.	KT344059	KT385640	EF	
<i>Goodyera seikomontana</i> Yamam.	KT344068	KT385645	E	
<i>Goodyera striata</i> Rchb.f.	AM778169	AM902104	B	
<i>Goodyera tessellata</i> Lodd.	HM141006	MK520148	A	
<i>Goodyera velutina</i> Maxim. ex Regel	KT344071	KT385651	E	
<i>Goodyera viridiflora</i> (Blume) Blume	JN166067	JN166027	CEF	B9
<i>Hetaeria heterosepala</i> (Rchb.f.) Summerh.	*MT271810	*MT265042	D	
<i>Hetaeria oblongifolia</i> Blume	JN166069	JN166029	EF	
<i>Hetaeria youngsayei</i> Ormerod	KY966608	KY966893	E	
<i>Hylophila lanceolata</i> (Blume) Miq.	JN166070	JN166030	E	
<i>Kreodanthus crispifolius</i> Garay	*MT271811	*MT265043	B	B4
<i>Kreodanthus simplex</i> (C.Schweinf.) Garay	FJ473328	-	B	B5, B7
<i>Kuhlhasseltia nakaiana</i> (F.Maek.) Ormerod	JN166071	JN166031	C	
<i>Lepidogyne longifolia</i> (Blume) Blume	JN166072	JN166032	F	
<i>Ludisia discolor</i> (Ker Gawl.) A.Rich.	AJ539483	AJ543911	ACE	
<i>Microchilus arietinus</i> (Rchb.f. & Warm.) Ormerod	*MN946472	*MN946486	B	B3, B4, B5, B6
<i>Microchilus austrobrasiliensis</i> (Porsch) Ormerod	*MN946473	*MN946487	B	B6
<i>Microchilus erythrodoides</i> (Schltr.) Ormerod	*MT271815	*MT265047	B	B3
<i>Microchilus luniferus</i> (Schltr.) Ormerod	*MT271816	*MT265048	B	B1, B2, B3
<i>Microchilus sparreorum</i> (Garay) Ormerod	*MT271817	*MT265049	B	B3
<i>Microchilus tridax</i> (Rchb.f.) Ormerod	-	FR832762	B	B2, B3
<i>Microchilus vesicifer</i> (Rchb.f.) Ormerod	*MT271818	*MT265050	B	B2, B7
<i>Odontochilus montanus</i> (Schltr.) J.M.H.Shaw	FJ473344	AJ310061	F	
<i>Pachyplectron arifolium</i> Schltr.	AJ539522	AJ310051	F	
<i>Pachyplectron neocaledonicum</i> Schltr.	*MT271819	*MT265051	F	
<i>Platylepis polyadenia</i> Rchb.f.	AJ539520 1	AJ543946	D	
<i>Vrydagzynea lancifolia</i> Ridl.	JN166074	JN166033	E	
<i>Zeuxine gracilis</i> (Breda) Blume	JN166075	JN166034	E	
<i>Zeuxine strateumatica</i> (L.) Schltr.	KY966688	AJ310080	ABCEF	

Table 1. Continued

Species	ITS	<i>matK</i>	Cranichideae Geography ^A	Neotropical Geography ^B
Manniellinae				
<i>Manniella gustavi</i> Rchb.f.	AJ539517	AJ543944	D	
Spiranthinae				
<i>Aulosepalum tenuiflorum</i> (Greenm.) Garay	AJ539491	AJ543919	B	
<i>Beloglottis costaricensis</i> (Rchb.f.) Schltr.	AJ539492.2	AJ543920	B	
<i>Brachystele guayanensis</i> (Lindl.) Schltr.	MG460367	MG460434	B	
<i>Deiregyne diaphana</i> (Lindl.) Garay	KU752292	KU752267	B	
<i>Dichromanthus aurantiacus</i> (Lex.) Salazar & Soto Arenas	FN996956	AJ543913	B	
<i>Cotylolabium lutzii</i> (Pabst) Garay	HG425360	HG425363	B	
<i>Eltroplectris calcarata</i> (Sw.) Garay & H.R.Sweet	MF464940	MG755123	B	
<i>Eurystyles cotyledon</i> Wawra	EF079172	HE575501	B	
<i>Funkiella hyemalis</i> (A.Rich. & Galeotti) Schltr.	AJ539495	AJ543923	B	
<i>Lankesterella ceracifolia</i> (Barb.Rodr.) Mansf.	FN556164	FN556174	B	
<i>Microthelys constricta</i> (Szlach.) Szlach.	AM778173	AM902108	B	
<i>Odontorrhynchus variabilis</i> Garay	AF348047	AJ543926	B	
<i>Pelexia orthosepala</i> (Rchb.f. & Warm.) Schltr.	MF464961	MG755145	B	
<i>Sacoila lanceolata</i> (Aubl.) Garay	MF464948	MG755151	B	
<i>Sarcoglottis acaulis</i> (Sm.) Schltr.	KJ472391	KJ472360	B	
<i>Sauroglossum elatum</i> Lindl.	KX421916	KX421973	B	
<i>Schiedeella garayana</i> R.González	LT600860	LT600878	B	
<i>Spiranthes romanzoffiana</i> Cham.	KMF465012	KF997301	AC	
<i>Svenkoeltzia congestiflora</i> (L.O.Williams) Burns-Bal.	AJ539493	AJ543921	B	
<i>Thelyschista ghillanyi</i> (Pabst) Garay	MF464937	MG755181	B	
<i>Veyretia rupicola</i> (Garay) F.Barros	MF465004	MG755182	B	
Pterostylidinae				
<i>Pterostylis curta</i> R.Br.	GQ866363	EF079298	CF	

Science Gateway (Miller, Pfeiffer & Schwartz, 2010) with the model GTR+I+G for both nrITS and *matK* based on the AIC criterion in jModelTest v.2.1.7 (Durriba *et al.*, 2012). Bayesian analyses started from random trees and employed Markov chain Monte Carlo (MCMC) runs over 10×10^6 generations, sampling trees every 1000 generations. We discarded 25% of the first generations as the burn-in after visual inspection of stabilisation of log-likelihood of the trees, as measured by the Stdev(s) and PSRF values (Gelman & Rubin, 1992) and the effective sample size (ESS) accessed by Tracer v.1.7 (Rambaut *et al.*, 2018). The remaining trees were used to produce the Bayesian probability tree with FigTree v.1.4.3 (Rambaut, 2009). Maximum parsimony (MP) analyses were performed with Fitch (1971) parsimony using PAUP v.4.0b10a (Swofford, 2002). Analyses included 1000 random taxon-addition replicates, holding ten trees per replicate and using the tree-bisection reconnection (TBR) swapping algorithm, followed by a second search to explore all

topologies from the previous search, limited to 10 000 trees. Support was estimated by 1000 bootstrap replicates (Felsenstein, 1985) using simple addition, the TBR algorithm and 20 trees held per replicate. Bootstrap percentages (> 50) were categorized as weak (51–75), moderate (76–90) and strong support (91–100, e.g. Whitten *et al.*, 2007). Individual shortest trees, strict consensus trees and bootstrap consensus trees were examined visually to assess congruence among data sets. In the absence of moderately to strongly supported phylogenetic incongruence between the plastid and nuclear markers (bootstrap percentages > 75; Wiens, 1998; Whitten *et al.*, 2005), the matrices were combined in Geneious Prime. The resulting phylogenetic trees were edited in FigTree and CorelDRAW v.18.0.0.448 (<http://www.coreldraw.com>). The aligned data matrix with 2875 characters (823 nrITS and 2052 plastid *matK*) is available in TreeBASE (<http://purl.org/phylo/treebase/phylo/study/TB2:25513>).

ESTIMATION OF DIVERGENCE TIMES

Ramírez *et al.* (2007) published a pollen fossil identified as a species of Goodyerinae, *Meliorchis caribea* S.R.Ramírez, Gravend., R.B.Singer., C.R.Marshall & N.E.Pierce, based on its morphology and dated to 15–20 Mya; the oldest and youngest age boundaries of Dominican amber. This fossil was used as a minimum-age calibration point for Goodyerinae and estimated age of 34–38 Myr for the subtribe (Ramírez *et al.*, 2007). Due to the diphyletic nature of Goodyerinae in the Neotropics and the partial overlap of their distribution in Central America, *Meliorchis caribea* (Ramírez *et al.*, 2007) could not be assigned to a specific node used for calibration in our study. Therefore, we used a secondary calibration of the crown age of Cranichideae from Givnish *et al.* (2015), 42.82 Myr (95% highest posterior density interval, 30.12–50.13, and normal distribution prior, mean = 42, standard deviation = 3.5). To estimate the divergence times of subclades of Cranichideae, we used the same matrix as above in BEAST v.1.8.3 (Drummond *et al.*, 2012) in the CIPRES server (Miller *et al.*, 2010).

We used an uncorrelated relaxed clock model with lognormal distribution and GTR+I+ Γ substitution model with empirical base frequencies for both partitions (nrITS and *matK*). The starting tree was the ML from IQ-TREE, with fixed tree topology to avoid incongruences between molecular dating and phylogenetics results. We tested two speciation models, Yule (Yule, 1925) and birth-death (Gernhard, 2008), which were compared in Tracer v.1.7 (Rambaut *et al.*, 2018) by tree likelihood under AIC estimated by method-of-moments (AICM; Baele *et al.*, 2012; see also Supporting Information, Table S1). MCMC chains were run for 100×10^6 generations, sampling every 10 000 steps. Convergence and ESS (≥ 300) were assessed in Tracer v.1.6 for all parameters. A maximum clade credibility tree was produced in TreeAnnotator v.1.8.4 (part of BEAST package) with 25% burn-in. Visualization and tree editing were performed in FigTree.

BIOGEOGRAPHICAL ANALYSES

Based on the current distribution of the species of American Goodyerinae (www.gbif.org, 10 April 2019), we infer the biogeographic history of the subtribe through two analyses. A first analysis aims to explore the origin of the group in the Americas included six operational areas (OAs) based on the terrestrial ecoregions of the World proposed by Olson *et al.* (2001): A, Nearctic; B, Neotropic; C, Palaearctic; D, Afrotropic; E, Indomalayan and F, Australasian. A second analysis was conducted to explore biogeographical patterns in the Neotropics using the OAs proposed

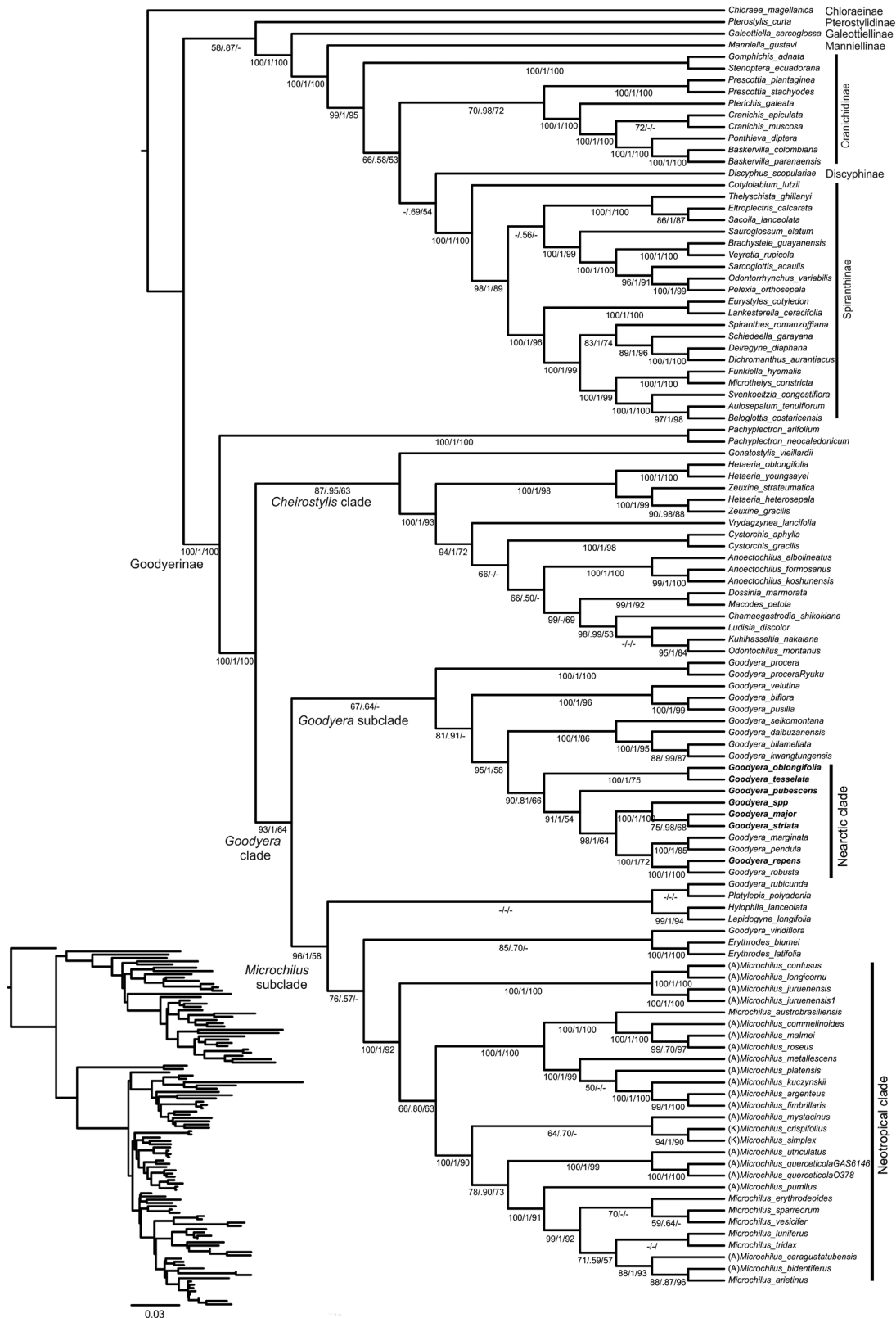
by Morrone (2014): B1, Mexican Transition Zone; B2, Mesoamerica; B3, Pacific Ocean; B4, northern Brazil; B5, southern Brazil; B6, Paraná (Atlantic Forest) B7, South American Transition Zone; B8, Chacoan and B9, Indomalayan, as outgroup, based in the sister group result from the first analysis (Table 1). The second analysis aims to explore if the dry diagonal, i.e. the separation of Amazon and Atlantic Forests by a north-east/south-west open vegetation diagonal (Werneck, 2011), created a vicariance pattern for this group and if dispersal events show latitudinal patterns. We used BioGeoBEARS (Matzke, 2013) as implemented in RASP v.4.0 (Yu *et al.*, 2015) to evaluate several biogeographic models to infer the contribution of various evolutionary processes (i.e. range expansion, range extinctions, vicariance, founder-event speciation, speciation despite sympatry) in explaining the current species distribution of Cranichideae. The highest AICc_wt value was used to select the biogeographic model best fitting the data.

RESULTS

PHYLOGENETIC RELATIONSHIPS

All methods produced a similar supported topology for the separate nrITS and *matK* analyses, and we present only results from the combined dataset (Fig. 3). Results of the phylogenetic analyses for each marker are shown in the Supporting Information (S1–S8) and parsimony statistics for each marker are presented in Table 2. Disagreements in the phylogenetic positions of some taxa are highlighted in the discussion. The aligned combined matrix for 110 species consisted of 2875 characters. Of these, 935 (32%) were potentially parsimony informative. The ML topology from the analysis of the combined dataset is presented with ML bootstrap percentages, Bayesian posterior probabilities and MP bootstrap percentages above the branches (Fig. 3).

Cranichideae have been proposed to comprise eight subtribes (Chase *et al.* 2015), with which the results presented here are compatible. Some inconsistencies in the relative positioning of these subtribes require more extensive sampling of species and molecular markers and are outside the focus of this study. Goodyerinae are monophyletic (100 MLBP, 1.0 PP and 100 MPBP), with *Pachyplectron* strongly supported as sister to the rest. The *Cheirostylis* clade (100 MPBP, 0.95 PP and 63 MLBP) is sister to the *Goodyera* clade, which in turn consists of two groups: the weakly supported *Goodyera* subclade (67 MLBP, 0.64 PP and < 50 MPBP) and the more strongly supported *Microchilus* subclade (96 MLBP, 1.0 PP and 58 MPBP; Fig. 3). *Goodyera sensu* Hu *et al.* (2016) and



Downloaded from https://academic.oup.com/botlinnean/article/197/3/322/6274654 by guest on 24 April 2024

Figure 3. Maximum-likelihood tree of Goodyerinae plus outgroups. Numbers on nodes represent ML bootstrap percentages (≥ 50), posterior probabilities from Bayesian inference (≥ 0.50) and maximum parsimony bootstrap percentages (≥ 50). See

Table 2. Results of the individual and combined parsimony analyses

	nrITS	plastid <i>matK</i>	combined
Number of taxa	109	106	110
Number of characters	823	2052	2875
Constant characters	359 (44%)	1139 (55%)	1498 (52%)
Potentially parsimony informative sites	378 (46%)	557 (27%)	935 (32%)
Number of most parsimonious trees	1740	10 000	3024
Tree steps	2349	2479	4953
Consistency index	0.36	0.50	0.43
Retention index	0.76	0.74	0.75
Percentage of resolved nodes	80%	80%	90%

Chen *et al.* (2019) is polyphyletic, with *G. rubicunda* (Blume) Lindl. and *G. viridiflora* (Blume) Blume recovered as members of the *Microchilus* subclade with weak to only moderate support. *Goodyera rubicunda* is the sister to *Platylepis polyadenia* Rchb.f. and *G. viridiflora* sister to *Erythrodes*. The North American *Goodyera* spp. occupy a derived position in the *Goodyera* clade, here termed the Nearctic clade (90 MLBP, 0.81 PP and 66 MPBP; Fig. 3) in which north temperate/Holarctic *G. repens* and Asian *G. marginata* Lindl., *G. robusta* Hook.f. and *G. pendula* Maxim. are nested. In the *Microchilus* subclade, all representatives of Neotropical Goodyerinae form a strongly supported group, i.e. the Neotropical clade (100 MLBP, 1.0 PP and 92 MPBP; Fig. 3). In this clade, three groups received strong support (100 MLBP, 1.0 PP and 90–100 MLBP), but none corresponds with previous generic limits. *Microchilus* and *Aspidogyne* (*sensu* Meneguzzo, 2012) are both polyphyletic, and *Kreodanthus* (monophyletic) is embedded among clades of the other two genera.

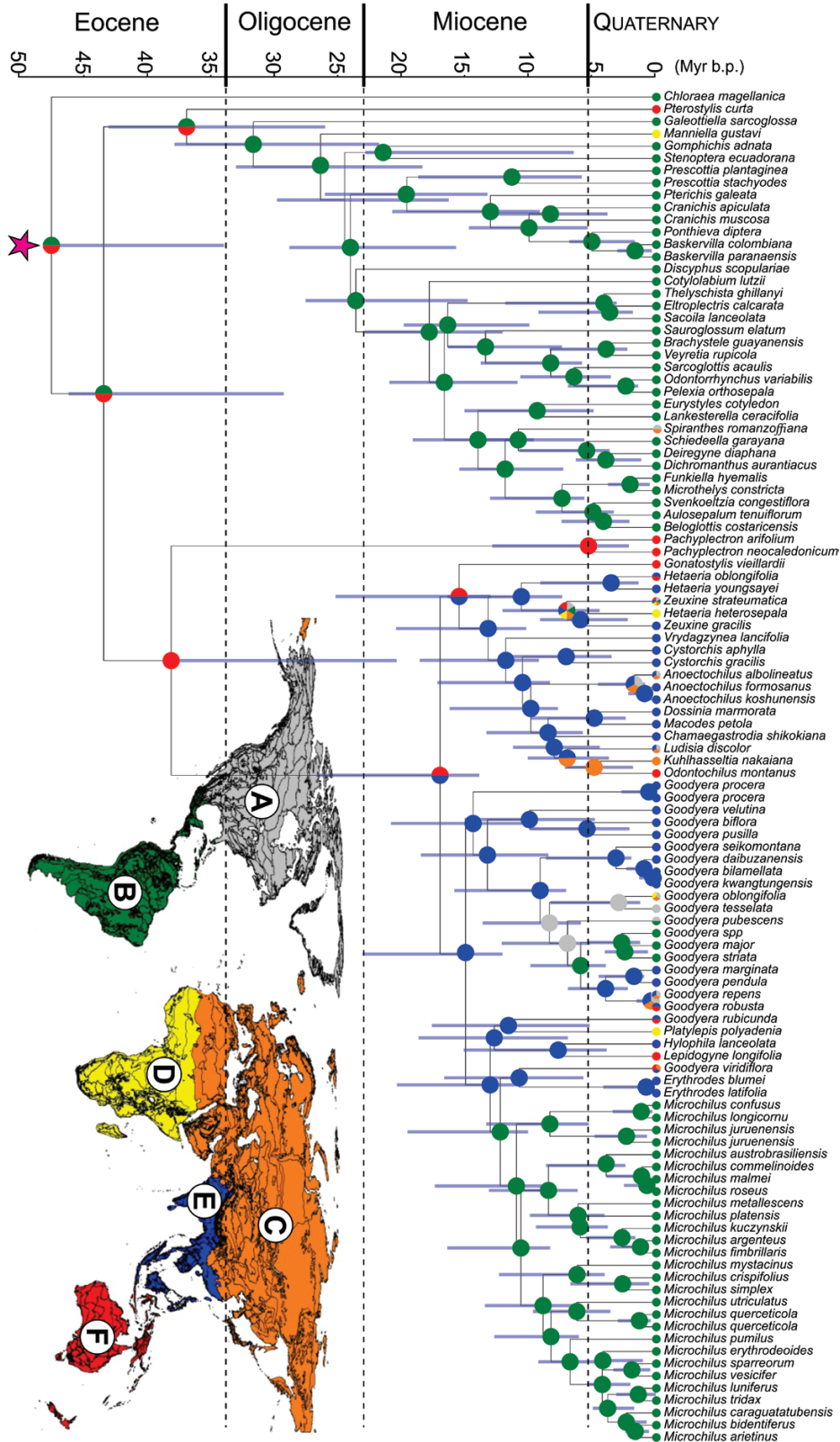
DIVERGENCE TIMES AND BIOGEOGRAPHY

The birth-death speciation model was chosen based on the AIC results (Supporting Information, Table S1). Slight differences were found between the ancestral area estimations using BAYAREALIKE, DEC and DIVALIKE, with or without the jump speciation parameter *j*. We report here estimates obtained using DEC+*j*, which was the model with the highest likelihood in AIC comparisons (Supporting Information, Table S1, Fig. 4, Supporting Information, Fig. S9). The origin of Goodyerinae occurred by a peripheral isolation event from Neotropics + Australasia to Australasia *c.* 38.3 Mya

(29–46 Mya, 95% HPD). After the divergence of *Pachyplectron* (Australasia), the remaining members of the subtribe probably diversified *c.* 17.09 Mya in Australasia + Indomalaya, where the *Cheirostylis* and *Goodyera* clades diversified *c.* 15.59 and 15.08 Mya, respectively. Our results suggest that in the *Cheirostylis* clade a first vicariance event explains the split of *Gonatostylis* (Australasian) from the rest of the clade (Indomalayan). The *Microchilus* and *Goodyera* subclades began to diversify *c.* 15.08 Mya. Dispersal from the Indomalayan region to North America took place 9.18 Mya, followed by a migration event via the Nearctic (*sensu* Olson *et al.* 2001) to create the Nearctic clade (American *Goodyera*). Their subsequent invasion of the Neotropics occurred 5.98 Mya, but at *c.* 4.0 Mya at least two species migrated back to Indomalayan region. The *Microchilus* subclade diversified in the Indomalayan ancestral area 13.13 Mya, followed by vicariance giving rise to the Neotropical clade 11.06 Mya in the northern Brazilian dominion, a result that may be biased by our sampling of Brazilian species. After that, only local speciation occurred up to the present (Fig. 5, Table 1, Supporting Information, Fig. S10). In the Neotropical clade, the three subclades originated at 8.4–8.9 Mya.

The first subclade dispersed from northern Brazil to the Pacific dominion 8.41 Mya and more recently (2.37 Mya) spread to other regions of South America. The remaining subclades experienced dispersal with vicariance from northern Brazilian to the Paraná dominion at 10.7 Mya. One clade diversified in the Paraná region at 8.52 Mya, and the other evolved in northern South America with a Mesoamerican and Mexican transition zone recently, 1.43 Mya (Fig. 5, Supporting Information, Fig. S10).

the Supporting Information for trees with support resulting from the three separate analyses. In detail, the ML tree is shown with proportional branch lengths. In the Nearctic clade, species in bold are present in the Americas. The letters before the name in the *Microchilus* species denote the previous classifications of A, *Aspidogyne* and K, *Kreodanthus*.



Downloaded from https://academic.oup.com/bol/advance-article/doi/10.1093/bol/obz017/54654 by guest on 24 April 2024

Figure 4. Time-calibrated tree for Goodyerinae plus outgroups using 110 species inferred under uncorrelated lognormal clock model in BEAST with biogeographic inference. The blue bar at the nodes indicates 95% highest posterior density

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

Relationships among the Cranichideae outgroups are outside the scope of this work, and the interested reader may refer to [Alvaréz-Molina & Cameron \(2009\)](#), [Cisternas et al. \(2012\)](#), [Chase et al. \(2015\)](#), [Givnish et al. \(2015\)](#) and [Salazar et al. \(2003, 2009, 2018\)](#). Recently, [Chen et al. \(2019\)](#) recovered subtribal relationships with only low support using seven molecular markers.

Our results agree with previous findings of [Hu et al. \(2016\)](#) and [Chen et al. \(2019\)](#) regarding potential polyphyly of *Goodyera* as previously delimited and a relationship between the Neotropical genera and some tropical Asian taxa, namely *Erythrodes*, *Hylophila*, *Lepidogyne* and *Platylepis*. Although we found good support for the Neotropical clade (composed of *Aspidogyne*, *Kreodanthus* and *Microchilus*), support for the *Microchilus* clade *sensu* [Chen et al. \(2019\)](#) is weak in our results as well as in [Hu et al. \(2016\)](#) and [Chen et al. \(2019\)](#). Based on the results of [Hu et al. \(2016\)](#) and [Chen et al. \(2019\)](#), [Pace \(2020\)](#) proposed a new taxonomic treatment for the polyphyletic elements of *Goodyera s.l.*, but we think these changes may be premature, and urge wider molecular sampling to add additional context and confidence to any proposed taxonomic transfers.

We also provide new evidence for a diphyletic origin for New World Goodyerinae (see Origin and Diversification of the Goodyerinae Clade). Although we were unable to sample the type species of these genera due to lack of fresh material, our expanded taxonomic sample permitted us to assess for the first time monophyly of the three Neotropical genera currently recognized, showing that *Aspidogyne sensu* [Meneguzzo \(2012\)](#) and *Microchilus sensu* [Omerod \(2002\)](#) are both polyphyletic. If we were proposing several new generic circumscriptions for these taxa, then it would be critical to include the type species of each concept (we include here only the type species of *Kreodanthus*), but for our taxonomic proposal to expand *Microchilus* sampling of the other type species is not necessary. The generic limits proposed by [Garay \(1977\)](#) fare no better in our phylogenetic results (not shown). Several successively diverging, strongly supported clades include mixtures of species assigned to *Aspidogyne* and *Microchilus*, which precludes any obvious way to recognize more than one genus without creating several morphologically undiagnosable new

genera. Therefore, we propose abandoning the highly artificial current generic arrangement, and in the interest of nomenclatural utility and stability provide the combinations required to recognise *Microchilus s.l.* (including *Aspidogyne* and *Kreodanthus*). If characters can eventually be found for diagnosing the three well-supported clades ([Fig. 3](#)) these could be recognized as subgenera of *Microchilus*. This circumscription of *Microchilus* is geographically coherent and includes all American members of Goodyerinae (except for *Goodyera*), which differ from the Asian members of the subtribe in their well-developed, long, unlobed spurs. Some Asian genera, such as *Erythrodes* and *Vrydagzynea* Blume, also have spurs, but these are typically shorter and bilobed. Details of the column have been the basis of previous generic concepts for both the Old World and New World genera, and these appear unlikely to be taxonomically useful, probably due to the fact that they are closely tied to pollination vectors (mostly unstudied, but see next). At present, few of the Asian genera related to *Erythrodes* have been included in phylogenetic studies, and thus it is premature to make any taxonomic conclusions based on such limited sampling. If column details and general floral shape perform as badly in phylogenetic studies of the Old World genera they have in the American genera, then a similar degree of generic synonymization might be the best taxonomic solution there as well.

[Singer & Sazima \(2001\)](#) demonstrated that at least two pollination mechanisms exist in Neotropical Goodyerinae. The two *Aspidogyne* spp. studied are similar to those in the *Pelexia* Poit. ex Lindl. clade of Spiranthinae, in which the adhesive portion of viscidium is dorsal (i.e. towards the anther), and thus the pollinarium adheres to the ventral surface of the labrum of the bees that pollinate them ([Singer & Sazima, 1999](#)). In contrast, in the studied *Microchilus* spp. (as *Erythrodes*), the adhesive surface of the viscidium is ventral (i.e. towards the labellum) and adheres to the dorsal part (galea) of the proboscis of the bee, a mechanism similar to that documented for North American *Goodyera* spp. and various Spiranthinae genera other than those belonging to the *Pelexia* clade (e.g. [Salazar et al., 2018](#)). Hence, differences in floral attributes among the genera of Neotropical Goodyerinae appear to be a reflection of pollination mechanisms, which is likely to result in conflicts between taxa based solely on floral morphological

intervals. Coloured circles represent the occurrence of that species in the delimited geographical areas depicted on the map of the World ([Olson et al. 2001](#)): A, Nearctic; B, Neotropic; C, Palearctic; D, Afrotropic; E, Indomalaya and F, Australasia. Pie charts at nodes indicate the most plausible geographic origin according to the with DEC+*j* model, and colour code follows the legend on the map. Calibration node for the molecular dating analysis is indicated by the star.

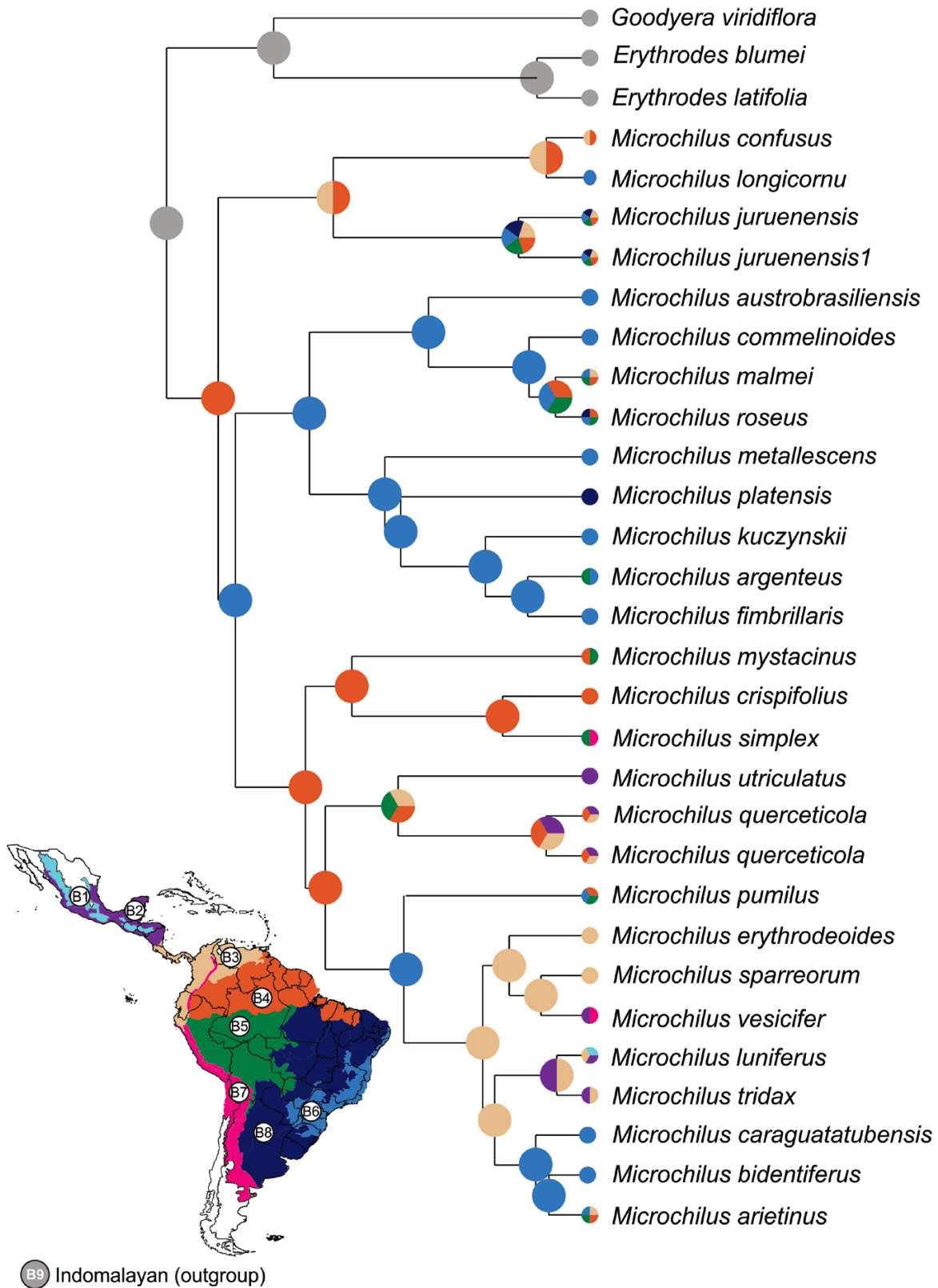


Figure 5. Maximum-likelihood tree of Neotropical Goodyerinae clade plus outgroups with ancestral area optimizations. Pie charts at nodes indicate the most plausible geographic origin according to the with BAYAREALIKE+J model, and coloured

characters and the DNA trees, as amply discussed for Spiranthinae (e.g. Salazar *et al.*, 2003, 2018; Salazar, Cabrera & Figueroa, 2011).

ORIGIN AND DIVERSIFICATION OF THE GOODYERINAE CLADE

We estimated the origin of Cranichideae *c.* 47 Mya, during the Eocene, in Australasia + Neotropics with the possible connection of both areas via Antarctica, as discussed in Givnish *et al.* (2016). The divergence of Goodyerinae from the rest of Cranichideae took place in the same epoch, *c.* 43 Mya, with diversification mostly in Indomalaya + Australasia, whereas other subtribes diversified mainly in the Neotropics (except Maniellinae in the Afrotropics and Pterostylidinae in Australasia). The probable origin of Goodyerinae occurred at the end of Eocene, *c.* 38 Mya, in the Australasian region. At that time, forests were widespread, and Antarctica became more isolated by the Antarctic Circumpolar Current, which produced increasingly cold temperatures (Pross *et al.*, 2012; Estrella *et al.*, 2019). This pattern has also been described for many genera and families that are known to exhibit disjunct distributions between Australia and South America, represented by extant lineages and fossil data (Conran *et al.*, 2015; Givnish *et al.*, 2016; Estrella *et al.*, 2019).

Goodyerinae remained stable in the Australasia region until the Mid-Miocene climatic optimum (*c.* 17 Mya) when grasslands expanded and forests dwindled, coinciding with a peak in regional plant diversity, as evidenced by the pollen record (Hoorn *et al.*, 2010). The major clades in Goodyerinae evolved between 17 and 13 Mya, nearly the same time as dispersal to the Neotropics, *c.* 13–11 Mya. At that time, Neotropical forests were continuous from the Amazon to the Atlantic coast (Antonelli *et al.*, 2009), but these forest biomes became partially fragmented by open vegetation such as Cerrado over time (Simon *et al.*, 2009). At present, the Amazon and Atlantic Forests are separated by a north-east/south-west open vegetation diagonal (Werneck, 2011), which has acted as a dispersal barrier resulting in disjunct distributions for various forest-dwelling groups, including epiphytic orchids such as the *Ornithocephalus* Hook. clade (Oncidiinae; Smidt *et al.*, 2018) and Pleurothallidinae (Pridgeon *et al.*, 2009). The same pattern is observed for several groups of animals and non-orchidaceous plants (Batalha-Filho *et al.*, 2013; Costa, 2003; Silva & Noll, 2014; Nihei & Carvalho, 2007; Pires & Marinoni, 2010; Galewski *et al.*, 2005; Pellegrino *et al.*, 2011;

Turchetto-Zolet *et al.*, 2012; Sobral-Souza, Lima-Ribeiro & Solferini, 2015). As a terrestrial group occurring in forested areas, Goodyerinae probably grew in the gallery forest that connected the Amazon and Atlantic Forests throughout the open vegetation diagonal. For this reason, no disjunct patterns are apparent between the Atlantic Forest and the Amazon or the Andes for Goodyerinae, given the numerous dispersals among the Neotropical members of this subtribe highlighted in this study during the late Miocene to Pliocene.

CONCLUSIONS

This study has revealed important aspects of the systematics and historical biogeography of New World Goodyerinae, supporting the existence of two independent American lineages. The Neotropical clade evolved in this region *c.* 13–11 Mya, derived from Indomalayan ancestors. The current taxonomic delimitation of the genera in the Neotropical clade, *Aspidogyne*, *Kreodanthus* and *Microchilus*, is inconsistent with the phylogenetic relationships recovered by our analyses, and we have decided to expand the circumscription of *Microchilus*, making the necessary nomenclatural transfers. *Microchilus s.l.* is strictly Neotropical, and with our proposal to expand its limits, the Neotropical clade becomes synonymous with *Microchilus*, even though the *Microchilus* clade *sensu* Chen *et al.* (2019) is different and includes many Old World genera, a situation that some readers will no doubt find confusing (we only used this clade name to remain consistent with Chen *et al.*, 2019). The second clade consists of a small set of Nearctic *Goodyera* spp. in which widespread north temperate/boreal *G. repens* and several eastern Asian species are nested; this group also dispersed from the Indomalaya region *c.* 8.45 Mya. Although we did not use it as a calibration in this study, the age of the fossil orchid *Meliorchis caribea* (Ramírez *et al.*, 2007) agrees well with our estimated ages of the two clades of New World Goodyerinae. From a systematic point of view, these results provide evidence that the previous taxonomy based mainly in the rostellum structure (Garay, 1977) is not useful to delimit evolutionary units in the Neotropical clade. Conflicts between floral morphology and clades recovered in our molecular analyses may be due to parallelisms/convergences or, more likely, retention of plesiomorphic morphological traits in successively diverging clades. Future work

circles represent the occurrence of that species in geographical areas depicted on the map of the Neotropics, following Morrone (2014) B1, Mexican Transition Zone; B2, Mesoamerican; B3, Pacific; B4, northern Brazilian; B5, southern Brazilian; B6, Paraná (Atlantic Forest) and B7, South American Transition Zone; B8, Chacoan and B9, Indomalaya (outgroup).

will benefit from the inclusion of many more species and additional, more variable markers (Smidt *et al.*, 2020), complemented with detailed comparative floral morphology studies to establish homologies and carry out total evidence analyses that permit the identification of synapomorphies for the clades recovered by the molecular data.

TAXONOMIC TREATMENT

MICROCHILUS C.PRESL, RELIQ. HAENK. I. 94 (1827).

Ligeophila Garay, Bradea 2: 194 (1977).
Platythelys Garay, Bradea 2: 196 (1977).
Rhamphorhynchus Garay, Bradea 2: 196 (1977).
Kreodanthus Garay, Bradea 2: 198 (1977).
Stephanothelys Garay, Bradea 2: 199 (1977).
Aspidogyne Garay, Bradea 2: 200 (1977).

1. ***Microchilus alajuelae*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Platythelys alajuelae* Ormerod, Harvard Pap. Bot. 11: 174 (–176; fig. 35). 2007.
 Homotypic synonym: *Aspidogyne alajuelae* (Ormerod) Meneguzzo, Orquidário 26: 89. 2012.
 Distribution: Costa Rica.

2. ***Microchilus amazonicus*** (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Ligeophila amazonica* Garay, Bradea 2: 194, fig. 2^a. 1977.
 Homotypic synonyms: *Erythrodes amazonica* (Garay) Carnevali & Dodson, Lindleyana 8: 101. 1993.
Aspidogyne amazonica (Garay) Meneguzzo, Orquidário 26: 89. 2012.
 Distribution: Venezuela.

3. ***Microchilus argenteus*** (Vell.) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Ophrys argentea* Vell., Fl. Flumin. Icon. 9: t. 61. 1831.
 Homotypic synonyms: *Physurus argenteus* (Vell.) K.Koch & Lauche, Berliner Allg. Gartenzeitung 25: 5. 1857. *Anoectochilus argenteus* (Vell.) Linden, Cat. Pl. Exot. (Linden) 14:13. 1859. *Aspidogyne argentea* (Vell.) Garay, Bradea 2: 203. 1977.
 Heterotypic synonyms: *Physurus pictus* var. *reticularis* Rchb.f. in Rchb., Cat. Orch.-Samml. Schiller ed. 3, 59. 1857. *Physurus pictus* Lindl., Gen. Sp. Orchid. Pl. 504 (1840). *Microchilus pictus* (Lindl.) D.Dietr. Syn. Pl. 5: 166. 1852. *Physurus callodictyus* Kraenzl.,

Kungl. Svenska Vetenskapsakad. Handl. n.f, 46: 40, t. 7. 1911. *Erythrodes picta* Ames, Orchidaceae (Ames) 5: 29. 1915. *Erythrodes picta* var. *reticularis* (Rchb.f.) Pabst in Angely, Flora Analit. Fitogeogr. Est. S. Paulo 6: 1279. 1972.

Distribution: Brazil, Paraguay.

4. ***Microchilus bicornutus*** (Cogn.) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Physurus bicornutus* Cogn., Fl. Bras. (Martius) 3(6): 547. 1906.
 Homotypic synonyms: *Erythrodes bicornuta* (Cogn.) Pabst, Orquídea (Rio de Janeiro) 18: 213. 1957. *Ligeophila bicornuta* (Cogn.) Garay, Bradea 2: 194 (1977). *Aspidogyne bicornuta* (Cogn.) Meneguzzo, Orquidário 26: 89. 2012.
 Distribution: Argentina, Brazil, Paraguay.

5. ***Microchilus bidentiferus*** (Schltr.) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Physurus bidentiferus* Schltr., Repert. Spec. Nov. Regni Veg. 16: 328. 1920.
 Homotypic synonyms: *Erythrodes bidentifera* (Schltr.) Garay, Comun. Inst. Nac. Invest. Ci. Nat., Ser. Ci. Bot. 1, number 6: 7. 1954. *Aspidogyne bidentifera* (Schltr.) Garay, Bradea 2: 203. 1977.
 Distribution: Argentina, Brazil, Paraguay.

6. ***Microchilus boliviensis*** (Cogn.) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Physurus boliviensis* Cogn. in Kuntze, Revis. Gen. Pl. 3[3]: 300. 1898.
 Homotypic synonyms: *Aspidogyne boliviensis* (Cogn.) Garay, Bradea 2(28): 201. 1977.
 Distribution: Bolivia, Colombia, Ecuador, Peru.

7. ***Microchilus brachyrrhynchus*** (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Physurus brachyrrhynchus* Rchb.f. in Rchb., Xenia Orchid. 2: 184. 1873.
 Homotypic synonyms: *Aspidogyne brachyrrhyncha* (Rchb.f.) Garay, Bradea 2(28): 204. 1977.
 Distribution: Venezuela.

8. ***Microchilus bruxelii*** (Pabst) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Erythrodes bruxelii* Pabst, Arch. Jard. Bot. Rio de Janeiro 12: 133. 1952.
 Homotypic synonyms: *Aspidogyne bruxelii* (Pabst) Garay, Bradea 2: 203. 1977.
 Distribution: Argentina, Brazil.

9. *Microchilus bugabae* (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Kreodanthus bugabae* Ormerod, Harvard Pap. Bot. 13: 59 (fig. 4). 2008.
 Distribution: Panama.
10. *Microchilus cajamarcae* (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Kreodanthus cajamarcae* Ormerod, Harvard Pap. Bot. 13: 59 (fig. 5). 2008.
 Distribution: Peru.
11. *Microchilus caraguatatubensis* (M.R.Miranda, Engels & E.C.Smidt) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne caraguatatubensis* M.R.Miranda, Engels & E.C.Smidt, Phytotaxa 289: 279. 2016.
 Distribution: Brazil.
12. *Microchilus carauchanus* (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne carauchana* Ormerod, Taiwania 54: 45 (–46; fig. 1). 2009.
 Distribution: Colombia.
13. *Microchilus casillasii* (R.González) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Kreodanthus casillasii* R.González, Acta Bot. Mex. 31: 33–37. 1995.
 Distribution: El Salvador, Mexico.
14. *Microchilus chocoensis* (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne chocoensis* Ormerod, Taiwania 54: 46 (–47; fig. 2). 2009.
 Distribution: Colombia.
15. *Microchilus claviger* (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus claviger* Rchb.f., Bonplandia 4: 211. 1856.
 Homotypic synonyms: *Ligeophila clavigera* (Rchb.f.) Garay, Bradea 2: 195. 1977. *Erythrodes clavigera* Ames, Orchidaceae (Ames) 7: 70. 1978. *Aspidogyne clavigera* (Rchb.f.) Meneguzzo, Orquidário 26: 89. 2012.
 Heterotypic synonyms: *Ligeophila clavigera* var. *rhodostachys* Ormerod, Harvard Pap. Bot. 13: 62 (fig. 8). 2008. *Aspidogyne clavigera* var. *rhodostachys* (Ormerod) Ormerod, Harvard Pap. Bot. 18: 52. 2013.
 Distribution: Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, Mexico, Nicaragua, Panamá, Peru, Suriname, Venezuela.
16. *Microchilus colombianus* (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Stephanothelys colombiana* Garay, Bradea 2: 199, fig. 3D. 1977.
 Homotypic synonyms: *Erythrodes colombiana* (Garay) P.Ortiz, Orquideas Colombia 265. 1995. *Aspidogyne colombiana* (Garay) Ormerod, Harvard Pap. Bot. 11: 146. 2007.
 Distribution: Colombia.
17. *Microchilus commelinoides* (Barb.Rodr.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus commelinoides* Barb.Rodr., Gen. Sp. Orchid. 1: 193. 1877.
 Homotypic synonyms: *Aspidogyne commelinoides* (Barb.Rodr.) Garay, Bradea 2: 201. 1977. *Erythrodes commelinoides* Ames, Orchidaceae (Ames) 7: 70. 1978.
 Distribution: Brazil.
18. *Microchilus confusus* (C.Schweinf.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes confusa* C.Schweinf., Fieldiana, Bot. 28: 174, fig. 29. 1951.
 Homotypic synonym: *Aspidogyne confusa* (C.Schweinf.) Garay, Bradea 2: 201. 1977.
 Distribution: Colombia, French Guiana, Guyana, Venezuela.
19. *Microchilus costaricensis* (Ormerod & M.A.Blanco) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne costaricensis* Ormerod & M.A.Blanco, Harvard Pap. Bot. 14: 111 (–113; fig. 1). 2009.
 Distribution: Costa Rica.
20. *Microchilus corniculatus* (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus corniculatus* Rchb.f., Flora 48: 274. 1865.

Homotypic synonyms: *Erythrodes corniculata* (Rchb.f.) Carabia, Mem. Soc. Cub. Hist. Nat. Felipe Poe y 17: 146. 1943. *Kreodanthus corniculatus* (Rchb.f.) Garay, Bradea 2: 198. 1977. *Goodyera corniculata* (Rchb.f.) Ackerman, Smithsonian Contr. Bot. 98: 638. 2012.
Distribution: Cuba.

21. ***Microchilus crispifolius*** (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Kreodanthus crispifolius* Garay, Fl. Ecuador 9: 288. 1978.

Homotypic synonym: *Erythrodes crispifolia* (Garay) Dodson & Carnevali, Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1257. 1993.

Distribution: Ecuador.

22. ***Microchilus cruciformis*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Aspidogyne cruciformis* Ormerod, Harvard Pap. Bot. 13: 55 (–57; fig. 1). 2008.

Distribution: Bolivia.

23. ***Microchilus curvatus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Kreodanthus curvatus* Ormerod, Harvard Pap. Bot. 13: 61 (fig. 6). 2008.

Distribution: Costa Rica.

24. ***Microchilus decorus*** (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Physurus decorus* Rchb.f., Gard. Chron. 1873: 177. 1873.

Homotypic synonym: *Aspidogyne decora* (Rchb.f.) Garay & G.A.Romero, Harvard Pap. Bot. 3: 53. 1998.

Heterotypic synonyms: *Physurus bicolor* Barb.Rodr., Gen. Sp. Orchid. 2: 290. 1882. *Erythrodes bicolor* Ames, Orchidaceae (Ames) 7: 67. 1922. *Aspidogyne bicolor* (Barb.Rodr.) Garay, Bradea 2: 203. 1977. *Aspidogyne decora* (Rchb.f.) Garay & G.A.Romero, Harvard Pap. Bot. 3: 53. 1998.

Distribution: Brazil, Paraguay.

25. ***Microchilus diaphanus*** (Szlach.) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Aspidogyne diaphana* Szlach. & Kolan., Pl. Biosystems 148: 896. 2014.

Distribution: Colombia.

26. ***Microchilus elatus*** (L.O.Williams) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Erythrodes elata* L.O.Williams Lilloa 6: 242. 1941.

Homotypic synonym: *Kreodanthus elatus* (L.O.Williams) Garay, Bradea 2: 199. 1977.

Distribution: Colombia, Venezuela.

27. ***Microchilus fimbriaris*** (B.S.Williams) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Physurus fimbriaris* Lindl., Gard. Chron.: 926, 1861. Homotypic synonym: *Aspidogyne fimbriaris* (B.S.Williams) Garay, Bradea 2(28): 203 (1977).

Heterotypic synonyms: *Physurus nobilis* Rchb.f., Gard. Chron. 1873: 177. 1873. *Physurus nobilis* var. *argyrocentrus* Hoehne, Fl. Bras. (Hoehne) 12, pt. 2: 360, t. 198, f. 2. 1945. *Erythrodes nobilis* (Rchb.f.) Pabst, Sellowia 7: 176. 1956. *Aspidogyne nobilis* (Rchb.f.) Baptista, Bol. CAOB 58: 50. 2005. *Aspidogyne nobilis* var. *argyrocentra* (Hoehne) J.M.H.Shaw, Orchid Rev. Suppl. 121 (1304): 76. 2013.

Distribution: Brazil.

28. ***Microchilus garayi*** E.C.Smidt & M.W.Chase, *nom. nov.*

Replaced synonyms: *Platythelys peruviana* Garay, Bot. Mus. Leaflet. 26: 24 (–25), pl. 1978. *Erythrodes peruviana* (Garay) Dodson, Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1257. 1993. *Aspidogyne peruviana* (Garay) Meneguzzo, Orquidário 26: 90. 2012.

Distribution: Peru.

Notes: not *Microchilus peruvianus* Szlach. & Kolan. Phyto (Horn) 56: 257. 2016.

The specific epithet honours Leslie Garay, for his contributions to the study of orchids in general and especially of Goodyerinae.

29. ***Microchilus gavilanensis*** (Ormerod & G.A.Romero) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Ligeophila gavilanensis* Ormerod & G.A.Romero, Lankesteriana 9: 513 (–514; figs. 1–2). 2010.

Homotypic synonym: *Aspidogyne gavilanensis* (Ormerod & G.A.Romero) Meneguzzo, Orquidário 26: 89. 2012.

Distribution: Venezuela.

30. ***Microchilus giganteus*** (Dodson) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Erythrodes gigantea* Dodson, Orquideologia 22: 196 (199, 219; fig. 126). 2003.

- Homotypic synonym: *Aspidogyne gigantea* (Dodson) Ormerod, *Taiwania* 50: 5. 2005.
Distribution: Ecuador.
31. ***Microchilus goaltalensis*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Aspidogyne goaltalensis* Ormerod, *Harvard Pap. Bot.* 13: 57 (fig. 2). 2008.
Distribution: Ecuador.
32. ***Microchilus grandis*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Ligeophila grandis* Ormerod, *Harvard Pap. Bot.* 9: 395 (397; fig. 6). 2005.
Homotypic synonym: *Aspidogyne grandis* (Ormerod) Ormerod, *Harvard Pap. Bot.* 11: 147. 2007.
Distribution: Panamá.
33. ***Microchilus grayumii*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Aspidogyne grayumii* Ormerod, *Harvard Pap. Bot.* 11: 147 (fig. 1). 2007.
Distribution: Costa Rica.
34. ***Microchilus harlingii*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Aspidogyne harlingii* Ormerod, *Harvard Pap. Bot.* 14: 115 (fig. 2). 2009.
Distribution: Ecuador.
35. ***Microchilus hylibates*** (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Physurus hylibates* Rchb.f., *Otia Bot. Hamburg.* 2: 83. 1881.
Homotypic synonyms: *Aspidogyne hylibates* (Rchb.f.) Garay, *Bradea* 2(28): 202 (1977). *Erythrodes hylibates* (Rchb.f.) Garay & Pabst, *Orquidea (Rio de Janeiro)* 18: 214. 1957.
Heterotypic synonyms: *Physurus lacteolus* Barb.Rodr., *Gen. Sp. Orchid.* ii. 292. 1882. *Erythrodes lacteola* Ames, *Orchidaceae (Ames)* 7: 71. 1922.
Distribution: Brazil.
36. ***Microchilus hyphaematicus*** (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Physurus hyphaematicus* Rchb.f. in *Rchb., Xenia Orchid.* 2: 184. 1873.
Homotypic synonym: *Aspidogyne hyphaematica* (Rchb.f.) Garay, *Bradea* 2: 204. 1977.
Distribution: Venezuela.
37. ***Microchilus jamesonii*** (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Ligeophila jamesonii* Garay, *Fl. Ecuador* 9 (225: 1): 274. 1978.
Homotypic synonyms: *Erythrodes jamesonii* (Garay) Dodson, *Orquideologia* 19: 149. 1994. *Aspidogyne jamesonii* (Garay) Meneguzzo, *Orquidário* 26: 89. 2012.
Distribution: Colombia, Ecuador.
38. ***Microchilus julianii*** E.C.Smidt & M.W.Chase, *nom. nov.*
Replaced synonyms: *Aspidogyne steyermarkii* Carnevali & Foldats, *Ann. Missouri Bot. Gard.* 76: 596, fig. 1989. *Erythrodes steyermarkii* (Carnevali & Foldats) Carnevali & Dodson, *Lindleyana* 8: 101. 1993.
Distribution: Venezuela.
Note: not *Microchilus steyermarkii* Szlach. & Kolan., *Phyton (Horn)* 56: 264 (2016). The new epithet honours the same person, Julian Alfred Steyermark (1909–1988), the famous American botanist who studied New World vegetation, collecting > 130 000 numbers in 26 countries and organised the *Flora of Missouri* project.
39. ***Microchilus juruenensis*** (Hoehne) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Physurus juruenensis* Hoehne, *Relat. Commiss. Linhas Telegr. Estratég. Matto Grosso Amazonas* 5 (Bot., pt. 1): 30. 1910.
Homotypic synonyms: *Erythrodes juruenensis* (Hoehne) Ames, *Orchidaceae (Ames)* 7: 70. 1922. *Ligeophila juruenensis* (Hoehne) Garay, *Bradea* 2: 195. 1977. *Aspidogyne juruenensis* (Hoehne) Meneguzzo, *Orquidário* 26: 89. 2012.
Distribution: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Venezuela.
40. ***Microchilus jussariensis*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Aspidogyne jussariensis* Ormerod, *Harvard Pap. Bot.* 21: 231. 2016.
Distribution: Brazil.
41. ***Microchilus kuczynskii*** (Porsch) E.C.Smidt & M.W.Chase, *comb. nov.*
Basionym: *Physurus kuczynskii* Porsch, *Oesterr. Bot. Z.* 55: 152. 1905.
Homotypic synonyms: *Erythrodes kuczynskii* (Porsch) Garay, *Comunic. Inst. Nac. Invest. Cienc. Nat.* [Buenos

- Aires], *Cienc. Bot* 1, number 6: 7. 1954. *Aspidogyne kuczynskii* (Porsch) Garay, *Bradea* 2: 203. 1977.
 Heterotypic synonyms: *Physurus dichopetalus* Kraenzl., *Kungl. Svenska Vetenskapsakad. Handl. n.f.* 46(10): 41, t. 7. 1911. *Erythrodes dichopetala* (Kraenzl.) L.O. Williams, *Bot. Mus. Leafl.* 6: 138. 1938.
 Distribution: Argentina, Brazil, Paraguay.
42. ***Microchilus lindleyanus*** (Cogn.) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus lindleyanus* Cogn., *Fl. Bras. (Martius)* 3(4): 238, t. 55, fig. 2. 1895.
 Homotypic synonym: *Aspidogyne lindleyana* (Cogn.) Garay, *Bradea* 2: 202. 1977.
 Heterotypic synonym: *Physurus lindmanii* Kraenzl., *Kungl. Svenska Vetenskapsakad. Handl. n.f.* 46(10): 38, t. 3, 7. 1911.
 Distribution: Argentina, Brazil, Paraguay.
43. ***Microchilus llanganetensis*** (Dodson) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes llanganetensis* Dodson, *Orquideologia* 19: 149, *nom. nov.* 1994.
 Distribution: Ecuador.
 Homotypic synonym: *Kreodanthus ecuadorensis* Garay, *Fl. Ecuador* 9(225: 1): 288. 1978.
 Note: not *Microchilus equadorensis* (Garay) Ormerod, *Lindleyana* 17: 216 (2002), basionym: *Erythrodes ecuadorensis* Garay, *Fl. Ecuador* 9(225: 1): 279 (1978). In his treatment in the *Flora of Ecuador*, Garay also described *Kreodanthus ecuadorensis* Garay, *Fl. Ecuador* 9(225: 1): 288 (1978). When Dodson moved this species to *Erythrodes*, it needed a new name, and here we make this combination in *Microchilus*.
44. ***Microchilus longibracteatus*** (Soroka) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Ligeophila longibracteata* Soroka, *Selbyana* 27: 8 (–12; figs. 1–3). 2006.
 Homotypic synonym: *Aspidogyne longibracteata* (Soroka) Ormerod, *Harvard Pap. Bot.* 13: 57. 2008.
 Distribution: Ecuador.
45. ***Microchilus longicornu*** (Cogn.) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus longicornu* Cogn., *Bull. Soc. Roy. Bot. Belgique* 43: 298. 1906.
 Homotypic synonyms: *Erythrodes longicornu* Ames, *Orchidaceae (Ames)* 5: 28. 1915. *Aspidogyne longicornu* (Cogn.) Garay, *Bradea* 2: 202. 1977.
 Heterotypic synonym: *Physurus longicalcaratus* Schltr., *Anexos Mem. Inst. Butantan, Secc. Bot.* 1: 35. 1922.
 Distribution: Brazil, French Guiana, Guyana, Suriname.
46. ***Microchilus loxoglottis*** (Rchb.f.) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus loxoglottis* Rchb.f., *Beitr. Orchid.-K. C. Amer.* 64. 1866.
 Homotypic synonyms: *Erythrodes loxoglottis* Ames, *Orchidaceae (Ames)* 7: 71. 1922. *Kreodanthus loxoglottis* (Rchb.f.) Garay, *Bradea* 2: 199. 1977.
 Distribution: Guatemala.
47. ***Microchilus luteus*** (Garay) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Ligeophila lutea* Garay, *Fl. Ecuador* 9 (225: 1): 274. 1978.
 Homotypic synonyms: *Erythrodes lutea* (Garay) Dodson, *Orquideologia* 19: 149. 1994. *Aspidogyne lutea* (Garay) Meneguzzo, *Orquidário* 26: 89. 2012.
 Distribution: Ecuador.
48. ***Microchilus macarenae*** (Ormerod) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Ligeophila macarenae* Ormerod, *Harvard Pap. Bot.* 11: 154 (fig. 7). 2007.
 Homotypic synonym: *Aspidogyne macarenae* (Ormerod) Meneguzzo, *Orquidário* 26: 90. 2012.
 Distribution: Colombia.
49. ***Microchilus malmei*** (Kraenzl.) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus malmei* Kraenzl., *Kungl. Svenska Vetenskapsakad. Handl. n.f.* 46: 41, t. 7. 1911.
 Homotypic synonyms: *Erythrodes malmei* (Kraenzl.) Pabst, *Orquídea (Rio de Janeiro)* 18: 215. 1957. *Aspidogyne malmei* (Kraenzl.) Garay, *Bradea* 2: 202. (1977).
 Distribution: Argentina, Brazil.
50. ***Microchilus mendoncae*** (Brade & Pabst) E.C.Smидt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes mendoncae* Brade & Pabst, *Orquídea (Rio de Janeiro)* 20: 5, fig. 1–8. 1958.
 Homotypic synonyms: *Rhamphorhynchus mendoncae* (Brade & Pabst) Garay, *Bradea* 2: 196. 1977. *Aspidogyne mendoncae* (Brade & Pabst) Ormerod, *Harvard Pap. Bot.* 13: 58. 2008.
 Distribution: Brazil.

51. ***Microchilus metallescens*** (Barb.Rodr.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus metallescens* Barb.Rodr., Gen. Sp. Orchid. 2: 291. 1882.
 Homotypic synonyms: *Erythrodes metallescens* Ames, Orchidaceae (Ames) 7: 72. 1922. *Aspidogyne metallescens* (Barb.Rodr.) Garay, Bradea 2: 204. 1977.
 Distribution: Brazil.
52. ***Microchilus miravalleanus*** (Szlach. & Kolan.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne miravalleana* Szlach. & Kolan., Mater. Orchid Fl. Colombia 1: 432. 2017.
 Distribution: Colombia.
53. ***Microchilus miserus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Ligeophila misera* Ormerod, Oasis Suppl. 3: 8 (14; fig.). 2004.
 Homotypic synonym: *Aspidogyne misera* (Ormerod) Ormerod, Harvard Pap. Bot. 11: 148. 2007.
 Distribution: Peru.
54. ***Microchilus mosaicus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne mosaica* Ormerod, Oasis Suppl. 3: 3 (5; fig.). 2004.
 Distribution: Colombia, Ecuador.
55. ***Microchilus multifolius*** (C.Schweinf.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes multifoliata* C.Schweinf., Bot. Mus. Leaflet. 9: 234, tab. 8. 1941.
 Homotypic synonym: *Aspidogyne multifoliata* (C.Schweinf.) Garay, Bradea 2: 202. 1977.
 Distribution: Ecuador, Guyana, Peru.
56. ***Microchilus mamex*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Kreodanthus mamex* Ormerod, Harvard Pap. Bot. 9: 394 (fig. 3). 2005.
 Distribution: Colombia.
57. ***Microchilus mystacinus*** (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus mystacinus* Rchb.f. in Rchb., Xenia Orchid. 2: 183. 1873.
 Homotypic synonyms: *Erythrodes mystacina* Ames, Orchidaceae (Ames) 7: 74. 1922. *Aspidogyne mystacina* (Rchb.f.) Garay, Bradea 2: 204. 1977.
 Distribution: Bolivia, Brazil, Ecuador, Peru, Suriname.
58. ***Microchilus ovatilabius*** (Ames & Correll) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes ovatilabia* Ames & Correll, Bot. Mus. Leaflet. 10: 70. 1942.
 Homotypic synonym: *Kreodanthus ovatilabius* (Ames & Correll) Garay, Bradea 2: 199. 1977.
 Distribution: Guatemala, Honduras and Mexico.
59. ***Microchilus pachysepalus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Platythelys pachysepala* Ormerod, Harvard Pap. Bot. 11: 176 (fig. 36). 2007.
 Homotypic synonym: *Aspidogyne pachysepala* (Ormerod) Meneguzzo, Orquidário 26: 90. 2012.
 Distribution: Colombia.
60. ***Microchilus pedicellatus*** (Cogn.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Spiranthes pedicellata* Cogn., Fl. Bras. (Martius) 3(4): 210. 1895.
 Homotypic synonyms: *Brachystele pedicellata* (Cogn.) Garay, Bot. Mus. Leaflet. 28: 304. 1982. *Platythelys pedicellata* (Cogn.) Szlach., Fragm. Florist. Geobot. Suppl. 3: 115. 1995. *Aspidogyne pedicellata* (Cogn.) Meneguzzo, Orquidário 26: 90. 2012.
 Heterotypic synonyms: *Wulfschlaegelia paranaensis* Kraenzl., Kungl. Svenska Vetenskapsakad. Handl. n.f. 46: 42, t. 8. 1911. *Physurus paranaensis* Schltr., Repert. Spec. Nov. Regni Veg. 16: 329. 1920. *Erythrodes paranaensis* (Kraenzl.) Pabst in Angely, Fl. Paraná number 6: 11. 1956. *Platythelys paranaensis* (Kraenzl.) Garay, Bradea 2: 197. 1977.
 Distribution: Brazil, Paraguay.
61. ***Microchilus peterianus*** (Cogn.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus peterianus* Cogn., Fl. Bras. (Martius) 3(4): 227, t. 52, fig. 1. 1895.
 Homotypic synonyms: *Erythrodes petersiana* (Cogn.) Ames, Orchidaceae (Ames) 7: 75 (1922). *Ligeophila peteriana* (Cogn.) Garay, Bradea 2: 195. 1977. *Aspidogyne peteriana* (Cogn.) Meneguzzo, Orquidário 26: 90. 2012.
 Distribution: Colombia, French Guiana, Guyana, Suriname.

62. ***Microchilus platensis*** (Hauman) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus platensis* Hauman, *Anales Mus. Nac. Hist. Nat. Buenos Aires* 29: 370, fig. 5. 1917.
 Homotypic synonym: *Aspidogyne platensis* (Hauman) Meneguzzo, *Orquidário* 26: 90. 2012.
 Distribution: Argentina, Brazil, Paraguay.
63. ***Microchilus popayanensis*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne popayanensis* Ormerod, *Harvard Pap. Bot.* 11: 150 (fig. 2). 2007.
 Distribution: Colombia.
64. ***Microchilus pumilus*** (Cogn.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus pumilus* Cogn., *Fl. Bras. (Martius)* 3(6): 545. 1906.
 Homotypic synonyms: *Erythrodes pumila* (Cogn.) Pabst, *Orquídea (Rio de Janeiro)* 18: 215. 1957. *Aspidogyne pumila* (Cogn.) Garay, *Bradea* 2: 204. 1977.
 Distribution: Brazil, French Guiana, Guyana, Peru, Suriname, Trinidad-Tobago, Venezuela.
65. ***Microchilus rariflorus*** (Lindl.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus rariflorus* Lindl., *Orchid. Linden.* 28. 1846.
 Homotypic synonyms: *Erythrodes rariflora* Ames, *Orchidaceae (Ames)* 7: 75. 1922. *Aspidogyne rariflora* (Lindl.) Garay, *Bradea* 2: 204. 1977.
 Distribution: Colombia, Venezuela.
66. ***Microchilus reddenii*** (Ormerod & Carnevali) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Aspidogyne reddenii* Ormerod & Carnevali, *Harvard Pap. Bot.* 23: 86. 2018.
 Distribution: Guyana.
67. ***Microchilus robustus*** (C.Schweinf.) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes robusta* C.Schweinf., *Bot. Mus. Leafl.* 20: 8, tab. 3. 1962.
 Homotypic synonym: *Aspidogyne robusta* (C.Schweinf.) Garay, *Bradea* 2: 203. 1977.
 Distribution: Colombia, Guyana, Venezuela.
68. ***Microchilus roseoalbus*** (Dressler) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes roseoalba* Dressler, *Orquidea (Mexico City)* 13: 257, 260 fig. 1993.
 Homotypic synonyms: *Platythelys roseoalba* (Dressler) Szlach., *Fragm. Florist. Geobot. Suppl.* 3: 115. 1995. *Aspidogyne roseoalba* (Dressler) Ormerod, *Harvard Pap. Bot.* 11: 150. 2007.
 Distribution: Costa Rica, Panamá.
69. ***Microchilus rotundifolius*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Kreodanthus rotundifolius* Ormerod, *Harvard Pap. Bot.* 9: 394 (-395; fig. 4). 2005.
 Homotypic synonym: *Aspidogyne rotundifolia* (Ormerod) Ormerod, *Harvard Pap. Bot.* 13: 58. 2008.
 Distribution: Peru.
70. ***Microchilus schlechterianus*** (Hoehne) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus schlechterianus* Hoehne, *Fl. Bras. (Hoehne)* 12(2): 354. 1945.
 Homotypic synonyms: *Platythelys schlechteriana* (Hoehne) Garay, *Bradea* 2(28): 198. 1977. *Aspidogyne schlechteriana* (Hoehne) Meneguzzo, *Orquidário* 26: 90. 2012.
 Heterotypic synonym: *Physurus foliosus* Schltr. ex Porto & Brade, *Anais Reunião Sul-Amer. Bot.* 1938, iii. 34. 1940, non Lindl.
 Distribution: Brazil.
71. ***Microchilus secundus*** (Ames) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Physurus secundus* Ames, *Orchidaceae (Ames)* 2: 260. 1908.
 Homotypic synonyms: *Erythrodes secunda* (Ames) Ames *Orchidaceae* 5: 28. 1915. *Kreodanthus secundus* (Ames) Garay, *Bradea* 2: 199. 1977.
 Distribution: El Salvador, Mexico, Panamá.
72. ***Microchilus serripetalus*** (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Erythrodes serripetala* Garay, *Bot. Mus. Leafl.* 21: 250. 1967.
 Homotypic synonym: *Aspidogyne serripetala* (Garay) Garay, *Bradea* 2: 204. 1977.
 Distribution: Colombia, Mexico.
73. ***Microchilus siberianus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
 Basionym: *Stephanothelys siberiana* Ormerod, *Harvard Pap. Bot.* 9: 422 (-423; fig. 40). 2005.

- Homotypic synonym: *Aspidogyne siberiana* (Ormerod) Ormerod, Harvard Pap. Bot. 11: 150. 2007.
Distribution: Bolivia.
74. ***Microchilus sororius*** (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Stephanothelys sororia* Garay, Bradea 2: 200, fig. 3B. 1977.
Homotypic synonym: *Aspidogyne sororia* (Garay) Ormerod, Harvard Pap. Bot. 11: 151. 2007.
Distribution: Bolivia, Peru.
75. ***Microchilus sparsiflorus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Stephanothelys rariflora* Garay, Bradea 2: 200. 1977.
Homotypic synonym: *Aspidogyne sparsiflora* Ormerod, Harvard Pap. Bot. 11: 151. 2007.
Distribution: Bolivia.
76. ***Microchilus simplex*** (C.Schweinf.) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Erythrodes simplex* C.Schweinf., Revista Acad. Colomb. Ci. Exact. 5: 348. 1943.
Homotypic synonym: *Kreodanthus simplex* (C.Schweinf.) Garay, Bradea 2: 199. 1977.
Distribution: Peru.
77. ***Microchilus stenocentron*** (Schltr.) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Physurus stenocentron* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 9: 60. 1921.
Homotypic synonyms: *Erythrodes stenocentron* Ames, Orchidaceae (Ames) 7: 75. 1922. *Aspidogyne stenocentron* (Schltr.) Szlach. & Kolan., Mater. Orchid Fl. Colombia 1: 409. 2017.
Distribution: Colombia, Peru.
78. ***Microchilus stictophyllus*** (Schltr.) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Physurus stictophyllus* Schltr., Repert. Spec. Nov. Regni Veg. 10: 249. 1911.
Homotypic synonym: *Aspidogyne stictophylla* (Schltr.) Garay, Bradea 2: 204. 1977.
Distribution: Costa Rica, Guatemala, Honduras, Mexico, Panamá.
79. ***Microchilus stigmatopterus*** (Rchb.f.) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Physurus stigmatopterus* Rchb.f. in Rchb., Xenia Orchid. 2: 185. 1873.
Homotypic synonyms: *Erythrodes stigmatoptera* (Rchb.f.) Pabst, Orquídea (Rio de Janeiro) 18: 215. 1957. *Aspidogyne stigmatoptera* (Rchb.f.) Meneguzzo, Orquidário 26: 90. 2012.
Distribution: Bolivia, Brazil, Colombia, French Guiana, Guyana, Peru, Suriname, Venezuela.
80. ***Microchilus sumacoensis*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Aspidogyne sumacoensis* Ormerod, Harvard Pap. Bot. 13: 58 (fig. 3). 2008.
Homotypic synonym: *Ligeophila sumacoensis* (Ormerod) Szlach. & Kolan., Mater. Orchid Fl. Colombia 1: 389. 2017.
Distribution: Ecuador.
81. ***Microchilus sytsmae*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Kreodanthus sytsmae* Ormerod, Harvard Pap. Bot. 13: 61 (fig. 7). 2008.
Distribution: Panamá.
82. ***Microchilus tribouillieri*** (Archila, Chiron & Slach.) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Aspidogyne tribouillieri* Archila, Chiron & Szlach., Richardiana 14: 178. 2014.
Distribution: Guatemala.
83. ***Microchilus tuerckheimii*** (Schltr.) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Physurus tuerckheimii* Schltr., Repert. Spec. Nov. Regni Veg. 2: 132. 1906.
Homotypic synonyms: *Erythrodes tuerckheimii* Ames, Orchidaceae (Ames) 7: 77. 1922. *Aspidogyne tuerckheimii* (Schltr.) Garay, Bradea 2: 204. 1977.
Heterotypic synonyms: *Physurus polygonatus* Ames, Orchidaceae (Ames) 2: 259, descr. 1908. *Erythrodes polygonata* Ames, Orchidaceae (Ames) 5: 28. 1915.
Distribution: Costa Rica, Ecuador, Guatemala, Honduras, Panamá.
84. ***Microchilus tulamengensis*** (Ormerod & Carnevali) E.C.Smidt & M.W.Chase, *comb. nov.*
- Basionym: *Aspidogyne tulamengensis* Ormerod & Carnevali, Harvard Pap. Bot. 23: 87. 2018.
Distribution: Guyana.

85. ***Microchilus umbraticola*** (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Ligeophila umbraticola* Garay, Bradea 2: 195 (-196; fig. 1C). 1977.

Homotypic synonym: *Aspidogyne umbraticola* (Garay) Meneguzzo, Orquidário 26: 90. 2012.

Distribution: Bolivia, Colombia, Peru.

86. ***Microchilus unicornis*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Ligeophila unicornis* Ormerod, Harvard Pap. Bot. 13: 63 (fig. 9). 2008.

Homotypic synonym: *Aspidogyne unicornis* (Ormerod) Meneguzzo, Orquidário 26: 90. 2012.

Distribution: Venezuela.

87. ***Microchilus utriculatus*** (Dressler) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Erythrodes utriculata* Dressler, Orquidea (Mexico City) 13: 260. 1993.

Homotypic synonym: *Aspidogyne utriculata* (Dressler) Szlach., Fragm. Florist. Geobot. Supp. 3: 115. 1995.

Distribution: Costa Rica.

88. ***Microchilus venustus*** (Ames) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Physurus venustus* Ames, Orchidaceae (Ames) 2: 261. 1908.

Homotypic synonyms: *Erythrodes venustula* Ames, Orchidaceae (Ames) 5: 28. 1915. *Erythrodes querceticola* var. *venustula* (Ames) Correll, Lloydia 10: 210. 1947. *Platythelys venustula* (Ames) Garay, Bradea 2: 198. 1977. *Aspidogyne venustula* (Ames) Meneguzzo, Orquidário 26: 91. 2012.

Heterotypic synonym: *Erythrodes cornejoi* Dodson, Orquideologia 20: 275. 1997.

Distribution: Costa Rica, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama.

89. ***Microchilus vesiculosus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Aspidogyne vesiculosa* Ormerod, Harvard Pap. Bot. 9: 392 (fig. 1). 2005.

Distribution: Venezuela.

90. ***Microchilus xystophylloides*** (Garay) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Erythrodes xystophylloides* Garay, Bot. Mus. Leaflet 18: 193, fig. 1958.

Homotypic synonyms: *Stephanothelys xystophylloides* (Garay) Garay, Bradea 2: 200. 1977. *Aspidogyne xystophylloides* (Garay) Ormerod, Harvard Pap. Bot. 11: 151. 2007.

Distribution: Colombia, Ecuador.

91. ***Microchilus zonatus*** (Ormerod) E.C.Smidt & M.W.Chase, *comb. nov.*

Basionym: *Aspidogyne zonata* Ormerod, Harvard Pap. Bot. 11: 152 (fig. 4). 2007.

Distribution: Colombia.

ACKNOWLEDGEMENTS

We would like to thank Tarcísio Magevski Rodrigues and Marcelo Miranda Rodrigues for helping in specimen collection and Sistema de Autorização e Informação em Biodiversidade/Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO/ICMBio) for the collecting permits. ECS would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for grant Bolsa de Produtividade em Pesquisa do CNPq-Nível 2 (proc. 308460/2017-0) and for PDE (proc. 203304/2018-7). We also thank two anonymous reviewers for helpful comments and suggestions and challenging us to better justify our taxonomic proposal.

REFERENCES

- Alvaréz-Molina I, Cameron KM. 2009. Molecular phylogenetics of Prescottiinae *s.l.* and their close allies (Orchidaceae, Cranichideae) inferred from plastid and nuclear ribosomal DNA sequences. *American Journal of Botany* 96: 1020–1040.
- Ames O. 1922. *Notes on Erythrodes. Orchidaceae* 7. Boston: Merrimount Press, 63–78.
- Antonelli A, Nylander JAA, Persson C, Sanmartín I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the United States of America* 106: 9749–9754.
- Baele G, Li WLS, Drummond A, Suchard M, Lemey P. 2012. Accurate model selection of relaxed molecular clocks in bayesian phylogenetics. *Molecular Biology and Evolution* 30: 239–243. <http://doi:10.1093/molbev/mss243>
- Batalha-Filho H, Fjeldså J, Fabre P, Miyaki CY. 2013. Connections between the Atlantic and the Amazonian forest avifaunas represent distinct historical events. *Journal of Ornithology* 154: 41–50.
- Bona C, Engels ME, Pieczak FS, Smidt EC. 2020. Comparative vegetative anatomy of Neotropical Goodyerinae Klotzsch (Orchidaceae Juss.: Orchidoideae Lindl.). *Brazilian Journal of Botany* 34: 530–539.

- Cameron, KM. 2004.** Utility of plastid *psaB* gene sequences for investigating intrafamilial relationships within Orchidaceae. *Molecular Phylogenetics and Evolution* **31**: 1157–1180.
- Cameron KM, Chase MW, Whitten WM, Kores PJ, Jarrell DC, Albert VA, Yukawa T, Hills HG, Goldman DH. 1999.** A phylogenetic analysis of the Orchidaceae: evidence from *rbcL* nucleotide sequences. *American Journal of Botany* **86**: 208–224.
- Cisternas MA, Salazar GA, Verdugo G, Novoa P, Calderón X, Negritto MA. 2012.** Phylogenetic analysis of Chloraeinae (Orchidaceae) based on plastid and nuclear DNA sequences. *Botanical Journal of the Linnean Society* **168**: 258–277.
- Chase MW, Cameron KM, Freudenstein JV, Pridgeon AM, Salazar G, van den Berg C, Schuiteman A. 2015.** An updated classification of Orchidaceae. *Botanical Journal of the Linnean Society* **177**: 151–174.
- Chen SP, Tian H, Guan QX, Junwen Z, Zhang GQ, Chen LJ, Lan SR, Li M. 2019.** Molecular systematics of Goodyerinae (Cranichideae, Orchidoideae, Orchidaceae) based on multiple nuclear and plastid regions. *Molecular Phylogenetics and Evolution* **139**: 106542.
- Chernomor O, von Haeseler A, Minh BQ. 2016.** Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* **65**: 997–1008.
- Conran JG, Bannister JM, Lee DE, Carpenter RJ, Kennedy EM, Reichgelt T, Fordyce RE. 2015.** An update of monocot macrofossil data from New Zealand and Australia. *Botanical Journal of the Linnean Society* **178**: 394–420.
- Costa LP. 2003.** The historical bridge between the Amazon and the Atlantic Forest of Brazil: a study of molecular phylogeography with small mammals. *Journal of Biogeography* **30**: 71–86.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012.** jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
- Desfeux C, Lejeune B. 1996.** Systematics of Euromediterranean *Silene* (Caryophyllaceae): evidence from a phylogenetic analysis using ITS sequences. *Comptes rendus de l'Académie des sciences Série III, Sciences de la vie* **319**: 351–358.
- Doyle J, Doyle J. 1987.** A rapid DNA isolation procedure for small amounts of leaf tissue. *Phytochemical Bulletin, Botanical Society of America* **19**: 810–815.
- Dressler, R. 1993.** *Phylogeny and classification of the orchid family*. Cambridge: Cambridge University Press.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012.** Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.
- Engels, ME, Barros F, Smidt ECS. 2016.** A subtribo Goodyerinae (Orchidaceae: Orchidoideae) no estado do Paraná, Brasil. *Rodriguésia* **67**: 917–952.
- Estrella M, Buerki S, Vasconcelos T, Lucas EJ, Forest F. 2019.** The role of Antarctica in biogeographical reconstruction: a point of view. *International Journal of Plant Sciences* **180**: 63–71.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach to using bootstrap. *Evolution* **39**: 783–791.
- Fitch WM. 1971.** Towards defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20**: 406–416.
- Ford CS, Ayres KL, Toomey N, Haider N, Stahl JVA, Kelly LJ, Wikstrom N, Hollingsworth PM, Duff RJ, Hoot SB, Cowan RS, Chase MW, Wilkinson MJ. 2009.** Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society* **159**: 1–11.
- Freudenstein JV, van den Berg C, Goldman DH, Kores PJ, Molvray M, Chase MW. 2004.** An expanded plastid DNA phylogeny of Orchidaceae and analysis of jackknife branch support strategy. *American Journal of Botany* **91**: 149–157.
- Galewski T, Mauffrey JF, Leite YLR, Patton JL, Douzery EJP. 2005.** Ecomorphological diversification among South American spiny rats (Rodentia; Echimyidae): a phylogenetic and chronological approach. *Molecular Phylogenetics and Evolution* **34**: 601–615.
- Garay LA. 1977.** Systematics of the Physurinae (Orchidaceae) in the New World. *Bradea* **28**: 191–204.
- Gelman A, Rubin DB. 1992.** Inference from iterative simulation using multiple sequences. *Statistical Science* **7**: 457–472.
- Gernhard T. 2008.** The conditioned reconstructed process. *Journal of Theoretical Biology*, **253**: 769–778.
- Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Doucette A, Caro GG, Mcdaniel J, Clements MA, Arroyo MTK, Endara L, Kriebel R, Williams NH, Cameron KM. 2016.** Orchid historical biogeography, diversification, Antarctica and the paradox of orchid dispersal. *Journal of Biogeography* **43**: 1905–1916.
- Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Iles WJ, Clements MA, Arroyo MT, Leebens-Mack J, Endara L, Kriebel R, Neubig KM, Whitten WM, Williams NH, Cameron KM. 2015.** Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society B* **282**: 20151553.
- Górnaiak M, Paun O, Chase MW. 2010.** Phylogenetic relationships within Orchidaceae based on a low-copy nuclear coding gene, *Xdh*: congruence with organellar and nuclear ribosomal DNA results. *Molecular Phylogenetics and Evolution* **56**: 784–795.
- Hoehne FC. 1945.** *Orchidaceas. Fasc. 8, Vol. 12(2)*. In: *Flora Brasílica 13–43*. São Paulo: Secretaria da Agricultura, Industria e Comércio.
- Hoorn C, Wesselingh FP, Ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A. 2010.** Amazonia through time: Andean uplift, climate change, landscape evolution and biodiversity. *Science* **330**: 927–931.
- Hu C, Tian H, Li H, Hu A, Xing F, Bhattacharjee A, Hsu T, Kumar P, Chung S. 2016.** Phylogenetic analysis of a 'jewel orchid' genus *Goodyera* (Orchidaceae) based on DNA sequence data from nuclear and plastid regions. *PLoS One* **11**: e0150366.

- Kalyaanamoorthy S, Minh B, Wong T, von Haeseler A, Jermiin L. 2017.** ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 4285.
- Katoh K, Standley DM. 2013.** MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Matzke NJ. 2013.** Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* **5**: 242–248.
- Meneguzzo TEC. 2012.** Mudanças nomenclaturais em Goodyerinae do Novo Mundo (Orchidaceae). *Orquidário* **26**: 86–91.
- Menini Neto L, Miranda MR, Cruz D. 2011.** *Zeuxine strateumatica* (Orchidaceae) goes south: a first record for Brazil. *Kew Bulletin* **66**: 155–158.
- Miller MA, Pfeiffer W, Schwartz T. 2010.** Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments 1–8. New Orleans. <http://doi:10.1109/GCE.2010.5676129>
- Minh BQ, Nguyen MAT, von Haeseler A. 2013.** Ultrafast approximation for phylogenetic bootstrap. *Molecular Phylogenetics and Evolution* **30**: 1188–1195.
- Morrone JJ. 2014.** Cladistic biogeography of the Neotropical region: identifying the main events in the diversification of the terrestrial biota. *Cladistics* **30**: 202–214.
- Nihei SS, Carvalho CJB. 2007.** Systematics and biogeography of the Neotropical genus *Polietina* Schnabl & Dziedzicki (Diptera, Muscidae): Neotropical areas relationships and Amazonia as a composite area. *Systematic Entomology* **32**: 477–501.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015.** IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274.
- Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR. 2001.** Terrestrial ecoregions of the World: a new map of life on Earth: a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience* **51**: 933–938.
- Ormerod P. 2002.** Taxonomic changes in Goodyerinae (Orchidaceae: Orchidoideae). *Lindleyana* **17**: 189–238.
- Ormerod P. 2005.** Studies of Neotropical Goodyerinae (Orchidaceae). *Harvard Papers in Botany* **9**: 391–423.
- Ormerod P. 2007.** Studies of Neotropical Goodyerinae (Orchidaceae) 2. *Harvard Papers in Botany* **11**: 145–177.
- Ormerod P. 2008.** Studies of Neotropical Goodyerinae (Orchidaceae) 3. *Harvard Papers in Botany* **13**: 55–87.
- Ormerod P. 2009.** Studies of Neotropical Goodyerinae (Orchidaceae) 4. *Harvard Papers in Botany* **14**: 111–128.
- Ormerod P. 2013.** Studies of Neotropical Goodyerinae (Orchidaceae) 5. *Harvard Papers in Botany* **18**: 51–60.
- Pace M. 2020.** A recircumscription of *Goodyera* (Orchidaceae), including the description of *Paorchis* gen. nov., and resurrection of *Cionisaccus*, *Eucosia*, and *Salacistis*. *Brittonia* **72**: 257–267.
- Paithankar KR, Prasad KS. 1991.** Precipitation of DNA by polyethylene glycol and ethanol. *Nucleic Acids Research* **19**: 1346.
- Pellegrino KCM, Rodrigues MT, Harris DJ, Yonenaga-Yassuda Y, Sites JW. 2011.** Molecular phylogeny, biogeography and insights into the origin of parthenogenesis in the Neotropical genus *Leposoma* (Squamata: Gymnophthalmidae): ancient links between the Atlantic forest and Amazonia. *Molecular Phylogenetics and Evolution* **61**: 446–459.
- Pires AC, Marinoni L. 2010.** Historical relationships among Neotropical endemic areas based on *Sepedonea* (Diptera: Sciomyzidae) phylogenetic and distribution data. *Zoologia* **27**: 681–690.
- POWO. 2020.** Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; <http://www.plantsoftheworldonline.org/>. Retrieved 30 November 2020.
- Presl C. 1827.** *Reliquia Haenkeanae, Vol. 1, fasc. II*. Prague: Calve.
- Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN. 2003.** *Genera orchidacearum, Vol. 3, Orchidoideae (part 2) Vanilloideae*. Oxford: Oxford University Press.
- Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN. 2009.** *Genera orchidacearum, Vol. 5, Epidendroideae (part 2)*. Oxford: Oxford University Press.
- Pross J, Contreras L, Bijl PK, Greenwood DR, Bohaty SM, Schouten S, Bendle JA, Rohl U, Tauxe L, Raine JI, Huck CE, van de Fliedert T, Jamieson SSR, Stickley CE, van de Schootbrugge B, Escutia C, Brinkhuis H. 2012.** Persistent near-tropical warmth on the Antarctic continent during the early Eocene epoch. *Nature* **488**: 73–77.
- Rambaut A. 2009.** FigTree v1.4.3: tree figure drawing tool. Available at: <http://tree.bio.ed.ac.uk/software/figtree>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018.** Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**: 901–904. <http://doi:10.1093/sysbio/syy032>
- Ramírez SR, Gravendeel B, Singer RB, Marshall CR, Pierce NE. 2007.** Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. *Nature Letters* **448**: 1042–1045.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Salazar GA, Batista JAN, Cabrera LI, van den Berg C, Whitten WM, Smidt EC, Buzatto CR, Singer RB, Gerlach G, Jiménez-Machorro R, Radins JA, Insaurralde IS, Guimarães LRS, De Barros F, Tobar F, Linares JL, Mújica E, Dressler RL, Blanco MA, Hågsater E, Chase MW. 2018.** Phylogenetic systematics of subtribe Spiranthinae (Orchidaceae: Orchidoideae: Cranichideae) based on nuclear and plastid DNA sequences of a nearly complete generic sample. *Botanical Journal of the Linnean Society* **186**: 273–303.

- Salazar GA, Cabrera LI, Figueroa C. 2011.** Molecular phylogenetics, floral convergence and systematics of *Dichromanthus* and *Stenorrhynchos* (Orchidaceae, Spiranthinae). *Botanical Journal of the Linnean Society* **167**: 1–18.
- Salazar GA, Cabrera LI, Madriñán S, Chase MW. 2009.** Phylogenetic relationships of Cranichidinae and Prescottiinae (Orchidaceae, Cranichideae) inferred from plastid and nuclear DNA sequences. *Annals of Botany* **104**: 403–416.
- Salazar GA, Chase MW, Soto Arenas MA, Ingrouille M. 2003.** Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae, Orchidoideae): evidence from plastid and nuclear DNA sequences. *American Journal of Botany* **90**: 777–795.
- Silva M, Noll FB. 2014.** Biogeography of the social wasp genus *Brachygastra* (Hymenoptera: Vespidae: Polistinae). *Journal of Biogeography* **42**: 833–842.
- Simon MF, Grether R, Queiroz LP, Skema C, Pennington RT, Hughes CE. 2009.** Recent assembly of the Cerrado, a Neotropical plant diversity hotspot, by *in situ* evolution of adaptations to fire. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 20359–20364.
- Singer RB, Sazima M. 1999.** The pollination mechanism in the ‘*Pelexia* alliance’ (Orchidaceae: Spiranthinae). *Botanical Journal of the Linnean Society* **131**: 249–262.
- Singer RB, Sazima M. 2001.** Flower morphology and pollination mechanism in three sympatric Goodyerinae orchids from south-eastern Brazil. *Annals of Botany* **88**: 989–997.
- Smidt EC, Engels ME, Miranda MR. 2016.** A new *Aspidogyne* (Orchidaceae: Goodyerinae) from the Brazilian Atlantic Forest. *Phytotaxa* **289**: 279–284.
- Smidt EC, Páez MZ, Vieira LN, Viruel J, Baura VA, Balsanelli E, Souza EM, Chase MW. 2020.** Characterization of sequence variability hotspots in Cranichideae plastomes (Orchidaceae, Orchidoideae). *PLoS One* **15**: e0227991.
- Smidt EC, Toscano de Brito ALV, Martins AC, Royer CA, Whitten WM, Chase MW. 2018.** Phylogenetics, biogeography and character evolution in the *Ornithocephalus* clade (Orchidaceae, Oncidiinae). *Botanical Journal of the Linnean Society* **188**: 339–354.
- Sobral-Souza T, Lima-Ribeiro MS, Solferini VN. 2015.** Biogeography of Neotropical rainforests: past connections between Amazon and Atlantic Forest detected by ecological niche modeling. *Evolutionary Ecology* **29**: 643.
- Soroka T. 2006.** *Ligeophila longibracteata* (Orchidaceae), a new jewel orchid of the Goodyerinae subtribe from the Neotropics. *Selbyana* **27**: 8–12.
- Swofford DL. 2002.** *PAUP*. Phylogenetic analysis using parsimony (* and other methods), version 4.0b10a*. Sunderland: Sinauer.
- Turchetto-Zolet AC, Cruz F, Vendramin GG, Simon MF, Salgueiro F, Margis-Pinheiro M, Margis R. 2012.** Large-scale phylogeography of the disjunct Neotropical tree species *Schizolobium parahyba* (Fabaceae-Caesalpinioideae). *Molecular Phylogenetics and Evolution* **65**: 74–182.
- Werneck FP. 2011.** The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. *Quaternary Science Reviews* **30**: 1630–1648.
- Whitten WM, Blanco MA, Williams NH, Köhler S, Carnevali G, Singer RB, Endara L, Neubig KM. 2007.** Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based on combined molecular data sets. *American Journal of Botany* **94**: 1860–1889.
- Whitten WM, Williams NH, Dressler RL, Gerlach G, Pupulin F. 2005.** Generic relationships of Zygopetalinae (Orchidaceae: Cymbidieae): combined molecular evidence. *Lankesteriana* **5**: 87–107.
- Wiens JJ. 1998.** Combining data sets with different phylogenetic histories. *Systematic Biology* **47**: 568–581.
- Yu Y, Harris AJ, Blair C, He X. 2015.** RASP (reconstruct ancestral state in phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution* **87**: 46–49.
- Yule GU. 1925.** A mathematical theory of evolution, based on the conclusions of Dr. JC Willis, FRS. *Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character* **213**: 21–87.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1. Strict consensus nrITS parsimony tree resulting from the analysis of 109 sequences from Goodyerinae plus outgroups. Numbers at nodes are bootstrap percentages > 50.

Figure S2. Maximum-likelihood nrITS tree resulting from the analysis of 109 sequences from the Goodyerinae plus outgroups. Numbers at nodes are bootstrap percentages > 50. In detail, the tree with proportional branch lengths.

Figure S3. Bayesian inference nrITS tree resulting from the analysis of 109 sequences from Goodyerinae plus outgroups. Numbers at nodes are Bayesian posterior probabilities. In detail, the tree with proportional branch lengths.

Figure S4. Plastid *matK* strict consensus parsimony tree resulting from the analysis of 106 sequences from Goodyerinae plus outgroups. Numbers at nodes are bootstrap percentages > 50.

Figure S5. Plastid *matK* maximum-likelihood tree resulting from the analysis of 106 sequences from Goodyerinae plus outgroups. Numbers at nodes are bootstrap percentages > 50. In detail, the tree with proportional branch lengths.

Figure S6. Plastid *matK* majority consensus Bayesian inference tree resulting from the analysis of 106 sequences from the Goodyerinae plus outgroups. Numbers at nodes are Bayesian posterior probabilities. In detail, the tree with proportional branch lengths.

Figure S7. Combined ITS + *matK* strict consensus parsimony tree resulting from the analysis of 110 sequences from Goodyerinae plus outgroups. Numbers at nodes are bootstrap percentages > 50.

Figure S8. Combined ITS + *matK* Bayesian inference tree resulting from the analysis of 110 sequences from Goodyerinae plus outgroups. Numbers at nodes are Bayesian posterior probabilities. In detail, the tree with proportional branch lengths.

Figure S9. Time-calibrated tree for Goodyerinae plus outgroups using 110 species inferred under uncorrelated lognormal clock model in BEAST with biogeographic inference. Grey bar at nodes indicates 95% highest posterior density intervals, and numbers above the nodes represent the age (Mya).

Figure S10. Maximum-likelihood tree of Goodyerinae plus outgroups with ancestral area optimizations. Pie charts at nodes indicate the most plausible geographic origin according to the with DEC+*j* model, and coloured circles represent the occurrence of that species in geographical areas depicted on the map of the World (Olson *et al.*, 2001): A, Nearctic; B, Neotropics; C, Palaeartic; D, Afrotropic; E, Indomalaya and F, Australasia.

Table S1. Results for Cranichidae from BioGeoBEARS ancestral area reconstruction analysis, and from BEAST divergence time estimation. For BioGeoBEARS: log-likelihood, parameters *d* (dispersion), *e* (extinction) and *j* (founder speciation event), and AIC values. For BEAST: likelihood mean (LM) and ESS (LESS), standard error (SE) and Akaike Criterion estimated under method-of-moments estimator (AICM). In bold, the preferred models.

Table S2. Results from BioGeoBEARS ancestral area reconstruction analysis, including log-likelihood, parameters *d* (dispersion), *e* (extinction) and *j* (founder speciation event), and AIC values. In bold, the preferred model for the Neotropical clade of Goodyerinae.