Molecular phylogenetics of Neotropical *Cyanaeorchis* (Cymbidieae, Epidendroideae, Orchidaceae): geographical rather than morphological similarities plus a new species

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Abstract

We investigated the phylogenetic placement of *Cyanaeorchis* and selected representatives of the tribe Cymbidieae based on nuclear (ITS) and plastid (*matK–trnK* and *rbcL*) DNA sequences. Bayesian and parsimony analyses of separate and combined datasets were largely congruent with each other and showed that the Neotropical *Cyanaeorchis* does not belongs in the predominantly Old World subtribe Eulophiinae, where it has previously been placed. Instead, it is strongly supported as a sister to *Grobya* in Catasetinae. Because Catasetinae are Neotropical and there are no unequivocal morphological similarities between *Cyanaeorchis* and other genera in the subtribe, this relationship reflects a geographical rather than morphological similarity and suggest habitat-driven local diversification. Specimens from central Brazil formerly identified as *Cyanaeorchis minor* are shown to be a distinct species, described here as *C. praetermissa*. Niche modeling indicates that *C. praetermissa* and *C. minor* have different distributions and ecological niches, whereas a third species, *C. arundinae* has broader climatic tolerances and a distribution that encompasses those of the other two species. The distribution of the genus is also predicted to include Bolivia, the states of Rio de Janeiro, Espirito Santo and several areas in northeastern Brazil, from where no collections are currently known.

Introduction

Cyanaeorchis Barbosa Rodrigues (1877: 112) is a small genus of two species distributed from the Espinhaço range in the state of Bahia in northeastern Brazil to central, southeastern and southern Brazil, Argentina and Paraguay. Species of *Cyanaeorchis* are terrestrial and found in humid grasslands, permanent swamps and marshes, usually from 600 to 1700 m, but also near sea level at latitudes greater than 27° S. Flowering is mainly from October to January, from the beginning to the peak of the rainy season. Plants of *Cyanaeorchis* have distinct growth and dormancy phases - a new vegetative shoot, leaves and terminal flowers are produced in the rainy season during the spring and summer. During the dry season or winter, the aerial parts wither and the plant survives as a short underground stem with roots, which makes possible a new cycle of growth in the following rainy season.

The first species of *Cyanaeorchis* was originally described as *Eulophia arundinae* Reichenbach (1850: 854). Barbosa Rodrigues recognized a series of differences with *Eulophia* and proposed the genus *Cyanaeorchis*. The main distinctive characters were the terminal inflorescence, four pollinia and the lack of a spur on the labellum in *Cyanaeorchis*. Cogniaux (1898–1902) and Hoehne (1942) accepted and summarized knowledge of the genus.

Phylogenetic placement of *Cyanaeorchis* has been unclear. Since Reichenbach (1850), most authors have considere it to have some relationship to *Eulophia* Brown in Lindley (1821a: 573) and the corresponding subtribe, usually Eulophiinae Bentham (1881: 287) (Dressler 1981, 1993, Chase *et al.* 2003, Pridgeon *et al.* 2009). Szlachetko (1995, 2003) included *Cyanaeorchis* in Cymbidiinae Bentham (1881: 287), but that version of Cymbidiinae partially corresponds to what was defined as Eulophiinae by other authors. In contrast to other authors, Schlechter (1915) placed *Cyanaeorchis* in Polystachyinae Schlechter (1915: 292), which included *Ansellia* Lindley (1844: 12), *Polystachya* Hooker (1824: 103) and *Galeandra* Lindley in Lindley & Bauer (1830: 8), whereas *Eulophia* was placed in Cyrtopodiinae Bentham (1881: 288) along with *Geodorum* Jackson (1811: 626), *Eulophiopsis* Pfitzer (1877: 105), now considered a synonym of *Graphorkis* Thouars (1809: 318), *Eulophiella* Linden & Rolfe (1892: 29), *Cyrtopodium* Brown (1813: 216), *Govenia* Lindley (1832: 153) and *Warrea* Lindley (1843a: 14).

Dressler (1993) was the first author to formally include *Cyanaeorchis* in Eulophiinae, but his morphological characterization of the subtribe did not match the morphological characters of *Cyanaeorchis*. Pridgeon *et al.* (2009) kept *Cyanaeorchis* in Eulophiinae and presented an expanded circumscription of the subtribe. However, beyond the general similarity between the flowers of *Cyanaeorchis* and some species of *Eulophia*, there are no clear, unequivocal morphological characters that link *Cyanaeorchis* to *Eulophia* or any other genus of Eulophiinae. For instance, Eulophiinae generally have pseudobulbs or an underground rhizomatous perennating organ (but not in *Acrolophia* Pfitzer 1887: 59), a lateral inflorescence (terminal in *Acrolophia*), two pollinia and a conspicuous stipe, whereas *Cyanaeorchis* has no pseudobulbs, a terminal inflorescence, four pollinia and an inconspicuous stipe. Another difference is found in distribution. *Cyanaeorchis* is restricted to the New World, in Brazil, Paraguay and northern Argentina, whereas Eulophiinae are predominantly Afro-Madagascan with extensions into tropical Asia, Australasia (Pridgeon *et al.* 2009) and a few (perhaps only three if *Cyanaeorchis* is excluded) in the American tropics. A few species of *Eulophia* and *Oeceoclades* (Eulophiinae) also occur in the New World, but these genera are mainly African, and in most cases, the same species, *Eulophia alta* (Linnaeus 1767: 594) Fawcett & Rendle (1910: 112) and *Eulophia ruwenzoriensis* Rendle (1895: 166), occurs in both continents, suggesting a recent long-distance dispersal from Africa to the New World. *Oeceoclades maculata* (Lindley 1821b: 15) Lindley (1833: 237) is a recently introduced invasive. These discrepancies raised doubts about phylogenetic placement of *Cyanaeorchis*.

Until recently, material of *Cyanaeorchis* had not been available for DNA studies, precluding a phylogenetic analysis of the genus using molecular data. Recent collections of the genus were few, and none had included samples for DNA extraction. Here, we report a phylogenetic analysis of *Cyanaeorchis* and selected representatives of tribe Cymbidieae Pfitzer (1887: 105) based on nuclear (ITS) and plastid (*matK–trnK* and *rbcL*) DNA sequences. Furthermore, specimens from central Brazil previously identified as *Cyanaeorchis minor* Schlechter (1920: 332) were found to represent a distinct new species, which is described here.

Material and methods

Taxonomic sampling for phylogenetic analysis

Because the phylogenetic position of *Cyanaeorchis* in Eulophiinae was uncertain, our ingroup consisted in selected species of most other subtribes of Cymbidieae. Exemplars of all species of *Cyanaeorchis* and representative species of most subtribes of Cymbidieae recognized by Pridgeon *et al.* (2009), namely Catasetinae Lindley (1843b: 22), Coeliopsidinae Szlachetko (1995: 97), Cymbidiinae, Cyrtopodinae, Eriopsidinae Szlachetko (1995: 94), Eulophiinae, Maxillariinae Bentham (1881: 288), Oncidiinae Bentham (1881: 288), Stanhopeinae Bentham (1881: 288) and Zygopetalinae Schlechter (1915: 417), except Vargasiellinae Schweinfurth in Romero & Carnevali (1993: 79), were analyzed in this study. Species of *Polystachya* (Vandeae Lindley (1826: 14), Polystachyinae) and *Phalaenopsis* Blume (1825: 294) (Vandeae, Aeridinae Pfitzer 1887: 108) were used as outgroups. Selection of outgroups was based on the phylogenetic analysis of Cameron *et al.* (1999), which placed the clade Epidendrae–Vandae as sister to Cymbidieae. The same outgroups were also used in the phylogenetic analyses of Cymbidieae of Pridgeon *et al.* (2009). Except for the *Cyanaeorchis* sequences obtained for this study, all sequences used in this analysis were downloaded from GenBank. Accession numbers are provided in Table 1.

TABLE 1. GenBank accession numbers for the sequences analysed in this study.

	ITS	matK–trnK	rbcL
Outgroup			
Phalaenopsis aphrodite Rchb.f.	AY391543	NC_007499	NC_007499
Polystachya cultriformis (Thouars) Lindl. ex Spreng.	GU556643	GQ145124	-
Polystachya pubescens (Lindl.) Rchb.f.	HM018554	GQ145180	AF074222
Catasetinae			
Catasetum expansum Rchb.f.	-	AF263637	AF074121
Catasetum fimbriatum (C.Morren) Lindl.	EU441210	-	-
Catasetum luridum (Link) Lindl.	EU877155	-	-
Catasetum planiceps Lindl.	-	EF079266	-
Catasetum saccatum Lindl.	EU441204	-	-
Catasetum sp.	-	EF065571	-
Clowesia warczewitzii (Lindl. & Paxton) Dodson	-	EU214146	-
Clowesia sp.	JF69204	-	-
Cyanaeorchis arundinae (Rchb.f.) Barb.Rodr. (RS)	KF771817	KF771821	KF771825
Cyanaeorchis arundinae (Rchb.f.) Barb.Rodr. (MG)	KF771816	KF771820	-
Cyanaeorchis minor Schltr.	KF771818	KF771822	KF771824
Cyanaeorchis praetermissa J.A.N.Bat. & Bianch.	KF771819	KF771823	KF771826
Cycnoches egertonianum Bateman	-	AY368401	AY368355
Cycnoches sp.	JF691909	-	-
Dressleria dilecta (Rchb.f.) Dodson	AF239411	EF079265	-
Dressleria eburnea (Rolfe) Dodson	-	-	AF074153
Dressleria sp.	-	AY368406	-
Galeandra beyrichii Rchb.f.	EU877151	-	-
Galeandra blanchetii E.S.Rand	EU877140	-	-
Galeandra devoniana M.R.Schomb. ex Lindl.	EU877142	AY368408	AF074171
Grobya amherstiae Lindl.	EU877158	-	-
Grobya galeata Lindl.	AF470487	AF470457	AY370655
Mormodes vinacea Hoehne	-	EF079252	-
Mormodes sp.	-	-	AF074196
Coeliopsidinae			
Coeliopsis hyacinthosma Rchb.f.	AF239344	EF065564	-
Lycomormium fiskei H.R.Sweet	AF239345	-	-
Lycomormium squalidum (Poepp. & Endl.) Rchb.f.	-	AY368414	AF074186
Peristeria elata Hook.	AF239346	-	-
Cymbidiinae			
Acriopsis javanica Reinw. ex Blume	AF470492	AF470462	-
Acriopsis sp.	-	-	AY368350
Ansellia africana Lindl.	AF470491	AF470461	EU213445
Cymbidium aloifolium (L.) Sw.	JN114485	AF470485	-
Cymbidium cyperifolium Wall. ex Lindl.	AF284719	-	-
Cymbidium eburneum Lindl.	JF729012	AF470470	AY368356

TABLE 1 (continued)

	ITS	matK–trnK	rbcL
Cymbidium elegans Lindl.	AF284712	AF470478	-
Cymbidium ensifolium (L.) Sw.	AF470512	AF470464	AF074141
Dipodium paludosum (Griff.) Rchb.f.	-	EF079261	-
Grammatophyllum speciosum Blume	AF470488	AF239510	AF074176
Grammatophyllum papuanum J.J.Sm.	-	EF079262	-
Graphorkis lurida (Sw.) Kuntze	-	AY368410	AY368359
Thecostele alata (Roxb.) E.C.Parish & Rchb.f.	-	-	AY368371
Thecostele sp.	-	AY368431	-
Cyrtopodiinae			
Cyrtopodium aliciae L.Linden & Rolfe	EU877156	-	-
Cyrtopodium andersonii (Lamb. ex Andrews) R.Br.	AF470490	EF079263	AF074143
Cyrtopodium punctatum (L.) Lindl.	AF239412	AF239508	AF074144
Eriopsidinae			
Eriopsis biloba Lindl.	DQ461788	DQ210866	AF074167
Eriopsis rutidobulbon Hook.	AF239410	-	-
Eriopsis sceptrum Rchb.f. & Warsz.	-	EF079253	-
Eulophiinae			
Cymbidiella pardalina (Rchb.f.) Garay	AF470489	AF470459	-
Eulophia alta (L.) Fawc. & Rendle	EU877157	-	-
Eulophia graminea Lindl.	FJ565666	FJ565159	-
Eulophia guineensis Lindl.	AF239413	AF239509	-
Eulophia petersii (Rchb.f.) Rchb.f.	-	EF079257	AF264167
Eulophia ruwenzoriensis Rendle	EU877159	-	-
Eulophia spectabilis (Dennst.) Suresh	-	JN004438	AF074170
Eulophia streptopetala Lindl.	-	EF079258	AM235039
Eulophia zollingeri (Rchb.f.) J.J.Sm.	AB306313	-	-
Eulophia flava (Lindl.) Hook.f.	-	JN004435	JN005457
Geodorum densiflorum (Lam.) Schltr.	AF284726	JN004443	JN005467
Oeceoclades rauhii (Senghas) Garay & P.Taylor	-	EF079260	-
Oeceoclades saundersiana (Rchb.f.) Garay & P.Taylor	-	AY368422	AY368366
Oeceoclades maculata (Lindl.) Lindl.	-	JQ588555	JQ593044
Maxillariinae			
Bifrenaria aureofulva Lindl.	AY063426	-	-
Bifrenaria tyrianthina (Lodd. ex Loudon) Rchb.f.	DQ210235	DQ210752	-
Bifrenaria harrisoniae (Hook.) Rchb.f.	-	-	AF074112
Bifrenaria tetragona (Lindl.) Schltr.	-	DQ210751	-
Cryptocentrum peruvianum (Cogn.) C.Schweinf.	DQ210321	DQ210820	AF074139
Lycaste cruenta (Lindl.) Lindl.	AF239342	AF239438	AF074185
Mormolyca polyphylla Garay & Wirth	DQ210534	DQ211009	-
Rudolfiella floribunda (Schltr.) Hoehne	DQ210394	DQ210881	-
Rudolfiella sp.	-	-	FJ534212

TABLE 1 (continued)

	ITS	matK–trnK	rbcL
Xylobium squalens (Lindl.) Lindl.	EF079427	EF079255	-
Xylobium sp.	-	-	AF074245
Oncidiinae			
Comparettia falcata Poepp. & Endl.	FJ565262	FJ563869	FJ534237
Gomesa crispa (Lindl.) Klotzsch ex Rchb.f.	FJ565415	FJ564906	-
Gomesa echinata (Barb.Rodr.) M.W.Chase & N.H.Williams	FJ565406	FJ564897	-
Gomesa flexuosa (Lodd.) M.W.Chase & N.H.Williams	-	-	FJ534252
Lockhartia amoena Endres &Rchb.f.	EF079418	FJ564686	-
Lockhartia micranta Rchb.f.	-	-	FJ534134
Miltonia flavescens (Lindl.) Lindl.	FJ565256	FJ563862	-
Oncidium altissimum (Jacq.) Sw.	FJ565295	FJ563914	FJ534170
*Ornithocephalus bicornis*Lindl.	-	FJ565126	FJ534228
Ornithocephalus inflexus Lindl.	AF350507	-	-
Trichocentrum cebolleta (Jacq.) M.W.Chase & N.H.Williams	FJ565669	-	-
Trichocentrum longicalcaratum Rolfe	FJ565524	FJ565007	-
Stanhopeinae			
Acineta superba (Kunth) Rchb.f.	AF239379	-	-
Acineta chrysantha (C.Morren) Lindl.	-	AF263619	AF074102
Coryanthes macrantha (Hook.) Hook.	AF239359	-	-
Coryanthes verrucolineata G.Gerlach	-	AY368398	AF074134
Gongora amparoana Schltr.	-	AY368409	AY368358
Gongora armeniaca (Lindl.) Rchb.f.	AF239386	-	-
Gongora galeata (Lindl.) Rchb.f.	-	EF079251	-
Gongora sphaerica Jenny	AF239388	-	-
Houlletia sanderi Rolfe	AF239371	EF065562	AF074178
Houlletia wallisii Linden & Rchb.f.	AF239369	-	-
Stanhopea cirrhata Lindl.	AF239368	-	-
Stanhopeae cornuta Lem.	-	-	AF074230
Stanhopea jenischiana F.Kramer ex Rchb.f.	-	FJ565129	-
Stanhopea tigrina Bateman ex Lindl.	FJ565224	AY368430	-
Zygopetalinae			
Cryptarrhena lunata R.Br.	AY870081	-	-
Cryptarrhena sp.	-	AY368399	AF074138
Dichaea riopalenquensis Dodson	EU123594	EU123657	AF074149
Huntleya wallisii (Rchb.f.) Rolfe	AY870074	EU123674	-
Huntleya heteroclita (Poepp. & Endl.) Garay	-	-	AF074179
Koellensteinia graminea (Lindl.) Rchb.f.	AY870102	AY870003	AF074182
Promenaea ovatiloba (Klinge) Cogn.	AY870100	AY368428	AY368369
Zygopetalum maculatum (Kunth) Garay	AY870097	AY869998	AF074246
Zygopetalum maxillare Lodd.	AY870095	EF079242	-

Molecular markers

Nucleotide sequences from one nuclear genome region (ITS) and three plastid regions (*matK*, *trnK* 3' intron and *rbcL*) were used in analyses. Amplifications of ITS were performed with primers 17SE and 26SE (Sun *et al.* 1994). The plastid DNA regions included the partial sequence of the *matK* gene, the complete sequence of the *trnK* 3' intron, and the complete sequence of the *rbcL* gene. The partial sequence of the *matK* gene and flanking region of the *trnK* 3' intron were amplified with primers 390F (5'-CGATCTATTCATTCAATATTTC-3') and 2R (5'-CCCGGAACTAGTCGGATG-3'). The *rbcL* gene was amplified with primers F1 (5'-ATGTCACCACAAACAGAAAC-3') and 1379R (5'-TCACAAGCAGCAGCTAGTTCAGGACTC-3'). Marker selection was based on availability of sequences from other genera of Cymbidieae in public databases and the general use of these markers for inferring phylogenetic relationships at various taxonomic levels in Cymbidieae and other Orchidaceae (Cameron *et al.* 1999, Whitten *et al.* 2000, Salazar *et al.* 2003, Freudenstein *et al.* 2004, van den Berg *et al.* 2005, Pridgeon *et al.* 2009). Genomic DNA was extracted from fresh or silica gel-dried material using the 2× CTAB method adapted from Doyle & Doyle (1987) or the protocol described by de la Cruz *et al.* (1997) and optimized for small-scale extraction by Sánchez-Hernández & Gaytán-Oyarzún (2006). Some samples that did not amplify well were further purified using Qiagen spin columns (DNeasy® Plant Mini Kit, Qiagen, Hilden, Germany). PCR amplifications were performed in a MJ96G (Biocycler) or Eppendorf Mastercycler thermal cycler. The general PCR system consisted of 2–3 µl genomic DNA (approximately 20–50 ng of DNA), 1× PCR buffer (Phoneutria Biotec., Belo Horizonte, Brazil),1.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM of each primer, 1.5 U of Taq DNA polymerase (Phoneutria Biotec., Belo Horizonte, Brazil) and water in a volume of 30 µl. The cycling conditions were as follows: an initial denaturation at 94 ºC for 4 min, 35 cycles of 94 ºC for 45 s, 58 ºC for 45 s, 72 ºC for 80 s, and a final extension of 5 min at 72 ºC. PCR products were purified by precipitation with polyethylene glycol and sequenced by Macrogen Inc. (Korea). Sequencing primers were the same as those used for amplification. Bidirectional sequence reads were obtained for all DNA regions, and the resulting electropherograms were edited and assembled using the Staden Package software (Bonfield *et al.* 1995).

Sequence alignment and phylogenetic analyses

The edited sequences were aligned with MUSCLE (Edgar 2004), and the resulting alignment was manually adjusted using MEGA4 software (Tamura *et al.* 2007) to maximize sequence similarity (Simmons 2004). No data were excluded from the analyses because of ambiguous alignments. Individual gap positions were treated as missing data. The data were analyzed with both parsimony and Bayesian inference. Phylogenetic analyses using maximum parsimony (MP) were performed in PAUP version 4 (Swofford 2002) with Fitch parsimony (Fitch 1971) as the optimality criterion. Each search consisted of 1,000 replicates of random taxon addition for the starting trees and branch swapping using the TBR (tree bisection-reconnection) algorithm, only saving up to ten trees per replicate to avoid extensive swapping on suboptimal islands. All characters were treated as unordered and equally weighted. Internal support was evaluated by nonparametric bootstrapping (Felsenstein 1985) with 1,000 replicates, simple addition and TBR branch swapping, saving up to ten trees per replicate. For bootstrap support levels, we considered bootstrap percentages (BP) of 50–70 as weak, 71–84 as moderate and > 85 as strong (Kress *et al.* 2002). Bayesian analysis of individual and combined datasets was implemented in MrBayes v. 3.1.2 (Ronquist *et al.* 2005), treating each DNA region (ITS, *matK–trnK* and *rbcL*) as a separate partition. An evolutionary model for each DNA region was selected with MrModeltest 2 (Nylander 2004). For all data sets, the GTR + I + G model was selected based on the Akaike information criterion (AIC) or hierarchical likelihood ratio tests (hLRTs). Each analysis consisted of two independent runs with four chains for 5,000,000 generations, sampling one tree every 1,000 generations. The temperature parameter for heating the chains was 0.2. Convergence between runs was evaluated by the average standard deviation of split frequencies (<0.01). After discarding the first 25% of the trees as burn-in, the remaining trees were used to assess topology and posterior probabilities (PP) in a majority-rule consensus. Because PP in Bayesian analysis are not equivalent to BP and are generally much higher (Erixon *et al.* 2003), we used criteria similar to a standard statistical test, considering groups with PP > 0.95 as strongly supported, PP ranging from 0.90–0.95 as moderately supported and PP < 0.90 as weakly supported.

Niche modeling

We assembled a database of 71 taxonomically verified, georeferenced, unique occurrence records of the species of *Cyanaeorchis*, including 58 records of *C. arundinae*, six of *C. minor* and seven of the new species, *C. praetermissa*, based on review of specimens from 24 herbaria (see taxonomic analysis below). Niche models were

generated for each species using Maxent and GARP (Phillips *et al.* 2004, Stockwell & Noble 1992) and a range of climatic and topographic variables, which have been shown to be useful for continental and regional scales (Thuiller *et al.* 2004). The following climatic variables were used: mean diurnal range, precipitation of driest quarter, precipitation of wettest quarter, precipitation of coldest quarter and precipitation seasonality (WorldClim; Hijmans *et al.* 2005). The topographic variables were as follows: elevation (from WorldClim; Hijmans *et al.* 2005), slope inclination and slope orientation (these two were obtained by processing the elevation variable of WorldClim using ArcGIS 9.3). Variables were selected by evaluating the correlation among variables by means of Pearson's correlation coefficient, as in Elith *et al.* (2006) and Murphy & Lovett (2007). We tested the correlation among all pairs of variables, and only those with r ≤ 0.7 were used.

Results of the modeling method can be affected by amount of available data; for instance, Papes & Gaubert (2007) showed that when modeling with small amounts of data, Maxent tended to over-predict the distribution more than GARP. Because the number of records of *C. arundinae* and *C. minor* were comparatively fewer (six and seven, respectively) than those of *C. arundinae* (58), we analyzed the data using both Maxent and GARP. Only in the case of *C. arundinae* was it possible to evaluate models using test points, i.e., other points distinct from those used to generate the model, that were posteriorly used to evaluate the model.

Model robustness was evaluated with the area under the curve (AUC) statistic, which is a descriptive index of the graph of the receiver operating characteristic. Furthermore, results from both algorithms were critically evaluated on the basis of everything that is known of the natural history of the three species.

Taxonomic analysis

Morphological analyses and descriptions were based on live, pickled or herbarium material. Details of flowers were examined from the pickled material under a stereoscopic microscope. Data on flowering times, habitats, and distribution were based on herbarium labels or field observations. Materials and images were examined from the following herbaria: AMES, BHCB, CEN, F, G, HB, HBG, HUEFS, HUFU, ICN, IPA, K, M, MBM, NY, P, R, RB, S, SI, SP, SPF, UB, US and W. The descriptive terminology used here is based on Stearn (1992) and Simpson (2006).

Results

Phylogenetic analyses

Initially, we performed separate analyses for each of the ITS and plastid data sets. Because no significant incongruence was detected between the plastid and nuclear data (i.e., no conflicting groups obtaining strong internal support), an analysis was performed with the combined matrix. Because the parsimony trees are largely congruent with the Bayesian trees but are less resolved and have weaker overall support, the Bayesian trees were chosen for presentation. General features of the datasets and parsimony statistics for each dataset and analysis are presented in Table 2.

TABLE 2. Taxon sampling and matrix and parsimony statistics for each of the parsimony analyses.

	Taxa	Aligned length	Variable, non-informative sites	Variable parsimony informative sites (%)	Trees	Fitch tree length	Consistency index	Retention index
ITS	72	892	114 (13%)	420 (47%)	61	2361	0.41	0.60
matK–trnK	74	1881	309 (16%)	378 (20%)	3573	1482	0.59	0.69
rbcL	61	1343	126 (9%)	121 (9%)	3090	450	0.60	0.70
Plastid	80	3247	406 (12%)	471 (14%)	3940	1783	0.60	0.68
Combined	45	4080	562 (14%)	700 (17%)	2	3377	0.52	0.50

Overall, the Bayesian tree and the parsimony strict consensus tree of the ITS dataset were congruent. Most subtribes were found to be monophyletic, except Cymbidiinae and Eulophiinae, because *Ansellia* and *Geodorum*, respectively, were not included in these subtribes, and Maxillariinae, which formed two major clades [(*Mormolyca, Cryptocentrum, Maxillaria, Xylobium, Lycaste*) and (*Bifrenaria, Rudolfiella, Scuticaria*)] that were paraphyletic to Coeliopsidinae and Stanhopeinae (Fig. 1). *Cyanaeorchis*, comprising the three species of the genus recognized
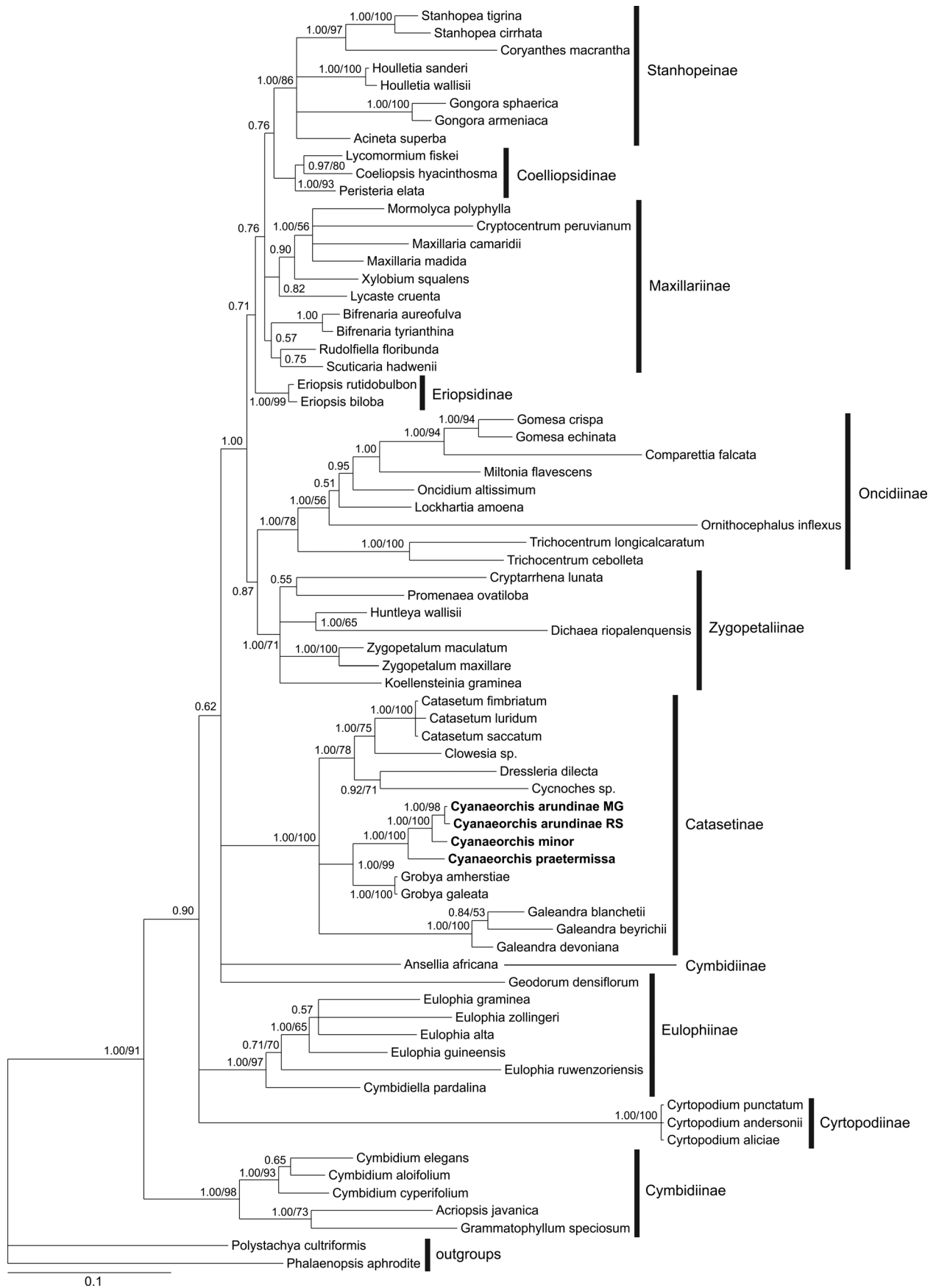
FIGURE 1. Bayesian tree from the ITS analysis. Posterior probabilities and bootstrap percentages ≥ 50% from the parsimony analysis are shown next to nodes. Subtribes of Cymbidieae are indicated by bars.

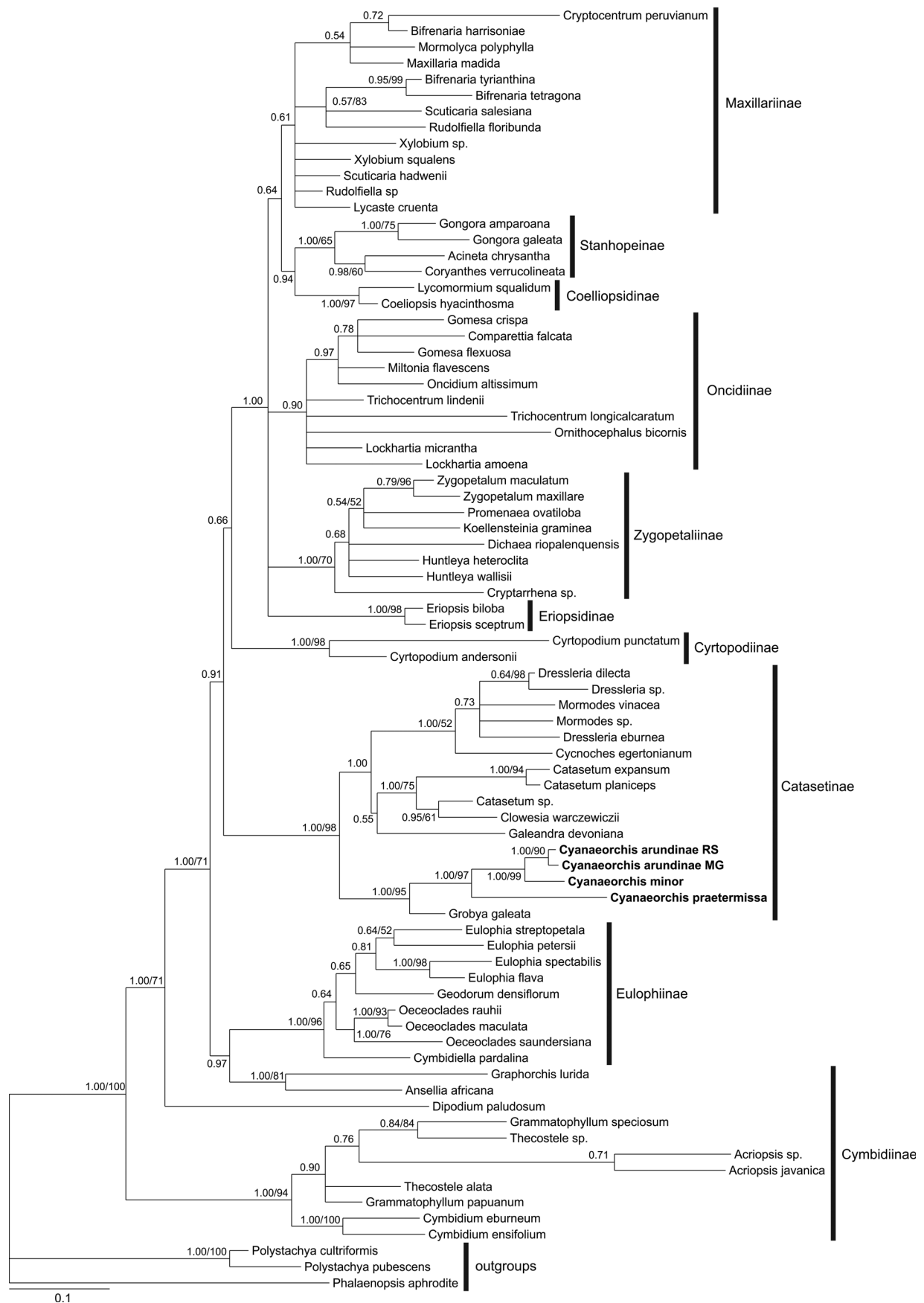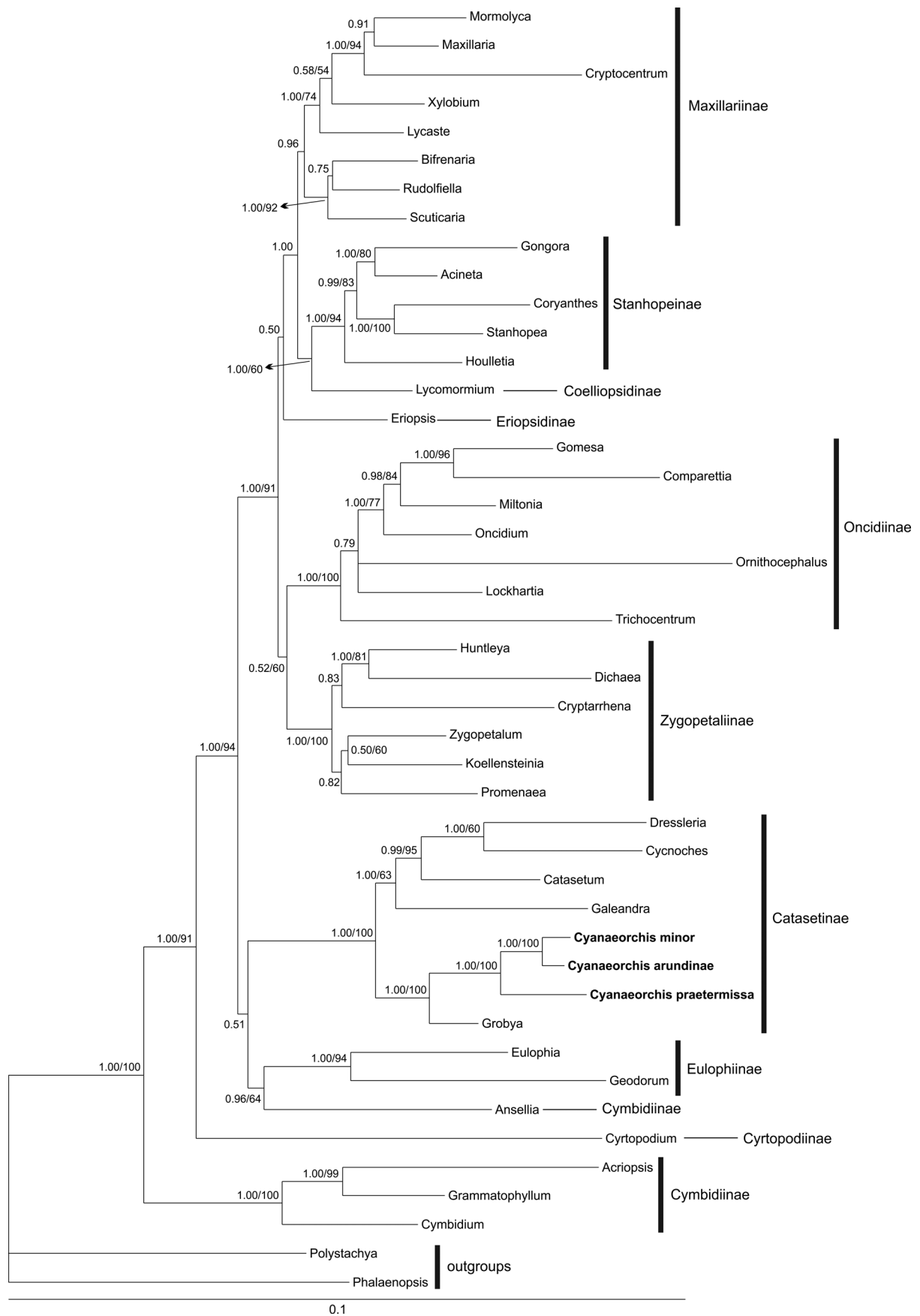**FIGURE 2.** Bayesian tree from the plastid (*matK–trnK*, *rbcL*) analysis. Posterior probabilities and bootstrap percentages ≥ 50% from the parsimony analysis are shown next to nodes. Subtribes of Cymbidieae are indicated by bars.

FIGURE 3. Bayesian tree from the combined ITS, *matK–trnK* and *rbcL* analysis. Posterior probabilities and bootstrap percentages ≥ 50% from the parsimony analysis are shown next to nodes. Subtribes of Cymbidieae are indicated by bars.

here, formed a strongly supported clade (1.00 PP; 100 BP) that was strongly supported (1.00 PP; 99 BP) as sister to *Grobya* Lindley (1835: 1740). The *Cyanaeorchis–Grobya* clade was strongly supported as a member of Catasetinae (1.00 PP; 100 BP), where it formed a polytomy with a *Catasetum–Clowesia–Dressleria–Cycnoches* and a *Galeandra* clade.

In analyses of the plastid dataset, similarly to that of ITS, most subtribes were recovered as monophyletic, with the exception of Cymbidiinae, because *Ansellia*, *Graphorkis*, and *Dipodium* Brown (1810: 330), were not included in this subtribe (Fig. 2). *Dipodium* was strongly to moderately supported (1.00 PP; 71 BP) as a sister of Cymbidieae, excluding Cymbidiinae, whereas *Ansellia* and *Graphrochis* formed a clade (1.00 PP; 81 BP) that was sister to Eulophiinae (0.97 PP). The three species of *Cyanaeorchis* formed a strongly supported clade (1.00 PP; 97 BP) that was a sister to *Grobya* (1.00 PP; 95 BP) and placed as a sister to other genera in a strongly supported Catasetinae (1.00 PP; 98 BP).

In the combined analysis, we used two approaches because the species with sequences available in public databases were not always the same in all datasets. First, we selected most of the sequences used in each of the individual analysis and treated the species absent in one of the datasets as missing data. Second we selected sequences in monophyletic genera common to the three datasets (ITS, *matK–trnK*, *rbcL*) and treated the taxa at the generic level, except for the *Cyanaeorchis* sequences, for which vouchers were the same for all datasets. In both cases, results were essentially the same, and there were no conflicting groups between the analyses with strong support. However, in the first case, when plastid and nuclear markers were from different species, resolution was lower and some genera were recovered as paraphyletic. Therefore, we choose for presentation the Bayesian tree from the second analysis, which was overall better resolved. With the exception of Cymbidiinae all other subtribes were recovered with moderate to high support (Fig. 3). Cymbidiinae, excluding *Ansellia*, were strongly supported (1.00 PP; 100 BP) as a sister to the remaining subtribes of Cymbidieae, whereas *Ansellia* was strongly to weakly supported as sister to Eulophiinae (0.96 PP; 64 BP). Similar to the other analyses, the *Cyanaeorchis* species formed a strongly supported clade (1.00 PP; 100 BP) that was a sister to *Grobya* (1.00 PP; 100 BP), the pair included in Catasetinae (1.00 PP; 100 BP).

Niche modeling

For *Cyanaeorchis arundinae*, there was no significant difference in the AUC statistic between the model generated with GARP (AUC = 0.901) and Maxent (AUC = 0.955), but the former grossly over-predicted the distribution, i.e., predicted a distribution area much larger than currently known, including several extensive suitable areas in the Amazon basin. The potential distribution of *C. arundinae* as modeled with Maxent is shown in Fig. 4C. The best model was mainly influenced by five variables (elevation, isothermality, precipitation of driest quarter, precipitation of wettest quarter and slope inclination), which explained over 90% of the model. In the cases of *C. minor* and *C. praetermissa*, Maxent resulted in large over-predictions, similarly to results obtained with GARP for *C. arundinae*, but the potential distributions inferred by GARP were much closer to what is known of the actual distribution of these species. The GARP models for both these species are shown in Fig. 4 A,B. However, GARP does not allow determination of which variables contributed more to the best models.

Discussion

Phylogenetic position of Cyanaeorchis

Our phylogenetic analysis clearly supports inclusion of *Cyanaeorchis* in Catasetinae, among which it is strongly supported as sister to *Grobya* (BP 100, PP 1.00). From this result, it is clear that all earlier phylogenetic positions of *Cyanaeorchis* among Eulophiinae based on an interpretation of its general floral morphology were mistaken. However, as with the previous inclusion of *Cyanaeorchis* in Eulophiinae, there are no obvious or unequivocal morphological characters shared by *Cyanaeorchis* and the other genera in Catasetinae. For instance, *Grobya* and most species of Catasetinae are epiphytic and have pseudobulbs, whereas *Cyanaeorchis* is terrestrial and lacks pseudobulbs. The only species of Catasetinae with some similarity to *Cyanaeorchis* are the terrestrial *Galeandra* species, which have a similar vegetative morphology. In accordance with this similarity, Schlechter (1915) placed *Cyanaeorchis* and *Galeandra* in Polystachyinae along with and a few other genera, but he defined this subtribe based mainly on pollinarium morphology. Like *Cyanaeorchis*, the terrestrial species of *Galeandra* are

found in grassland habitats, but this similarity may be, however, just convergence due to similar habitats. Accordingly, the molecular phylogenetic analysis of *Galeandra* (Monteiro *et al.* 2010) indicated that the terrestrial habit is a derived character in this genus.
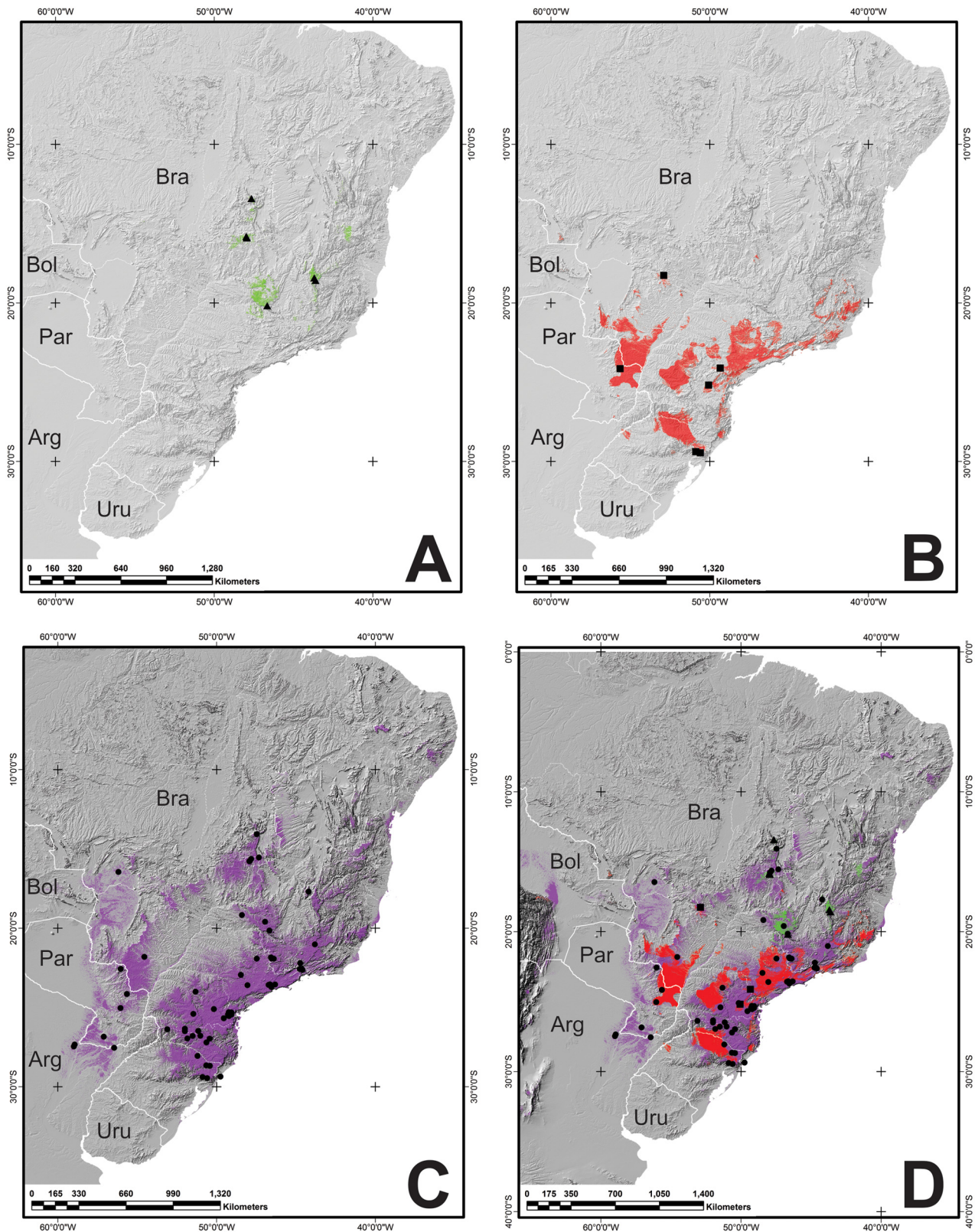

FIGURE 4. Occurrence records and niche modeling inferred with GARP (A–B) and Maxent (C). **A.** *Cyanaeorchis praetermissa.* **B.** *Cyanaeorchis minor.* **C.** *Cyanaeorchis arundinae.* **D.** *Cyanaeorchis* spp. Political divisions are highlighted in white. Country abbreviations are as follows: Arg, Argentina; Bol, Bolivia; Bra, Brazil; Par, Paraguay; Uru, Uruguay.

Because Catasetinae are restricted to the Neotropics, our results reveal a geographical pattern, not one based on morphological similarity. The geographical structure of Catasetinae along with the habit and habitat of the species suggests divergent local diversification of the group in the Neotropics from a common American ancestor. Core Catasetinae (*Catasetum, Clowesia, Cycnoches, Dressleria, and Mormodes*), *Grobya* and one group of *Galeandra* species are mainly epiphytic in tropical forests, whereas *Cyanaeorchis* and another group of *Galeandra* species are grassland terrestrial. There are a few exceptions, such as terrestrial *Galeandra beyrichii* Reichenbach (1850: 854) that occurs in forests, and a few terrestrial species of *Catasetum* Richard in Kunth (1822: 330). Because most species of Catasetinae and Cymbidieae have pseudobulbs, the lack of pseudobulbs in *Cyanaeorchis* is most likely a derived character and an adaptation to permanently water-saturated soil. In contrast, the grassland terrestrial species of *Galeandra* grow in seasonally humid or dry soils and have pseudobulbs.

Beyond the lack of pseudobulbs and terminal inflorescence, four pollinia in *Cyanaeorchis* is another distinctive feature that differs from other genera in Catasetinae and Eulophiinae. Nevertheless, the number of pollinia is a variable character in other genera in the tribe; for example, *Cymbidium* Swartz (1799: 70) can have two or four pollinia (Seidenfaden 1983). In summary, our results suggest that the modified vegetative morphology of *Cyanaeorchis* compared to other Catasetinae reflects an adaption to moist grasslands. Differences in flower features are most likely also adaptations to specific pollinators. Nothing is known about pollination of *Cyanaeorchis*, but the three species in the genus have papillae in the labellum midlobe. This feature is also found in several species of *Eulophia*. Pollination by carpenter bees of the genus *Xylocopa* has been reported for *Eulophia*, and it is possible that *Cyanaeorchis* may have a similar pollinator.

Inclusion of *Cyanaeorchis* in Catasetinae requires a morphological change to circumscription in the latter, but this was true also of inclusion of *Grobya* and *Galeandra* in this subtribe. However, there are no apparent common morphological characters shared among genera in the subtribe, except for homoblastic pseudobulbs, which also occur in other subtribes and tribes (Pridgeon *et al.* 2009). Consequently more detailed comparisons, including additional data from anatomy, cytogenetics, palynology, phytochemistry etc. will be necessary to reassess the phylogenetic position of *Cyanaeorchis*, *Grobya* and *Galeandra* in Catasetinae, which is supported by molecular but not morphological characters.

Relationships within Catasetinae were not completely resolved, particularly in relation to the position of *Galeandra*, which varied in each analysis. In the analysis of the combined datasets *Galeandra* was a sister to core Catasetinae, in the analyses of the plastid dataset it was a sister to a *Catasetum–Clowesia* clade, whereas in the analyses of the ITS dataset it formed a polytomy with *Grobya–Cyanaeorchis* and core Catasetinae.

Phylogenetic relationships in Cymbidieae

Although our study was not designed to resolve relationships in Cymbidieae, some results concerning major relationships in the tribe and differences from the literature deserve some mention. In all our analyses, Cymbidiinae were recovered as a sister to the remaining subtribes of Cymbidieae, with the exception of the parsimony analysis of the ITS dataset, in which *Geodorum* (Eulophiinae) was a sister to the remainder of the tribe. In the combined analysis, Cyrtopodiinae were sister to Cymbidieae excluding Cymbidiinae. All analyses also recovered the clade composed of Eriopsidinae, Zygopetalinae, Oncidiinae, Maxillariinae, Coeliopsidinae and Stanhopeinae, but relationships between subtribes were not resolved. In the ITS and combined analyses, Zygopetalinae were a sister to Oncidiinae, Coeliopsidinae sister to Stanhopeinae, and Coeliopsidinae-Stanhopeinae sister to Maxillariinae. The position of Eriopsidinae was not resolved.

Some results obtained were not in agreement with the literature. In our analyses, *Ansellia* and *Graphorkis* were more closely related to Eulophiinae, whereas in *Genera orchidacearum* (Pridgeon *et al.* 2009), they were placed in Cymbidiinae. The two first genera are from Africa, and in a biogeographical context, their inclusion in Eulophiinae makes more sense because Eulophiinae are predominantly Afro-Madagascan whereas Cymbidiinae are almost entirely from tropical and subtropical Asia. In this context, the monospecific genus *Imerinaea* Schlechter (1925: 152), from Madagascar, is most likely more related to Eulophiinae than to Cymbidiinae. The position of *Dipodium* warrants further investigation. In our analysis of the plastid dataset, it was strongly to moderately supported (1.00 PP; 71 BP) as sister to Cymbidieae, excluding Cymbidiinae. The same result was obtained in the parsimony analysis of Górniak *et al.* (2010) using the low-copy nuclear gene *Xdh*. However, in *Genera orchidacearum* (Pridgeon *et al.* 2009), *Dipodium* was placed in the subtribe Cymbidiinae, indicating that the phylogenetic position of the genus is not clear and requires further investigation.

Distribution of the species of Cyanaeorchis

There is no overlap between the known or potential distribution areas of *C. praetermissa* and *C. minor* (Fig. 4A, B). *Cyanaeorchis praetermissa* occurs in the cerrado biome in the highlands of central-western and southeastern Brazil at an elevation of 1000–1400 m, whereas *C. minor* occupies the southernmost part of the Mata Atlântica domain at elevations of 700–900 m. Although the method (GARP) used to model potential distribution of these species does not specify which variables contributed more to the model, a comparison of the climatic data for the predicted areas suggests that the species have different ecological niches. *Cyanaeorchis praetermissa* occurs in locations with a marked seasonality, the highest precipitation of wettest quarter (mean of 863 mm vs. 594 mm for *C. minor*), lowest precipitation of the coldest quarter (mean of 53 mm vs. 200 mm for *C. minor*) and higher isothermality. On the other hand, the known and potential distribution of *C. arundinae* encompasses those of *C. minor* and *C. praetermissa* over an elevation range of 4–1800 m. The mean temperature of the coldest quarter (15.1°C for the sampled locations vs. 16.7 °C for *C. minor* and 18.2 °C for *C. praetermissa*) and precipitation of warmest quarter were the bioclimatic variables that most contributed to the niche model of *C. arundinae*, but its wide distribution indicates that this species likewise has broader climatic tolerance. Overall, the mean values of the bioclimate variables were more similar for the sampled sites of *C. arundina*e and *C. minor*, and sites of *C. praetermissa* were different from that of those two.

Our field observations also indicate differences in the specific habitat preferences of *C. praetermissa* and the other two species. All three species occur in hydromorphic, water-saturated soil, but *C. praetermissa* occurs in areas where the herb cover is thinner and less developed and the soil is stable. In contrast, *C. arundinae* and *C. minor* occur in areas with a taller herb cover, usually over soft, unstable soil, and such areas are generally referred to as "brejos." Although the known (and predicted) distribution of *C. arundinae* encompasses the distribution of *C. praetermissa*, we are not aware of any place in which the two species co-occur. Conversely, *C. arundinae* and *C. minor* co-occur in some sites.

Distribution models predicted larger areas of occurrence for the three species, particularly for *C. arundinae*. According to our results, this species should also be expected in Bolivia, the southeastern Brazilian states of Rio de Janeiro and Espirito Santo and several areas in northeastern Brazil, including the Borborema Plateaux and Chapada do Araripe in the states of Pernambuco and Ceará. The only known record of this species from northeastern Brazil so far is from the Chapada Diamantina in Bahia (Toscano de Brito *et al.* 2005).

Taxonomy

Two species of *Cyanaeorchis* are accepted, *C. arundinae* and *C. minor* (Hoehne 1942, Pabst & Dungs 1975, Barros *et al.* 2013, Govaerts *et al.* 2013). Differences between these two species are size of plants and flowers and colour of the labellum. Plants of *C. arundinae* are usually taller with larger flowers that are completely yellow, whereas *C. minor* has smaller plants and flowers with a whitish perianth and wine-coloured lateral lobes of the labellum. However, there are no unique characteristics for either of the species because these characteristics overlap (Table 3), and the exact separation between them is not clear. Hoehne (1942) accepted the existence of two species but suggested that *C. minor* could correspond to stunted specimens of *C. arundinae*.

In terms of distribution, *C. arundinae* is the most widespread species, occurring from the Espinhaço range in the state of Bahia in northeastern Brazil to central (Distrito Federal, Goiás, Mato Grosso, Mato Grosso do Sul), southeastern (Minas Gerais, São Paulo), southern Brazil (Paraná, Santa Catarina, Rio Grande do Sul), Argentina (Chaco and Buenos Aires) and Paraguay (Amambay, Caaguazú, Canindeyú and Misiones). *Cyanaeorchis minor* is found in southern (Paraná and Rio Grande do Sul), southeastern (São Paulo) and central (Goiás) Brazil, and Paraguay (Caaguazú). Some authors have also recorded *C. arundinae* in the Amazon region and the state of Rio de Janeiro in southeastern Brazil (Hoehne 1942, Pabst & Dungs 1975, Barros *et al.* 2013), but there are no herbarium specimens known from these regions.

Small plants of *Cyanaeorchis* from central Brazil were previously identified as *C. minor* by Pabst & Dungs (1975), Menezes (1992, 2004), Batista & Bianchetti (2003) and Batista *et al.* (2005) because of the small size of the plants and flowers. However, examination of images of syntypes of *C. minor* in the herbarium of the Swedish Museum of Natural History (S) and several specimens of *C. minor* from other herbaria (AMES, BHCB, G, HBG, M, NY, SP, SPF) has shown that plants from central Brazil are a new species distinct from *C. minor*, described here as *C. praetermissa*.

TABLE 3. Diagnostic characteristics for *Cyanaeorchis* species.

	C. praetermissa	*C. minor*	*C. arundinae*
Plant size, including inflorescence (cm)	14–41	20–52	31–123
Nº leaves	(2–)3–4(–5)	3–4	3–7
Leaves size: length × width (cm)	3.5–10.0 × 0.3–0.7	13.0–24.0 × 0.4–0.6	5.0–25.0(–38.0) × 0.4–1.0
Nº flowers	(1–)2–3(–6)	(1–)3–5(–6)	(2–)3–7(–13)
Dorsal sepal: length × width (mm)	12–16 × 3–5	(9–)11–17(–23) × 3–6	15–27 × 5–9
Petals: length × width (mm)	(9.0–)11.0(–12.0) × 3.0–4.0(–5.0)	10.5–12.0(–16.0) × 3.5–5.0	12.0–14.0(–20.0) × 5.0–7.0
Sepals and petals colour	yellowish	whitish	yellowish or whitish
Labellum: length × width (mm)	6.0–8.0 × 4.5–6.5	8.0–12.0 × 9.0	9.0–15.0 × 9.0–12.0
Labellum lateral lobes: length × width (mm)	5.5–7.0 × 1.5–2.5	8.0–10.0 × 3.0	9.0–12.0 × 3.0–6.0
Labellum midlobe: length × width (mm)	2.0–3.0 × 2.0–2.5	3.2–5.0 × 3.0–4.0	(3.0–)5.0–7.0 × 3.0–5.0
Papillae length	minute	conspicuous	conspicuous
Labellum colour	yellow	midlobe and callus yellow, lateral lobes wine-coloured	usually completely yellow, lateral lobes occasionally whitish or wine-coloured
Column length (mm)	5.7–7.2	about 9.0	9.0–13.0
Column colour	yellow	base dorsally white, ventrally yellow, middle and apex wine	mostly white, apex slightly wine
Anther	yellow	wine-coloured	wine-coloured
Biome	Cerrado	Atlantic Forest	Atlantic Forest, Cerrado
Distribution	Brazil (DF, GO, MG)	Brazil (GO, PR, RS, SP), Paraguay (Caaguazú)	Brazil (BA, DF, GO, MG, MT, MS, PR, RS, SC, SP), Argentina (Chaco, Buenos Aires), Paraguay (Amambay, Caaguazú, Canindeyú, Misiones)

Key to the species of *Cyanaeorchis*

1. Petals slightly falcate; labellum 4.5–6.5 mm wide between the outer margins of side lobes; lateral lobes 5.5–7.0 mm long, 1.5–2.5 mm wide; midlobe 2.0–3.0 mm long, 2.0–2.5 mm wide; column 5.7–7.2 mm .... *C. praetermissa*
1. Petals straight; labellum 9–12 mm wide between the outer margins of side lobes; lateral lobes 8–12 mm long, 3–6 mm wide; midlobe 3–7 mm long, 3–5 mm wide; column 9–13 mm 2
2. Plants 31–123 cm tall, including inflorescence; dorsal sepal 15–27 × 5–9 mm; petals 12–14(–20) × 5–7 mm; labellum 9–15 × 9–12 mm, usually completely yellow, lateral lobes occasionally whitish or wine-coloured .... *C. arundinae*
2. Plants 20–52 cm tall, including inflorescence; dorsal sepal (9–)11–17(–23) × 3–6 mm; petals 10.5–12.0(–16.0) × 3.5–5.0 mm; labellum 8–12× 9 mm, midlobe and callus yellow, lateral lobes conspicuously wine-coloured .... *C. minor*

Cyanaeorchis praetermissa J.A.N.Bat. & Bianch., *sp. nov.* (Figs. 5B–D, 6H–M).

Similar to *C. minor* in plant size, number and size of leaves and number of flowers but with distinct completely yellow flowers, a smaller labellum (6.0–8.0 × 4.5–6.5 mm) with a smaller midlobe (2.0–3.0 × 2.0–2.5 mm) and less developed papillae, and a distribution restricted to the cerrado biome in central and southeastern Brazil.
Type:—BRAZIL. Minas Gerais: Presidente Kubitschek, BR-239 no sentido para Datas, cerca de 2.8 km após a entrada para

Presidente Kubitschek, 18º34'24.8" S, 43º36'37.6" W, 1108 m, 8 December 2010 (fl), *Batista, Fernandes, Ramalho & Vale 3041* (holotype BHCB!; isotype CEN!).
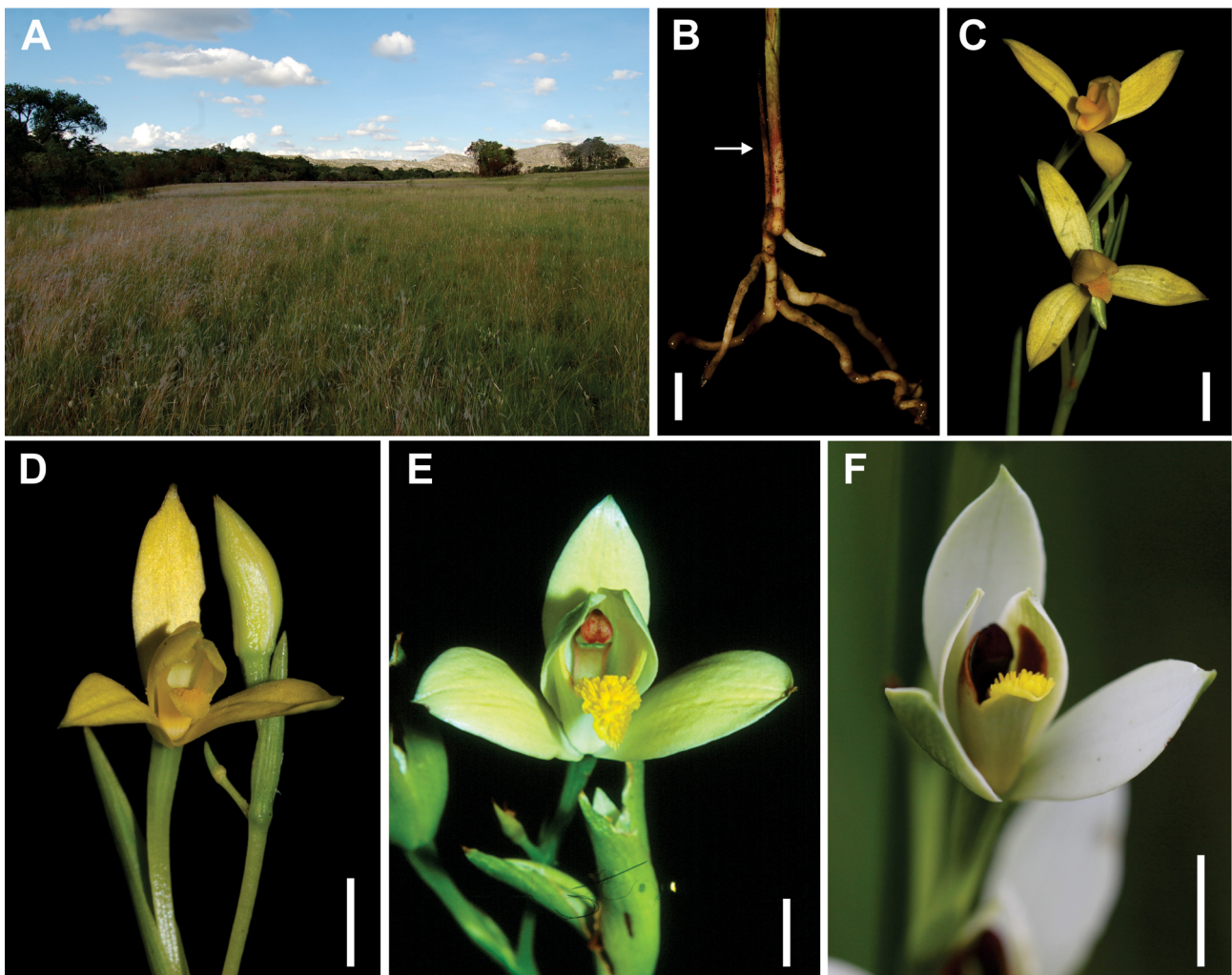

FIGURE 5. *Cyanaeorchis praetermissa*. **A.** Habitat: permanently wet grassland (*campo limpo úmido*) at the margin of gallery forest in Presidente Kubitschek, Minas Gerais, at the beginning of the rainy season. **B.** Uprooted plant, showing roots and stem base; remnants of the withered stem from the previous growth season are indicated by an arrow (*Batista & Bianchetti 3175*, BHCB). **C.** Inflorescence (*Batista et al. 3041*, BHCB). **D.** Flower, partial lateral view (*Batista et al. 2218*, BHCB). *Cyanaeorchis arundinae.* **E.** Flower, partial lateral view (*Batista 312*, CEN). *Cyanaeorchis minor.* **F.** Flower, partial lateral view (*Klein 124*, BHCB). Scale bars = 5 mm.

Terrestrial, sympodial herbs with a few roots. Stem erect, unbranched, glabrous, cylindrical, not modified into pseudobulb, 14–41 cm high, including inflorescence, 0.6–1.8 mm wide, base with 1–2 sheaths. Leaves (2–)3–4(–5), grass-like, erect, rigid, linear to lanceolate, acuminate, mostly sheathing, 3.5–10.0 × 0.3–0.7 cm, largest at the center of stem, shorter to slightly longer than internodes, green. Inflorescence terminal, erect, racemose, few flowered, lax, 2.5–7.5 cm high; floral bracts persistent, erect, rigid, linear to lanceolate, acuminate, 7–39 × 2–5 mm, longer, about the same size or shorter than the pedicel and ovary, green. Flowers (1–)2–3(–6), ascending, resupinate, mostly yellow, ovary and pedicel 15–30 mm, fusiform, green. Sepals spreading, free, external side greenish, internal side yellowish; dorsal sepal, 12–16 × 3–5 mm, lanceolate, reflexed, acute to slightly obtuse, mucronate; lateral sepals, (11–)12–15 × 4–6 mm, obliquely oblanceolate, slightly reflexed, apex acute to slightly obtuse. Petals (9–)11(–12) × 3–4(–5) mm, yellowish, base sessile to shortly unguiculate, 0.5–0.7 mm, obliquely oblong, slightly falcate, partially superimposed to one another, forming a hood over the column and labellum, apex obtuse to rounded, sometimes slightly apiculate. Labellum trilobed, conduplicate-concave, 6–8 mm long, when spread 4.5–6.5 mm wide between the outer margins of side lobes, yellow; base shortly unguiculate to almost sessile, 0.5–0.8 mm; lateral lobes curved forward, parallel, transversally elliptic, 5.5–7.0 × 1.5–2.5 mm, mostly glabrous, pubescent at base, apex obtuse; median part between lateral lobes with five prominent parallel veins that
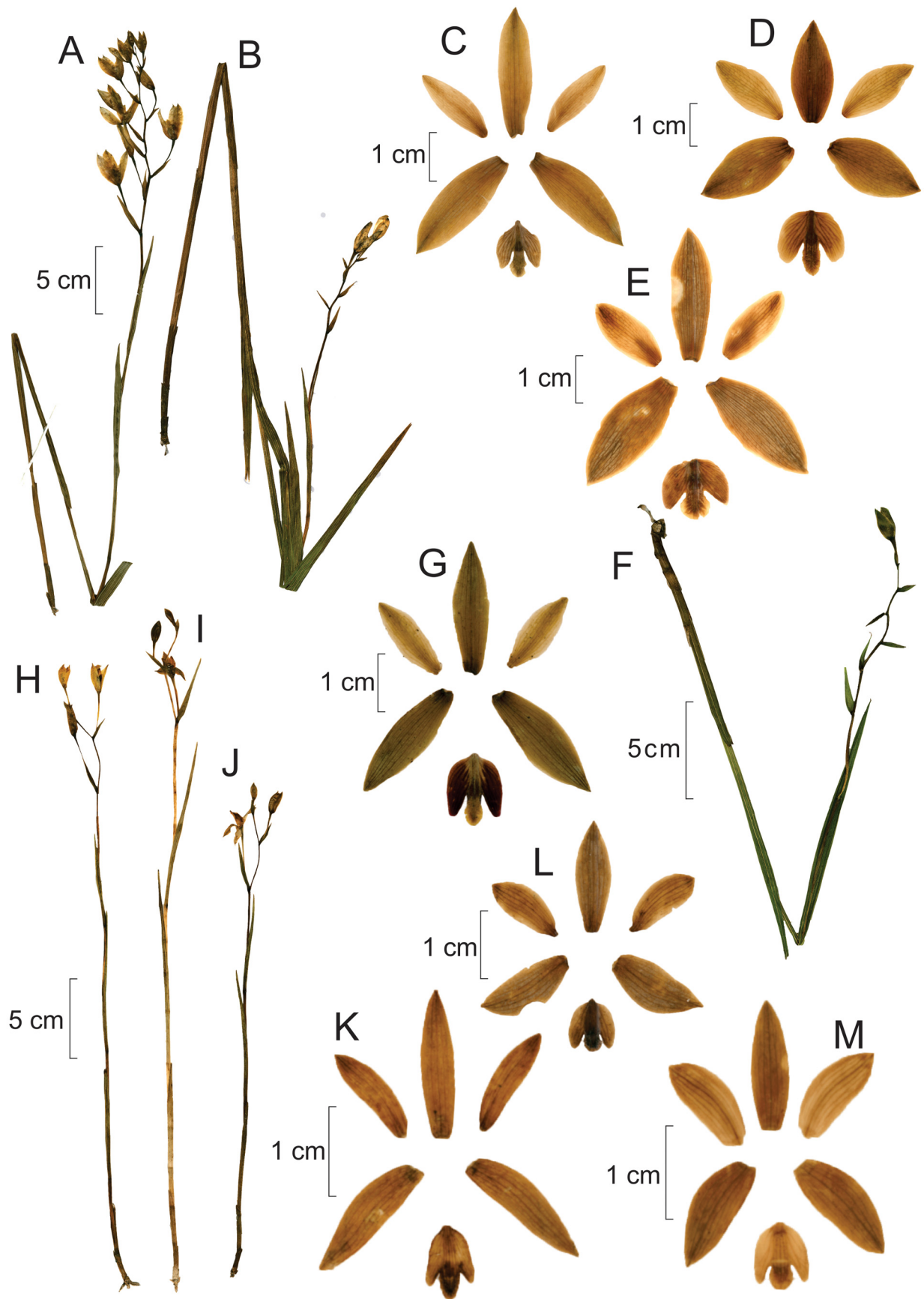
FIGURE 6. *Cyanaeorchis arundinae.* **A–B.** Habit. **C–E.** perianth. *Cyanaeorchis minor.* **F.** Habit. **G.** Perianth. *Cyanaeorchis praetermissa.* **H–J.** Habit. **K–M.** Perianth. A. *Mota & Giacomin 2337* (BHCB). B–C. *Batista et al. 2769* (BHCB). D. *Batista s.n.* (CEN). E. *Klein 128* (BHCB). F–G. *Klein 124* (BHCB). H. *Batista et al. 2218* (BHCB). I. *Salles & Bianchetti s.n.* (CEN). J and M. *Batista et al. 3041* (BHCB). K. *Batista 130* (CEN). L. *Batista & Carvalho 2342* (BHCB).

extend onto the midlobe from the base to about the middle of the median part with numerous minute papillae, immediately before the midlobe with a flat, short, glabrous, longitudinally slightly grooved callus; midlobe 2.0–3.0 × 2.0–2.5 mm, base attenuate, apex orbicular to suborbicular, rounded, curved downwards, verrucose, verrucae concentrated at base or middle of midlobe, margins crenulate. Gynostemium erect, half-terete, slightly arcuate, 5.7–7.2 × 1.3–1.5 mm, foot absent, base reddish with small, rounded, lateral wings on each side, greenish or yellowish toward apex; anther terminal, incumbent, deciduous, membranous, ovate, transversally depressed, ca. 1.3 × 1.5 mm, yellowish; pollinia four, waxy, yellow, in two pairs, each pair with one larger, ca. 0.85 × 0.70 mm, flat, roundish, concave, forming a groove that fits the smaller pollinia, the other smaller, 0.70 × 0.60 mm; viscidium not seen; stigma ventral, entire, oblong, yellowish, slightly convex. Fruit fusiform, at one month old 2.5 × 0.6 cm.

Distribution and Conservation Status:—Restricted to the cerrado biome in central and southeastern Brazil in the states of Goiás and Minas Gerais and the Federal District (Fig. 4A). The species is known from 13 collections at seven localities. Most collections and some of the larger known populations of the species are from the Federal District, where the species seem to be most threatened. The population at Setor Mansões Park Way has been destroyed, whereas large populations at Santuário Ecologico do Riacho Fundo and Reserva Ecológica do Guará have been severely depleted in the last 20 years due to alterations in habitat quality. Based on the World Conservation Union Red List Categories and Criteria (IUCN 2001), *C. praetermissa* can be tentatively classified as Vulnerable (VU) (criteria A2ac; B2ab (ii, iii, iv); C2a(i)).

Habitat and Ecology:—*Cyanaeorchis praetermissa* grows in permanently wet grassland, *campo limpo úmido*, according to the classification of Ribeiro & Walter (2008), over dark, water-saturated, hydromorphic soil, usually near the border of flooded gallery forests (Fig. 5A) or in *veredas* (grasslands with *buriti* palms), 1000–1400 meters above sea level. Vegetative and reproductive growth are associated with rainfall, and blooming occurs at the beginning of the rainy season, from October to early December. Blooming appears to be strictly related to brushfires that occur during the dry season. All collections of the species made by the authors (12 of the 13 known collections) were made in recently burned areas. The species is sympatric with, and flowers at about the same time as, other orchid species, such as *Veyretia simplex* (Grisebach 1864: 641) Szlachetko (1995: 116), *Habenaria edwallii* Cogniaux (1906 publ. 1907: 275), *H. balansae* Cogniaux (1906 publ. 1907: 271), *Gomesa hydrophila* (Barbosa Rodrigues 1877: 92) Chase & Williams (2009: 397), *G. barbaceniae* (Lindley 1855: 32) Chase & Williams (2009: 395), *Epidendrum dendrobioides* Thunberg (1818: 17), *Cleistes castaneoides* Hoehne (1939: 42) and *Cyrtopodium fowliei* Menezes (1995: 17), all also typically found in habitats with water-saturated soil. Populations of *Cyanaeorchis praetermissa* usually form compact groups with large number of specimens, whereas *C. arundinae* and *C. minor* are usually found as scattered individuals or a few plants.

Etymology:—From Latin, meaning overlooked or neglected, in reference to the new species having been overlooked by previous taxonomists.

Illustrations:—Pabst & Dungs (1975, pg. 381, fig. 1645, based on *Heringer 9202*, HB), Menezes (1992, pg.18–19, colour photographs; 2004, pg. 120, colour photograph); all identified as *C. minor*.

Additional specimens examined:—BRAZIL. Distrito Federal: Guará, Reserva Ecológica do Guará, campo úmido, 20 October 1990 (fl), *Batista 130* (CEN!); Núcleo Bandeirante, Santuário Ecológico do Riacho Fundo, campo limpo permanentemente úmido, solo escuro, hidromórfico, 18 October 1996 (fl), *Batista 630* (CEN!); Guará, Reserva Ecológica do Guará, campo limpo permanentemente úmido a borda de mata ciliar inundada, 29 October 1997 (fl), *Batista 745* (CEN!); Reserva Ecológica do Guará, 30 November 1997 (fr), *Batista 755* (CEN!); Brasília, Setor Mansões Park Way, Campo as margens do Córrego do Cedro, na altura em que este é cortado pela pista, um pouco antes do conjunto 1 da Quadra 19, campo limpo inundado (permanentemente úmido), solo escuro, hidromórfico, com água sobre a superfície, 30 October 1999 (fl), *Batista et al. 952* (CEN!); Guará, Reserva Ecológica do Guará, campo limpo permanentemente úmido na borda de mata ciliar inundada, 10 December 2002 (fl), *Batista et al. 1318* (CEN!); Núcleo Bandeirante, Santuário Ecológico do Riacho Fundo, em mancha de campo limpo inundável (permanentemente úmido), 12 November 2011 (fl), *Batista & Bianchetti 3175* (BHCB!); Brasília, Parque Zoobotânico, brejo inundável, 30 October 1963 (fl), *Heringer 9202* (HB!, K!, M!, NY!); Núcleo Bandeirante, Santuário Ecológico do Riacho Fundo, campo limpo úmido, 3 October 1990 (fl), *Salles & Bianchetti s.n.* (CEN 26597!). Goiás: Cavalcante, cerca de 64 km da cidade, na estrada de terra em direção a Minaçu, Cachoeiras do Rio Prata, campo limpo permanentemente úmido, solo escuro, hidromórfico, com água sobre a superfície, 27 November 2004 (fl), *Batista et al. 1536* (BHCB!, CEN!). Minas Gerais: Gouveia, BR 259 cerca de 7 km NE de Gouveia em direção a Diamantina, campo limpo estacionalmente úmido, na borda de mata de galeria,

solo escuro, hidromórfico, 13 November 2007 (fl), *Batista & Martins 2218* (BHCB!); São Roque de Minas, Parque Nacional da Serra da Canastra, 37 km a partir da portaria principal de São Roque de Minas, em direção a Sacramento, campo limpo úmido, 12 December 2007 (fl), *Batista & Carvalho 2342* (BHCB!).

Notes:—As far as we could determine, *C. praetermissa* was first collected by Heringer in Brasília in 1963. Material of this collection was examined by Pabst, who misidentified it as *C. minor* and published a sketch of a flower from this collection in his seminal work of Brazilian orchids, *Orchidaceae Brasilienses* (Pabst & Dungs 1975). This mistake was followed by all subsequent authors who examined and identified material of the species (Menezes 1992, 2004, Batista & Bianchetti 2003, Batista *et al.* 2005) or compiled data on the distribution of the genus (Barros *et al.* 2013, Govaerts *et al.* 2013).

Cyanaeorchis praetermissa is the smallest of the three species in the genus (Fig. 6). In terms of size, number and size of leaves and number of flowers, *C. praetermissa* overlaps *C. minor* and small individuals of *C. arundinae*. In size of sepals and petals, *C. praetermissa* overlaps *C. minor*. The main distinctive characteristics are size of the labellum and papillae. In *C. praetermissa*, the labellum is shorter and straighter (6.0–8.0 × 4.5–6.5 mm, versus 8.0–12.0× 9.0 mm in *C. minor* and 9.0–15.0 × 9.0–12.0 mm in *C. arundinae*), and papillae are shorter. Another difference in *C. minor* is floral colour. *Cyanaeorchis praetermissa* has completely yellow flowers, whereas *C. minor* has whitish sepals and petals and wine-coloured lateral lobes of the labellum. Other differences between the three species are outlined in Table 3.

Acknowledgments

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Additional specimens examined:—*Cyanaeorchis arundinae*: *Amaral s.n.* (HUFU 18370); *Amaral 1224* (SP); *Barroso et al. 509-68* (IPA, UB); *Batista s.n.* (BHCB), *312* (CEN), *Batista et al. 2769* (BHCB); *Boechat s.n.* (ICN 44273); *Brade 6855A* (RB), *7575* (HB); *Carvalho et al. 40* (RB); *Duarte 2291* (RB), *4303* (HB, RB), *13911* (HB, HBG, M, NY); *Dusen 7500* (HBG, K); *Dutra 1015* (ICN), *1098* (ICN); *Gaudichaud 175a* (P), *176* (P); *Glaziou s.n.* (P 436668); *Harley et al. 24924* (K, SPF); *Hassler 5323* (G), *9677* (G, NY, W), *11406* (G); *Hatschbach 22888* (F, K), *23285* (NY), *25107* (NY), *35541* (M), *43437* (NY, UB); *Irwin et al. 8597* (HB, NY), *9007* (HB, NY, UB); *Kirsten s.n.* (CEN 46501, SP 28749); *J. Klein 126* (BHCB); *R.M. Klein 3344* (HB), *3505* (HB), *3562* (HB); *Leinig s.n.* (HB 41038); *Leitão et al. 1350* (UEC); *Lutz s.n.* (HB 10733); *Markgraf & Borges 10337* (HB); *Mota & Giacomin 2337* (BHCB); *Oliveira 435* (HUFU); *Pedersen 5230* (K); *Regnell ser. II 264* (S); *Reitz & Klein 14195* (HB, RB); *Ribas et al. 5769* (RB); *Roth s.n.* (ICN 68316); *Sello 462-1341* (K); *Smith & Klein 13627* (HB), *13669* (HB), *13971* (HB, NY, P); *Sobral 9460* (RB); *Stutzel s.n.* (ICN 51518); *Ule 7029* (HBG); *Waechter 2000* (ICN); *Weddell s.n.* (P 436670). *Cyanaeorchis minor*: *Dusen 15681* (S), *15708* (S), *15840* (AMES, NY); *Hassler 9677a* (G); *Hatschbach 32806* (HBG, M); *Hoehne 661* (M, NY); *Jonsson 1307a* (AMES, F); *Klein 124* (BHCB); *Paula-Souza et al. 8301* (SPF); *Souza et al. 7230* (ESA, SP).