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# Molecular phylogeny of the Neotropical sections of *Bulbophyllum* (Orchidaceae) using nuclear and plastid spacers

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**Abstract** The systematic utility of sequences from two non-coding regions of plastid DNA, *psbA-trnH* and *trnS-trnG*, and one nuclear region, nrITS, was examined in an assessment of phylogenetic relationships among Neotropical sections of *Bulbophyllum* Thouars (Orchidaceae, Epidendroideae, Dendrobieae). The nrITS region was 6 to 7 times more variable than the two cpDNA regions. No major incongruencies between the nuclear and cpDNA datasets were detected. The combined analysis revealed a well-resolved phylogeny at sectional level based on both maximum parsimony and Bayesian approaches. Six sections of *Bulbophyllum* are recognized for the Neotropics. Five of these were pre-existent but needed to be recircumscribed and one is proposed as new. We did not find disagreements between maximum parsimony and Bayesian analyses, but the latter showed better resolved relationships between the sections, which are supported by morphological features. Of the two main clades of Neotropical *Bulbophyllum* species, one has two lineages and occurs predominantly north of the Equator. The second contains four lineages which are highly diverse in southeastern Brazil. Our results suggest a single colonization event in the Neotropics, from Africa to northern South America, followed by dispersion through the Andes to southeastern Brazil.

**Keywords** biogeography; *Bulbophyllum*; Neotropics; Orchidaceae; phylogeny; taxonomy

**Supplementary Material** Figures S1–S3 (in the Electronic Supplement) and the alignment are available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

## ■ INTRODUCTION

Numerous papers have been published in the last decade examining the phylogeny of Orchidaceae using molecular data (e.g., Cameron & al., 1999, utilizing *rbcL*; Cameron & Chase, 2000, 18S; Freudenstein & Chase, 2001, intron *nad1b-c*; Angiosperm Phylogeny Group, 2003, *rbcL* and *matK* among others; Cameron, 2004, *psaB*; Freudenstein & al., 2004, *rbcL* and *matK* among others). The variation found in these works permitted conclusions concerning delimitations and relationships between subfamilies, but contributed little to elucidating the relationships between tribes and subtribes established previously based on morphological data (Dressler, 1993) because of the low levels of support received in the analyses. Van den Berg & al. (2005), for example, analyzed five molecular regions (four plastid and one nuclear) and obtained high bootstrap support (i.e., above 85%) only for 8 of 17 clades in the subfamily Epidendroideae. Bayesian analyses generated similar clades performed for the same taxa, although with a more consistent resolution (i.e., a larger number of clades with more than 95 PP). This was explained in part by the fact that Bayesian analyses are more robust in identifying clades with few substitutions (Alfaro & al., 2003).

*Bulbophyllum* Thouars is probably the largest Pantropical genus of Orchidaceae, with ca. 1500 species, although its distribution is not homogeneous over its entire range. The Paleotropical region is the richest in species of this genus, with hundreds occurring in Asia, followed by Africa and then the Neotropics (Vermeulen, 1991; Dressler, 1993; Sieder & al., 2007). The genus *Bulbophyllum* was originally described by Thouars in 1822, and the first Neotropical species was described in 1838 by John Lindley (*B. setigerum* Lindl.) from a plant collected in the Guyanas. The relationships among the higher Epidendroid taxa, including *Bulbophyllum* and its closest relatives (i.e., the subtribe Bulbophyllinae and the tribe Dendrobieae), are still unresolved due in part to the low levels of sampling of these taxa in previous analyses. Many works have simply not included these genera (e.g., Freudenstein & al., 2000, utilizing the mitochondrial intron *nad1b-c*), or have sampled only a single species of *Bulbophyllum* and *Dendrobium* Sw. in combined analyses of nuclear and plastid data (Van den Berg & al., 2005), not permitting the establishment of the monophyletic nature of these genera.

In spite of our lack of knowledge concerning the relationships between the genera close to *Bulbophyllum*, recent studies have shown that this genus belongs to subfamily Epidendroideae, tribe Dendrobieae, subtribe Bulbophyllinae Schltr. (Cameron & al., 1999; Chase & al., 2003; Van den Berg & al., 2005). The elucidation of the phylogenic relationships of the genera of tribe Dendrobieae and within Bulbophyllinae will still require more detailed study, as the few works published until recently have concentrated only on the genus *Dendrobium* and related genera (Yukawa & al., 1996; Clements, 2003).

Gravendeel & al. (2004) presented the first phylogenetic study based on plastid (*matK*) genome data in examining species of *Bulbophyllum* from all the continents where this genus occurs. Their work demonstrated the genus probably arose on the Asian continent, and the African and Neotropical species form two monophyletic groups sister to each other, and this entire group is sister to the Asian clade. This result is quite plausible, because the closely related genus *Dendrobium* has its distribution almost totally restricted to Asia, with a few representatives in Australasia. In this study Gravendeel & al. (2004) included ca. 150 species, eight of which Neotropical, selected to include the range of morphological variation in this region. Fischer & al. (2007) using nuclear ITS and four plastid regions in an expanded matrix (ca. 150 species, five Neotropical) found 95 percent of bootstrap support and 0.99 posterior probability for the monophyletic status of the Neotropical group, and for the sister relationship with the African group as well.

Approximately 60 species of *Bulbophyllum* are currently accepted in the Neotropics (Smidt, 2007). Historically, five sections have been proposed for the Neotropical species based on floral characters of species occurring in Brazil only (ca. 80% of the Neotropical species; Cogniaux, 1902, updated by Pabst & Dungs, 1975). In the latter work, the authors maintained the sections recognized by Cogniaux (1902), and created numerous informal "alliances" to accommodate the morphological heterogeneity observed among species. In these works, systematics was based on characters of the inflorescence and flowers, orientation and fleshiness of the rachis, correlation between the width and the length of the sepals, the degree of fusion between the lateral sepals, and the number of ventral and apical appendices of the column (referred to here as "teeth" [ventral appendices], not related to the structure the pollinia are attached to) and the "stelidia", here considered as projections of the staminodes (regions originating from the filaments; Dressler, 1993).

Recently, Azevedo & al. (2007) used allozymes to study the genetic structure of seven species of *Bulbophyllum* occurring in southeastern Brazil. This study revealed that the intrapopulational genetic variation of the species examined was relatively high, as also reported for some species of *Acianthera* Scheidw. (Borba & al., 2001), which belong to the other large group of myophilous Orchidaceae in the Neotropics, Pleurothallidinae. This study also included a phenetic analysis among the species, based on 14 allozymic loci. As a result, some species which were very similar vegetatively but had been placed in different sections based on their floral morphology, were grouped together, while other apparently closely related species were not observed to form distinct clusters, indicating a possible introgression or a close phylogenetic proximity (Azevedo & al., 2007). The incongruities between allozyme data and the systematics of this group based on morphological characteristics indicated the need for phylogenetic work to clarify the relationships between the species.

In the present study, phylogenetic analyses were undertaken to evaluate (1) the relationships between the Neotropical species of *Bulbophyllum* and (2) to test previous infrageneric delimitations among New World species based upon morphology. To do this, we collected DNA sequence data from one nuclear ribosomal spacer plus 5.8S coding region (nrITS), and two plastid spacers (*psbA-trnH*, *trnS-trnG*). The choice of these regions was based on nrITS studies in Orchidaceae (e.g., Ryan & al., 2000; Van den Berg & al., 2000; Williams & al., 2001a, b; Koehler & al., 2002) and Shaw & al. (2005) for plastid spacers.

## ■ MATERIALS AND METHODS

**Taxon sampling. —** We sampled 50 accessions, of which 42 belong to Neotropical species of *Bulbophyllum*, including representatives from all sections and alliances previously published, and nine were used as outgroups (Appendix 1). We included three outgroups from subtribe Dendrobiinae Lindl. (*Dendrobium kingianum* Bidwill ex Lindl., *D. crumenatum* Sw. and *Epigeneium tricallosum* (Ames & C. Schweinf.) J.J. Wood), and six of subtribe Bulbophyllinae with *Bulbophyllum* species from the Paleotropics, including *B. nutans* Thouars (the type of the generic name), *B. newportii* (F.M. Bailey) Rolfe from Australia, *B. lobbii* Lindl., and *B. clandestinum* Lindl. from Asia, and the African species *B. falcatum* Rchb. f. In all analyses *D. kingianum* was defined as outgroup. Complete sampling of all species for DNA regions was not possible due to difficulties in amplification and sequencing, and for four species of the ingroup and one of the outgroup two accessions available were included in the analysis.

The sampling represents 70% of the species currently accepted for the Neotropics (Smidt, 2007) and comprises essentially all morphological diversity found in this region and almost all species not previously included in any group. Samples were obtained from plants in living collections at São Paulo and Rio de Janeiro Botanical Garden, Feira de Santana State University, Embrapa/CENARGEN (Distrito Federal, Brazil), Museu Paraense Emílio Goeldi (Amazon, Brazil), and from collaborator researchers and field trips in Brazil. Vouchers of plant material used in this study are indicated in Appendix 1.

**DNA extraction, amplification, and sequencing. —** DNA was extracted from fresh leaf material using the 2× CTAB (cetyltrimethyl ammonium bromide) procedure of Doyle & Doyle (1987). PCR amplification of all fragments was performed in 25 µl reactions (1× buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 mM of each primer, 10 ng BSA, 1 unit of Taq DNA polymerase [Phoneutria Biotec. Ltda, Belo Horizonte, Brazil]). For the nrITS mix, we added Betaine at 1 M, 0.5% of BSA and 2% DMSO.

The nrITS region was amplified and sequenced with the primers 92 (5′ AAG GTT TCC GTA GGT GAA C 3′) and 75 (5′ TAT GCT TAA ACT CAG CGG G 3′) (Desfeaux & al., 1996), or the primers 17SE and 26SE (Sun & al., 1994). For the primers of Desfeaux & al. (1996), the amplification was conducted with the following PCR program: an initial 1 min premelt at 94°C and 40 cycles of 30 s denaturation at 94°C, 40 s annealing at 49°C–55°C, and 40 s extension at 72°C followed by a final extension at 72°C for 5 min. With the Sun & al. (1994) primers, the same PCR program was utilized, but with 28–30 cycles.

The cpDNA *trnS-trnG* spacer was amplified with the primers trnS$^{GCU}$ (5′ AGA TAG GGA TTC GAA CCC TCG 3′) and trnG$^{UUC}$ (5′ GTA GCG GGA ATC GAA CCC GCA TC 3′) (Shaw & al., 2005). Amplification was carried out with an initial 1 min premelt at 94°C and 30 cycles of 30 s denaturation at 94°C, 40 s annealing at 52°C–55°C, 40 s extension at 72°C followed by a final extension for 5 min at 72°C.

The cpDNA *psbA-trnH* spacer was amplified with the primers trnH$^{GUG}$ (5′ CGC GCA TGG TGG ATT CAC AAT CC 3′) (Tate & Simpson, 2003) and psbA (5′ GTT ATG CAT GAA CGT AAT GCT C 3′) (Sang & al., 1997). Amplification was carried out with an initial 3 min premelt at 95°C and 35 cycles of 30 s denaturation at 95°C, 1 min annealing at 52°C–55°C, 1 min 30 s extension at 72°C followed by a final extension for 4 min at 72°C.

All amplifications were performed in a Applied Biosystems GeneAmp 9700 thermocycler. PCR products were purified using enzymatic reaction with Exonuclease I and Shrimp Alkaline Phosphatase enzymes (GE Healthcare, Cleveland, Ohio, U.S.A.). The sequencing reaction was carried out with the kit Big Dye Terminator version 3.1 (Applied Biosystems, Foster City, California, U.S.A.). The same primers were utilized by amplification and sequencing reaction. The sequencing was realized in both directions in an automatic sequencer SpectruMedix SCE2410, following the manufacturer's protocols. Some PCR products were sequenced at Macrogen Inc., Korea.

**DNA sequencing alignment. —** The sequences were superimposed and edited with the Staden Package software (Staden & al., 2003). Multiple sequence alignments were performed using ClustalW (Thompson & al., 1994) with default settings, and visually inspected and manually adjusted using PAUP v.4.0b10a (Swofford, 2002). The indels (insertion/deletion markers) were treated as missing data. Sequences are deposited in GenBank (Appendix 1); the aligned matrix is available as supplementary data.

**Phylogenetic analysis. —** Maximum parsimony (MP) analyses were performed with Fitch (1971) parsimony using the software PAUP v.4.0b10a (Swofford, 2002). Bayesian analyses (BA) were performed using MRBAYES v.3.1 (Ronquist & Huelsenbeck, 2003).

In the MP analyses a heuristic search was performed with 2000 replicates of random taxon-addition, holding 20 trees per replication, TBR algorithm, followed by a second search to explore all topologies from the previous search, limited to 10,000 trees. The support was estimated by 2000 bootstrap replications (Felsenstein, 1985), simple addition, TBR algorithm, holding 20 trees per replication and by Decay indices (Bremer Support; Bremer, 1988). ACCTRAN assumption was used for branch length optimizations.

For BA, the model of nucleotide substitution was chosen based on hierarchical likelihood ratio tests (hLRTs) conducted with MrModeltest v.2.2 (Nylander, 2004). Bayesian analyses started from random trees and employed Markov chain Monte Carlo (MCMC) runs over two million generations, sampling trees every 100 generations. We discarded 25% of the initial generations as burn-in period, after visual inspection of the stabilization of the log-likelihood of the trees, as measured by the Stdev(s) and PSRF values (Gelman & Rubin, 1992). The remaining 15,000 trees were used to produce a 50% majority-rule consensus tree with the posterior probabilities of the clades, visualized with TREEVIEW (Page, 1996).

After the analyses of each DNA region separately, the congruence between the combined cpDNA spacers, and nrITS and the combined cpDNA datasets were tested using the incongruence length difference (ILD) test (Farris & al., 1995) as implemented by the partition homogeneity test in PAUP for 100 replicates (heuristic search, simple addition, TBR branching swapping), each saving a maximum of 1000 most parsimonious trees per replicate. Barker & Lutzoni (2002) and Zelwer & Daubin (2004) casted doubt on the usefulness of the ILD test for quantifying incongruence. We adopted this test nonetheless because we focused on a sectional classification only and not so much on determining the exact phylogenetic position of all species sampled. Both the combined cpDNA dataset (*psbA-trnH+trnS-trnG*—referred here as plastid) and the combined plastid+nrITS (referred here as combined) data were analyzed in the standard procedure for MP without weight attributions. For BA, the data were partitioned following the evolutionary model for each region and analyzed together using the mixed model of Ronquist & Huelsenbeck (2003).

## ■ RESULTS

As the ILD test (Farris & al., 1995) revealed no detectable incongruence between the two plastid spacers datasets, the results of these analyses were combined. The ILD test, however, strongly rejected the combined analysis of the plastid and nuclear data. Taxa with incongruent positions were detected through visual examination of the trees (*B. glutinosum* (Barb. Rodr.) Cogn., *B. mentosum* Barb. Rodr.). After the exclusion of these species from the analyses, the test accepted the congruency of all the datasets in a combined analysis, and so the tree of the combined analysis is presented without these two taxa. The possible causes of the incongruence are discussed below.

In all trees presented here clades are labeled as A to H, with A referring to subtribe Bulbophyllinae, B to the Neotropical species of *Bulbophyllum*, and C–H to the Neotropical clades (formally treated as sections at the end of the discussion). Details on DNA regions and the phylogenetic analyses are provided in Table 1.

**nrITS: Maximum parsimony analysis. —** The aligned matrix of nrITS sequences was 749 base pairs (bp) long, of which 384 characters were constant and 218 (29%) were parsimony-informative (non-autapomorphic). The analysis produced 10,000 equally parsimonious trees (according to the limitation

**Table 1.** Results of analyses of the different datasets.

| | nrITS | *trnS-trnG* | *psbA* | Plastid | Combined |
|---|---|---|---|---|---|
| Number of taxa | 50 | 41 | 43 | 46 | 48 |
| Number of characters | 749 | 901 | 1199 | 2100 | 2849 |
| Variable characters | 367 | 94 | 141 | 234 | 600 |
| Parsimony-informative characters | 218 | 36 | 51 | 81 | 298 |
| % informative characters | 29.1 | 3.9 | 4.25 | 3.8 | 10.45 |
| Tree length | 721 | 121 | 202 | 332 | 1053 |
| Number of MP trees retained | 10,000 | 16 | 10,000 | 10,000 | 10,000 |
| Consistency index (CI) | 0.664 | 0.876 | 0.806 | 0.804 | 0.707 |
| Retention index (RI) | 0.755 | 0.899 | 0.825 | 0.817 | 0.767 |
| Model suggested | GTR+G | HKY+G | F81+I+G | MIXED | MIXED |

established by the analysis), of 721 steps length, consistency index (CI) of 0.664 and retention index (RI) of 0.755. The strict consensus tree with the bootstrap percentages (BP) and Decay Index (DI) is presented in Fig. S1 (Electronic Supplement). In this analysis, the clade referring to Bulbophyllinae (A) received strong support (96 BP, 11 DI), the Neotropical species (B) moderate support (65 BP, 2 DI), while six monophyletic lineages could be recognized within the Neotropical group. Except for clade C, all other five lineages received moderate to strong support (71–100 BP, 2–8 DI). In this analysis, the relationships between the clades F, G, and H could not be identified as they collapsed in the strict consensus tree.

**nrITS: Bayesian analysis. —** MrModeltest v.2.2 (Nylander, 2004) selected the GTR+G evolutionary model (Fig. S2 in the Electronic Supplement) for the nrITS data. In this analysis, the Bulbophyllinae clade (A) and the Neotropical species (B) received high posterior probability support (1.00 PP), and six monophyletic lineages within the Neotropical clade could be identified. Four lineages received high support (1.00 PP) each, but the clades C and D received weak support with (0.80 and 0.91 PP, respectively; only PP values >95 were considered well-supported).

***psbA-trnH+trnS-trnG*: Maximum parsimony. —** The matrix resulting from the combined analysis of the plastid spacers contained 2100 base pairs, of which 1866 were constant and only 81 (3.8%) were parsimony-informative. The analysis produced 10,000 equally parsimonious trees (according to the limitations established by the analysis) with 332 steps, CI = 0.804, RI = 0.817. The strict consensus tree results in a complete polytomy (trees not presented here). In general, the resolution of the groups was low; using these markers, not even the monophyletic nature of the Neotropical species could be established by the analysis of bootstrap and DI support. Only three clades had bootstrap values >50% and DI 1–2 (E–G), and only two small groups of very similar looking species obtained high support (BP 100%, DI 6 and 10), corresponding to the F clade. In spite of generally low support, the clades were the same as obtained in the nrITS analysis. The only significant incongruence was the position of *B. mentosum*, which in this analysis appeared in the G clade with moderate support, but received strong support in the F clade of the nrITS analysis. Additionally, incongruence occurred in relation to the positioning of species of clades C and D by the nrITS data that did not have support and moved around in this analysis, appearing within other clades without support (e.g., *B. nagelii* L.O. Williams).

***psbA-trnH+trnS-trnG*: Bayesian analysis. —** In the combined analysis, the strategy of a mixed-model with partitioned data was employed, so that a partition was used for each spacer employing model parameters obtained separately for each (HKY+G model for *trnS-trnG* and F81+I+G model for *psbA-trnH*). The majority-rule consensus for this group of trees is presented in Fig. S3 (Electronic Supplement). The resolution of this tree was significantly better in relation to the analyses of the two spacers considered separately (trees not presented here) and in relation to MP analysis. In spite of the positioning of *B. glutinosum* together with the African species *B. falcatum*, the clade with the remaining Neotropical species had high support (0.99 PP). Among the remaining Neotropical species, the species of clades C and D were not supported as monophyletic, but the four other clades (E–H), with the exception of clade H, had high posterior probability support (i.e., above 0.95 PP). The relationships between these clades were also similar to those found for nrITS data, with clade E appearing separated from clades F and G, which together form a well-supported group with a 0.97 PP.

**Combining nuclear and plastid datasets. —** After the ILD test (Farris & al., 1995) rejected the possibility of a combined analysis with all taxa, analyses were performed excluding two species (*B. glutinosum*, *B. mentosum*) identified as the source of incongruence; the results are presented in Figs. 1 and 2. Analyses were also carried out including these two species (data not presented here) in an attempt to understand the reasons behind this incongruence.

**Combined dataset: Maximum parsimony analysis. —** The matrix resulting from the combined analysis of the plastid spacers and the nrITS contained 2849 base pairs, of which 2249 were constant and 298 (10.45%) were parsimony-informative. The analysis produced 10,000 trees (according the limitation established by the analysis) that were equally parsimonious with 1053 steps, CI = 0.707, and RI = 0.767. The strict consensus tree is presented in Fig. 1. Resolution of the strict consensus trees was relatively high: the clade referring to Bulbophyllinae (A) received strong support (97 BP, 18 DI) and the Neotropical species (B) moderate support (83 BP, 1 DI). Six clades are recognized in the Neotropical region (C–H), in spite of clade C lacking bootstrap support and clade D receiving moderate support (75 BP, DI 3). The other four clades had moderate to high bootstrap support (97–100 BP) and high DI (1–8).

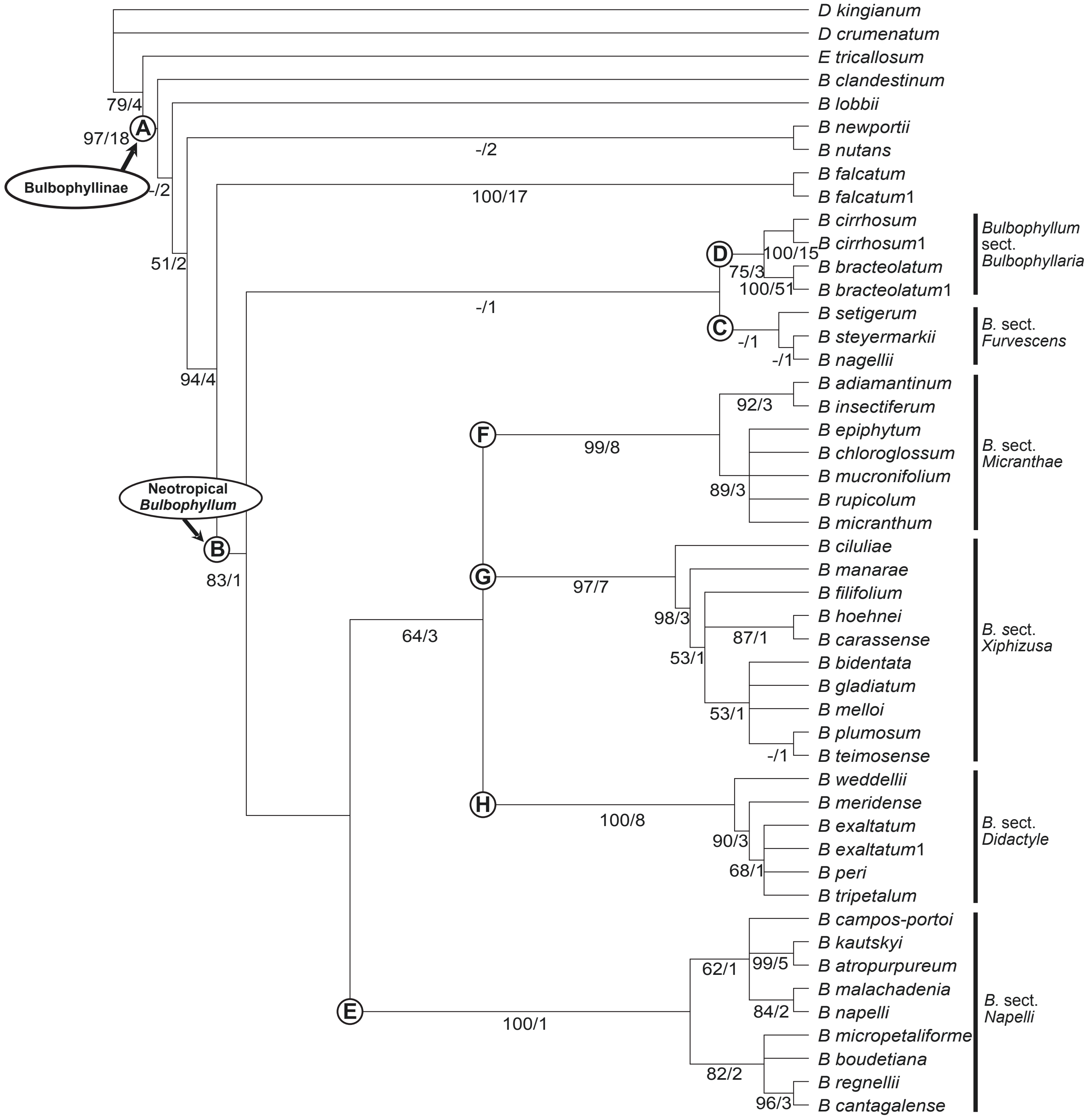

**Fig. 1.** Strict consensus of 10,000 most parsimonious trees obtained after analysis of the combined data (plastid *psbA-trnH+trnS-trnG* and nrITS). The bootstrap percentages above 50% and Decay index are presented below the branches (Length = 1053, CI = 0.707, RI = 0.767). **A,** Bulbophyllinae; **B,** Neotropical clade; **C,** sect. *Furvescens*; **D,** sect. *Bulbophyllaria*; **E,** sect. *Napelli;* **F,** sect. *Micranthae*; **G,** sect. *Xiphizusa*: **H,** sect. *Didactyle*.

**Combined dataset: Bayesian analysis. —** The majority-rule consensus resulting from Bayesian analysis is presented in Fig. 2. In this analysis, resolution of the trees based on individual plastid or nrITS markers was improved. The Bulbophyllinae clade (A) and the Neotropical species (B) received high posterior probability support (1.00 PP), and six monophyletic lineages within the Neotropical clade could be identified. Four lineages received high support (1.00 PP) each, but the clades C and D received weak support (0.51 and 0.92 PP, respectively). Clade support is provided in Table 2.

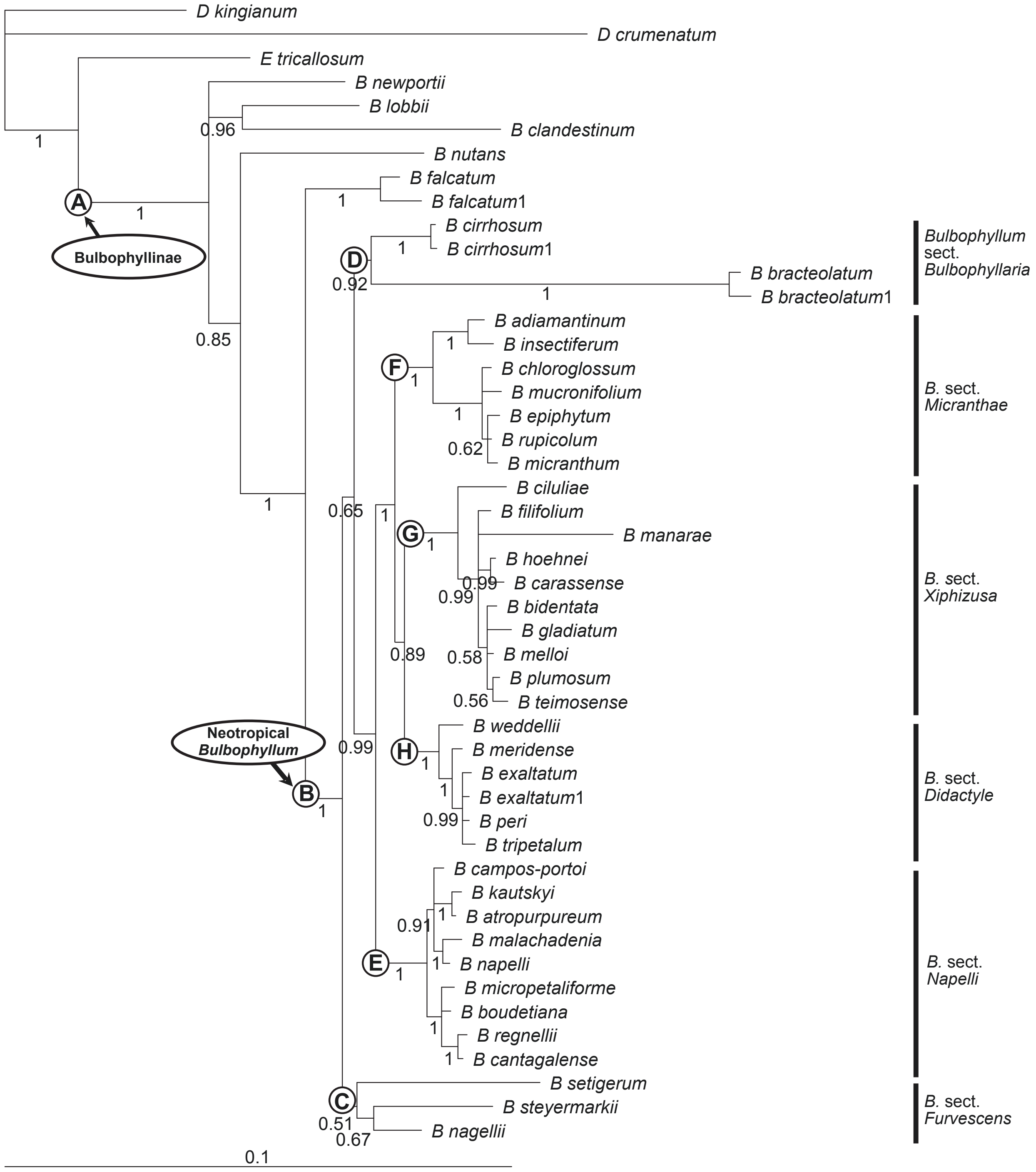

**Fig. 2.** Majority-rule consensus tree of the 15,000 trees from Bayesian analysis with mixed model, i.e., with individual model for each partition of the combined data (plastid *psbA-trnH+trnS-trnG* and nrITS). Posterior probability values are indicated below the nodes. For further explanation see Fig. 1.

**Table 2.** Results of support of the different clades in the combined analysis.

| | Bootstrap support | Deday index | Posterior probability |
|---|---|---|---|
| Bulbophyllinae (A) | 97 | 18 | 1.00 |
| Neotropical clade (B) | 83 | 1 | 1.00 |
| *B.* sect. *Furvescens* (C) | – | 1 | 0.51 |
| *B.* sect. *Bulbophyllaria* (D) | 75 | 3 | 0.92 |
| *B.* sect. *Napelli* (E) | 100 | 1 | 1.00 |
| *B.* sect. *Micranthae* (F) | 99 | 8 | 1.00 |
| *B.* sect. *Xiphizusa* (G) | 97 | 7 | 1.00 |
| *B.* sect. *Didactyle* (H) | 100 | 8 | 1.00 |

## ■ DISCUSSION

Our analysis with a broad sampling of New World species confirmed the monophyly of Neotropical *Bulbophyllum* previously established by Gravendeel & al. ( 2004) and Fischer & al. (2007). Bulbophyllinae received high support in all analyses except in the MP analysis of the plastid dataset. The African *B. falcatum* was recovered as sister to the Neotropical sections of *Bulbophyllum*, thus supporting colonization of the New World from Africa (Gravendeel & al., 2004). All analyses recovered six monophyletic lineages in the Neotropics with high support of posterior probabilities and moderate bootstrap support. Plastid analyses, however, collapsed due to the low differentiation among Neotropical species and in relation to the African *B. falcatum*. Exceptions were the poor resolution of two clades (C, D) in the MP analyses. The results of the Bayesian analyses demonstrated better performance in supporting these lineages in all analyses, mainly in the identification of relationships among sections. Although PP values of the Bayesian analyses cannot be directly compared with bootstrap values in MP, Alfaro & al. (2003) were able to demonstrate that PP are more powerful to detect clades, mainly when there are only a few characters supporting them. With a correctly chosen model, Erixon & al. (2003) found that Bayesian posterior probabilities are significantly higher than corresponding nonparametric bootstrap frequencies for true clades. According to these authors, erroneous conclusions would be made more often when the models used for analyses are underparameterized. When data are analyzed under the correct model, nonparametric bootstrapping is conservative, and Bayesian posterior probabilities as well, but less so with respect to the identification of the correct clades.

The plastid spacers utilized in this study did not show the monophyly of the Neotropical group, offering very little phylogenetic value. Therefore, the discussion of relationships among the Neotropical clades is based solely on the results of MP and BA of the combined data, which provided a much better resolution. Additional and faster evolving plastid markers are currently being retrieved from fully assembled chloroplast genomes of different species of *Bulbophyllum* using Next Generation sequencing (Jaros & al., in prep.)

The species belonging to clade C have never been placed in any of the sections proposed for the Neotropics. These species have a combination of morphological features in common that distinguish them from the other groups, such as fleshy flowers placed spirally on a fleshy rachis, and unifoliate pseudobulbs. The column of these species has a short foot in relation to column length and lacks defined teeth on the ventral portion. All these species have restricted distributions and occur mainly north of the Equator, with the exception of *B. setigerum* which occurs in the eastern portion of the Brazilian Amazon (Figs. 3A, 4A). The new section *B.* sect. *Furvescens* (described formally at the end of this paper) is proposed here for these species. The type species of the sectional name, *B. nagellii* L.O. Williams, occurs in Mexican oak forests and is the northernmost species of the genus in the Neotropics. In addition, *B. setigerum* and *B. steyermarkii* Foldats (both included in the analyses) belong to this new section, as well as *B. quadrisetum* Lindl., a species close to *B. setigerum* and displaying the characteristic morphological features of the section.

Clade D corresponds to *B.* sect. *Bulbophyllaria* (Rchb. f.) Griseb. The main diagnostic characters of this section are the presence of ovoid to pyriform bifoliate pseudobulbs, coriaceous leaves, and a flat fleshy rachis. Flowers are essentially sessile and arranged in a spiral, with free erect trinervate sepals, erect petals, an entire and fleshy labellum, and a column without stelidia or teeth and short column-foot. The species of this section are found from Mexico throughout Central America, the Amazon basin, and throughout the tropical Andes south to Bolivia (Figs. 3B, 4B).

The next four sections received high support in both MP and BA. Among these sections, the first group of species (clade E) was attributed by both Cogniaux (1902) and Pabst & Dungs (1975) to various sections, and has never been recognized as a distinct group. Reichenbach filius described *B.* sect. *Napelli* in 1861 as part of a group he called "Uniflora" which included species from various regions and was considered by other authors to belong to the genera *Sarcopodium* Lindl. and *Oxysepala* Wight, among others. *Bulbophyllum* sect. *Napelli* as defined by Reichenbach, and accepted by Cogniaux (1902) and Pabst & Dungs (1977), is monotypic, including only *B. napelli* Lindl., and recognized by conical pseudobulbs, oblong and obtuse leaves, a one-flowered inflorescence of the same length as the leaves, ovate concave and acute sepals, reduced petals, a linear obtuse and unguiculate labellum, and a column with teeth. However, according to our data, *B. napelli* Lindl. is included within a larger species group. The circumscription of sect. Napelli should therefore be extended to include species previously scattered among various sections, such as *B.* sect. *Bulbophyllaria–B. micropetaliforme* alliance, *B.* sect. *Didactyle–B. glutinosum* alliance, and *B.* sect. *Micrantha–B. micranthum* alliance (Pabst & Dungs, 1975). The main diagnostic characters of this section are unifoliate, pyriform pseudobulbs, coriaceous flat leaves, a thin rachis, and flowers placed opposite one another along the inflorescence (distichous). Flowers have free, erect sepals, with normally evident apices, and elongated, trinervate, erect and normally very reduced petals, an entire labellum with a smooth disk or lamella, and a column without teeth and long column-foot. The 12 species of this section are restricted to Brazil, with exception of *B. dunstervillei* Garay from Venezuela (Figs. 3C, 4C–E). This group is extremely consistent in its morphology and geographic distribution, with essentially all the species being restricted to the Atlantic rain forest of southeastern Brazil and the gallery forests of the Cerrado region of central Brazil.

Clade F contains species previously attributed to *B.* sect. *Micranthae* Barb. Rodr., first described in 1877. The type of the sectional name is *B. micranthum* Barb. Rodr. (Smidt, 2007). The main diagnostic features are unifoliate, pyriform to fusiform pseudobulbs, flat or aciculate fleshy leaves, rachis normally thin, flowers with pedicel, arranged spirally. The flowers have free erect sepals, normally uninervate, erect petals, a trilobed labellum with dentiform lateral lobes, no callus, a labellum disk with ridges or lamellae, and a column without teeth and a short column-foot. The 10 species in this section are predominantly Brazilian, with only one species occurring in Bolivia and a few occurring along the Brazilian border with Paraguay, mainly in the cerrado with *campo rupestre* (rocky field) formations (Figs. 3D, 4F–H). *Bulbophyllum mentosum* Barb. Rodr. is the sister species of the rest of the entire section, which is divided into two clades, one of them highly supported (formed by *B. adiamantinum* Brade and *B. insectiferum* Barb. Rodr.) and the other constituting the core of the section as a collapsed clade of very closely related species (Fig. S2). The core clade of this section has predominantly white flowers, yellow labellum, and sepals with a single vein, which is different from the rest of the Neotropical species which have sepals with three veins.

Clade G refers to the species placed by Lindley in the genus *Didactyle* Lindl., subsequently reduced to a section of *Bulbophyllum* by Cogniaux. The type of the sectional name, *B. exaltatum* Lindl., is from the Brazilian-Venezuelan border. Main diagnostic characters of this section are unifoliate, ovoid pseudobulbs, flat coriaceous leaves, a slender rachis, and flowers placed opposite each other in the inflorescences (distichous).

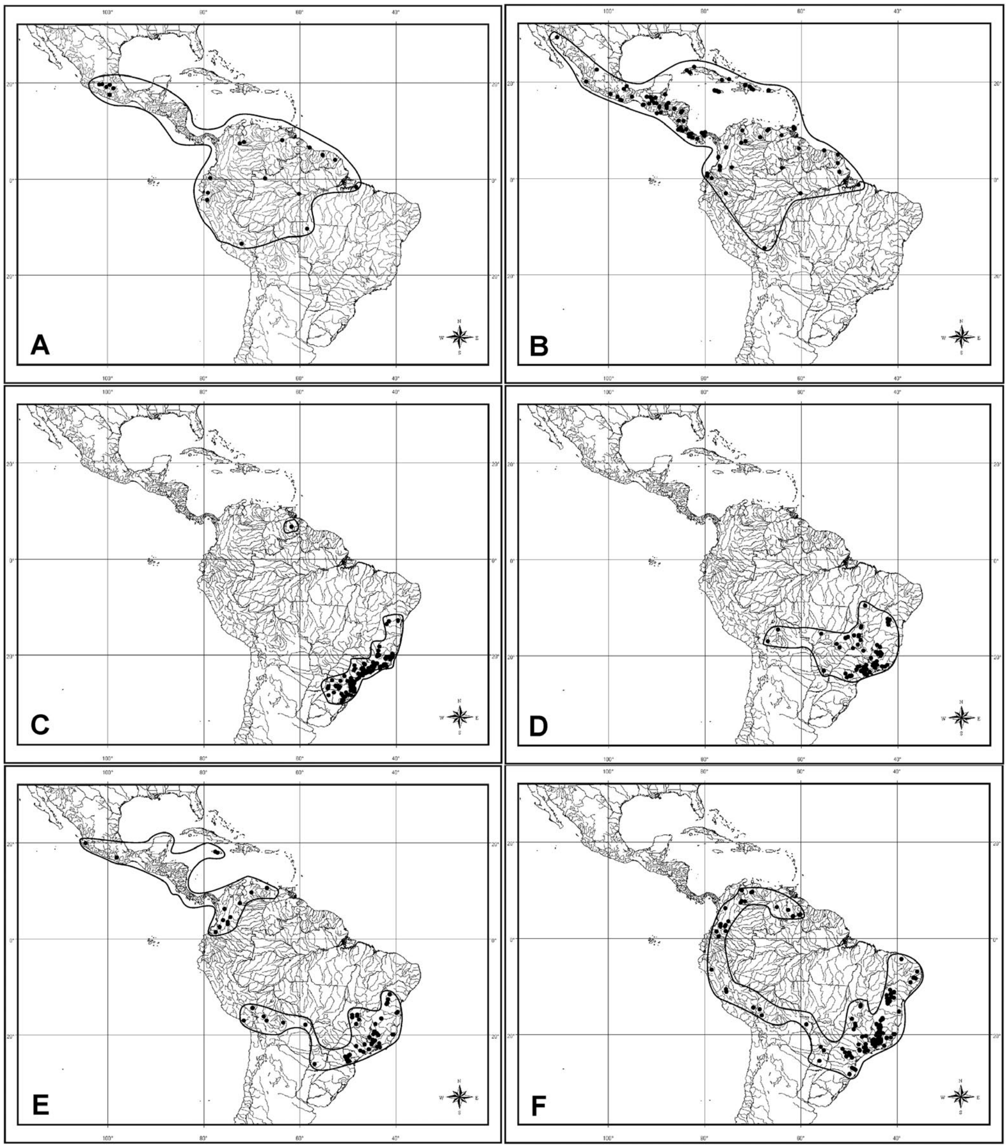

**Fig. 3.** General geographical distribution of Neotropical *Bulbophyllum* sections. Dots represent localities where herbarium specimens were collected. **A,** *Bulbophyllum* sect. *Furvescens*; **B,** *B.* sect. *Bulbophyllaria*; **C,** *B.* sect. *Napelli*; **D,** *B.* sect. *Micranthae*; **E,** *B.* sect. *Xiphizusa*; **F,** *B.* sect. *Didactyle*.

Flowers have free sepals, with the dorsal sepal being erect and the lateral sepals normally patent and trinervate, the petals patent, the labellum trilobed, with orbicular lateral lobes and a pronounced callus between the lobes, and a column with stelidia and teeth and short column-foot with bilobed end. The seven species and a natural hybrid among the species of this section are found throughout South America, although the greatest diversity is in the mountains in the interior of Brazil between the states of São Paulo and Bahia (Figs. 3F, 4J–L). This section presents the greatest problems in specific delimitation, with species demonstrating highly variable morphologies, having resulted in the description of many geographically restricted "species" (Ribeiro & al., 2007; Smidt, 2007).

Clade H refers to species in *B.* sect. *Xiphizusa*, described by Reichenbach filius as the genus *Xiphizusa* Rchb. f. and subsequently reduced to a section of *Bulbophyllum* by Cogniaux. The type of the sectional name is *B. chloropterum* Rchb. f., described in 1849 for Brazil. The main diagnostic characters of this section are unifoliate, deltoidic pseudobulbs, coriaceous flat leaves, and a thin rachis with distichous flowers. The sepals are erect, trinervate and normally fused, forming a synsepal, the petals are erect, the labellum is trilobed, the lateral lobes are erect, with a pronounced callus between the lobes, the column has stelidia and teeth and a short foot. The 23 species of this section are distributed from Mexico to the southern Brazilian state of Paraná, with a majority of the species being micro-endemic; the greatest species diversity is found in the mountains in the interior of Brazil between the states of São Paulo and Bahia (Figs. 3E, 4I).

**Morphological aspects related to the evolution of the Neotropical sections of *Bulbophyllum*. —** In spite of the monophyly of Neotropical *Bulbophyllum* established by Gravendeel & al. (2004), and confirmed in the present work, morphological characters supporting this monophyly are lacking. The only morphological feature indicating relationship with the taxa from other parts of the world is the presence of bifoliate pseudobulbs, found in *B.* sect. *Bulbophyllaria* and in some African and Asian species. However, this section is restricted to the Neotropics and not related to taxa from other continents. According to our data and those of Gravendeel & al. (2004) and Fischer & al. (2007), this character may thus be homoplasic. The relationship of clades C and D in relation to the remaining group requires additional study, both in terms of increasing the number of taxa examined as well as the number of regions sequenced, in order to establish the ancestry of the sections on the basis of the Neotropical clade.

Traditionally, the taxonomy of Neotropical *Bulbophyllum* was based on vegetative and floral features, such as the presence of one or two leaves per pseudobulb, the consistency of the rachis, the length of the pedicels, fusion of the lateral sepals, presence of lateral lobes on the labellum, and the presence of stelidia and teeth on the column. Our study underlines the value of these characteristics in delimiting the phylogenetically recovered sections. The majority of the incongruences with traditionally established groups appear to be due to erroneous interpretations of these characters. The main exception to this statement is *B.* sect. *Bulbophyllaria*, which is based on the plesiomorphic presence of bifoliate pseudobulbs, a fleshy rachis, and spirally arranged flowers—although in the Neotropical clade these characteristics are synapomorphies for this section. Other characteristics, such as the appearance of a trilobed labellum probably occurred only once, which is a synapomorphy for the clade which groups clades F, G, and H. In the same way, distichous and non-spiraled inflorescence probably also evolved only once, being shared by the clades E, G, and H, with a reversion in clade F to a spiraled form. However, in this case the rachis is thin and not fleshy as in clades C and D.

The presence of stelidia and teeth on the ventral portion of the column is common only in clades G and H, with all of the species demonstrating these features. Fused sepals occur only in clade G, fleshy leaves are only observed in clade F, and patent petals are mainly seen in clade H. The traditional morphology-based and the molecular-based classifications for the species used in this work are presented in Appendix 2. As Azevedo & al. (2007) noted, some species that presented high similarity revealed by isozymes were historically grouped in different sections or alliances (Cogniaux, 1902; Pabst & Dungs, 1975) despite their similar vegetative features. This result agrees with our results suggesting that, in this genus, vegetative characters may be more informative to identify the genetic relatedness between species than floral characters, as observed in other orchid groups (Van den Berg & al., 2000; Cameron, 2005; Chase & al., 2009).

**Incongruence between molecular phylogeny and morphological taxonomic units. —** The positions of some species demonstrate that morphological modifications between one clade to another occurred by gradual transitions in the characters that define them, creating difficulty in interpreting the positions of some sister species in relation to main clades, such as *B. ciluliae* Bianch. & J.A.N. Bat., *B. weddellii* (Lindl.) Rchb. f., and *B. mentosum* Barb. Rodr. *Bulbophyllum ciluliae*, described from central Brazil occurring throughout central Bahia (Smidt, 2007), was highly supported in all analyses as sister to remaining species in clade G (sect. *Xiphizusa*). This species has vegetative characters typical of this section, such as unifoliate and deltoidic pseudobulbs, and coriaceous flat leaves. However, its flowers possess features of sect. *Micranthae*, having a trilobed labellum with erect and dentiform lobes which are not erect and obtuse as it is typical for sect. *Xiphyzusa*, and patent petals characteristic of sect. *Didactyle*. A clear gradation of floral characteristics from "early diverging" to "recently derived" species occurs in sect. *Xiphyzusa*. The lateral sepals of the early diverged species of the section are initially free but always connate and forming a synsepal in the more derived species, and the labellum is initially fleshy and little differentiated into hypochile and epichile, but clearly differentiated with a membranous epichile in the more recently derived species, representing two important diagnostic morphological characteristics of this group.

A labellum divided into hypo- and epichile occurs in clade G+H, being a synapomorphy for this group. However, *B. wedellii*, which has a divided labellum with a membranaceous epichile, is sister to all species of clade H (sect. *Didactyle*) which have a fleshy and very short epichile. This clade also has many other

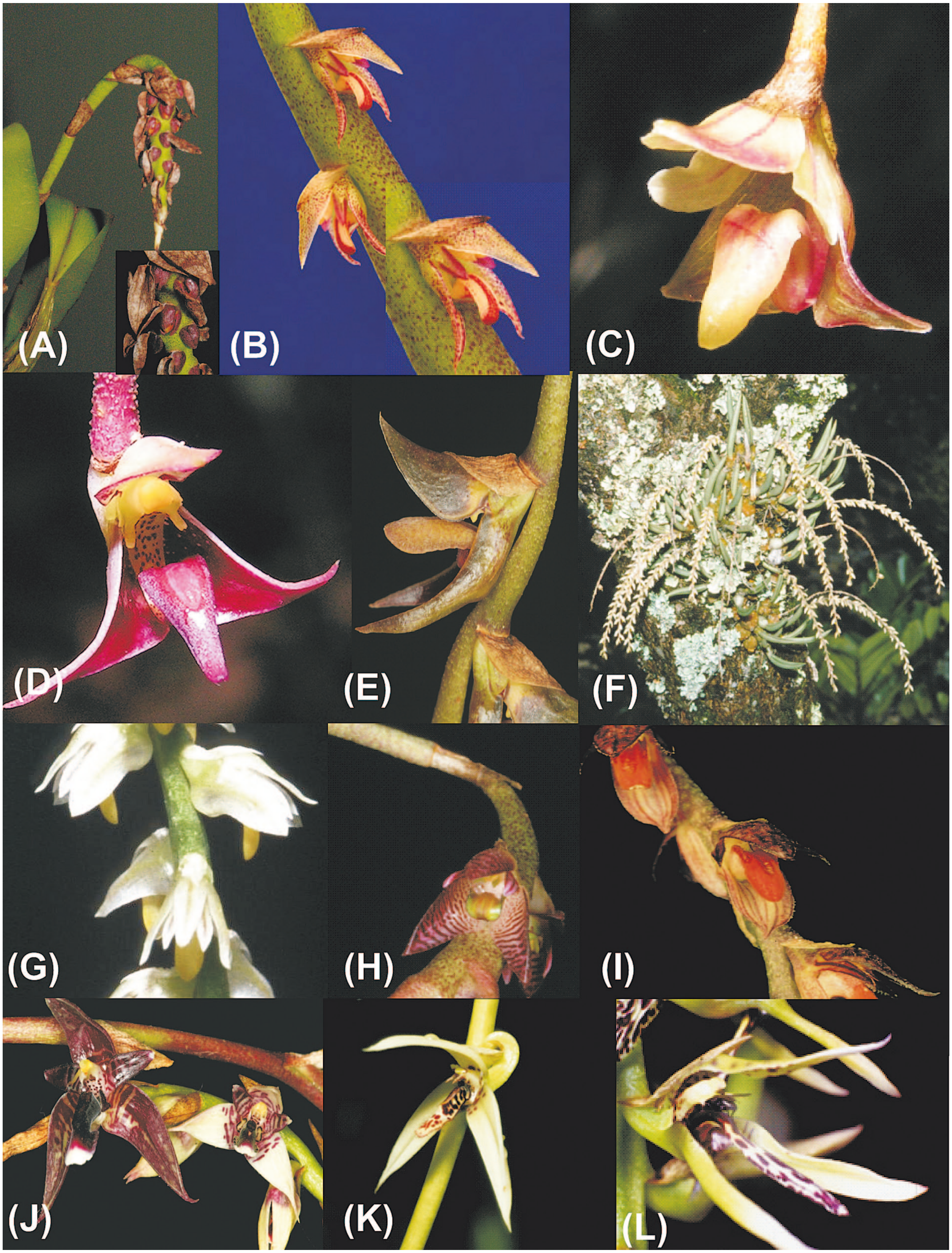

**Fig. 4.** Flower diversity in the Neotropical *Bulbophyllum* clade. **A,** *Bulbophyllum steyermarkii*; **B,** *B. bracteolatum*; **C,** *B. napelli*; **D,** *B. kautskyi*; **E,** *B. atropurpureum*; **F–G,** *B. epiphytum*; **H,** *B. mentosum*; **I,** *B. manarae*; **J,** *B. involutum* and *B. exaltatum*; **K,** *B. tripetalum*; **L,** *B. weddellii*.

synapomorphic characters, such as patent petals and widely spaced 4-angled pseudobulbs, while sect. *Xiphizusa* has discoid aggregated pseudobulbs, and erect petals. However, the characters that clearly define these two groups are not observed in the early diverging species (*B. ciluliae*, *B. weddellii*), which possess flowers arranged spirally, typical of sect. *Micranthae*.

One of the most interesting features revealed by these analyses is related to the position of *B. mentosum*. This species has some unique characters, such as a ribbon-like scape, that is cylindrical in all the other species. Vegetatively, *B. mentosum* is similar to the species of sect. *Didactyle*, but presenting fleshy leaves, typical for sect. *Micranthae*. Its flowers are arranged in spirals with a very short pedicel, and have an entire labellum without lateral lobes, with only the margin erect, flattened and ciliated, as in sect. *Furvescens* (clade C). In the plastid analyses, *B. mentosum* is placed in clade G with 60 PP, 68 PB and DI 1, however in the nrITS analysis it appears in clade F with 100 PP, 88 PB and DI 3. In the combined analysis it appears at the base of the sect. *Micranthae* with 95 PP (not shown, because de IDL test rejected its inclusion in the combined analysis), but this is probably due to the high difference in the number of informative sites between the plastid and nrITS data.

A mosaic of plesiomorphic characters seems to be part of a pattern that occurs in the evolution of several groups within Orchidaceae. This is especially true in Oncidiinae, in which plesiomorphic morphological characters exist at the base of various clades that should ultimately be recognized as distinct genera (in spite of being morphologically highly heterogeneous; Williams & al., 2001a, b; Chase & al., 2009). Another group in which this has been observed is Pleurothallidinae. Pridgeon & al. (2001) analyzed Pleurothallidinae using molecular data and placed *Dilomilis* Raf. and *Neocogniauxia* Schltr. at the base of this subtribe, denying the articulation between the ovary and pedicel as a synapomorphic character for the whole subtribe. The same pattern has been observed in Malaxideae (Cameron, 2005), in terms of the transition of the leaf from conduplicate to plicate in the terrestrial clade. Although causing disagreements between classifications based on morphology and those derived from molecular phylogenies, these patterns are important sources of information for understanding the processes involved in morphological modification of lineages over time.

**Origin of *Bulbophyllum* in the Neotropics. —** Although the monophyly of Neotropical *Bulbophyllum* (Figs. 1, 2) was already shown by Gravendeel & al. (2003, 2004) and Fischer & al. (2007), this is the first phylogenetic study on the relationships between the New World species of *Bulbophyllum*. The question may be posed whether the diversity of *Bulbophyllum* in the Neotropics originated due to vicariance following the splitting of Pangaea or due to dispersal by the "Boreotropical" route. Sanmartin & Ronquist (2004) argued for a hybrid origin of the South American flora, with many dispersal events occurring between southern South America, Australia, and New Zealand, and rare dispersal events between Africa and northern South America. Our data indicate that the present distribution in *Bulbophyllum* is better explained by the latter events, i.e., exchange between tropical Africa and northern South America, since these two regions are sister groups in the phylogeny and because the sister clades within the Neotropics have their diversity center above the Equator line.

The origin of Orchidaceae is estimated to date to approximately 70–80 Ma (Wikström & al., 2001; Van den Berg, 2003, 2007; Ramírez & al., 2007), being much later than the splitting of northern South America and Africa (ca. 100 Ma; Sanmartin & Ronquist, 2004). *Bulbophyllum* belongs to one of the most derived orchid subfamilies, and the split of the node between Dendrobiinae and Bulbophyllinae was estimated to ca. 30–32 Ma (Van den Berg, 2003, 2007). Because *Bulbophyllum* probably first evolved in Asia and arrived in the Americas through Africa (Gravendeel, 2004), its distribution might be explained by the Tropical Gondwanan Pattern (TGP, Sanmartin & Ronquist, 2004). More studies, however, are necessary to determine whether its arrival in the Neotropics was due to the TGP or to more recent dispersal events as described by Givnish & al. (2004), Li & al. (2009) and others. Within the Neotropics, another biogeographical pattern can be visualized. Utilizing the *Bulbophyllum* species' distributions and the biomes described for the Neotropics, Smidt & al. (2007) identified a connection between the biomes of North America, Central America, and the Amazon and the Andean region linked with the cerrado region and Atlantic rain forest by parsimony analysis of endemism. The two patterns identified (TGP and exchange of species between the northern to southern regions of the American continent through the Andes) were probably common in Orchidaceae (Chase, 2001) and particularly in the subfamily Epidendroideae (Van den Berg & al., 2005).

## TAXONOMIC CONCLUSIONS

A sectional classification of Neotropical *Bulbophyllum*, with sections in chronological order, is presented here. A full taxonomic treatment with keys, maps, illustration, and synonymies of all recognized species is in progress and will be published elsewhere.

### Artificial key to the Neotropical sections of *Bulbophyllum*

1\. Bifoliate pseudobulbs ............ ***B.* sect. *Bulbophyllaria***
1\. Unifoliate pseudobulbs .................................... 2
2\. Inflorescences spike, rachis fleshy (more than 4 mm diam.) ................................ ***B.* sect. *Furvescens***
2\. Inflorescences raceme, rachis thin (less than 3 mm diam.) ...................................................... 3
3\. Rachis with flowers spirally arranged ..................... ........................................ ***B.* sect. *Micranthae***
3\. Rachis with flowers distichously arranged ............... 4
4\. Petals patent, column foot with bi-lobed apices .......... .......................................... ***B.* sect. *Didactyle***
4\. Petals erect, column foot with entire apices ............. 5
5\. Column foot shorter than column length, lateral sepals entirely united to form a synsepal ........ ***B.* sect. *Xiphizusa***
5\. Column foot longer than column length, lateral sepals totally free ................................... ***B.* sect. *Napelli***

***Bulbophyllum*** sect. ***Xiphizusa*** (Rchb. f.) Cogn. in Martius, Fl. Bras. 3(5): 607. 1902 – Type: *Bulbophyllum chloropterum* Rchb. f. in Linnaea 22: 835. 1849.

Pseudobulbs unifoliate. Inflorescence a raceme with a thin rachis. Flowers distichously arranged. Lateral sepals totally united to form a synsepal. Petals erect. Column foot with entire apex, shorter than column length. Twenty-three species, occurring in Bolivia, Colombia, Brazil, Jamaica, Mexico, Paraguay, Peru, and Venezuela (Figs. 3E, 4I). *Bulbophyllum amazonicum* L.O. Williams; *B. antioquiense* Kraenzl.; *B. arianeae* C.N. Fraga & E.C. Smidt; *B. barbatum* Barb. Rodr.; *B. bidentatum* (Barb. Rodr.) Cogn.; *B. carassense* R.C. Mota, F. Barros & Stehmann; *B. chloropterum* Rchb. f.; *B. ciluliae* Bianch. & J.A.N. Bat.; *B. dusenii* Kraenzl.; *B. fendlerianum* E.C. Smidt & Cribb; *B. filifolium* Borba & E.C. Smidt; *B. gehrtii* E.C. Smidt & Borba; *B. gladiatum* Lindl.; *B. hatschbachianum* E.C. Smidt & Borba; *B. hoehnei* E.C. Smidt & Borba; *B. jamaicense* Cogn.; *B. manarae* Foldats; *B. melloi* Pabst; *B. plumosum* (Barb. Rodr.) Cogn.; *B. solteroi* R. González; *B. teimosense* E.C. Smidt & Borba; *B. vareschii* Foldats; *B. weberbauerianum* Kraenzl.

***Bulbophyllum*** sect. ***Napelli*** Rchb. f. in Ann. Bot. Syst. 6: 249. 1861 – Type: *Bulbophyllum napelli* Lindl. in Ann. Mag. Nat. Hist. 10: 185. 1842.

Pseudobulbs unifoliate. Inflorescence a raceme with thin rachis. Flowers distichously arranged. Lateral sepals totally free. Petals erect. Column foot with entire apex and longer than column length. Twelve species, occurring in Argentina, Brazil, and Venezuela (Figs. 3C, 4C–E). *Bulbophyllum atropurpureum* Barb. Rodr.; *B. boudetianum* Fraga; *B. campos-portoi* Brade; *B. cantagallense* Barb. Rodr.; *B. dunstervillei* Garay & Dunst.; *B. glutinosum* (Barb. Rodr.) Cogn.; *B. granulosum* (Barb. Rodr.) Cogn.; *B. kautskyi* Toscano; *B. malachadenia* (Lindl.) Cogn.; *B. micropetaliforme* Leite; *B. napelli* Lindl.; *B. regnellii* Rchb. f.

***Bulbophyllum*** sect. ***Bulbophyllaria*** (Rchb. f.) Griseb., Fl. Brit. W. I.: 613. 1864 – Type: *Bulbophyllum bracteolatum* Lindl. in Edwards's Bot. Reg. 24: t. 57, fig. 3. 1838.

Pseudobulbs bifoliate. Inflorescence in a spike with fleshy rachis. Flowers spirally arranged. Lateral sepals totally free. Petals erect. Column foot with entire apex and shorter than column length. Three species, occurring in Cuba, Colombia, Costa Rica, Dominican Republic, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, and Venezuela (Figs. 3B, 4B). *Bulbophylum aristatum* (Rchb. f.) Hemsl.; *B. bracteolatum* Lindl.; *B. cirrhosum* L.O. Williams.

***Bulbophyllum*** sect. ***Micranthae*** Barb. Rodr., Gen. Sp. Orchid. 2: 117. 1882 – Type: *Bulbophyllum micranthum* Barb. Rodr., Gen. Sp. Orchid. 1: 39, t. 352. 1877.

Pseudobulbs unifoliate. Inflorescence a raceme with thin rachis. Flowers spirally arranged. Lateral sepals totally free. Petals erect. Column foot with entire apex and shorter than column length. Twelve species, occurring in Bolivia, and Brazil (Fig. 3D, Fig. 4F–H). *Bulbophyllum adiamantinum* Brade; *B. chloroglossum* Rchb. f. & Warm.; *B. epiphytum* Barb. Rodr.; *B. insectiferum* Barb. Rodr.; *B. macroceras* Barb. Rodr.; *B. mentosum* Barb. Rodr.; *B. micranthum* Barb. Rodr.; *B. mucronifolium* Rchb. f. & Warm.; *B. pitengoense* Campacci; *B. rupicolum* Barb. Rodr.; *B. tricolor* Smith & Harris; *B. uhl-gabrielianum* Chiron & V.P. Castro.

***Bulbophyllum*** sect. ***Didactyle*** (Lindl.) Cogn. in Martius, Fl. Bras. 3(5): 595. 1902 – Type: *Bulbophyllum exaltatum* Lindl., Ann. Mag. Nat. Hist. 10: 186. 1842.

Pseudobulbs unifoliate. Inflorescence a raceme with thin rachis. Flowers distichously arranged. Lateral sepals totally free. Petals patent. Column foot with bilobed apex and shorter than column length. Seven species, occurring in Bolivia, Brazil, Colombia, Ecuador, Guyana, Paraguay, Peru, and Venezuela (Figs. 3F, 4J–L). *Bulbophyllum exaltatum* Lindl.; *B. involutum* Borba, Semir & F. Barros; *B. meridense* Rchb. f.; *B. perii* Schltr.; *B. popayanense* F. Lehm. & Kraenzl.; *B. tripetalum* Lindl.; *B. weddellii* (Lindl.) Rchb. f.

***Bulbophyllum*** sect. ***Furvescens*** E.C. Smidt, Borba & Van den Berg, **sect. nov.** – Type: *Bulbophyllum nagelii* L.O. Williams in Bot. Mus. Leafl. 7: 144. 1939 – Holotype: Mexico, Morelos, Vulcao Popocatepetl, *Williams, L.O. & Nagel, O. 3864*, 21 May 1938 (AMES!).

Haec sectio Bulbophyllo sectione Bulbophyllaria similis, sed pseudobulbis unifoliatis differt.

Pseudobulbs unifoliate. Inflorescence a spike with fleshy rachis. Flowers spirally arranged. Lateral sepals totally free. Petals erect. Column foot with entire apex and shorter than column length. Five species, occurring in Brazil, Ecuador, French Guiana, Guyana, Mexico, Peru, Suriname, and Venezuela (Figs. 3A, 4A). *Bulbophyllum meristorhachis* Garay & Dunst.; *B. nagelii* L.O. Williams; *B. quadrisetum* Lindl.; *B. setigerum* Lindl.; *B. steyermarkii* Foldats.

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**Appendix 1.** Sample taxa with voucher information and GenBank accession numbers of the sequences included in the present analyses. Sequences of GenBank are marked with asterisk (*).

Taxon; voucher, herbaria, origin, GenBank accession number for ITS/*trnS-trnG*/*psbA-trnH*

**INGROUP:** ***Bulbophyllum adiamantinum*** Brade; *E.C. Smidt & al., 723;* HUEFS; Brazil; GQ339691/GQ339656/GQ339621. ***B. atropurpureum*** Barb. Rodr.; Jd. Bot. SP. 12016; Brazil; GQ339706/GQ339668/GQ339632. ***B.*** aff. ***atropurpureum*** 1 Barb. Rodr.; *L. Menini, s.n.*; Brazil; GQ862815/GQ862817/GQ862818. ***B. bidentatum*** (Barb. Rodr.) Cogn.; *E.C. Smidt & al., 777*; HUEFS; Brazil; GQ339701/GQ339663/GQ339627. ***B. boudetianum*** Fraga; *L. Kullman, s.n.*; MBML; Brazil; GQ339723/GQ339682/GQ339650. ***B. bracteolatum*** Lindl.; *B. Gravendeel, s.n.*; L; México; GQ339686/–/–. ***B. bracteolatum*** 1 Lindl.; *B. Gravendeel, s.n.*; L; México; GQ339687/–/–. ***B. campos-portoi*** Brade; *E.C. Smidt & al., 790*; HUEFS; Brazil; GQ339721/GQ339681/GQ339648. ***B. cantagallense*** Barb. Rodr.; *L. Kullman, s.n.*; MBML; Brazil; GQ339722/–/GQ339649. ***B. carassense*** R.C. Mota, F. Barros & Stehmann; *R. Custodio, 2819*; BHCB; Brazil; GQ339717/GQ339677/GQ339643. ***B. chloroglossum*** Rchb. f. & Warm.; *M. Campacci, s.n.* (spirit); Brazil; GQ339694/GQ339659/GQ339624. ***B. ciluliae*** Bianch. & J.A.N. Bat.; *E.C. Smidt & al., 805*; HUEFS; Brazil; GQ339698/–/–. ***B. cirrhosum*** L.O. Williams; *B. Gravendeel, s.n.*; L; México; GQ339684/GQ339652/GQ339617. ***B. cirrhosum*** 1 L.O. Williams; *G. Salazar, s.n.*; AMO; México; GQ339685/–/–. ***B. epiphytum*** Barb. Rodr.; *E.C. Smidt & al., 737*; HUEFS; Brazil; GQ339693/GQ339658/GQ339623. ***B. exaltatum*** Lindl.; *E.C. Smidt & al., 753*; HUEFS; Brazil; GQ339714/GQ339675/GQ339640. ***B. exaltatum*** 1 Lindl.; *E.C. Smidt & al., 309*; HUEFS; Brazil; GQ339715/GQ339676/GQ339641. ***B. filifolium*** Borba & E.C. Smidt; *E.C. Smidt & al., 793*; HUEFS; Brazil; GQ339699/–/–. ***B. gladiatum*** Lindl.; *Toscano, 1905*; HUEFS; Brazil; GQ339718/GQ339678/GQ339644. ***B. glutinosum*** (Barb. Rodr.) Cogn.; *L. Menini, 125* Brazil; GQ339707/–/GQ339633. ***B. hoehnei*** E.C. Smidt & Borba; *E.C. Smidt & al., 700*; HUEFS; Brazil; GQ339700/–/–. ***B. insectiferum*** Barb. Rodr.; *E.C. Smidt & al., s.n.*; HUEFS; Brazil; GQ339692/GQ339657/GQ339622. ***B. kautskyi*** Toscano; *C. Azevedo, 183*; HUEFS; Brazil; GQ339705/GQ339667/GQ339631. ***B. malachadenia*** (Lindl.) Cogn.; *E.C. Smidt & al., 750*; HUEFS; Brazil; GQ339708/GQ339669/GQ339634. ***B. manarae*** Foldats; *E.C. Smidt & al., 747*; HUEFS; Brazil; GQ339704/GQ339666/GQ339630. ***B. melloi*** Pabst; *Mota & Marques, 656*; BHCB; Brazil; GQ339719/GQ339679/GQ339645. ***B. mentosum*** Barb. Rodr.; *Cardoso, 312*; HUEFS; Brazil; GQ339690/GQ339655/GQ339620. ***B. meridense*** Rchb. f.; *E.C. Smidt & al., s.n.*; HUEFS; Brazil; GQ339712/GQ339673/GQ339638. ***B. micranthum*** Barb. Rodr.; *E. Saddi, 82*; RB; Brazil; GQ339697/GQ339661/GQ339626. ***B. micropetaliforme*** Leite; *E.L. Borba, 2127*; HUEFS; Brazil; GQ339709/GQ339670/GQ339635. ***B. mucronifolium*** Rchb. f. & Warm.; *E.C. Smidt & al., 742*; HUEFS; Brazil; GQ339695/–/–. ***B. nagelii*** L.O. Williams; *G. Salazar, s.n.*; AMO; México; GQ339720/GQ339680/GQ339647. ***B. napelli*** Lindl.; *E.C. Smidt & al., 727*; HUEFS; Brazil; GQ339711/GQ339672/GQ339637. ***B. peri*** Schltr.; *Bento, s.n.* (spirit); GQ862980/GQ862816/–. ***B. plumosum*** (Barb. Rodr.) Cogn.; *E.C. Smidt, 726*; HUEFS; Brazil; GQ339702/GQ339664/GQ339628. ***B. regnellii*** Rchb. f.; *E.C. Smidt & al., 773*; HUEFS; Brazil; GQ339710/GQ339671/GQ339636. ***B. rupicolum*** Barb. Rodr.; *E. C. Smidt & al., 766*; HUEFS; Brazil; GQ339696/GQ339660/GQ339625. ***B. setigerum*** Lindl.; *J. Batista, s.n.*; HUEFS; Brazil; GQ339689/GQ339654/GQ339619. ***B. steyermarkii*** Foldats; *E.C. Smidt, 780* (spirit); Ecuador; GQ339688/GQ339653/GQ339618. ***B. teimosense*** E.C. Smidt & Borba; *E.C. Smidt, 308*; HUEFS; Brazil; GQ339703/GQ339665/GQ339629. ***B. tripetalum*** Lindl.; *Brieger Coll., 9783* (ESA); GQ339716/–/GQ339642. ***B. weddellii*** (Lindl.) Rchb. f.; *C. Azevedo, 188*; HUEFS; Brazil; GQ339713/GQ339674/GQ339639. **OUTGROUPS:** ***B. clandestinum*** Lindl.; JF706719/JF693822/JF693814. ***B. falcatum*** Lindl.; *E.C. Smidt, 772*; HUEFS; cult.; GQ339683/GQ339651/GQ339616. ***B. falcatum*** 1 Lindl.; *Vienna 2394*; EF195927.1*/JF693818/JF693811; ***B. lobbii*** Lindl.; EF195931*/JF693819/JF693812. ***B. nutans*** Thouars; EF196038*/JF693821/–. ***B. newportii*** (F.M. Bailey) Rolfe; JF706720/JF693820/JF693813. ***Dendrobium kingianum*** Bidwill ex Lindl.; EU430386*/JF693826/JF693810. ***D. crumenatum*** Sw.; EU840700*/JF693823/JF693815. ***Epigeneium tricallosum*** (Ames & C. Schweinf.) J.J. Wood; JF706721/JF693825/JF693817.

**Appendix 2.** List of Neotropical *Bulbophyllum* species studied, taxonomic position following Pabst & Dungs (1975) and taxonomic position following this study.

| Species | Section, following Pabst & Dungs (1975) | Section, following this study |
|---|---|---|
| *B. adiamantinum* Brade | *Micranthae* | *Micranthae* |
| *B. atropurpureum* Barb. Rodr. | *Didactyle* | *Napelli* |
| *B. bidentatum* (Barb. Rodr.) Cogn. | *Xiphizusa* | *Xiphizusa* |
| *B. boudetiana* Fraga | incertae sedis | *Napelli* |
| *B. bracteolatum* Lindl. | *Bulbophyllaria* | *Bulbophyllaria* |
| *B. campos-portoi* Brade | *Didactyle* | *Napelli* |
| *B. cantagalense* Barb. Rodr. | *Didactyle* | *Napelli* |
| *B. carassense* R.C. Mota, F. Barros & Stehmann | *Xiphizusa* | *Xiphizusa* |
| *B. chloroglossum* Rchb. f. & Warm. | *Micranthae* | *Micranthae* |
| *B. ciluliae* Bianch. & J.A.N.Bat. | *Xiphizusa* | *Xiphizusa* |
| *B. cirrhosum* L.O. Williams | *Bulbophyllaria* | *Bulbophyllaria* |
| *B. epiphytum* Barb. Rodr. | *Micranthae* | *Micranthae* |
| *B. exaltatum* Lindl. | *Didactyle* | *Didactyle* |
| *B. filifolium* Borba & E.C. Smidt | *Didactyle* | *Xiphizusa* |
| *B. gladiatum* Lindl. | *Xiphizusa* | *Xiphizusa* |
| *B. glutinosum* (Barb. Rodr.) Cogn. | *Didactyle* | *Napelli* |
| *B. hoehnei* E.C. Smidt & Borba | incertae sedis | *Xiphizusa* |
| *B. insectiferum* Barb. Rodr. | *Bulbophyllaria* | *Micranthae* |
| *B. kautskyi* Toscano | *Didactyle* | *Napelli* |
| *B. malachadenia* (Lindl.) Cogn. | *Didactyle* | *Napelli* |
| *B. manarae* Foldats | insertae sedis | *Xiphizusa* |
| *B. melloi* Pabst | *Xiphizusa* | *Xiphizusa* |
| *B. mentosum* Barb. Rodr. | *Bulbophyllaria* | *Micranthae* |
| *B. meridense* Rchb. f. | *Didactyle* | *Didactyle* |
| *B. micranthum* Barb. Rodr. | *Micranthae* | *Micranthae* |
| *B. micropetaliforme* Leite | *Bulbophyllaria* | *Napelli* |
| *B. mucronifolium* Rchb. f. & Warm. | *Micranthae* | *Micranthae* |
| *B. nagellii* L.O. Williams | insertae sedis | *Furvescens* |
| *B. napelli* Lindl. | *Napelli* | *Napelli* |
| *B. peri* Schltr. | *Didactyle* | *Didactyle* |
| *B. plumosum* (Barb. Rodr.) Cogn. | *Xiphizusa* | *Xiphizusa* |
| *B. regnellii* Rchb. f. | *Bulbophyllaria* | *Napelli* |
| *B. rupicolum* Barb. Rodr. | *Micranthae* | *Micranthae* |
| *B. setigerum* Lindl. | *Micranthae* | *Furvescens* |
| *B. steyermarkii* Foldats | insertae sedis | *Furvescens* |
| *B. teimosense* E.C. Smidt & Borba | *Xiphizusa* | *Xiphizusa* |
| *B. tripetalum* Lindl. | *Didactyle* | *Didactyle* |
| *B. weddellii* (Lindl.) Rchb. f. | *Xiphizusa* | *Didactyle* |