

MOLECULAR PHYLOGENETICS OF VANDEAE (ORCHIDACEAE) AND THE EVOLUTION OF LEAFLESSNESS¹

BARBARA S. CARLSWARD,^{2,3} W. MARK WHITTEN,² NORRIS H. WILLIAMS,² AND BENNY BYTEBIER⁴

²Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611-7800 USA; ³Department of Botany, University of Florida, Gainesville, Florida 32611-8526 USA; and ⁴Department of Biochemistry, University of Stellenbosch, Private Bag x1, 7602 Matieland, South Africa

Members of tribe Vandae (Orchidaceae) form a large, pantropical clade of horticulturally important epiphytes. Monopodial leafless members of Vandae have undergone extreme reduction in habit and represent a novel adaptation to the canopy environment in tropical Africa, Asia, and America. To study the evolution of monopodial leaflessness, molecular and structural evidence was used to generate phylogenetic hypotheses for Vandae. Molecular analyses used sequence data from ITS nrDNA, *trnL-F* plastid DNA, and *matK* plastid DNA. Maximum parsimony analyses of these three DNA regions each supported two subtribes within monopodial Vandae: Aeridinae and a combined Angraecinae + Aerangidinae. Adding structural characters to sequence data resulted in trees with more homoplasy, but gave fewer trees each with more well-supported clades than either data set alone. Two techniques for examining character evolution were compared: (1) mapping vegetative characters onto a molecular topology and (2) tracing vegetative characters onto a combined structural and molecular topology. In both cases, structural synapomorphies supporting monopodial Vandae were nearly identical. A change in leaf morphology (usually reduced to a nonphotosynthetic scale), monopodial growth habit, and aeration complexes for gas exchange in photosynthetic roots seem to be the most important characters in making the evolutionary transition to leaflessness.

Key words: ITS; *matK*; monopodial leaflessness; Orchidaceae; phylogeny; *trnL-F*; Vandae.

Orchidaceae are one of the largest, most diverse plant families with more than 24 000 species (Board of Trustees of the Royal Botanic Gardens Kew, 2004). With the recent spate of molecular data, evolutionary relationships among orchids are being reestablished and recircumscribed (Chase et al., 2003). Surprisingly, Vandae, a large horticulturally important group with almost 2000 species, has been essentially untouched by molecular taxonomists. Traditional classification of taxa within Vandae is based primarily on floral morphology, especially for leafless taxa with few vegetative characteristics.

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⁵ Author for correspondence (e-mail: bcarlsward@flmnh.ufl.edu)

However, floral characteristics are highly homoplasious within the family and have proven to be treacherous in making hypotheses of phylogenetic relationships (Chase and Palmer, 1989; Bateman et al., 1997).

Vandae were first circumscribed by Lindley during the early 1800s in his treatment of the orchid family (1830–1840). Members of Vandae sensu Lindley were grouped based on the presence of distinct caudicles of the pollinarium. In addition to Vandae sensu Dressler (1993; e.g., *Vanda* and *Angraecum*), a significant part (40%) of Lindley's group was of tropical American taxa that are now placed in Maxillarieae. However, after describing Vandae as a new tribe, Lindley admitted his circumscription of Vandae could probably be even further subdivided into well-defined groupings, given further study.

More than a century later, Dressler and Dodson (1960) placed subtribe Sarcanthinae within a large tribe Epidendreae. They noted that although this monopodial subtribe may be difficult to distinguish from the tropical American Oncidiinae using floral morphology, they are probably not closely related. However, Garay (1960, 1972a) reinstated tribe Vandae based on the presence of an incumbent anther, porrect rostellum, and well-developed stipes. Vandae sensu Garay included tropical American subtribes Cyrtopodiinae, Zygopetalinae, and Oncidiinae as well as Vandinae (Garay, 1972a).

Dressler (1981) elevated units of the original tribe Epidendreae delimited by Dressler and Dodson (1960) to form two subfamilies: Epidendroideae and Vandoideae. Although Vandoideae had long been recognized (at varying taxonomic levels) on the basis of floral morphology, there were few consistent characters to delimit Vandoideae from Epidendroideae. Dressler admitted that the only clear character delimiting these two subfamilies was anther development and several years later (1989) reinstated members of Vandoideae into a broadly defined Epidendroideae, much like his original systematic treatment with Dodson (Dressler and Dodson, 1960). In either case, Vandae formed a well-defined group

of orchids split into three subtribes: Sarcanthinae, Angraecinae, and Aerangidinae.

In Dressler's most recent classification (1993), Vandaeae were essentially unchanged from his previous work (1981) with two exceptions: Vandaeae were allied with Dendrobieae and Podochileae in a dendrobioid subclade of Epidendroideae, and the illegitimate subtribal name Sarcanthinae (Bentham, 1881) was replaced with Aeridinae.

Unlike any previous workers, Szlachetko (1995) provided a much more subdivided classification in which he split orchids into three families and subdivided Orchidaceae sensu stricto (s.s.) into eight subfamilies, including Vandoideae. Szlachetko's system was primarily based on overall similarity in floral morphology, not on shared derived characters. In his organization of Vandaeae (based primarily on rostellum morphology), Szlachetko created 10 new subtribes to complement the three existing subtribes at the time (Aeridinae, Angraecinae, and Aerangidinae). Current molecular phylogenetic evidence often conflicts with Szlachetko's extremely subdivided classification system, and it has been essentially disregarded by most taxonomists today (Pridgeon et al., 1999).

The most comprehensive cladistic morphological study of orchid systematics was carried out by Freudenstein and Rasmussen (1999). In their analyses, Vandaeae formed a monophyletic tribe with several synapomorphies: isodiametric exodermal cell shape, monopodial growth habit, spherical stigmata, and seeds with laterally compressed walls. *Aerangis* and *Angraecum* were united by the reduction of four pollinia to two, and they were sister to a paraphyletic grade of Aeridinae (*Acampe* and *Phalaenopsis*). Their study also indicated a sister relationship with Polystachyinae (*Polystachya* and *Neobenthamia*).

Existing molecular evidence for relationships among members of tribe Vandaeae is scarce. This tribe has often been referred to as the "black box" of Orchidaceae (R. Dressler, Florida Museum of Natural History, personal communication). Molecular analyses of Vandaeae included sequence data from several plastid-encoded DNA regions: Jarrell and Clegg (1995) using *matK*; Neyland and Urbatsch (1996) using *ndhF*; Cameron et al. (1999) using *rbcl*; and Cameron (2001) using *atpB* and *psaB*. The only study to find a nonmonophyletic Vandaeae sensu Dressler (1993) was the one performed by Neyland and Urbatsch (1996) in which *Angraecum philippinense* Ames (= *Amesiella philippinense* (Ames) Garay, Aeridinae) was erroneously used as a representative of Angraecinae.

In the most recent classification of Orchidaceae (Chase et al., 2003), Vandaeae form a monophyletic tribe within a large polytomy of advanced epidendroid groups (Cymbidieae and Agrostophyllinae) and include the sympodial subtribe Polystachyinae. The sister relationship of Vandaeae sensu Dressler (1993) and Polystachyinae is well-supported by analyses of Cameron (2001) as well as van den Berg (2005).

Vandaeae sensu Chase et al. (2003) represent the most complete classification based on a modern phylogenetic approach. However, we chose to focus on the morphologically diagnosable clade of monopodial Vandaeae (Vandaeae s.s.) for our study, which includes approximately 158 genera with 1968 species of monopodial epiphytes. Distinguishing characteristics of Vandaeae s.s. include velamen of the *Vanda* type (Porembski and Barthlott, 1988); monopodial growth habit; tegular stipe (Rasmussen, 1986); pollinia reduced from eight to four or two

(Rasmussen, 1986); pollinia superposed (Rasmussen, 1986); and *Vanda*-type seeds (Ziegler, 1981).

Subtribal relationships—Lower-level phylogenetic studies of Vandaeae are even more uncommon than higher-level studies of the tribe. Topik et al. (2005) studied the subtribe Aeridinae, using African members of Vandaeae sensu Dressler (1993) as outgroups. The horticulturally important genus *Phalaenopsis* was also examined by several workers from Taiwan (Tsai et al., 2003), and Carlsward et al. (2003) performed detailed studies of the New World genus *Dendrophylax* (including *Harrisella* and *Polyradicion*).

Within Vandaeae s.s., three monopodial subtribes are defined by floral morphology and biogeography. Aeridinae form a monophyletic group (Topik et al., 2005) and make up the largest subtribe, with 103 genera (1253 species) primarily distributed throughout Asia, Australia, and a few species in Africa. They are distinguished from members of the other two subtribes by having an entire rostellum, a relatively small spur formed by the lip, and four (or two) pollinia. Several genera, such as *Acampe* and *Renanthera*, may also possess a prominent column foot.

Aerangidinae contain 36 genera (307 species) from tropical Africa and Madagascar. Members of Aerangidinae typically possess an elongate rostellum, elongate spur, and two pollinia. Their appearance closely resembles that of members of subtribe Angraecinae, which contain 19 genera (408 species). Angraecinae are distributed throughout Madagascar, the Mascarene and Comoros Islands, and tropical Africa, with two genera in tropical America. Angraecinae possess an apron-like rostellum, elongate spur, and two pollinia. Other than the shape of the rostellum, Aerangidinae and Angraecinae are similar in appearance and have collectively been referred to as the "angraecoids."

Leaflessness—Although leaflessness within angiosperms has arisen several times, the type of leaflessness found in monopodial Vandaeae is unique to this specialized orchid tribe (Fig. 1). Most leafless vascular plants are typically either succulent and xeric-adapted (e.g., Cactaceae, Euphorbiaceae) or in some way parasitic on other plants or fungi (e.g., Orobanchaceae, Orchidaceae). In almost all cases, the shoot system of these leafless plants is well developed and forms the main body (as it does in leafless *Vanilla* species in which the stem has taken over the role of photosynthesis). However, the shoot system of leafless monopodial Vandaeae is extremely reduced, contributing no net carbon gain to the plant (Benzing and Ott, 1981). Instead, the roots form the main body of the plant and have assumed the role of food assimilation using a Crassulacean Acid Metabolism (CAM)-like system of recycling CO₂ (Cockburn et al., 1985). The degree of reduction is so extreme that these leafless orchids have even been referred to as "shootless" and "semishootless" by some workers (Benzing and Ott, 1981; Benzing et al., 1983). Benzing and Ott (1981) hypothesized that these extreme epiphytes have reduced their carbon investment in vegetative organs by limiting shoot formation, while increasing their potential fitness by allocating more resources for flower and fruit production.

Species of monopodial leafless orchids occur in all three subtribes of Vandaeae s.s. and across the globe. Aeridinae contain the greatest diversity of leafless taxa, with four genera (230 species) found primarily throughout Asia. *Taeniophyllum*



Fig. 1. Representative leafless taxa of Vandaeae. (A) *Chiloschista parishii* plants showing the deciduous type of monopodial leaflessness. On the left is a plant with short-lived vestigial leaves, and on the right is a leafless plant of the same species. (B) *Campylocentrum* sp. from Ecuador is leafless with an abbreviated stem and nonphotosynthetic scale leaves. (C) *Dendrophylax fumlalis* is leafless with an abbreviated stem and nonphotosynthetic scale leaves. (D) *Phalaenopsis wilsonii* with the deciduous type of leaflessness. (E) *Solenangis aphylla* with an elongate stem and nonphotosynthetic scale leaves.

is the largest exclusively leafless genus within Vandaeae, with approximately 185 species that possess brown scale leaves. The remaining three genera (*Chiloschista*, *Microtatorchis*, and *Phalaenopsis* section *Aphyllae*) together contain approximately 45 species with caducous, vestigial leaves.

Three genera within Aerangidinae contain leafless species. *Microcoelia* is an exclusively leafless genus with approximately 26 species found throughout tropical Africa and Madagascar. *Chauliodon* is a monotypic African genus that resembles *Microcoelia* (Jonsson, 1979). *Solenangis* is mainly a leafy genus with two leafless members: *S. aphylla* (Thouars) Summerh. and *S. cornuta* (Rchb.f.) Summerh. Both *Microcoelia* and *Solenangis* have leaves reduced to small, non-photosynthetic scales. The main vegetative difference between these genera is the length of their stems. Species of *Microcoelia* all possess abbreviated stems (<3 cm long), whereas *S. aphylla* and *S. cornuta* possess elongate stems (>4 cm long).

The primarily paleotropical Angraecinae contain two leafless genera restricted to the neotropics (Carlswald et al., 2003). *Dendrophylax* is an exclusively leafless genus with 13 species bearing brown scales along an abbreviated stem axis. The larger genus, *Campylocentrum* Benth. (35 species), is primarily leafy with approximately 12 leafless species, ranging from plants with abbreviated stems bearing nonphotosynthetic scales to one species [*C. poeppigii* (Rchb.f.) Rolfe] with elongate stems bearing minute, falcate, caducous leaves.

MATERIALS AND METHODS

Molecular techniques—Specimens were obtained from cultivated material, herbarium specimens, and wild-collected plants. Taxon names and authorities of sampled taxa (Appendix) follow Brummitt and Powell (1992) and the Royal Botanic Gardens, Kew (2004) *World Checklist of Monocotyledons*. When possible, the type species for each genus was sampled (Appendix S1, see Supplemental Data with the online version of this article). The internal

transcribed spacer (ITS) nrDNA region for representatives of all subtribes was sequenced to obtain an overall hypothesis of relationships within the monopodial Vandaeae. Polystachyinae were used as outgroup taxa, based on results of previous analyses (Neyland and Urbatsch, 1996; Cameron et al., 1999; Freudenstein and Rasmussen, 1999; Cameron, 2001; Chase et al., 2003; van den Berg et al., 2005). Two additional plastid regions were sequenced for all available Aerangidinae and Angraecinae and a few representative Aeridinae: *matK* (part of the exon encoding maturase K) and *trnL-F* (*trnL* intron + *trnL* 3' exon + *trnL-trnF* intergenic spacer).

DNA from fresh, silica-dried, and herbarium material was extracted using a modified cetyl trimethylammonium bromide (CTAB) technique from Doyle and Doyle (1987), scaled down to a 1-mL volume. For DNA extracted from fresh or silica-dried specimens, the entire internal transcribed spacer region (ITS 1 + 5.8S + ITS 2) was amplified using primers of Sun et al. (1994) designed for *Sorghum* (17SE and 26SE). Polymerase chain reactions (PCRs) were carried out in 50- μ L volumes using an initial denaturation of 98°C for 6 min (for more complete PCR thermocycler protocols, see Appendix S2 in Supplemental Data with the online version of this article). For degraded DNA extracted from herbarium specimens, ITS 1 and ITS 2 were amplified separately using primers of Blattner (1999) designed for general angiosperms (ITS 1: ITS-A + ITS-C and ITS 2: ITS-D + ITS-B). These PCRs were carried out in a 50- μ L volume using a hot start of 80°C and an initial denaturation of 94°C for 2 min.

In some Vandaeae, apparent paralogy in ITS was detected as a large deletion (approximately 40 bp long) at the beginning of the ITS 2 region (plus numerous base substitutions). In our study, paralogy was restricted to genera of Angraecinae. These paralogous sequences were checked using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) to eliminate the possibility of fungal contamination; results of the BLAST search indicated all sequences were from vandaceous orchids. Apparent paralogy has also been detected in Aeridinae by other workers (J. Schulman, University of Texas at Austin and A. Kocyan, Ludwig-Maximilians-University Munich, personal communications). The PCR products of *Angraecum calceolus* were initially cloned to separate orthologous and paralogous DNA regions, but only one copy (presumably a paralogous one) was ever detected. Taxa with putatively paralogous sequences were therefore excluded from all analyses and are indicated with an asterisk in the Appendix.

For DNA extracted from fresh or silica-dried plant material, the plastid-encoded *matK* and *trnL-F* regions were amplified using a hot start of 80°C and an initial denaturation at 94°C for 3 min. Primers for part of *matK* were those of Whitten et al. (2000), and primers for *trnL-F* were those of Taberlet et al. (1991). Cycle sequence products were directly sequenced, and all sequences (including paralogous sequences) were submitted to GenBank (see Appendix for accession numbers).

Phylogenetic analyses—The ITS region was sequenced for all three monopodial subtribes of Vandaeae. Multiple individuals were sequenced for several species, as material was available. This large ITS matrix was used to examine relationships among all monopodial Vandaeae. To more closely examine relationships among the angraecoids, we collected additional sequence data from the plastid regions *trnL-F* and *matK*. Therefore, two sets of analyses were performed: (1) ITS analyses of all monopodial Vandaeae and (2) three-region analyses (ITS, *matK*, and *trnL-F*) of mainly angraecoids and a reduced subset of Aeridinae.

Sequence data for each region were aligned manually within Se-AL version 2.0a11 (Rambout, 1996) and imported into PAUP* version 4.0b10 (Swofford, 2003) for phylogenetic analysis. All characters were weighted equally, and indels were coded as missing data. No sequence data were excluded from ITS and *matK* regions. However, due to difficulties with alignment and multiple repeat regions within the *trnL-F* matrix, all analyses excluded 356 aligned base pairs from the central region (preceding the *trnL* exon). Maximum parsimony (MP) analyses were run using a heuristic search strategy of branch swapping by subtree pruning and regrafting (SPR), stepwise addition with 1000 random-addition replicates holding 10 trees at each step, saving multiple trees (MULTREES on). The maximum number of trees saved for all analyses was limited to 20000. The resulting shortest trees from this initial analysis were then swapped to completion. Levels of support were estimated with 1000 bootstrap replicates, using the SPR algorithm of branch swapping for 10 random-addition replicates per bootstrap replicate.

Phylogenetic analyses were run separately for each region (ITS, *matK*, and *trnL-F*). Bootstrap trees generated from each region were then manually compared for congruence, as described by Whitten et al. (2000). When there

were no conflicting, well-supported clades (bootstrap percentage [BP] > 74) between regions, data were combined. Data congruence was also tested using the partition homogeneity test (HT_P) in PAUP* version 4.0b10 (Swofford, 2003) as described by Johnson and Soltis (1998). Heuristic searches for the HT_P tests were performed using 100 replicates and an SPR algorithm. Five random addition replicates were performed per HT_F replicate. Probability values (*p*) greater than 0.05 were used to identify data sets that were not significantly different from one another and could therefore be combined. Combined analyses were performed with the same heuristic search strategies as described earlier.

Character mapping—To compare consistent data sets and perform combined analyses of Angraecinae and Aerangidinae, taxon sampling from the anatomical data set of Carlsward et al. (in press) and from our molecular data set were pruned so that each contained the same species (Appendix S3, see Supplemental Data with the online version of this article). However, corresponding species from each data set were not necessarily from the same specimen, and anatomical data from several specimens of the same species were often combined. A total of 112 ingroup (monopodial Vandaeae) and four outgroup species (Polystachyinae) were used in all analyses.

Twenty-four structural characters (Appendix S4, see Supplemental Data with the online version of this article) from Carlsward et al. (in press) were used to make a character matrix for this subset of Vandaeae (Appendix S5, see Supplemental Data with the online version of article). For combined structural and molecular analyses, sequence data from ITS, *matK*, and *trnL-F* were used. All MP analyses were run with the same search strategies described.

Evolution of structural characters was examined in two ways using MacClade version 4.06 for Mac OS X (Maddison and Maddison, 2000): (1) molecular data were used to create a topology onto which anatomical characters were mapped and (2) anatomical characters were traced onto a combined structural and molecular topology.

RESULTS

In all of the analyses, monopodial Vandaeae formed a strongly supported clade (>90 BP). Within this clade, Aeridinae also formed a well-supported clade with >90 BP in all analyses.

ITS analyses of Vandaeae—The large ITS matrix of all Vandaeae included 207 individuals representing 193 species. Of the 777 aligned sequence positions, 394 were potentially parsimony informative. Heuristic analysis produced 20 000+ trees (Figs. 2, 3) with length (L) = 3205 steps, consistency index (CI₁) = 0.29, consistency index excluding uninformative characters (CI₂) = 0.26, retention index (RI) = 0.76, and rescaled consistency index (RC) = 0.22.

Within Vandaeae, Aeridinae were supported with 98 BP, whereas Angraecinae + Aerangidinae formed a well-supported (92 BP) clade (Figs. 2, 3). However, there was little resolution within each of these large monophyletic groups.

ITS and plastid analyses of Angraecinae and Aerangidinae—The ITS submatrix included 116 ingroup species. Most genera of Angraecinae possessed paralogous ITS sequences and were therefore excluded from phylogenetic analyses. Results of the Angraecinae + Aerangidinae ITS submatrix (Fig. 4A, Table 1) were similar to those of the larger Vandaeae matrix (Figs. 2, 3).

The *matK* submatrix included 137 ingroup species (*Bolusiella batesii* and *Rangaeris rhipsalisocia* were excluded due to amplification difficulties). A majority of the Old World Angraecinae formed a clade (82 BP) sister to the remaining polytomy of Angraecinae + Aerangidinae (for the *matK* bootstrap tree see Appendix S6 in Supplemental Data with the online version of this article). There was little resolution within this Old World Angraecinae clade.

The *trnL-F* submatrix included 138 ingroup species

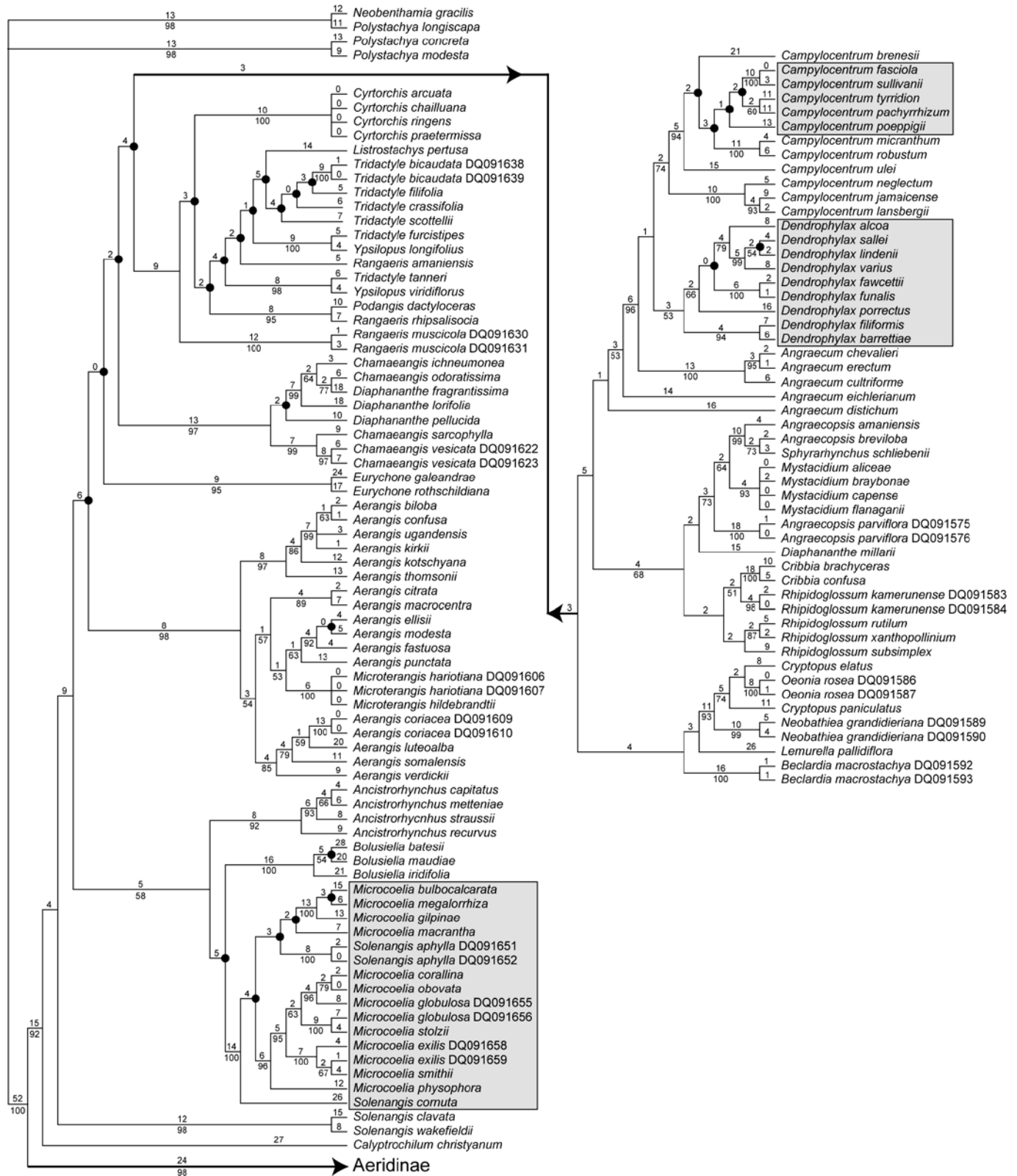


Fig 2. Angraecoid portion of one of 20 000+ most parsimonious trees using the larger ITS matrix for Vandeae. Filled circles represent clades not present in the strict consensus. Grey boxes indicate leafless taxa. Fitch lengths are shown above branches; bootstrap percentages (1000 replicates) are shown below branches. Numbers next to taxon names represent GenBank accession numbers (Appendix).

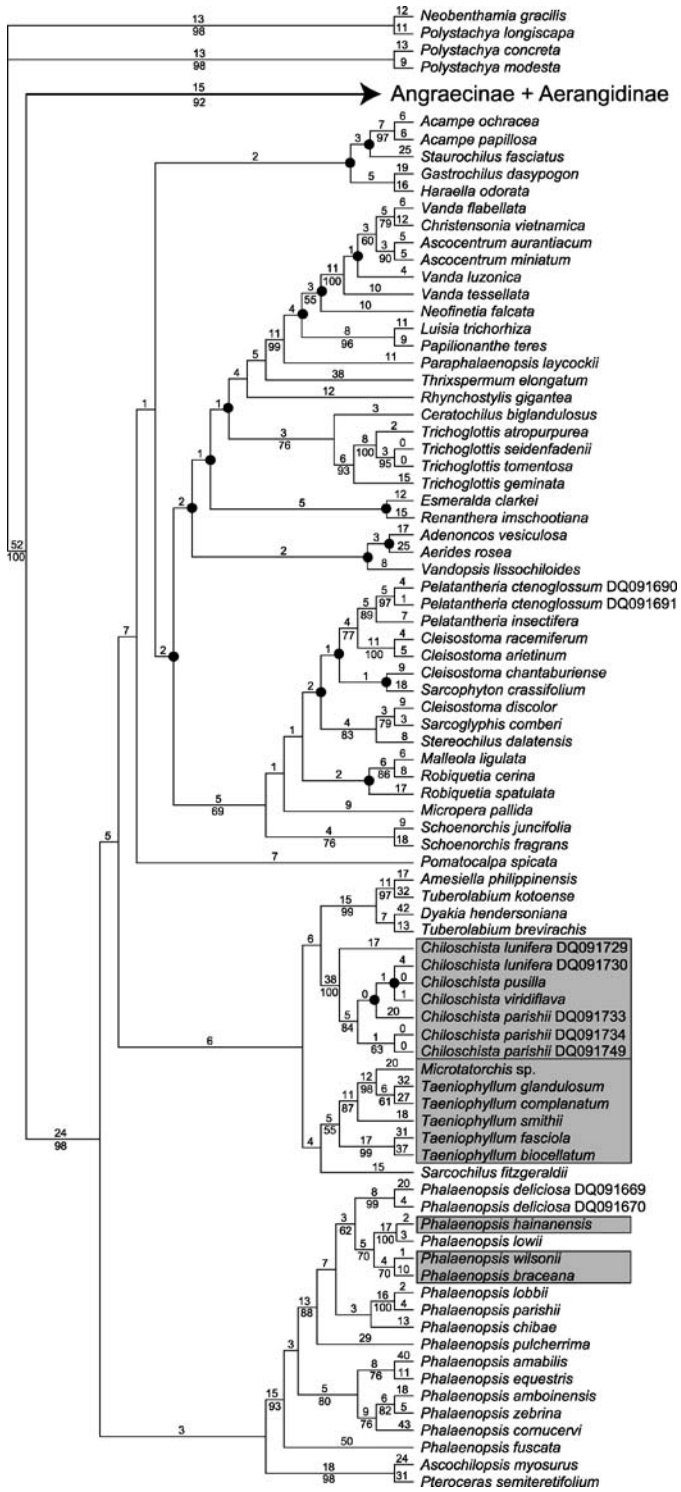


Fig. 3. One of 20 000+ most parsimonious trees using the larger ITS matrix for Vandaeae, emphasizing Aeridinae. Filled circles represent clades not present in the strict consensus. Grey boxes surround leafless taxa. Fitch lengths are shown above branches; bootstrap percentages (1000 replicates) are shown below branches. Numbers next to taxon names represent GenBank accession numbers (Appendix).

(*Ypsilopus viridiflorus* was excluded due to amplification difficulties). The data from the *trnL-F* region gave similar results to those of the *matK* region; the primary difference was that *trnL-F* generally gave more resolution among taxa (for the *trnL-F* bootstrap tree see Appendix S7 in Supplemental Data with the online article).

The combined plastid submatrix included 139 ingroup species. There were no conflicting, well-supported clades between the *matK* and *trnL-F* topologies, which was supported by the partition homogeneity test ($p = 0.18$). The topologies of the combined plastid trees (Fig. 4B) were similar to both the *matK* and *trnL-F* topologies, and in most cases were more resolved with better support than either region separately (Table 1).

There were only three minor instances in which the ITS data conflicted with the plastid data (indicated with arrowheads in Fig. 4). To eliminate the possibility of taxon sampling incongruence, reduced plastid analyses were performed in which taxa with paralogous ITS sequences were removed from the matrix. Topologies and BP for the conflicting taxon groupings were the same in the reduced and complete plastid analyses.

Probability values from the partition homogeneity tests between *trnL-F*/ITS ($p = 0.01$) and *matK*/ITS ($p = 0.01$) had significant heterogeneity between both plastid and ITS data sets. However, this statistical test has been shown to be overly sensitive (Graham et al., 1998; Reeves et al., 2001), and given the infrequency of topology conflicts between the plastid and ITS data sets and the minor position of these topologies within the overall tree, this incongruence is more likely due to technical issues such as taxon sampling or phylogenetic signal and homoplasy (Wendel and Doyle, 1998) than they are to real genealogy differences. Therefore, the data sets were combined to give a more robust phylogenetic tree of all Aerangidinae and Angraecinae than would otherwise be attainable with individual data sets (Soltis et al., 1998).

The combined three-region matrix included 139 ingroup species. Topologies of the resulting trees were more similar to the plastid trees than the ITS trees (Figs. 4, 5). This combined three-region matrix resulted in fewer trees with better resolution and support than any of the three data sets alone (Table 1).

Structural characters mapped onto molecular topologies—Heuristic analyses resulted in 20 000+ equally parsimonious trees using anatomical data and 20 000+ equally parsimonious trees using combined sequence data from ITS, *matK*, and *trnL-F* (Table 2). The bootstrap consensus of the anatomical data set only revealed three clades with greater than 50 BP (Appendix S8, see Supplemental Data with online version of this article). Monopodial Vandaeae were well supported (94 BP) by the following anatomical characters (Carlsward et al., in press): (1) loss of tilosomes, (2) presence of aeration complexes, (3) presence of spherical stegmata, (4) loss of mucilage, and (5) presence of a monopodial stem. *Angraecopsis amaniensis* and *A. breviloba* were weakly supported as sister taxa (65 BP) by the presence of deciduous leaves. *Cyrtorchis praetermissa* and *C. ringens* were moderately supported (72 BP) by the presence of ridged endovelamen thickenings, an adaxial hypodermis, and fibrous, foliar idioblasts.

Results of the molecular analyses were similar to those obtained in analyses of Vandaeae above (Appendix S8, see Supplemental Data with online version of this article). Angraecinae + Aerangidinae were monophyletic (100 BP) and sister to Aeridinae (100 BP). Within Angraecinae, a large

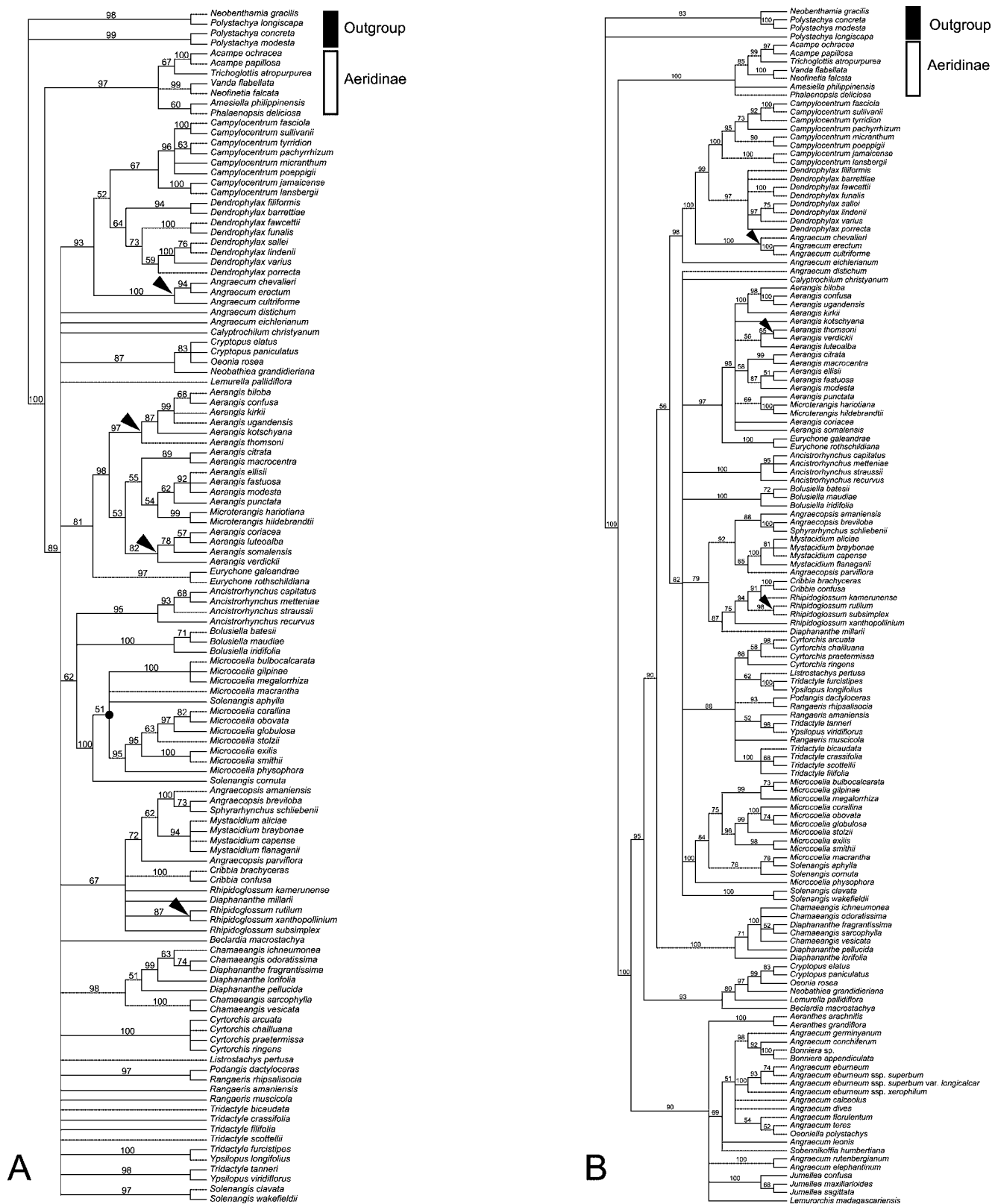


Fig. 4. Bootstrap consensus for (A) ITS and (B) plastid (*matK* + *trnL-F*) data sets. Bootstrap percentages, shown above branches, are based on 1000 replicates. Arrowheads indicate points of incongruence in the ITS and plastid trees. Filled circles represent clades not present in the strict consensus.

TABLE 1. Comparison of tree statistics for maximum parsimony analyses of sequence data for Vandaeae.

Tree statistics	ITS	matK	trnL-F	matK + trnL-F	ITS + matK + trnL-F
Length (aligned)	1767	1235	1918	3173	5013
Informative characters	320	314	435	749	1069
Trees saved	126	20 000+	20 000+	20 000+	2688
CI ₁	0.38	0.54	0.55	0.54	0.48
CI ₂	0.34	0.44	0.43	0.43	0.39
RI	0.71	0.75	0.72	0.73	0.72
RC	0.27	0.40	0.40	0.40	0.34
Clades with >85 BP	46	37	42	71	77

Notes: CI₁, consistency index; CI₂, consistency index excluding uninformative characters; RI, retention index; RC, rescaled consistency index; BP, bootstrap percentage.

clade of primarily Malagasy taxa (93 BP) was sister to an unresolved clade of Old and New World angraecoids (95 BP).

Mapping the 24 structural characters onto a representative molecular tree (showing all most parsimonious states at each node) revealed few patterns of anatomical character evolution because of the extreme homoplasy in the structural data set (for mapping of each character see Appendix S9 in Supplemental Data with the online version of this article). The synapomorphies of Vandaeae s.s. supported by character mapping (Appendix S10, see Supplemental Data with the online version of this article) were character 2, loss of tilosomes; character 6, presence of aeration complexes; character 14, loss of a hypodermis; character 18, presence of stigmata; character 21, loss of mucilage; character 22, presence of a monopodial stem.

Structural characters traced on combined topologies—Bootstrap consensus topologies of the structural and molecular data sets were manually compared for homogeneity assessment. Because there were no hard incongruencies among the data sets, the structural data were combined with the molecular data for phylogenetic analysis. Heuristic searches produced 690 most parsimonious trees with only slightly lower CI and RI values than molecular data alone (Table 2). However, there were more clades with BP > 85 and many fewer trees (Table 2) when the combined data set was used than when molecular data alone was used.

Results of tracing 24 anatomical and morphological characters along one of the 690 most parsimonious trees of the combined analysis were almost identical to that of mapping the same structural characters onto the molecular tree (Fig. 6 and Appendix S10, see Supplemental Data with the online version of this article). The synapomorphies supporting Vandaeae s.s. were almost the same in both instances, with the exception of hypodermal composition (character 15). However, when ACCTRAN optimization was used to map hypodermal composition onto a molecular topology, it also formed a synapomorphy of Vandaeae s.s. Most differences in the patterns of character evolution between mapping and tracing were due to the slightly different topologies of the combined and molecular trees.

DISCUSSION

Taxonomy of Vandaeae—The two African and Malagasy subtribes of monopodial Vandaeae, Angraecinae and Aerangidinae, were originally circumscribed by Summerhayes (1966) based on rostellum shape and chromosome number. These differences, however, seem to be phylogenetically misleading.

Chromosome counts from several sources (Charard, 1963; Jones, 1967; Arends et al., 1980; Jonsson, 1981; Arends and Van der Laan, 1983, 1986) support a general trend of $x=25$ for Aerangidinae and $x=19$ for Angraecinae, as indicated by Summerhayes (1966), but within each subtribe there is tremendous variation. This chromosomal variation is most prominent in large genera such as *Angraecum* ($x=19, 20, 21, 23, 24, 25$) and *Aerangis* ($x=21, 23, 25, 27$).

Morphologically, members of these two subtribes have similar vegetative and floral features and are often referred to collectively as “angraecoids.” *Angraecum distichum*, which is sister to the *Angraecopsis amaniensis*/*Diaphananthe millarii* clade (traditionally Aerangidinae) in the combined three-region analysis of our study, has a chromosome number of $x=25$. Several other African species of *Angraecum* not included in our study (*A. bancoense* Burg, *A. podochiloides* Schltr., and *A. subulatum* Lindl.) also have been reported as having $x=25$ (Arends et al., 1980). The chromosome number common to many Aerangidinae is $x=25$, suggesting affinities between Aerangidinae (which is primarily African) and the African species of *Angraecum*.

Individually, Aerangidinae and Angraecinae are polyphyletic, but together they form a well-supported monophyletic group in all molecular analyses. Therefore, we propose recognizing a broadly circumscribed subtribe Angraecinae (Summerh., *Kew Bulletin* 20: 188, 1966) that includes Aerangidinae (Summerh., *Kew Bulletin* 20: 188, 1966).

Unlike Angraecinae or Aerangidinae, Aeridinae are a well-supported subtribe in all analyses with a consistent chromosome number of $x=19$ (Woodard, 1951; Storey, 1952; Sagawa, 1962; Charard, 1963; Shindo and Kamemoto, 1963; Storey et al., 1963; Kamemoto, 1965; Jones, 1967; Tara and Kamemoto, 1970; Arends and Van der Laan, 1983). Therefore, our current circumscription of monopodial Vandaeae consists of only two subtribes: (1) the primarily Asian Aeridinae and (2) the African, Malagasy, and American Angraecinae.

For the majority of Aeridinae in our study, only data from the ITS region were gathered, and few species were collected for each genus. Therefore, many of our taxonomic conclusions are speculative. Based on our sampling, *Acampe*, *Chiloschista*, *Pelatantheria*, *Phalaenopsis*, *Taeniophyllum* (including *Microtatorchis*), and *Trichoglottis* are monophyletic (Fig. 3). *Cleisostoma*, *Robiquetia*, and *Vanda* (as currently circumscribed) are not monophyletic. In the strict consensus, *Chiloschista* and *Taeniophyllum* (both leafless) are part of one large clade. *Taeniophyllum* is sister to the leafy genus *Sarcochilus*, and *Chiloschista* is sister to a leafy clade of *Amesiella*, *Dyakia*, and *Tuberolabium*. This would suggest that

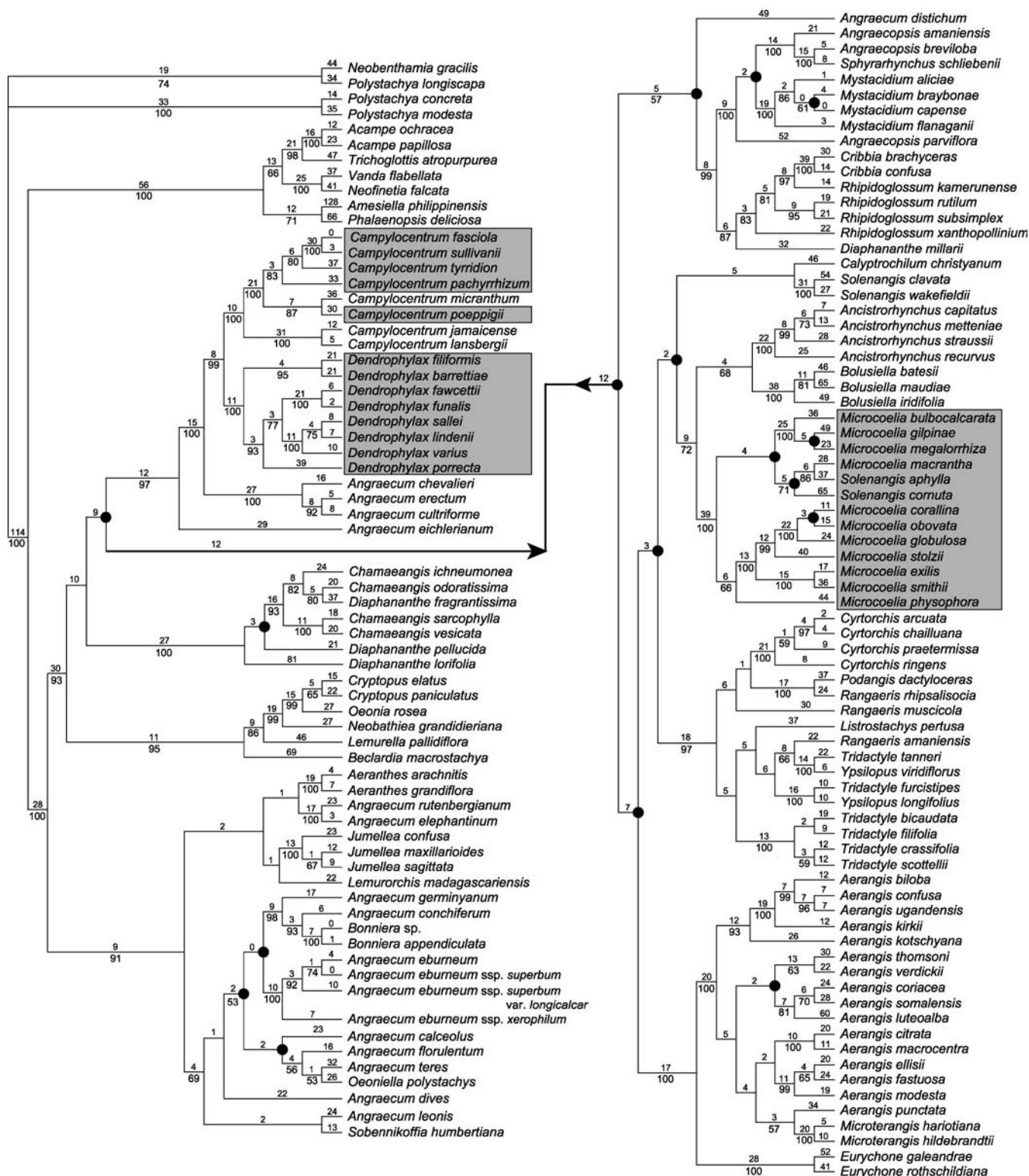


Fig. 5. One of 2688 trees resulting from a heuristic search of the combined molecular matrix (ITS, *trnL-F*, and *matK*). Filled circles represent clades not present in the strict consensus. Grey boxes surround leafless taxa. Fitch lengths are shown above branches; bootstrap percentages (1000 replicates) are shown below branches.

TABLE 2. Comparison of tree statistics for molecular and structural phylogenetic analyses for Vandaeae.

Tree statistics	Structural characters	Molecular characters	Combined structural + molecular
Length	171	4390	4677
Informative characters	24	993	1017
Trees saved	20 000+	20 000+	690
CI ₁	0.19	0.50	0.48
CI ₂	0.18	0.41	0.39
RI	0.72	0.70	0.69
RC	0.14	0.35	0.33
Clades with >85 BP	1	63	64

Notes: Molecular characters = combined sequence data from ITS, *matK*, and *trnL-F*. CI₁, consistency index; CI₂, consistency index excluding uninformative characters; RI, retention index; RC, rescaled consistency index; BP, bootstrap percentage.

leaflessness probably evolved at least three times within Aeridinae (once in each of the leafy ancestors of *Taeniophyllum*, *Chiloschista*, and *Phalaenopsis*).

Within *Phalaenopsis* (including *Doritis*, *Kingidium*, and *Kingiella*), two main clades exist (subgeneric classification following Christenson, 2001): (1) subgenus *Phalaenopsis* including sections *Aphyllae*, *Deliciosae*, *Esmeralda*, *Parishianae*, and *Proboscidioides* and (2) subgenus *Phalaenopsis* including sections *Phalaenopsis* and *Stauroglossis* as well as subgenus *Polychilos* including sections *Amboinensis*, *Polychilos*, and *Zebrinae*. Section *Aphyllae* is monophyletic (72 BP) if *P. lowii* (section *Proboscidioides*) is included. *Phalaenopsis lowii* is morphologically similar to section *Aphyllae* (Christenson, 2001), which would support its inclusion within section *Aphyllae*. The monophyly of section *Deliciosae* is questionable because *P. deliciosa* is more closely related to section *Aphyllae* (65 BP) than to *P. chibae*. Subgenus *Polychilos* is weakly supported as monophyletic (78 BP), excluding *P. fuscata*, which is unresolved in a clade with *P. deliciosa*/*P. pulcherrima* and *P. amabilis*/*P. cornucervi*.

Gussonea was originally circumscribed by Richard (1828) for *Angraecum aphyllum* Thouars. The genus was adopted by Schlechter (1918) and Perrier de la Bâthie (1941) to include all leafless African and Malagasy angraecoids. Schlechter (1918) divided *Gussonea* into subgenus *Eugussonea* with elongate stems and loosely spaced roots (i.e., *Solenangis aphylla* (Thouars) Summerh.) and subgenus *Taeniophylloides* with shortened stems and densely tufted roots (i.e., *Microcoelia* spp.). As noted by Summerhayes (1943), *Gussonea* A.Rich. is actually a later homonym of the Euphorbiaceae genus *Gussonia* (now a synonym of *Sebastiania*) previously described by Sprengel. In his treatment of the leafless angraecoids, Summerhayes (1943) split *Gussonea* into two genera, which are comparable to Schlechter's subgenera (1918), based on floral morphology and overall habit: the long-stemmed *Solenangis* and the short-stemmed *Microcoelia*. Currently, *Solenangis* includes two leafless species [*S. aphylla* and *S. cornuta* (Rchb.f.) Summerh.], one intermediate species with reduced leaves [*S. conica* (Schltr.) L. Jonss.], and three species with well-developed leaves [*S. clavata* (Rolfe) Schltr., *S. scandens* (Schltr.) Schltr., and *S. wakefieldii* (Rolfe) P.J. Cribb & J. Stewart]. Based on sequence data (Fig. 5), the leafless *S. aphylla* and *S. cornuta* are more closely related to other species of *Microcoelia* than to the leafy species of *Solenangis* (*S. clavata* and *S. wakefieldii*). In the strict consensus of the

combined analyses, *S. clavata* + *S. wakefieldii* are sister to *Microcoelia* + leafless *Solenangis* (although there is no support for this relationship). *Solenangis aphylla* was originally transferred from *Gussonea* into the genus *Microcoelia* by Summerhayes (1936), and the results of our molecular phylogenetic analyses support the transfer of *S. aphylla* back to *M. aphylla* (Thouars) Summerh.

***Microcoelia aphylla* (Thouars) Summerh., Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew: 233, 1936.**

Basionym—*Angraecum aphyllum* Thouars, Histoire Particulière des Plantes Orchidées: 72, 1822.

Synonyms—*Gussonea aphylla* A. Rich., Mémoires de la Société d'Histoire Naturelle de Paris 4: 68, 1828. *Saccolabium aphyllum* Lindl., Genera and Species of Orchidaceous Plants: 223, 1833. *Mystacidium aphyllum* T. Durand & Schinz, *Conspectus Florae Africae* 5: 51, 1894. *Rhaphidorhynchus aphyllus* Finet, *Mémoires de la Société Botanique de France*, Paris 9: 35, 1907. *Angraecum defoliatum* Schltr., *Annales du Musée d'Histoire Naturelle de Marseille* 1: 191, 1913. *Gussonea defoliata* Schltr., *Beihefte zum Botanischen Centralblatt. Kassel* 33: 425, 1915. *Solenangis aphylla* (Thouars) Summerh., *Botanical Museum Leaflets* 11: 159, 1943.

Solenangis cornuta was a new combination by Summerhayes (1942), transferred from *Gussonea cornuta* Ridl. The results of our molecular phylogenetic analyses support the transfer of *S. cornuta* to *M. cornuta* (Fig. 5). The type species of *Solenangis*, *S. scandens*, possesses well-developed photosynthetic leaves. Further sampling of *S. scandens* (the type species) and *S. conica* (an intermediate species with small, deciduous, conical leaves) would be needed before conclusions about the monophyly of the leafy species of *Solenangis* can be made.

***Microcoelia cornuta* (Ridl.) Carlsward, comb. nov.**

Basionym—*Gussonea cornuta* Ridl., *Journal of Botany, London* 23: 310, 1885.

Synonyms—*Angraecum cornutum* Rchb.f., *Flora* 68: 538, 1885. *Angraecum cyclochilum* Schltr., *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 38: 160, 1906. *Rhaphidorhynchus cornutus* Finet, *Mémoires de la Société Botanique de France*, Paris 9: 34, 1907. *Solenangis cornuta* (Ridl.) Summerh., *Botanical Museum Leaflets* 11: 160.

Two other African leafless genera that have been recognized as segregates of *Microcoelia* are *Chauliodon* Summerh. and *Encheiridion* Summerh. Summerhayes transferred *Microcoelia macrorrhynchia* (Schltr.) Summerh. to *Encheiridion macrorrhynchium* (Schltr.) Summerh. based on the trilobed lip and horizontally elongate rostellum (Summerhayes, 1943). Jonsson (1981) in his revision of *Microcoelia* found several intermediate species [namely *M. leptostele* (Summerh.) L. Jonss., *M. nyungwensis* L. Jonss., and *M. sanfordii* L. Jonss.] that formed a continuum in lip morphology with *Encheiridion macrorrhynchium*. Given this floral evidence, Jonsson logically transferred *Encheiridion* back into *Microcoelia* (Jonsson, 1981).

In the same publication on leafless angraecoids in which

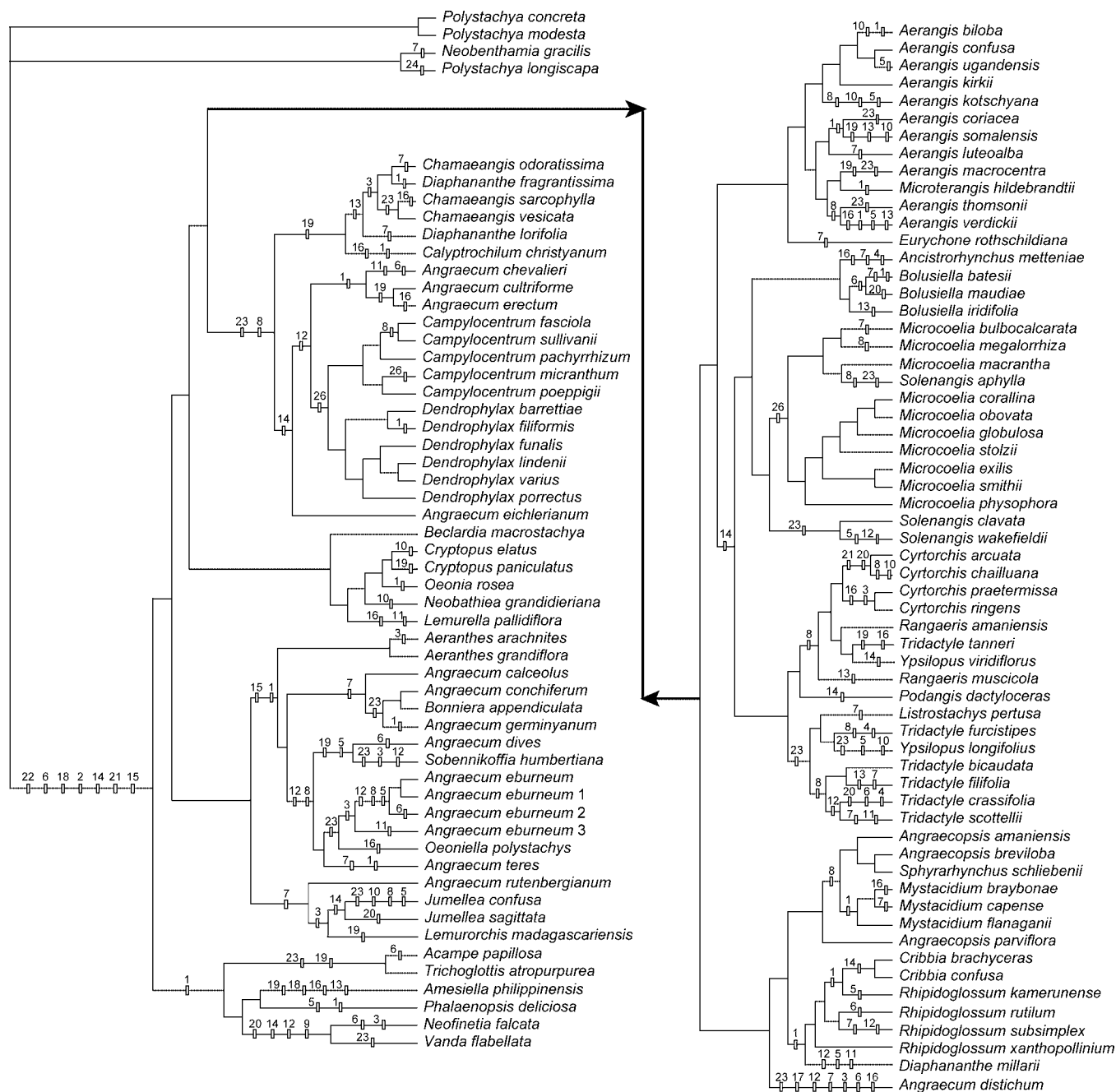


Fig. 6. Hypothesis of structural character evolution with characters traced on a representative (one of 690) combined molecular + structural phylogeny. Numbers represent character states as delimited in Appendix S3 (see Supplemental Data with the online version of this article).

Summerhayes (1943) introduced *Encheiridion*, he also created a new genus and species, *Chauliodon buntingii* Summerh., based on callus morphology in the lip. Summerhayes mentioned the resemblance of this species to *Microcoelia*, especially in vegetative habit and pollinarium structure. During his monographic work on *Microcoelia*, Jonsson (1981) transferred Summerhayes' *Chauliodon buntingii* (Summerhayes, 1943) to *Chauliodon deflexicalcaratum* (DeWild.) L. Jonss. *Chauliodon deflexicalcaratum* was originally described by De Wildeman (1916) as *Angraecum deflexicalcaratum* and

was later transferred to the short-stemmed section of *Gussonea* by Schlechter (1918). Summerhayes (1943) transferred *Gussonea deflexicalcarata* (DeWild.) Schltr. to *Microcoelia deflexicalcarata* (DeWild.) Summerh. in the same publication in which he described the new genus *Chauliodon*. Jonsson (1979) attributed Summerhayes' confusion to not having access to De Wildeman's original material of *Angraecum deflexicalcaratum*. In examining type material for both *M. deflexicalcarata* and *Chauliodon buntingii*, Jonsson felt that these species were conspecific and should be lumped together under the older name of *C. deflexicalcarata*. Although

Chauliodon and *Encheiridion* were not sampled in our study, their vegetative and floral similarity to *Microcoelia* is a strong indication that they should be included in *Microcoelia*.

The only remaining leafless Old World member of Aerangidinae is *Taeniorrhiza* Summerh., a monotypic genus native to Africa. In his description of the genus, Summerhayes (1942) separated *Taeniorrhiza* from the remaining leafless Aerangidinae by its flattened roots, single-flowered inflorescences, winged column, and *Eulophia*-like lip. Unfortunately, *Taeniorrhiza* was not sampled in our study, and because it is so morphologically distinct, conclusions of its phylogenetic position cannot be made.

Rhipidoglossum has traditionally been segregated from *Diaphananthe* by two key features: a column foot and a distinct conical projection or “tooth-like” callus at the opening of the spur (Summerhayes, 1960; Garay, 1972b; Senghas, 1986). However, Summerhayes (1960) and Cribb (1989) observed a complete continuum in these floral character states within *Diaphananthe* and *Rhipidoglossum*. Sequence data support the inclusion of *Rhipidoglossum* with *Cribbia* and *Diaphananthe millarii*, whereas most species of *Diaphananthe* in our study are most closely related to *Chamaeangis* (Fig. 5). It is clear from these preliminary analyses that *Rhipidoglossum* and *Diaphananthe* do not form a monophyletic group. However, given the relatively small percentage of *Diaphananthe* and *Rhipidoglossum* species sampled in our study (Appendix S1, see Supplemental Data with the online version of this article), no nomenclatural changes are made here.

In all molecular analyses, species of *Aerangis* form a monophyletic group with the inclusion of *Microterangis hariatiana* (the type species) and *M. hildebrandtii*. Before Senghas erected the genus *Microterangis* (1985), most of its members were included in *Chamaeangis* section *Microterangis* (Schlechter, 1918). Morphologically, flowers of *Microterangis* look more similar to *Chamaeangis* than to *Aerangis*, but molecular data clearly indicate this to be convergence. The type species (*M. hariatiana*) and *M. hildebrandtii* should both be transferred to *Aerangis*, but doing this would leave the five species not sampled for our study in a state of nomenclatural limbo (with no genus name). The remaining five species of *Microterangis* may form part of the large *Aerangis* clade (along with *M. hariatiana* and *M. hildebrandtii*); they may form a clade that will need a new generic name; or they may be closely related to other genera of Angraecinae. We feel the genus needs further study before name changes can be made.

Paralogous ITS sequences were never found in New World Angraecinae or their African *Angraecum* relatives, but paralogy was common in the Malagasy Angraecinae (especially *Aerantes*, *Angraecum*, *Oeoniella*, *Sobennikoffia*, *Jumellea*, and *Lemurorchis*). *Cryptopus*, *Oeonia*, *Neobathiea*, and *Lemurella* (also Malagasy Angraecinae containing orthologous ITS sequences) are most closely related to *Beclardia* (Malagasy Aerangidinae), and this group is embedded in a large, poorly resolved angraecoid clade (Fig. 5). The topology of the combined tree suggests that duplication events creating paralogy probably originated in Madagascar.

Angraecum is clearly polyphyletic in all analyses. With over 200 species (only 15 of which were included in our study) and distribution in America (*Campylocentrum* and *Dendrophyllax*) as well as Madagascar and Africa (*Aerantes*, *Jumellea*, *Lemurorchis*, and Aerangidinae), its polyphyletic nature is not surprising. *Bonnieria*, *Oeoniella*, and *Sobennikoffia* are embedded within a clade composed primarily of *Angraecum* (Fig. 5).

Jumellea and *Aerantes* each seem to be monophyletic. However, without more extensive taxon sampling, taxonomic decisions about most traditional Angraecinae are premature.

The clade of *Cyrtorchis*, *Listrostachys*, *Podangis*, *Rangaeris*, *Tridactyle*, and *Ypsilopus* (Fig. 5) show that generic circumscriptions for many of these genera may need to be reevaluated with denser taxon sampling. *Cyrtorchis* is clearly monophyletic, whereas *Listrostachys* and *Podangis* may also be distinct genera (only one of the two species of *Listrostachys* was sampled and *Podangis* is monotypic). Some species of *Rangaeris* are more closely related to other genera (*Podangis* and *Tridactyle*) than they are to one another, but no clear pattern of relationships was found. The two species of *Ypsilopus* sampled are more closely related to *Tridactyle* than they are to one another, so *Ypsilopus* should probably be transferred to *Tridactyle*. However, more taxon sampling within *Tridactyle* (only six of the 43 species were sampled) would be needed to make any taxonomic conclusions about the generic concept of *Tridactyle*.

Mystacidium forms a monophyletic group in the combined bootstrap analysis, but it is embedded in a larger clade that includes several species of *Angraecopsis* and one species of *Sphyrarhynchus* (Fig. 5). However, more extensive taxon sampling of *Mystacidium* and *Angraecopsis* is needed for generic circumscription.

Although there are problems with the monophyly of several of the genera mentioned earlier, many angraecoid genera are monophyletic in the combined bootstrap consensus (Fig. 5). The New World *Campylocentrum* and *Dendrophyllax*, as redefined by Carlswald et al. (2003), form well-supported clades. Old World angraecoid genera that also form monophyletic groups are *Cryptopus*, *Aerantes*, *Jumellea*, *Eurychone*, *Ancistrorhynchus*, *Bolusiella*, and *Cyrtorchis*. *Oeonia*, *Neobathiea*, *Lemurella*, *Beclardia*, and *Lemurorchis* are all small genera with only one species sampled from each, and they are likely monophyletic as well.

Evolution of leaflessness—Sequence data derived from the ITS region for all monopodial subtribes (Aeridinae, Aerangidinae, and Angraecinae) supported a monophyletic Vandaeae s.s. (Figs. 2, 3). With this overall tree, it appears that leaflessness has arisen six to seven times within Vandaeae, depending on the occurrence of reversals to a leafy condition: three to four times in Asia with *Taeniophyllum*, *Chiloschista*, and *Phalaenopsis*; once in Africa and Madagascar with *Microcoelia* (including leafless *Solenangis*); and at least twice in the New World with *Dendrophyllax* and *Campylocentrum*. From examining the pattern of variation in anatomical and morphological characters among leafy and leafless Vandaeae, a monopodial growth habit, a reduction to nonphotosynthetic leaves, and the presence of aeration complexes in photosynthetic roots (the cortical stomatal complexes of Carlswald et al., in press) may be important structural precursors to the leafless habit. This extremely reduced epiphytic habit probably represents an adaptation for carbon conservation (Benzing and Ott, 1981) and/or water conservation in a canopy environment.

Our molecular phylogenetic work not only represents a foundation for examining evolutionary questions within a horticulturally important plant group, it also represents an integrated approach to answering a general question of parallelism that has occurred systematically and geographically in different lineages.

LITERATURE CITED

- ARENDS, J. C., W. J. VAN DER BURG, AND F. M. VAN DER LAAN. 1980. Notes on African orchids. In J. C. Arends and H. C. D. de Wit [eds.], *Liber gratulatorius in honorem H.C.D. de Wit*, 449. H. Veenman & Zonen, Wageningen, Netherlands.
- ARENDS, J. C., AND F. M. VAN DER LAAN. 1983. Cytotaxonomy of the monopodial orchids of the African and Malagasy regions. *Genetica* 62: 81–94.
- ARENDS, J. C., AND F. M. VAN DER LAAN. 1986. Cytotaxonomy of the Vandaeae. *Lindleyana* 1: 33–41.
- BATEMAN, R. M., A. M. PRIDGEON, AND M. W. CHASE. 1997. Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 2. Infrageneric relationships and reclassification to achieve monophyly of *Orchis sensu stricto*. *Lindleyana* 12: 113–141.
- BENTHAM, G. 1881. Notes on Orchideae. *Journal of the Linnean Society, Botany* 18: 281–360.
- BENZING, D. H., W. E. FRIEDMAN, G. PETERSON, AND A. RENFROW. 1983. Shootlessness, velamentous roots, and the pre-eminence of Orchidaceae in the epiphytic biotope. *American Journal of Botany* 70: 121–133.
- BENZING, D. H., AND D. W. OTT. 1981. Vegetative reduction in epiphytic Bromeliaceae and Orchidaceae: its origin and significance. *Biotropica* 13: 131–140.
- BLATTNER, F. R. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *BioTechniques* 27: 1180–1186.
- BOARD OF TRUSTEES OF THE ROYAL BOTANIC GARDENS, KEW. 2004. World checklist of monocotyledons. [Online, accessed Dec 2004] Website, <http://www.rbgb.org.uk/monocotChecklist/>.
- BRUMMITT, R. K., AND C. E. POWELL. 1992. Authors of plant names. Royal Botanic Gardens, Kew, UK.
- CAMERON, K. M. 2001. An expanded phylogenetic analysis of Orchidaceae using three plastid genes: *rbcL*, *atpB*, and *psaB*. *American Journal of Botany* 88: 104.
- CAMERON, K. M., M. W. CHASE, W. M. WHITTEN, P. J. KORES, D. C. JARRELL, V. A. ALBERT, T. YUKAWA, H. G. HILLS, AND D. H. GOLDMAN. 1999. A phylogenetic analysis of the Orchidaceae: evidence from *rbcL* nucleotide sequences. *American Journal of Botany* 86: 208–224.
- CARLSWARD, B. S., W. L. STERN, AND B. BYTEBIER. In press. Comparative vegetative anatomy and systematics of the angraecoids (Vandaeae, Orchidaceae) with an emphasis on the leafless habit. *Botanical Journal of the Linnean Society*.
- CARLSWARD, B. S., W. M. WHITTEN, AND N. H. WILLIAMS. 2003. Molecular phylogenetics of neotropical leafless Angraecinae (Orchidaceae): reevaluation of generic concepts. *International Journal of Plant Sciences* 164: 43–51.
- CHARARD, R. 1963. Contributions a l'étude cyto-taxonomique des Orchidées. *Revue de Cytologie et de Biologie Végétales* 26: 1–58.
- CHASE, M. W., J. V. FREUDENSTEIN, K. M. CAMERON, AND R. L. BARRETT. 2003. DNA data and Orchidaceae systematics: a new phylogenetic classification. In K. W. Dixon, S. P. Kell, R. L. Barrett, and P. J. Cribb [eds.], *Orchid conservation*, 69–89. Natural History Publications, Kota Kinabalu, Malaysia.
- CHASE, M. W., AND J. D. PALMER. 1989. Chloroplast DNA systematics of lilioid monocots: resources, feasibility, and an example from the Orchidaceae. *American Journal of Botany* 76: 1720–1730.
- CHRISTENSON, E. A. 2001. *Phalaenopsis*: a monograph. Timber Press, Portland, Oregon, USA.
- COCKBURN, W., J. C. GOH, AND P. N. AVADHANI. 1985. Photosynthetic carbon assimilation in a shootless orchid, *Chiloschista usneoides* (Don) Lindl. *Plant Physiology* 77: 83–86.
- CRIBB, P. 1989. Flora of tropical east Africa, Orchidaceae, part 3. A.A. Balkema, Rotterdam, Netherlands.
- DE WILDEMAN, E. 1916. Additions à la flore du Congo. *Bulletin du Jardin Botanique de l'État (Bruxelles)* 5: 117–268.
- DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- DRESSLER, R. L. 1981. The orchids: natural history and classification. Harvard University Press, Cambridge, Massachusetts, USA.
- DRESSLER, R. L. 1989. The vandoid orchids: a polyphyletic grade? *Lindleyana* 4: 89–93.
- DRESSLER, R. L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press, Portland, Oregon, USA.
- DRESSLER, R. L., AND C. H. DODSON. 1960. Classification and phylogeny in the Orchidaceae. *Annals of the Missouri Botanical Garden* 47: 25–68.
- FREUDENSTEIN, J. V., AND F. N. RASMUSSEN. 1999. What does morphology tell us about orchid relationships? A cladistic analysis. *American Journal of Botany* 86: 225–248.
- GARAY, L. A. 1960. On the origin of the Orchidaceae. *Botanical Museum Leaflets, Harvard University* 19: 57–95.
- GARAY, L. A. 1972a. On the origin of the Orchidaceae II. *Journal of the Arnold Arboretum* 53: 202–215.
- GARAY, L. A. 1972b. On the systematics of the monopodial orchids I. *Botanical Museum Leaflets, Harvard University* 23: 149–212.
- GRAHAM, S. W., J. R. KOHN, B. R. MORTON, J. E. ECKENWALDER, AND S. C. H. BARRETT. 1998. Phylogenetic congruence and discordance among one morphological and three molecular data sets from Pontederiaceae. *Systematic Biology* 47: 545–567.
- HOLMGREN, P. K., N. H. HOLMGREN, AND L. C. BARNETT. 1990. Index herbariorum, part I, The herbaria of the world. New York Botanical Garden, New York, New York, USA.
- JARRELL, D. C., AND M. T. CLEGG. 1995. Systematic implications of the chloroplast-encoded *matK* gene on the tribe Vandaeae (Orchidaceae). *American Journal of Botany* 82: 137.
- JOHNSON, L. A., AND D. E. SOLTIS. 1998. Assessing congruence: empirical examples from molecular data. In D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], *Molecular systematics of plants II: DNA sequencing*, 297–343. Kluwer Academic, Boston, Massachusetts, USA.
- JONES, K. 1967. The chromosomes of orchids, II. *Kew Bulletin* 21: 151–156.
- JONSSON, L. 1979. New combinations in the African genera *Chauliodon* and *Solenangis* (Orchidaceae). *Botaniska Notiser* 132: 381–384.
- JONSSON, L. 1981. A monograph of the genus *Microcoelia* (Orchidaceae). Borgströms Tryckeri AB, Motala, Sweden.
- KAMEMOTO, H. 1965. Chromosomes and species relationships in the *Vanda* alliance. Straits Times Press, Singapore, Malaysia.
- LINDLEY, J. 1830–1840. The genera and species of orchidaceous plants. Ridgways, London, UK.
- MADDISON, D. R., AND W. P. MADDISON. 2000. MacClade 4: analysis of phylogeny and character evolution, version 4.06. Sinauer, Sunderland, Massachusetts, USA.
- NEYLAND, R., AND L. E. URBATSCH. 1996. Phylogeny of subfamily Epidendroideae (Orchidaceae) inferred from *ndhF* chloroplast gene sequences. *American Journal of Botany* 83: 1195–1206.
- PERRIER DE LA BATHIE, H. 1941. Orchidées. In HUBERT, H. [ed.], *Flore de Madagascar*, 49 famille, 1–542. Imprimerie Officielle, Tananarive, Madagascar.
- POREMBSKI, S., AND W. BARTHOLOTT. 1988. Velamen radicum micromorphology and classification of Orchidaceae. *Nordic Journal of Botany* 8: 117–137.
- PRIDGEON, A. M., P. J. CRIBB, M. W. CHASE, AND F. N. RASMUSSEN. 1999. *Genera Orchidacearum*. I. General introduction, Apostasioideae, Cyripedioideae. Oxford University Press, New York, New York, USA.
- RAMBOUT, A. 1996. Se-Al: Sequence Alignment editor, version 2.0a11. University of Oxford. [Online]. Website <http://evolve.zoo.ox.ac.uk/>.
- RASMUSSEN, F. N. 1986. On the various contrivances by which pollinia are attached to viscidia. *Lindleyana* 1: 21–32.
- REEVES, G., M. W. CHASE, P. GOLDBLATT, P. RUDALL, M. F. FAY, A. V. COX, B. LEJEUNE, AND T. SOUZA-CHIES. 2001. Molecular systematics of Iridaceae: evidence from four plastid regions. *American Journal of Botany* 88: 2074–2087.
- RICHARD, A. 1828. Monographie des Orchidées des Iles de France et de Bourbon. *Mémoires de la Société d'Histoire Naturelle de Paris* 4: 1–83.

- SAGAWA, Y. 1962. Cytological studies of the genus *Phalaenopsis*. *American Orchid Society Bulletin* 31: 459–465.
- SCHLECHTER, R. 1918. Versuch einer natürlichen Neuordnung der afrikanischen agraekoiden Orchidaceen. *Beihefte zum Botanischen Centralblatt Abteilung II*. 36: 62–181.
- SENGHAS, K. 1985. *Cribbia* und *Microterangis*, zwei neue Orchideen Gattungen. *Die Orchidee* 36: 19–22.
- SENGHAS, K. 1986. *Rhipidoglossum*. *Die Orchidee* 18: 1110–1112.
- SHINDO, K., AND H. KAMEMOTO. 1963. Karyotype analysis of some Sarcanthine orchids. *American Journal of Botany* 50: 73–79.
- SOLTIS, D. E., P. S. SOLTIS, M. E. MORT, M. W. CHASE, V. SAVOLAINEN, S. B. HOOT, AND C. M. MORTON. 1998. Inferring complex phylogenies using parsimony: an empirical approach using three large DNA data sets for angiosperms. *Systematic Biology* 47: 32–42.
- STOREY, W. B. 1952. Chromosome numbers of some *Vanda* species and hybrids. *American Orchid Society Bulletin* 21: 801–806.
- STOREY, W. B., H. KAMEMOTO, AND K. SHINDO. 1963. Chromosomes of *Vanda spatulata* and its hybrids. *American Orchid Society Bulletin* 32: 703–709.
- SUMMERHAYES, V. S. 1936. African Orchids. VIII. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)* 1936: 221–233.
- SUMMERHAYES, V. S. 1942. African Orchids. XII. *Botanical Museum Leaflets (Harvard University)* 10: 257.
- SUMMERHAYES, V. S. 1943. African Orchids. XIII. *Botanical Museum Leaflets (Harvard University)* 11: 137–170.
- SUMMERHAYES, V. S. 1960. African Orchids. XXVII. *Kew Bulletin* 14: 126–157.
- SUMMERHAYES, V. S. 1966. African Orchids. XXX. *Kew Bulletin* 20: 165–199.
- SUN, Y., D. Z. SKINNER, G. H. LIANG, AND S. H. HULBERT. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32.
- SWOFFORD, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.
- SZLACHETKO, D. L. 1995. Systema Orchidialium. *Fragmenta Floristica et Geobotanica Supplementum* 3: 1–152.
- TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- TARA, M., AND H. KAMEMOTO. 1970. Karyotype relationships in Sarcanthinae (Orchidaceae). *American Journal of Botany* 57: 176–182.
- TOPIK, H., T. YUKAWA, AND M. ITO. 2005. Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): insights from plastid *matK* and nuclear ribosomal ITS sequences. *Journal of Plant Research* 118: 271–284.
- TSAL, C. C., S. C. HUANG, AND C. H. CHOU. 2003. Phylogeny of the genus *Phalaenopsis* (Orchidaceae) with emphasis on the subgenus *Phalaenopsis* based on the sequences of the internal transcribed spacers 1 and 2 of rDNA. *Journal of Horticultural Science and Biotechnology* 78: 879–887.
- VAN DEN BERG, C., D. H. GOLDMAN, J. V. FREUDENSTEIN, A. M. PRIDGEON, K. M. CAMERON, AND M. W. CHASE. 2005. An overview of the phylogenetic relationships within Epidendroideae inferred from multiple DNA regions and circumscription of the Epidendreae and Arethuseae (Orchidaceae). *American Journal of Botany* 92: 613–624.
- WENDEL, J. F., AND J. J. DOYLE. 1998. Phylogenetic incongruence: window into genome history and molecular evolution. In D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], *Molecular systematics of plants II: DNA sequencing*, 265–296. Kluwer Academic, Boston, Massachusetts, USA.
- WHITTEN, M. W., N. H. WILLIAMS, AND M. W. CHASE. 2000. Subtribal and generic relationships of Maxillarieae (Orchidaceae) with emphasis on Stanhopeinae: combined molecular evidence. *American Journal of Botany* 87: 1842–1856.
- WOODARD, J. W. 1951. Some chromosome numbers in *Phalaenopsis*. *American Orchid Society Bulletin* 20: 356–358.
- ZIEGLER, B. 1981. Mikromorphologie der Orchideensamen unter Berücksichtigung taxonomischer Aspekte. Ph.D. dissertation, Ruprecht Karls Universität, Heidelberg, Germany.

APPENDIX. Taxa of Vandaeae for molecular study. Taxon names and authorities follow the Royal Botanic Gardens (RBG), *Kew World Checklist of Monocotyledons* (2004). Author abbreviations follow Brummitt and Powell (1992). Herbarium abbreviations follow Holmgren et al. (1990). “ver. La Croix” are those specimens with no voucher that have been identified by Isobyl La Croix. PCP, Plant Conservation Programme of the East African National Museum; OIC, Orchid Identification Center spirit collection at The Marie Selby Botanical Gardens. “Hort. specimens” are cultivated plants from various individual and commercial orchid growers: FLMNH, Florida Museum of Natural History greenhouse collection; PCP, Plant Conservation Programme Living Collection; SEL, Marie Selby Botanical Gardens (numbers represent living plant accession numbers); ABG, Atlanta Botanical Garden; and SBG, Singapore Botanic Gardens. GenBank accession numbers are listed in the following order: ITS, *trnL-F*, *matK*. An asterisk denotes a paralogous ITS sequence. A dash indicates the region was not sampled.

Taxon; Voucher (location); Source; ITS, *trnL-F*, *matK*.

Tribe Vandaeae, Subtribe Aerangidinae

Aerangis biloba (Lindl.) Schltr.; Whitten 1938 (FLAS); FLMNH hort.; DQ091594; DQ091455; DQ091331. *A. citrata* (Thouars) Schltr.; Whitten 1788 (FLAS); Countryside Orchids; DQ091600; DQ091461; DQ091337. *A. confusa* J.Stewart; Bytebier s.n. (EA); PCP hort.; DQ091595; DQ091456; DQ091332. *A. coriacea* Summerh.; No voucher; Hoosier Orchid Co.; DQ091610; —; —. *A. coriacea*; Bytebier 562 (EA); PCP hort.; DQ091609; DQ091469; DQ091345. *A. ellisii* var. *grandiflora* J.Stewart; Carlsward 401 (FLAS); Hoosier Orchid Co.; DQ091602; DQ091463; DQ091339. *A. fastuosa* (Rchb. f.) Schltr.; Carlsward 402 (FLAS); Countryside Orchids; DQ091604; DQ091464; DQ091340. *A. kirkii* (Rchb.f.) Schltr.; Bytebier 637 (EA); PCP hort.; DQ091596; DQ091457; DQ091333. *A. kotschyana* (Rchb.f.) Schltr.; Bytebier 671 (EA); PCP hort.; DQ091598; DQ091459; DQ091335. *A. luteoalba* var. *rhodosticta* (Kraenzl.) J. Stewart; Bytebier 691 (EA); PCP hort.; DQ091611; DQ091471; DQ091347. *A. macrocentra* (Schltr.) Schltr.; Kew 779 (K); J. Hermans hort.; DQ091601; DQ091462; DQ091338. *A. modesta* (Hook.f.) Schltr.; Carlsward 242 (FLAS); ABG hort.; DQ091603;

DQ091465; DQ091341. *A. punctata* J.Stewart; Carlsward 328 (FLAS); Hoosier Orchid Co.; DQ091605; DQ091466; DQ091342. *A. somalensis* (Schltr.) Schltr.; Bytebier 1549 (EA); PCP hort.; DQ091612; DQ091470; DQ091346. *A. thomsonii* (Rolfe) Schltr.; Kirika 968 (EA); PCP hort.; DQ091599; DQ091460; DQ091336. *A. ugandensis* Summerh.; Bytebier 681 (EA); PCP hort.; DQ091597; DQ091458; DQ091334. *A. verdickii* (DeWild.) Schltr.; No voucher; Countryside Orchids; DQ091613; DQ091472; DQ091348. *Ancistrorhynchus capitatus* (Lindl.) Summerh.; Carlsward 276 (FLAS); Uzumara Orchids; DQ091643; DQ091475; DQ091351. *A. metteniae* (Kraenzl.) Summerh.; Carlsward 398 (FLAS); Uzumara Orchids; DQ091644; DQ091476; DQ091352. *A. recurvus* Finet; No voucher, ver. La Croix; Uzumara Orchids; DQ091646; DQ091478; DQ091354. *A. straussii* (Schltr.) Schltr.; Carlsward 285 (FLAS); Uzumara Orchids; DQ091645; DQ091477; DQ091353. *Angraecopsis amaniensis* Summerh.; No voucher, ver. La Croix; Uzumara Orchids; DQ091568; DQ091482; DQ091357. *A. breviflora* Summerh.; Bytebier 307 (EA); PCP hort.; DQ091569; DQ091483; DQ091358. *A. parviflora* (Thouars) Schltr.; Carlsward

- 291 (FLAS); Uzumara Orchids; DQ091575; DQ091489; DQ091364. *A. parviflora*; *Kew* 4363 (K); J. Hermans hort.; DQ091576; —; —.
- Beclardia macrostachya* (Thouars) A.Rich.; *Carlsward* 289 (FLAS); Uzumara Orchids; DQ091592; DQ091497; DQ091372. *B. macrostachya*; *Kew* 3536 (K); J. Hermans hort.; DQ091593; —; —.
- Bolusiella batesii* (Rolfe) Schltr.; *Carlsward* 152 (FLAS), *Nkongmeneck* 2087 (SEL); SEL hort. (1997–0173A); DQ091663; DQ091479; —. *B. iridifolia* (Rolfe) Schltr.; *Bytebier* 1113 (EA); PCP hort.; DQ091665; DQ091481; DQ091356. *B. maudiae* (Bolos) Schltr.; *Bytebier* 485 (EA); PCP hort.; DQ091664; DQ091480; DQ091355.
- Chamaeangis ichneumonea* (Lindl.) Schltr.; La Croix 1101 (?), *Carlsward* 286 (FLAS); Uzumara Orchids; DQ091616; DQ091498; DQ091373. *C. odoratissima* (Rchb.f.) Schltr.; No voucher, ver. La Croix; Uzumara Orchids; DQ091617; DQ091499; DQ091374. *C. sarcophylla* Schltr.; *Bytebier* 339 (EA); PCP hort.; DQ091621; DQ091503; DQ091378. *C. vesicata* (Lindl.) Schltr.; *Bytebier* 796 (EA); PCP hort.; DQ091622; DQ091504; DQ091379. *C. vesicata*; *Kew* 399 (K); J. Hermans hort.; DQ091623; —; —.
- Cribbia brachyceras* (Summerh.) Senghas; *Bytebier* 361 (EA); PCP hort.; DQ091577; DQ091490; DQ091365. *C. confusa* P.J.Cribb; *Kew* 3936 (K); J. Hermans hort.; DQ091578; DQ091491; DQ091366.
- Cyrtorchis arcuata* (Lindl.) Schltr.; *Bytebier* 676 (EA); PCP hort.; DQ091624; DQ091505; DQ091380. *C. chailluana* (Hook.f.) Schltr.; *Carlsward* 156 (SEL); SEL hort. (1996–0294); DQ091625; DQ091506; DQ091381. *C. praeternissa* Summerh.; No voucher; Countryside Orchids; DQ091627; DQ091507; DQ091382. *C. ringens* (Rchb.f.) Summerh.; *Carlsward* 226 (FLAS), *Nkongmeneck* 1388 (SEL); SEL hort. (1997–0154A); DQ091626; DQ091508; DQ091383.
- Diaphanathe fragrantissima* (Rchb.f.) Schltr.; *Kirika* 536 (EA); PCP hort.; DQ091618; DQ091500; DQ091375. *D. lorifolia* Summerh.; *Bytebier* 346 (EA); PCP hort.; DQ091619; DQ091501; DQ091376. *D. millarii* (Bolos) H.P. Linder; *Carlsward* 346 (FLAS); Countryside Orchids; DQ091579; DQ091493; DQ091368. *D. pellucida* (Lindl.) Schltr.; *Carlsward* 241 (FLAS); ABG hort.; DQ091620; DQ091502; DQ091377.
- Eurychone galeandrae* (Rchb.f.) Schltr.; *Carlsward* 293 (FLAS); Uzumara Orchids; DQ091614; DQ091473; DQ091349. *E. rothschildiana* (O'Brien) Schltr.; *Carlsward* 407 (FLAS); Sunset Orchids; DQ091615; DQ091474; DQ091350.
- Listrostachys pertusa* (Lindl.) Rchb.f.; *Carlsward* 399 (FLAS); Uzumara Orchids; DQ091637; DQ091509; DQ091384.
- Microcoelia bulbocalcarata* L.Jonss.; No voucher, ver. La Croix; Uzumara Orchids; DQ091647; DQ091521; DQ091396. *M. corallina* Summerh.; No voucher, ver. La Croix; Uzumara Orchids; DQ091653; DQ091527; DQ091402. *M. exilis* Lindl.; *Whitten* 1937 (FLAS); Sunset Orchids; DQ091658; DQ091531; DQ091406. *M. exilis*; *Bytebier* 1255 (EA); PCP hort.; DQ091659; —; —. *M. gilpiniae* (Rchb.f. & S. Moore) Summerh.; *Carlsward* 290 (FLAS); Uzumara Orchids; DQ091649; DQ091522; DQ091397. *M. globulosa* (Hochst.) L.Jonss.; *Carlsward* 259 (FLAS); Uzumara Orchids; DQ091655; DQ091529; DQ091404. *M. globulosa*; *PCP* 488 (EA); PCP hort.; DQ091656; —; —. *M. macrantha* (H.Perrier) Summerh.; *Kew* 5391 (K); J. Hermans hort.; DQ091650; DQ091524; DQ091399. *M. megalorrhiza* (Rchb.f.) Summerh.; *Bytebier* 1250 (EA); PCP hort.; DQ091648; DQ091523; DQ091398. *M. obovata* Summerh.; *Bytebier* 1256 (EA); PCP hort.; DQ091654; DQ091528; DQ091403. *M. physophora* (Rchb.f.) Summerh.; *Bytebier* 629 (EA); PCP hort.; DQ091661; DQ091533; DQ091408. *M. smithii* (Rolfe) Summerh.; *Bytebier* 1248 (EA); PCP hort.; DQ091660; DQ091532; DQ091407. *M. stolzii* (Schltr.) Summerh.; *Carlsward* 287 (FLAS); Uzumara Orchids; DQ091657; DQ091530; DQ091405.
- Microterangis hariotiana* (Kraenzl.) Senghas; *Carlsward* 227 (FLAS); Andy's Orchids; DQ091606; DQ091467; DQ091343. *M.s hariotiana*; *Carlsward* 292 (FLAS); Uzumara Orchids; DQ091607; —; —. *M. hildebrandtii* (Rchb.f.) Senghas; *Kew* 2616 (K); J. Hermans hort.; DQ091608; DQ091468; DQ091344.
- Mystacidium aliciae* Bolus; *Whitten* 1787 (FLAS); Countryside Orchids; DQ091571; DQ091485; DQ091360. *M. braybonae* Summerh.; *Carlsward* 179 (FLAS); Andy's Orchids; DQ091572; DQ091486; DQ091361. *Mystacidium capense* (L.f.) Schltr.; *Whitten* 1781 (FLAS); Countryside Orchids; DQ091573; DQ091487; DQ091362. *M. flanaganii* (Bolos) Bolus; *Kew* 5084 (K); J. Hermans hort.; DQ091574; DQ091488; DQ091363.
- Podangis dactyloceras* (Rchb.f.) Schltr.; *Kew* 4999 (K); J. Hermans hort.; DQ091628; DQ091510; DQ091385.
- Rangaeris amaniensis* (Kraenzl.) Summerh.; *Bytebier* & *Kirika* 26 (EA); PCP hort.; DQ091634; DQ091512; DQ091386. *R. muscicola* (Rchb. f.) Summerh.; *Carlsward* 169 (SEL); SEL hort. (1997–0177A); DQ091630; DQ091513; DQ091387. *R. muscicola*; *Carlsward* 400 (FLAS); Countryside Orchids; DQ091631; —; —. *R. rhipsalisocia* (Rchb.f.) Summerh.; *Carlsward* 235 (SEL); SEL hort. (1997–0172D); DQ091629; DQ091511; —.
- Rhipidoglossum kamerunense* (Schltr.) Garay; *Carlsward* 248 (FLAS), *Nkongmeneck* 3030 (SEL); SEL hort. (1997–0188A); DQ091583; DQ091492; DQ091367. *R. kamerunense*; No voucher, ver. La Croix; Uzumara Orchids; DQ091584; —; —. *R. rutilum* (Rchb.f.) Schltr.; *Carlsward* 288 (FLAS); Uzumara Orchids; DQ091581; DQ091494; DQ091369. *R. subsimplex* (Summerh.) Garay; *Bytebier* 546 (EA); PCP hort.; DQ091580; DQ091496; DQ091371. *R. xanthopollinium* (Rchb.f.) Schltr.; *Carlsward* 384 (FLAS); Uzumara Orchids; DQ091582; DQ091495; DQ091370.
- Solenangis aphylla* (Thouars) Summerh.; *Carlsward* 341 (FLAS); Andy's Orchids; DQ091651; DQ091525; DQ091400. *S. aphylla*; *Kew* 2389 (K); J. Hermans hort.; DQ091652; —; —. *S. clavata* (Rolfe) Schltr.; *Carlsward* 396 (FLAS); Uzumara Orchids; DQ091666; DQ091534; DQ091409. *S. cornuta* (Rchb.f.) Summerh.; *OIC* 14302 (SEL); Rick's Tropica, Madagascar; DQ091662; DQ091526; DQ091401. *S. wakefieldii* (Rolfe) P.J.Cribb & J.Stewart; *Bytebier* 627 (EA); PCP hort.; DQ091667; DQ091535; DQ091410.
- Sphyrarhynchus schliebenii* Mansf.; *Bytebier* 393 (EA); PCP hort.; DQ091570; DQ091484; DQ091359.
- Tridactyle bicaudata* (Lindl.) Schltr.; *Carlsward* 396 (FLAS); Countryside Orchids; DQ091639; —; —. *T. bicaudata*; *Bytebier* 348 (EA); PCP hort.; DQ091638; DQ091514; DQ091388. *T. crassifolia* Summerh.; *Carlsward* 174 (FLAS), *Nkongmeneck* 2076 (SEL); SEL hort. (1997–0165A); DQ091640; DQ091515; DQ091389. *T. filifolia* (Schltr.) Schltr.; *Bytebier* 707 (EA); PCP hort.; DQ091641; DQ091516; DQ091390. *T. furcistipes* Summerh.; *Bytebier* 1731 (EA); PCP hort.; DQ091635; DQ091518; DQ091392. *T. scottellii* (Rendle) Schltr.; *Bytebier* 497 (EA); PCP hort.; DQ091642; DQ091517; DQ091391. *T. tanneri* P.J.Cribb; *PCP* 198 (EA); PCP hort.; DQ091632; DQ091520; DQ091394.
- Ypsilopus longifolius* (Kraenzl.) Summerh.; *Bytebier* 609 (EA); PCP hort.; DQ091636; DQ091519; DQ091393. *Y. viridiflorus* P.J.Cribb & J.Stewart; *Bytebier* 402 (EA); PCP hort.; DQ091633; —; DQ091395.

Tribe Vandaeae, Subtribe Angraeacinae

- Aeranthus arachnitis* (Thouars) Lindl.; *Carlsward* 198 (FLAS); Cal-Orchid; DQ091759*; DQ091536; DQ091411. *A. grandiflora* Lindl.; *Carlsward* 238 (FLAS); Cal-Orchid; DQ091760*; DQ091537; DQ091412.
- Angraecum calceolus* Thouars; No voucher; SEL hort. (1996–0480); —; DQ091546; DQ091421. *A. chevalieri* Summerh.; *Carlsward* 208 (FLAS); SEL hort. (1997–0160); AF506320; AF506339; AF506363. *A. conchiferum* Lindl.; *Bytebier* 616 (EA); PCP hort.; DQ091748*; DQ091539; DQ091414. *A. cultriforme* Summerh.; *Carlsward* 298 (FLAS); Countryside Orchids; AF506321; AF506340; AF506364. *A. distichum* Lindl.; *Carlsward* 237 (FLAS); Cal-Orchid; DQ091567; DQ091448; DQ091324. *A. dives* Rolfe; *Marimoto* 42 (EA); PCP hort.; DQ091756*; DQ091547; DQ091422. *A. eburneum* Bory; *Carlsward* 335 (FLAS); FLMNH hort.; DQ091737*; DQ091542; DQ091417. *A. eburneum*; *Carlsward* 182 (FLAS); FLMNH hort.; DQ091742*; —; —. *A. eburneum* subsp. *superbum* (Thouars) H. Perrier; *Carlsward* 282 (FLAS); W. L. Stern hort.; DQ091738*; DQ091543; DQ091418. *A.*

- eburneum* subsp. *superbum* var. *longicalcar* Bosser; No voucher; Countryside Orchids; DQ091739*; DQ091544; DQ091419. *A. eburneum* subsp. *xerophilum* H.Perrier; *Carlsward* 275 (FLAS); W. L. Stern hort.; —; DQ091545; DQ091420. *A. eichlerianum* Kraenzl.; *Carlsward* 284 (FLAS); FLMNH hort.; AF506322; AF506341; AF506365. *A. elephantinum* Schltr.; *Carlsward* 187 (FLAS); Cal-Orchid; DQ091744*; —; — *A. elephantinum*; *Carlsward* 251 (FLAS); W. L. Stern hort.; DQ091751*; DQ091549; DQ091424. *A. erectum* Summerh.; *Bytebier* 801 (EA); PCP hort.; DQ091566; DQ091447; DQ091323. *A. florulentum* Rchb.f.; *Carlsward* 321 (FLAS); Hoosier Orchid Co.; DQ091741*; DQ091550; DQ091425. *A. germinyanum* Hook.f.; No voucher; Santa Cruz Orchids; DQ091754*; DQ091538; DQ091413. *A. leonis* (Rchb.f.) André; *Carlsward* 390 (FLAS); Cal-Orchid; DQ091735*; DQ091551; DQ091426. *A. rutenbergianum* Kraenzl.; *Carlsward* 300 (FLAS); Santa Cruz Orchids; DQ091743*; DQ091548; DQ091423. *A. teres* Summerh.; *Bytebier* 675 (EA); PCP hort.; DQ091755*; DQ091552; DQ091427.
- Bonniera* sp.; *Carlsward* 391 (FLAS); Cal-Orchid; DQ091745*; DQ091540; DQ091415. *B. appendiculata* (Frapp. ex Cordem.) Cordem.; *Kew* 4232 (K); J. Hermans hort.; DQ091752*; DQ091541; DQ091416.
- Calyptochilum christyanum* (Rchb.f.) Summerh.; *Carlsward* 194 (SEL); SEL hort. (1997–0239); DQ091668; DQ091449; DQ091325.
- Campylocentrum brenesii* Schltr.; *Blanco* 2139 (USJ); M. Blanco, Costa Rica; DQ091563; —; —. *C. fasciola* (Lindl.) Cogn.; *Carlsward* 185 (FLAS); Hamlyn Orchids, Jamaica; AF506294; AY147226; AF506342. *C. jamaicense* (Rchb.f. & Wullschl.) Benth. ex Rolfe; *Whitten* 1934 (FLAS); Jamaica; AF506299; AF506326; AF506348. *C. lansbergii* (Rchb.f.) Schltr.; *Carlsward* 272 (FLAS); Brazil; AF506297; AF506324; AF506345. *C. micranthum* (Lindl.) Rolfe; *Carlsward* 180 (FLAS); Cal-Orchid (Mexico); AF506298; AY147227; AF506347. *C. neglectum* (Rchb.f. & Warm.) Cogn.; *Zardini* 14995 (MO); Herbarium specimen, Paraguay; AF506300; —; —. *C. pachyrrhizum* (Rchb.f.) Rolfe; *Ackerman* s.n. (UPRRP); J. Ackerman, Puerto Rico; AF506301; AF506328; AF506350. *C. poeppigii* (Rchb.f.) Rolfe; *Carnevali* 4507 (CICY, FLAS); Mexico; AF506302; AF506329; AF506351. *C. robustum* Cogn.; *Hoeijer & Dalstroem* 839 (MO); Herbarium specimen, Ecuador; DQ091565; —; —. *C. sullivanii* Fawc. & Rendle; *Carlsward* 301 (FLAS); Hamlyn Orchids, Jamaica; DQ091564; DQ091445; DQ091321. *C. tyrridion* Garay & Dunst.; *Carnevali* 5145 (CICY); Mexico; AF506305; DQ091446; DQ091322. *C. ulei* Cogn.; *Chagas & Silva* 1333 (MO); Herbarium specimen, Brazil; AF506306; —; —.
- Cryptopus elatus* (Thouars) Lindl.; *Carlsward* 403 (FLAS); Uzumara Orchids; DQ091585; DQ091450; DQ091326. *C. paniculatus* H. Perrier; *Kew* 5392 (K); J. Hermans hort.; DQ091588; DQ091451; DQ091327.
- Dendrophyllax alcoa* Dod; *Ackerman* 2773 (UPRRP); Herbarium specimen, Dominican Republic; AF506307; —; —. *D. barrettiae* Fawc. & Rendle; *Carlsward* 199 (FLAS); Hamlyn Orchids, Jamaica; AF506308; AF506330; AF506353. *D. fawcettii* Rolfe; *Whitten* 1939 (FLAS); Grand Cayman; AF506309; AF506331; AF506354. *D. filiformis* (Sw.) Carlsward & Whitten; *Whitten* 1842 (FLAS); J. Ackerman, Puerto Rico; AF506296; AF506323; AF506344. *D. funalis* (Sw.) Benth. ex Rolfe; *Carlsward* 302 (FLAS); FLMNH hort., Jamaica; AY147221; AY147229; AF506355. *D. lindenii* (Lindl.) Benth. ex Rolfe; *Carlsward* 394 (FLAS); Hamlyn Orchids, Jamaica; AF506318; AF506338; AF506362. *D. porrectus* (Rchb.f.) Carlsward & Whitten; *Carlsward* 329 (FLAS); Fish-eating Creek, Florida; AY147223; AY147232; AY147237. *D. sallei* (Rchb.f.) Benth. ex Rolfe; *Whitten* 1945 (JBSD); Dominican Republic; AY147225; AY147234; AY147239. *D. varius* (Gmel.) Urb.; *Whitten* 1960 (JBSD); Dominican Republic; AY147222; AY147230; AY147236.
- Jumellea confusa* (Schltr.) Schltr.; *Carlsward* 228 (FLAS); W. L. Stern hort.; DQ091758*; DQ091553; DQ091428. *J. maxillarioides* (Ridl.) Schltr.; No voucher; Hoosier Orchid Co.; DQ091757*; DQ091554; DQ091429. *J. sagittata* H.Perrier; *Carlsward* 232 (SEL); SEL hort. (1981–1182); DQ091740*; DQ091555; DQ091430.
- Lemurella pallidiflora* Bosser; *Kew* 4958 (K); J. Hermans hort.; DQ091591; DQ091454; DQ091330.
- Lemurorchis madagascariensis* Kraenzl.; *Kew* 5383 (K); J. Hermans hort.; DQ091747*; DQ091556; DQ091431.
- Neobathiea grandidieriana* (Rchb.f.) Garay; *Carlsward* 395 (FLAS); Uzumara Orchids; DQ091589; DQ091453; DQ091329. *N. grandidieriana*; *Kew* 3450 (K); J. Hermans hort.; DQ091590; —; —.
- Oeonia rosea* Ridl.; *Whitten* 1813 (FLAS); FLMNH hort.; DQ091586; DQ091452; DQ091328. *O. rosea*; *Kew* 3222 (K); J. Hermans hort.; DQ091587; —; —.
- Oeoniella polystachys* (Thouars) Schltr.; *Carlsward* 221 (FLAS); Cal-Orchid; DQ091736*; DQ091557; DQ091432.
- Sobennikoffia humbertiana* H.Perrier; *Carlsward* 304 (FLAS); P. Simon hort.; DQ091750*; DQ091558; DQ091433.
- Tribe Vandaeae, Subtribe Aeridinae
- Acampe ochracea* (Lindl.) Hochr.; *Carlsward* 206 (SEL); SEL hort. (1983–0253); DQ091707; DQ091438; DQ091314. *A. papillosa* (Lindl.) Lindl.; *Carlsward* 191 (SEL); SEL hort. (1991–0066); DQ091708; DQ091439; DQ091315.
- Adenoncos vesiculosa* Carr; *Carlsward* 258 (FLAS); W. L. Stern hort.; DQ091688; —; —.
- Aerides rosea* Lodd. ex Lindl. & Paxton; *Carlsward* 254 (FLAS); Unknown garden origin; DQ091687; —; —.
- Amesiella philippinensis* (Ames) Garay; *Carlsward* 295 (FLAS); Countryside Orchids; DQ091718; DQ091443; DQ091319.
- Ascocentrum aurantiacum* (J.J.Sm.) Schltr.; *Carlsward* 217 (SEL); SEL hort. (1987–0134); DQ091677; —; —. *A. miniatum* (Lindl.) Schltr.; *Carlsward* 273 (SEL); SEL hort. (1985–0839); DQ091678; —; —.
- Ascochilopsis myosurus* (Ridl.) Carr; *Carlsward* 239 (FLAS); ABG hort.; DQ091721; —; —.
- Ceratochilus biglandulosus* Blume; No voucher; Andy's Orchids; DQ091712; —; —.
- Chiloschista lunifera* (Rchb.f.) J.J.Sm.; *Carlsward* 305 (FLAS); R. F. Orchids; DQ091729; —; —. *C. lunifera*; No voucher; SEL hort. (s. n.); DQ091730; —; —. *C. parishii* Seidenf.; *Carlsward* 222 (FLAS); W. L. Stern hort.; DQ091733; —; —. *C. parishii*; *Carlsward* 250 (FLAS); TropicOne; DQ091734; —; —. *C. parishii*; *Carlsward* 267 (FLAS); Banjong Orchids; DQ091749; —; —. *C. pusilla* (J.König) Schltr.; *Carlsward* 303 (FLAS); Camp-Lot-A-Noise Tropicals; DQ091731; —; —. *C. viridiflava* Seidenf.; *Carlsward* 312 (FLAS); R. Ing hort.; DQ091732; —; —.
- Christensonia vietnamica* Haager; *Carlsward* 349 (FLAS); Mountain Orchids; DQ091676; —; —.
- Cleisostoma arietinum* (Rchb.f.) Garay; *Carlsward* 211 (SEL); SEL hort. (1995–0045); DQ091694; —; —. *C. chantaburiense* Seidenf.; *Carlsward* 155 (SEL); SEL hort. (1988–0303); DQ091695; —; —. *C. discolor* Lindl.; *Carlsward* 253 (FLAS); W. L. Stern hort.; DQ091696; —; —. *C. racemiferum* (Lindl.) Garay; *Carlsward* 299 (SEL); SEL hort. (s.n.); DQ091693; —; —.
- Dyalkia hendersoniana* (Rchb.f.) Christenson; *Carlsward* 252 (FLAS); Gypsy Glen Orchids; DQ091753; —; —.
- Esmeralda clarkei* Rchb.f.; *Carlsward* 189 (SEL); SEL hort. (1997–0233); DQ091711; —; —.
- Gastrochilus dasypogon* (Sm.) Kuntze; *Carlsward* 183 (FLAS); R. F. Orchids; DQ091685; —; —.
- Haraella odorata* Kudô; *Carlsward* 240 (FLAS); ABG hort.; DQ091686; —; —.
- Luisia trichorhiza* (Hook.) Blume; *Carlsward* 203 (SEL); SEL hort. (1986–0372); DQ091681; —; —.
- Malleola ligulata* (J.J.Sm.) J.J.Sm.; *Carlsward* 246 (FLAS); Andy's Orchids; DQ091700; —; —.
- Micropera pallida* (Roxb.) Lindl.; *Carlsward* 162 (FLAS); Motes Orchids; DQ091703; —; —.
- Microtatorchis* sp.; *Carlsward* 387 (FLAS); J. Watts hort.; DQ091723; —; —.

- Neofinetia falcata* (Thunb.) Hu; *Carlsward 163* (SEL); SEL hort. (1993–0377); DQ091684; DQ091442; DQ091318.
- Papilionanthe teres* (Roxb.) Schltr.; *Carlsward 332* (FLAS); Motes Orchids; DQ091682; —; —.
- Paraphalaenopsis laycockii* (M.R.Hend.) A.D.Hawkes; *Carlsward 225* (SEL); SEL hort. (1993–0318); DQ091683; —; —.
- Pelatantheria ctenoglossum* Ridl.; *Carlsward 220* (FLAS); Motes Orchids; DQ091690; —; —. *P. ctenoglossum*; *Carlsward 244* (SEL); SEL hort. (871–1); DQ091691; —; —. *P. insectifera* (Rchb.f.) Ridl.; *Carlsward 164* (SEL); SEL hort. (1986–0712); DQ091692; —; —.
- Phalaenopsis amabilis* (L.) Blume; *KDAIS KC-329*; NCBI; AY391535; —; —. *P. amboinensis* J.J.Sm.; No voucher; NCBI; AF537006; —; —. *P. braceana* (Hook.f.) Christenson; No voucher; NCBI; AY228495; —; —. *P. chibae* T.Yukawa; No voucher; NCBI; AF536996; —; —. *P. cornu-cervi* (Breda) Blume & Rchb.f.; No voucher; NCBI; AF536994; —; —. *P. deliciosa* Rchb.f. (syn. = *Kingidium deliciosum*); *Carlsward 160* (SEL); SEL hort. (1997–0330); DQ091669; DQ091444; DQ091320. *P. deliciosa* (syn. = *Kingiella decumbens*); *Carlsward 274* (FLAS); W. L. Stern hort.; DQ091670; —; —. *P. equestris* (Schauer) Rchb.f.; No voucher; NCBI; AF537012; —; —. *P. fuscata* Rchb.f.; No voucher; NCBI; AY228498; —; —. *P. hainanensis* T.Tang & F.T.Wang; No voucher; SBG hort.; DQ091671; —; —. *P. lobbii* (Rchb.f.) H.R. Sweet; *Carlsward 261* (FLAS); Andy's Orchids; DQ091673; —; —. *P. lowii* Rchb.f.; No voucher; NCBI; AF537019; —; —. *P. parishii* Rchb.f.; No voucher; NCBI; AF537037; —; —. *P. pulcherrima* (Lindl.) J.J.Sm.; No voucher; NCBI; AF536993; —; —. *P. wilsonii* Rolfe; *Carlsward 331* (FLAS); Andy's Orchids; DQ091672; —; —. *P. zebrina* Teijsm. & Binn.; *KDAIS KC-231*; NCBI; AY390252; —; —.
- Pomatocalpa spicata* Breda; *Carlsward 214* (SEL); SEL hort. (1986–0172); DQ091706; —; —.
- Pteroceras semiteretifolium* H.A.Pedersen; *Carlsward 257* (FLAS); W. L. Stern hort.; DQ091722; —; —.
- Renanthera imschootiana* Rolfe; *Carlsward 306* (FLAS); W. L. Stern hort.; DQ091710; —; —.
- Rhynchostylis gigantea* (Lindl.) Ridl.; *Carlsward 201* (SEL); SEL hort. (1981–1033); DQ091717; —; —.
- Robiquetia cerina* (Rchb.f.) Garay; *Carlsward 210* (SEL); SEL hort. (1990–0199); DQ091701; —; —. *R. spatulata* (Blume) J.J.Sm.; *Carlsward 262* (FLAS); W. L. Stern hort.; DQ091702; —; —.
- Sarcophilus fitzgeraldii* F.Muell.; *Carlsward 231* (FLAS); Andy's Orchids; DQ091728; —; —.
- Sarcoglyphis comberi* (J.J.Wood) J.J.Wood; *Carlsward 296* (FLAS); Countryside Orchids; DQ091697; —; —.
- Sarcophyton crassifolium* (Lindl. & Paxton) Garay; *Carlsward 236* (SEL); SEL hort. (1990–0552); DQ091699; —; —.
- Schoenorchis fragrans* (C.S.P.Parish & Rchb.f.) Seidenf. & Smitinand; *Carlsward 249* (FLAS); Andy's Orchids; DQ091705; —; —. *S. juncifolia* Reinw. ex Blume; *Whitten 1783* (FLAS); Countryside Orchids; DQ091704; —; —.
- Staurochilus fasciatus* (Rchb.f.) Ridl.; *Carlsward 223* (SEL); SEL hort. (1981–1550); DQ091709; —; —.
- Stereochilus dalatensis* (Guillaumin) Garay; No voucher; Andy's Orchids; DQ091698; —; —.
- Taeniophyllum biocellatum* J.J.Sm.; *Carlsward 317* (FLAS); J. Watts hort.; DQ091746; —; —. *T. complanatum* Fukuy; No voucher; Rick's Tropica, Thailand; DQ091724; —; —. *T. fasciola* (G.Forst.) Seem.; *Kores 0652364* (?), *Molvray 7997130* (?); Kores & Molvray, Fiji; DQ091727; —; —. *T. glandulosum* Blume; No voucher; Rick's Tropica, Thailand; DQ091725; —; —. *T. smithii* Kores & L.Jonss.; *Molvray s.n.* (?); Kores & Molvray, Fiji; DQ091726; —; —.
- Thrixspermum elongatum* Ames; *Carlsward 170* (SEL); SEL hort. (1985–1735); DQ091674; —; —.
- Trichoglottis atropurpurea* Rchb.f.; *Carlsward 173* (FLAS); Motes Orchids; DQ091713; DQ091440; DQ091316. *T. geminata* (Teijsm. & Binn.) J.J.Sm.; *Carlsward 197* (SEL); SEL hort. (1992–0404); DQ091716; —; —. *T. seidenfadenii* Aver.; *Carlsward 172* (SEL); SEL hort. (1981–1413); DQ091714; —; —. *T. tomentosa* Seidenf.; *Carlsward 270* (FLAS); W. L. Stern hort.; DQ091715; —; —.
- Tuberolabium brevirachis* L.O.Williams & J.J.Wood; *Carlsward 202* (SEL); SEL hort. (1987–0212); DQ091720; —; —. *T. kotoense* Yamam.; *Carlsward 205* (SEL); SEL hort. (1986–0724); DQ091719; —; —.
- Vanda flabellata* (Rolfe ex Downie) Christenson; *Carlsward 192* (SEL); SEL hort. (1996–0223); DQ091675; DQ091441; DQ091317. *V. luzonica* Loher ex Rolfe; *Carlsward 245* (SEL); SEL hort. (1984–0565); DQ091680; —; —. *V. tessellata* (Roxb.) Hook. ex G.Don.; *Carlsward 175* (SEL); SEL hort. (1985–0965); DQ091679; —; —.
- Vandopsis lissochiloides* (Gaudin) Pfitzer; *Carlsward 177* (SEL); SEL hort. (1983–0249); DQ091689; —; —.

Tribe Vandeeae, Subtribe Polystachyinae

- Neobenthamia gracilis* Rolfe; *Carlsward 311* (FLAS); Unknown garden origin; DQ091559; DQ091434; DQ091310.
- Polystachya concreta* (Jacq.) Garay & H.R.Sweet; *Carlsward 213* (SEL); SEL hort. (1996–0140); DQ091561; DQ091436; DQ091312. *P. longiscapa* Summerh.; No voucher; W. L. Stern hort.; DQ091560; DQ091435; DQ091311. *P. modesta* Rchb.f.; *Carlsward 219* (SEL), *Heaney 984* (FLAS); SEL hort. (1994–0078); DQ091562; DQ091437; DQ091313.