Biodiv. Res. Conserv. 3-4: 205-209, 2006

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Tribal and subtribal relationship of Epidendroideae Lindl. (Orchidaceae) with emphasis on Epidendreae Humb., Bonpl. & Kunth based on *matK* gene

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Abstract: The Epidendroideae (Orchidaceae) is one of the orchids largest subfamilies with substantially larger number of genera and species than all other subfamilies altogether.Taxa incorporated in the subfamily Epidendroideae are characterized by the largest variety and diversity of forms. Once in a while, the classification inside the Epidendroideae subfamily undergoes modifications. It is because different scientists base their classification system on different features. This paper shows is to show relationship between genera within the subfamily Epidendroideae (with the particular consideration of the tribes Dendrobieae and Epidendreae) on the basis of the analysis of the nucleotide sequence *matK* gene.

Key words: Orchidaceae, Epidendroideae, Epidendreae, Dendrobieae, molecular phylogenetics, PCR, DNA sequencing, matK gene

1. Introduction

Dressler (1993) singled out within Epidendroideae Epidendroid phylad to which he rated 4 tribes: Arethuseae, Coelogyneae, Epidendreae I – that is Orchids from the New World, and Epidendreae II – Orchids from the Old World and also Cymbidioid phylad. To the group of Epidendroid phylad there has also been allocated a subclade of Dendrobioids to which Dressler rated 3 tribes, including the Dendrobieae tribe. The epidendroid phylad is distinguished by the presence of eight pollinia in its primitive members, and includes most of the plants with a reed-stem habit of growth. Most of the Cymbidioid phylad are vandoid, with superposed pollinia, viscidia and usually stipes (Dressler 1993). Epidendroideae *sensu* Szlachetko (1995) is a much smaller subfamily than that of Dressler's system. Szlachetko separeted from the Epidendroideae Cymbidioid phylad (excluding tribe Calypsoeae), Vandeae and Polystachyinae *sensu* Dressler. In his opinion Epidendroideae is clearly defined by 'the organization of the pollen mass and ultrastructure of the pollen walls'. Among the Epidendroideae he recognized several clades: (1) Bletieae, Podochileae, Adrorhizeae; (2) Malaxideae; (3) Calypsoeae, Coelogyneae; (4) Dendrobieae; (5) Elleantheae, Epidendreae. Other researchers also proposed systems with Vandoideae as a separate subfamily (Dressler 1981; Rasmussen 1985). The main basis for classification according to Szlachetko (1995) were the similarities and differences in the structure of column, mainly the features of viscidium and rostellum but also type of leaves, seed and velamen.

Recently, van den Berg *et al*. (2005) published an overview of the phylogenetic relationships within Epidendroideae, based on nuclear ITS and plastid genes and introns. The results of the study mentioned are quite similar to those presented in this article.

In our study we used chloroplast *matK-trnK* region for resolving relationship among subtribes of the Epidendroideae *sensu* Szlachetko. The gene *matK* is located in the large single-copy region of the chloroplast genome and it encodes a maturase involved in splicing type II introns from RNA transcripts. The rate of evolution of *matK* makes this gene appropriate for resolving infrageneric relationship and also has a great potential for retrieving phylogeny within subtribes of seed plants (Soltis & Soltis 1998). The gene *matK* appears as a pseudo-gene both in Orchidaceae and probably also in majority of angiosperms (Kores *et al*. 2000). Analysis

of *matK* gene was successfully conducted to determinate a degree of relationship between particular of Orchidaceae: Stanhopeinae (Whitten *et al*. 2000) Pleurothallidinae (Pridgeon *et al*. 2001), Arethuseae (Goldman *et al*. 2001), Spiranthinae (Salazar *et al*. 2003), Laeliinae (van den Berg *et al*. 2000) and Epidendreae (van den Berg *et al*. 2005).

2. Material and methods

Plant material (see Appendix 1). Sources of plants and vouchers are available upon request from M. Górniak. Representatives of the subfamily Epidendroideae were used to analysis. Outgroup taxa were taken to analysis under permission from the data matrices (Kores *et al*. 2000) from M. W. Chase. Ingroup taxa come from M. Górniak PhD thesis (unpublished).

DNA isolation. Total genomic DNA was extracted from 100 mg of fresh-frozen or 20 mg of silica dried leaves (Chase & Hills 1991) using the DNA Mini Plant (A&A Biotechnology, Poland) following manufacturer protocol.

Amplification and sequencing. MatK region (mostly *matK* gene and 3' of *trnK* intron) was amplified via Polymerase Chain Reaction (PCR) using the primers-19F (Molvray *et al*. 2000) and *trnK*2R (Johnson & Soltis 1994). Both strands were sequenced (two internal primers and PCR primers were used for sequencing) to assure accuracy in base calling. 'Sequence Navigator' was used to edit the sequences and each individual base position was examined for agreement of the two strands using AutoAssembler. Before alignment, sequence of each taxon was checked using blast on NCBI web site.

Phylogenetics analysis. DNA sequences were aligned by 'ClustalX™' (Thompson *et al*. 1997) and adjusted by eye. MatK region was analyzed using heuristic search method of PAUP* (Phylogenetic Analysis Using Parsimony* and Other Methods) version 4.0b10 (Swofford 2000). Optimality criterion was parsimony with tree-bisection-reconnection (TBR) branch swapping and the MULTREES option in effect, simple addition and ACCTRAN optimization. Gaps were treated as missing value. Internal support of clades was evaluated by the bootstrap (Felsenstein 1985) with 400 bootstrap replicates. All characters were unordered and equally weighted (Fitch 1971).

3. Results and discussion

The aligned *matK-trnK* matrix consisted of 1983 bp of which 798 were variable and 424 were potentially parsimony informative. The strict consensus of 5000 trees, with length of 2030 steps CI of 0.558 and RI of 0.575 is shown in Fig. 1. Bootstrap (BP) percentage >50% are shown above the branches. Tree shows relationships between members of subfamily Epidendroideae (Fig. 1). Clade A embracing taxa from tribe Dendrobieae *sensu* Szlachetko (1995). The members which belong to this tribe are characterized by naked pollinia, without caudicles and other appendages, duplicate leaves, pseudobulbs and lateral inflorescence. Dressler (1993) placed Dendrobieae with tribes Podochileae and Vandeae in Dendrobioid subclade on the basis of spherical silica bodies. Also Bayesian analysis (PP 55) show that Dendrobieae were sister to Vandeae (van den Berg *et al*. 2005). On the other hand morphological study of Rasmussen (1985) and Szlachetko (1995) and molecular researches of Chase *et al*. (2003) placed Vandeae far away from Dendrobieae. In this study relationship between Dendrobieae and other tribes from Epidendroideae is unresolved. Epidendreae *sensu* Szlachetko (1995) (clad D2 – Fig. 1) embracing mixture of taxa from subtribe Laeliine, Meiracyllinae and Epidendriinae (clade D4), Pleurothalidinae (clade D3) and well supported by (BP 94) Ponera – Isochilus clade with Chysis as a sister group. Dressler (1993) placed Chysis (Chysinae) into tribe Arethuseae, but Schlechter (1926), Szlachetko (1995) and van de Berg *et al*. (2000) placed Chysis in a separate subtribe Chysinae (Epidendreae). Our analysis (Fig. 1) and also van den Berg *et al*. (2005) researche shows, that Chysis (Chysinae) should belong to the Epidendreae. Some morphological features support molecular analysis: ligulate rostellum, incumbent anther and laterally flattened pollinia, with the sticky caudicules, present in other members of Epidendreae. Van den Berg *et al*. (2000, 2005) suggested that Laeliinae *sensu* Dressler (1993) are monophyletic after transferring some taxa to the other subtribes: *Dilomilis*, *Neocogniauxia* to Pleurothalidinae and *Ponera*, *Helleriella*, *Isochilus* to Ponerinae. Szlachetko (1995) placed *Ponera* and *Helleriella* in Ponerinae but *Isochilus* in Laeliinae. Szlachetko (1995) divided also Laeliinae *sensu* Dressler (1993) into two subtribes: Laeliinae and Epidendriinae. Taxa which belong to Laeliinae have terminal inflorescence, reduced or lacking column foot, ligulate and short rostellum, bent forward anther and dorsiventrally flattened pollinia with sticky caudicules. The subtribe Epidendriinae characterized by lateral inflorescence, absent column foot, short, shelf-like and erect rostellum, erect anther and pollinia, with sticky caudicles, attached to the viscidium. This study indicates that Epidendrinae *sensu* Szlachetko 1995 is deeply embedded in Laeliinae. Except for Epidendrum, Oerstedella, Nanodes and Porpax all Epidendrinae are closely related, and form a well supported clade (BP 94, Fig. 1). Molecular data also do not support Meiracyllinae as a distinct subtribe. Pleurothalidinae *sensu* Szlachetko (1995) form a well supported clade (D3) (BP 91) within Epidendreae. These taxa are characterized by velamen

of Pleurothallis type, terminal inflorescence, short column with foot, erect to incumbent anther and pollinia with sticky and granular caudicules. Subclade D1 (Fig. 1) is the sister group to the Epidendreae and consists of the subtribe Eriinae (*Eria, Ceratostylis, Mediocalcar*), Podochillinae (*Appendicula*) and Glomerinae (*Earina*), all Podochileae *sensu* Szlachetko (1995). These taxa are characterized by *Calanthe* type of velamen, *Elleanthus* type of seed and conical silica bodies. *Arundina*, *Bletilla* and *Coelogyne* form moderately supported clade B (BP 79). Dressler (1995) indicated that Coelogyneae may share a common ancestry

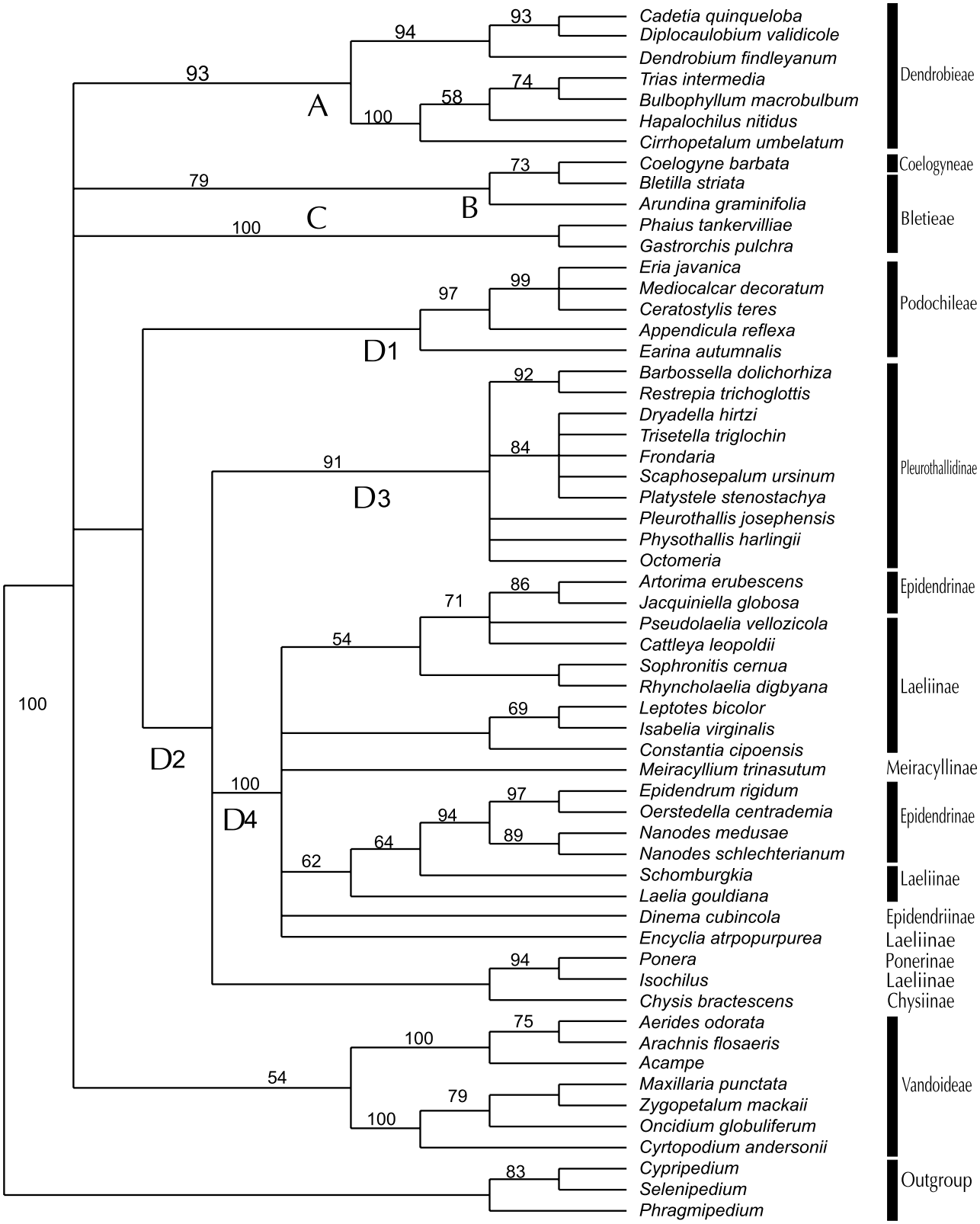

Fig. 1. Strict consensus of 5000 trees obtained from the maximum parsymony analysis of the *matK-trnK* region. Bootstrap percentages >50% are shown above the branches

with the Arethuseae where he placed *Bletilla*. „The flowers of the Coelogyneae resemble those of the Arethuseae in the petaloid column and emergent, cam-shell stigma". This study confirms close relationship between *Coelogyne* and *Bletilla*. Van den Berg *et al*. (2005) used more taxa in their analyses of Coelogyninae and Arethusinae and moved *Bletilla* to Coelogynineae in Arethuseae. Clade C (BP 100) embraces *Phaius thankervilliae* and *Gastrorchis pulchra*. But relationship between those taxa and other clades are not analyzed in this paper. Only increasing the number of taxa and DNA regions in analysis could determine relationship among the different tribes of Epidendroideae.

Acknowledgments. We would like to express our great appreciation to Prof. D. L. Szlachetko for inspiring discussions. The authors would like to thank Prof. Mark W. Chase, David Dockrell for their help during our visit in the Jodrell Laboratory, Royal Botanic Gardens, Kew. Scientific work financed from the resources earmarked for science in years 2004-2007 as the Research Project no. 2 P04C 051 27.

References

Chase M. W. & Hills H. H. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. Taxon 40: 215-220.

Chase M. W., Cameron K. M., Barrett R. L. & Freudenstein J. V. 2003. DNA data and Orchidaceae systematics: a new phylogenetic classification. In: K. M. Dixson, S. P. Kell, R. L. Barrett & P. J. Cribb (eds.). Orchid Conservation, pp. 69-89. Natural History Publications.

Dressler R. L. 1981. The Orchids: Natural History and Classification. 332 pp. Harvard University Press, Cambridge.

Dressler R. L. 1993. Phylogeny and classification of the orchid family. 312 pp. Dioscorides Press, Portland-Oregon.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

Fitch W. M. 1971. Towards defining the course of evolution: Minimum change for a specific tree topology. Systematic Zoology 20: 406-416.

Goldman D. H., Freudenstein J. V., Kores P. J., Molvray M., Jarrell D. C., Whitten W. M., Cameron K. M. & Chase M. W. 2001. Phylogenetics of Arethuseae (Orchidaceae) based on plastid matK and rbcL sequences. Systematic Botany 26: 670-695.

Johnson L. A. & Soltis D. E. 1994. MatK DNA sequences and phylogenetic reconstruction in Saxifragaceae. Systematic Botany 19: 143-156.

Kores P. J., Weston P. H., Molvray M. & Chase M. W. 2000. Phylogenetic relationships within the Diurideae (Orchidaceae): inferences from platid matK DNA sequences. In: K. L. Wilson & Morrison D. A. (eds.). Monocots: systematics and evolution, pp. 449-456. CSIRO Publishing, Collingwood, Victoria, Australia.

Molvray M., Kores P. J. & Chase M. W. 2000. Polyphyly of mycoheterotrophic orchids and functional influences on floral and molecular characters. In: K. L. Wilson & Morrison D. A. (eds.). Monocots: systematics and evolution, pp. 441-448. CSIRO Publishing, Collingwood, Victoria, Australia.

Pridgeon A. M., Solano R. & Chase M. W. 2001. Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. Am. J. Bot. 88(12): 2286-2308.

Rasmussen F. N. 1985. Orchids. In: R. M. Dahlgren, H. T. Clifford & P. F. Yeo (eds.). The families of the monocotyledons, pp. 249-274. Springer Verlag, Berlin.

Salazar G. A., Chase M. W., Soto Arenas M. A. & Ingrouille M. 2003. Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae, Orchidoideae): evidence from plastid and nuclear DNA sequences. Am. J. Bot. 90: 777-795.

Schlechter R. 1926. Das System der Orchidaceen. Notizbl. Bot. Gart. Mus. Berlin-Dahlem 9(88): 563-591.

Soltis D. E. & Soltis P. S. 1998. Choosing an approach and appropriate gene for phylogenetic analysis. In: D. E. Soltis, P. E. Soltis & J. J. Doyle (eds.). Molecular Systematics of Plants II: DNA sequencing, pp. 1-42. Kluwer Academic Publishers, Boston Massachusetts, USA.

Swofford D. L. 2000. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b2. Sinauer Associates, Sunderland MA.

Szlachetko D. L. 1995. Systema Orchidalium. Fragm. Flor. Geobot. Suppl. 3: 1-137.

Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F. & Higgins D. G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuclei Acids Research 24: 4876-4882.

van den Berg C., Goldman D. H., Freudenstein J. V., Pridgeon A. M., Cameron K. M. & Chase M. W. 2005. An overview of the phylogenetic relationships within Epidendroideae inferred from multiple DNA regions and recircumscription of Epidendreae and Arethuseae (Orchidaceae). Am. J. Bot. 92(4): 613-624.

van den Berg C., Higgins W. E., Dressler R. L., Whitten W. M., Soto Arenas M. A., Culham A. & Chase M. W. 2000. A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA. Lindleyana 15: 96-114.

Whitten W. M., Williams N. H. & Chase M. W. 2000. Subtribal and generic relationships of Maxillarieae (Orchidaceae) with emphasis on Stanhopeinae: combined molecular evidence. Am. J. Bot. 87(12): 1842-1856.

Appendix 1

Epidendroideae

BLETIEAE,
Bletiinae: *Bletilla striata* EF079331 [GB] ex cult. Szlachetko s.n. [V], *Gastrorchis pulchra* EF079305 [GB] Heidelberg BG 104634 [V], *Phaius tankervilliae* EF079306 [GB] ex cult. Szlachetko s.n. [V]; **Arundiinae:** *Arundina graminifolia* EF079333 [GB] ex cult. Szlachetko s.n. [V];

PODOCHILEAE,
Glomerinae: *Earina autumnalis* EF079336 [GB] Heidelberg BG 124446[V]; **Eriinae:** *Eria javanica* EF079354 [GB] ex cult. Szlachetko s.n. [V], *Ceratostylis teres* EF079356 [GB] Heidelberg BG 120023 [V], *Mediocalcar decoratum* EF079355 [GB] Heidelberg BG 123319 [V]; **Podochilinae:** *Appendicula reflexa* EF079357 [GB] Heidelberg BG 120017 [V];

COELOGYNEAE,
Coelogyninae: *Coelogyne barbata* EF079332 [GB] Schoenbrunn [V];

DENDROBIEAE,
Dendrobiinae: *Cadetia quinqueloba* EF079346 [GB] Heidelberg BG 120066 [V], *Dendrobium findleyanum* EF079348 [GB] ex cult. Szlachetko s.n. [V], *Diplocaulobium validicolle* EF079345 [GB] Heidelberg BG 123424 [V]; **Bulbophyllinae:** *Bulbophyllum macrobulbum* EF065596 [GB] Wien BG [V], *Cirrhopetalum umbellatum* EF079343 [GB] ex cult. Szlachetko s.n. [V], *Hapalochilus nitidus* EF065598 [GB] Heidelberg BG 123418 [V], *Trias intermedia* EF065595 [GB] Heidelberg BG 122330 [V];

EPIDENDREAE,
Chysiinae: *Chysis bractescens* EF079351 [GB] Wien BG [V]; **Meiracyllinae:** *Meiracyllium trinasutum* EF079317 [GB] Heidelberg BG 120031 [V]; **Laeliinae:** *Cattleya leopoldii* EF065586 [GB] ex cult. Szlachetko s.n. [V], *Constantia cipoensis* EF079322 [GB] Heidelberg BG 105289 [V], *Dinema cubincola* EF079321 [GB] Heidelberg BG 103394 [V], *Encyclia atropurpurea* EF079318 [GB] ex cult. Szlachetko s.n. [V], *Isabelia virginalis* EF079320 [GB] Heidelberg BG 120030 [V], *Isochilus* EF079350 [GB] ex cult. Szlachetko s.n. [V], *Laelia gouldiana* EF079315 [GB] ex cult. Szlachetko s.n. [V], *Leptotes bicolor* EF079319 [GB] Heidelberg BG 121368 [V], *Pseudolaelia vellozicola* EF065589 [GB] Heidelberg BG 121730 [V], *Rhyncholaelia digbyana* EF079309 [GB] ex cult. Szlachetko s.n. [V], *Schomburgkia* EF079316 [GB] ex cult. Szlachetko s.n. [V], *Sophronitis cernua* EF079310 [GB] Heidelberg BG 120065 [V]; **Epidendrinae:** *Artorima erubescens* EF065587 [GB] Heidelberg BG 121493 [V], *Epidendrum rigidum* EF079311 [GB] Wien BG [V], *Jacquiniella globosa* EF065588 [GB] Heidelberg BG 120088 [V], *Nanodes medusae* EF079313 [GB] Szlachetko s.n. [V], Ecuador, *Nanodes schlechterianum* EF079314 [GB] Heidelberg BG 121366 [V], *Oerstedella centradenia* EF079312 [GB] Heidelberg BG 101762 [V]; **Ponerinae:** *Ponera* EF079323 [GB] Heidelberg BG 121987 [V]; **Pleurothallidinae:** *Barbosella dolichorhiza* EF079328 [GB] Heidelberg BG 123410 [V], *Dryadella hirtzii* EF079327 [GB] Heidelberg BG 123364 [V], *Frondaria* EF079324 [GB] Szlachetko s.n., Ecuador [V], *Octomeria* EF079352 [GB] Heidelberg BG 125079 [V], *Physothallis harlingii* EF065591 [GB] Heidelberg BG 124671 [V], *Platystele stenostachya* EF079326 [GB] ex cult. Szlachetko s.n. [V], *Pleurothallis josephensis* EF079330 [GB] Heidelberg BG 120676 [V], *Restrepia trichoglottis* EF079329 [GB] Heidelberg BG 123495 [V], *Scaphosepalum ursinum* EF079325 [GB] Heidelberg BG 124283 [V], *Trisetella triglochin* EF065592 [GB] Heidelberg BG 103324 [V].

Vandoideae

CYMBIDIEAE,
Cyrtopodiinae: *Cyrtopodium andersonii* EF079263 [GB] Szlachetko s.n., French Guyana [V];

VANDEAE,
Vandinae: *Arachnis flosaeris* EF079275 [GB] Wien BG [V]; **Gastrochilinae:** *Acampe* EF079278 [GB] ex cult. Szlachetko s.n. [V]; **Aeridinae:** *Aerides odorata* EF079276 [GB] ex cult. Szlachetko s.n. [V];

MAXILLARIEAE,
Maxillariinae: *Maxillaria punctata* EF079249 [GB] Ecuagenera [V];

ZYGOPETALEAE,
Zygopetalinae: *Zygopetalum mackaii* EF079243 [GB] ex cult. Szlachetko s.n. [V];

ONCIDIEAE,
Oncidiinae: *Oncidium globuliferum*

Outgroup: *Cypripedium*, *Phragmipedium*, *Selenipedium*

Explanations: GB – GeneBank *matK*, V – Voucher