

# Phylogeny of Subtribe Aeridinae (Orchidaceae) Inferred from DNA Sequences Data : Advanced Analyses Including Australasian Genera

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## Graphical abstract

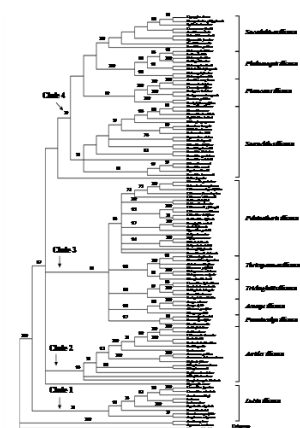


Fig. 1. Maximum likelihood phylogenetic tree of combined dataset (single 1000 boot CI: 94, 92-93). Bootstrap values at 95% confidence level.

## Abstract

Advanced phylogenetic analyses of the orchid subtribe Aeridinae has been conducted using DNA sequences of ITS region of nrDNA and *matK* of cpDNA. In the preliminary work, we only involved the most representative Asian genera of the subtribe. Further, to establish more robust relationships in the Aeridinae, in this study we have extended the sampling to include Australasian specimens. Our analyses revealed that: (1) the subtribe is reorganised by four major groups with 11 subgroups (This is inconsistent with previous classification systems of the subtribe); (2) the Australasian region is a secondary center of diversification of the subtribe; (3) vegetative features have shown to have greater value than reproductive one in determining major groups in the subtribe; and (4) at genus level, some genera, i.e. *Phalaenopsis*, *Cleisostoma*, *Sarcochilus*, and *Aerides* are shown to be non-monophyletic. This study also resolved the taxonomic status of *Aerides flabellata* Rolfe ex Downie, a species with a debatable generic position.

**Keywords:** Aeridinae; Australasian genera; ITS region; *matK* gene; Orchidaceae; Phylogenetic analyses

## Abstrak

Analisis filogenetik lanjut pada orkid subtribe Aeridinae telah dijalankan menggunakan jujukan DNA iaitu ITS daripada nrDNA dan *matK* daripada cpDNA. Dalam kerja-kerja awal, kami hanya melibatkan genus yang mewakili Asia. Selanjutnya, untuk mewujudkan hubungan yang lebih mantap dalam Aeridinae, dalam kajian ini kami telah melanjutkan persampelan untuk memasukkan spesimen daripada Australasia. Hasil analisis kami menunjukkan bahawa: (1) subtribe yang disusun semula oleh empat kumpulan utama dengan 11 kumpulan kecil (Ini adalah konsisten dengan sistem klasifikasi terdahulu subtribe itu); (2) Australasia merupakan pusat sekunder kepelbagaian daripada subtribe; (3) ciri vegetatif telah menunjukkan mempunyai nilai yang lebih besar daripada satu pembiakan dalam menentukan kumpulan-kumpulan utama dalam subtribe; dan (4) di peringkat genus, beberapa genera, iaitu *Phalaenopsis*, *Cleisostoma*, *Sarcochilus*, dan *Aerides* ditunjukkan bukan-monophyletic. Kajian ini juga memutuskan status taksonomi *Aerides flabellata* Rolfe ex Downie, satu spesies dengan kedudukan generik yang masih diperdebatkan.

**Kata kunci:** Aeridinae; Australasian genera; ITS region; *matK* gene; Orchidaceae; Phylogenetic analyses

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## 1.0 INTRODUCTION

The Aeridinae is one of the largest and most diverse subtribes of the Orchidaceae, which is estimated to be made up of 103 genera with 1,350 species. Along with the other two monopodial subtribes, Angraecinae and Aerangidinae, the Aeridinae have been placed in tribe Vandae (Dressler 1993), forming a large

horticulturally important group in the family Orchidaceae. The members of subtribe Aeridinae are mostly epiphytes distributed primarily throughout warm-temperate and tropical regions of Asia and Australasia with a very much smaller number of genera occurring in Africa. The subtribe Aeridinae is characterized by having two or four hard pollinia with a well-developed stipe and a viscidium. Several genera are further characterized by a column-

foot and a spurred lip. Taxonomic treatments by several workers such as Garay (1972), Senghas (1988), Seidenfaden (1988), Dressler (1993), and Chase (2005) have circumscribed the subtribe Aeridinae. However, owing to remarkable morphological diversification and parallelism, generic relationships in the subtribe remain unresolved.

A number of generic classifications have been proposed in the subtribe, which were based mainly on presence or absence of the column-foot (Schlechter 1926) and number and aperture type of pollinia (Smith 1934; Holttum 1958; Senghas 1988; Dressler 1993). Karyotypes and chromosome number have been used to identify one group in the subtribe which consists of *Vanda*, *Ascocentrum*, *Neofinetia*, and *Aerides* (Tanaka & Kamemoto 1961; Kamemoto & Shindo 1962; Shindo & Kamemoto 1962, 1963; Kamemoto 1963). However, Tara & Kamemoto (1970) and Tanaka & Kamemoto (1984) suggested that karyotypes and chromosome numbers are highly uniform in subtribe Aeridinae and that these characters are not informative in a phylogenetic context. Moreover, as described by Topik *et al.* (2005, 2006), the column-foot and pollinarium are highly diverse in the subtribe, and are not always phylogenetically informative characters.

To clarify phylogenetic relationships among the members of the subtribe, DNA sequences data have been employed: Jarrell and Clegg (1995) for ITS region and *matK* sequences; Cameron *et al.* (1999) for *rbcL*; van den Berg *et al.* (2005) for ITS region, *matK*, *rbcL*, and *trnL-F*; Topik *et al.* (2005) for ITS region and *matK*; Carlswald *et al.* (2006) for ITS region; and more recently Kocyan *et al.* (2008) for ITS region, *matK*, and *trnL-F*. The three last-

mentioned studies used the greatest number of representative genera in the subtribe. In these three analyses, the monophyletic nature of the Aeridinae is clearly demonstrated. However, satisfactory conclusions about phylogenetic relationships within subtribe Aeridinae could not be produced. This is perhaps because sampling size in these analyses remains insufficient (using mainly Asian Aeridinae). In our study, therefore, phylogenetic analyses based on DNA sequences of the nuclear ITS region and the plastid *matK* region were conducted, using a more extensive sampling in which genera distributed in the Australasian region were included. It was aimed to address generic relationships in subtribe Aeridinae and the biogeographic history of Australasian Aeridinae.

## 2.0 MATERIALS AND METHODS

### 2.1 Plant Materials

In this study, specimens from the Asian and Australasian region were examined. Members of subtribe Angraeacinae and Aerangidinae were used as outgroup because these subtribes have been recognized as the sister group to subtribe Aeridinae on the basis of morphological (Dressler 1993) and macromolecular characters (Jarrell & Clegg 1995; Cameron *et al.* 1999; Chase 2005; van den Berg *et al.* 2005; Topik *et al.* 2005). Voucher specimens were deposited at TNS; see Table 1.

**Table 1** Plant materials examined in this study. The materials were collected from Tsukuba Botanical Garden-Japan (TBG), Bogor Botanical Garden Indonesia (BBG), Thailand (THAI), Malaysia (MAL), and Australia (AU)

| TAXON                                                   | SOURCE | VOUCHER            |
|---------------------------------------------------------|--------|--------------------|
| <b>Tribe Vandae</b>                                     |        |                    |
| <b>Subtribe Angraeacinae</b>                            |        |                    |
| <i>Angraecum scottianum</i> Rchb.f.                     | TBG    | TBG102594          |
| <i>Jumellea sagittata</i> H. Perrier.                   | TBG    | TBG140595          |
| <b>Subtribe Aerangidinae</b>                            |        |                    |
| <i>Microterangis hariotiana</i> (Kraenzl.) Senghas      | TBG    | TBG126670          |
| <b>Subtribe Aeridinae</b>                               |        |                    |
| <i>Abdominea minimiflora</i> J.J.Sm.                    | BBG    | B200107222         |
| <i>Acampe ochracea</i> Hochr.                           | TBG    | TBG180168          |
| <i>Acampe rigida</i> (Buch.-Ham. ex Sm.) P.F. Hunt      | TBG    | TBG56086           |
| <i>Adenoccos parviflora</i> Ridl.                       | TBG    | TBG142425          |
| <i>Aerides flabellata</i> Rolfe ex Downie               | TBG    | TBG144183          |
| <i>Aerides odorata</i> Lour.                            | TBG    | TBG118480          |
| <i>Amesiella monticola</i> J.E. Cootes & D.P. Banks     | TBG    | TBG123790          |
| <i>Arachnis flosaeris</i> Rchb.f.                       | TBG    | TBG118482          |
| <i>Armadorum sulingi</i> Schltr.                        | BBG    | 983.III.130        |
| <i>Ascocentrum christensonianum</i> J.R. Haager         | TBG    | TBG145826          |
| <i>Ascocentrum pusillum</i> Averyanov                   | TBG    | TBG130215          |
| <i>Ascochilus emarginatus</i> (Blume) Schuit.           | TBG    | TBG142222          |
| <i>Ascoglossum calopterum</i> (Reichb. f.) Schltr.      | TBG    | TBG144580          |
| <i>Biermannia decipiens</i> (J.J.Sm.) Garay             | TBG    | TBG145838          |
| <i>Bogoria raciborskii</i> J.J.Sm.                      | BBG    | B200207370         |
| <i>Brachypeza indusiata</i> (Reichb.f.) Garay           | BBG    | B995112733         |
| <i>Brachypeza zamboangensis</i> (Ames) Garay            | TBG    | TBG145835          |
| <i>Ceratocentron fessellii</i> Senghas                  | TBG    | TBG133203          |
| <i>Ceratochilus biglandulosus</i> Blume                 | TBG    | TBG144188          |
| <i>Cleisocentron merrillianum</i> (Ames) Christenson    | TBG    | TBG137038          |
| <i>Cleisomeria pilosulum</i> (Gagnep.) Seidenf. & Garay | TBG    | TBG140482          |
| <i>Chiloschista viridiflava</i> Seidenf.                | THAI   | OR-2392002239      |
| <i>Christensonia vietnamica</i> J.R. Haager             | TBG    | TBG118224          |
| <i>Cleisostoma aff. gjellerupii</i> (J.J.Sm.) Garay     | TBG    | Cult. K. Tsukahara |
| <i>Cleisostoma fuerstenbergianum</i> Kranzlin           | AU     | PW012004           |
| <i>Cleisostoma scolopendrifolium</i> (Makino) Garay     | TBG    | TBG134570          |
| <i>Cleisostoma williamsonii</i> (Rchb.f.) Garay         | AU     | PW022004           |
| <i>Cryptopylos clausus</i> (J.J.Sm.) Garay              | THAI   | TBG145845          |
| <i>Dimorphorchis lowii</i> Rolfe                        | TBG    | TBG118871          |
| <i>Diploprora truncata</i> Rolfe ex Downie              | TBG    | TBG133822          |
| <i>Doritis pulcherrima</i> Lindl.                       | TBG    | TBG118342          |

|                                                                          |      |            |
|--------------------------------------------------------------------------|------|------------|
| <i>Dryadorchis singularis</i> (J.J.Sm) Christenson & Schuit.             | BBG  | B200009216 |
| <i>Drymoanthus minimus</i> (Schltr.) Garay                               | TBG  | TBG125168  |
| <i>Dyakia hendersoniana</i> (Rchb.f.) Christenson                        | TBG  | TBG133581  |
| <i>Esmeralda clarkei</i> Rchb. f.                                        | TBG  | TBG132983  |
| <i>Gastrochilus calceolaris</i> D.Don                                    | TBG  | TBG142434  |
| <i>Gastrochilus japonicus</i> Schltr.                                    | TBG  | TBG126600  |
| <i>Grosourdia callifera</i> Seidenf.                                     | THAI | TBG145840  |
| <i>Gunnarella begaudii</i> (N. Halle) Senghas                            | TBG  | TBG125182  |
| <i>Haraella retrocalla</i> Kudo                                          | TBG  | TBG133078  |
| <i>Holcoglossum amesianum</i> (Rchb. f.) Christenson                     | TBG  | TBG128927  |
| <i>Holcoglossum tsi</i> T. Yukawa                                        | TBG  | TBG124467  |
| <i>Hygrochilus parishii</i> Pfitzer                                      | TBG  | TBG118479  |
| <i>Hymenorchis javanica</i> (Teijsmann & Bien.) Schltr.                  | TBG  | TBG144228  |
| <i>Lesliea mirabilis</i> Seidenf.                                        | THAI | TBG145844  |
| <i>Luisia amesiana</i> Rolfe.                                            | TBG  | TBG128939  |
| <i>Luisia teres</i> Bl.                                                  | TBG  | TBG56127   |
| <i>Macropodanthus philippinensis</i> Williams                            | TBG  | TBG128821  |
| <i>Malleola baliensis</i> J.J.Sm.                                        | TBG  | TBG127481  |
| <i>Micropera pallida</i> Lindl.                                          | TBG  | TBG118444  |
| <i>Microsaccus griffithii</i> (Par. & Rchb.f.) Seidenf.                  | TBG  | TBG129769  |
| <i>Mobilabium hamatum</i> Rupp                                           | AU   | PW032004   |
| <i>Monantochilus chrysanthus</i> (Schltr.)R. Rice comb. nov              | TBG  | TBG145831  |
| <i>Neofinetia falcata</i> Hu.                                            | TBG  | TBG140668  |
| <i>Nothodoritis zhejiangensis</i> Z.H. Tsi                               | TBG  | TBG137501  |
| <i>Omoea philippinensis</i> Ames                                         | TBG  | TBG133261  |
| <i>Ornithochilus difformis</i> (Wall. ex Lindl.) Schltr.                 | TBG  | TBG127885  |
| <i>Papilionanthe subulata</i> (Willd.) Garay                             | TBG  | TBG118901  |
| <i>Papillilabium beckeri</i> (Benth.) Dockrill                           | AU   | PW042004   |
| <i>Paraphalaenopsis labukensis</i> P.S.Shim,A.L.Lamb & C.L.Chan          | TBG  | TBG137305  |
| <i>Pelatantheria ctenoglossum</i> Ridl.                                  | TBG  | TBG130214  |
| <i>Pennilabium struthio</i> Carr                                         | MAL  | TBG144490  |
| <i>Peristeranthus hillii</i> (F. Muell.) Hunt                            | AU   | PW052004   |
| <i>Phalaenopsis amabilis</i> Blume                                       | TBG  | TBG145847  |
| <i>Phalaenopsis chibae</i> T. Yukawa                                     | TBG  | TBG115846  |
| <i>Phalaenopsis deliciosa</i> Rchb.f.                                    | TBG  | TBG145842  |
| <i>Phalaenopsis fasciata</i> Rchb.f.                                     | TBG  | TBG145726  |
| <i>Phalaenopsis wilsonii</i> Rolfe                                       | TBG  | TBG144214  |
| <i>Plectorrhiza brevilabris</i> (F. Muell.) Dockrill                     | AU   | PW062004   |
| <i>Plectorrhiza erecta</i> (Fitzg.) Dockrill                             | AU   | PW072004   |
| <i>Plectorrhiza tridentata</i> (Lindl.) Dockrill                         | AU   | PW082004   |
| <i>Pomatocalpa diffusa</i> Breda                                         | TBG  | TBG145837  |
| <i>Pomatocalpa kunstleri</i> J.J.Sm.                                     | TBG  | TBG145833  |
| <i>Pteroceras pallidum</i> (Bl.) Holttum                                 | TBG  | TBG140670  |
| <i>Renanthera angustifolia</i> (Bl.) Hook.f.                             | TBG  | TBG124337  |
| <i>Renanthera isosepala</i> Holttum                                      | AU   | PW092004   |
| <i>Rhyncostylis retusa</i> (L.) Blume                                    | TBG  | TBG118423  |
| <i>Rhinerrhiza divitiflora</i> (Benth.) Rupp                             | AU   | PW102004   |
| <i>Rhinerrhiza moorei</i> (Rchb.f) M.A. Clem., B.J. Wallace & D.L. Jones | BBG  | B20000912  |
| <i>Robiquetia bertholdii</i> Schltr.                                     | TBG  | TBG125177  |
| <i>Robiquetia mooreana</i> J.J.Sm.                                       | AU   | PW112004   |
| <i>Saccolabiopsis armitii</i> (F. Muell.) Dockrill                       | AU   | PW132004   |
| <i>Saccolabium pusillum</i> Bl.                                          | TBG  | TBG144220  |
| <i>Sarcochilus hartmannii</i> F. Mueller                                 | TBG  | TBG145793  |
| <i>Sarcochilus hirticalcar</i> (Dockrill) M.A. Clem. & B.J. Wallace      | AU   | PW142004   |
| <i>Sarcochilus moorei</i> Schltr.                                        | AU   | PW152004   |
| <i>Sarcochilus spathulatus</i> R.S. Rogers                               | AU   | PW162004   |
| <i>Sarcochilus weinthalii</i> F.M. Bailey                                | AU   | PW172004   |
| <i>Sarcoglyphis comberi</i> (J.J.Wood) J.J.Wood                          | TBG  | TBG144127  |
| <i>Schistotylus purpuratus</i> (Rupp) Dockrill                           | AU   | PW182004   |
| <i>Schoenorchis paniculata</i> Bl.                                       | TBG  | TBG140487  |
| <i>Sedirea japonica</i> (L. Linden & Rchb.f.) Garay & HR. Sweet          | TBG  | TBG145832  |
| <i>Seidenfadenia mitrata</i> (Rchb. f.) Garay                            | TBG  | TBG141188  |
| <i>Smitinandia helferi</i> (Hk. f.) Garay                                | TBG  | TBG140484  |
| <i>Smitinandia micrantha</i> (Lindl.) Holttum                            | TBG  | TBG118427  |
| <i>Staurochilus ionosma</i> Schltr.                                      | TBG  | TBG130159  |
| <i>Stereochilus aff. dalatensis</i> (Guill.) Garay                       | TBG  | TBG127489  |
| <i>Taeniophyllum aphyllum</i> Makino                                     | TBG  | TBG145829  |
| <i>Thrixspermum centipeda</i> Lour.                                      | TBG  | TBG118459  |
| <i>Thrixspermum subulatum</i> Rchb.f.                                    | TBG  | TBG113211  |
| <i>Trichoglottis latisepala</i> Ames var. tricarinata T. Hashimoto       | TBG  | TBG79675   |
| <i>Trudelia pumila</i> (Hook.f.) Senghas                                 | TBG  | TBG118899  |
| <i>Tuberolabium escritorii</i> (Ames) Garay                              | TBG  | TBG141159  |
| <i>Vanda coerulea</i> Griff. ex Lindl.                                   | TBG  | TBG133816  |
| <i>Vanda hindsii</i> Lindl.                                              | AU   | PW192004   |
| <i>Vanda tricolor</i> "Planilabre"                                       | AU   | PW202004   |
| <i>Vandopsis lissochiloides</i> (Gaud.) Pfitzer                          | TBG  | TBG56108   |
| <i>Ventricularia tenuicaulis</i> (Hk. f.) Garay                          | THAI | TBG145846  |

## 2.2 Amplification and Sequencing

The ITS region and the *matK* gene of Asian genera have previously been sequenced for our preliminary phylogenetic

analyses of the subtribe (Topik *et al.* 2005). Total DNA of Australasian specimens was extracted from silica-gel dried plant tissue with a QIAGEN DNeasy Mini Plant Kit following the manufacturer's instructions. Experimental methods for the ITS

and *matK* amplification and sequencing are described in Topik et al. (2005). In case of ITS amplification, we performed single strand conformation polymorphism (SSCP) analysis to confirm homogeneity of amplification product. SSCP performed was based on the method developed by Orita et al. (1989).

### 2.3 Phylogenetic Analyses

DNA sequences obtained from *matK* and ITS were aligned with Clustal X and were then adjusted manually following the guidelines in Kelchner (2000). The aligned data file is available from the first author upon request. Phylogenetic analyses based on the maximum parsimony criterion were performed using PAUP\* version 4.0b10 (Swofford 1998) for three data sets, *matK*, ITS and a combination of the two. Insertions and deletions were treated as missing data. All characters were equally weighted and unordered (Fitch 1971). All the data sets were analyzed by the heuristic search method with tree bisection-reconnection (TBR) branch swapping and the MULTREES option on, ten replications of random addition sequences with the stepwise addition option, and most parsimonious trees (MPTs) were saved. Evaluation of internal support of clades was conducted by the bootstrap analysis (Felsenstein 1985) utilizing 1,000 replicates with TBR branch swapping and the MULTREES option off. Number of steps, consistency indices (CI) and retention indices (RI) were calculated on one of the MPTs in each analysis with the TREE SCORES command in PAUP\*.

## 3.0 RESULTS

In our preliminary analyses, combined data of ITS and *matK* sequences revealed much more robust topologies of phylogenetic trees than those generated from the separate data sets (Topik et al. 2005). Therefore, in this paper we utilised only the combined data.

The aligned matrix for the combined analysis comprised 2,407 characters, of which 1,110 (46%) were constant and 757 (32%) were potentially informative. This analysis resulted in 560 MPTs with the length 3,989 steps, CI (excluding autapomorphies) of 0.46 and RI of 0.59. Although some nodes have low bootstrap percentages, a strict consensus tree (Fig. 1) clearly recognized four major clades with 11 subclades. Most monophyletic subclades recognized in our previous study (Topik et al. 2005) were similar in this analysis with some differences. In this analysis, bootstrap percentages (BP) are very high for several alliances such as *Aerides* alliance (BP 96), *Pomatocalpa* (BP 97), *Acampe* (BP 99), and *Phalaenopsis* (BP 100). Low bootstrap support is given for some alliances such as *Trichoglottis* (BP 68), *Sarcochilus* (BP 54), and *Pteroceras* (BP 60). Additionally, the positions of *Sedirea* and *Rhynchostylis* were undetermined as in all previous results.

## 4.0 DISCUSSION

### 4.1 Generic Relationships in Subtribe Aeridinae

The analysis of the combined data set revealed that subtribe Aeridinae is composed of four major clades with 11 subclades (Fig. 1). The component of four major clades with 11 subclades is not in agreement with previous subdivisions in the subtribe such as Schlechter (1926) and Senghas (1988). Although several recognisable morphological groups are found in this study, such

as the *Phalaenopsis* and *Aerides* alliances, many unexpected relationships appeared.

Clade 1 (*Luisia* alliance) comprises *Luisia*, *Dryadorchis*, *Armadorum*, *Esmeralda*, *Renanthera*, *Ascoglossum*, and *Gastrochilus*. This group is characterized by long leafy stems, a triangular viscidium, and a strap-like stipe (Topik et al. 2006). However, pollinia numbers are not consistent in this group. The phylogenetic positions of *Sedirea* and *Rhynchostylis* remain unclear but the shortest trees place them each as sister to the rest of clade 2 and 3, and clade 4, respectively. This problem may be resolved through increasing the sample (Zwickl & Hillis 2002), or by using different markers.

Clade 2 (*Aerides* alliance) comprises *Holcoglossum*, *Papilionanthe*, *Ascocentrum*, *Vanda*, *Neofinetia*, *Aerides*, *Seidenfadenia*, *Trudelia*, and *Christensonia*. This grouping is consistent with Christenson's (1987, 1994) view based on morphological characters such as a long leafy stem, broad epichile of the lip, and stout column. Based on pollinarium structure, putative synapomorphic characters of the group are a quadrangular viscidium (Topik et al. 2006). Additionally, as mentioned before, chromosomal analyses (Tanaka & Kamemoto 1961; Kamemoto & Shindo 1962; Shindo & Kamemoto 1962, 1963; Tara & Kamemoto 1970) revealed that *Vanda*, *Ascocentrum*, *Neofinetia*, and *Aerides* are closely related to each other.

Clade 3 has low bootstrap percentages (BP 68) and consists of the following seven subclades: *Pomatocalpa*, *Acampe*, *Trichoglottis*, *Thrixspermum*, *Diploprora*, *Arachnis*, and *Pelatantheria* alliances. According to Dressler (1993), this clade is defined by long leafy stem, non-broad epichile, and non-stout column. Monophyletic nature of *Pomatocalpa* and *Haraella* (the *Pomatocalpa* alliance) has not been reported in previous studies. A pollinarium character shared by the two genera is an oval viscidium (Topik et al. 2006). This character state is apomorphic in the subtribe, but it is also found in the *Saccolabium*, *Acampe*, *Thrixspermum*, *Diploprora*, and *Arachnis* alliances (Topik et al. 2006). The two genera have different pollinium numbers: two in *Haraella* and four in unequal pairs in *Pomatocalpa*. Watthana et al. (2006) conducted a detailed analysis of this alliance.

Three genera, *Acampe*, *Adenoncos*, and *Micropera*, constitute the *Acampe* alliance with high bootstrap support (BP 99). An unmovable lip characterizes this group (Dressler 1993). Pollinia characters differ among the genera. *Adenoncos* has four pollinia in equal pairs, a state similar to *Doritis*, *Nothodoritis*, *Taeniophyllum*, and *Microsaccus*. On the other hand, *Micropera* and *Acampe* share four pollinia in unequal pairs.

The *Trichoglottis* alliance, which has low bootstrap percentages (BP 68), comprises *Trichoglottis*, *Staurochilus*, *Vandopsis*, *Ceratochilus*, and *Ventricularia*. Previously, Holtum (1958) recognized the affinity of the first three genera but did not include *Ceratochilus* and *Ventricularia*. This alliance shares a raising tongue across the spur from the column base to the spur base (Seidenfaden 1988) and a quadrangular viscidium (Topik et al. 2006).

The *Thrixspermum* alliance comprises six genera: *Thrixspermum*, *Dimorphorchis*, *Abdominea*, *Microsaccus*, *Cleisomeria*, and one sampled species of *Sarcochilus* (*S. chrysanthus*). Although this grouping is highly supported (BP 99), no morphological synapomorphies are available for the subclade at this time. With the exception of *Dimorphorchis*, all of these genera have four pollinia.

Clade 4, which has moderate bootstrap support (BP 70), is defined by a putative synapomorphic state, short leafy stem. The following four subclades are recognized within this clade: *Sarcochilus*, *Pteroceras*, *Phalaenopsis*, and *Saccolabium*

alliances. The *Sarcochilus* alliance includes several genera distributed mainly in Australasian region, such as *Plectorrhiza*, *Papillilabium*, *Peristeranthus*, *Sarcochilus*, and *Mobilabium*. This alliance shares a fleshy root, a stem with dry leaf bases and a fan of channeled leaves (Garay 1972).

The *Pteroceras* alliance appears to be a monophyletic group. The alliance shares an elongated column (Seidenfaden 1988; Garay 1972) and a putative synapomorphic character in their pollinarium, a prominent caudicle from which the pollinia are attached to the apex of a strap-like stipe (Topik et al. 2006).

Monophyly of the *Phalaenopsis* alliance was strongly supported (BV 100). The alliance, consisting of *Phalaenopsis*, *Lesliea*, *Doritis*, and *Nothodoritis*, is defined by a putative synapomorphy of the pollinarium, i.e., an apically expanded stipe to which the pollinia are ventrally attached (Topik et al. 2006). Yukawa et al. (2005) have carried out a more detailed analysis of this alliance.

The *Saccolabium* alliance includes *Dyakia*, *Saccolabium*, *Macropodanthus*, *Ceratocentron*, *Amesiella*, *Tuberolabium*, *Pennilabium*, *Cryptopylos*, and *Hymenorchis*. An inflorescence with the pedicel of each flower emerging from the base of a crater-like structure is a putative synapomorphy for this group (Christenson 1986b).

### 4.2 Significance of Vegetative Characters

Floral morphology has received great attention in major subdivision of subtribe Aeridinae. The most recent classification

scheme of subtribe Aeridinae, by Dressler (1993), is based mainly on pollinia characters (number and aperture). However, Topik et al. (2005) showed that these characters, and other characters such as presence or absence of column foot, are not good characters for determining major relationships of the subtribe. In contrast, length of the stem appears to be straightforward character as seen in this study.

To trace evolutionary trends and systematic utility of the length stem, we mapped the character states onto one of four MPTs using MacClade 3.05 under accelerated character transformation (ACCTRAN) optimization (Fig 2). The result displayed in Fig 2 shows that a short stem is the apomorphic state in the subtribe. Moreover, this also indicates that this vegetative feature may deserve recognition as an indicator of phylogenetic relationships in subtribe Aeridinae.

Vegetative features have shown to have greater value than reproductive features in determining major groups in some groups of orchids. Some examples are as follows: number of leaves is used in major subdivision of *Cattleya* (van den Berg et al. 2000); a combination of stem, sheath, and leaf characters have united florally disparate taxa such as *Scaphosepalum* and *Platystele* into a monophyletic group (Pridgeon et al. 2001); based upon pseudobulb and number of foliaceous bract characters, two genera, *Cyrtorchilum* and *Odontoglossum*, can be distinguished from each other (Williams et al. 2001); and based on life form of its members, tribe Malaxideae is clearly split into two major groups, one with mainly terrestrial species and the other mainly comprising epiphytes (Cameron 2005).

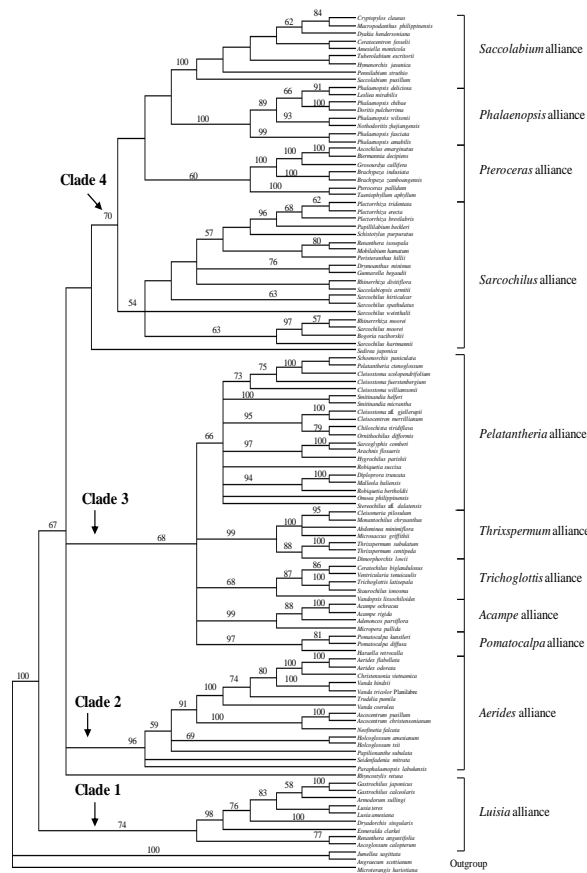


Fig. 1. Strict consensus of 560 MPTs derived from the parsimony analysis of a combined data set (Length= 3,989 steps; CI= 0.46; RI= 0.59). Bootstrap percentages of > 50 are shown above each branch.

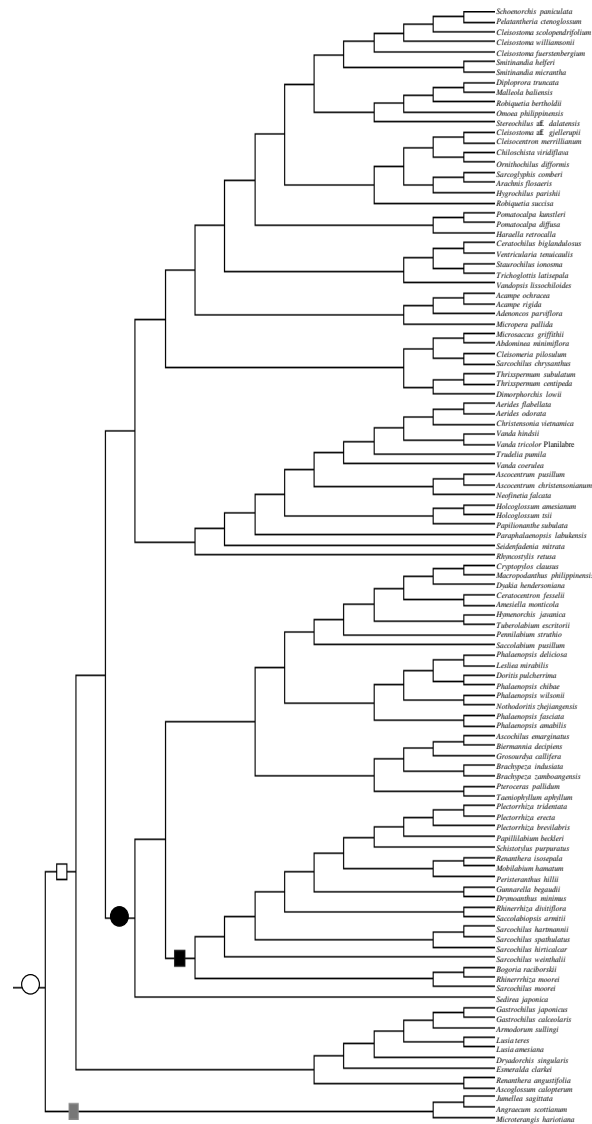


Fig. 2. Area (boxes) and stem length (circles) state reconstruction of subtribe Aeridinae under ACCTAN optimization. White box is Asian clade and black box is Australasian clade. Gray box represents African clade for outgroup. White and black circles are long and short stem respectively.

Floral traits, on the other hand, are frequently found to show considerable levels of homoplasy (e.g., Pridgeon *et al.* 1997; Bateman *et al.* 1997, 2003). Orchids are well known for their elaborate relationships with pollinators (e.g., Dodson 1962), which are capable of repeatedly driving the evolution of similar floral forms in lineages without necessarily sharing a recent common ancestor (Cameron 2005). Floral morphology of three distantly related lineages of Orchidaceae: *Sobralia* (lower Epidendroideae), *Cattleya* (higher Epidendroideae), and *Epistephium* (Vanilloideae), represents convergence evolution. They superficially look very much alike and probably share similar or closely related pollinators. Morphological convergence of flowers may also be pointed out in the case of *Angraecum* (Angraecoids group) and two genera of subtribe Aeridinae, *Amesiella* and *Neofinetia* (Topik *et al.* 2005).

Concerning its important role in orchid taxonomy, only few authors have used length of the stem as a main character in their systems. Schlechter (1913) used length of the stem as one important character in his infrageneric system of the genus *Sarcocochilus*. Rice (2004) utilized this character as one of the

most important characters in the separation of *Sarcocochilus chrysanthus* and related species from other *Sarcocochilus* species in the new genus *Monantochilus*. In terms of its basic function, length of the stem determines energy efficiency in the transport of fluids between the roots and the leaves and vice versa. The consequence of this is that those with a long(er) stem will need more energy to transport the fluids than those with a short one. The change of length of the stem may influence mode of growth, development, and adaptation, because the process of fluid transportation is associated with these factors in many cases (Evans 1975).

### 4.3 Biogeographic Implications

Species of subtribe Aeridinae cover mainly the Asian and Australasian regions, and these areas are considered to be the center of diversification of this subtribe (e.g., Holttum 1958; Dressler 1981, 1993; Seidenfaden 1988). In this study, the Australasian genera were nested within clade 4, and formed a monophyletic group together with part of the Asian genera. To

trace the center of origin and biogeographic history of the subtribe, character-state mapping onto one of 560 MPTs was performed using MacClade under ACCTRAN optimization. The result as depicted in Fig. 2 supports the hypothesis that subtribe Aeridinae originated from Asia and subsequently spread out to Australasian regions.

The result also suggests that the Australasian region is a secondary center of diversification of the subtribe. It is likely that a part of ancestral stocks in clade 4 had migrated to the Australasian region. The migration from Asia to Australasia may have occurred through New Guinea when polar glaciation was at its peak and New Guinea was connected to Australia (White 1990). Without a doubt, land connection between these two regions has acted as corridors for this group. A similar pattern of migration has also been suggested for *Nothofagus* (Setoguchi et al. 1997), *Araucaria* (Setoguchi et al. 1999), *Amaryllidaceae* (Ito et al. 1999), and *Dendrobium* (Yukawa et al. 2000).

Several genera such as *Sarcochilus*, *Rhinerrhiza*, and *Bogoria* can be considered as candidates of ancestors that migrated to the Australasian region as some species of these genera are also distributed in Asian region. Thus their occurrence in the Asian region provides good evidence of such an assumption. It is natural that migration to a new region may be accompanied by several morphological changes to adapt to new environmental conditions before they are subsequently divergence.

#### 4.4 Taxonomic Implications

Previous classifications have based subdivisions of subtribe Aeridinae mainly on pollinia characters (number and aperture) and the presence or absence of the column foot. Topik et al. (2005), however, suggested that the repeated evolution of these character states masked their true phylogenetic relationships. Several characters described above, such as stem length (in this study) and shapes of the stipe and viscidium (Topik et al. 2005), were found to be more useful diagnostic characters of major clades.

At generic level, some genera such as *Phalaenopsis*, *Cleisostoma*, and *Sarcochilus* are found to be non-monophyletic. In *Phalaenopsis*, pollinium number, shape of the rostellum, spur length, and shape and processes of the lip are greatly diversified. Novel combinations of characters in the alliance resulted in several monotypic genera, such as *Nothodoritis* and *Lesliea*, and the status of *Doritis* and *Kingidium* is still not settled. Yukawa et al. (2005) conducted a detailed analysis of *Phalaenopsis* alliance and advocated a revised classification on the basis of molecular and morphological characters.

*Cleisostoma* exhibits remarkable diversification in the shape of leaves, the septum of spur, stipe, viscidium, and the apex of lip. The genus was revised by Seidenfaden (1975) and is divided into seven sections. The type section has a bilobed leaf, a linear, tapering or clavate stipe, and an oval viscidium. The three sampled species of *Cleisostoma*: *C. scolopendrifolium* (section *Paniculatum*), *C. fuerstenbergium* (section *Pilearia*), and *C. williamsonii* (section *Mitriiformes*), have mitre-shaped, larger stipe and broad viscidium, whereas a tapering, strap-shape stipe is found in *C. aff. gjellerupii* (section *Cleisostoma*). Owing to its possession of similar characteristics to the type section, *C. aff. gjellerupii* should remain in *Cleisostoma*.

Surprisingly, the results are strong enough to suggest that, in its present circumscription, *Vanda* is not monophyletic. The nomenclatural consequences are unpleasant: whether to sink *Vanda* into *Aerides* or to split it into several genera. In the last

option, more detailed taxonomic sampling to include other likely segregates such as *Euanthe* is required. Further, the last option seems likely to be the best choice because the former option will make *Aerides* become non-monophyletic.

This study resolved the taxonomic status of *Aerides flabellata* Rolve ex Downie, a species with a debatable generic position. For example, Garay (1972), Seidenfaden (1973), and Christenson (1986a) advocated its placement in genus *Vanda* because it exhibits the short spur and broad lip that characterize *Vanda*. In contrast, results of this study suggested its placement in *Aerides*, which is found to be characterized by two apomorphic states: a long column foot and a movable lip.

#### 4.5 Future Challenges

The present study indicates that subtribe Aeridinae is one of the most taxonomically complex groups in the orchids. The phylogenetic relationships presented here provide framework for further systematic and taxonomic investigations of the subtribe. In particular, some unexpected relationships were found in this study, and putative morphological synapomorphies for these relationships are not understood. Further phylogenetic analyses with more molecular markers and greater taxon sampling are desirable to establish more robust phylogenetic hypotheses for the subtribe.

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