

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

Topik Hidayat^{a,b*}, Peter H. Weston^c, Tomohisa Yukawa^d, Motomi Ito^e, Rod Rice^f

^aDepartment of Biological Sciences, Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia (UTM); 81310 UTM Skudai, Johor Bahru, Johor, Malaysia

^bDepartment of Biology Education, Faculty of Mathematic and Natural Science Education, Indonesia University of Education (UPI); JL. Dr. Setiabudi 229 Bandung 40154 Indonesia

^cNational Herbarium of NSW, Mrs. Macquaries Road, Sydney NSW 2000, Australia

^dTsukuba Botanical Garden, National Science Museum; 1-1, Amakubo 4, Tsukuba, Japan

^eDepartment of General Systems Sciences, Graduate School of Arts and Science, The University of Tokyo; Komaba 3-8-1, Meguro-ku, Tokyo, Japan

^fOrchid Scholar/Honorary Research Associate to the Orchid Herbarium; Sydney Botanical Gardens. Mrs Macquaries Road Sydney NSW 2000 Australia.

*Corresponding author: topik28@yahoo.com

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Graphical abstract

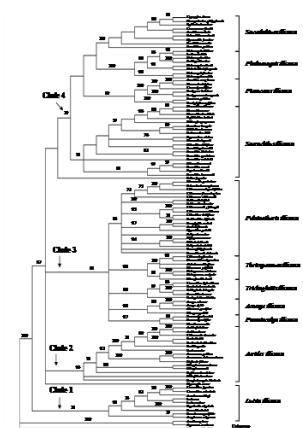


Fig. 1. Maximum likelihood phylogenetic tree of subtribe Aeridinae (Orchidaceae) based on ITS and matK DNA sequences. The tree is rooted with the outgroup of Vandeae. Bootstrap values are shown at the nodes.

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*, *Sarcocylus*, and *Aerides* are shown to be non-monophyletic. This study also resolved the taxonomic status of *Aerides flabellata* Rolve 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*, *Sarcocylus*, dan *Aerides* ditunjukkan bukan-monophyletic. Kajian ini juga memutuskan status taksonomi *Aerides flabellata* Rolve 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 Vandeae (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 Angraecinae 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
Tribe Vandae		
Subtribe Angraecinae		
<i>Angraecum scottianum</i> Rchb.f.	TBG	TBG102594
<i>Jumellea sagittata</i> H. Perrier.	TBG	TBG140595
Subtribe Aerangidinae		
<i>Microterangis hariotiana</i> (Kraenzl.) Senghas	TBG	TBG126670
Subtribe Aeridinae		
<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>Adenoncos 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>Ceratocentrum fessellii</i> Senghas	TBG	TBG133203
<i>Ceratocentrum biglandulosum</i> Blume	TBG	TBG144188
<i>Cleisocentrum 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>Grosourdyia 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 tsii</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>Pelatanthera 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 latiseppala</i> Ames var. <i>tricarinata</i> 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, Holttum (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 *Sarcophilus* alliance includes several genera distributed mainly in Australasian region, such as *Plectorhiza*, *Papillilabium*, *Peristeranthus*, *Sarcophilus*, and *Mobilabium*. This alliance shares a fleshy root, a stem with dry leaf bases and a fan of channelled 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).

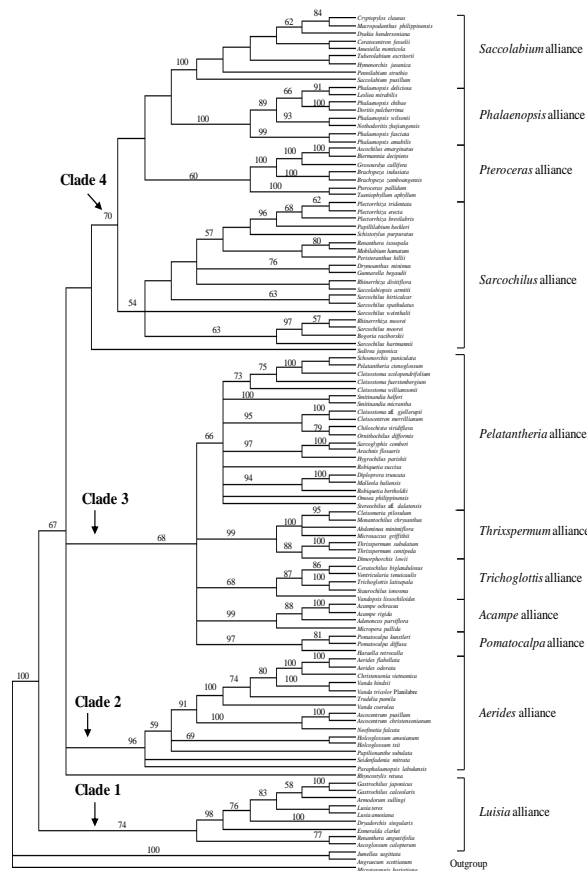
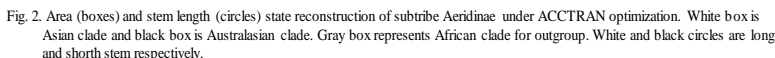


Fig. 1. Strict consensus of 560 MPTs derived from the parsimony analysis of a combined data set (Length= 3,989 steps; CI= 0.46; RI= 0.59). Bootstrap percentages of > 50 are shown above each branch.



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 *Sarcophilus*. Rice (2004) utilized this character as one of the

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., Holtum 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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