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Molecular phylogeny of *Phalaenopsis* Blume (Orchidaceae) based on the internal transcribed spacer of the nuclear ribosomal DNA

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Abstract. The internal transcribed spacer (ITS1, 5.8S rDNA, and ITS2) region of nuclear ribosomal DNA (nrDNA) was sequenced from 53 species, which represent most of the living species diversity in the genus *Phalaenopsis* (Orchidaceae). A phylogeny was developed for the genus based on the neighbor-joining and maximum parsimony analyses of molecular data. Results of these analyses provided support for the monophyly of the genus *Phalaenopsis* and concurred in that the genera *Doritis* and *Kingidium* should be treated as being parts of the genus *Phalaenopsis* as suggested by Christenson (2001). Within the genus *Phalaenopsis*, neither subgenera *Aphyllae* nor *Parishianae* were monophyletic, and they were highly clustered with subgenus *Proboscidioides* plus sections *Esmeralda* and *Deliciosae* of subgenus *Phalaenopsis* based on ITS data. Those species also have the same characters of morphology of four pollinia and similar biogeographies. Furthermore, neither subgenus *Phalaenopsis* nor *Polychilos* was monophyletic. Within the subgenus *Phalaenopsis*, only section *Phalaenopsis* was highly supported as being monophyletic. As for the subgenus *Polychilos*, only section *Polychilos* was moderately supported as being monophyletic. In conclusion, the present molecular data obtained from the ITS sequence of nrDNA of the genus *Phalaenopsis* provide valuable information for elucidating the phylogeny of this genus.

Key words: *Phalaenopsis*, phylogeny, rDNA, internal transcribed spacer.

The genus *Phalaenopsis* Blume (Orchidaceae), a beautiful and popular orchid, comprises approximately 66 species according to the latest classification of Christenson (2001), who divided this genus into five subgenera, namely *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos*, and *Phalaenopsis*. Of these, subgenus *Polychilos* was subdivided into four sections, namely *Polychilos*, *Fuscatae*, *Amboinenses*, and *Zebrinae*. In addition, subgenus *Phalaenopsis* was also subdivided into four sections, namely *Phalaenopsis*, *Deliciosae*, *Esmeralda*, and *Stauroglottis*.

Species of the genus *Phalaenopsis* are found throughout tropical Asia and the larger islands of the Pacific Ocean. The western distribution of *Phalaenopsis* is in Sri Lanka and South India. The eastern limit of the range is in Papua New Guinea. To the north, they are distributed in Yunnan Province (southern

China) and Taiwan. The southern limit is in northern Australia (Christenson 2001). Different subgenera of *Phalaenopsis* have distinct geographic distributions. Subgenera *Aphyllae*, *Parishianae*, and *Proboscidioides* are distributed in southern China and India extending to northern Vietnam, Myanmar, and Thailand, respectively. The subgenus *Polychilos* has a few species distributed as far west as northeastern India, but it is primarily centered in Indonesia and the Philippines (Christenson 2001). Subgenus *Phalaenopsis* is centered in the Philippines with two species extending to Taiwan (*P. aphrodite* subsp. *formosana* and *P. equestris*) and one wide-ranging species (*P. amabilis*) found from the Philippines and Indonesia to northern Australia (Christenson 2001).

All *Phalaenopsis* species (with the exception of the natural tetraploid species, *P. buyssoniana*) have 38 chromosomes (2n = 38) (Woodard 1951, Shindo and Kamemoto 1963, Tanaka and Kamemoto 1984, Christenson 2001). Furthermore, the chromosome number of the subtribe *Aeridinae* (syn. *Sarcanthinae*) (with only a few exceptions of tetraploid or hexaploid species) is consistent with that of *Phalaenopsis* (Christenson 2001). Crosses between *Phalaenopsis* species and other close genera, namely *Aerides*, *Arachnis*, *Ascocentrum*, *Doritis*, *Neofinetia*, *Renanthera*, *Rhynchostylis*, *Vanda*, and *Vandopsis*, also show various degrees of fertility (Sweet 1980).

Based on the number of pollinia, traditional species of *Kingidium* (which have four pollinia) were segregated from the genus *Phalaenopsis* (which has two pollinia) (Sweet 1980, Seidenfaden 1988b). Christenson (2001) treated the traditional genus *Kingidium* as *Phalaenopsis* and split it into different parts of *Phalaenopsis*, placing some species into the subgenus *Aphyllae* (*P. braceana*, *P. minus*, and *P. taenialis*) and some into the section *Deliciosae* (*P. chibae* and *P. deliciosa*) of the subgenus *Phalaenopsis*. Another group, the *P. parishii* complex, having four pollinia, was proposed as a segregated genus *Grafia*, by Hawkes (see Christenson 2001). This complex was first treated as section *Parishianae* of the genus *Phalaenopsis* by Sweet (1968). Christenson (2001) agreed with Sweet's treatment and placed this complex in the subgenus *Parishianae* of *Phalaenopsis*. Furthermore, the other four-pollinia species, *P. lowii*, was also placed in the section *Proboscidioides* of the genus *Phalaenopsis* (Sweet 1980) and in the subgenus *Proboscidioides* of the genus *Phalaenopsis* by Christenson (2001). Shim (1982), however, disagreed with Sweet's concept (1980) and separated sections *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos*, *Zebrinae*, *Fuscatae,* and *Amboinenses* as the genus *Polychilos* from a narrowly defined *Phalaenopsis*. In addition, the genus *Doritis* was traditionally separated from *Phalaenopsis* because of its pollinium number, lip structure, and adaptations to a terrestrial habitat (Sweet 1980, Seidenfaden 1988a). This group was also treated as the genus *Phalaenopsis* and placed in the section *Esmeralda* of the subgenus *Phalaenopsis* (Christenson 2001).

The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) has provided valuable information for resolving phylogenetic relationships at different taxonomic levels (e.g. Baldwin 1992, 1993; Suh et al. 1993; Sun et al. 1994; Bayer et al. 1996; Cox et al. 1997), particularly at the infrageneric level because of relatively rapid evolutionary rates. In this study, we followed the systematics proposed and references described by Christenson (2001). In order to reconstruct the phylogeny within the genus, the nucleotide sequences of the ITS region of the nrDNA from 53 taxa of *Phalaenopsis* plus 13 outgroup species were analyzed to address the phylogeny of the genus.

Materials and methods

Taxonomy and nomenclature of *Phalaenopsis* species in this study followed Sweet (1980) and Christenson (2001). Plant materials used include 53 taxa of the genus *Phalaenopsis*. In addition, specimens of each of seven related genera, namely *Aerides*, *Ascocentrum*, *Neofinetia*, *Renanthera*,

Rhynchostylis, *Vanda*, and *Vandopsis*, which show various degrees of fertility with *Phalaenopsis* (Sweet 1980), *Paraphalaenopsis*, which was once treated as *Phalaenopsis* (see Sweet 1980), as well as of five other genera of the subtribe *Aeridinae*, namely *Amesiella*, *Gastrochilus*, *Haraella*, *Thrixspernum*, and *Tuberolabium*, were selected as outgroup species (Table 1). All leaf material was taken from living plants in the Kaohsiung District Agricultural Improvement Station (KDAIS) in Taiwan and from the collection of C. C. Tsai. The flower pictures of those species in this study are available from the author (tsaicc@mail.kdais.gov.tw). Using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987), total DNA was extracted from fresh etiolated leaves. Ethanol-precipitated DNA was dissolved in TE (Tris-EDTA) buffer and stored at −20°C. Qiagen (Valencia, CA, USA) columns were used to clean the DNA of samples which was difficult to amplify by PCR. The approximate DNA yields were then determined using a spectrophotometer (model U-2001, Hitachi).

The ITS region, which includes the ITS1, 5.8S rDNA, and ITS2, was amplified by PCR with primers referenced to Tsai and Huang (2001). The protocols for the PCR were as follows: we used a 50-μl mixture containing 40 mM Tricine-KOH (pH 8.7), 15 mM KOAc, 3.5 mM $Mg(OAc)_2$, 3.75 μg/ml BSA, 0.005% Tween 20, 0.005% Nonidet-P40, four dNTPs (0.2 mM each), primers (0.5 μM each), 2.5 of Advantage 2 DNA polymerase (Clontech), 10 ng genomic DNA, and a 50-μl volume of mineral oil. The PCR mixture for amplifying the ITS region included 10% dimethyl sulfoxide (DMSO) to reduce problems related to the secondary structure and efficiency of PCR primer binding. Amplification reactions were completed in a dry-block with two-step thermal cycles (Biometra). In the first step, the mixture was incubated at 94°C for 3 min, then it underwent 10 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min. The second step was carried out by the following process: 30 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 45 s, and extension at 72°C for 1 min, with a final extension for 10 min at 72°C. These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained with 0.5 μg/ml ethidium bromide, and finally photographed under UV light exposure. The PCR products of ITS sequences from the plant material in this study were recovered using glassmilk (BIO 101, California).

These DNAs were directly sequenced following the method of dideoxy chain-termination using an ABI377 automated sequencer with the Ready Reaction Kit (PE Biosystems, California) of the BigDye™ Terminator Cycle Sequencing. Sequencing primers were the same as those used for PCR. Each sample was sequenced two or three times to confirm the sequences. These reactions were performed as recommended by the manufacturers.

The sequence alignment was determined using the program Clustal W multiple alignment in BioEdit (Hall 1999). The alignment was then checked, and apparent alignment errors were corrected by hand. Genetic relationships were then determined using the program MEGA version 2.1 (Kumar et al. 2001). The genetic distance matrix was calculated by use of the two-parameter method of Kimura (1980), and then it was used to construct phylogenetic trees using the Neighbor-joining (NJ) method (Saitou and Nei 1987). Maximum parsimony (MP) analyses (Fitch 1971) were done using code modified from the Close-Neighbor-Interchange (CNI) algorithm (Rzhetsky and Nei 1992) in MEGA (Kumar et al. 1993). Bootstrapping (1025 replicates) was carried out to estimate the support for both NJ and MP topologies (Felsenstein 1985, Hillis and Bull 1993). The strict consensus parsimonious tree was then constructed using the program MEGA version 2.1 (Kumar et al. 2001). The aligned data matrix and tree files are available from the author (tsaicc@mail.kdais.gov.tw).

Results

Sequence alignment and ITS characteristics. PCR products of each sample studied were directly sequenced except that of *P. viridis*. Since the ITS repeat sequences of *P. viridis* were not homogeneous, the sequences of this species were finally sequenced after T-vector cloning. Three separate clones were selected for sequencing. These three ITS sequences were quite variable. Sequence variation among these three clones ranged from 2.8 to 6.7%. Furthermore, *P. cochlearis* showed two separate PCR products for the ITS region of PCR amplification. Their lengths were approximately 460 and 700 bp,

Table 1. Names of specimens, geographical distribution, source, and GenBank accession numbers for the sequences of the internal transcribed spacer of nuclear ribosomal DNA

Taxa and systematic classification [a]	Geographical distribution	Source	GenBank accession no.
Genus *Phalaenopsis*			
Subgenus *Proboscidioides* (Rolfe) E. A. Christ.			
Phalaenopsis lowii Rchb.f. [b]	Myanmar, and adjacent western Thailand	KDAIS KC-88	AY912236
Subgenus *Aphyllae* (Sweet) E. A. Christ.			
Phalaenopsis wilsonii Rolfe	China (Sichuan, Yunnan, and eastern Tibet)	KDAIS KC-130	AY912257
Phalaenopsis minus (Seidenf.) E. A. Christ.	endemic to Thailand	KDAIS KC-227	AY912241
Phalaenopsis braceana (J. D. Hook.) E. A. Christ.	Bhutan and China	KDAIS KC-289	AY228495
Phalaenopsis honghenensis F. Y. Liu	China (Yunnan)	KDAIS KC-305	AY912229
Subgenus *Parishianae* (Sweet) E. A. Christ.			
Phalaenopsis gibbosa Sweet	Vietnam and Laos	KDAIS KC-52	AY912228
Phalaenopsis lobbii (Rchb.f.) Sweet	India, Bhutan, Myanmar, and Vietnam	KDAIS KC-21	AY912235
Phalaenopsis parishii Rchb.f.	eastern Himalayas, India, Myanmar, and Thailand	KDAIS KC-316	AY912245
Phalaenopsis appendiculata C. E. Carr	Endemic to Malaysia (Malay Peninsula)	KDAIS KC-411	AY912218
Subgenus *Polychilos* (Breda) E. A. Christ.			
Section *Polychilos* (Breda) Rchb.f.			
Phalaenopsis mannii Rchb.f.	northeast India, Nepal, and China to Vietnam	KDAIS KC-22	AY912238
Phalaenopsis cornu-cervi (Breda) Bl. & Rchb.f.	northeast India and the Nicobar Islands to Java and Borneo	KDAIS KC-450	AY912222
Phalaenopsis borneensis Garay	endemic to Borneo	KDAIS KC-109	AF537024
Phalaenopsis pantherina Rchb.f.	endemic to Borneo	KDAIS KC-573	AY912244
Phalaenopsis lamelligera Sweet	endemic to Borneo	KDAIS KC-263	AY912233
Section *Fuscatae* Sweet			
Phalaenopsis cochlearis Holtt.	Malaysia (Malay Peninsula) and Indonesia (Sarawak)	KDAIS KC-484	AY912221
Phalaenopsis viridis J. J. Sm.	endemic to Indonesia (Sumatra)	KDAIS KC-127	

Table 1. (Continued)

Taxa and systematic classification [a]	Geographical distribution	Source	GenBank accession no.
		"clone 1"	AY912254
		"clone 2"	AY912255
		"clone 3"	AY912256
Phalaenopsis kunstleri J. D. Hook.	Myanmar and Malay Peninsula	KDAIS KC-540	AY912232
Section *Amboinenses* Sweet			
Phalaenopsis pulchra (Rchb.f.) Sweet	endemic to the Philippines (Luzon and Leyte)	KDAIS KC-579	AY912248
Phalaenopsis bellina (Rchb.f.) E. A. Christ.	Malaysia (Malay Peninsula) and East Malaysia (Sarawak)	KDAIS KC-107	AY900290
Phalaenopsis violacea Witte	Indonesia (Sumatra) and Malaysia (Malay Peninsula)	KDAIS KC-153	AY390229
Phalaenopsis micholitzii Rolfe	Philippines (Mindanao)	KDAIS KC-382	AY912240
Phalaenopsis fimbriata J. J. Sm.	Indonesia (Java, Sarawak, and Sumatra)	KDAIS KC-62	AF537013
Phalaenopsis floresensis Fowlie	endemic to the island of Flores	KDAIS KC-241	AY912227
Phalaenopsis fasciata Rchb.f.	endemic to the Philippines (Luzon, Bohol, and Mindanao)	KDAIS KC-191	AY912226
Phalaenopsis doweryensis Garay & E. A. Christ.	east Malaysia, Sabah, without a precise locality	KDAIS KC-516	AY912224
Phalaenopsis modesta J. J. Sm.	endemic to the island of Borneo in East Malaysia (Sabah) and Indonesia (Kalimantan)	KDAIS KC-353	AY912242
Phalaenopsis maculata Rchb.f.	Malaysia (Pahang), East Malaysia (Sabah and Sarawak), and Indonesia (Kalimantan Timur)	KDAIS KC-49	AF537008
Phalaenopsis javanica J. J. Sm.	endemic to Indonesia (Java)	KDAIS KC-39	AY912231
Phalaenopsis mariae Burb. ex Warn. & B. S. Wms.	endemic to the Philippines and Indonesia (Kalimantan and Borneo)	KDAIS KC-11	AY912239
Phalaenopsis amboinensis J. J. Sm.	Indonesia (Molucca Archipelago and Sulawesi)	KDAIS KC-157	AY912217
Phalaenopsis lueddemanniana Rchb.f.	endemic to the Philippines	KDAIS KC-262	AY912237
Phalaenopsis venosa Shim & Fowlie	endemic to Indonesia (Sulawesi)	KDAIS KC-220	AY912253
Phalaenopsis pallens (Lindl.) Rchb.f.	endemic to the Philippines	KDAIS KC-258	AY912243
Phalaenopsis bastianii Gruss & Rollke	endemic to the Philippines	KDAIS KC-245	AY912219
Phalaenopsis hieroglyphica (Rchb.f.) Sweet	endemic to the Philippines	KDAIS KC-33	AF537000

Table 1. (Continued)

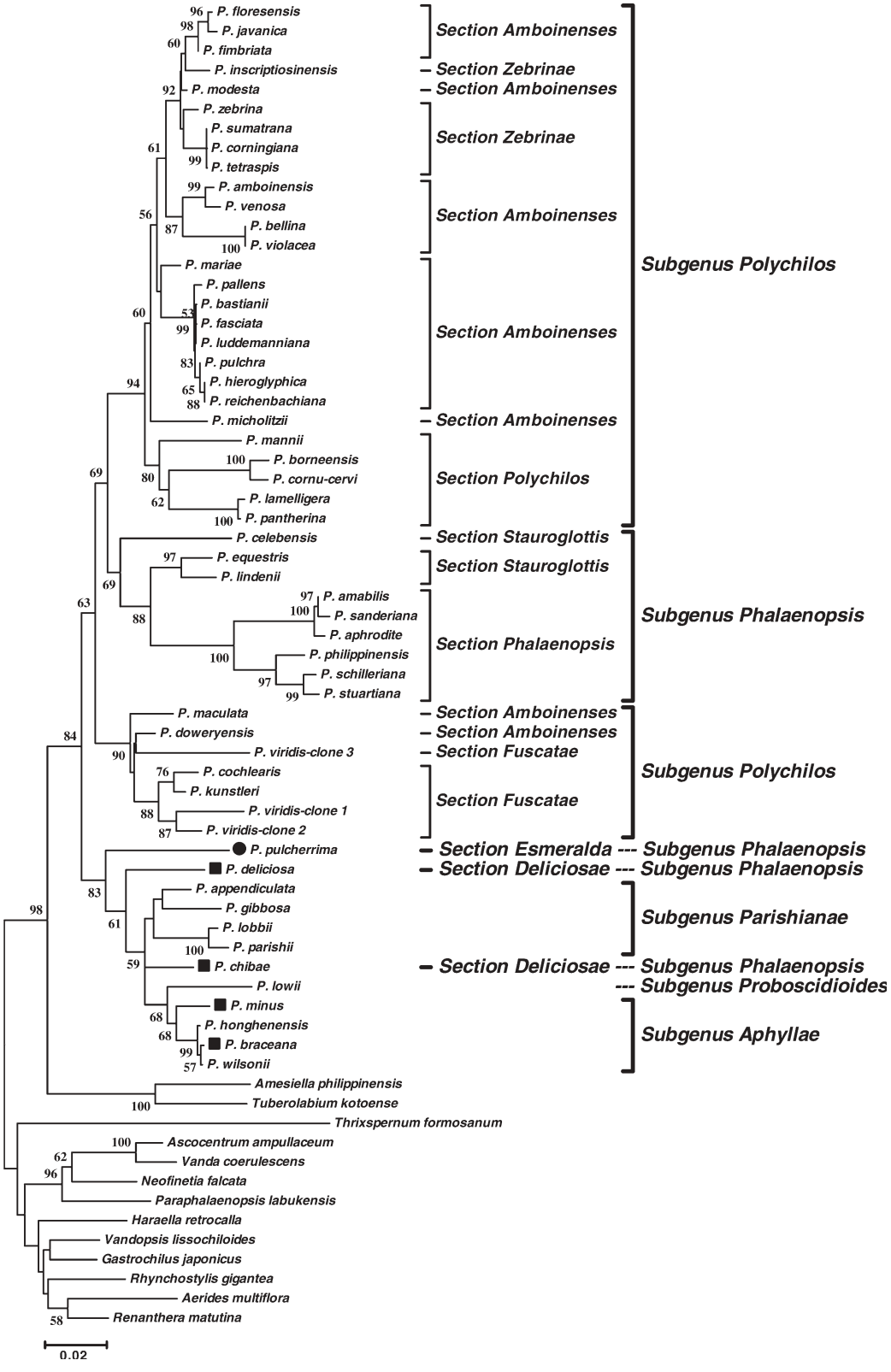
Taxa and systematic classification [a]	Geographical distribution	Source	GenBank accession no.
Phalaenopsis reichenbachiana Rchb.f. & Sander	endemic to the Philippines	KDAIS KC-389	AY912249
Section *Zebrinae* Pfitz.			
Phalaenopsis inscriptiosinensis Fowlie	endemic to Indonesia (Sumatra)	KDAIS KC-298	AY912230
Phalaenopsis tetraspis Rchb.f.	India (Andaman and Nicobar Islands) and Indonesia (Sumatra)	KDAIS KC-531	AY912252
Phalaenopsis corningiana Rchb.f.	Borneo (Sarawak and elsewhere on the island)	KDAIS KC-346	AY390247
Phalaenopsis sumatrana Korth. & Rchb.f.	widespread from Myanmar, Thailand, Vietnam, to Indonesia (Java and Sumatra), Malaysia (Perak and Johore), East Malaysia (Sabah), and the Philippines (Palauan)	KDAIS KC-161	AY390241
Phalaenopsis zebrina Witte		KDAIS KC-257	AY390251
Subgenus *Phalaenopsis*			
Section *Phalaenopsis* Benth.			
Phalaenopsis philippinensis Golamco ex Fowlie & Tang	endemic to the Philippines	KDAIS KC-534	AY912246
Phalaenopsis amabilis (L.) Blume	widespread from Sumatra and Java to the southern Philippines, and east to New Guinea and Queensland, Australia	KDAIS KC-327	AY391524
Phalaenopsis aphrodite Rchb.f.	northern Philippines and southeastern Taiwan	KDAIS KC-173	AY391537
Phalaenopsis sanderiana Rchb.f.	endemic to the Philippines	KDAIS KC-175	AY391552
Phalaenopsis schilleriana Rchb.f.	endemic to the Philippines	KDAIS KC-429	AY912250
Phalaenopsis stuartiana Rchb.f.	endemic to the island of Mindanao in the southern Philippines	KDAIS KC-528	AY912251
Section *Deliciosae* E. A. Christ.			
Phalaenopsis chibae Yukawa	endemic to Vietnam	KDAIS KC-488	AY912220
Phalaenopsis deliciosa Rchb.f.	widespread from Sri Lanka and India to the Philippines and Sulawesi	KDAIS KC-255	AY912223
Section *Esmeralda* Rchb.f.			
Phalaenopsis pulcherrima (Lindl.) J. J. Sm.	widespread from northeast India and southern China throughout Indochina to Malaysia (Malay Peninsula), Indonesia (Sumatra), and East Malaysia (Sabah)	KDAIS KC-256	AY912247

Table 1. (Continued)

Taxa and systematic classification [a]	Geographical distribution	Source	GenBank accession no.
Section *Stauroglottis* (Schauer) Benth.			
Phalaenopsis equestris (Schauer) Rchb.f.	Philippines and Taiwan	KDAIS KC-75	AY912225
Phalaenopsis celebensis Sweet	endemic to Indonesia (Sulawesi)	KDAIS KC-482	AF537014
Phalaenopsis lindenii Loher	endemic to the Philippines	KDAIS KC-119	AY912234
Outgroups			
Aerides multiflora Roxbury	widespread from Nepal to Indochina	KDAIS Van-10	AY912258
Amesiella philippinensis (Ames) Garay	endemic to the Philippines	KDAIS KC-46	AY912259
Ascocentrum ampullaceum (Lindl.) Schltr.	endemic to Nepal and Thailand	KDAIS Van-24	AY912260
Gastrochilus japonicus (Makino) Schltr.	endemic to Taiwan and Japan	KDAIS KC-69	AY228503
Haraella retrocalla (Hayata) Kudo	endemic to Taiwan	KDAIS KC-70	AY912261
Neofinetia falcata (Thunb.) H.H. Hu	from Japan and Korea to the Ryukyu Is. (Jpn.)	KDAIS Aer-36	AY912262
Paraphalaenopsis labukensis (P.S. Shim) A. Lamb & C.L. Chan.	endemic to Borneo	KDAIS KC-121	AF537028
Renanthera matutina (Blume)Lindl.	widespread from Thailand, Sumatra, Java and Malaysia	KDAIS Van-33	AY912263
Rhynchostylis gigantea (Lindl.) Ridley	Indochinese peninsula	KDAIS KC-45	AY912264
Thrixspermum formosanum (Hayata) Schltr.	endemic to Taiwan	KDAIS KC-78	AY912265
Tuberolabium kotoense Yamam.	Taiwan and the Philippines	KDAIS KC-68	AF537003
Vanda coerulescens Griffith	China, Burma, and Thailand	KDAIS Van-29	AY912266
Vandopsis lissochiloides (Gaudich) Pfitzer	Thailand to Indonesia and the Philippines	KDAIS Van-7	AY912267

[a] The systematics of *Phalaenopsis* are based on Christenson (2001).
[b] Plant materials were cultivated at the Kaohsiung District Agricultural Improvement Station, Taiwan.

respectively. The sequence of the long PCR product was unique from other *Phalaenopsis* species as well as the 13 outgroup species studied after phylogenetic analyses based on both the distance method and maximum parsimony method (data not shown). Therefore, the short PCR product of *P. cochlearis* was selected for analysis in the following study. The short ITS sequence of *P. cochlearis* showed a 237-bp deletion. Parts of the deleted fragment included the ITS1 region, and parts of it included 5.8S rDNA. The boundaries of the internal transcribed spacers (ITS1 and ITS2) and nrDNA coding regions in the 66 taxa studied were determined by comparison to several published sequences obtained from a range of angiosperms (Takaiwa et al. 1984, 1985; Kiss et al. 1989a; Kiss et al. 1989b; Baldwin 1992). The accession numbers of the 53 species of


P. floresensis
P. javanica
P. fimbriata
P. inscriptiosinensis
P. modesta
P. zebrina
P. sumatrana
P. corningiana
P. tetraspis
P. amboinensis
P. venosa
P. bellina
P. violacea
P. mariae
P. pallens
P. bastianii
P. fasciata
P. luddemanniana
P. pulchra
P. hieroglyphica
P. reichenbachiana
P. micholitzii
P. mannii
P. borneensis
P. cornu-cervi
P. lamelligera
P. pantherina
P. celebensis
P. equestris
P. lindenii
P. amabilis
P. sanderiana
P. aphrodite
P. philippinensis
P. schilleriana
P. stuartiana
P. maculata
P. doweryensis
P. viridis-clone 3
P. cochlearis
P. kunstleri
P. viridis-clone 1
P. viridis-clone 2
P. pulcherrima
P. deliciosa
P. appendiculata
P. gibbosa
P. lobbii
P. parishii
P. chibae
P. lowii
P. minus
P. honghenensis
P. braceana
P. wilsonii
Amesiella philippinensis
Tuberolabium kotoense
Thrixspernum formosanum
Ascocentrum ampullaceum
Vanda coerulescens
Neofinetia falcata
Paraphalaenopsis labukensis
Haraella retrocalla
Vandopsis lissochiloides
Gastrochilus japonicus
Rhynchostylis gigantea
Aerides multiflora
Renanthera matutina
0.02
Section Amboinenses
Section Zebrinae
Section Amboinenses
Section Zebrinae
Section Amboinenses
Section Amboinenses
Section Amboinenses
Section Amboinenses
Section Polychilos
Section Stauroglottis
Section Stauroglottis
Section Phalaenopsis
Section Amboinenses
Section Amboinenses
Section Fuscatae
Section Fuscatae
Section Esmeralda --- Subgenus Phalaenopsis
Section Deliciosae --- Subgenus Phalaenopsis
Section Deliciosae --- Subgenus Phalaenopsis
--- Subgenus Proboscidioides
Subgenus Polychilos
Subgenus Phalaenopsis
Subgenus Polychilos
Subgenus Parishianae
Subgenus Aphyllae

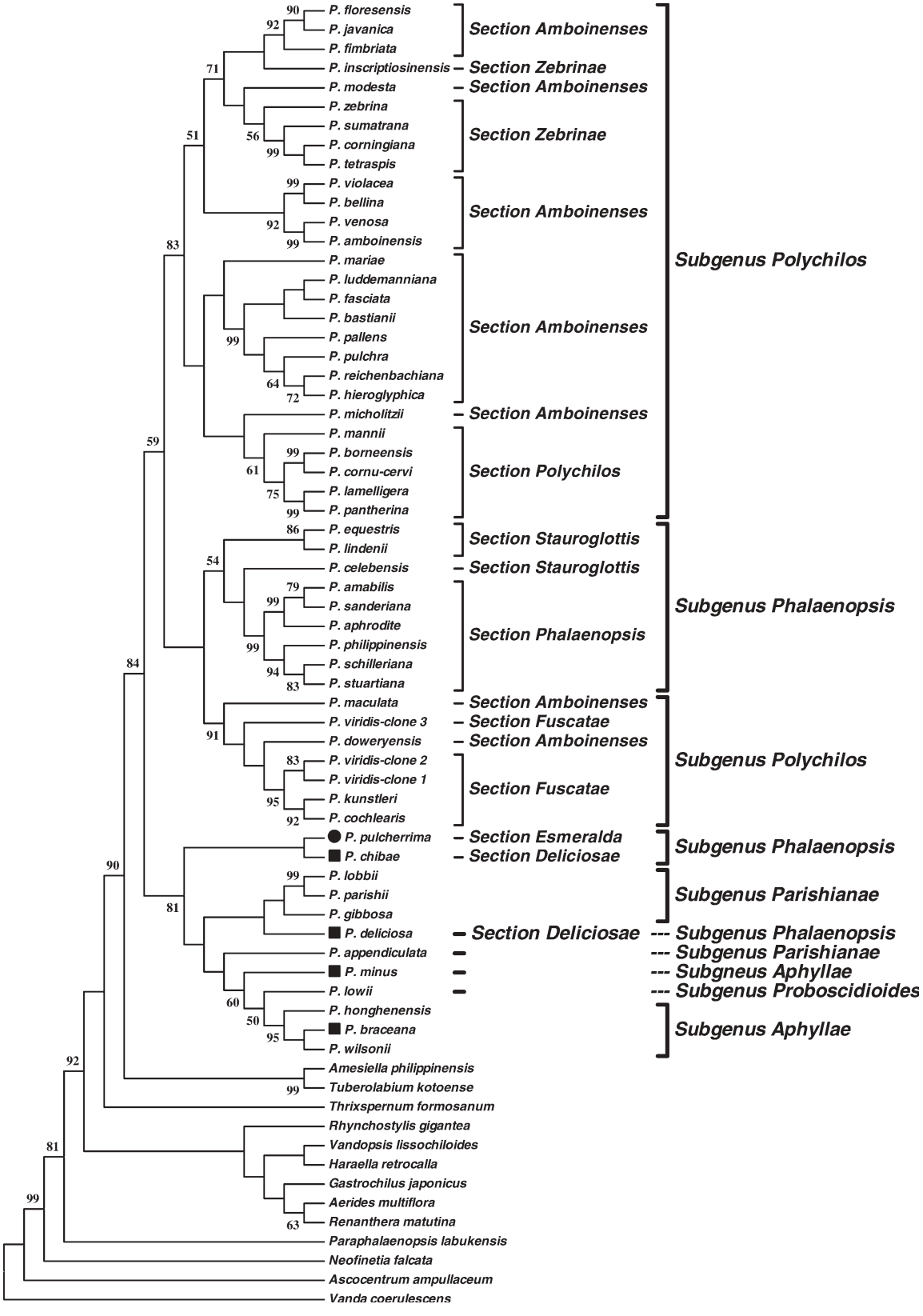
the genus *Phalaenopsis* plus 13 outgroup species are shown in Table 1. The ITS lengths obtained from *Phalaenopsis* species and outgroup species were similar to those reported from a broad sample of angiosperms (Baldwin et al. 1995). Sequences from the 53 *Phalaenopsis* species plus 13 outgroup species of related genera were aligned and resulted in 719 characters which were subdivided as follows: the ITS1 spacer region comprised 262 characters among which 165 were variable sites and 113 were potentially parsimony informative sites, the ITS2 spacer region comprised 294 characters among which 154 were variable sites and 109 were potentially parsimony informative sites, and 5.8S rDNA comprised 163 characters among which 16 were variable sites and 8 were potentially parsimony informative sites. The genetic distances among the 53 *Phalaenopsis* species were in the range of 0.00 between *P. bellina* and *P. violacea* to 0.137 between *P. aphrodite* and *P. viridis* (clone 3) with an average of 0.073 using the 2-parameter method of Kimura (1980) (data not shown).

ITS phylogeny. The phylogenetic tree of regions of the ITS1, 5.8S rDNA, and ITS2 used character state changes which were equally weighted. The phylogenetic tree constructed following the NJ method is shown in Fig. 1. Based on the MP method, the analysis yielded 118 equally parsimonious trees with a length of 911 steps, a consistency index (CI) of 0.50, and a retention index (RI) of 0.76. One of the 118 most parsimonious trees and the strict consensus tree are shown in Figs. 2 and 3, respectively. Bootstrap values higher than 50% are shown below/above the supported branches for both the NJ and MP trees. Among *Phalaenopsis* species, the NJ tree and the MP strict consensus tree constructed from ITS data were generally congruent. Based on the phylogenetic trees (Figs. 1, 3), none of the outgroup species was nested within the genus *Phalaenopsis*. This results support that the genus *Phalaenopsis* as described by Christenson (2001) is a monophylum. Within the genus *Phalaenopsis*, two major clades were shown. The first clade comprised the subgenus *Polychilos* and sections *Phalaenopsis* and *Stauroglottis* of the subgenus *Phalaenopsis* (with bootstrap values of 63% in the NJ tree and 59% in the MP strict consensus tree); and the second clade consisted of sections *Esmeralda* and *Deliciosae* of the subgenus *Phalaenopsis*, as well as subgenera *Proboscidioides*, *Parishianae*, and *Aphyllae* (Figs. 1, 3) (with bootstrap values of 83% in the NJ tree and 81% in the MP strict consensus tree).

The monophyly of the subgenus *Polychilos* was not supported, since parts of the subgenus *Phalaenopsis*, namely sections *Phalaenopsis* and *Stauroglottis*, were nested within the subgenus *Polychilos*. Within the subgenus *Polychilos*, only the section *Polychilos* showed monophyly supported with bootstrap values of 80% in the NJ tree and 61% in the MP strict consensus tree. Parts of section *Amboinenses*, namely *P. hieroglyphica*, *P. reichenbachiana*, *P. bastianii*, *P. pallens*, *P. lueddemanniana*, *P. fasciata*, and *P. pulchra*, formed a clade supported with bootstrap values of 99% in both NJ and MP strict consensus trees. In addition, parts of the section *Amboinenses*, namely *P. doweryensis* and *P. maculata*, formed a clade with the section *Fuscatae* supported by bootstrap values of 90% in the NJ tree and 91% in the MP strict consensus tree (Figs. 1, 3).

Furthermore, the monophyly of the subgenus *Phalaenopsis* was also not supported in this study. Within the subgenus *Phalaenopsis*,

Fig. 1. Neighbor-joining tree of 53 *Phalaenopsis* species plus 13 outgroups obtained from sequence comparisons of the ITS region of rDNA. Numbers above the internodes indicate bootstrap values from 1025 replicates. Bootstrap values > 50% are shown on each branch. A solid circle (●) on the tree indicates that this species was traditionally treated as the genus *Doritis*. Solid squares (■) on the tree indicate that these species were traditionally treated as the genus *Kingidium*


P. floresensis
P. javanica
P. fimbriata
P. inscriptiosinensis
P. modesta
P. zebrina
P. sumatrana
P. corningiana
P. tetraspis
P. violacea
P. bellina
P. venosa
P. amboinensis
P. mariae
P. luddemanniana
P. fasciata
P. bastianii
P. pallens
P. pulchra
P. reichenbachiana
P. hieroglyphica
P. micholitzii
P. mannii
P. borneensis
P. cornu-cervi
P. lamelligera
P. pantherina
P. equestris
P. lindenii
P. celebensis
P. amabilis
P. sanderiana
P. aphrodite
P. philippinensis
P. schilleriana
P. stuartiana
P. maculata
P. viridis-clone 3
P. doweryensis
P. viridis-clone 2
P. viridis-clone 1
P. kunstleri
P. cochlearis
P. pulcherrima
P. chibae
P. lobbii
P. parishii
P. gibbosa
P. deliciosa
P. appendiculata
P. minus
P. lowii
P. honghenensis
P. braceana
P. wilsonii
Amesiella philippinensis
Tuberolabium kotoense
Thrixspernum formosanum
Rhynchostylis gigantea
Vandopsis lissochiloides
Haraella retrocalla
Gastrochilus japonicus
Aerides multiflora
Renanthera matutina
Paraphalaenopsis labukensis
Neofinetia falcata
Ascocentrum ampullaceum
Vanda coerulescens
Section Amboinenses
Section Zebrinae
Section Amboinenses
Section Zebrinae
Section Amboinenses
Section Amboinenses
Section Amboinenses
Section Polychilos
Section Stauroglottis
Section Stauroglottis
Section Phalaenopsis
Section Amboinenses
Section Fuscatae
Section Amboinenses
Section Fuscatae
Section Esmeralda
Section Deliciosae
Section Deliciosae
Subgenus Polychilos
Subgenus Phalaenopsis
Subgenus Polychilos
Subgenus Phalaenopsis
Subgenus Parishianae
--- Subgenus Phalaenopsis
--- Subgenus Parishianae
--- Subgneus Aphyllae
--- Subgenus Proboscidioides
Subgenus Aphyllae

the monophyly of the section *Phalaenopsis* was supported with bootstrap values of 100% in the NJ tree and 99% in MP strict consensus tree. Section *Stauroglottis* was shown not to be monophyletic. Parts of the section *Stauroglottis*, namely *P. equestris* and *P. lindenii*, showed a sister group relationship to the section *Phalaenopsis* supported by bootstrap values of 88% in the NJ tree and 67% in the MP strict consensus tree. Furthermore, section *Deliciosae* was not monophyletic (Figs. 1, 3). Sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis* were both separated from most species of the subgenus *Phalaenopsis*, namely sections *Phalaenopsis* and *Stauroglottis*. Instead they were to be shown closely related to the subgenera *Parishianae*, *Aphyllae*, and *Proboscidioides*.

In addition, subgenus *Parishianae* was not shown to be monophyletic either since the bootstrap value in the NJ tree was low ($< 50\%$) (Fig. 1), and its members did not form a clade in the MP strict consensus tree (Fig. 3). Furthermore, there was conflict between the phylogenies of the subgenus *Aphyllae* depicted by the NJ and MP strict consensus trees based on ITS data. The NJ tree showed it to be monophyletic supported with a moderate bootstrap value (of 68%), but its members did not form a clade in the MP strict consensus tree (Figs. 1, 3). As for the monotypic subgenus *Proboscidioides*, *P. lowii* had a sister relationship to the subgenus *Aphyllae* in NJ tree, and was nested within the subgenus *Aphyllae* in the MP strict consensus tree (Figs. 1, 3).

Discussion

Monophyly of the genus *Phalaenopsis*

Phalaenopsis pulcherrima was previously treated as a member of the genus *Doritis* (Seidenfaden 1988a), while members of the section *Deliciosae* of the subgenus *Phalaenopsis* and parts (*P. minus*, *P. braceana*, and *P. taenialis*) of the subgenus *Aphyllae* were treated as parts of the genus *Kingidium* (Seidenfaden 1988b). Recently, Christenson (2001) presented a generic revision of *Phalaenopsis* and treated *Doritis* as a synonym of the genus *Phalaenopsis* based on the high hybrid fertility and similar morphology of microspores between *P. pulcherrima* (syn. *Doritis pulcherrima*) and parts (*P. lobbii* or *P. parishii*) of *Phalaenopsis* (Aoyama et al. 1994, Christenson 2001). In addition, the genus *Kingidium* was also treated as a synonym of the genus *Phalaenopsis* based on the fact that it shares small subsaccate lip bases with parts of *Phalaenopsis*, namely the subgenus *Aphyllae*, as well as it has four pollinia as have parts of *Phalaenopsis*, namely the subgenera *Proboscidioides*, *Aphyllae*, and *Parishianae* (Christenson 2001). Molecular data from the ITSs of nrDNA showed that outgroup species were not nested within the genus *Phalaenopsis*. In addition, traditional *Kingidium* and *Doritis* species were not separated from *Phalaenopsis* species. Therefore, the monophyly of the genus *Phalaenopsis* as described by Christenson (2001) was supported in the present study.

Infrageneric relationships within *Phalaenopsis*

(1) Subgenera *Proboscidioides*, *Aphyllae*, and *Parishianae*

Molecular data from ITS sequences showed that the monotypic subgenus *Proboscidioides*, namely *P. lowii*, formed a clade with the subgenera *Aphyllae* and *Parishianae* plus the sections *Esmeralda* and *Deliciosae* of subgenus *Phalaenopsis*. The result is in agreement with the morphological characteristics of this clade, bearing four separate pollinia, as well as with the fact that they are geographically distributed in close proximity to one another (Fig. 4,

Fig. 2. One of 118 most parsimonious trees of 53 *Phalaenopsis* species plus 13 outgroups obtained from sequence comparisons of the ITS region of rDNA. Bootstrap values $> 50\%$ are shown on each branch. A solid circle (●) on the tree indicates that this species was traditionally treated as the genus *Doritis*. Solid squares (■) on the tree indicate that these species were traditionally treated as the genus *Kingidium*


P. floresensis
P. javanica
P. fimbriata
P. inscriptiosinensis
P. modesta
P. zebrina
P. tetraspis
P. sumatrana
P. corningiana
P. amboinensis
P. venosa
P. bellina
P. violacea
P. mariae
P. pallens
P. luddemanniana
P. fasciata
P. bastianii
P. pulchra
P. hieroglyphica
P. reichenbachiana
P. micholitzii
P. mannii
P. borneensis
P. cornu-cervi
P. lamelligera
P. pantherina
P. celebensis
P. equestris
P. lindenii
P. amabilis
P. sanderiana
P. aphrodite
P. philippinensis
P. schilleriana
P. stuartiana
P. maculata
P. viridis-clone 3
P. doweryensis
P. cochlearis
P. kunstleri
P. viridis-clone 1
P. viridis-clone 2
P. pulcherrima
P. chibae
P. lobbii
P. parishii
P. gibbosa
P. deliciosa
P. appendiculata
P. minus
P. lowii
P. honghenensis
P. braceana
P. wilsonii
Amesiella philippinensis
Tuberolabium kotoense
Thrixspernum formosanum
Rhynchostylis gigantea
Aerides multiflora
Renanthera matutina
Haraella retrocalla
Vandopsis lissochiloides
Gastrochilus japonicus
Paraphalaenopsis labukensis
Neofinetia falcata
Ascocentrum ampullaceum
Vanda coerulescens
Section Amboinenses
Section Zebrinae
Section Amboinenses
Section Zebrinae
Section Amboinenses
Section Amboinenses
Section Amboinenses
Section Polychilos
Section Stauroglottis
Section Stauroglottis
Section Phalaenopsis
Section Amboinenses
Section Fuscatae
Section Amboinenses
Section Fuscatae
Section Esmeralda --- Subgenus Phalaenopsis
Section Deliciosae --- Subgenus Phalaenopsis
Section Deliciosae --- Subgenus Phalaenopsis
--- Subgenus Aphyllae
--- Subgenus Proboscidioides
Subgenus Polychilos
Subgenus Phalaenopsis
Subgenus Polychilos
Subgenus Parishianae
--- Subgenus Parishianae
--- Subgenus Parishianae
Subgenus Aphyllae

Table 1) (Sweet 1980, Christenson 2001). This four-pollinia clade, namely subgenera *Aphyllae* and *Parishianae*, plus the sections *Esmeralda* and *Deliciosae* of subgenus *Phalaenopsis*, is separate from two-pollinia clade, namely subgenus *Polychilos* and sections *Phalaenopsis* and *Stauroglottis* of subgenus *Phalaenopsis* based on phylogenetic tree derived from ITS data. Based on the biogeography of *Phalaenopsis*, the four-pollinia clade is separated from the two-pollinia clade by oceans/straits. As for *Phalaenopsis* species of the Malay Peninsula, the border between the two-pollinia clade and the four-pollinia clade is approximately located at the Isthmus of Kra. This region is a famous phytogeographical break between SE Asia and Malaysia (Van Steenis 1950).

Within the four-pollinia clade, neither subgenus *Aphyllae* nor *Parishianae* were monophyletic based on the ITS sequences. Furthermore, the monotypic subgenus *Proboscidioides*, namely *P. lowii*, had a close relationship to the subgenus *Aphyllae*. Therefore, the unique characters in *P. lowii*, namely the long beak-like rostellum and lateral lobes of the lip in the form of recurved hooks, might have been overemphasized in the systematics of *Phalaenopsis* proposed by Christenson (2001). In addition, section *Deliciosae* of the subgenus *Phalaenopsis* was not monophyletic either.

(2) Subgenus *Phalaenopsis*

This subgenus is subdivided into four sections, namely sections *Phalaenopsis*, *Deliciosae*, *Esmeralda*, and *Stauroglottis*, based on the Christenson's systematics of *Phalaenopsis*. This subgenus is characterized by having a single callus (with the exception of the three species of the section *Deliciosae*) and smooth lateral lobes of the lip (Christenson 2001). Monophyly of the subgenus *Phalaenopsis* was not supported in this study. Excluding sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis*, the rest of subgenus *Phalaenopsis*, namely sections *Phalaenopsis* and *Stauroglottis*, formed a monophyletic group supported by moderate bootstrap values based on the ITS sequences. The close relationship between sections *Phalaenopsis* and *Stauroglottis* was also supported by morphological characters of sharing similar geographic ranges, small chromosome sizes, and flowers lacking transversely barred patterns (Christenson 2001), RAPD analyses (Chen et al. 1995), and the IGS sequence of 5S rDNA (Kao 2001).

Within subgenus *Phalaenopsis*, the monophyly of section *Phalaenopsis* was supported in the study. The section *Phalaenopsis* bears flowers with broad petals, which are much broader than the sepals. Additionally, they bear prominent, erect, somewhat glossy calli (Christenson 2001). Furthermore, based on geographical distributions (Sweet 1980, Christenson 2001), and molecular data of the intergenic spacer (IGS) of 5S nrDNA (Kao 2001), the monophyly of section *Phalaenopsis* was supported as well. Monophyly of section *Stauroglottis* was not supported in this study, since *P. celebensis* was unique from other members of the section *Stauroglottis*. According to the geographical distribution of this section, *P. celebensis* (distributed in Sulawesi, Indonesia) was also separated from other species of the section *Stauroglottis* (distributed in the Philippines) (Christenson 2001). Furthermore, section *Deliciosae* was not shown to be monophyletic based on ITS sequence either.

(3) Subgenus *Polychilos*

This large subgenus is subdivided into the four sections *Polychilos*, *Fuscatae*, *Amboinenses*, and *Zebrinae* based on the systematics of Christenson (2001). The subgenus is characterized by having fleshy, long-lasting flowers with two pairs of calli on the lip (biseriate), the lateral lobes of the lip producing a raised tooth along

Fig. 3. The strict consensus parsimonious tree of 53 *Phalaenopsis* species plus 13 outgroups obtained from sequence comparisons of the ITS region of rDNA. Bootstrap values > 50% are shown on each branch. A solid circle (●) on the tree indicates that this species was traditionally treated as the genus *Doritis*. Solid squares (■) on the tree indicate that these species were traditionally treated as the genus *Kingidium*

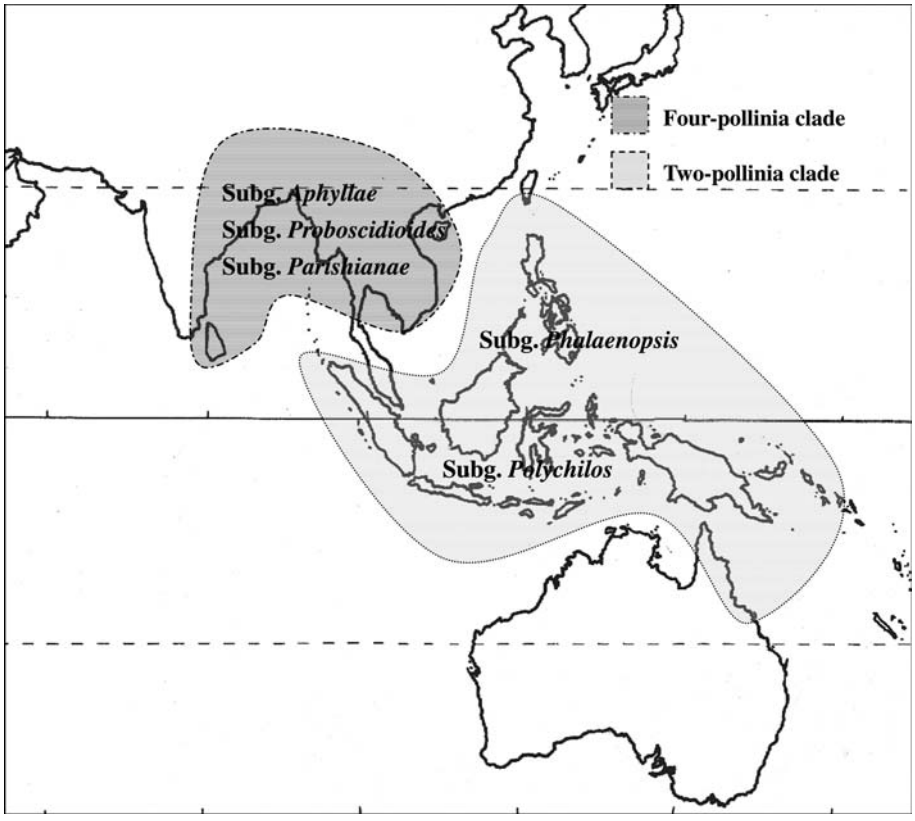


Fig. 4. Correlation between the biogeography and phylogenetic clades among five subgenera of *Phalaenopsis*

the leading edge, and by two pollinia (Christenson 2001). In the present study, the monophyly of subgenus *Polychilos* was not supported, since parts of the subgenus *Phalaenopsis*, namely sections *Phalaenopsis* and *Stauroglottis*, were nested within subgenus *Polychilos*. Within subgenus *Polychilos*, section *Polychilos* was monophyletic. The result is in agreement with the morphological characters of this section, which features a fleshy flattened rachis (with the exception of *P. mannii*), non-fragrant flowers, petals narrower than sepals, a triseriate callus, a slightly saccate lip base, a transversely lunate mid-lobe of the lip, a lip base continuous with the column foot, and a pair of knee-like projections at the base of the column (Christenson 2001). However, other sections of subgenus *Polychilos*, namely *Amboinenses*, *Zebrinae*, and *Fuscatae*, were not monophyletic. Based on ITS data, the section *Fuscatae* and two species of the section *Amboinenses*, namely *P. doweryensis* and *P. maculata*, formed a unique clade and were separated from other members of the subgenus *Polychilos*. Furthermore, ITS data also revealed no differentiation between sections *Amboinenses* and *Zebrinae*. In addition, within section *Amboinenses*, seven species, namely *P. bastianii*, *P. pallens*, *P. hieroglyphica*, *P. reichenbachiana*, *P. lueddemanniana*, *P. fasciata*, and *P. pulchra*, distributed in the Philippines, formed a subclade and were separated from other members of the clade. The result is in agreement with the geographical distribution of those species which are distributed in the Philippines (Sweet 1980, Christenson 2001) as well as the traditional classification of seven species (with the exception of *P. bastianii*, a newly discovered species) which were treated as one species, *P. lueddemanniana* (see Christenson 2001). Until Sweet (1968, 1969), this highly variable species was fully resolved and treated as different species.

Conclusions

In conclusion, our molecular data offer new perspectives on the phylogeny among species of *Phalaenopsis*. The ITS region of nrDNA appears to be adequate for resolving infrageneric relationships in this group. The results from this study concurred in that the genera *Doritis* and *Kingidium* can be treated as part of the genus *Phalaenopsis*. Neither subgenus *Aphyllae* nor *Parishianae* were monophyletic, and they clustered with subgenus *Proboscidioides* plus sections *Esmeralda* and *Deliciosae* of subgenus *Phalaenopsis*. This clade has the same characters of morphology with four pollinia and similar biogeographies. In addition, neither subgenus *Phalaenopsis* nor *Polychilos* were monophyletic based on this study. Within subgenus *Phalaenopsis*, only section *Phalaenopsis* was highly supported as being monophyletic. As for the subgenus *Polychilos*, only section *Polychilos* was moderately supported as being monophyletic. In short, based on ITS data we support the systematics of the genus *Phalaenopsis* on the generic level but not on the subgeneric or sectional levels for most groups as described by Christenson (2001).

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References

Aoyama M., Kojima K., Kobayashi M. (1994) Morphology of microspores in *Phalaenopsis* hybrids. Kinki Chugoku Agr. Res. 88: 49–53.

Baldwin B. G. (1992) Phylogenetic utility of the internal transcribed sequences of nuclear ribosomal DNA in plants: an example from the Compositae. Molec. Phylogenet. Evol. 1: 3–16.

Baldwin B. G. (1993) Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. Amer. J. Bot. 80: 222–238.

Baldwin B. G., Sanderson M. J., Porter J. M., Wojciechowski M. F., Campbell C.S., Donoghue M. J. (1995) The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann. Missouri Bot. Gard. 82: 247–277.

Bayer R. J., Soltis D. E., Soltis P. S. (1996) Phylogenetic inferences in *Antennaria* (Asteraceae: Gnaphalieae: Cassiniinae) based on sequences from nuclear ribosomal DNA internal transcribed spacers (ITS). Amer. J. Bot. 83: 516–527.

Chen W. H., Fu Y. M., Hsieh R. M., Tsai W. T., Chyou M. S., Wu C. C., Lin Y. S. (1995) Application of DNA amplification fingerprinting in the breeding of *Phalaenopsis* orchid. In: Terzi M., Cella R., Falavigna A. (eds.) Current issues in plant molecular and cellular biology. Kluwer Academic Publishers, Dordrecht, Boston, and London, pp. 341–346.

Christenson E. A. (2001) *Phalaenopsis*. Timber Press, Portland, OR.

Cox A. V., Pridgeon A. M., Albert V. A., Chase M. W. (1997) Phylogenetics of the slipper orchids (Cypripedioideae, Orchidaceae): nuclear rDNA ITS sequences. Pl. Syst. Evol. 208: 197–223.

Doyle J., Doyle J. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

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. Syst. Zool. 20: 406–416.

Hall T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41: 95–98.

Hillis D. M., Bull J. J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42: 182–192.

Kao Y. H. (2001) Phylogeny of *Phalaenopsis* species based on 5S rDNA intergenic sequences. Institute of Botany National Taiwan University, Taipei, Taiwan, Master's thesis. (in Chinese).

Kimura M. (1980) A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. Molec. Evol. 16: 111–120.

Kiss T., Kis M., Solymosy F. (1989a) Nucleotide sequence of a 25S rRNA gene from tomato. Nucl. Acids Res. 17: 796.

Kiss T., Szkukalek A., Solymosy F. (1989b) Nucleotide sequence of a 17S (18S) rRNA gene from tomato. Nucl. Acids Res. 17: 2127.

Kumar S., Tamura K., Jakobsen I. B., Nei M. (2001) MEGA 2.1: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe, AZ.

Rzhetsky A., Nei M. (1992) Statistical properties of the ordinary least-squares, generalized least-squares, and minimum-evolution methods of phylogenetic inference. J. Molec. Evol. 35: 367–375.

Saitou N., Nei M. (1987) The Neighbor-joining method: a new method for reconstructing phylogenetic trees. Molec. Biol. Evol. 4: 406–425.

Seidenfaden G. (1988a) *Doritis*. Opera Bot. 95: 31–34.

Seidenfaden G. (1988b) *Kingidium*. Opera Bot. 95:182–189.

Shim P. S. (1982) A new generic classification in the *Phalaenopsis* complex (Orchidaceae). Malayan Nat. J. 36: 1–28.

Shindo K., Kamemoto H. (1963) Karyotype analysis of some species of *Phalaenopsis*. Cytologia 28: 390–398.

Suh Y., Thien L. B., Reeve H. E., Zimmer E. A. (1993) Molecular evolution and phylogenetic implications of internal sequences of nuclear ribosomal DNA in Winteraceae. Amer. J. Bot. 80: 1042–1055.

Sun Y., Skinner D. Z., Liang G. H., Hulbert S. H. (1994) Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor. Appl. Genet. 89: 26–32.

Sweet H. R. (1968) Revision of the genus *Phalaenopsis*, pt. 2. Amer. Orchid Soc. Bull. 37: 1089–1104.

Sweet H. R. (1969) Revision of the genus *Phalaenopsis*, pts. 3 and 4. Amer. Orchid Soc. Bull. 38: 33–43, 225–239.

Sweet H. R. (1980) The genus *Phalaenopsis*. The Orchid Digest, Pomona, CA.

Takaiwa F., Oono K., Sugiura M. (1984) The complete nucleotide sequence of a rice 17S rRNA gene. Nucl. Acids Res. 12: 5441–5448.

Takaiwa F., Oono K., Sugiura M. (1985) The complete nucleotide sequence of a rice 25S rRNA gene. Gene 37: 255–289.

Tanaka R., Kamemoto K. (1984) Chromosomes in orchids: counting and numbers. In: Arditti J. (Ed.) Orchid biology: reviews and perspectives. Vol. III. Comstock Publishing, Cornell University Press, Ithaca, N.Y.

Tsai C. C., Huang S. C. (2001) The internal transcribed spacer of ribosomal DNA as a marker for identifying species and hybrids of the Oncidinae. J. Hort. Sci. Biotech. 76: 674–680.

Van Steenis C. G. G. J. (1950) The delimitation of Malaysia and its main plant geographical divisions. Flora Malesiana. Noordhoff-Kolff (Jakarta) 1(1): 70–75.

Woodard J. W. (1951) Some chromosome numbers in *Phalaenopsis*. Amer. Orchid Soc. Bull. 20: 356–358.

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