Molecular phylogeny of *Phalaenopsis* Blume (Orchidaceae) based on plastid and nuclear DNA

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Abstract. The internal transcribed spacers (ITS) of nuclear ribosomal DNA (nrDNA) and plastid DNA, including the *trn*L intron, the *trn*L-F spacer and the *atp*B-*rbc*L spacer, were sequenced from most of the living species in the genus *Phalaenopsis*. The monophyly of the genus described by Christenson (2001) was supported that *Doritis*

and *Kingidium* are synonyms of *Phalaenopsis* based on these molecular data. Within the genus, subgenus *Polychilos* was monophyletic, and the species were divided into two subclades. The subgenus *Phalaenopsis* was shown to be non-monophyletic because the sections *Esmeralda* and *Deliciosae* appeared separated from sections *Phalaenopsis* and *Stauroglottis*. Meanwhile, subgenera *Aphyllae* and *Parishianae* were also shown to be non-monophyletic based on molecular data. Furthermore, the monotypic species of subgenus *Proboscidioides*, *P. lowii*, formed a clade with subgenus *Aphyllae*. According to geographical distribution, historical geography of Southeast Asia due to the periodic glacial epochs and molecular phylogeny, two evolutionary trends of *Phalaenopsis* from the original center in South China to the Philippines, Indonesia and Malaysia were suggested. First, using Indochina and some older parts of the Philippines (e.g., Mindoro and Palawan) as stepping stones, *Phalaenopsis* species dispersed from South China to the Philippines, in which the sections *Phalaenopsis* and *Stauroglottis* of subgenus *Phalaenopsis* developed. Second, using the Malay Peninsula as a stepping stone, *Phalaenopsis* species dispersed from South China to Indonesia and Malaysia, in which the subgenus *Polychilos* developed.

Key words: *Phalaenopsis*; phylogeny; biogeography; plastid DNA.

The genus *Phalaenopsis* Blume (Orchidaceae) comprises approximately 66 species worldwide according to the latest classification of Christenson (2001), who divided this genus into five subgenera, namely *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos* and *Phalaenopsis*. This classification was mainly based on the plant size and floral morphology (callus, lip structure and pollinium number). Subgenus *Polychilos* was subdivided into four sections (*Polychilos*, *Fuscatae*, *Amboinenses* and *Zebrinae*), while subgenus *Phalaenopsis* was subdivided into four sections (*Phalaenopsis*, *Deliciosae*, *Esmeralda* and *Stauroglottis*).

Species of *Phalaenopsis* are found throughout tropical Asia and the larger islands of the Pacific Ocean, and ranges from Sri Lanka and southern India in the west, to Papua New Guinea in the east, to the Yunnan Province (southern China) and Taiwan in the north, and to northern Australia in the south (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. 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 into 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).

Sweet (1980) divided *Phalaenopsis* into eight sections (Table 1). Shim (1982), however, disagreed with Sweet's concept (1980) and treated sections *Proboscidioides*, *Aphyllae*, *Parishianae, Polychilos*, *Zebrinae*, *Fuscatae* and *Amboinenses* as the genus *Polychilos*, leaving a narrowly defined *Phalaenopsis*. Christenson (2001) basically agreed with Sweet's treatment and raised five subgenera. In addition, he treated the traditional genera *Kingidium* and *Doritis* as synonyms of *Phalaenopsis* and split *Kingidium* into different parts of *Phalaenopsis*, placing some species (i.e., *P. braceana*, *P. minus* and *P. taenialis*) into subgenus *Aphyllae* and some (i.e., *P. chibae* and *P. deliciosa*) into section *Deliciosae* of subgenus *Phalaenopsis* (Table 1). The generic-level treatment was supported by several lines of molecular evidence (Padolina et al. 2005; Yukawa et al. 2005; Tsai et al. 2006). Furthermore, two genera of subtribe Aeridinae, *Nothodoritis* and *Lesliea,* were nested within *Phalaenposis* based on molecular data (Topik et al. 2005; Yukawa et al. 2005). Yukawa et al. (2005) split *Phalaenopsis* into two genera, *Phalaenopsis* and *Doritis,* in accordance with a narrow genus concept. According to the systematics of *Phalaenopsis* described by Christenson (2001), the subgenus *Aphyllae* was not monophyletic, forming a clade with *P. lowii* of subgenus *Proboscidioides* and the sections *Esmeralda* and *Deliciosae* of subgenus *Phalaenopsis* based on several molecular evidence (Padolina et al. 2005; Yukawa et al.

2005; Tsai et al. 2006). Therefore, the subgenus *Phalaenopsis* was not monophyletic based on molecular data. Within subgenus *Phalaenopsis*, section *Phalaenopsis* was shown to be monophyletic based on nrITS data (Yukawa et al. 2005; Tsai et al. 2006) but not based on the plastid *mat*K, *atp*H-F and *trn*D-E spacers (Padolina et al. 2005) and the plastid *mat*K and *trn*K introns (Yukawa et al. 2005). The subgenus *Polychilos* was shown to be polyphyletic according to nrITS data (Yukawa et al. 2005; Tsai et al. 2006) but not based on plastid DNA (Padolina et al. 2005; Yukawa et al. 2005). Within subgenus *Polychilos*, only section *Polychilos* was shown to be monophyletic based on ITS data (Yukawa et al. 2005; Tsai et al. 2006), and section *Fuscatae* was shown to be monophyletic according to plastid DNA (Padolina et al. 2005; Yukawa et al. 2005).

The aim of the present study was to further elucidate the phylogeny of *Phalaenopsis* with DNA sequence data, including the *trn*L intron, the *trn*L-F and *atp*B-*rbc*L spacers and nrITS data. Nearly all *Phalaenopsis* species were sampled to evaluate in detail the treatments of Christenson (2001) on the generic, subgeneric and sectional levels using both nuclear and plastid DNA. Furthermore, the biogeographic and evolutionary trends of *Phalaenopsis* were discussed in the light of the molecular data, geographical distribution and historical geology of Southeast Asia.

Materials and methods

The taxonomy and nomenclature of the *Phalaenopsis* species in this study followed Sweet (1980) and Christenson (2001). The sampled plants included 54 taxa of *Phalaenopsis* and plants of seven related genera having different degrees of hybridization with *Phalaenopsis*, including *Aerides*, *Ascocentrum*, *Neofinetia*, *Renanthera*, *Rhynchostylis*, *Vanda* and *Vandopsis* (Sweet 1980). In addition, *Paraphalaenopsis,* once treated as *Phalaenopsis* (Sweet 1980), and five other genera of subtribe Aeridinae, *Amesiella*, *Gastrochilus*, *Haraella*, *Thrixspermum* and *Tuberolabium*, were selected as outgroup species (Table 2). Leaf materials were collected from living plants cultivated in the Kaohsiung District Agricultural Improvement Station (KDAIS) in Taiwan; their voucher specimens were deposited at the herbarium of the National Museum of Natural Science, Taiwan (TNM). Flower photos of the species sampled for this study are available from the first author (tsaicc@mail.kdais.gov.tw). Using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987), total DNA was extracted from fresh leaves. Ethanol-precipitated DNA was dissolved in TE (Tris-EDTA) buffer and stored at –20 ºC. Qiagen (Qiagen, Geschäftsführer, Germany) columns were used to clean DNA samples that were difficult to amplify.

The amplification protocols were as follows: 50-µl reactions 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 units of Advantage 2 DNA polymerase (Clontech, CA., USA), 10 ng genomic DNA, and a 50-µl volume of mineral oil. The amplification reactions were completed in a dry-block with two-step thermal cycles (Biometra, Goettingen, Germany). The *trn*L intron and *trn*L-F spacer primers were those of Taberlet et al. (1991). For the *atp*B-*rbc*L spacer, two primers were designed from conserved regions at the 3' end of the *atp*B gene and the 5' end of the *rbc*L gene from GenBank sequences, i.e., 5' CATCTAGGATTTACATATAC 3' and 5' GTCAATTTGTAATCTTTAAC 3', respectively. The PCR conditions for the *trn*L intron were as follows: incubation at 94 ºC for 3 min, followed by 10 cycles of denaturation at 94 ºC for 30 s, annealing at 68 ºC for 10 s, and extension at 72 ºC for 45 s, followed by 30 cycles of denaturation at 94 ºC for 30 s, annealing at 66 ºC for 10 s, extension at 72 ºC for 45 s, with a final extension for 5 min at 72 ºC. The PCR condition for the *trn*L-F spacer were as follows: incubation at 94 ºC for 3 min, followed by 10 cycles of denaturation at 94 ºC for 30 s, annealing at 68 ºC for 30 s, and extension at 72 ºC for 30 s, followed by 30 cycles of denaturation at 94 ºC for 30 s, annealing at 66 ºC for 30 s, extension at 72 ºC for 30 s, with a final extension for 5 min at 72 ºC. The PCR reaction condition for the *atp*B-*rbc*L spacer were as follows: incubation at 94 ºC for 3 min, followed by 10 cycles of

denaturation at 94 ºC for 45 s, annealing at 54 ºC for 30 s, and extension at 72 ºC for 1 min, followed by 30 cycles of denaturation at 94 ºC for 45 s, annealing at 52 ºC for 30 s, extension at 72 ºC for 1 min, with a final extension for 5 min at 72 ºC. The PCR products were stained with 0.5 μg/ml ethidium bromide, detected by agarose gel electrophoresis (1.0%, w/v in TBE), and photographed under UV light. The PCR products were purified using Glassmilk (BIO 101, CA., USA).

The amplicons were sequenced in both directions using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA., USA). The sequencing primers were the same as those used for PCR. The sequencing was performed on an Applied Biosystems 3730 automated sequencer. Each sample was sequenced two or three times for confirmation. Cloning was used to clarify the intra-individual variation (additive peaks) found in some samples. The recovered PCR products were ligated into a pGEM-T Easy vector (Promega, WI., USA), and the resulting recombinants were transformed into *Escherichia coli*. Five to seven clones for each individual were randomly selected, and the plasmid DNA was purified with Qiagen spin mini-prep kits. The plasmid DNA was sequenced with vector-specific primers (SP6 and T7). In addition, nuclear ITS sequences of *Phalanopsis* and outgroup species studied were obtained from Tsai et al. (2006).

The sequence alignment was determined using the Clustal W multiple alignment

program in BioEdit ver. 7.0.5 (Hall 1999). The alignment was subsequently visually checked. Maximum parsimony (MP) analyses (Fitch 1971) were performed using code modified from the Close-Neighbor-Interchange (CNI) algorithm (Rzhetsky and Nei 1992) in MEGA ver. 4 (Tamura et al. 2007). The strict consensus of the most parsimonious trees was constructed using MEGA ver. 4 (Tamura et al. 2007), and bootstrap consensus values were calculated using 1000 replicates (Felsenstein 1985; Hillis and Bull 1993). The phylogenetic relationships among the haplotypes were also evaluated using Bayesian inference using MRBAYES ver. 3.1.2 (Ronquist and Huelsenbeck 2003). The general time-reversible GTR model with the gamma-distributed rate variation across sites and a proportion of invariable sites was determined to be the most suitable model by jModelTest ver. 0.1.1 (Posada 2008). This model was used for the subsequent nucleotide analyses, coded as the MrBayes likelihood settings hLset nst = 6 rates = invgammai. Two parallel runs and four Markov Chain Monte Carlo (MCMC) chains were run for 10,000,000 generations, and a tree was sampled every 1,000 generations after a burn-in period of 3,000,000 generations, after which the standard deviation of the split frequencies was below 0.01 as suggested in the manual. The aligned data matrix and tree files are available from the first author (tsaicc@mail.kdais.gov.tw).

Results

Sequence alignment and characteristics. All PCR products were directly sequenced except for those of the *trn*L intron of *Thrixspermum formosanum*, the *trn*L-F spacers of *Haraella retrocalla* and *T. formosanum*, and the *atp*B-*rbc*L spacers of *Ascocentrum ampullaceum*, *Haraella retrocalla*, *Renanthera matutina*, *Rhynchostylis gigantea*, and *Vandopsis lissochiloides*. Five separate clones were randomly selected for sequencing within an individual (Table 2). In addition, *Phalaenopsis lowii* showed PCR products of different lengths for both the plastid *trn*L intron and *atp*B-*rbc*L spacer, and *P. gibbosa* for the *atp*B-*rbc*L spacer. These PCR products were separately cloned. Comparing the long and short form of the *trn*L intron and *atp*B-*rbc*L spacer in *P. lowii*, there were showed that the short forms had long deletions of 139 bp (data not shown) and 244 bp plus nine nucleotide substitutions, respectively (Fig. 1). For *P. gibbosa*, the short form had a long deletion of 158 bp, four short deletions and 16 nucleotide substitutions (Fig. 2). In this study, the molecular phylogeny of *Phalaenopsis* was clarified using the long form of the plastid DNA fragment. The GenBank accession numbers of those plastid DNA sequences from the 54 species of *Phalaenopsis* plus the 13 outgroup species are shown in Table 2. Four sequences (the *trn*L intron from *Aerides multiflora*, *Amesiella philippinensis*, and *Tuberolabium kotoense* were 283, 353 and 365 bp, respectively, and

the *atp*B-*rbc*L spacer from *Gastrochilus japonicus* was 486 bp) were extremely short compared with the others and were therefore excluded from further analyses.

Heterogeneous sequences were found in six outgroup species as described above, and the first clone sequenced from each of these samples was selected for the phylogenetic analyses because the individual sequences had high nucleotide sequence identity to each other within a sample (data not shown). The three plastid DNA regions were combined following the removal of 95 bp within the *trn*L intron due to an unalignable TA-rich region caused by differences in the P8 stem loop region, as described by Kocyan et al. (2008). The resulting alignment was 2,107 bp in length, of which 527 sites were variable (*trn*L intron, *trn*L-F spacer and *atp*B-*rbc*L spacer accounted for 166, 107 and 254 variable sites, respectively) and 193 were potentially parsimony-informative sites (*trn*L intron, *trn*L-F spacer and *atp*B-*rbc*L spacer accounted for 66, 35 and 92, respectively). The sequence alignment of the combined nuclear and plastid DNA data matrix was 2,886 bp, of which 963 were variable and 498 were potentially parsimony-informative sites.

Phylogenetic reconstruction. The Bayesian inference (BI) tree derived from the nrITS data is shown in Fig. 3. Using the plastid DNA, the MP analysis yielded 425 equally most-parsimonious trees, each of length (L) = 868 steps, with a consistency index (CI) of 0.71 and a retention index (RI) of 0.76. The strict consensus tree is shown

in Fig. 4. The BI tree is generally congruent with the MP strict consensus tree (Fig. 5). Based on the combined data matrix, the MP analysis yielded 13 equally most-parsimonious trees, each of length (L) = 1,913 steps, with a CI of 0.64 and an RI of 0.78. The MP strict consensus tree is shown in Fig. 6 and is generally congruent with the BI tree (Fig. 7).

The monophyly of *Phalaenopsis* was supported by the nrITS, plastid DNA, and the combined DNA data sets (84%, 99%, and 99% bootstraps in the MP tree, 100%, 100%, and 100% in the BI tree) (Figs. 3-7). Within *Phalaenopsis*, the monophyly of subgenus *Polychilos* was supported by the plastid DNA and the combined data (55% and 72% bootstraps in the MP tree, 100% and 100% in the BI tree). Within subgenus *Polychilos*, section *Fuscatae* was monophyletic based on the plastid DNA, combined data, and the nrITS, excluding one clone of ITS in *P. viridis* (91%, 88% and 95% bootstrap in the MP tree, 100%, 100% and 100% in the BI tree) (Figs. 3-7). Section *Polychilos* was monophyletic according to the nrITS (75% bootstrap in the MP tree) (Tsai et al. 2006) but not in the trees based on the plastid DNA and combined DNA data due to the position of *P. mannii* (Figs. 4-7). Species of both sections *Amboinenses* and *Zebrinae* were not monophyletic in all the analyses (Figs. 3-7). Parts of section *Amboinenses*, namely *P. bastianii*, *P. fasciata*, *P. hieroglyphica*, *P. lueddemanniana*, *P. pallens*, *P. pulchra*, and *P. reichenbachiana,* formed a clade according to the nrITS, plastid DNA,

and combined data (99%, 76% excluding *P. reichenbachiana*, and 99% bootstrap in the MP tree, 100%, 93%, and 100% in the BI tree). *Phalaenopsis mariae* was sister to the aforementioned group (Figs. 3-7). Furthermore, parts of section *Amboinenses*, namely *P. doweryensis*, *P. gigantea* and *P. maculata*, formed a strongly supported clade with section *Fuscatae* in all the phylogenetic trees (Figs. 3-7).

The monophyly of subgenus *Phalaenopsis* was not supported in all the analyses (Figs. 3-7). Within subgenus *Phalaenopsis*, section *Phalaenopsis* was monophyletic, but section *Stauroglottis* was not, based on the nrITS and combined DNA data (99% and 99% bootstraps in the MP tree, 100% and 100% in the BI tree) (Figs. 3, 6 and 7). Section *Deliciosae* did not form a clade based on the nrITS data (Fig. 3) but formed a clade in plastid DNA analyses (Figs. 4, 5). *Phalaenopsis pulcherrima* (section *Esmeralda*) formed a clade with the members of subgenera *Parishianae*, *Aphyllae* and *Proboscidioides* based on the nrITS and combined data (81% and 70% bootstraps in the MP tree, 100% and 93% in the BI tree) (Figs. 3, 6 and 7). Subgenus *Parishianae* appeared monophyletic in the BI tree derived from plastid DNA only (Fig. 5). Subgenus *Aphyllae* was not monophyletic since *P. lowii* (subgenus *Proboscidioides*) was nested within subgenus *Aphyllae* in all the obtained phylogenetic trees (Figs. 3-7).

Discussion

Heterogeneous plastid DNA within individuals of two *Phalaenopsis* species

The short forms of DNA fragments in both the *trn*L intron and the *atp*B-*rbc*L spacer had also indels based on the sequence alignment. There are at least two alternative explanation for the different copies of plastid DNA within an individual: (1) These different copies are all retained in the plastid genome due to the occasional biparental inheritance of plastid genomes (Second et al. 1989; Liu et al. 2004; Matsushima et al. 2008); or (2) the short form of the plastid DNA might be retained in the nuclear or mitochondrial genome through horizontal gene transfer (Ellis 1982; Timmis and Scott 1983; Cheung and Scott 1989; Ayliffe and Timmis 1992; Ayliffe et al. 1998). Horizontal transfer can occur in large fragments (>30 kb) coming from the organellar genome into the nuclear genome (Yuan et al. 2002; Huang et al. 2005). In the present study, the sequence of the short form of DNA was different from that of the other species of *Phalaenopsis*. Thus, we suggest that these short forms were not retained through biparental inheritance, but were retained through horizontal gene transfer. In addition, the mutation rate in nuclear DNA is higher than that in plastid DNA in plants (Wolfe et al. 1987). This could explain the high mutation rate for the short form of plastid DNA in the nuclear genome in both *P. lowii* and *P. gibbosa*.

The phylogeny of the genus *Phalaenopsis*

Phalaenopsis, as described by Christenson (2001), was monophyletic based on the nrITS data (Tsai et al. 2006, Fig. 3), plastid *trn*L intron, *trn*L-F and *atp*B-*rbc*L spacers (Figs. 4, 5), and combined data (Figs. 6, 7). These results are consistent with those from previous phylogenetic analyses based on other molecular evidence, including the plastid *mat*K, *atp*H-F, and *trn*D-E (Padolina et al. 2005) and the plastid *mat*K and *trn*K introns as well as the nrITS (Yukawa et al. 2005). Excluding the monotypic subgenus *Proboscidioides*, only subgenus *Polychilos* was monophyletic in the plastid DNA and combined data analyses, and the same patterns have been shown in other studies (Padolina et al. 2005; Yukawa et al. 2005). Based on the phylogenetic tree from the combined data, three major clades are shown within genus *Phalaenopsis*. The first clade, which includes species of subgenus *Polychilos*, was also found in previous studies (Padolina et al. 2005; Yukawa et al. 2005), as was the second clade, including subgenus *Phalaenopsis* sections *Phalaenopsis* and *Stauroglottis* (Kao 2001; Padolina et al. 2005; Yukawa et al. 2005). The third clade, comprising subgenera *Parishianae*, *Aphyllae* and *Proboscidioides* and sections *Deliciosae* and *Esmeralda* of subgenus *Phalaenopsis*, was also retrieved in other molecular phylogenetic studies (Padolina et al. 2005; Yukawa et al. 2005; Carlsward et al. 2006). The species included in this latter clade have also similar morphology (four pollinia, Sweet 1980; Seidenfaden 1988a) and

distributions (mainly distributed in South China and Indochina, Christenson 2001).

Two monotypic genera, *Nothodoritis* and *Lesliea,* were nested within *Phalaenopsis* based on the molecular phylogeny of subtribe Aeridinae (Topik et al. 2005). Yukawa et al. (2005) re-evaluated *Phalaenopsis* at the generic level. The results suggested that *Nothodoritis* and *Lesliea* belong in *Phalaenopsis*, and that there are two main genera, *Phalaenopsis* (including subgenera *Polychilos* and *Phalaenopsis* sections *Phalaenopsis* and *Stauroglottis*; the two-pollinium clade) and *Doritis* (including subgenera *Parishianae*, *Aphyllae*, *Proboscidioides* and *Phalaenopsis* sections *Emeralda* and *Deliciosae* plus genera *Nothodoritis* and *Lesliea*; the four-pollinia clade). This result was only moderately supported by the nrITS sequences (Yukawa et al. 2005, Tsai et al. 2006) but not supported by plastid data from the *mat*K, *atp*H-F and *trn*D-E spacers (Padolina et al. 2005), *mat*K and *trn*K introns (Yukawa et al. 2005), or by the *trn*L intron, *trn*L-F and *atp*B-*rbc*L spacers of this study (Figs. 4, 5). This supports clear incongruences between plastid DNA and nrITS data in the lineage of subgenus *Phalaenopsis*, sections *Phalaenopsis* and *Stauroglottis*. Because plastid DNA is maternally inherited and nrITS is biparentally inherited, the inconsistency might derive from an ancient hybridization event or maybe due to the paralogous ITS sequences.

Intrageneric relationships within *Phalaenopsis*

(1) Subgenus *Polychilos*

The plastid DNA and combined data support the monophyly of subgenus *Polychilos* with weak or moderate support. However, this group cannot be separated from sections *Phalaenopsis* and *Stauroglottis* of subgenus *Phalaenopsis* based on the nrITS data (Yukawa et al. 2005; Tsai et al. 2006). Furthermore, within subgenus *Polychilos*, only section *Fuscatae* was shown to be monophyletic based on the plastid DNA and combined data. Section *Polychilos* appeared polyphyletic based on the position of *P. mannii* in the plastid DNA and combined data phylogenies, but formed a clade with moderate support in the MP tree derived from the nrITS data (Tsai et al. 2006). Those molecular data are in agreement with morphological data, which show that species in section *Polychilos* show a fleshy, flattened rachis, with the exception of *P. mannii* having a fleshy, rounded rachis (Christenson 2001). In addition, one species of section *Amboinenses*, *P. micholitzii*, grouped with section *Polychilos*. These results are consistent with other molecular phylogenetic results (Goh et al. 2005; Yukawa et al. 2005).

According to all the phylogenetic trees of this study, section *Fuscatae* grouped with parts of section *Amboinenses*, namely *P. doweryensis*, *P. gigantea* and *P. maculata*. These results are congruent with the 5S spacer data (Kao 2001), nrITS + *mat*K-*trn*K intron data (Yukawa et al. 2005), and the RAPD data of Goh et al. (2005).

Morphologically, the aforementioned three species of section *Amboinenses* and the species of section *Fuscatae* all exhibit striped lips with a longitudinal keel (Christenson 2001). In addition, we also have observed that the species of this clade do not exhibit post-pollination chlorophyll in the perianth, as opposed to the remaining species of subgenus *Polychilos* described by Christenson (2001). Therefore, the molecular, morphological and physiological data all support that *P. doweryensis*, *P. gigantea*, and *P. maculata* are closely related to section *Fuscatae*.

Sweet (1980) separated section *Amboinenses* from section *Zebrinae* based on the shape of the perianth and the lip midlobe. However, Christenson (2001) disagreed, distinguishing section *Zebrinae* from section *Amboinenses* by the presence of a hooded anther bed. In this study, the molecular evidence shows that section *Amboinenses* cannot be separated from the species of section *Zebrinae* of both Sweet's (1980) and Christenson's (2001) treatment. Within section *Amboinenses*, several species from the Philippines, including *P. bastianii*, *P. fasciata*, *P. hieroglyphica*, *P. lueddemanniana*, *P. pallens*, *P. pulchra*, *P. reichenbachiana*, and *P. mariae*, formed a clade separating them from the remainder of section *Amboinenses*. Excluding *P. mariae* and one newly described species, *P. bastianii* (Gruss and Rollke 1991), this group was treated as one highly variable species, *P. lueddemanniana,* in traditional classifications (see Christenson 2001). Therefore, species of sections *Zebrinae* and *Amboinenses* should

not be placed in separate section according to molecular data.

(2) Subgenus *Phalaenopsis*

The subgenus was shown to be non-monophyletic because the sections *Esmeralda* and *Deliciosae* appeared separated from sections *Phalaenopsis* and *Stauroglottis*, in agreement with other molecular evidence (Chen et al. 1995; Kao 2001; Goh et al. 2005; Padolina et al. 2005; Yukawa et al. 2005). These results can partly explain why species of section *Deliciosae* (i.e., *P. chibae* and *P. deliciosa*) and section *Esmeralda* (i.e., *P. pulcherrima*) were traditionally treated as separate genera, *Kingidium* (Seidenfaden 1988a) and *Doritis* (Seidenfaden 1988b), respectively.

In our study, section *Phalaenopsis* formed a clade with section *Stauroglottis*, and this relationship was also supported by other molecular evidence (Chen et al. 1995; Kao 2001; Goh et al. 2005; Padolina et al. 2005; Yukawa et al. 2005), floral morphology (flowers lack transversely barred patterns) (Christenson 2001), geographic distributions (most of both sections are distributed in the Philippines) (Christenson 2001; Table 2), cytology (species of both sections have small chromosome sizes) (Shindo and Kamemoto 1963; Kao et al. 2001), and DNA content (species of both sections have a low DNA content) (Lin et al. 2001). The monophyly of section *Phalaenopsis* was supported by nrITS and combined data in this study and in Yukawa et al. (2005), but

not in the plastid DNA analyses of this study and other plastid DNA studies (Padolina et al. 2005; Yukawa et al. 2005). According to the nrITS, this section was divided into two subclades. One includes *P. schilleriana*, *P. stuartiana* and *P. philippinensis*, which have marbling on the upper surface of their leaves, and the other includes *P. amabilis*, *P. aphrodite* and *P. sanderiana*, which lack this marbling (Sweet 1980; Christenson 2001). Section *Stauroglottis* did not form a clade in this study or any other molecular studies (Tsai et al. 2003; Padolina et al. 2005; Yukawa et al. 2005). All data showed that *P. celebensis* was separated from *P. equestris* and *P. lindenii*. This result is also consistent with the geographic distribution of the section, in which both *P. equestris* and *P. lindenii* are distributed in the Philippines, but *P. celebensis* is distributed in Sulawesi (Christenson 2001; Table 2).

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

Subgenus *Parishianae* was not monophyletic but formed a clade with section *Deliciosae* using plastid DNA data in this and prior molecular studies (Padolina et al. 2005; Yukawa et al. 2005; Carlsward et al. 2006). This subgenus formed a clade with section *Deliciosae* in the plastid DNA data, but the aforementioned relationship was not supported in the nrITS tree (Tsai et al. 2006; Yukawa et al. 2005). Supporting the plastid data, species of both section *Deliciosae* and subgenus *Parishianae* share a

similar geographical distribution (Indochina) and exhibit four pollinia (Christenson 2001; Table 2). Subgenus *Aphyllae* was not monophyletic based on the plastid DNA and nrITS data since the monotypic subgenus *Proboscidioides* (*P. lowii*) was nested within it. With the exception of *P. lowii*, molecular data support that *P. minus* (syn. *K. minus*) and *P. braceana* (syn. *K. braceana*) could be placed in the subgenus *Aphyllae* proposed by Christenson (2001). *Phalaenopsis lowii* was traditionally considered as unique based on its extremely long beak-like rostellum and the possession of lateral lobes of the lip in the form of recurved hooks (Sweet 1980; Christenson 2001). However, *P. lowii* is embedded within subgenus *Aphyllae* in this and prior studies (Goh et al. 2005; Padolina et al. 2005; Yukawa et al. 2005; Carlsward et al. 2006). These molecular data are in agreement with several morphological (four pollinia), ecological (deciduous habit), and geographic distribution (Sweet 1980; Christenson 2001).

Biogeography

According to the evolutionary trend seen in pollinia number (Holttum 1959; Dressler 1993), the four-pollinium clade was suggested as the basal group in the genus *Phalaenopsis*. The four-pollinium clade of *Phalaenopsis* developed in South China and Indochina and then dispersed into Indonesia, Malaysia and the Philippines. Thereafter, the two-pollinium clade developed. Within the two-pollinium clade, there are two

major groups, sections *Phalaenopsis* and *Stauroglottis* of subgenus *Phalaenopsis* and subgenus *Polycholis*. According to the present geographical distribution of *Phalaenopsis*, sections *Phalaenopsis* and *Stauroglottis* are only distributed in the Philippines with the exception of the widespread species, *P. amabilis* and *P. celebensis,* which are distributed in Sulawesi, and *P. aphrodite* subsp. *formosana* and *P. equestris*, which are distributed in Taiwan. In contrast, subgenus *Polychilos* is distributed in Indonesia and Malaysia with the exception of the *P. luddemanniana* complex and *P. mitcholitzii,* which are distributed in the Philippines. Therefore, the evolutionary trend between subgenus *Phalaenopsis* sections *Phalaenopsis* and *Stauroglottis* and subgenus *Polychilos* seem to be different.

During the Pleistocene (about 0.01~1.8 Mya), when sea levels were low, the Malay Peninsula, Borneo, Sumatra, Java, Bali, and various parts of the Philippines would have been interconnected. The sea level was approximately 120 m below the current level in the Last Glacial Maximum (Hanebuth et al. 2000) when the Sunda Shelf connected the Thai-Malay Peninsula and Borneo, forming Sundaland (0.02 Mya) (Sathiamurthy and Voris 2006). This would have made crossings relatively easy among these regions (van Oosterzee 1997). Therefore, the *Phalaenopsis* species might have dispersed from South China, Indochina to Indonesia and Malaysia, using the Malay Peninsula as a steppingstone, from which the subgenus *Polychilos* developed. In addition, most of the

Philippine islands are young (< 5 Mya) with the exception of Palawan, Mindoro, Zamboanga and parts of the western Philippines based on historical geology (Aurelio et al. 1991; Quebral et al. 1994). The older islands of the Philippines, including Palawan and Mindoro, are on the margin of the Eurasian Plate and may have begun to slide away from the main mass in the middle Oligocene (~30 Mya) (Fig. 8). Therefore, the *Phalaenopsis* species might have dispersed from South China and Indochina to the Philippines, using some older lands of the Philippines (e.g., Mindoro and Palawan) as stepping stones, from which the sections *Phalaenopsis* and *Stauroglottis* developed (Fig. 9).

Until 5-10 Mya, the crust of the older plate, Palawan, was combined with Borneo (Karig et al. 1986; Stephan et al. 1986; Hall 1996). This provided an opportunity for interchange between the Philippines species and Borneo species, and thereafter, *P. amabilis* dispersed into Borneo, Java, New Guinea, and Australia, and species of subgenus *Polychilos* dispersed into the Philippines during the glacial period. Because *P. mariae* is distributed in both Borneo and Palawan (Christenson 2001) and belongs to the basal species of the *P. luddemanniana* complex, according to the combined data of the nrITS and plastid DNA, *P. mariae* was suggested as a basal species in the *P. luddemanniana* complex. This species dispersed from Borneo into the Philippines, and thereafter, the *P. luddemanniana* complex developed. Two *Phalaenopsis* taxa, *P.*

aphrodite subsp. *formosana* and *P. equestris,* respectively belonging to sections *Phalaenopsis* and *Stauroglottis,* are found in Taiwan. Therefore, these two Taiwanese *Phalaenopsis* species must have separately come from the Philippines.

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References

Aurelio M. A., Barrier E., Rangin C., Muller C. (1991) The Philippine Fault in the late Cenozoic tectonic evolution of the Bondoc-Masbate-N. Leyte area, central Philippines. J. SE Asian Earth Sci. 6: 221-238.

Ayliffe M. A., Scott N. S., Timmis J.N. (1998) Analysis of plastid DNA-like sequences within the nuclear genomes of higher plants. Mol. Biol. Evol. 15: 738-745.

Ayliffe M. A., Timmis J. N. (1992) Tobacco nuclear DNA contains long tracts of homology to chloroplast DNA. Theor. Appl. Genet. 85: 229-238.

Carlsward B. S., Whitten W. M., Williams N. H., Bytebier B. (2006) Molecular

phylogenetics of Vandeae (Orchidaceae) and the evolution of leaflessness. Amer. J. Bot. 93: 770-786.

Chen W. H., Fu Y. M., Hsieh R. M., Tsai W. T., Chou 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. pp. 341-346. Kluwer Academic Publishers, Dordrecht, Boston, and London.

Cheung W. Y., Scott N. S. (1989) A contiguous sequence in spinach nuclear DNA is homologous to three separated sequences in chloroplast DNA. Theor. Appl. Genet. 77: 625-633.

Christenson E. A. (1986) Nomenclatural changes in the Orchidaceae subtribe Sarcanthinae. Selbyana 9: 167-170.

Christenson E. A. (2001) *Phalaenopsis*. Timber Press, Portland, OR, USA, 330 pp.

Christenson E. A., Whitten M. W. (1995) *Phalaenopsis bellina* (Rchb.f.) Christenson, a segregate from *P. violacea* Witte (Orchidaceae: Aeridinae). Brittonia 47: 57-60.

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

Dressler R. L. (1993) Phylogeny and classification of the orchid family. Dioscorides Press, Portland, Oregon. 314p.

Ellis J. (1982) Promiscuous DNA—chloroplast genes inside plant mitochondria. Nature 299: 678-679.

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.

Fowlie J. A. (1983) A new *Phalaenopsis* species of the section *Zebrinae* from central Sumatra, *Phalaenopsis inscriptiosinensis* Fowl. Orchid Dig.47: 11-12.

Fowlie J. A. (1993) A new species of *Phalaenopsis* from Flores Island, Indonesia: *Phalaenopsis floresensis* Fowl., sp. nov. Orchid Dig. 57: 35-36.

Garay L. A., Hamer F., Siegerist E. S. (1995) Inquilina orchidacea: Orchidaceae plaerumque Levyanae. Lindleyana 10: 174-182.

Goh M. W. K., Kumar P. P., Lim S. H., Tan H. T. W. (2005) Random amplified polymorphic DNA analysis of the moth orchids, *Phalaenopsis* (Epidendroideae: Orchidaceae). Euphytica 141: 11-22.

Gruss O., Rollke L. (1991) Eine weitere *Phalaenopsis* von den Philippinen-*P. bastianii* Gruss and Rollke. Die Orchidee 42: 76-79.

Hall R. (1996) Reconstructing Cenozoic SE Asia. *In* Hall, R, and D. J. Blundell (eds.) Tectonic Evoultion of SE Asia. Geological Society of London Special Publication. No. 106, pp. 153-184.

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.

Hanebuth T., Stattegger K., Grootes P.M. (2000) Rapid flooding of the Sunda Shelf: A late-glacial sea-level record. Science 288: 1033-1035.

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.

Holttum R. E. (1959) Evolutionary trends in the Sarcanthiine orchids. Am. Orchid Soc. Bull. 5: 399-423.

Huang C. Y., Grunheit N., Ahmadinejad N., Timmis J. N., Martin W. (2005) Mutational Decay and Age of Chloroplast and Mitochondrial Genomes Transferred Recently to Angiosperm Nuclear Chromosomes. Plant Physiol. 138: 1723-1733.

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 with English abstract).

Kao Y. Y., Chang S. B., Lin T. Y., Hsieh C. H., Chen Y. H., Chen W. H., Chen C. C. (2001) Differential accumulation of heterochromatin as a cause for karyotype variation in *Phalaenopsis* orchids. Ann. Bot. 87: 387-395.

Karig D. E., Sarewitz D. R., Iiaeck G. D. (1986) Role of strike-slip faulting in the evolition of allochthonous terrances in the Philippines. Geology 14: 852-855.

Kocyan A., de Vogel E. F., Conti E., Gravendeel B. (2008) Molecular phylogeny of *Aerides* (Orchidaceae) based on one nuclear and two plastid markers: A step forward in understanding the evolution of the Aeridinae. Mol. Phylogenet. Evol. 48: 422-443.

Lin S., Lee H. C., Chen W. H., Chen C. C., Kao Y. Y. Fu Y. M., Chen Y. H., Lin T. Y. (2001) Nuclear DNA contents of *Phalaenopsis* species and *Doritis pulcherrima*. J. Amer. Soc. Hort. Sci. 126: 195-199.

Liu F. (1988) A new species of *Phalaenopsis* from Yunnan. Acta Bot. Yunnan. 10: 119-120.

Liu Y., Zhang Q., Hu Y., Sodmergen (2004) Heterogeneous pollen in *Chlorophytum comosum*, a species with a unique mode of plastid inheritance intermediate between the maternal and biparental modes. Plant Physiol. 135: 193-200.

Matsushima R., Hu Y., Toyoda K., Sodmergen, Sakamoto W. (2008) The model plant *Medicago truncatula* exhibits biparental plastid inheritance. Plant Cell. Physiol. 49: 81-91.

Padolina J., Linder C. R., Simpson B. B. (2005) A phylogeny of *Phalaenopsis* using multiple chloroplast markers. Selbyana 26: 23-27.

Posada D. 2008. jModelTest: Phylogenetic Model Averaging. Mol. Biol. Evol. 25: 1253-1256.

Quebral R. D., Pubellier M., Rangin C. (1994) The Mindanao: a transition from collision to strike-slip environment. Tectonics 15: 713-726.

Ronquist F., Huelsenbeck J. P. (2003) MRBAYES 3: Bayesian phylogenetic inferenceunder mixed models. Bioinformatics 19: 1572-1574.

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

Sathiamurthy E., Voris H. K. (2006) Maps of Holocene sea transgression and submerged lakes on the Sunda Shelf. Nat. Hist. J. Chulalongkorn Univ., Supplement 2: 1-43.

Second G., Dally A, Zhang S. H. (1989) Occasional biparent inheritance of chloroplast DNA in rice. Rice Genet. Newsl. 6: 150-152.

Seidenfaden G. (1988a) *Kingidium*. Opera Bot. 95:182-189.

Seidenfaden G. (1988b) *Doritis*. Opera Bot. 95: 31-34.

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

Shim P. S., Fowlie J. A. (1983) A new species of *Phalaenopsis* from Sulawesi (Celebes) formerly confused with *Phalaenopsis psilantha* Schltr., *Phalaenopsis venosa* Shim and Fowl. Orchid Dig. 47: 124-128.

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

Siegerist E. S. (1989) *Kingidium deliciosum*. Amer. Orchid Soc. Bull. 56: 228-231.

Stephan J. F., Blanchet R., Rangin C., Pelletir B., Letouzey J., Muller C. (1986) Geodynamic evolution of the Taiwan-Luzon-Mindoro belt since the Late Eocene. Tectonophysics 125: 245-268.

Sweet H. R. (1980) The genus *Phalaenopsis*. The Orchid Digest, Pomona, CA, USA, 128 pp.

Taberlet P., Gielly L., Pautou G., Bouvet J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17: 1105-1109.

Tamura K., Dudley J., Nei M., Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Tharp A. G., Fowlie J. A., Tang C. Z. (1987) A recently described *Phalaenopsis* species from the Philippines: *Phalaenopsis philippinensis* Golamco ex Fowl. and Tang. Orchid Dig. 51: 87-92.

Timmis J. N., Scott N. S. (1983) Sequence homology between spinach nuclear and chloroplast genomes. Nature 305: 65-67.

Topik H., Yukawa T., Ito M. (2005) Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): insights from plastid *mat*K and nuclear ribosomal ITS sequences. J. Plant Res. 118: 271-284.

Tsai C. C., Huang S. C., Chou C. H. (2006) Molecular phylogeny of *Phalaenopsis* Blume (Orchidaceae) based on the internal transcribed spacer of the nuclear ribosomal DNA. Plant Syst. Evol. 256: 1-16.

Tsai C. C., Huang S. C., Huang P. L., Chou C. H. (2003) Phylogeny of the genus *Phalaenopsis* (Orchidaceae) with emphasis on the subgenus *Phalaenopsis* based on the sequences of the internal transcribed spacers 1 and 2 of rDNA. J. Hort. Sci. Biotech. 78: 879-887.

van Oosterzee P. (1997) Where Worlds Collide: The Wallace Line. Cornell Univ. Press, Ithaca, NY.

Wolfe K. H., Li W. H., Sharp P. M. (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc. Nati. Acad. Sci. USA 84: 9054-9058.

Yuan Q., Hill J., Hsiao J., Moffat K., Ouyang S., Cheng Z., Jiang J., Buell C. R. (2002) Genome sequencing of a 239-kb region of rice chromosome 10L reveals a high

frequency of gene duplication and a large chloroplast DNA insertion. Mol. Genet. Genomics 267: 713-720.

Yukawa T. (1996) *Phalaenopsis chibae* (Orchidaceae) – A new species from Vietnam. Ann. Tsukuba Bot. Gard. 15: 19-22.

Yukawa T., Kita K., Handa T., Topik H. (2005) Molecular phylogenetics of Phalaenopsis (Orchidaceae) and allied genera: Re-evaluation of generic concepts. Acta Phytotax. Geobot. 56: 141-161.

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Figure legends

Fig. 1. The sequence alignment of the different forms of *atp*B-*rbc*L spacers of plastid DNA from a *P. lowii* individual.

Fig. 2. The sequence alignment of the different forms of *atp*B-*rbc*L spacers of plastid DNA from a *P. gibbosa* individual.

Fig. 3. The Bayesian inference (BI) tree resulting from the analysis of 53 *Phalaenopsis* and 13 outgroup ITS sequences. Posterior probabilities > 50% are shown above each branch for the BI tree and below each branch for the MP tree. A solid circle (●) indicates that the species was traditionally treated as the genus *Doritis*. A solid square (■) indicates that the species was traditionally treated as the genus *Kingidium*. Redrawn from Tsai et al. 2006.

Fig. 4. The strict consensus tree of the most parsimonious trees resulting from the analysis of the combined plastid DNA (including the plastid *trn*L intron, *trn*L-F spacer, and *atp*B-*rbc*L spacer) from 54 *Phalaenopsis* and 9 outgroup species. Bootstrap values > 50% are shown above each branch. A solid circle (●) indicates that the species was traditionally treated as the genus *Doritis*. A solid square (■) indicates that the species was traditionally treated as the genus *Kingidium*.

Fig. 5. The Bayesian inference tree resulting from the analysis of the combined plastid DNA (including the plastid *trn*L intron, *trn*L-F spacer, and *atp*B-*rbc*L spacer) from 54 *Phalaenopsis* and 9 outgroup species. Posterior probabilities > 50% are shown above each branch. A solid circle (●) indicates that the species was traditionally treated as the genus *Doritis*. A solid square (■) indicates that the species

was traditionally treated as the genus *Kingidium*.

Fig. 6. The strict consensus tree of the most parsimonious trees resulting from the analysis of the combined data matrix (the nuclear ribosomal ITS, plastid *trn*L intron, *trn*L-F spacer, and *atp*B-*rbc*L spacer) from 52 *Phalaenopsis* and 9 outgroup species. Bootstrap values > 50% are shown above each branch. A solid circle (●) indicates that the species was traditionally treated as the genus *Doritis*. A solid square (■) indicates that the species was traditionally treated as the genus *Kingidium*.

Fig. 7. The Bayesian inference tree resulting from the analysis of the combined data matrix (the nuclear ribosomal ITS, plastid *trn*L intron, *trn*L-F spacer, and *atp*B-*rbc*L spacer) from 52 *Phalaenopsis* and 9 outgroup species. Posterior probabilities > 50% are shown above each branch. A solid circle (●) indicates that the species was traditionally treated as the genus *Doritis*. A solid square (■) indicates that the species was traditionally treated as the genus *Kingidium*.

Fig. 8. The putative map of Southeast Asia 30 Mya (modified from Hall 1996).

Fig. 9. The evolutionary trends and biogeography of the *Phalaenopsis* based on molecular data from this study.