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Molecular Phylogenetics of *Phalaenopsis* Taxa: An Updated Review

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ABSTRACT

Phalaenopsis is one of the most popular and beautiful orchids, exhibiting an amazing flower morphology looking like moths. The *Phalaenopsis* species was first described by Linnaeus and was placed in the genus *Epidendrum* as *Epidendrum amabile* in 1753. Blume (1825) erected the genus *Phalaenopsis* and placed all of moth orchids into this genus. This genus was confused with its related genus, *Kingidium*, *Doritis*, *Polychilos*, for a long time. Sweet (1980) treated *Polychilos* as a synonym of *Phalaenospis*. Until recently, Christenson (2001) treated *Kingidium* and *Doritis* as synonym of *Phalaenopsis*, and divided into five subgenera, *Phalaenopsis*, *Polychilos*, *Parishianae*, *Proboscidioides* and *Aphyllae*. Molecular techniques are used to clarify the phylogeny of *Phalaenopsis* recently. First analyses of internal transcribed spacer (ITS) of ribosomal DNA supported that *Kingidium* and *Doritis*, *Polychilos* could be treated as a synonym of *Phalaenopsis* as suggested by Christenson (2001). However, the phylogeny of subgenera *Phalaenopsis*, *Polychilos*, *Aphyllae* was not supported nature groups. In addition, plastid DNAs, including *atp*B-*rbc*L intergenic spacer (IGS), *trn*L-F IGS, and *trn*L intron, were to clarify the molecular phylogeny of *Phalaenospis* since its maternal inheritance is separated from biparental inheritance of nuclear ITS of rDNA. Plastid DNAs also supported Christenson's treatment on generic level but not on subgeneric and sectional levels. Some incongruence between nuclear and plastid DNA are found. This is usually ascribed to a number of biological effects, such as hybridization, introgression, horizontal gene transfer, and lineage sorting. Furthermore, molecular data was also revealed the phylogeny of species complex and natural *Phalaenospis* hybrid as well as the identification of closely related *Phalaenopsis* cultivars.

Keywords: DNA sequences, moth orchids, molecular marker, RAPD, SSRs

CONTENTS

INTRODUCTION

Moth orchids (*Phalaenopsis*) have been examined in taxonomy, systematics, physiology, ecology, phytochemistry, and tissue culture (e.g., Slaytor 1977; Arditti 1977; Sweet 1980; Christenson 2001). More recently, several molecular techniques have been developed to examine molecular information of *Phalaenopsis*. It provides new opportunities to do new *Phalaenopsis* research, e.g., to address unresolved taxa, phylogeny of *Phalaenopsis*. Up to date, molecular data have been used to examine phylogenetic relationship in *Phalaenopsis* and population structure based on different kinds of macromolecules, specially in DNA information, such as random amplified polymorphic DNA [RAPD], inter simple sequence repeat [ISSR], and sequences data.

Phalaenopsis plants are epiphytic habit with the exception of few species living at terrestrial habit. Plants of *Phalaenopsis* all have short stem; aerial, prostrate and substrate roots; succulent, fleshy leaves (Sweet 1980; Christenson 2001). More recently, the latest classification of *Phalaenopsis* have been introduced by Christenson (2001), who divided this genus into five subgenera, namely *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos* and *Phalaenopsis*. Of them, 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*. Christenson (2001) treated *Kingidium* and *Doritis* as synonyms of *Phalaenopsis*.

Species of *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

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Invited Mini-Review

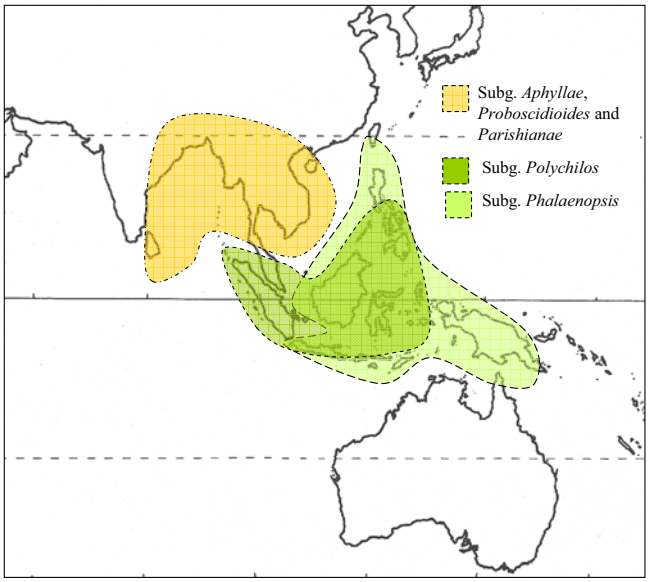

Fig. 1 The distribution pattern of different subgenera of *Phalaenopsis*.

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) (**Fig. 1**).

Although isoenzyme pattern has been widely applied to study population structure or to reconstruct phylogenetic relationship in plants during the last decade (Crawford *et al*. 1990). However, isoenzymes still not be applied for reconstructing the phylogeny or inspecting population structure of *Phalaenopsis* plants. Most of isoenzymes pattern in *Phalaenopsis* study focus on physiological studies (e.g., Trippi 1971). Since most of *Phalaenopsis* species are endemic in the wild, it is not easy to collect enough specimens for inspecting population structure.

Recently, DNA data provides an index to reconstruct the phylogenetic relationships. First DNA fingerprinting technique is restriction fragment length polymorphism (RFLP). The detection of polymorphic DNA is based on various restriction enzymes isolated from various bacteria. Those enzymes can recognize specific DNA sequences, generally from four to eight base pairs, and cut it. Different genetic background might be detected different lengths of DNA fragments as a result of different cutting sites. This molecular marker is shown as DNA band pattern. More recently, another technology for DNA band pattern, RAPD was revealed by Williams *et al*. (1990). This technology is based on polymerase chain reaction (PCR) with an arbitrary short DNA primer (generally 10 mer). Different amplified DNA fragments can be obtained from different genetic backgrounds. The DNA band pattern developed more recently, such simple sequence repeats (SSR) (Lagercrantz *et al.* 1993), inter-simple sequence repeats (ISSR) (Zietkiewicz *et al*. 1994), and amplified fragment length polymorphisms (AFLP) (Vos *et al*. 1995) have been widely applied in DNA fingerprinting for various plant taxa. Some of those technologies have been applied in *Phalaenopsis* research.

In this review, we have focused in recent years on the phylogenetic relationship on inter-, intra-generic, and species complex levels.

DNA AS MOLECULAR MARKERS TO CONSTRUCT THE PHYLOGENY OF *PHALAENOPSIS*

RAPD

RAPD has been conducted for revealing the phylogenetic relationship of 16 *Phalaenopsis* species (Fu *et al*. 1997). 381 RAPD makers derived from 20 primers were obtained. Chuang (2002) examined several accessions of *Phalaenopsis aphrodite* subsp. *formosana* and several related *Phalaenopsis* species from Philippines based on RAPD and ISSR molecular Markers. The result showed that these two molecular techniques could offer informative markers to separate those of samples which have close relationship.

Another RAPD analysis was conducted by Goh *et al*. (2005). This study examined 149 accessions representing 46 species in the genus *Phalaenopsis* and four *Paraphalaenopsis* species as outgroups. A total of 20 random primers were screened. Of them, six random primers were selected and provided 123 polymorphic bands. Clustering analysis derived from those RAPD molecular markers showed that those *Phalaenospis* form seven groups and are mostly congruent with those based on morphological characters erected by previous workers. According to banding patterns, *P. doweryensis* is suspected to be a hybrid of *P. gigantea* and *P. kunstleri* or *P. cochlearis*.

SSRs

Young (2004) examined DNA fingerprinting of 89 accessions of *Phalaenopsis amabilis* based on microsatellite DNA (SSRs). Three SSR loci were cloned and evaluated those accessions of *P. amabilis*. The result has been proven to be a good molecular marker to identify intraspecific variation of *Phalaenopsis*. Han (2005) obtained 42 loci of microsatellite DNA in both *Phalaenopsis aphrodite* subsp. *formosana* and *P. equestris* based on cDNA-SSRs technology. Some of those SSR loci can be used to identify closely related *Phalaenopsis* hybrids. Those preliminary results indicated that microsatellites will have potential applications in *Phalaenopsis* cultivar identification.

DNA sequences

Kao (2001) examined the molecular phylogeny of 28 species of *Phalaenopsis* species derived from intergenic spacers of 5S rDNA. The result supported that *Phalaenopsis* species was monophyletic. Seven clades were revealed based on 5S intergenic spacer (IGS) data.

Internal transcribed spacer (ITS) of ribosomal DNA (rDNA) in nuclear DNA is widely applied in reconstructing the phylogenetics in both plants and animals. This DNA region has been proposed to identify species in plants and has potentially to apply barcoding to flower plants (Kress *et al*. 2005). Tsai *et al*. (2003) first examined the molecular phylogeny of *Phalaenopsis* based on ITS sequences. Phylogenies of 17 species of the genus *Phalaenopsis* and two related species were examined. Seventeen *Phalaenopsis* species were shown to be a monophyletic group as supported by molecular data analysis from ITS1 and ITS2 sequences. This result agreed that the genera *Doritis* and *Kingidium* could be treated as the genus *Phalaenopsis*. In addition, members of subgenus *Phalaenopsis* are not a monophyletic group. Within the subgenus *Phalaenopsis*, members of section *Phalaenopsis* are a monophyletic group. Both section *Stauroglottis* and *Deliciosae* are not monophyletic groups. Furthermore, members of the section *Deliciosae* and *Esmeralda* of subgenus *Phalaenopsis* are closer to subgenera *Parishianae*, *Proboscidioides* and *Aphyllae* and separated from the other *Phalaenopsis* species. In conclusion, this study supports the systematics of the genus *Phalaenopsis* on the generic level but not on the subgeneric level.

Recently, Carlsward *et al*. (2006) examined the molecular phylogenetics of Vandeae derived from both ITS nuclear ribosomal DNA (nrDNA) and plastid DNAs. Within *Pha-*

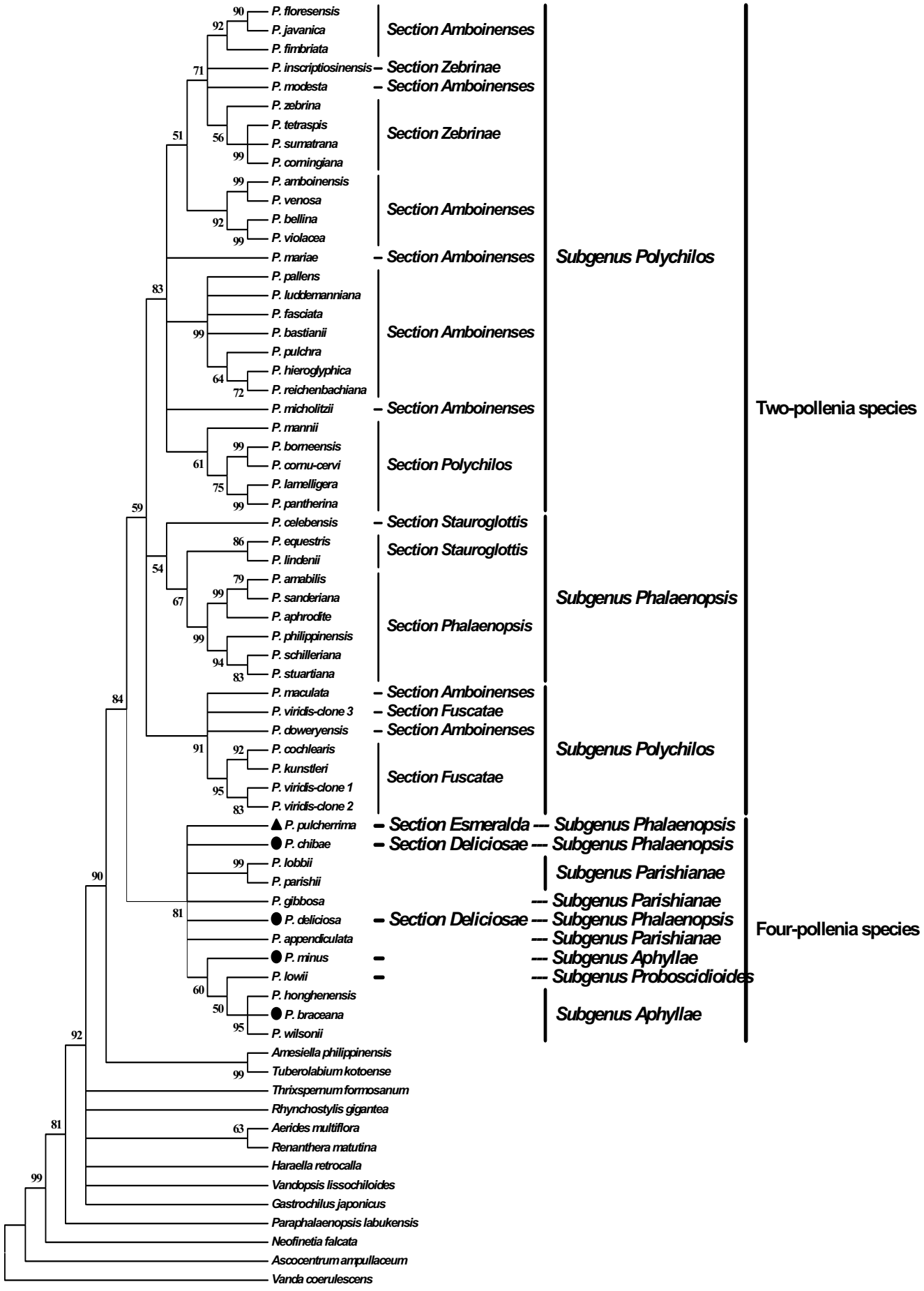

Fig. 2 The strict consensus parsimonious tree of 53 *Phalaenopsis* species plus 13 outgroups obtained from sequence comparisons of the ITS region of rDNA. Bootstrap values > 50% are shown on each branch. A solid triangle (▲) on the tree indicates that this species was traditionally treated as the genus *Doritis*. Solid circles (●) on the tree indicate that these species were traditionally treated as the genus *Kingidium*. (redrawn from Tsai *et al*. 2006a)

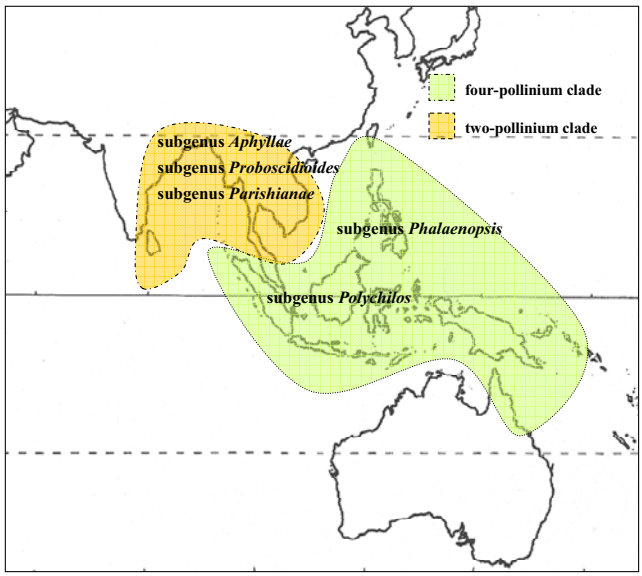

Fig. 3 Correlation between the distribution pattern and pollinia number of different subgenera of *Phalaenopsis*. (redrawn from Tsai *et al.* 2006a)

laenopsis (including *Doritis*, *Kingidium*), 17 species were examined based on ITS nrDNA. The result showed two main clades: (1) subgenus *Phalaenopsis* including sections *Aphyllae*, *Deliciosae*, *Esmeralda*, *Parishianae*, and *Proboscidioides* and (2) subgenus *Phalaenopsis* including sections *Phalaenopsis* and *Stauroglottis* as well as subgenus *Polychilos* including sections *Amboinensis*, *Polychilos*, and *Zebrinae*. Section *Aphyllae* is monophyletic if *P. lowii* (section *Proboscidioides*) is included. *Phalaenopsis lowii* is morphologically similar to section *Aphyllae* (Christenson 2001), which would support its inclusion within section *Aphyllae*. The monophyly of section *Deliciosae* is questionable because *P. deliciosa* is more closely related to section *Aphyllae* than to *P. chibae*. Subgenus *Polychilos* is weakly supported as monophyletic, excluding *P. fuscata*, which is unresolved in a clade with *P. deliciosa*/*P. pulcherrima* and *P. amabilis*/*P. cornu-cervi*.

More recently, Tsai *et al*. (2006) examined the molecular phylogeny of *Phalaenopsis*. The internal transcribed spacer (ITS1, 5.8S rDNA, and ITS2) region of nrDNA was sequenced from 53 species, which represent most of the living species diversity in the genus. 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*. 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* was monophyletic, but they were highly clustered with subgenus *Proboscidioides* plus sections *Esmeralda* and *Deliciosae* of the subgenus *Phalaenopsis* based on ITS data (**Fig. 2**). Those species also have the same characters of morphology of four pollinia and similar biogeographies (**Fig. 3**). 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 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.

Chloroplast DNAs

Padolina *et al*. (2005) demonstrated that a phylogeny of *Phalaenopsis* was reconstructed using three chloroplast markers, *mat*K, *atp*H-*atp*F, and *trn*D-*trn*E which a total of 2177 base pairs. The result supports the placement of the species of *Doritis* and *Kingidium* into a more broadly defined *Phalaenopsis,* as proposed in a revision of the genus by Christenson, and is in agreement with previous report of Tsai *et al*. (2006).

More recently, Tsai *et al*. (unpublished) examined the molecular phylogeny of *Phalaenopsis* base on multiple plastid DNAs, including the intron of *trn*L, the intergenic spacer (IGS) of *trn*L-*trn*F and the IGS of *atp*B-*rbc*L, 2202 bp in total. Fifty four *Phalaenopsis* species representing most of the living species diversity in the genus were examined. The result provided support for the monophyly of the genus and concurred in that *Doritis* and *Kingidium* are synonym of *Phalaenopsis* as suggested by Christenson (2001). The result is also in agreement with ITS data of the previous study (Tsai *et al*. 2006). Within the genus, subgenus *Polychilos* was monophyletic based on plastid DNA analysis, but not monophyletic in ITS analysis. Basically, species of subgenus *Polychilos* were divided into two subclades based on both plastid DNA and ITS analysis. One of subclades includes the section *Fuscatae* plus parts of the section *Amboinenses*, which have concave striped lips with a longitudinal keel. The other subclade includes the remaining species of the section *Amboinenses* plus the section *Zebrinae*. The subgenus *Phalaenopsis* was not monophyletic, since sections *Esmeralda* and *Deliciosae* were separated from sections *Phalaenopsis* and *Stauroglottis* based on both plastid DNA and ITS data. Subgenera *Aphyllae* and *Parishianae* were not shown to be monophyletic based on both plastid DNA and ITS data. Furthermore, the monotypic species of subgenus *Proboscidioides*, *P. lowii*, formed a clade with the subgenus *Aphyllae* based on both plastid DNA and ITS data. In conclusion, molecular phylogeny of *Phalaenopsis* derived from plastid DNA is partially in agreement with that derived from ITS data. The main incongruent phylogenetic pattern between plastid DNA and ITS trees is the four-pollinia *Phalaenopsis* species did not form a clade based on plastid DNA data. The incongruence of the four-pollinia *Phalaenopsis* species between plastid DNA and ITS data may be caused by the inheritance of plastid DNA from maternal species and homogenization of ITS of rDNA from both parental species.

DNA AS MOLECULAR MARKERS TO REVEAL SPECIES COMPLEX OF *PHALAENOPSIS*

Tsai (2003a) examined *Phalaenopsis lueddemanniana* species complex, including *P. bastianii*, *P. pallens*, *P. hieroglyphica*, *P. reichenbachiana*, *P. lueddemanniana*, *P. fasciata*, *P. pulchra*, and *P. mariae*, as well as others of section *Amboinenses* based on ITS sequences and chloroplast DNA. The result is in agreement with the treatment of Sweet (1980) that raised this complex into seven species. It also showed that *P. mariae* is a basal species of the *P. lueddemanniana* complex (**Fig. 4**). Since the Philippines did not find any species of the section *Amboinenses* with exceptions of *P. lueddemanniana* complex plus *P. micholitzii* (distributed in Mindanao, the Philippines), suggesting that species of the *P. lueddemanniana* complex in the Philippines descended from species of the section *Amboinenses* distributed in Borneo and developed to be a unique lineage (Tsai 2003a) (**Fig. 5**). Based on the historical geology, combination of both plates of the Philippines and Borneo was young (5~10 Mya) (Karig *et al*. 1986; Stephan *et al*. 1986; Hall 1996), it makes both lands of Borneo and Palawan (the Philippines) have much chance to be interconnected during glacial times. Therefore, this evolutionary trend of the *P. lueddemanniana* complex was reasonable based on the historical geology of both the Philippines and Borneo.

Furthermore, Tsai (2003b) examined *Phalaenopsis amabilis* species complex, including *P. amabilis*, *P. amabilis* subsp. *moluccana*, *P. amabilis* subsp. *rosenstromii*, *P. aphrodite*, *P. aphrodite* subsp. *formosana*, and *P. sanderiana*. The internal transcribed spacers 1 and 2 (ITS1+ITS2) re-

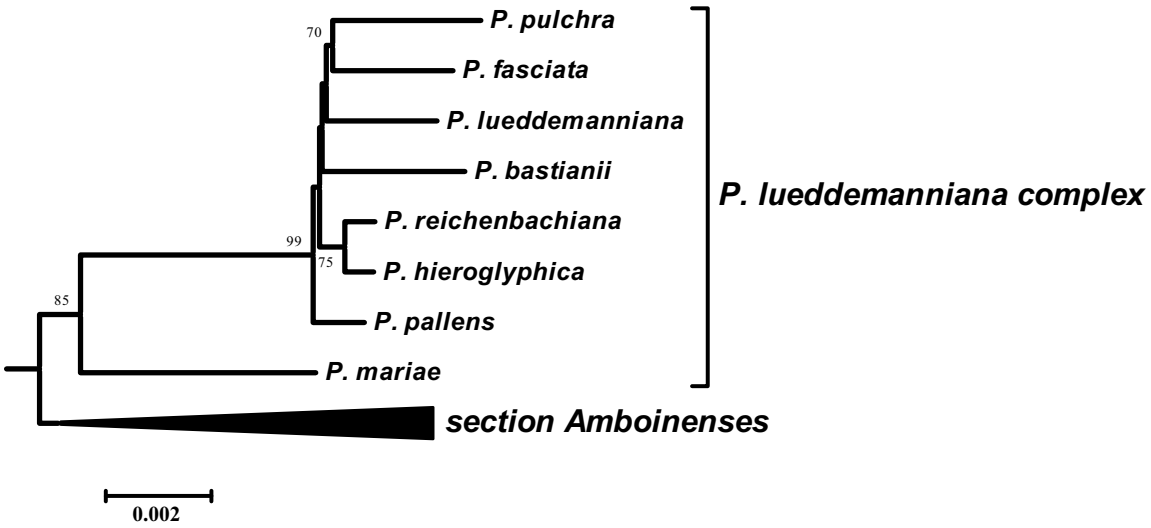

Fig. 4 The evolutionary phylogenetic subtree of both the section *Amboinenses* and the *Phalaenopsis lueddemanniana* complex inferred from combined data of the ITS of nrDNA and chloroplast DNA data contructed by Minimum evolution. (redrawn from Tsai *et al*. 2003a). Species of the section *Amboinenses* were compressed and shown in bold branches.

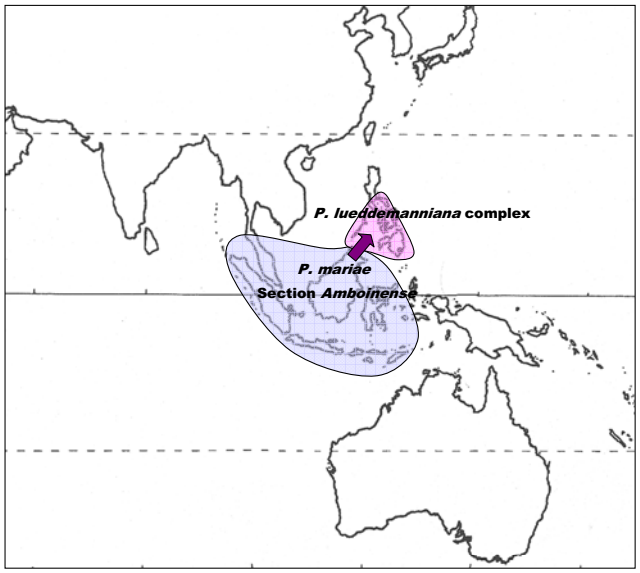

Fig. 5 The evolutionary trend of the *Phalaenopsis lueddemanniana* complex was suggested based on the historical geology and molecular DNA.

gion of nrDNA was applied to reconstruct the phylogeny of this complex. Rooted at outgroups, monophyly of the species complex was significantly supported in the Neighbor-joining tree. Within accessions of *P. amabilis* and its subspecies, different locations of *P. amabilis* and its subspecies formed different separated clades with exceptions of Palawan and Borneo populations plus Timor population and *P. amabilis* subsp. *rosenstromii*. Furthermore, *P. aphrodite* from different locations and its subspecies could not be separated from each other, but all of them were separable from others of *P. amabilis* complex. In addition, accessions of *P. sanderiana* were nested within accessions of both *P. amabilis* and its subspecies. This result does not support *P. sanderiana* being treated as a separate species from *P. amabilis*. According to the phylogenetic tree derived from ITSs of nrDNA, the Palawan population of *P. amabilis* was suggested as being the origin group of the *P. amabilis* complex. *P. aphrodite* and *P. sanderiana* were suggested to be descended from *P. amabilis* (or their most recent common ancestor). In addition, the evolutionary trend of the *P. amabilis* complex included three different lineages corresponding to three different dispersal pathways. First, *P. amabilis* distributed in Palawan dispersed into southern Mindanao and evolved into *P. sanderiana*, thereafter further dispersing into Sulawesi and New Guinea, from which *P. amabilis* subsp. *moluccana* and *P. amabilis* subsp. *Rosenstromii* developed respectively. *P. amabilis* subsp. *rosenstromii* further dispersed into Northern Australia and Timor. Second, *P. amabilis* distributed in Palawan dispersed into Borneo, thereafter further dispersing into Mentawai Is. Third, the Palawan population of *P. amabilis* dispersed into other islands of the Philippines, *P. aphrodite* evolved. And then, *P. aphrodite* is distributed throughout the Philippines with the exceptions of Palawan and southern Mindanao.

The other species complex, *Phalaenopsis sumatrana* complex, was conducted by Tsai (2003c). Phylogenetic trees inferred from the internal transcribed spacers 1 and 2 (ITS1+ITS2) region of nrDNA, the intron of *trn*L, and the intergenic spacer of *atp*B-*rbc*L of chloroplast DNA (cpDNA) were used to clarify the phylogenetics and evolutionary trends of the *Phalaenopsis sumatrana* complex. The *P. sumatrana* complex includes the two species of *P. sumatrana* and *P. corningiana*, as well as a problem species, *P. zebrina*, according to the concepts of Sweet (1980) and Christenson (2001). Based on the phylogenetic tree inferred from the ITS sequence, accessions of *P. sumatrana* cannot be separated from those of *P. corningiana*. Furthermore, accessions of *P. zebrina* can be separated from those of both *P. sumatrana* and *P. corningiana*. In addition, analyses of both sequences of the *trn*L intron and *atp*B-*rbc*L IGS of cpDNA apparently cannot discriminate among these three species of the *P. sumatrana* complex. Inspection of the morphological characters of plants of the *P. sumatrana* complex, floral fragrances of *P. zebrina*, can be used to separate it from both *P. sumatrana* and *P. corningiana*. Based on the molecular and morphological data of this study, plants of *P. zebrina* might not be suitable to be treated as a synonym of *P. sumatrana*. In the evolutionary trend of the *P. sumatrana* complex, *P. zebrina* were suggested to be the relative origin group of the *P. sumatrana* complex based on the phylogenetic tree and biogeography. In addition, *P. sumatrana* and *P. corningiana* might have evolved from *P. zebrina*.

Furthermore, *Phalaenopsis violacea* species complex also was examined by Tsai (2003d). The *P. violacea* complex includes two species, namely *P. violacea* Witte and *P. bellina* (Rchb.f.) E. A. Christ. In addition, three forms of *P. violacea* were found based on different distributions, including Sumatra, Malay Peninsula, and Mentawai Island. The phylogenetic trees inferred from ITS region of nrDNA, the intron of *trn*L, and the IGS of *atp*B-*rbc*L of plastid DNA,

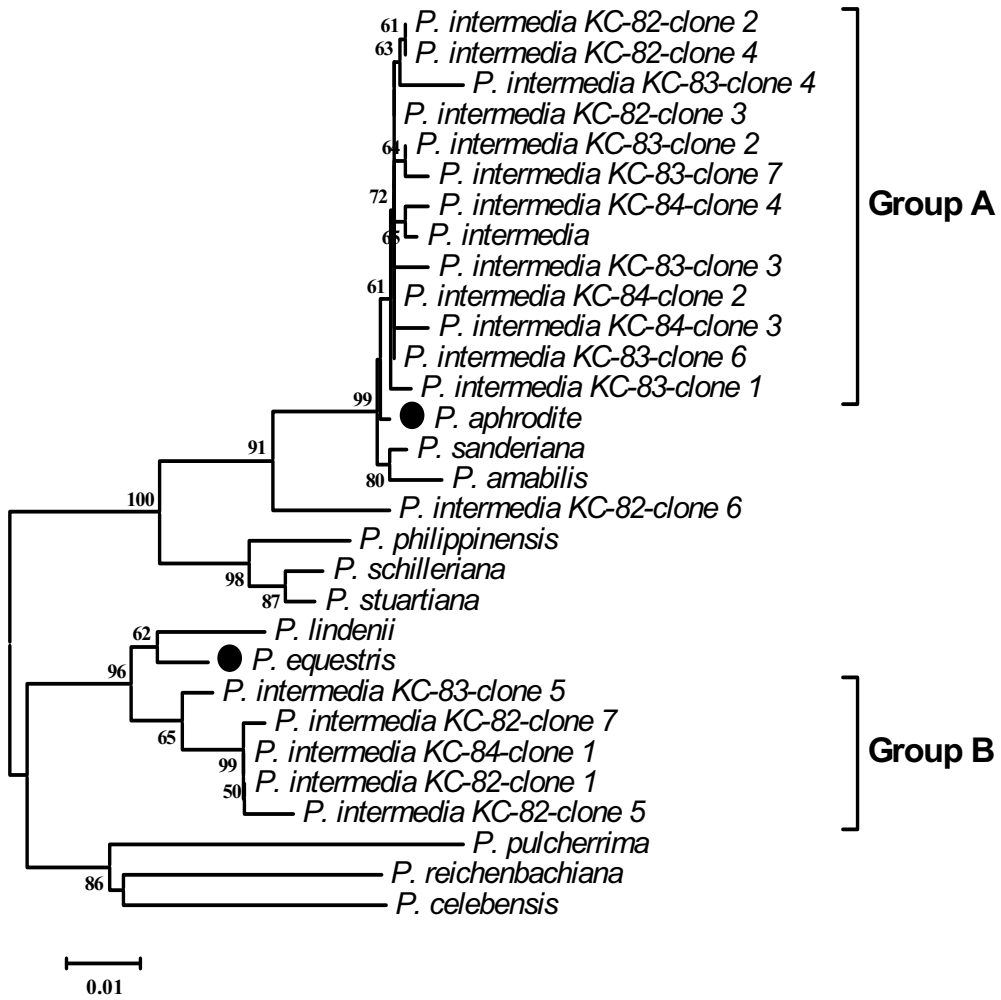

Fig. 6 Phylogenetic tree of 19 clones of *Phalaenopsis* × *intermedia*, 11 species from the sections *Phalaenopsis* and *Stauroglottis* inferred from the ITS data. Values of the interior branch test > 50% are shown on the branches. Branch lengths are proportional to the number of base changes along each branch. The scale bar indicates the genetic distance of Kimura (1980). Solid circles (●) indicate the putative parents of *P.* × *intermedia*. (redrawn from Tsai *et al*. 2006b)

were used to clarify the phylogenetics and biogeography of the *P. violacea* complex. The sequences of the IGS of *atp*B-*rbc*L of plastid DNA among the accessions of *P. violacea* complex are identical. No substitution of the intron of *trn*L was found from this complex. In contrast, a hot spot region of insertion/deletion was found within the introns of *trn*L of plastid DNA among those accessions. However, this hot spot region cannot offer valuable information to discriminate these two species of the *P. violacea* complex. Two valuable polymorphic sites were found within ITS1 regions of nrDNA. Based on the phylogenetic tree inferred from ITS sequence, *P. bellina* cannot separate from accessions from *P. violacea* with the exception of the population distributed on Mentawai Is., Indonesia. Furthermore, based on the morphological characters, *P. violacea* distributed on Mentawai Is. is having a long and roundish rachis and separates from the other groups of the *P. violacea* complex described by Christenson (2001). Therefore, the results in this study have a trend to support the population of Mentawai Is. of the *P. violacea* complex as a separated species from *P. violacea*. Mentawai Is. are located nearby Sumatra, therefore Mentawai plants of this complex might be descended from those of Sumatra/Malay Peninsula.

DNA AS MOLECULAR MARKERS TO IDENTIFY NATURAL HYBRIDS

DNA markers from both nuclear and plastid DNAs have been used to study natural hybrids. Since chloroplast DNA of *Phalaenopsis* species has been proved as maternal inheritance (Chang *et al*. 2000). Tsai *et al*. (2006) examined the natural hybrid, *Phalaenopsis* × *intermedia* Lindl. In order to confirm the hybrid origin of this natural hybrid, ITSs of rDNA and three fragments of cpDNA were studied. Nineteen clones of ITS sequences from three accessions of *P.* × *intermedia* Lindl. were aligned with nine species of sections of *Phalaenopsis* and *Stauroglottis* that were candidate parents. A phylogenetic tree, derived from the ITS data, was constructed by the Neighbor-joining (NJ) method. Two major groups were shown for 19 clones of ITS sequences of *P.* × *intermedia* based on the phylogenetic tree (**Fig. 6**). The average genetic distance between the aforementioned two groups and the candidate parents was calculated based on the Kimura 2-parameter method. One group (Group A) had the lowest genetic distance from the ITS repeat sequences of *P. aphrodite* Rchb.f., and another group (Group B) had the lowest distance from that of *P. equestris* (Schauer) Rchb.f. The results showed that both *P. aphrodite* and *P. equestris* are parents of *P.* × *intermedia* based on the ITS data. In addition, analysis of three fragments of chloroplast DNA, namely the *trn*L intron, the *trn*L-*trn*F intergenic spacer (IGS), and the *atp*B-*rbc*L IGS, showed the phylogenetic tree (**Fig. 7**). Based on genetic distance, *P.* × *intermedia* had the lowest genetic distance from *P. aphrodite*. Both the ITS and cpDNA data, as well as reference to the effects of maternal inheritance of cpDNA suggest that *P. aphrodite* is the maternal parent and *P. equestris* is the paternal parent of *P.* × *intermedia*. Therefore, molecular evidence supports *Phalaenopsis* × *intermedia* being a natural hybrid derived from *P. aphrodite* and *P. equestris*.

FUTURE OUTLOOK

Molecular data, especially DNA markers, have been used tremendously to reconstruct phylogenetic relationships in plants during the last decade. More recently, such DNA data are now being applied to study *Phalaenopsis*. There are several topics for the phylogenetic relationship of *Phalaenopsis* should be focused in the future, e.g., *P. wilsonii* complex, *P. cornu-cervi* complex. Furthermore, reconstruc-

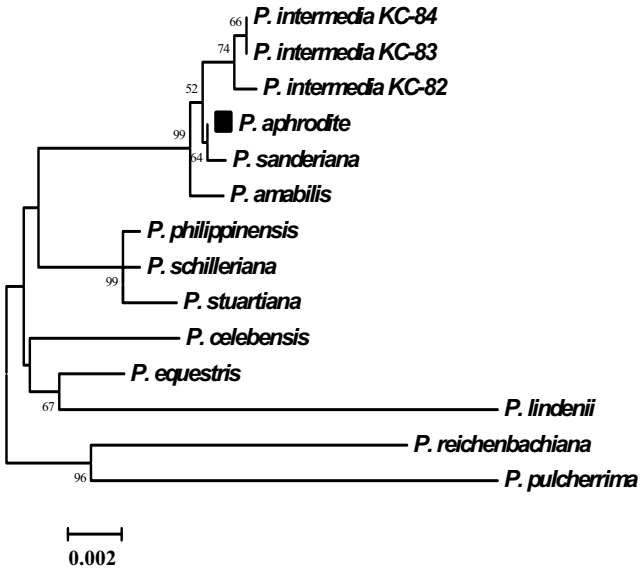

Fig. 7 Phylogenetic tree of three accessions of *Phalaenopsis* × *intermedia* (KC-82, KC-83 and KC-84), 11 species from the sections *Phalaenopsis* and *Stauroglottis,* and two groups (A and B) inferred from chloroplast DNA sequence data. Values of the interior branch test > 50% are shown on the branches. Branch lengths are proportional to the number of base changes along each branch. The scale bar indicates the genetic distance of Kimura (1980). The solid square (■) indicates most likely the maternal parent of *P.* × *intermedia.* (redrawn from Tsai *et al*. 2006b)

tion of phylogenetic relationships of *Phalaenopsis* plants will provide new concepts of the history and geography of *Phalaenopsis* lineages and offer insights into the processes of speciation, extinction, and migration. Molecular phylogeny also offers new concepts to help us to understand the evolution of morphological and physiological characters of *Phalaenopsis* taxa.

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