CHAPTER 1

Molecular Phylogeny and Biogeography of *Phalaenopsis* Species

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Christenson (2001) treated *Kingidium* and *Doritis* as synonyms of *Phalaenopsis*, and divided the latter into five subgenera, *Phalaenopsis*, *Polychilos*, *Parishianae*, *Proboscidioides* and *Aphyllae*. Molecular techniques are used to clarify the phylogeny of *Phalaenopsis*. Based on the internal transcribed spacer (ITS) of ribosomal DNA, *Kingidium* and *Doritis*, as well as *Polychilos* could be treated as synonyms of *Phalaenopsis*, as suggested by Christenson (2001). However, the phylogeny of the subgenera *Phalaenopsis*, *Polychilos* and *Aphyllae* was not supported by evidence in nature. According to the geographical distribution of the genus *Phalaenopsis*, the phylogenetic relationship of the genus *Phalaenopsis* is basically congruent with its biogeographies. In addition, there were two evolutionary trends of *Phalaenopsis* emanating from its center of origin to Southeast Asia. In one trend, the *Phalaenopsis* species dispersed and evolved from southern China to Southeast Asia, while in the other, the *Phalaenopsis* species dispersed and evolved from southern China to Southeast Asia. Furthermore, the molecular phylogenetics and biogeography of four species complexes, including *P. amabilis* complex, *P. sumatrana*, *P. violacea* complex, and *P. lueddemanniana* complex were also described based on molecular evidence.

1.1. Introduction

Genus *Phalaenopsis* Blume (Orchidaceae), a beautiful and popular orchid, comprises approximately 66 species, according to the latest classification (Table 1.1). Species of the genus *Phalaenopsis* are found

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Table 1.1 Taxon Names, Systematics and Geographical Distribution

Taxa and Systematic Classification[a]	Geographical Distribution
Subgenus *Proboscidioides* (Rolfe) E. A. Christ.	
Phalaenopsis lowii Rchb.f.	Myanmar, adjacent to western Thailand
Subgenus *Aphyllae* (Sweet) E. A. Christ.	
Phalaenopsis braceana (J. D. Hook.) E. A. Christ.	Bhutan and China at 1,100–1,700 m elevation
Phalaenopsis hainanensis Tang and Wang	China (Hainan, Yunnan)
Phalaenopsis honghenensis F. Y. Liu	China (Yunnan)
Phalaenopsis minus (Seidenf.) E. A. Christ.	Endemic to Thailand
Phalaenopsis taenialis (Lindl.) E. A. Christ. and U. C. Pradhan	Nepal, northeast India, Bhutan, Myanmar, and Thailand at 1,000–2,500 m elevation
Phalaenopsis wilsonii Rolfe	China (Szechuan, Yunnan) and eastern Tibet at 800–2,200 m elevation
Subgenus *Parishianae* (Sweet) E. A. Christ.	
Phalaenopsis appendiculata C. E. Carr	Endemic to Malaysia (Malay Peninsula)
Phalaenopsis gibbosa Sweet	Vietnam and Laos
Phalaenopsis lobbii (Rchb.f.) Sweet	India, Bhutan, Myanmar and Vietnam
Phalaenopsis parishii Rchb.f.	Eastern Himalayas, India, Myanmar and Thailand
Subgenus *Polychilos* (Breda) E. A. Christ.	
Section *Polychilos* (Breda) Rchb.f.	
Phalaenopsis borneensis Garay	Endemic to Borneo
Phalaenopsis cornu-cervi (Breda) Bl. and Rchb.f.	Northeast India and the Nicobar Islands to Java and Borneo from sea level to 800 m elevation
Phalaenopsis lamelligera Sweet	
Phalaenopsis mannii Rchb.f.	Northeast India, Nepal and China to Vietnam at 500–1,400 m elevation
Phalaenopsis pantherina Rchb.f.	Endemic to Borneo from sea level to 800 m elevation
Section *Fuscatae* Sweet	
Phalaenopsis cochlearis Holtt.	Malaysia (Malay Peninsula) and Indonesia (Sarawak) at 500–700 m elevation
Phalaenopsis fuscata Rchb.f.	Malaysia (Malay Peninsula), Borneo (West Koetai)

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Table 1.1 (*Continued*)

Taxa and Systematic Classification[a]	Geographical Distribution
Phalaenopsis kunstleri J. D. Hook.	Myanmar and Malay Peninsula
Phalaenopsis viridis J. J. Sm.	Endemic to Indonesia (Sumatra) at 1,000 m elevation
Section *Amboinenses* Sweet	
Phalaenopsis ×*gersenii* (Teijsm. and Binn.) Rolfe	Indonesia (Sumatra)
Phalaenopsis ×*singuliflora* J. J. Sm.	Western Borneo
Phalaenopsis amboinensis J. J. Sm.	Indonesia (Molucca Islands and Sulawesi)
Phalaenopsis bastianii Gruss and Rollke	Endemic to the Philippines
Phalaenopsis bellina (Rchb.f.) E. A. Christ.	Malaysia (Malay Peninsula) and East Malaysia (Sarawak)
Phalaenopsis doweryensis Garay and E. A. Christ.	East Malaysia, Sabah, without precise locality
Phalaenopsis fasciata Rchb.f.	Endemic to the Philippines (Luzon, Bohol and Mindanao)
Phalaenopsis fimbriata J. J. Sm.	Indonesia (Java, Sarawak and Sumatra)
Phalaenopsis floresensis Fowlie	Endemic to the island of Flores at 300–500 m
Phalaenopsis gigantea J. J. Sm.	Endemic to Sabah in East Malaysia and adjacent to Kalimantan Timur, from sea level to 400 m elevation
Phalaenopsis hieroglyphica (Rchb.f.) Sweet	Endemic to the Philippines
Phalaenopsis javanica J. J. Sm.	Endemic to Indonesia (Java)
Phalaenopsis lueddemanniana Rchb.f.	Endemic to the Philippines
Phalaenopsis maculata Rchb.f.	Malaysia (Pahang), East Malaysia (Sabah, Sarawak), Indonesia (Kalimantan Timur)
Phalaenopsis mariae Burb.ex Warn. and B. S. Wms.	Endemic to the Philippines and Indonesia (Kalimantan, Borneo)
Phalaenopsis micholitzii Rolfe	The Philippines (Mindanao)
Phalaenopsis modesta J. J. Sm.	Endemic to the island of Borneo in East Malaysia (Sabah) and Indonesia (Kalimantan)
Phalaenopsis pallens (Lindl.) Rchb.f.	Endemic to the Philippines
Phalaenopsis pulchra (Rchb.f.) Sweet	Endemic to the Philippines (Luzon, Leyte) at 100–650 m elevation
Phalaenopsis reichenbachiana Rchb.f. and Sander	Endemic to the Philippines
Phalaenopsis robinsonii J. J. Sm.	Endemic to Indonesia (Ambon)

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Table 1.1 (*Continued*)

Taxa and Systematic Classification[a]	Geographical Distribution
Phalaenopsis venosa Shim and Fowlie	Endemic to Indonesia (Sulawesi)
Phalaenopsis violacea Witte	Indonesia (Sumatra) and Malaysia (Malay Peninsula)
Section *Zebrinae* Pfitz.	
Phalaenopsis corningiana Rchb.f.	Borneo (Sarawak and elsewhere on the island)
Phalaenopsis inscriptiosinensis Fowlie	Endemic to Indonesia (Sumatra)
Phalaenopsis speciosa Rchb.f.	Endemic to India (Andaman and Nicobar Islands)
Phalaenopsis sumatrana Korth. and Rchb.f.	Widespread from Myanmar, Thailand, Vietnam, to Indonesia (Java, Sumatra), Malaysia (Perak, Johore), East Malaysia (Sabah), and the Philippines (Palawan)
Phalaenopsis tetraspis Rchb.f.	India (Andaman and Nicobar Islands) and Indonesia (Sumatra)
Phalaenopsis zebrina Witte	Borneo, the Philippines (Palawan)
Subgenus *Phalaenopsis* E. A. Christ.	
Section *Phalaenopsis* Benth.	
Phalaenopsis amabilis	Widespread from Sumatra and Java to the southern Philippines, and east to New Guinea and Queensland, Australia
Phalaenopsis aphrodite Rchb.f.	The Northern Philippines and southeastern Taiwan
Phalaenopsis intermedia Lindl.	The Philippines
Phalaenopsis philippinensis Golamco ex Fowlie and Tang	Endemic to the Philippines
Phalaenopsis sanderiana Rchb.f.	Endemic to the Philippines
Phalaenopsis schilleriana Rchb.f.	Endemic to the Philippines
Phalaenopsis stuartiana Rchb.f.	Endemic to the island of Mindanao in the southern Philippines
Phalaenopsis ×*amphitrite* Kraenzl.	Endemic to the Philippines
Phalaenopsis ×*leucorrhoda* Rchb.f.	Endemic to the southern Philippines
Phalaenopsis ×*veitchiana* Rchb.f.	Endemic to the Philippines
Section *Deliciosae* E. A. Christ.	
Phalaenopsis chibae Yukawa	Endemic to Vietnam at 400–600 m elevation
Phalaenopsis deliciosa Rchb.f.	Widespread from Sri Lanka and India to the Philippines and Sulawesi
Phalaenopsis mysorensis Saldanha	Endemic to southern India

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Table 1.1 (*Continued*)

Taxa and Systematic Classification[a]	Geographical Distribution
Section *Esmeralda* Rchb.f.	
Phalaenopsis buyssoniana Rchb.f.	Thailand and Indochina
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)
Phalaenopsis regnieriana Rchb.f.	Endemic to Thailand
Section *Stauroglottis* (Schauer) Benth.	
Phalaenopsis celebensis Sweet	Endemic to Indonesia (Sulawesi)
Phalaenopsis equestris (Schauer) Rchb.f.	The Philippines and Taiwan
Phalaenopsis lindenii Loher	Endemic to the Philippines

[a] The classification of *Phalaenopsis* based on Christenson (2001).

throughout tropical Asia, namely southern China, Indochina, India and Southeast Asia, and in Australia. The western distribution of *Phalaenopsis* is in Sri Lanka and southern 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). Traditonally, the systematics of *Phalaenopsis* is confused with those of both genera of *Kingidium* and *Doritis*. A systematics of genus *Phalaenopsis* introduced by Sweet (1980) still treated *Doritis* and *Kingidium* as separate genera from *Phalaenopsis*. According to the latest systematics of *Phalaenopsis* suggested by Christenson (2001), *Kingidium* and *Doritis* are treated as synonyms of *Phalaenopsis*, which is divided into five subgenera, namely *Aphyllae*, *Parishianae*, *Proboscidioides*, *Phalaenopsis* and *Polychilos*.

Different subgenera of *Phalaenopsis* have distinct geographic distributions. The 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). Furthermore, the 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). Furthermore, as we know, *Phalaenopsis* is only distributed in tropical Asia, namely southern China, Indochina, India, and Southeast Asia (including Malaysia, Indonesia, the Philippines, New Guinea, etc.) (Christenson, 2001). In these regions, Southeast Asia in particular, there have been numerous biogeographical studies on flora or fauna. Since Southeast Asia was created from the collisions between several oceanic plates (Pacific, Indian, Philippine) and land plates (Eurasian, Indian, Australian) (Hall, 1996), the flora and fauna of these regions are highly complicated and have characteristics shared between Asia and Australia. Several biogeographical boundaries have been introduced in these regions, such as the Wallace Line and Weber's Line, since deep straits limit the dispersal of flora and fauna (Van Oosterzee, 1997; Pianka, 1994).

1.2. Biogeographical Pattern of Genus *Phalaenopsis*

In the late 19th century, six major biogeographic regions in the world were recognized, namely Palaearctic, Nearctic, Neotropical, Ethiopian, Oriental, and Australian regions (Pianka, 1994) (Fig. 1.1). Lyell suggested that these six regions were separated based on dispersal barriers such as oceans and high mountain ranges (Van Oosterzee, 1997). In the Oriental region, there are several deep straits in Southeast Asia that separate the Oriental from the Australian region. On the other hand, the Himalayas divides the Oriental and the Palaearctic region (Pianka, 1994). All of the distribution regions of the genus *Phalaenopsis*, namely southern China, India, Indochina, Southeast Asia (Malaysia, Indonesia and the Philippines) (Sweet, 1980; Christenson, 2001) belong to the Oriental region. Southeast Asia was created from the collisions between several oceanic plates (Pacific, Indian, Philippine) and land plates (Eurasian, Indian, Australian). The process may already have started in the early Palaeozoic (about 400 Mya) (Hall, 1996). Furthermore, one widespread species of *Phalaenopsis*, *P. amabilis*, extended to the Australian region, namely New Guinea and northern Australia.

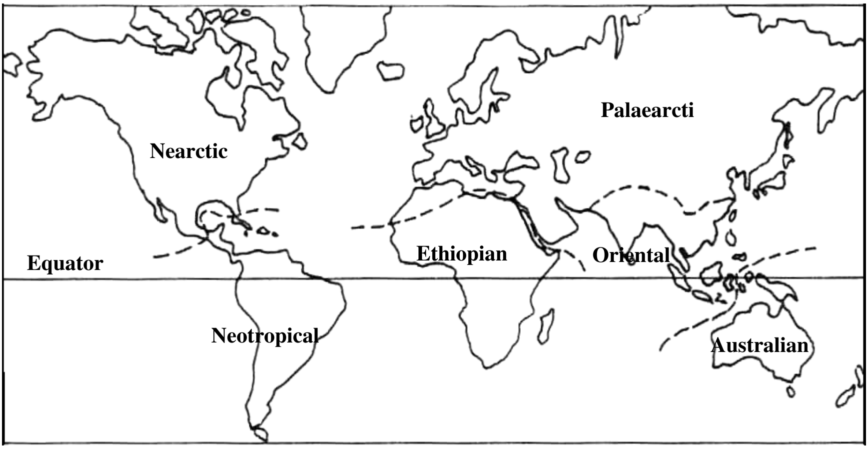

Fig. 1.1. The world's six major biogeographical regions. [Redrawn from Pianka (1994).]

Within the distributions of genus *Phalaenopsis*, there are ten different geographical regions, namely India, Sri Lanka, southern China, Indochina, the Malay Peninsula, Borneo, Sumatra, Java, Flores, Sulawesi, the Moluccan Islands, New Guinea, the Philippines, Taiwan, and Australia. According to the distribution matrix of the *Phalaenopsis* species (Tsai, 2003a), a biogeographical tree has been constructed based on the neighbor-joining method (Fig. 1.2). In that tree, New Guinea and Australia form a group. The second group includes Sulawesi and the Moluccas. The third includes Taiwan and the Philippines; the fourth, the Malay Peninsula, Borneo, Sumatra, Java and Flores; and the fifth, India and Indochina. Furthermore, southern china and Sri Lanka are in independent groups (Fig. 1.2). This scheme is in agreement with biogeographical regions of the world (Pianka, 1994), since the first group belonging to the Australian region is not nested within the groups of the Oriental region (Figs. 1.1 and 1.2). Furthermore, the group comprising the Malay Peninsula, Borneo, Sumatra and Java belonging to Sunda Shelf show the close relationship between one another based on the biogeographical tree. This is in agreement with the historical geology of these regions, which were interconnected during Pleistocene times (about 0.01∼1.8 Mya)

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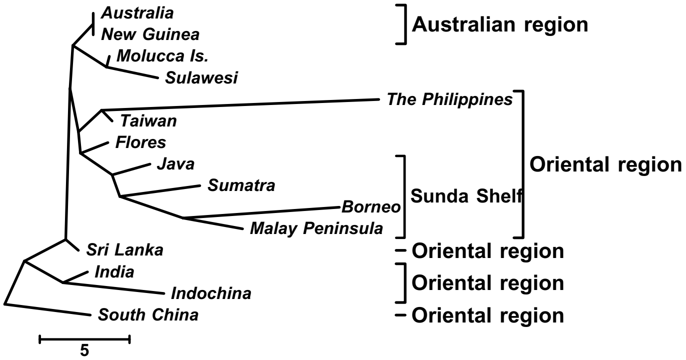

Fig. 1.2. Biogeographical tree of the genus *Phalaenopsis* constructed using the Neighbor-joining method.

(Van Oosterzee, 1997). This allowed easy species migration among these regions. In addition, the high divergence of species between Sulawesi and Borneo is also in agreement with the historical geology of both regions, as they were isolated from each other at least 14 Mya or 50 Mya by the Makassar Strait (Morley, 1998). Studies of the flora and fauna in both regions led to the introduction of the Wallace Line in the late 19th century (Van Oosterzee, 1997). However, the biogeography of the genus *Phalaenopsis* was not in agreement with the natural barriers of Weber's Line between Sulawesi and the Moluccan Islands, since the biogeographical relationship of the *Phalaenopsis* species distributed in both those regions were close (Fig. 1.2).

1.3. Molecular Phylogeny and Biogeography of the *Phalaenopsis* Species

1.3.1. *Phylogenetics and biogeography of the genus Phalaenopsis*

Based on the number of pollinia, traditional species of *Kingidium* (which have four pollinia) have been distinguished from the genus *Phalaenopsis* (which has two pollinia) (Sweet, 1980; Seidenfaden, 1988b). Christenson (2001) treated the traditional genus *Kingidium* as

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Phalaenopsis and split it into different parts of *Phalaenopsis*, placing some species into the subgenus *Aphyllae* (*P. braceana*, *P. minus* and *P. taenialis*) and others 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 separate genus, *Grafia*, by Hawkes [see Christenson (2001)]. This complex was first treated as the 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). A molecular phylogenetic tree of genus *Phalaenopsis* was reconstructed based on the nucleotide sequences of the ITS region of the nrDNA by Tsai *et al.* (2006a) (Fig. 1.3).

1.3.1.1. *Molecular phylogenetics of Phalaenopsis*

1.3.1.1.1. Monophyly of the genus *Phalaenopsis*

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

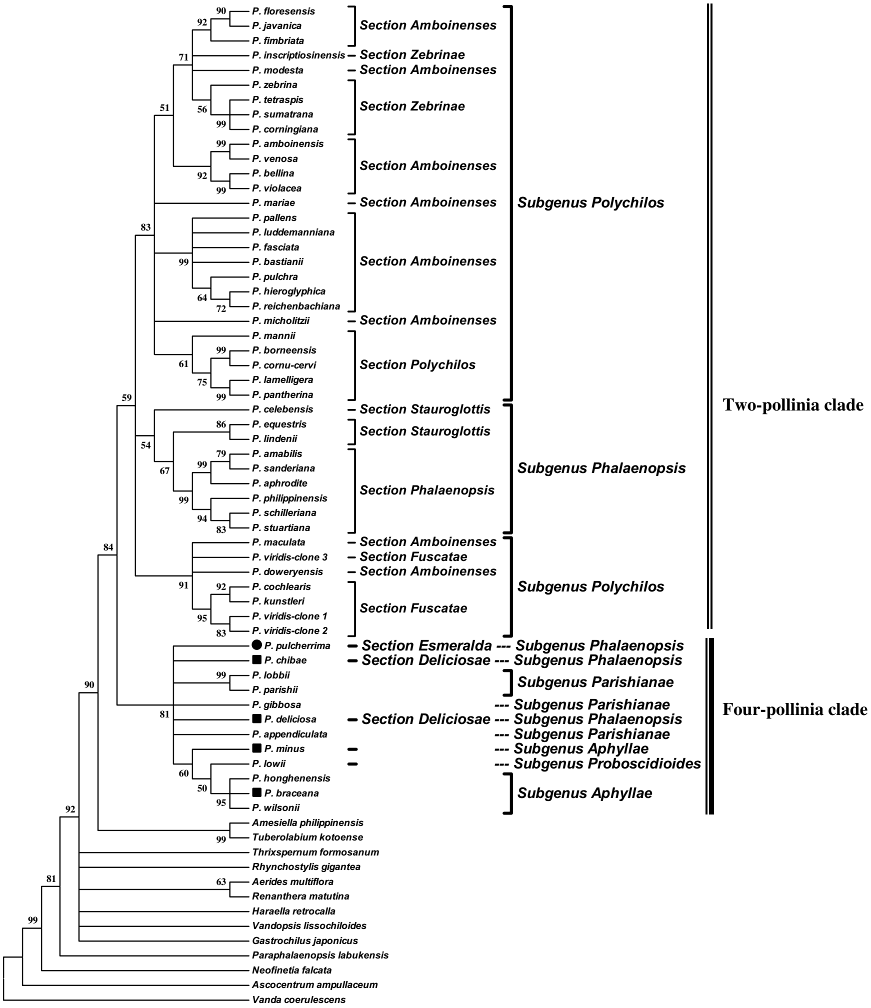

Fig. 1.3. The strict consensus parsimonious tree of 53 *Phalaenopsis* species plus 13 outgroups obtained from sequence comparisons of the ITS region of rDNA. Bootstrap values greater than 50% are shown on each branch. A solid circle (•) on the tree indicates that this species was traditionally treated as the genus *Doritis*. Solid squares (■) on the tree indicate that these species were traditionally treated as the genus *Kingidium*. [Redrawn from Tsai *et al.* (2006).]

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Aphyllae, as well as having 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.

1.3.1.1.2. Infrageneric relationships within *Phalaenopsis*

(i) 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 the 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 (Table 1.1) (Sweet, 1980; Christenson, 2001). This four-pollinia clade, namely subgenera *Aphyllae* and *Parishianae*, plus the sections *Esmeralda* and *Deliciosae* of the subgenus *Phalaenopsis*, is separate from the two-pollinia clade, namely the subgenus *Polychilos* and sections *Phalaenopsis* and *Stauroglottis* of the subgenus *Phalaenopsis*, based on phylogenetic tree derived from ITS data.

Within the four-pollinia clade, neither subgenus *Aphyllae* nor *Parishianae* were a monophyletic group based on the ITS sequences. Furthermore, the monotypic subgenus *Proboscidioides*, namely *P. lowii*, had a close relationship to the subgenus *Aphyllae*. Therefore, the unique characteristics 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.

(ii) Subgenus *Phalaenopsis*

The monophyly of the subgenus *Phalaenopsis* was not supported based on ITS sequences. Excluding sections *Deliciosae* and *Emeralda* of the subgenus *Phalaenopsis*, the rest of the 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 characteristics sharing similar geographic ranges, small chromosome sizes, and flowers lacking transversely barred patterns (Christenson, 2001), RAPD analyses (Chen *et al.*, 1995), and the intergenic spaces (IGS) sequence of 5S rDNA (Kao, 2001).

Within subgenus *Phalaenopsis*, the monophyly of the section *Phalaenopsis* was supported in this 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 IGS of 5S nrDNA (Kao, 2001), the monophyly of the section *Phalaenopsis* was supported as well. The monophyly of the 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 also not shown to be monophyletic based on ITS sequences.

(iii) Subgenus *Polychilos*

This large subgenus is subdivided into the four sections *Polychilos*, *Fuscatae*, *Amboinenses* and *Zebrinae* based on the systematics of Christenson (2001). The monophyly of the 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. This finding is in agreement with the morphological characteristics 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 the 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*.

1.3.1.2. *Biogeography*

According to the evolutionary trend of the pollinia number (Holttum, 1959; Dressler, 1993), the four-pollinia clade was suggested as the basal clade of the genus *Phalaenopsis*. The four-pollinium clade of *Phalaenopsis* developed in southern China and Indochina and then dispersed into Indonesia, Malaysia and the Philippines. Thereafter, the two-pollinia clade developed. Within the two-pollinia clade, two major groups were shown, 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 trends of subgenus *Phalaenopsis* sections *Phalaenopsis* and *Stauroglottis* and subgenus *Polychilos* might be different.

During Pleistocene times (about 0.01∼1.8 Mya), and when sea levels were low, the Malay Peninsula, Borneo, Sumatra, Java, Bali, and various parts of the Philippines would have been interconnected. For example, the sea level was approximately 120 meters below the current level in the Last Glacial Maximum (Hanebuth *et al.*, 2000). 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 southern China and Indochina to Indonesia and Malaysia, using the Malay Peninsula as a stepping stone, from which the subgenus *Polychilos* developed. In addition, historical geology suggests that most of the Philippine islands are young (<5 Mya) with the exception of Palawan, Mindoro, Zamboanga and parts of the western Philippines (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). Therefore, the *Phalaenopsis* species might have dispersed from southern 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. 1.4).

1.3.2. *Phylogenetics and biogeography of the Phalaenopsis amabilis complex*

Tsai (2003b) examined the *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 and ITS2) region of nuclear ribosomal DNA (nrDNA) was applied to reconstruct the phylogeny of this complex. Rooted at outgroups, the 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 the exceptions of Palawan and Borneo populations plus the 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 the *P. amabilis* complex. In addition, accessions of *P. sanderiana* were nested within accessions of both *P. amabilis* and its subspecies.

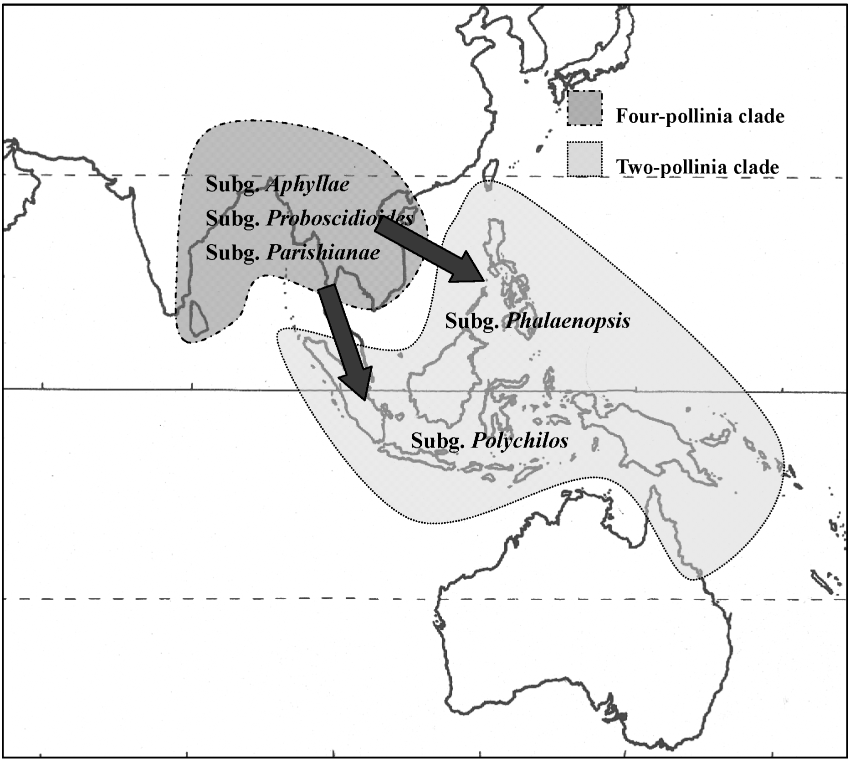

Fig. 1.4. The evolutionary trends of the genus *Phalaenopsis* obtained from this study plotted on a map of the geographical distribution of this genus.

This result does not support *P. sanderiana* being treated as a separate species from *P. amabilis*. According to the phylogenetic tree derived from the 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* descended from *P. amabilis* (or their most recent common ancestor) were suggested. 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*

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subsp. *rosenstromii* respectively developed. *P. amabilis* subsp. *rosenstromii* further dispersed into northern Australia and Timor. Second, *P. amabilis* distributed in Palawan dispersed into Borneo, thereafter further dispersing into the Mentawai Islands. Third, the Palawan population of *P. amabilis* dispersed into other islands of the Philippines and evolved into *P. aphrodite*. *P. aphrodite* is distributed throughout the Philippines with the exceptions of Palawan and southern Mindanao.

1.3.3. *Phylogenetics and biogeography of the Phalaenopsis sumatrana complex*

The *Phalaenopsis sumatrana* complex includes *P. sumatrana* and *P. corningiana* plus one questionable species, *P. zebrina*. Of these, *P. sumatrana* is widely distributed in Sumatra, Java, Borneo, the Mentawei Islands, Malaysia, Perak, Johor and Thailand. *P. corningiana* and *P. zebrina* are restricted to Borneo and Sarawak and to Borneo and Palawan, respectively (Sweet, 1980; Masaaki, 2002). Based on their morphology, the characteristics of these three species of this complex are not easily differentiated, even recently. *P. zebrina* was treated as a synonym of *P. sumatrana* by Sweet (1968). Fowlie (1982) attempted to raise plants of the *P. sumatrana* complex with a white ground color into a separate species, *P. zebrina*, from plants of *P. sumatrana* with a yellowish ground color. Christenson (2001), however, disagreed with Fowlie's separation based on the treatment being contrary to the type specimen of *P. zebrina* with a yellowish ground color. Therefore, *P. zebrina* is still retained as a synonym of *P. sumatrana*. Nevertheless, *P. zebrina* is still used in horticulture to represent plants with narrower brown markings on a white or yellowish ground color (Masaaki, 2002). Furthermore, Sweet (1968), Fowlie (1969), and Christenson (2001) are in agreement that *P. sumatrana* and *P. corningiana* both have species-level status, are separate from each other, and are treated as two separate species.

The molecular phylogenetics of this complex was examined based on the ITS of nrDNA and the IGS of *atp*B-*rbc*L of chloroplast DNA by Tsai *et al.* (2009) as described in the following section.

1.3.3.1. *Molecular phylogenetics*

P. sumatrana and *P. corningiana* cannot be separated from each other from the molecular phylogenetic tree derived from ITS sequences (Fig. 1.5). These results do not support *P. corningiana* and *P. sumatrana* being treated as two separate species, as described by Sweet (1968), Fowlie (1969) and Christenson (2001). However, the characteristics of the callus morphology and marking pattern on the petals could distinguish between *P. sumatrana* and *P. corningiana*, as described by Sweet (1980) and Christenson (2001). Accessions of *P. zebrina* in this complex can be separated from both *P. sumatrana* and *P. corningiana* based on the ITS sequences of nrDNA. In analyses of sequences of IGS of *atp*B-*rbc*L, neither species of this complex could be separated from the other. Therefore, the molecular data of this study only support *P. zebrina* being separated from both *P. corningiana* and *P. sumatrana*. This result supports the treatment of *P. zebrina* described by Fowlie

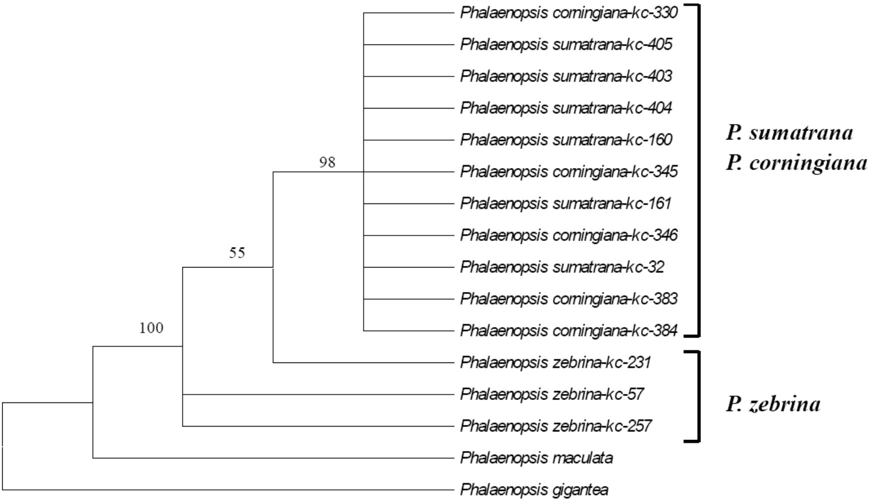

Fig. 1.5. The MP tree of the 14 accessions from the *Phalaenopsis sumatrana* complex plus the outgroups *P. maculata* and *P. gigantea*, obtained from sequence comparisons of the ITS region of nrDNA. The numbers above the internodes indicate the values of the bootstrap test from 1,000 replicates. Bootstrap values greater than 50% are shown. [Redrawn from Tsai *et al.* (2009).]

(1982). However, the floral ground color of *P. zebrina* is not a characteristic of this species, as described by Fowlie (1982). Inspection of the plants of *P. zebrina* revealed that both white and yellowish ground colors in the flower of this species were apparent, as described by Masaaki (2002). Furthermore, all sepals and petals of these three species are retained after pollination (Christenson, 2001). We found that the marking pattern of sepals and petals in both *P. zebrina* and *P. sumatrana* was partly retained after pollination, but not in that of *P. corningiana*. In short, plants of *P. zebrina* could be separated from both *P. sumatrana* and *P. corningiana* based on floral fragrances and molecular data. Therefore, it is not appropriate to treat *P. zebrina* as a synonym of *P. sumatrana*, as described by Sweet (1968, 1980) and Christenson (2001).

1.3.3.2. *Biogeography and evolutionary trends*

The distributions of these three species of the species complex overlap in Borneo. This creates a much greater chance for the three species to hybridize in their natural environment; which makes it difficult to discriminate among the three species. In fact, some naturally hybridized plants between species of *P. sumatrana* and *P. corningiana* have been collected (Christenson, 2001). Apparently, the three species share a common ancestor. Using the phylogenetic tree inferred from the ITS sequences of nrDNA, the evolutionary trend of the *P. sumatrana* complex was deduced. Since accessions of *P. zebrina* were located as the basal group within the *P. sumatrana* complex, *P. zebrina* was suggested to be the relative origin group of the *P. sumatrana* complex. Based on the evolutionary trend derived from molecular data, the dispersal pathway of the *P. sumatrana* complex was deduced and is shown in Fig. 1.6.

P. zebrina developed in Borneo and dispersed into Palawan, the Philippines. *P. corningiana* and *P. sumatrana* might have evolved from *P. zebrina* in Borneo. Thereafter, *P. sumatrana* dispersed into Sumatra, the Malay Peninsula and the Andaman Islands via land bridges in glacial times and it has been shown to be a widespread species. Land bridges between Sumatra, the Malay Peninsula and Borneo could have been formed, since Borneo, Sumatra, the Malay Peninsula and Java

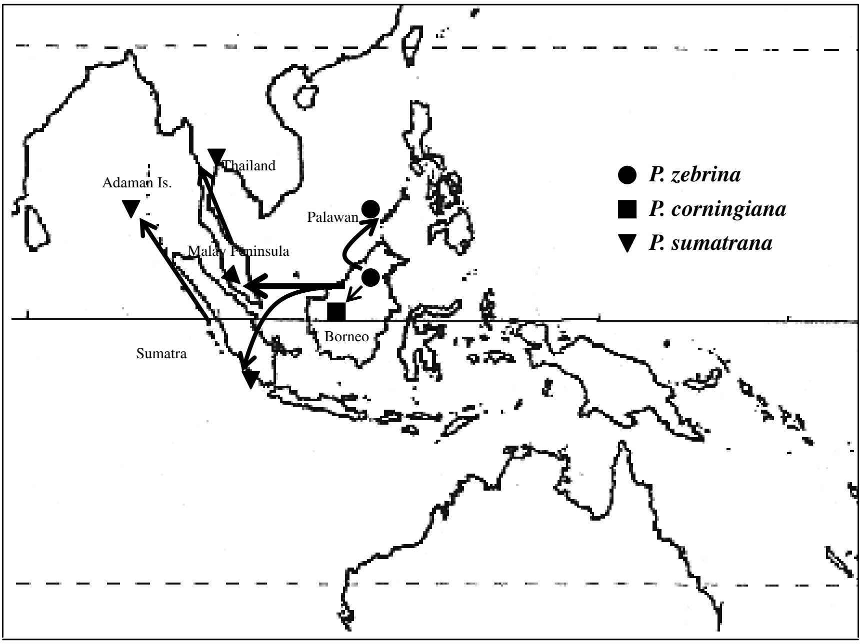

Fig. 1.6. The evolutionary trend of the *P. sumatrana* complex based on the phylogenetic tree. [Redrawn from Tsai *et al.* (2009).]

comprised the Sunda Shelf (Van Oosterzee, 1997). However, to date *P. sumatrana* has not been found in Java. This result is in agreement with the biogeography of the genus *Phalaenopsis*, with *Phalaenopsis* species found in Java differing from those of other lands of the Sunda Shelf (Christenson, 2001; Tsai, 2003a). Furthermore, evidence suggests that the Andaman Islands might have been connected to Sumatra in ancient times.

1.3.4. *Phylogenetics and biogeography of the Phalaenopsis violacea complex*

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* have been found according to different distributions, including Sumatra, the Malay Peninsula and the Mentawai Islands.

The *P. violacea* species complex was examined by Tsai (2003c) using molecular data. The phylogenetic trees inferred from the ITS region of nuclear ribosomal DNA (nrDNA), the *trn*L intron, and IGS of *atp*B-*rbc*L of plastid DNA, 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 the *P. violacea* complex are identical. No substitution of the *trn*L intron was found from this complex. In contrast, a hot spot region of insertion/deletion was found within the *trn*L introns 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 the ITS1 region of nrDNA. Based on the phylogenetic tree inferred from ITS sequences, *P. bellina* cannot be separated from accessions of *P. violacea* with the exception of the population distributed on the Mentawai Islands, Indonesia. Furthermore, in terms of morphological characteristics, the *P. violacea* distributed on the Mentawai Islands has a long and roundish rachis and has been separated from the other groups of the *P. violacea* complex described by Christenson (2001). Hence, the results of this study tend to support the population on the Mentawai Islands of the *P. violacea* complex being a separate species from *P. violacea*. The Mentawai Islands are located near Sumatra, so Mentawai plants of this complex might be descended from those of Sumatra and the Malay Peninsula.

1.3.5. *Phylogenetics, biogeography and evolutionary trends of the Phalaenopsis lueddemanniana complex*

The *Phalaenopsis lueddemanniana* species complex includes *P. bastianii*, *P. pallens*, *P. hieroglyphica*, *P. reichenbachiana*, *P. lueddemanniana*, *P. fasciata*, *P. pulchra* and *P. mariae*. This complex as well as others of the section *Amboinenses* was examined using ITS sequences and chloroplast DNA by Tsai (2003a). Those findings are in agreement with the treatment of Sweet (1980), which differentiated this complex into seven species. It also showed that *P. mariae* is a

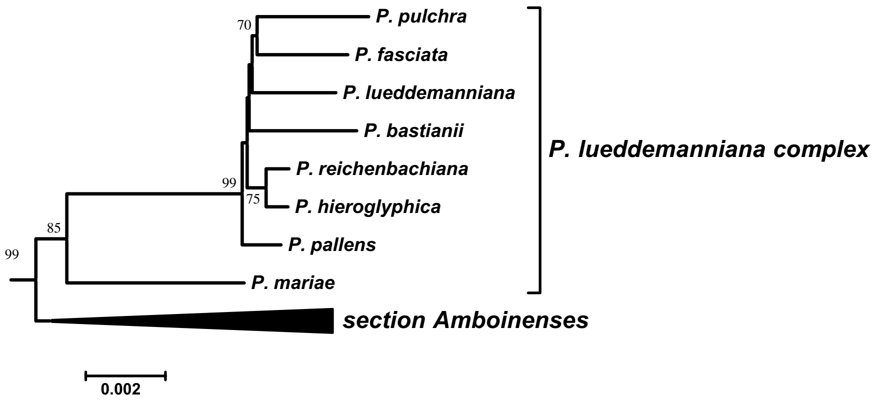

Fig. 1.7. The evolutionary phylogenetic subtree of both the section *Amboinenses* and the *P. lueddemanniana* complex inferred from combined data of the ITS sequences of nrDNA and chloroplast DNA data constructed by the minimum-evolution method [Redrawn from Tsai (2003a)]. Species of the section *Amboinenses* are compressed and shown in a bold branch.

basal species of the *P. lueddemanniana* complex (Fig. 1.7). With the exceptions of the *P. lueddemanniana* complex and *P. micholitzii* (distributed in Mindanao), no species of the section *Amboinenses* has been found in the Philippines. This suggests that the species of the *P. lueddemanniana* complex in the Philippines descended from species of the section *Amboinenses* distributed in Borneo and developed into a unique lineage (Tsai, 2003a) (Fig. 1.8).

Until 5 ~ 10 Mya, the crust of an 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. lueddemanniana* complex according to the combined data of the nrITS and plastid DNA, *P. mariae* has been suggested as a relative origin species of the *P. lueddemanniana* complex. This species dispersed from Borneo into the Philippines, and thereafter, the *P. lueddemanniana* complex

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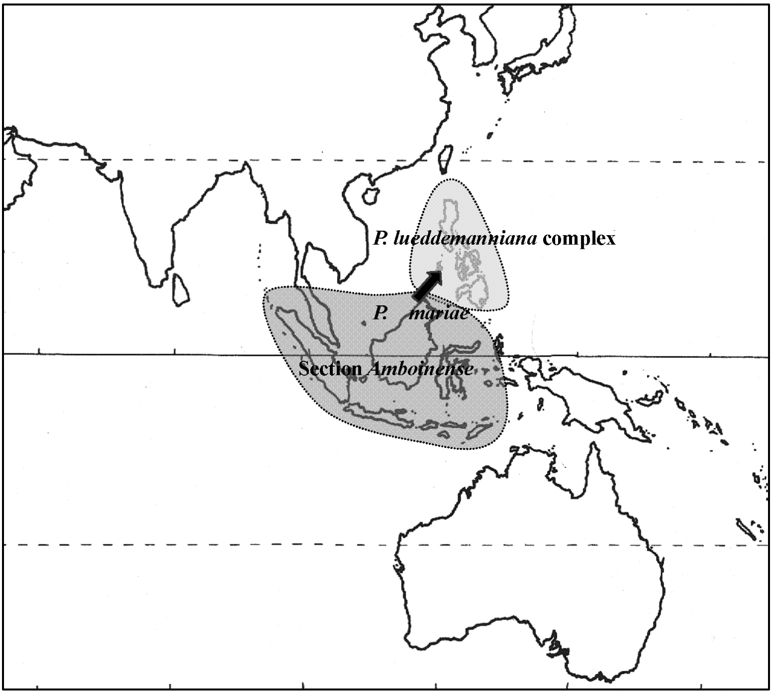

Fig. 1.8. The evolutionary trend of the *P. lueddemanniana* complex as suggested by historical geology and molecular DNA.

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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