國立中山大學 生物科學系

博士論文

蝴蝶蘭屬植物之分子親緣、生物地理及演化趨勢之研究

Molecular phylogeny, biogeography, and evolutionary trends of the genus *Phalaenopsis* (Orchidaceae)

研究生：蔡奇助 撰

指導教授：周昌弘 博士

中華民國 九十二 年 十二 月

Molecular phylogeny, biogeography, and evolutionary trends of the genus *Phalaenopsis* (Orchidaceae)

by

Chi-Chu Tsai

A Dissertation Presented to National Sun Yat-sen University in Partial Fulfillment of the Requirements for the Degree of

Ph. D. in Biological Sciences

Dissertation Committee

Dr. Zin-Huang Liu, Chairperson
Dr. Tzen-Yuh Chiang
Dr. Chang-Hung Chou
Dr. Hao-Jen Huang
Dr. Yuen-Po Yang

國立中山大學研究生學位論文審定書

本校生物科學系博士班

研究生蔡奇助 (學號：8921803) 所提論文

蝴蝶蘭屬植物之分子親緣、生物地理及演化趨勢之研究
Molecular Phylogeny, Biogeography and Evolutionary Trends of *Phalaenopsis* (Orchidaceae)

經本委員會審查並舉行口試，符合博士學位論文標準。

學位考試委員簽章：

(召集人) 孫景煌

蔣鎮宇　　周昌弘

楊遠波　　黃浩仁

指導教授 周昌弘

系主任/所長 楊遠波

Molecular phylogeny, biogeography, and evolutionary trends of the genus *Phalaenopsis* (Orchidaceae)

Chi-Chu Tsai, Doctor of Philosophy
National Sun Yat-sen University, 2003

Dissertation directed by:

Dr. Chang-Hung Chou

Proffessor

Department of Biological Sciences

National Sun Yat-sen University

Kaohsiung, Taiwan

December, 2003

誌　　謝

凡走過，必留痕跡。回想起博士班生涯的點點滴滴，感激之心油然而生。周遭的親友、師長、同事、實驗室伙伴及好友們的鼓勵與支持，讓我能盡我最大的努力去完成我的論文，在此由衷的感謝他們。

承蒙恩師　周昌弘教授不嫌棄我的資質平庸，仍於百忙之中給我諸多研究觀念的指導與為人處事的諄諄教誨，翩翩的學者風範與治學態度令我印象深刻。啟發式的指導方式，讓我能培養出獨立思考及研究的能力，我深深慶幸能拜於周教授的門下。論文研究期間，承蒙劉景煌教授、楊遠波教授、蔣鎮宇教授及黃浩仁教授百忙中撥冗指導我的研究方向，並於論文初稿完成時給予細心的審閱，以及提供許多寶貴的意見，讓我的論文能更加充實。此外，也要感謝帶我與植物結緣的陳擎霞教授，大學時代跟隨陳教授深入原始林山區，開啟我對植物領域的好奇之心，並從中培養觀察植物與自然對話的能力。卓忠隆教授在分生實驗技術上亦給我諸多的寶貴意見，讓我減少許多摸索的時間。

在論文的研究階段，承蒙高雄區農業改良場林前場長富雄博士及邱運全課長的鼓勵與支持，以及研究室伙伴黃柄龍先生、黃文理博士、勤珍姊、小么、文璘、炫達、育彥、家棋、素銘、幸姿、屏合、藝芸及惠榕等熱心的幫忙，尤以小么、文璘及家棋在實驗上給我的協助最多。此外，中山大學周教授實驗室的伙伴紀伶、慧雅、姿秀、英美、玉燕及碩士班研究生國洋、承裕、子媛及瑞麟在各方面的幫忙。撰寫論文期間，承蒙許秋容博士提供部分研究文獻、成大蔣鎮宇教授的研究生志宏傳授 r8s 程式的操作流程。另外，同門師兄李宗徽博士、江友中博士及陳志輝先生，以及美國進修博士的綠野社友人王旭坤先生在生活及研究上也我諸多的鼓勵與協助。此外，農試所花卉研究中心黃勝忠博士及台中科博館陳志雄博士亦提供我諸多的寶貴意見。中研院實驗室的伙伴曾梅珠小姐亦幫忙我找許多的研究資料。林務局屏東林管處廖政麒先生在這段日子裡亦不時給我鼓勵。另外，劉厚志醫師、黃明宏先生、劉耕良先生、陳谷仲先生、廖世華先生、阿諾等蘭友亦傳授我不少關於蝴蝶蘭的知識，開啟我對蝴蝶蘭愛好之心。在論文口試期間，感謝中山大學生科系系秘書雅惠姊協助安排相關的事宜，使口試得以順利進行，特此一併申謝。另外，也要感謝高雄縣五甲龍成宮提供優良的讀書環境，讓我能在極佳的環境下撰寫我的論文。千言萬語，要感謝的人太多，最後要感謝一路陪我渡過博士班研究生涯的老婆-淑芬及我的父母。博士班研究生涯的結束，代表另一個研究生涯的開始，我會自我砥礪、孜孜不倦，好好將所學發揮，以不辜負這麼多曾經幫助過我的人。

謹以本論文獻給愛我、體諒我的老婆淑芬，以及關心我、愛護我的父母親及家人，謝謝您們。

摘　　要

蝴蝶蘭屬植物之分子親緣、生物地理及演化趨勢之研究

Molecular Phylogeny, Biogeography, and Evolutionary Trends of the genus *Phalaenopsis* (Orchidaceae)

蔡奇助
國立中山大學 生物科學系哲學博士
指導教授 周昌弘博士

蝴蝶蘭屬 (*Phalaenopsis*) 植物是蘭科植物的成員之一，主要分佈於南中國 (South China)、印度中國 (Indochina)、印度 (India)、東南亞 (Southeast Asia) 及澳洲等熱帶亞洲地區。根據最新的分類資料顯示，本屬約有 66 種原生種。這些植物也都極具有觀賞價值，目前已有成千上萬的雜交品種經由人工的方法被培育出來。雖然蝴蝶蘭屬植物是那樣的漂亮且受歡迎，但是蝴蝶蘭親緣關係方面的研究卻相當缺乏。

Christenson (2001) 最近將朵麗蘭屬 (*Doritis*) 及金氏蝶蘭屬 (*Kingidium*) 處理為蝴蝶蘭屬的同物異名 (synonym)，並將所有的成員分成五個亞屬 (subgenus)，分別為 *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos* 及 *Phalaenopsis*。本研究擬以分子的證據來進一步闡釋由 Christenson (2001) 所修訂的蝴蝶蘭屬植物的系統分類及親緣關係，並進一步探討蝴蝶蘭屬植物的演化趨勢。首先藉由分析核基因組 (nuclear genome) 的核糖體核酸 (ribosomal DNA, rDNA) 內轉錄間隔區 (internal transcribed spacer, ITS) 及葉綠體基因組 *trn*L 的 intron, *trn*L-*trn*F 的基因間隔區 (intergenic spacer, IGS) 及 *atp*B-*rbc*L 的基因間隔區，來探討現今尚存活的大部分蝴蝶蘭屬植物的親緣關係及演化趨勢。在親緣關係的研究方面，結果支持 Christenson (2001) 將朵麗蘭屬 (*Doritis*) 及金氏蝶蘭屬 (*Kingidium*) 處理為蝴蝶蘭屬的同物異名 (synonym)的觀點；然而在屬內的五個亞屬中，僅有 *Parishianae* 亞屬為單支系類群 (monophyletic group)。並且也發現同為具有四個花粉塊 (pollinia) 的分類群具有相近的親緣關係 (phylogenetic

relationship)，這些類群包含 *Proboscidioides, Aphyllae, Parishianae* 及蝴蝶蘭亞屬內的兩個節，分別為 *Deliciosae* 及 *Esmeralda*。這一群具有四個花粉塊的蝴蝶蘭植物亦有地理分佈相近的共同點，他們主要分佈於南中國、印度中國及印度等地區；有別於分佈於印尼、菲律賓及馬來西亞等地具有兩個花粉塊的蝴蝶蘭類群。在整個蝴蝶蘭屬植物的演化趨勢方面，藉由核基因組及葉綠體基因組的分子證據, 配合花粉塊的演化趨勢推估分佈於南中國喜馬拉雅山山區的 *Aphyllae* 亞屬這一群植物為蝴蝶蘭屬植物的起源類群, 向印度中國及印度發展出其它具有四個花粉塊的類群。再經由兩個途徑往東南亞分佈及演進，發展出具有二個花粉塊的蝴蝶蘭類群，其一是經由印度中國到達菲律賓的古老島嶼發展出 *Phalaenopsis* 亞屬；另一是經由馬來半島向婆羅州分佈及演進，發展出 *Polychilos* 亞屬。此外，由分子資料及地質歷史的訊息可以估算 DNA 序列的取代速率 (substitution rate)，結果顯示蝴蝶蘭 ITS 及葉綠體 DNA 序列每年每個位置的取代速率 (substitutions/site/year) 分別為 $2.4\sim4.7 \times 10^{-9}$ 及 $3.9\sim7.8 \times 10^{-10}$。依上述 DNA 取代速率可以估算蝴蝶蘭其它類群的分離時間，初步估算分佈於菲律賓的 *P. lueddemanniana* 複合種群的形成約在 Pleistocene 期間；而 *Deliciosae* 節與 *Stauroglottis* 節的分離時間約在 21~10.5 百萬年前 (Mya)。

另外，也利用分子證據來探討蝴蝶蘭屬內三個複合種群之分子親緣、生物地理及演化趨勢。首先針對 *P. amabilis* 複合種群 (*P. amabilis* complex)進行分析，其成員計有 *P. amabilis, P. amabilis* subsp. *moluccana, P. amabilis* subsp. *rosenstromii, P. aphrodite, P. aphrodite* subsp. *formosana* 及 *P. sanderiana*，這些成員在分類上界定不易，因此常會因觀點不同而有新的修正。本研究藉由分析核基因組的核糖體核酸內轉錄間隔區 (ITS) 來探討此一相近分類群的分子親緣、生物地理及其演化趨勢。結果顯示，除了 *P. aphrodite* 及 *P. aphrodite* subsp. *formosana* 無法明顯區分外，其它上述的分類群皆可以加以區分，並且顯示不同地區的 *P. amabilis* 族群已有分化，僅有分佈於菲律賓巴拉望及印尼沙巴地區的族群無法明顯區分。分子證據也顯示，目前被處理為物種階級的 *P. sanderiana* 無

法與 *P. amabilis* 的不同族群加以區隔，因此並不支持將 *P. sanderiana* 處理為獨立的物種。在 *P. amabilis* 複合種群的演化趨勢方面，經由蝴蝶蘭屬的親緣關係樹得知，同樣分佈於菲律賓群島的 *P. schilleriana* 及其相近類群與 *P. amabilis* 類群具有共同的祖先，因此以 *P. schilleriana* 的類群為外群，可以推估出 *P. amabilis* 類群的起源類群為 *P. aphrodite*。*P. aphrodite* 向北有一分支推進至台灣南部，目前處理為 *P. aphrodite* subsp. *formosana*；有兩個途徑向南推演，其一是經由菲律賓巴拉望 (Palawan)，演化出 *P. amabilis*，再向婆羅州及蘇門達臘推進；另外經由菲律賓名達那峨 (Mindanao)，演化出 *P. sanderiana*，再往新幾內亞或蘇拉威西演化出 *P. amabilis* subsp. *rosenstromii* 或 *P. amabilis* subsp. *moluccana*，其中 *P. amabilis* subsp. *rosenstromii* 再往澳洲北部推進。

其次探討 *P. sumatrana* 的複合種群，這一群包含 *P. sumatrana* 及 *P. corningiana*，及一群已被處理為 *P. sumatrana* 的同物異名之物種 *P. zebrina*。藉由分析核基因組的核糖體核酸內轉錄間隔區 (ITS)、及葉綠體基因組 *trn*L 的 intron, *trn*L-*trn*F 的基因間隔區及 *atp*B-*rbc*L 的基因間隔區來探討此類群的親緣關係及演化趨勢。葉綠體的資料顯示，此類群的物種無法明顯的加以區分；在核基因組 (ITS) 的證據顯示, *P. sumatrana* 及 *P. corningiana* 依然無法區分, 但 *P. zebrina* 可以與上述兩物種加以區分。因此，由分子證據建議將這一群稱為 *P. zebrina* 的植物獨立出來。在 *P. violacea* 複合種群的演化趨勢方面，經由親緣關係樹，可以推估出 *P. sumatrana* 複合種群的起源類群可能為 *P. zebrina*, 此物種在婆羅州演化出來，並演變出 *P. corningiana* 及 *P. sumatrana* 二個物種，其中 *P. sumatrana* 藉由冰河所形成的陸橋，漸漸擴展至蘇門達臘、馬來半島及巴拉望等。

最後研究 *P. violacea* 的複合種群，這一群包含 *P. violacea* 及 *P. bellina* 二個物種。其中 *P. violacea* 又有分為兩個型(馬來亞型及蘇門達臘型)。本研究針對核基因組的核糖體核酸內轉錄間隔區 (ITS) 及葉綠體基因組 *trn*L 的 intron, *trn*L-*trn*F 的基因間隔區及 *atp*B-*rbc*L 的基因間隔區來探討此類群的親緣關係及演化趨勢。葉綠體的資料顯示，此類群的物種無法明顯的加以區分；在核基因組

(ITS) 的證據顯示，*P. bellina* 及 *P. violacea* 的兩個型亦無法加以區分，僅有分佈於印尼蘇門達臘西南的小島-蒙達威 (Mentawai Is.) 的 *P. violacea* 與其它物種或族群可以加以區分。由分子證據建議，產於蒙達威的 *P. violacea* 可以處理為另一物種。以目前的證據並無法推測此複合種群的起始類群為何？但可以初步推測產於蒙達威的 *P. violacea* 植物為較近期演變出來的類群。

Abstract

Molecular Phylogeny, Biogeography, and Evolutionary Trends of the genus *Phalaenopsis* (Orchidaceae)

by
Chi-Chu Tsai
Doctor of Philosophy in Biological Sciences
National Sun Yat-sen University
Professor Chang-Hung Chou

Species of *Phalaenopsis* Blume (Orchidaceae) are found throughout tropical Asia, namely South China, Indochina, India, Southeast Asia, and Australia. This genus is comprised of approximately 66 species according to the latest classification. Most of them possess commercial value. Thousands of *Phalaenopsis* cultivars have been grown for commercial goals. Although this orchid is very beautiful and popular throughout the world, studies on the molecular systematics and phylogenetic relationships among these orchids are still deficient.

Phylogenetic trees inferred from the internal transcribed spacers 1 and 2 (ITS1+ITS2) region of nuclear ribosomal DNA (nrDNA) and chloroplast DNAs (cpDNAs), including the intron of *trn*L, the IGS of *trn*L-*trn*F, and the IGS of *atp*B-*rbc*L, were used to clarify the phylogenetics and evolutionary trends of the genus *Phalaenopsis* (Orchidaceae). Molecular data are provided to clarify the latest systematics of the genus *Phalaenopsis* as suggested by Christenson (2001). He treated the genera of *Doritis* and *Kingidium* as synonyms of the genus *Phalaenopsis* and divided it into the five subgenera of *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos*, and *Phalaenopsis*. The results concurred that the genera *Doritis* and *Kingidium* should be treated as synonyms of the genus *Phalaenopsis* as suggested by Christenson (2001). The subgenera of *Aphyllae* and *Parishianae* were both shown to be monophyletic groups, and to be highly clustered with the subgenus *Proboscidioides* and two sections (including sections *Esmeralda* and *Deliciosae*) of

the subgenus *Phalaenopsis*, which have the same morphological characters of four pollinia as well as similar biogeographies. Furthermore, neither the subgenus *Phalaenopsis* nor *Polychilos* was found to be a monophyletic group in this study. In addition, the phylogenetic tree indicates that *Phalaenopsis* is monophyletic and does not support the existing subgeneric and sectional classification.

The phylogenetic tree of the genus *Phalaenopsis* is basically congruent with the geographical distributions of this genus. Based on the tree, two major clades were separated within the genus *Phalaenopsis*. The first clade, having four pollinia, included sections *Proboscidiodes*, *Parishianae*, and *Esmeralda*, of which are distributed in South China, India, and Indochina. The second clade, bearing two pollinia, included the sections *Phalaenopsis*, *Polychilos*, and *Fuscatae*, of which are distributed in Malaysia, Indonesia, and the Philippines. In addition, the biogeography of the genus *Phalaenopsis* is congruent with the historical geology of the distribution regions of this genus as well. According to molecular evidences, biogeography, historical geology, and the evolutionary trend of pollinia number of orchid, evolutionary trends of the genus *Phalaenopsis* were deduced. The subgenus *Aphyllae* was suggested to be the origin of *Phalaenopsis* and South China was suggested to be the origin center of *Phalaenopsis*. In addition, there were two dispersal pathways of *Phalaenopsis* from the origin center to Southeast Asia. In one pathway, *Phalaenopsis* species dispersed from South China to Southeast Asia, in particular the Philippines, using Indochina, older lands of the Philippines (Mindoro, Palawan, Zamboanga, etc.) as steppingstones, from which the subgenus *Phalaenopsis* developed. In the other pathway, *Phalaenopsis* species dispersed from South China to Southeast Asia, in particular Indonesia and Malaysia, using the Malay Peninsula as a steppingstone, from which the subgenus *Polychilos* developed.

Furthermore, molecular data and geological dating were used to estimate the

substitution rates of DNA from the genus *Phalaenopsis* based on the hypothesis of the molecular clock. The substitution rates of both ITS and cpDNA data from the genus *Phalaenopsis* were 2.4~4.7 x 10^{-9} and 3.9~7.8 x 10^{-10} substitutions/site/year, respectively. The substitution rates of ITS data of the genus *Phalaenopsis* are approximately six times those of cpDNA. Based on the substitution rates, the divergence time among most of the *P. lueddemanniana* complex was estimated to have been during the Pleistocene. The section *Deliciosae* separated from the section *Stauroglottis* at 21~10.5 Mya.

Furthermore, the phylogenetics of the close species of *Phalaenopsis* will be evaluated based on molecular data, involving three groups of close *Phalaenopsis* species, namely the *P. amabilis* complex, *P. sumatrana* complex, and *P. violacea* complex. For the first complex, the internal transcribed spacer 1 and 2 (ITS1+ITS2) regions of nuclear ribosomal DNA (nrDNA) were applied to evaluate the phylogenetics of the *P. amabilis* complex, namely *P. amabilis*, *P. amabilis* subsp. *moluccana*, *P. amabilis* subsp. *rosenstromii*, *P. aphrodite*, *P. aphrodite* subsp. *formosana*, and *P. sanderiana*. Based on molecular data, each of species/subspecies from the *P. amabilis* complex with the exception of *P. aphrodite* and its subspecies could be separated from each other. *Phalaenopsis aphrodite* from different locations and its subspecies could not be separated from each other, but all of them were separable from different populations/subspecies of *P. amabilis*. In addition, *P. sanderiana* was nested within both *P. amabilis* and its subspecies. These results do not support *P. sanderiana* being treated as a separate species from *P. amabilis*. In addition, I suggest that *P. aphrodite* is the origin of the *P. amabilis* complex and originated in the Philippines. *Phalaenopsis amabilis* and *P. sanderiana* descended from *P. aphrodite* (or its ancestor). Based on the phylogenetic tree, evolutionary trends of the *P. amabilis* complex were suggested. Within evolutionary trends of P.

amabilis complex, two different lineages with different dispersal pathways were suggested. First, *P. aphrodite*, dispersed into Palawan and evolved to be *P. amabilis*, thereafter further dispersing into Borneo and Sumatra. Second, *P. aphrodite* 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.

For the second complex, the phylogenetic relationship of the *P. sumatrana* complex, namely *P. sumatrana*, *P. corningiana*, and *P. zebrina*, was detected based on the ITS1 and ITS2 regions of nrDNA, the intron of *trn*L, and the IGS of *atp*B-*rbc*L of cpDNA. 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. Based on the molecular data of this study, plants of *P. zebrina* might be treated as a separate species from both *P. sumatrana* and *P. corningiana*. In the evolutionary trend of the *P. sumatrana* complex, plants of *P. zebrina* were deduced to be the relative origin group of the *P. sumatrana* complex based on the phylogenetic tree and biogeography. In addition, plants of both *P. sumatrana* and *P. corningiana* might have descended from plants of *P. zebrina*.

For the third complex, the phylogenetic trees inferred from the internal transcribed spacer 1 and 2 (ITS1+ITS2) regions of nuclear ribosomal DNA (nrDNA), the intron of *trn*L, and the intergenic spacer of *atp*B-*rbc*L of chloroplast DNA (cpDNA) were used to clarify the phylogenetic relationships of the *P. violacea*

complex. The complex includes the two species of *P. violacea* and *P. bellina*, according to the concept of Christenson (2001). Based on the phylogenetic tree inferred from the ITS sequence, *P. bellina* could not be separated from most populations from *P. violacea* with the exception of the population distributed on Mentawai Is., Indonesia. In addition, analyses of both the intron of *trn*L and the IGS of *atp*B-*rbc*L of cpDNA apparently could not discriminate among the three species of the *P. sumatrana* complex. Based on the morphological characters, *P. violacea* from Mentawai Is. bears a long floral rachis and was separable from the other groups of the *P. violacea* complex. Therefore, the results in this study have a trend to treat the population of Mentawai Is. of the *P. violacea* complex as a separate species from *P. violacea*. In the evolutionary trend of the *P. violacea* complex, Mentawai plants of this complex might be descended from those of Sumatra/the Malay Peninsula according to the phylogenetic analysis and biogeography.

Tables of Contents

Chapter 4

Phylogenetics, Biogeography, and Evolutionary Trends of the *P. violacea* Complex Inferred from Nuclear DNA and Chloroplast DNA

List of Tables

Chapter 1

Chapter 4.

List of Figures

Chapter 1.

Chapter 2.

Introduction in General

Genus *Phalaenopsis* Blume (Orchidaceae), a beautiful and popular orchid, comprises approximately 66 species according to the latest classification. Species of the genus *Phalaenopsis* are found throughout tropical Asia, namely South China, Indochina, India, Southeast Asia and Australia. 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 (South China) and Taiwan. The southern limit is in northern Australia (Christenson, 2001). Traditonally, the systematics of *Phalaenopsis* is confused to those of both genera of *Kingidium* and *Doritis*. A systematics of genus *Phalaenopsis* introduced by Sweet (1980), which was 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*, and divided them 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 South China, and India extending to northern Vietnam, Myanmar and Thailand, respectively. The subgenus *Polychilos* has a few species distributed in 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* only distributes in tropical Asia, namely South China, Indochina, India, Southeast Asia including Malasia, Indonesia, the Philippines, and New Guinea etc. (Christenson, 2001). These regions, in particular Southeast Asia, have been studied lots of biogeography on flora or fauna. Since Southeast Asia is created from the collisions between several ocean plates (Pacific, Indian, Philippine) and land plates (Eurasian, Indian, Australian) (Hall, 1996), the flora and fauna of these regions are so complicate and intermediate between Asia and Australia. Several biogeographical boundaries have been introduced in these regions, for instances Wallace's Lines and Weber's Lines, since deep straits limit the dispersal of flora and fauna (Van Oosterzee, 1997; Moss and Wilson, 1998).

To the present, the systematics of *Phalaenopsis* and its alliance, namely members of the subtribe *Aeridinae* (syn. *Sarcanthinae*), are only described based on the basical

morphological data as Linneaus times. So far, there is not any report of *Phalaenopsis* focusing on the ontogeny. The cytology of *Phalaenopsis* also did not use to clarify the systematics of *Phalaenopsis* due to each *Phalaenopsis* speices (with the exception of the natural tetraploid species, *P. buyssoniana*) having the same chromosome number of 38 (Woodard, 1951; Shindo and Kamemoto, 1963; Tanaka and Kamemoto, 1984). Although several reports had described the relationship of different taxa of *Phalaenopsis* based on the chromosome size (Shindo and Kamemoto, 1963; Arends, 1970), it also cannot obtain the relationships for most of plant taxa of *Phalaenopsis* based on it. Furthermore, since the other members of subtribe *Aeridinae* have the same chromosome of 38 as *Phalaenopsis*. The genetic barriers of *Phalaenopsis* are unclear. It can cross to its related genera, for instances, *Aerides*, *Arachnis*, *Ascocentrum*, *Doritis*, *Neofinetia*, *Paraphalaenopsis*, *Renanthera*, *Rhynchostylis*, *Vanda*, and *Vandopsis*, and hybrids show various degrees of fertility (cf. Sweet, 1980). Furthermore, only a report described the discrimination of *Phalaenopsis* species, namely *P. violacea* and *P. bellina*, based on the chemical taxonomy. The biochemical method, i.e., isozyme analysis, has not been used to clarify the systematics of *Phalaenopsis* until today. It is surprising to me that only a few researches of phylogeny from *Phalaenopsis* had been examined.

In the present study, molecular evidences obtained from data of internal transcribed spacer (ITS) of ribosomal DNA (rDNA) of nuclear DNA and chloroplast DNAs, including the *trn*L intron, the *trn*L-*trn*F intergenic spacer (IGS), and the *atp*B-*rbc*L IGS, were be used to clarify the phylogenetics, biogeography, and evolutionary trends of the genus *Phalaenopsis* (Chapter 1) as well as the three groups of close *Phalaenopsis* species, namely the *P. amabilis* complex (Chapter 2), the *P. sumatrana* complex (Chapter 3), and the *P. violacea* complex (Chapter 4).

Literature Cited

Arends, J. C. 1970. Cytological observations on genome homology in eight interspecific hybrids of *Phalaenopsis*. Genetica 41: 88-100.

Christenson E. A. 2001. *Phalaenopsis*. Timber Press, Portland, Oregon. 330p.

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

Moss, S. J., and M. E. J. Wilson. 1998. Biogeographic implications from the Tertiary

Paleaeogeographic evolution of Sulawesi and Borneo. *In* Hall, R. and Holloway, J. D. (eds.). Biogeography and geological evolution of SE Asia. Backhuys, Leiden.

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

Sweet H. R. 1980. The genus *Phalaenopsis*. The Orchid Digest, Pomona, California.

Tanaka R., Kamemoto K. 1984. Chromosome in orchids: counting and numbers. *In* Arditti, J. (ed.), Orchid Biology: Reviews and Perspectives. Cornell Univ. Press, Ithaca, NY.

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

Woodard J. W. 1951. Some chromosome numbers in *Phalaenopsis*. Am. Orchid Soc. Bull. 20: 356-358.

Chapter 1
Molecular Phylogeny, Biogeography, and Evolutionary Trends of the Genus *Phalaenopsis* (Orchidaceae)

Abstract

Phylogenetic trees inferred from the internal transcribed spacers 1 and 2 (ITS1+ITS2) region of nuclear ribosomal DNA (nrDNA) and chloroplast DNA (cpDNA), including the *trn*L intron, the *trn*L-*trn*F intergenic spacer (IGS), and the *atp*B-*rbc*L IGS, were used to clarify the phylogenetics and evolutionary trends of the genus *Phalaenopsis* (Orchidaceae). Molecular evidence derived from this study supports the monophyly of the genus *Phalaenopsis*. This result concurs that the genera *Doritis* and *Kingidium* should be treated as synonyms of the genus *Phalaenopsis*. Both subgenera of *Aphyllae* and *Parishianae* were shown to be monophyletic groups, and to be highly clustered with the subgenus *Proboscidioides* and the sections *Esmeralda* and *Deliciosae* of the subgenus *Phalaenopsis*, which have the same morphological character of four pollinia as well as proximal distributions. Furthermore, neither of the subgenera, *Phalaenopsis* or *Polychilos*, was determined to be a monophyletic group in this study. In addition, the phylogenetic relationship of the genus *Phalaenopsis* is basically congruent with its biogeography. In the evolutionary trend of the genus *Phalaenopsis*, the subgenus *Aphyllae* was suggested to be the relative origin group of the genus *Phalaenopsis*. Therefore, the species diversity center of the subgenus *Aphyllae*, South China (the Himalayas), was shown to be the origin center of *Phalaenopsis*. In addition, there were two evolutionary trends of *Phalaenopsis* from the origin center to Southeast Asia. In one trend, *Phalaenopsis* species dispersed from South China to Southeast Asia, in particular the Philippines, using Indochina and some older lands of the Philippines (e.g., Mindoro and Palawan) as steppingstones, from which the subgenus *Phalaenopsis* developed. In the other trend, *Phalaenopsis* species dispersed from South China to Southeast Asia, in particular Indonesia and Malaysia, using the Malay Peninsula as a steppingstone, from which the subgenus *Polychilos* developed. Furthermore, based on molecular data and geological dating, the substitution rates of both ITS and cpDNA data from the genus *Phalaenopsis* were determined to be 2.4~4.7 x 10^{-9} and 3.9~7.8 x 10^{-10} substitutions/site/year, respectively. The substitution rates of ITS data of the genus *Phalaenopsis* are approximately six times those of cpDNA. Based on these substitution rates, the divergence time among most of the *P. lueddemanniana* complex was estimated to have occurred during the Pleistocene. The section *Deliciosae* was separated from the section

Stauroglottis 21~10.5 million years ago.

Introduction

The genus *Phalaenopsis* Blume (Orchidaceae), beautiful and popular orchids, comprises approximately 66 species according to the latest classification of Christenson (2001). The first *Phalaenopsis* species was 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 the moth orchids into it. In 1827, a new genus, *Polychilos*, which was related to *Phalaenopsis*, was erected by Breda (cf. Christenson 2001). Plants of *Phalaenopsis* bear poly-calli which was treated as the genus *Polychilos*. *Doritis* was treated as a separate genus from the genus *Phalaenopsis* based on habitation and the four pollinia by Lindley in 1833. Furthermore, Smith (1933) treated the genus *Doritis* as a synonym of *Phalaenopsis* and placed it in the section *Esmeralda* of the subgenus *Phalaenopsis*. *Kingiella* (syn. *Kingidium*) was treated as a separate genus from *Phalaenopsis* based on the four pollinia and the flower with a small saccate spur by Rolfe (1917) (cf. Christenson 2001). Several decades later, series of revisions of the genus *Phalaenopsis* were undertaken by Sweet (1968, 1969). Systematics of the genus *Phalaenopsis* introduced by Sweet (1980) treated *Doritis* and *Kingidium* as separate genera from *Phalaenopsis*. Based on the concept of Sweet (1980), the genus *Phalaenopsis* was divided into nine sections, namely *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos*, *Fuscatae*, *Amboinenses*, *Zebrinae*, *Phalaenopsis*, and *Stauroglottis*. Among these, the section *Zebrinae* was subdivided into four subsections, namely *Glabrae*, *Hirsutae*, *Lueddemannianae*, and *Zebrinae*. Seidenfaden (1988c) clarified many problematic species of the section *Aphyllae* according to Sweet's systematics and led the way for deemphasizing the pollinium number as a generic character. Until recently, Christenson (2001) treated *Kingidium* and *Doritis* as synonyms of the genus *Phalaenopsis*, which had been divided into five subgenera, namely *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos*, and *Phalaenopsis*. Of these, the subgenus *Polychilos* was subdivided into the four sections of *Polychilos*, *Fuscatae*, *Amboinenses*, and *Zebrinae*, and the subgenus *Phalaenopsis* was subdivided into the four sections of *Phalaenopsis*, *Deliciosae*, *Esmeralda*, and *Stauroglottis* (Christenson, 2001).

Plants of *Kingidium* having four pollinia were traditionally segregated from plants of the genus *Phalaenopsis* (two pollinia) based on the number of pollinia (Sweet, 1980;

Seidenfaden, 1988b). Christenson (2001) treated the genus *Kingidium* as a synonym of *Phalaenopsis* and split it into different parts of *Phalaenopsis*, some as part of the subgenus *Aphyllae* (*P. braceana*, *P. minus*, and *P. taenialis*) and some as the section *Deliciosae* of the subgenus *Phalaenopsis* (*P. chibae* and *P. deliciosa*). Furthermore, another group, the *P. parishii* complex, which has four pollinia, was proposed as a separate genus, *Grafia*, by Hawkes (cf. Christenson, 2001). This group had first been treated as a synonym of the genus *Phalaenopsis* and placed in the section *Parishianae* by Sweet (1968). Shim (1982), however, disagreed with Sweet's concept (1980) and treated members of the sections *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos*, *Zebrinae*, *Fuscatae*, and *Amboinenses* of the genus *Phalaenopsis* as the genus *Polychilos* based on a narrowly defined *Phalaenopsis*. Two decades later, Christenson (2001) accepted Sweet's concept (1980) and placed the monotypic species, *P. lowii*, in the subgenus *Proboscidioides* of the genus *Phalaenopsis*. In addition, plants of the genus *Doritis* were traditionally separated from plants of *Phalaenopsis* because of their characters of the number of pollinia, the lip structure, and adaptations to terrestrial habitats (Sweet, 1980; Seidenfaden, 1988a). Until recently, this group was treated as a synonym of the genus *Phalaenopsis* and placed in the section *Esmeralda* of the subgenus *Phalaenopsis* by Christenson (2001). Compared to Sweet's systematics (1980), Christenson (2001) treated the genera *Kingidium* and *Doritis* as synonyms of the genus *Phalaenopsis* and raised the sections *Proboscidioides*, *Aphyllae*, and *Parishianae* into subgeneric levels. A portion of the members of *Kingidium* were placed in the subgenus *Phalaenopsis* as the section *Deliciosae*, and all the others were placed in the subgenus *Aphyllae*. All of the members of *Doritis* were placed in the subgenus *Phalaenopsis* as the section *Esmeralda*. In addition, he placed sections *Phalaenopsis* and *Stauroglottis* as well as sections *Amboinenses*, *Polychilos*, *Fuscatae*, and *Zebrinae* in the subgenera *Phalaenopsis* and *Polychilos*, respectively. Moreover, species of the subsections *Lueddemannianae*, *Hirsutae*, and *Glabrae* of the section *Zebrinae* were moved to the section *Amboinenses* by Christenson (2001). Comparisons between Sweet's (1980) and Christenson's systematics (2001) are shown in Table 1.

All *Phalaenopsis* species (with the exception of the natural tetraploid species, *P. buyssoniana*) have 38 chromosomes (2n=38) (Woodard, 1951; Shindo and Kamemoto, 1963; Arends, 1970; Tanaka and Kamemoto, 1984; Christenson, 2001). In addition, the chromosome number of the subtribe *Aeridinae* (syn. *Sarcanthinae*) is consistent with that of *Phalaenopsis*, which displays a uniform diploid number of 38 chromosomes, with only a few

tetraploid or hexaploid species (Christenson, 2001). Furthermore, crosses between *Phalaenopsis* species and other close genera, namely *Aerides*, *Arachnis*, *Ascocentrum*, *Doritis*, *Neofinetia*, *Paraphalaenopsis*, *Renanthera*, *Rhynchostylis*, *Vanda*, and *Vandopsis*, also show various degrees of fertility (Sweet, 1980).

Species of the genus *Phalaenopsis* are found throughout tropical Asia and the larger islands of the Pacific Ocean. The western distribution of *Phalaenopsis* is 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, while the southern limit is in northern Australia (Christenson, 2001). 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. 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. (Christenson, 2001) (Fig. 1).

In the late 19th century, six major biogeographic regions in the world were recognized, namely the Palaearctic, Nearctic, Neotropical, Ethiopian, Oriental, and Australian regions (Pianka, 1994) (Fig. 2). 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, several deep straits in Southeast Asia separate the Oriental from the Australian region. On the other hand, the Himalayas divide the Oriental and the Palaearctic regions (Pianka 1994). Most of the geographical distributions of the genus *Phalaenopsis*, namely South China, India, Indochina, and Southeast Asia (Malaysia, Indonesia, and the Philippines) (Sweet, 1980; Christenson, 2001), belong to the Oriental biogeographic region. Of these areas, Southeast Asia was created from collisions between several ocean plates (the Pacific, Indian, and Philippine Plates) and land plates (the Eurasian, Indian, and Australian Plates). These processes may already have started by the early Paleozoic (~400 million years ago (Mya); Hall, 1996). According to the historical geology of Southeast Asia, the Malay Peninsula, Borneo, Sumatra, Java, western Sulawesi, and parts of the Philippines (namely Palawan, Mindoro, and Zamboanga) belong to the Eurasian Plate, while east Sulawesi, Flores, the Molucca Is., New Guinea, Australia, and the other parts of the Philippines belong to the Indian-Australian Plate (Hall, 1996) (Fig. 3). The deep Makassar Strait, dividing western Sulawesi from Borneo, formed in the Paleogene (~50 Mya), when western Sulawesi moved

away from the Sunda Shelf (including the Malay Peninsula, Borneo, Sumatra, and Java). Therefore, lands of Borneo and western Sulawesi were interconnected some 50 Mya. The new strait prevented any further dispersal of Bornean species to Sulawesi. This historical geology can explain why Sulawesi's terrestrial organisms are closely related to those of Borneo at the family level and higher. This also accounts for the high degree of endemism among Sulawesi's faunal and floral species (Moss and Wilson, 1998). Furthermore, 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, namely Palawan and Mindoro, Zamboanga, etc., 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. 3). Until 5~10 Mya, the crust of the older plate was combined with Borneo (Karig et al., 1986; Stephan et al., 1986; Hall, 1996). 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. This would have made crossings relatively easy among these regions (Van Oosterzee, 1997) (Fig. 4). In addition, Flores, Indonesia is an island of the Lesser Sunda Islands which belongs to a newer volcanic island arc (< 5 Mya), and the Lombok Strait and volcanism have formed a dispersal barrier between there and the Sunda Shelf. In fact, fauna and flora of the region of the Lesser Sunda Islands have also been shown to be separated from those of the Sunda Shelf (Van Oosterzee, 1997; Hall, 1998).

The evolutionary rates of molecular data from plant taxa have been estimated based on fossil records (Baldwin and Sanderson, 1998; Sanderson and Doyle, 2001; Vinnersten and Bremer, 2001), geological datings (e.g., Richardson et al., 2001), and paleoclimatic data (e.g., Baldwin and Sanderson, 1998). To the present, heterogeneous evolutionary rates in the genomes of both plant and animal groups have been demonstrated in several studies (e.g., Wu and Li, 1985; Britten, 1986; Bousquet et al., 1992; Eyre-Walker and Gaut, 1997). However, the causes of rate heterogeneity are still unclear. To the present, these differences in DNA substitution rates between plants of contrasting life histories have been explained in various ways, e.g., by generation time or speciation rate effects or by varying efficiencies of DNA replication or repair in combination with selection against heterozygosity and differences in population sizes between annuals and perennials (Bousquet et al., 1992; Eyre-Walker and Gaut, 1997; Gaut et al., 1997; Laroche and Bousquet, 1999). Comparisons of more than one DNA region can yield significant insights into processes affecting

molecular evolutionary rates and may allow discrimination among different possible explanations for rate heterogeneity (Eyre-Walker and Gaut, 1997; Gaut et al., 1997; Muse, 2000). To identify evolutionary forces that are expected to affect substitution rates at the organismal level, e.g., the impact of generation time or speciation rate, investigation of functionally independent DNA regions from more than one genome is ideal. Organismal-level factors should cause correlated patterns of rate heterogeneity between DNA regions from different genomes within a lineage. Comparisons between DNA regions from the same organelle can be useful for distinguishing between gene-specific factors (e.g., selection) and genome-specific factors (e.g., DNA replication rate) (Andreasen and Baldwin, 2001).

Ribosomal DNAs (rDNAs) with repeated sequences are organized as families in tandem arrays in nucleolar organizer regions of chromosomes in all eukaryotes. Copy numbers of rDNA in different species vary from a few hundred to several thousand. Each repeated unit is comprised of a non-transcribed spacer known as an intergenic spacer (IGS) and the transcription unit coding for the precursor of rRNA. Ribosomal DNA is affected by rapid concerted evolution, which results in homogeneity among copies (Maynard, 1989). The basic rDNA repeat unit contains the following segment: 5'-IGS-ETS-18S-ITS1-5.8S-ITS2-26S-ETS-3'. The 18S rRNA gene of each transcription unit is separated from 26S rRNA by an internal transcribed spacer (ITS1), the 5.8S rRNA gene, and ITS2 (Takaiwa et al., 1985a; Barker et al., 1988). Different regions of rDNA can be used to examine lineages with different levels of divergence. Sequences of 18S and 26S rRNA are useful for elucidating phylogenetic relationships among diverse organisms (Suh et al., 1993). In contrast, the IGS and ITS regions show great divergence. Several studies have shown that the spacer regions can be used to infer phylogenies among closely related taxa and to identify species or strains (Hillis and Dixon, 1991). In fact, the ITS regions of nuclear ribosomal DNA (nrDNA) have been widely applied and can offer valuable information for resolving phylogenetic relationships at different taxonomic levels (e.g., Baldwin, 1992, 1993; Suh et al., 1993; Sun et al., 1994; Bayer et al., 1996; Cox et al., 1997; Hodkinson et al., 2002; Yang et al., 2002), particularly at the intrageneric level because of relatively rapid evolutionary rates.

Generally, chloroplast DNA (cpDNA) and plant mitochondrial DNA (mtDNA) have slowly evolved relative to both nrDNA and animal mtDNA (Palmer et al., 1988; Schaal et al., 1998). Uniparental inheritance was introduced for cpDNA (Derepas and Dulieu 1992).

Therefore, gene recombination between chloroplasts does not normally occur (Chiu and Sears, 1985). Nevertheless, cpDNA has also been extensively applied to evolutionary and phylogenetic research (Palmer, 1987). Compared to gene regions of the chloroplast genome, introns and the IGS of cpDNA should have evolved faster and may be useful for resolving phylogenies at lower taxonomic levels, such as tribes and genera (Golenberg et al., 1993). Taberlet et al. (1991) developed a series of universal primers for numbers of non-coding plastid regions. Of them, the *trn*L (UAA) intron and *trn*L (UAA)-*trn*F (GAA) IGS have successfully been used as phylogenetic tools at intrageneric levels, such as with *Miscanthus* and *Saccharum* (Poaceae) (Hodkinson et al., 2002), *Brassica* (Brassicaceae) (Yang et al., 2002), *Moraea* (Iridaceae) (Goldblatt et al., 2002), *Adenophorus* (Grammitidaceae) (Ranker et al., 2003), *Allium* (Liliaceae) (Van Raamsdonk et al., 2003), *Ehrharta* (Poaceae) (Verboom et al., 2003), and the tribe *Exaceae* (Gentianaceae) (Yuan et al., 2003). Furthermore, the *atp*B-*rbc*L IGS has also been used to reconstruct the phylogeny of different plants at the generic or tribal level, such as *Moraea* (Iridaceae) (Goldblatt et al., 2002), the tribe *Rubieae* (Rubiaceae) (Manen and Natali, 1995), and the subgenus *Ceratotropis* (genus *Vigna*) (Doi et al., 2002).

Although a few species (approximately 20 species) of the genus *Phalaenopsis* have been examined using the RAPD technique (Chen et al., 1995) and IGS regions of 5S rDNA (Kao, 2001), those studies could not provide insights into the entire phylogeny of the genus *Phalaenopsis*. In this study, I followed the systematics of *Phalaenopsis* proposed and described by Christenson (2001) to reveal the phylogeny of this genus. In order to reconstruct the phylogeny of the genus *Phalaenopsis*, nucleotide sequences of the ITS regions (ITS1 and ITS2) of nrDNA and three fragments of cpDNA, namely the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS, from 52 taxa of *Phalaenopsis* and several outgroups were analyzed to address the phylogeny of the genus. The sequence data generated in this study were used to evaluate (1) the monophyly of the genus *Phalaenopsis* and (2) relationships among species of this genus. Furthermore, biogeography, evolutionary trends, and substitution rates of the genus *Phalaenopsis* were evaluated based on the phylogenetic tree obtained from molecular data, geographical distributions of the genus *Phalaenopsis*, historical geology, and the evolutionary trend of pollinium number.

Materials and Methods

Plant materials

The taxonomy and nomenclature of *Phalaenopsis* at the species level followed Sweet (1980) and Christenson (2001). Fifty-two taxa of the genus *Phalaenopsis* plus five taxa from related genera as outgroups, namely *Paraphalaenopsis laycockii*, *Par. labukensis*, *Par. serpentilingua*, *Gastrochilus japonicus*, and *Tuberolabium kotoense*, were used for this study and are summarized in Table 2 (Pictures of *Phalaenopsis* species see Appendix 1).

DNA extraction

Using the CTAB (cetyltrimethylammonium bromide) method, total DNA was extracted from fresh etiolated leaves (Doyle and Doyle, 1987). The approximate DNA yields were then determined using a spectrophotometer (Hitachi U-2001).

PCR amplification

Internal transcribed spacers of nrDNA

One of primers was designed from conserved regions of the 5' end of 18S rDNA of rice (Takaiwa et al. 1984), melon (Kavanagh and Timmis, 1988), tomato (Kiss et al., 1989a), mustard (Rathgeber and Capesius, 1989), mungbean (Schiebel and Hemleben, 1989), and strawberry (Simovic et al., 1992). Another primer was designed from complementary conserved regions of the 3' end of 26S rDNA of rice (Takaiwa et al., 1985b), tomato (Kiss et al., 1989b), *Lithophragma trifoliata*, and *Drimys winteri* (Kuzoff et al., 1998). Two primers were designed to amplify the internal transcribed spacer (ITS) of nrDNA of *Phalaenopsis* taxa, i.e., IT1: 5' AGTCGTAACAAGGTTTCC 3' and IT2: 5' GTAAGTTTCTTCTCCTCC 3' (Fig. 5). The protocol for manipulating PCR was as follows. I used a 50-µl mixture containing 40 mM Tricine-KOH (pH 8.7), 15 mM KOAc, 3.5 mM $Mg(OAc)_2$, 3.75 µg/ml BSA, 10% DMSO, 0.005% Tween 20, 0.005% Nonidet-P40, with four dNTPs (0.2 mM each), primers (0.5 µM each), 2.5 units of Advantage 2 DNA polymerase (Clontech), 10 ng genomic DNA, and a 50-µl volume of mineral oil. Amplification reactions were performed in a dry block with two-step thermal cycles (Biometra). In the first step, the mixture was incubated at 94 ºC for 5 min, then it underwent 10 cycles of denaturation at 94 ºC for 45 s, annealing at 62 ºC for 20 s, and extension at 72 ºC for 1 min. The second step was carried out by the following process: 30 cycles of denaturation at 94 ºC for 45 s, annealing at 58 ºC for 20 s, extension at 72 ºC for 1 min, with a final extension for 7 min at 72 ºC. These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained by 0.5 µg/ml ethidium bromide, and finally photographed under UV light exposure.

The trnL intron of chloroplast DNA (cpDNA)

Two universal primers for amplifying the *trn*L intron of cpDNA were referenced from Taberlet et al. (1991)(Fig. 6). The PCR reactions were carried out in 50-µl mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 0.01% BSA, with four dNTPs (0.2 mM each), primers (0.5 µM each), 2.5 units of *Taq* DNA polymerase (Virogene) and 10 ng genomic DNA, and 50-µl volume mineral oil. The amplification reactions were done in a dry-block with two-step thermal cycles (Biometra). The first step, the mixture was incubated at 94 ºC for 3 min, then it underwent 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. The second step was carried out by the following process: 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. These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained by 0.5 µg/mL ethidium bromide, and finally photographed under UV light exposure.

The trnL-trnF IGS of cpDNA

Two universal primers for amplifying the *trn*L-*trn*F IGS of cpDNA were also referenced from Taberlet et al. (1991) (Fig. 6). The PCR reactions were carried out in 50-µl mixture, containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 0.01% BSA, with four dNTPs (0.2 mM each), primers (0.5 µM each), 2.5 units of *Taq* DNA polymerase (Virogene) and 10 ng genomic DNA, and 50-µl volume mineral oil. The amplification reactions were done in a dry-block with two-step thermal cycles (Biometra). The first step, the mixture was incubated at 94 ºC for 3 min, then it underwent 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. The second step was carried out by the following process: 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. These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained by 0.5 µg/mL ethidium bromide, and photographed under UV light exposure.

The atpB-rbcL IGS of cpDNA

Two primers were designed from conserved regions of the 3’ end of *atp*B gene and of the 5’end of *rbc*L gene of cpDNA from different species of GenBank, i.e., 5’ CATCTAGGATTTACATATAC 3' and 5’ GTCAATTTGTAATCTTTAAC 3’, respectively

(Fig. 7). The PCR reactions were carried out in 50-µl mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 0.01% BSA, with four dNTPs (0.2 mM each), primers (0.5 µM each), 2.5 units of *Taq* DNA polymerase (Virogene) and 10 ng genomic DNA, and 50-µl volume mineral oil. The amplification reactions were done in a dry-block with two-step thermal cycles (Biometra). The first step, the mixture was incubated at 94 ºC for 3 min, then it underwent 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. The second step was carried out by the following process: 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. These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), and stained by 0.5 µg/mL ethidium bromide, and finally photographed under UV light exposure.

DNA recovery and sequencing

PCR products of different DNA fragments from the plant material studied were recovered by glassmilk (BIO 101, California) and directly sequenced using the dideoxy chain-termination method with an ABI377 automated sequencer and a Bigdye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, California). Sequencing primers were the same as those used for PCR. These reactions were performed based on the recommendations of the manufacturers.

Phylogenetic reconstructions

Boundaries of the ITS regions, the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS were determined by reference to published ITSs of nrDNA (Takaiwa et al., 1984, 1985b; Kiss et al., 1989a, b; Baldwin, 1992; Tsai and Huang, 2001), sequences of the *trn*L intron, the *trn*L-*trn*F IGS (Bayer et al., 2000; Espelund et al., 2000; Brouat et al., 2001), and the *atp*B-*rbc*L IGS (Schwarzbach et al., 2000; Chiang et al., 2001; Doi et al., 2002), respectively. The sequences were entered into BioEdit. Sequence lengths and base compositions were determined using the program Clustal W multiple alignment in BioEdit (Hall, 1999). Genetic relationships were determined using the program MEGA version 2.1 (Kumar et al., 2001). Genetic distances were calculated by the two-parameter method of Kimura (1980), and were used to reconstruct phylogenetic trees by the Neighbor-joining method (NJ) (Saitou and Nei, 1987) with interior branch tests of 1000 replicates (Sitnikova et al., 1995).

Biogeographical analyses

The geographical distributions of the genus *Phalaenopsis* in this study were referenced based on the monograph of the genus *Phalaenopsis* written by Christenson (2001). According to the geographical distributions of the genus *Phalaenopsis*, I divided them into 14 different geographical regions, namely India/Sri Lanka, South China, Indochina, the Malay Peninsula, Borneo, Sumatra, Java, Flores, Sulawesi, Molucca Is., New Guinea, the Philippines, Taiwan, and Australia. Furthermore, the matrix of the geographical distributions of the genus *Phalaenopsis* was obtained based on the distribution of each *Phalaenopsis* species. According to the matrix, the distances among these 15 regions were calculated by the number of differences (Nei and Kumar, 2000), and a dendrogram was constructed using the Neighbor-joining method (Saitou and Nei, 1987).

Evolutionary trends

The evolutionary phylogenetic tree of the genus *Phalaenopsis* inferred from combined data of ITS and cpDNA was reconstructed based on the minimum-evolution method (Edwards and Cavalli-Sforza, 1963; Rzhetsky and Nei, 1992, 1993). According to the phylogenetic relationships obtained from the tree, species of the genus *Phalaenopsis* were divided into several groups. In order to reveal the relative origin group, each group divided by molecular data was respectively placed in the root and tested. Genetic distances were calculated by the two-parameter method of Kimura (1980) from the rooted group being tested to the remaining groups of the genus *Phalaenopsis*. The rooted group with the smallest sum of genetic distances was suggested to be the relative origin group of the genus *Phalaenopsis* according to the concepts of minimum evolution (Edwards and Cavalli-Sforza, 1963). Therefore, evolutionary trends of the genus *Phalaenopsis* are shown based on the evolutionary phylogenetic tree of the genus *Phalaenopsis* rooted by the relative origin group.

Substitution rates

Since there is no fossil record for the genus *Phalaenopsis*, substitution rates of molecular data from the genus *Phalaenopsis* were estimated based on geological dating of the combination between Palawan, the Philippines, and Borneo (5~10 Mya; Karig et al., 1986; Stephan et al., 1986; Hall, 1996). Palawan might have formed a steppingstone when species of the genus *Phalaenopsis* dispersed between Borneo and the Philippines. Before the date of combination, these two islands were separated by an oceanic barrier. If I assume that

the dispersal first occurred 5~10 Mya, I can estimate substitution rates by comparing nucleotide substitution rates between related *Phalaenopsis* species of Philippine and Bornean species. According to the evolutionary trends of the genus *Phalaenopsis* in this study, the *P. lueddemanniana* complex (including *P. mariae*, *P. pallens*, *P. hieroglyphica*, *P. fasciata*, *P. lueddemanniana*, *P. bastianii*, *P. pulchra*, and *P. reichenbachiana*) distributed in the Philippines descended from species of the section *Amboinenses* distributed on the Sunda Shelf (Borneo, Sumatra, Malay Peninsula, Java, etc.). The calibration point was based on the split between the *P. lueddemanniana* complex distributed in the Philippines and most of the section *Amboinenses* (with the exception of the *P. lueddemanniana* complex) distributed on the Sunda Shelf. In order to estimate the substitution rates of the calibration point, the average number of nucleotide substitutions between the above two groups was respectively calculated. Based on the nucleotide substitutions and the geological dating (5~10 My), the substitution rates of the genus *Phalaenopsis* could be estimated: the substitution rate = X/2T, where X is the no. of differences/no. of total sites and T is the divergence time (year) (Li, 1997). The substitution rates were able to estimate the divergence times within the remaining groups of the genus *Phalaenopsis* when molecular data in this study were not rejected by the hypothesis of the molecular clock, which assumes constancy of evolutionary rates over time (Zuckerkandl and Pauling, 1965). Nucleotide mutations of neutrality were tested based on Tajima's test of neutrality (Tajima, 1989). The constancy of the evolutionary rate of molecular data was tested based on Tajima's relative rate test (Tajima, 1993). This test involves comparing two sequences relative to a third sequence (the reference group). All characters showing a gap in at least one taxon were eliminated. For each pair of sequences, every position was counted for which only one of the pair differed from the reference sequence. The difference in the number of informative changes in the sequence pair was squared and divided by the sum of those changes. The quotient was then rated within the chi-square distribution with one degree of freedom (df) and a 5% probability value ($p < 0.05$).

Results

ITS sequence data by sequence analysis

Sequence analyses

The accession numbers of the 52 species of the genus *Phalaenopsis* plus the five outgroups are shown in Table 3. The sequence lengths of ITS1 ranged from 213 to 255 bp

and those of ITS2 were from 256 to 270 bp. The ITS lengths obtained from *Phalaenopsis* species and the outgroups were similar to those reported from a broad sample of angiosperms (Baldwin et al., 1995). Percentages of the G+C content across the ITS1 region of *Phalaenopsis* species varied from 63.1% in *P. kunstleri* to 78.2% in *P. stuartiana*. On the other hand, percentages of the G+C content across the ITS2 region of *Phalaenopsis* species varied from 62.8% in *P. kunstleri* and *P. fuscata* to 77.9% in both *P. violacea* and *P. bellina* (Table 3). Combining ITS1 and ITS2, those sequences of the 52 *Phalaenopsis* species plus the five outgroups were aligned and resulted in 581 characters (data not shown). Conserved sites and variable sites among the 52 *Phalaenopsis* species were 236 and 303, respectively. The ratio of variable sites in the alignment was 56.2% (Table 4). Among the 57 taxa studied, on the other hand, they were 211 and 330, respectively. The ratio of variable sites in this alignment was 60.1% (Table 5).

Genetic distances

Genetic distances of ITS1 and ITS2 among the 57 taxa of this study were in the range of from 0.000 to 0.242 with an average of 0.117 using the two-parameter method of Kimura (1980). The outgroup, *Tuberolabium kotoense*, and *Phalaenopsis amabilis* were more divergent since they had a genetic distance of 0.242. Among the 52 species of the genus *Phalaenopsis*, the ranges of genetic distances were from 0.000 to 0.225 with an average of 0.109. *P. gigantea* and *P. schilleriana* were more divergent since they had a genetic distance of 0.225. Within the subgenus *Phalaenopsis*, the ranges of genetic distances were also from 0.014 to 0.176 with an average of 0.108. *P. philippinensis* and *P. schilleriana* were more closely related since they had a genetic distance of 0.014. In contrast, *P. amabilis* and *P. deliciosa* were more divergent since they had a genetic distance of 0.176. Within the subgenus *Polychilos*, the ranges of genetic distances were from 0.000 to 0.206 with an average of 0.082. *P. corningiana* and *P. sumatrana* as well as *P. bellina* and *P. violacea* were more closely related since they had a genetic distance of 0.000. In contrast, *P. gigantea* and *P. cornu-cervi* were more divergent since they had a genetic distance of 0.206. Within the subgenus *Parishianae*, genetic distances ranged from 0.010 to 0.053 with an average of 0.037. *P. parishii* and *P. lobbii* were more closely related since they had a genetic distance of 0.010. In contrast, *P. parishii* and *P. gibbosa* were more divergent since they had a genetic distance of 0.053. Within the subgenus *Aphyllae*, the ranges of genetic distances were from 0.006 to 0.033 with an average of 0.022. *P. wilsonii* and *P. braceana* were more closely

related since they had a genetic distance of 0.006. In contrast, *P. wilsonii* and *P. minus* were more divergent since they had a genetic distance of 0.033 (Table 6).

The phylogenetic reconstruction

The phylogenetic tree was reconstructed following the NJ method is shown in Fig. 8. Based on the phylogenetic trees, the genera *Paraphalaenopsis*, *Tuberolabium*, and *Gastrochilus* were not nested within the genus *Phalaenopsis*. It shows that the genus *Phalaenopsis* described by Christenson (2001) is a monophyly. Within the genus *Phalaenopsis*, two major clades were shown. The first clade comprised the subgenera *Polychilos* and *Phalaenopsis* (in part); and the second clade consisted of the subgenera *Phalaenopsis* (the other part), *Proboscidioides*, *Parishianae*, and *Aphyllae*.

The monophyly of the subgenus *Polychilos* was not supported in this study. Parts of the subgenus *Phalaenopsis* were nested within the subgenus *Polychilos*. Two separate groups were revealed within the subgenus *Polychilos*. Within the subgenus *Polychilos*, only the section *Polychilos* showed a monophyly supported by a 78% interior branch test. Parts of the section *Amboinenses*, namely *P. hieroglyphica*, *P. reichenbachiana*, *P. bastianii*, *P. pallens*, *P. lueddemanniana*, *P. fasciata*, and *P. pulchra*, formed a clade supported by a 99% interior branch test. In addition, most of the section *Zebrinae*, namely *P. corningiana*, *P. sumatrana*, and *P. tetraspis* and *P. venosa* (section *Amboinenses*) formed a clade supported by a 99% interior branch test. Parts of the section *Amboinenses*, namely *P. gigantea*, *P. doweryensis* and *P. maculata*, were nested within the section *Fuscatae* supported by a 99% interior branch test. Excluding the clade of *P. gigantea*, *P. doweryensis*, *P. maculata*, and the section *Fuscatae*, the remaining species of the subgenus *Polychilos* formed a clade supported by a 98% interior branch test.

The monophyly of the subgenus *Phalaenopsis* was also not supported in this study. Within the subgenus *Phalaenopsis*, the section *Phalaenopsis* showed a monophyly supported by a 99% interior branch test. Parts of the section *Stauroglottis*, namely *P. equestris* and *P. lindenii*, showed a sister relationship to the section *Phalaenopsis* supported by an 80% interior branch test. Within the section *Stauroglottis*, *P. celebensis* showed a divergence to the remaining species. In addition, the section *Deliciosae* was not shown to be a monophyly in this study. Besides, sections *Deliciosae* and *Esmeralda* were separated from sections *Phalaenopsis* and *Stauroglottis* of the subgenus *Phalaenopsis*.

The subgenus *Parishianae* was shown to be a monophyly supported by a 90% interior

branch test. Furthermore, the subgenus *Aphyllae* was also shown to be a monophyly supported by an 80% interior branch test. The monotypic subgenus *Proboscidioides* showed a sister relationship to the subgenus *Aphyllae*. *P. pulcherrima* (section *Esmeralda* of the subgenus *Phalaenopsis*) showed a sister relationship to the common ancestor of both subgenera of *Proboscidioides* and *Aphyllae*, and parts of the subgenus *Phalaenopsis* (section *Deliciosae*) supported by a 97% interior branch test.

Chloroplast DNA data by sequence analysis

Sequence length and composition

The accession numbers of the *trn*L intron, the *trn*L-*trn*F intergenic spacer (IGS), and the *atp*B-*rbc*L IGS from the 52 species of the genus *Phalaenopsis* plus the five outgroups are shown in Table 7. Within the genus *Phalaenopsis*, sequence lengths of the *trn*L intron ranged from 525 bp in *P. minus* to 652 bp in *P. lamelligera*, those of the *trn*L-*trn*F IGS were from 393 bp in *P. cornu-cervi* and *P. pantherina* to 412 bp in *P. amabilis*, and those of the *atp*B-*rbc*L IGS were from 627 bp in *P. pulcherrima* to 721 bp in *P. mannii*. Percentages of the G+C content for the *trn*L intron across *Phalaenopsis* species varied from 23.8% in *P. lamelligera* to 28.2% in *P. lowii*. Percentages of G+C content of the *trn*L-*trn*F IGS across *Phalaenopsis* species varied from 29.5% in *P. lobbii* and *P. parishii* to 31.1% in *P. aphrodite* and *P. sanderiana*. In addition, percentages of the G+C content of the *atp*B-*rbc*L IGS across *Phalaenopsis* species varied from 20.7% in *P. lindenii* to 24.3% in *P. minus* (Table 7).

Sequence alignment

The *trn*L introns from the 52 *Phalaenopsis* species and the five species of related genera were aligned and resulted in 820 characters (data not shown). In the alignment sequences of the *trn*L intron, there were 536 conserved sites and 174 variable sites among the 52 *Phalaenopsis* species. The ratio of variable sites was 24.5%. Among the 57 taxa studied, these values were 470 and 283, respectively, and the ratio of variable sites was 37.6%. The IGS regions of *trn*L-*trn*F from the 52 *Phalaenopsis* species and the five outgroups were aligned and resulted in 466 characters (data not shown). In the alignment sequences of the *trn*L-*trn*F IGS, there were 376 conserved sites and 50 variable sites among the 52 *Phalaenopsis* species. The ratio of variable sites in the alignment of the *trn*L-*trn*F IGS was 11.7%. Among the 57 taxa studied, these values were 348 and 79, respectively, and the ratio of variable sites was 18.5%. The IGS regions of *atp*B-*rbc*L from the 52 *Phalaenopsis* species

plus the five outgroups (with the exception of *Gastrochilus japonicus*) were aligned and resulted in 838 characters (data not shown). In the alignment sequences of the *atp*B-*rbc*L IGS, there were 614 conserved sites and 128 variable sites among the 52 *Phalaenopsis* species. The ratio of variable sites in the alignment of the *atp*B-*rbc*L IGS was 17.3% (Table 4). Among the 57 taxa with the exception of *Gastrochilus japonicus* studied, these values were 587 and 172, respectively, and the ratio of variable sites was 22.7% (Table 5). The *trn*L intron and the *trn*L-*trn*F IGS were combined and aligned, and this combination resulted in 1282 characters (data not shown). In addition, the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS were combined and aligned, and this combination resulted in 2103 characters (data not shown).

Sequence variations in length within an individual

Based on analyses of the aforementioned cpDNA sequences from the 52 *Phalaenopsis* studied, the sequence variation within an individual could not be found using direct sequencing of the PCR product (data not shown). However, I found two *Phalaenopsis* species, namely *P. gibbosa* and *P. lowii*, with different sequence lengths within an individual. In *P. gibbosa*, there were two different lengths of the DNA fragment of the *atp*B-*rbc*L IGS within an individual. The sequence lengths of two types of the *atp*B-*rbc*L IGS were 675 and 503 bp, respectively. Alignment of the two types of DNA fragments of the *atp*B-*rbc*L IGS from *P. gibbosa* is shown in Fig. 9. The short type of DNA fragment had a long deletion of 158 bp. In *P. lowii*, different lengths of DNA fragments were revealed in both the *trn*L intron and the *atp*B-*rbc*L IGS within an individual. Sequence lengths of the two types of the *trn*L intron were 554 and 415 bp, respectively. Comparing these two DNA fragments of the *trn*L intron from *P. lowii* with those of the other *Phalaenopsis* species, the long type of DNA fragment was determined to be the normal type. The alignment of these two types of the *trn*L intron from *P. lowii* showed a long deletion of 139 bp in the short type of DNA fragment (Fig. 10). In the *atp*B-*rbc*L IGS, the normal type of sequence length was 662 bp and short type of DNA fragment was 419 bp. The alignment of the two types of DNA sequences showed a long deletion of 244 bp in the shorter DNA fragment (Fig. 11).

Genetic distances

The *trn*L intron—Genetic distances of the *trn*L intron obtained from the 57 taxa of this study were in the range of from 0.000 to 0.311 with an average of 0.040 using the

two-parameter method of Kimura (1980). The outgroups, *Tuberolabium kotoense*, and *Phalaenopsis fimbriata*, were more divergent since they had a genetic distance of 0.311. Among the 52 species of the genus *Phalaenopsis*, the ranges of genetic distances were from 0.000 to 0.087 with an average of 0.028. *P. minus* and *P. fimbriata* were more divergent since they had a genetic distance of 0.087. Within the subgenus *Phalaenopsis*, the ranges of genetic distances were also from 0.000 to 0.029 with an average of 0.017. *P. philippinensis* and *P. schilleriana* were more closely related since they had a genetic distance of 0.000. In contrast, *P. pulcherrima* and *P. sanderiana* as well as *P. pulcherrima* and *P. stuartiana* were more divergent since they had a genetic distance of 0.029. Within the subgenus *Polychilos*, the ranges of genetic distances were from 0.000 to 0.082 with an average of 0.020. In contrast, *P. viridis* and *P. fimbriata* were more divergent since they had a genetic distance of 0.082. Within the subgenus *Parishianae*, genetic distances ranged from 0.007 to 0.009 with an average of 0.0085. *P. parishii* and *P. lobbii* were more closely related since they had a genetic distance of 0.007. In contrast, *P. gibbosa* and *P. parishii* (or *P. lobbii*) were more divergent since they had a genetic distance of 0.009. Within the subgenus *Aphyllae*, the ranges of genetic distances were from 0.005 to 0.043 with an average of 0.029. *P. wilsonii* and *P. braceana* were more closely related since they had a genetic distance of 0.005. In contrast, *P. wilsonii* and *P. minus* were more divergent since they had a genetic distance of 0.043 (Table 8).

The *trn*L-*trn*F IGS—Genetic distances of the *trn*L-*trn*F IGS obtained from the 57 taxa studied were in the range of from 0.000 to 0.074 with an average of 0.017 using the two-parameter method of Kimura (1980). The outgroup, *Tuberolabium kotoense*, and *Phalaenopsis viridis* were more divergent since they had a genetic distance of 0.074. Among the 52 species of the genus *Phalaenopsis*, the ranges of genetic distances were from 0.000 to 0.034 with an average of 0.012. *P. celebensis* and *P. viridis* were more divergent since they had a genetic distance of 0.034. Within the subgenus *Phalaenopsis*, the ranges of genetic distances were also from 0.000 to 0.026 with an average of 0.010. *P. deliciosa* and *P. celebensis* as well as *P. pulcherrima* and *P. celebensis* were more divergent since they had a genetic distance of 0.026. Within the subgenus *Polychilos*, the ranges of genetic distances were from 0.000 to 0.026 with an average of 0.008. *P. viridis* and *P. micholitzii* as well as *P. viridis* and *P. lamelligera*/*P. borneensis*) were more divergent since they had a genetic distance of 0.026. Within the subgenus *Parishianae*, genetic distances ranged from 0.000 to

0.003 with an average of 0.002. *P. parishii* and *P. lobbii* were more closely related since they had a genetic distance of 0.000. In contrast, *P. gibbosa* and *P. lobbii* as well as *P. gibbosa* and *P. parishii* were more divergent since they had a genetic distance of 0.003. Within the subgenus *Aphyllae*, the ranges of genetic distances were from 0.000 to 0.010 with an average of 0.007. *P. wilsonii* and *P. braceana* were more closely related since they had a genetic distance of 0.000. In contrast, *P. wilsonii* and *P. minus* as well as *P. braceana* and *P. minus* were more divergent since they had a genetic distance of 0.010 (Table 9).

Combined data of sequences of the *trn*L intron and the *trn*L-*trn*F IGS－Genetic distances of combined data of sequences of the *trn*L intron and the *trn*L-*trn*F IGS obtained from the 57 taxa were in the range of from 0.001 to 0.167 with an average of 0.029 using the two-parameter method of Kimura (1980). The outgroup, *Tuberolabium kotoense*, and *Phalaenopsis fimbriata* were more divergent since they had a genetic distance of 0.167. Among the 52 species of the genus *Phalaenopsis*, the ranges of genetic distances were from 0.001 to 0.046 with an average of 0.021. *Phalaenopsis minus* and *P. borneensis* were more divergent since they had a genetic distance of 0.021. Within the subgenus *Phalaenopsis*, the ranges of genetic distances were also from 0.001 to 0.028 with an average of 0.017. *P. aphrodite* and *P. sanderiana* were more closely related since they had a genetic distance of 0.001. In contrast, *P. sanderiana* and *P. pulcherrima* were more divergent since they had a genetic distance of 0.028. Within the subgenus *Polychilos*, the ranges of genetic distances were from 0.000 to 0.063 with an average of 0.015. *P. viridis* and *P. fimbriata* were more divergent since they had a genetic distance of 0.063. Within the subgenus *Parishianae*, genetic distances ranged from 0.004 to 0.006 with an average of 0.0056. *P. parishii* and *P. lobbii* were more closely related since they had a genetic distance of 0.004. Within the subgenus *Aphyllae*, the ranges of genetic distances were from 0.003 to 0.028 with an average of 0.019. *P. wilsonii* and *P. braceana* were more closely related since they had a genetic distance of 0.003. In contrast, *P. wilsonii* and *P. minus* were more divergent since they had a genetic distance of 0.028 (Table 10).

The *atp*B-*rbc*L IGS－Genetic distances among the 57 taxa with the exception of *Gastrochilus japonicus* were in the range of from 0.000 to 0.091 with an average of 0.025 using the two-parameter method of Kimura (1980). The outgroup, *Tuberolabium kotoense*, and *Phalaenopsis lindenii* were more divergent since they had a genetic distance of 0.091.

Among the 52 species of the genus *Phalaenopsis*, the ranges of genetic distances were from 0.000 to 0.067 with an average of 0.022. *P. pulcherrima* and *P. lindenii* were more divergent since they had a genetic distance of 0.067. Within the subgenus *Phalaenopsis*, the ranges of genetic distances were also from 0.000 to 0.067 with an average of 0.026. *P. aphrodite* and *P. sanderiana* as well as *P. schilleriana* and *P. philippinensis* were more closely related since they had a genetic distance of 0.000. In contrast, *P. lindenii* and *P. pulcherrima* were more divergent since they had a genetic distance of 0.067. Within the subgenus *Polychilos*, the ranges of genetic distances were from 0.000 to 0.034 with an average of 0.013. *P. pallens*, *P. hieroglyphica*, and *P. lueddemanniana* were more closely related since they had a genetic distance of 0.000 from one other. In contrast, Between *P. cornu-cervi* and *P. cochlearis*, *P. parishii* or *P. kunstleri* were more divergent since they had a genetic distance of 0.034. Within the subgenus *Parishianae*, genetic distances ranged from 0.000 to 0.008 with an average of 0.005. *P. parishii* and *P. lobbii* were more closely related since they had a genetic distance of 0.000. In contrast, *P. parishii* and *P. gibbosa* as well as *P. lobbii* and *P. gibbosa* were more divergent since they had a genetic distance of 0.008. Within the subgenus *Aphyllae*, the ranges of genetic distances were from 0.012 to 0.042 with an average of 0.030. *P. wilsonii* and *P. braceana* were more closely related since they had a genetic distance of 0.012. In contrast, *P. wilsonii* and *P. minus* were more divergent since they had a genetic distance of 0.042 (Table 11).

Combined data of sequences of the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS—Genetic distances among the 57 taxa with the exception of *Gastrochilus japonicus* were in the range of from 0.004 to 0.130 with an average of 0.028 using the two-parameter method of Kimura (1980). Two outgroups, *Tuberolabium kotoense* and *Paraphalaenopsis labukensis*, were more divergent since they had a genetic distance of 0.130. Among the 52 species of the genus *Phalaenopsis*, the ranges of genetic distances were from 0.000 to 0.054 with an average of 0.022. *P. pallens*, *P. hieroglyphica*, and *P. lueddemanniana* were more closely related since they had a genetic distance of 0.000 one another. In contrast, *Phalaenopsis lamelligera*, and *P. pulcherrima* were more divergent since they had a genetic distance of 0.054. Within the subgenus *Phalaenopsis*, the ranges of genetic distances were also from 0.001 to 0.050 with an average of 0.024. *P. philippinensis* and *P. schilleriana* as well as *P. aphrodite* and *P. sanderiana* were more closely related since they had a genetic distance of 0.001. In contrast, *P. pulcherrima* and *P. lindenii* as well as *P. pulcherrima* and *P.*

sanderiana were divergent since they had a genetic distance of 0.050. Within the subgenus *Polychilos*, the ranges of genetic distances were from 0.000 to 0.037 with an average of 0.015. *P. pallens*, *P. hieroglyphica*, and *P. lueddemanniana* were more closely related since they had a genetic distance of 0.000 one another. *Phalaenopsis viridis* and *P. fimbriata* were more divergent since they had a genetic distance of 0.037. Within the subgenus *Parishianae*, genetic distances ranged from 0.002 to 0.007 with an average of 0.005. *P. parishii* and *P. lobbii* were more closely related since they had a genetic distance of 0.002. In contrast, *P. parishii* and *P. gibbosa* as well as *P. lobbii* and *P. gibbosa* were more divergent since they had a genetic distance of 0.007. Within the subgenus *Aphyllae*, the ranges of genetic distances were from 0.007 to 0.029 with an average of 0.021. *P. wilsonii* and *P. braceana* were more closely related since they had a genetic distance of 0.007. In contrast, *P. wilsonii* and *P. minus* were more divergent since they had a genetic distance of 0.029 (Table 12).

Phylogenetic reconstructions

The *trn*L intron—The phylogenetic tree inferred from the *trn*L intron is shown in Fig. 12. Based on the phylogenetic tree, the outgroups of the three *Paraphalaenopsis* species and *Gastrochilus japonicus* were nested within the clade of the genus *Phalaenopsis*. This result does not support the monophyly of the genus *Phalaenopsis* described by Christenson (2001). Excluding *P. lowii* (subgenus *Proboscidioides*), the remaining species of the genus *Phalaenopsis* showed a monophyly supported by a 92% interior branch test.

Based on the analysis of the *trn*L intron, the monophyly of the subgenus *Polychilos* was supported by a 95% interior branch test. Within the subgenus *Polychilos*, none of sections showed to be a monophyly. Two major separate groups were revealed within the subgenus *Polychilos*. For the first clade, sections *Amboinenses* (with three exceptions of *P. gigantea*, *P. doweryensis* and *P. maculata*), *Zebrinae*, and *Polychilos* formed a clade supported by a 96% interior branch test. Another clade included three species of the section *Amboinenses*, namely *Phalaenopsis gigantea*, *P. doweryensis* and *P. maculata*, and the section *Fuscatae* supported by a 96% interior branch test. Furthermore, *P. lowii*, *P. minus* (subgenus *Aphyllae*), *P. pulcherrima* (section *Esmeralda*), *P. chibae* and *P. deliciosa* (section *Deliciosae*), and *P. sumatrana* (subgenus *Polychilos*) did not form a clade with the other *Phalaenopsis* species showing to be unique taxa. Of them, *P. deliciosa* had a sister relationship to the remaining of the genus *Phalaenopsis* with the exception of *P. lowii*.

Members of the subgenus *Phalaenopsis* did not show to be a monophyletic group based

on the analysis of the *trn*L intron. Within the subgenus *Phalaenopsis*, the section *Deliciosae* did not show to be a monophyly. In contrast, both sections of *Phalaenopsis* and *Stauroglottis* were shown to be monophyletic groups. These monophyletic groups, however, did not have highly statistical support (< 50%).

Furthermore, the subgenus *Parishianae* was shown to be a monophyly supported by a 96% interior branch test. On the other hand, the subgenus *Aphyllae* was not shown to be a monophyly. Within the subgenus *Aphyllae*, *Phalaenopsis minus* was separated from other species of the subgenus *Aphyllae*, namely *P. wilsonii* and *P. braceana*. In contrast, it formed a clade with *P. pulcherrima* with weak support (< 50%).

The *trn*L-*trn*F IGS—The phylogenetic tree inferred from the *trn*L-*trn*F IGS is shown in Fig. 13. Based on the phylogenetic tree, the monophyly of the genus *Phalaenopsis* described by Christenson (2001) was supported by a 94% interior branch test.

Within the genus *Phalaenopsis*, low resolution of the topology has been shown based on the phylogenetic tree inferred from the *trn*L-*trn*F IGS. The monophyly of the subgenus *Polychilos* was not supported due to the sections *Phalaenopsis* and *Stauroglottis* of the subgenus *Phalaenopsis* were nested within the subgenus *Polychilos*. Furthermore, none of the sections of the subgenus *Polychilos* was shown to be a monophyly.

The subgenus *Phalaenopsis* (with the exception of sections *Esmeralda* and *Deliciosae*), namely sections *Phalaenopsis* and *Stauroglottis*, showed a monophyly supported by a 92% interior branch test. These two sections, however, could not be separated from each other. In addition, species of the section *Deliciosae* formed a clade with weak support and had a sister relationship to the subgenus *Parishianae*. Moreover, *P. pulcherrima* (section *Esmeralda*) formed a clade with both *P. minus* and *P. lowii* with weak support.

In addition, the subgenus *Parishianae* was shown to be a monophyly supported by an 87% interior branch test. Furthermore, the subgenus *Aphyllae* was not shown to be a monophyly. Within the subgenus *Aphyllae*, *P. minus* was separated from other members of the subgenus *Aphyllae*, namely *P. wilsonii* and *P. braceana*. In contrast, it formed a clade with *P. pulcherrima* with weakly supported (< 50%).

Combined data of sequences of the *trn*L intron and the *trn*L-*trn*F IGS — The phylogenetic tree inferred from the combined data of sequences of the *trn*L intron and the *trn*L-*trn*F IGS is shown in Fig. 14. Based on the phylogenetic tree, the genera

Paraphalaenopsis, *Tuberolabium*, and *Gastrochilus* were not nested within the genus *Phalaenopsis*. It also shows that the genus *Phalaenopsis* described by Christenson (2001) is a monophyly supported by a 99% interior branch test.

The monophyly of the subgenus *Polychilos* was weakly supported (< 50%) based on the combined data of sequences of the *trn*L intron and the *trn*L-*trn*F IGS. Within the subgenus *Polychilos*, none of sections was shown to be a monophyly. Two major separate groups were revealed within the subgenus *Polychilos*. Sections *Amboinenses* (with three exceptions of *P. gigantea*, *P. doweryensis*, and *P. maculata*), *Zebrinae*, and *Polychilos* formed a clade supported by a 98% interior branch test. Another clade included the three species of the section *Amboinenses*, namely *Phalaenopsis gigantea*, *P. doweryensis* and *P. maculata*, and the section *Fuscatae* supported by a 99% interior branch test. Within the clade, *P. gigantea* was nested within the section *Fuscatae*.

The subgenus *Phalaenopsis* with the exception of *P. pulcherrima* (section *Esmeralda*), showed a monophyly supported by a 91% interior branch test. *P. pulcherrima* showed a sister relationship to the subgenus *Polychilos* supported by a 98% interior branch test. Within the subgenus *Phalaenopsis*, the section *Deliciosae* showed a monophyly supported by an 82% interior branch test. Neither of the sections of *Phalaenopsis* and *Stauroglottis* was shown to be a monophyly due to *P. lindenii* (section *Stauroglottis*) was nested within the section *Phalaenopsis*.

In addition, the subgenus *Parishianae* was shown to be a monophyly supported by a 99% interior branch test. Furthermore, the subgenus *Aphyllae* was not shown to be a monophyly. Within the subgenus *Aphyllae*, *P. minus* was separated from the other members of the subgenus *Aphyllae*, namely *P. wilsonii* and *P. braceana*. In contrast, it formed a clade with the monotypic subgenus *Proboscidioides*, *P. lowii*, supported by a 61% interior branch test.

The *atp*B-*rbc*L IGS—The phylogenetic tree inferred from the *atp*B-*rbc*L IGS is shown in Fig. 15. Based on the phylogenetic tree, it shows that the genus *Phalaenopsis* described by Christenson (2001) is not a monophyly. A *Phalaenopsis* species, *P. pulcherrima* (section *Esmeralda*of the subgenus *Phalaenopsis*), was separated from the other *Phalaenopsis* species. In addition, the three *Paraphalaenopsis* species (outgroups) were formed a clade and nested within members of the genus *Phalaenopsis*.

In the subgenus *Polychilos*, it did not show a monophyly based on the phylogenetic tree

inferred from the *atp*B-*rbc*L IGS. Most species of the subgenus *Polychilos*, namely sections *Polychilos*, *Zebrinae*, and *Amboinenses* with three exceptions of *Phalaenopsis gigantea*, *P. doweryensis*, *P. maculata*, formed a clade supported by a 93% interior branch test. Two species of the section *Amboinenses*, namely *Phalaenopsis gigantea* and *P. doweryensis* (section *Amboinenses*) and the section *Fuscatae* formed a clade supported by a 97% interior branch test. In addition, *Phalaenopsis maculata* (section *Amboinenses*) had a sister relationship to aforementioned clade. In short, none of the sections of the subgenus *Polychilos* was shown to be a monophyly.

The genus *Phalaenopsis* was not a monophyly based on the phylogenetic tree inferred from the *atp*B-*rbc*L IGS. The subgenus *Phalaenopsis* also did not show to be a monophyly since sections *Deliciosae* and *Esmeralda* were separated from sections of *Phalaenopsis* and *Stauroglottis*. Within the subgenus *Phalaenopsis*, each of the sections of *Deliciosae*, *Phalaenopsis*, and *Stauroglottis* was shown to be a monophyly. Of them, the section *Deliciosae* was shown to be a monophyly supported by a 92% interior branch test and formed a clade with subgenus *Parishianae* supported weakly by an interior branch test (< 50%). Members of the section *Phalaenopsis* also formed a clade with weak support by an interior branch test. Members of the section *Stauroglottis* formed a clade supported by a 79% interior branch test. In particular, *P. pulcherrima* (section *Esmeralda*) showed a unique species and formed a clade with the outgroup, *Tuberolabium kotoense*, with weak support.

Furthermore, the subgenus *Parishianae* was shown to be a monophyly supported by a 74% interior branch test. The monotypic subgenus *Proboscidioides*, *Phalaenopsis lowii*, was shown to be having a sister relationship to parts of the subgenus *Aphyllae*, namely *P. wilsonii* and *P. braceana*. The subgenus *Aphyllae* was not shown to be a monophyly because that *Phalaenopsis minus* was separated from the remaining species of the subgenus *Aphyllae*. In contrast, *P. minus* had a sister relationship to the clade, which included the section *Fuscatae* and the three species of the section *Amboinenses*, namely *P. gigantea*, *P. doweryensis*, and *P. maculata*.

Combined molecular data of chloroplast DNA—The phylogenetic tree inferred from the combined data of cpDNAs is shown in Fig. 16. Based on the phylogenetic tree, the species of genera *Paraphalaenopsis*, *Tuberolabium*, and *Gastrochilus* were not nested within the genus *Phalaenopsis*. It also shows that the genus *Phalaenopsis* described by Christenson (2001) is a monophyly supported by a 94% interior branch test.

The monophyly of the subgenus *Polychilos* was supported based on combined data of cpDNAs with weak support (of a 77% interior branch test). Within the subgenus *Polychilos*, none of sections was shown to be a monophyly. Two major separate groups were revealed within the subgenus *Polychilos*. The sections *Amboinenses* (with three exceptions of *Phalaenopsis gigantea*, *P. doweryensis*, and *P. maculata*), *Zebrinae*, and *Polychilos* formed a clade supported by a 99% interior branch test. Another clade included the three species of the section *Amboinenses*, namely *Phalaenopsis gigantea*, *P. doweryensis* and *P. maculata*, and the section *Fuscatae* supported by a 99% interior branch test. Within the clade, *P. gigantea* and *P. doweryensis* were nested within the section *Fuscatae*.

With three exceptions of *P. pulcherrima* (section *Esmeralda*), *P. chibae*, and *P. deliciosa* (section *Deliciosae*), the subgenus *Phalaenopsis* showed a monophyly supported by a 77% interior branch test. Neither of the sections of *Phalaenopsis* and *Stauroglottis* was showed to be a monophyly due to members of the section *Stauroglottis* were nested within the section *Phalaenopsis*. Furthermore, *P. pulcherrima* (section *Esmeralda*) formed a clade with *P. minus* (subgenus *Aphyllae*) with weak support by a 74% interior branch test. Members of the section *Deliciosae* was shown to be a monophyly supported by a 94% interior branch test and formed a clade with members of the subgenus *Parishianae* supported by a 98% interior branch test.

In addition, the subgenus *Parishianae* was shown to be a monophyly supported by an 82% interior branch test. Furthermore, the subgenus *Aphyllae* was not shown to be a monophyly. Within the subgenus *Aphyllae*, *Phalaenopsis minus* was separated from the other members of the subgenus *Aphyllae*, namely *P. wilsonii* and *P. braceana*. In contrast, it formed a clade with *P. pulcherrima* (section *Esmeralda* of the subgenus *Phalaenopsis*) and separated from the remaining species of *Phalaenopsis*. In monotypic subgenus *Proboscidioides*, *P. lowii*, formed a clade with both *P. wilsonii* and *P. braceana* (subgenus *Aphyllae*) supported by a 94% interior branch test.

Combined data of nuclear DNA and chloroplast DNA

Sequence analyses and genetic distances

Combined data of the ITS of nrDNA and cpDNAs (including the *trn*L intron, the *trn*L-*trn*F IGS, and the IGS *atp*B-*rbc*L) from the 52 *Phalaenopsis* species plus the four outgroups of related genera, namely *Paraphalaenopsis laycockii*, *Par. labukensis*, *Par. serpentilingua*, and *Tuberolabium kotoense*, were aligned and resulted in 2872 characters

(data not shown). Genetic distances among the 56 taxa of this study were in the range of from 0.001 to 0.136 with an average of 0.043 using the two-parameter method of Kimura (1980). *Tuberolabium kotoense* and *P. kunstleri* were more divergent since they had a genetic distance of 0.136. Among the 52 species of the genus *Phalaenopsis*, the ranges of genetic distances were from 0.001 to 0.067 with an average of 0.038. *Phalaenopsis kunstleri* and *P. cornu-cervi* were more divergent since they had a genetic distance of 0.067. Within the subgenus *Phalaenopsis*, the ranges of genetic distances were also from 0.003 to 0.051 with an average of 0.035. *Phalaenopsis aphrodite* and *P. sanderiana* were more closely related since they had a genetic distance of 0.003. In contrast, *P. amabilis* and *P. pulcherrima* were more divergent since they had a genetic distance of 0.051. Within the subgenus *Polychilos*, the ranges of genetic distances were from 0.001 to 0.067 with an average of 0.028. *Phalaenopsis tetraspis* and *P. sumatrana* as well as *P. violacea* and *P. bellina* were more closely related since they had a genetic distance of 0.001. In contrast, *Phalaenopsis kunstleri* and *P. cornu-cervi* were more divergent since they had a genetic distance of 0.067. Within the subgenus *Parishianae*, genetic distances ranged from 0.005 to 0.017 with an average of 0.013. *Phalaenopsis parishii* and *P. lobbii* were more closely related since they had a genetic distance of 0.005. In contrast, *P. parishii* and *P. gibbosa* as well as *P. lobbii* and *P. gibbosa* were more divergent since they had genetic distances of 0.017. Within the subgenus *Aphyllae*, the ranges of genetic distances were from 0.007 to 0.031 with an average of 0.022. *Phalaenopsis wilsonii* and *P. braceana* were more closely related since they had a genetic distance of 0.007. In contrast, *P. wilsonii* and *P. minus* were more divergent since they had a genetic distance of 0.031 (Table 13).

Phylogenetic reconstruction

The phylogenetic tree of combined data of ITSs of nrDNA and cpDNA constructed following the NJ method is shown in Fig. 17. Based on the phylogenetic tree, species of the genera *Paraphalaenopsis* and *Tuberolabium* were not nested within the genus *Phalaenopsis*. This shows that the genus *Phalaenopsis* described by Christenson (2001) is a monophyly supported by a 99% interior branch test. Two major clades were shown within the genus *Phalaenopsis*. The first clade comprised the subgenus *Polychilos* and the sections *Phalaenopsis* and *Stauroglottis* of the subgenus *Phalaenopsis*. The second clade consisted of the subgenera *Proboscidioides*, *Parishianae*, and *Aphyllae* and the sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis*.

The monophyly of the subgenus *Polychilos* studied was weakly supported by the interior branch test based on the phylogenetic tree obtained from combined data of the ITSs of nrDNA and cpDNA. Within the subgenus *Polychilos*, none of sections was shown to be a monophyly. In contrast, two separate groups were revealed within the subgenus. The first group included the sections *Zebrinae* and *Polychilos*, and the section *Amboinenses* with the exception of *Phalaenopsis gigantea, P. doweryensis*, and *P. maculata,* as supported by a 99% interior branch test. The second group included the section *Fuscatae* and the three species of the section *Amboinenses*, namely *P. gigantea, P. doweryensis*, and *P. maculata,* as supported by a 99% interior branch test. In addition, parts of the section *Amboinenses*, namely *P. hieroglyphica, P. reichenbachiana, P. bastianii, P. pallens, P. lueddemanniana, P. fasciata*, and *P. pulchra,* formed a clade supported by a 99% interior branch test, and *P. mariae* had a sister relationship to this clade. In addition, most of the section *Zebrinae* (*P. corningiana, P. sumatrana,* and *P. tetraspis*) and one species of the section *Amboinenses* (*P. venosa*) formed a clade supported by a 98% interior branch test. The section *Polychilos* was not shown to be a monophyly since *P. mannii* was separated from the remaining species of the section *Polychilos*.

The monophyly of the subgenus *Phalaenopsis* was also not supported based on the molecular evidence. Sections *Deliciosae* and *Esmeralda* were separated from the remaining members of the subgenus *Phalaenopsis*. Within the subgenus *Phalaenopsis,* the section *Phalaenopsis* showed a monophyly supported by a 99% interior branch test. Within the section *Stauroglottis, P. celebensis* showed divergence with the other species of this section. As to the remaining species of the section *Stauroglottis,* namely *P. equestris* and *P. lindenii*, they were shown to be closely related to each other. In addition, the section *Deliciosae* was shown to be a monophyly with weak support (of a 67% interior branch test).

The subgenus *Parishianae* was shown to be a monophyly supported by an 88% interior branch test. The section *Deliciosae* of the subgenus *Phalaenopsis* showed a sister relationship to the subgenus *Parishianae*. Furthermore, the subgenus *Aphyllae* was shown not to be a monophyly, due to *P. lowii* (subgenus *Proboscidioides*) being nesting within this group. In addition, the subgenera *Aphyllae, Parishianae*, and *Proboscidioides,* and the section *Deliciosae* of the subgenus *Phalaenopsis* formed a clade supported by a 91% interior branch test. In addition, *P. pulcherrima* (section *Esmeralda*) showed a sister relationship to this clade.

Biogeography

The matrix of the geographical distributions of the genus *Phalaenopsis* was summarized and is shown in Fig. 18. According to the matrix, the biogeographical tree of the genus *Phalaenopsis* was constructed based on the Neighbor-joining method (Fig. 19). Based on the tree, New Guinea and Australia formed a clade. The second clade included Sulawesi and the Molucca Is. The third clade included Taiwan and the Philippines; the fourth clade included the Malay Peninsula, Borneo, Sumatra, and Java; and the fifth clade included India/Sri Lanka, Indochina, and South China. Furthermore, Flores was shown to be an independent clade (Fig. 19). In addition, the phylogenetic tree inferred from the combined data of ITSs and cpDNA was labeled by the geographical distributions of each *Phalaenopsis* species to show the connection between the phylogenetic relationship and geographical distribution of the genus *Phalaenopsis* (Fig. 20).

Evolutionary trends

The evolutionary phylogenetic tree derived from combined data of nuclear DNA and chloroplast DNA was constructed using the minimum-evolution method and is shown in Fig. 21. According to the molecular phylogeny of the genus *Phalaenopsis*, two major clades were shown, namely the four-pollinium clade and the two-pollinium clade. Based on molecular data, the genus *Phalaenopsis* can be divided nine groups, namely the subgenera *Proboscidioides*, *Aphyllae*, and *Parishianae*; sections *Conspicuum*, *Deliciosae*, and *Esmeralda* (belonging to four-pollinium species); subgenera *Polychilos* (with the exception of the section *Fuscatae* plus *P. gigantea*, *P. doweryensis*, and *P. maculata*) and *Phalaenopsis* (with the exception of sections *Deliciosae* and *Esmeralda*); and the section *Fuscatae* plus *P. gigantea*, *P. doweryensis*, and *P. maculata* (belonging to two-pollinium species). According to the evolutionary trend of pollinium number (Holttum, 1959; Dressler, 1993), the four-pollinium clade was the relative origin clade of the genus *Phalaenopsis*. To evaluate the relative origin group within the four-pollinium clade, namely subgenera *Proboscidioides*, *Aphyllae*, and *Parishianae* as well as sections *Conspicuum*, *Deliciosae*, and *Esmeralda*, genetic distances were calculated. Each group of the four-pollinium clade was respectively placed at the root for testing. The sum of the genetic distances of the subgenus *Aphyllae* when it was rooted was shown to be the smallest (at 0.147) (Table 14). These results therefore suggest that the subgenus *Aphyllae* was the relative origin group among the four-pollinium clade. According to the evolutionary trend of pollinium number (Holttum,

1959; Dressler, 1993) and the concept of minimum evolution (Edwards and Cavalli-Sforza, 1963), the subgenus *Aphyllae* was suggested to be the relative origin group of the genus *Phalaenopsis*. In order to provide insights into the evolutionary trends of the genus *Phalaenopsis*, the evolutionary phylogenetic tree (Fig. 21) derived from combined data of nrDNA and cpDNA of this study was rooted based on the putative origin group, the subgenus *Aphyllae*, and species within consistent subgenera/sections were compressed (Fig. 22). Finally, I mapped the rooted-compressed evolutionary phylogenetic tree onto the geographical distributions of *Phalaenopsis* (Fig. 23).

Substitution rates of the genus Phalaenopsis

Tajima's test of neutrality was examined for both ITS data and cpDNA data from the genus *Phalaenopsis*. The results showed that nucleotide mutations of both ITS and cpDNA data were neutral (Table 15). Tajima's relative rate tests for both ITS and cpDNA data between the species of the sections *Amboinenses* (with the exception of *P. gigantea*, *P. doweryensis*, and *P. maculata*) and *Zebrinae*, namely *P. bellina*, *P. corningiana*, *P. fimbriata*, *P. inscriptiosinensi*, *P. javanica*, *P. modesta*, *P. sumatrana*, *P. tetraspis*, and *P. violacea* distributed on the Sunda Shelf and species of the *P. lueddemanniana* complex distributed in the Philippines, were evaluated using the hypothesis of equal evolutionary rates in comparison with the reference taxon, *P. lobbii* (a species of the subgenus *Parishianae*), at a significance level of 5%. The results showed that no species was rejected in the ITS data (Table 16), while *P. bellina*, *P. fimbriata*, and *P. violacea* were rejected in the cpDNA data (Table 17). The number of differences in the ITS and cpDNA data between the group of the section *Amboinenses* (with the exception of the *P. lueddemanniana* complex) and each species of the *P. lueddemanniana* complex is shown in Tables 18 and 19, respectively. According to geological events concerning the combination between the Philippines and Borneo (5~10 Mya) and molecular data, the average substitution rates of both the ITS and cpDNA data were shown to be 2.4~4.7 x 10^{-9} and 3.9~7.8 x 10^{-10} substitutions/site/year, respectively (Tables 18, 19). Based on the above substitution rates of the genus *Phalaenopsis*, the divergence times of species of the *P. lueddemanniana* complex could be calculated. First, *P. bellina* (a species of the section *Amboinenses* distributed in Borneo) was set as the reference group, and Tajima's relative rate tests for both the ITS and cpDNA data among species of the *P. lueddemanniana* complex were evaluated to test the molecular clock hypothesis. For ITS data, this complex, with the exception of *P. hieroglyphica*, was in

agreement with the hypothesis of an equal evolutionary rate (Table 20). Each species of this complex, however, was in agreement with the hypothesis of an equal evolutionary rate for cpDNA data (Table 21). The number of differences for both ITS and cpDNA data among the species of the *P. lueddemanniana* complex is shown in Tables 22 and 23, respectively. Based on the substitution rates of both the ITS and cpDNA data, the divergence times among species of the *P. lueddemanniana* complex could be estimated and are shown in Tables 24 and 25, respectively. Excluding two divergent species of the *P. lueddemanniana* complex, namely *P. mariae* and *P. reichenbachiana*, the average nucleotide mutations of both the ITS and cpDNA data among this complex were 6.60 ± 1.57 and 1.67 ± 0.72, respectively. The average divergence times obtained from the substitution rates of the ITS and cpDNA data among this complex were 2.9 ± 0.69 to 1.5 ± 0.35 million years (my) and 1.3 ± 0.56 to 0.65 ± 0.28 my, respectively. Furthermore, the divergence time between the sections *Deliciosae* and *Stauroglottis* can be estimated based on the substitution rate. Tajima's relative rate tests for both the ITS and cpDNA data between species of the section *Stauroglottis* and the *P. lueddemanniana* complex were evaluated using the hypothesis of equal evolutionary rates in comparison with the reference taxon, *P. lobbii* (subgenus *Parishianae*), at a significance level of 5%. The results showed that *P. lindenii* of the section *Stauroglottis* was rejected by the test of the hypothesis of equal evolutionary rates in cpDNA data (Table 26). Therefore, that species was excluded from the calculation of the divergence time between groups of the sections *Deliciosae* and *Stauroglottis* in cpDNA data. Numbers of differences for both the ITS and cpDNA data between these two groups were 48.17 ± 4.90 and 26.50 ± 3.83, respectively. According to the substitution rates of both the ITS and cpDNA data, the divergence times between the sections *Deliciosae* and *Stauroglottis* were 20.9 ± 2.1 to 10.7 ± 1.1 and 21.2 ± 2.1 to 10.6 ± 1.5 Mya, respectively (Table 27).

Discussion

Sequence characteristics

ITS data

Lengths of the ITS1 and ITS2 regions in various angiosperms range from 187 to 298 bp and from 187 to 252 bp, respectively (Baldwin et al., 1995). In the genus *Phalaenopsis*, lengths of ITS1 and ITS2 ranged from 213 to 255 bp and from 256 to 270 bp, respectively. These ranges of the genus *Phalaenopsis* are within the ITS variation of angiosperms. In addition, the G+G contents of ITS1 and ITS2 in the genus *Phalaenopsis* ranged

63.1%~78.2% and 62.8%~77.9%, respectively. These ranges are a little higher than those of other diverse angiosperms (Tsai and Chou, 2000).

The ITSs of nrDNA from *Phalaenopsis* species were directly sequenced based on the PCR products. Excluding *P.* ×*intermedia*, the ITS repeat sequences of the other *Phalaenopsis* species studied were homogeneous within an individual. This indicates that concerted evolution of the ITS repeat sequence of *Phalaenopsis* species does work. As to *P.* ×*intermedia*, a natural hybrid species, showed heterogeneous sequences of the ITSs of nrDNA within an individual. This result indicates that *P.* ×*intermedia* bears different types of ITS sequences within an individual. In addition, the ITSs of nrDNA of the other three accessions of *P.* ×*intermedia* were analyzed. The results support the ITS repeat sequences of *P.* ×*intermedia* being heterogeneous (data not shown). In fact, this natural hybrid has been proven between *P. aphrodite* and *P. equestris* using artificial hybridization of both species (cf. Christenson, 2001). In order to obtain the molecular evidences, the heterogeneous repeat sequences of ITSs from one plant of *P.* ×*intermedia* were amplified by PCR and sequenced by T-vector based sequencing. The ITS sequences (of five clones) of *P.* ×*intermedia* was aligned with those of species of the sections *Phalaenopsis* and *Stauroglottis* (Fig. 24) and the phylogenetic tree was reconstructed by the NJ method (Fig. 25). The results indicate that ITS repeat sequences of nrDNA from *P.* ×*intermedia* include similar ITS sequences of both *P. aphrodite* and *P. equestris*. Therefore, the molecular evidences support *P.* ×*intermedia* being a natural hybrid between *P. aphrodite* and *P. equestris*. Furthermore, the blooming periods of *P. aphrodite* and *P. equestri*s overlap in the wild. Therefore, I suggest that natural hybridization could easily occur between *P. aphrodite* and *P. equestri*s. In addition, the natural hybrid of *P.* ×*intermedia* might be recently derived from the hybridization between *P. aphrodite* and *P. equestri*s, and concerted evolution had not homogenized the sequence divergence of ITS repeat sequences. Generally, concerted evolution of those repeat DNA sequences is considered to be controlled by the mechanisms of unequal crossing-over (Smith, 1976) and gene conversion (Baltimore, 1981). As to the other repeat sequences of nrDNA, Kao (2001) analyzed the IGS repeat sequences of 5S rDNA from *Phalaenopsis* and found that concerted evolution of the regions was apparently not evident. The sequence divergence of IGS repeat sequences within an individual ranged from 0.061% to 14.84% among 29 *Phalaenopsis* species. Furthermore, similar results were also found in the IGS of 5S rDNA from different plants (Allaby and Brown, 2001; Liu et al., 2003). These results indicate that the IGS repeat sequences of 5S rDNA are heterogeneous within an individual. This is why

Allaby and Brown (2001) proposed that 5S rDNA arrays contain a large store of mutant variations in both genes and IGS regions.

Chloroplast DNA data

The size range of the *trn*L intron in the genus *Phalaenopsis* (from 525 to 652 bp) fell in the range of several diverse taxa described by Gielly and Taberlet (1994). Therefore, the sequence lengths of the *trn*L intron were more variable than those of the *trn*L-*trn*F IGS (from 393 to 412 bp) among the 52 taxa of the genus *Phalaenopsis*. Previous reports had suggested that the *trn*L intron had a slightly lower resolution than other non-coding plastid DNA regions (Gielly and Taberlet, 1994, 1996). McDade and Moody (1999) also showed that the *trn*L-*trn*F IGS offered more-variable information than the *trn*L intron. In the present study of the genus *Phalaenopsis*, however, the *trn*L intron (with 174 variable sites, 24.5% in total) offered more-variable information than did the *trn*L-*trn*F IGS (with 50 variable sites, 11.7% in total). Therefore, the phylogenetic tree of the genus *Phalaenopsis* based on the *trn*L-*trn*F IGS had a lower resolution than that of the *trn*L intron. Furthermore, the phylogenetic tree based on the *trn*L intron was not very congruent with that of the *trn*L-*trn*F IGS. Within the genus *Phalaenopsis*, sequencing of both the *trn*L intron and the *trn*L-*trn*F IGS produced a sufficient number of variable sites for distinguishing most of the taxa studied. On the other hand, the *atp*B-*rbc*L IGS (with 128 variable sites, 17.3% in total) obtained from the genus *Phalaenopsis*, offered moderately variable information. Therefore, the phylogenetic tree obtained from the *atp*B-*rbc*L IGS had a higher resolution than that of *trn*L-*trn*F, but lower than that of the *trn*L intron.

Among the 52 *Phalaenopsis* species, two species, namely *P. lowii* and *P. gibbosa*, showed different types of sequence lengths in both DNA regions of the *trn*L intron and the *atp*B-*rbc*L IGS of cpDNA. Of these two types of sequences, the long type of DNA fragment is the normal type. Therefore, the molecular phylogeny of the genus *Phalaenopsis* was clarified based on the normal type of cpDNA fragment. The short types of DNA fragment in both the *trn*L intron and the *atp*B-*rbc*L IGS had a long deletion based on the sequence alignment. As with the previous description, short types of the *atp*B-*rbc*L IGS were found in both *P. gibbosa* and *P. lowii*. However, those of the *trn*L intron were only found in *P. lowii*. Until the present, I therefore can draw no conclusion as to whether the other *Phalaenopsis* species bear different types of DNA lengths in other DNA regions of the chloroplast genome within an individual. Perhaps, different lengths of DNA fragments might be found in other

Phalaenopsis species when we inspect other cpDNA. Generally, there are at least two available alternatives for clarifying the different types of cpDNA (including sequence and length variations) within an individual: (1) the different types of chloroplast DNA are all retained in the chloroplast genome, because occasional biparental inheritance of chloroplast genomes might occur (Second et al., 1989) or (2) the variant type of cpDNA might have been retained in the nuclear genome through gene transfer from the chloroplast genome to the nuclear genome during evolutionary times (Huang et al., 2003). In the present study, the sequence of the short type of DNA was not similar to that of other species of *Phalaenopsis*. This suggests that these short types of chloroplast DNA sequences in the present study were not retained through biparental inheritance. Nevertheless, I can draw no conclusion about how these short types of chloroplast DNA in this study have been retained in the nuclear genome to the present time.

Congruence or incongruence between phylogenetic trees

Among analyses of the trnL intron, the trnL-trnF IGS, and the atpB-rbcL IGS

In a focus on the phylogeny of the genus *Phalaenopsis*, the phylogenetic tree obtained from the *trn*L-*trn*F IGS supports the monophyly of the genus *Phalaenopsis*. In contrast, neither of the trees obtained from data of sequences of the *trn*L intron or the *atp*B-*rbc*L IGS supported the monophyly of the genus *Phalaenopsis*, since *P. lowii* was separated from the other *Phalaenopsis* species in the tree inferred from data of the *trn*L intron (Fig. 12), and *P. pulcherrima* was separated from the other *Phalaenopsis* species in the tree inferred from data of the *atp*B-*rbc*L IGS (Fig. 15). However, analyses of the combined data of sequences of the *trn*L intron and the *trn*L-*trn*F IGS showed monophyly of the genus *Phalaenopsis* with high support (99%; Fig. 14). Since the location of the *trn*L intron in the chloroplast genome is next to the *trn*L-*trn*F IGS (Fig. 6), I prefer the analysis of the combined data rather than those of independent data. In addition, the phylogenetic tree obtained from the *atp*B-*rbc*L IGS was partially incongruent with those of the independent data of the *trn*L intron and the *trn*L-*trn*F IGS, and that of the combined data of the *trn*L intron and the *trn*L-*trn*F IGS. The topology of the phylogenetic tree inferred from the *atp*B-*rbc*L IGS had four remarkable incongruences with those of the independent data of the *trn*L intron and the *trn*L-*trn*F IGS, and with the combined data of the *trn*L intron and the *trn*L-*trn*F IGS. First, *P. minus*, a species of the subgenus *Aphyllae*, was separated from both the subgenus *Aphyllae* and the section *Deliciosae* of the subgenus *Phalaenopsis* instead of showing a close relation to the section

Fuscatae of the subgenus *Polychilos*. Second, the three outgroups of the genus *Paraphalaenopsis* were nested within the genus *Phalaenopsis*. Third, *P. pulcherrima* was shown to have a sister relationship to the outgroup, *T. kotoense*, and was separated from the remaining species of *Phalaenopsis*. Fourth, the phylogeny within the subgenus *Phalaenopsis* was not congruent among analyses using the *atp*B-*rbc*L IGS, the *trn*L intron, or the *atp*B-*rbc*L IGS. Analysis of the *trn*L intron did not show monophyly because of the sections *Deliciosae* and *Esmeralda* being separated from the sections *Phalaenopsis* and *Stauroglottis* of the subgenus *Phalaenopsis*. In contrast, trees of both the *trn*L intron and the *trn*L-*trn*F IGS supported the subgenus *Phalaenopsis*, with the exception of sections *Esmeralda* and *Deliciosae*, being a monophyletic group. Excluding the aforementioned incongruent branches, the topology of the phylogenetic tree reconstructed from data of the *atp*B-*rbc*L IGS was very similar to those reconstructed from the independent data of the *trn*L intron and the *trn*L-*trn*F IGS, and the combined data of the *trn*L intron and the *trn*L-*trn*F IGS.

In the present study, phylogenies of the genus *Phalaenopsis* inferred from different fragments of cpDNA showed variable incongruences. Therefore, the phylogeny obtained from specific DNA fragments just shows this gene tree, not the evolutionary tree. In order to make the gene tree closer to the evolutionary tree, additional DNA fragments are required. Since DNA fragments of the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS are located at the chloroplast genome, which is inherited through maternal inheritance, I thus discuss the phylogeny of the genus *Phalaenopsis* in the following paragraphs based on the combined data of sequences of the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS data.

Differences between analyses of data of ITSs of nrDNA and cpDNA

Chloroplast DNA evolves slowly relative to both nuclear DNA and animal mitochondrial DNA (Palmer et al., 1988). In the present study, sequences of ITS1 and ITS2 of nrDNA (with 303 variable sites, 56.2% of the total) from the genus *Phalaenopsis* offered more-variable information than data of each of the sequences of the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS individually. The ratio of variable sites in the sequences of ITS1+ITS2 among the 52 *Phalaenopsis* species was over twice that of the *trn*L intron, three times that of the *atp*B-*rbc*L IGS, and nearly five times that of the *trn*L-*trn*F IGS from the same group (Table 4). These results are in agreement with previous reports, which have shown higher sequence divergences between taxa in ITS regions than of the cpDNA region

from the same angiosperm group (Baldwin, 1992; Sang et al., 1994, 1995; Baldwin et al., 1995). Uniparental inheritance has been introduced in cpDNA (Derepas and Dulieu, 1992). Therefore, gene recombination between chloroplasts does not normally occur (Chiu and Sears, 1985). In contrast, nrDNA with biparental inheritance provides different insights into the phylogenetic reconstruction than does cpDNA. In the present study, the ITSs of nrDNA occur in multiple copies and are affected by rapid concerted evolution, which results in homogeneity among copies (Marynard, 1989; Tan et al., 1993). In the present study, compared to the ITS of nrDNA, the cpDNAs (including the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS) are relatively conserved. Furthermore, clades obtained from the combined analysis with lower statistical support might indicate conflicting data (Smedmark and Eriksson, 2002). The ITS of nrDNA and cpDNA were combined to investigate how a combined analysis compared to the topologies of the independent data, and where they agreed and disagreed. Congruence between the ITS of nrDNA and cpDNA was shown in some clades of this study. For example, the statistical support (99%) of the monophyly of the genus *Phalaenopsis* obtained from the analysis of the combined data was higher than that obtained from the independent data of both nrDNA ITS (88%) and cpDNA (94%). On the other hand, the incongruence between trees inferred from data of the ITS of nrDNA and cpDNA also was shown for several clades. For example, species of the section *Phalaenopsis* of the subgenus *Phalaenopsis* showed a monophyly with high statistical support (99%) as inferred from data of the ITS of nrDNA. This group, however, was not shown to be a monophyly based on the analysis of cpDNA, due to the section *Stauroglottis* being nested within the section *Phalaenopsis*. In addition, several conflicts were revealed in both *P. mannii* and *P. minus* between phylogenetic trees inferred from data of both the ITS of nrDNA and cpDNA. *Phalaenopsis mannii* had a close relationship to its allies (section *Polychilos*) in the tree inferred from data of the ITSs of nrDNA with weak support (77%). In contrast, it was separated from species of the section *Polychilos* and showed a sister relationship to a clade which included sections *Polychilos* and *Zebrinae*, and most of the section *Amboinenses*. *Phalaenopsis minus* was closely related to its allies (subgenus *Aphyllae*) in the tree inferred from data of the ITSs of nrDNA with weak support (79%). In contrast, it was separated from the other species of the subgenus *Aphyllae*, and instead showed a sister relationship to *P. pulcherrima* (section *Esmeralda* of the subgenus *Phalaenopsis*) in the tree inferred from data of cpDNA with weak support (74%). The different results between the trees inferred from data of nrDNA ITS and from data of cpDNA might be an indication of reticulated evolution

(Smedmark and Eriksson, 2002). Therefore, incongruences between sequences of different genes as well as between molecular and morphological characters are usually ascribed to a number of biological effects, such as hybridization, introgression, horizontal gene transfer, and lineage sorting (Larson, 1994). In fact, complete congruence among data sets rarely occurs (Swofford, 1991).

Although Slowinski and Page (1999) suggested that combining genes with different evolutionary histories and numbers of nucleotides can potentially produce trees that differ from the true species trees, the main focus of this study, however, was to define broad relationships between groups of taxa rather than of individual species. Rossetto et al. (2002)

suggested that combined analyses of two data sets representing different evolutionary rates should provide the greatest explanatory power for phylogenetic reconstructions.

Molecular phylogenetic analyses

Monophyly of the genus Phalaenopsis

In former research, *P. pulcherrima* (syn. *Doritis pulcherrima*) was treated as a member of the genus *Doritis* (Seidenfaden, 1988a), while members of the section *Deliciosae* of the subgenus *Phalaenopsis* and portions (*P. minus*, *P. braceana*, *P. taenialis*, and *P. stobartiana*) of the subgenus *Aphyllae* were treated as being in the genus *Kingidium* (Seidenfaden, 1988b). Christenson (2001) treated *Doritis* and *Kingidium* as synonyms of the genus *Phalaenopsis* based on high hybrid fertility between *P. pulcherrima* and *P. lobbii* (Christenson, 2001), the similar morphology of microspores between *P. pulcherrima* and *P. parishii* (Aoyama et al., 1994), and the similar column foot between *Doritis* and the *P. parishii* group of *Phalaenopsis* (Christenson, 2001). Furthermore, since members of the genus *Kingidium* and the section *Aphyllae* of the genus *Phalaenopsis* (Sweet's systematics) shared small subsaccate lip bases and four pollinia in the flower, the genus *Kingidium* was treated as a synonym of the genus *Phalaenopsis* by Christenson (2001). In this study, each of the phylogenetic analyses inferred from independent data of both the ITSs of nrDNA and cpDNA as well as the combined data supports the genus *Phalaenopsis* as described by Christenson (2001) being a monophyly. The results support *Doritis* and *Kingidium* being treated as synonyms of the genus *Phalaenopsis* as proposed by Christenson (2001).

Molecular data inferred from both data of nrDNA and cpDNA support *Doritis* and

Kingidium being treated as synonyms of *Phalaenopsis*. However, species of both traditional genera were placed in the sections of *Deliciosae* (parts of *Kingidium*) and *Esmeralda* (all of *Doritis*) of the subgenus *Phalaenopsis* or the subgenus *Aphyllae* (the other portions of *Kingidium*) by Christenson (2001), which is not completely supported by the molecular data. I discuss this in the following paragraphs.

Intrageneric relationships in Phalaenopsis

Subgenera *Proboscidioides*, *Aphyllae*, and *Parishianae*—The monotypic subgenus *Proboscidioides*, namely *Phalaenopsis lowii*, is treated as a unique species and separated from the remaining species of *Phalaenopsis* by having an extremely long beak-like rostellum and the lateral lobes of the lip in the form of recurved hooks (Sweet, 1980; Christenson, 2001). However, this species formed a clade with the subgenera *Aphyllae* and *Parishianae*, and portions (sections *Deliciosae* and *Esmeralda*) of the subgenus *Phalaenopsis* based on the independent data of the ITS of nrDNA as well as the combined data. In addition, analyses of cpDNA data showed that *P. lowii* formed a clade with the subgenus *Aphyllae* as well. In short, molecular data support *P. lowii* having a close relationship to the subgenera *Aphyllae* and *Parishianae*, and to the sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis*. In fact, this species also shares the same characters with the aforementioned *Phalaenopsis* species, namely the subgenera *Aphyllae* and *Parishianae*, and the sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis*. First, this species having four pollinia is congruent with those of the subgenera *Aphyllae* and *Parishianae*, and those of the sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis*. Second, this species having a deciduous habit is congruent with those of the subgenera *Aphyllae* and *Parishianae*. Third, the geographical distribution of this species is closely related to those of the subgenera *Aphyllae* and *Parishianae*, and those of the sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis* (Sweet, 1980; Christenson, 2001). Based on the aforementioned characters and molecular evidence, the extremely long beak-like rostellum of this species, therefore, has likely been overemphasized.

The subgenus *Aphyllae* is characterized as small deciduous plants with strongly flattened roots, small scarious floral bracts, lateral lobes of the lip with flap-like flanges, biseriate calli, and four pollinia (Christenson, 2001). Analyses of neither independent data of cpDNA nor of the combined data supported this subgenus being a monophyly, since one species of the subgenus *Aphyllae*, *P. minus*, was separated from the other species of the

subgenus *Aphyllae*. In contrast, *P. minus* formed a clade with *P. pulcherrima* (section *Esmeralda* of the subgenus *Phalaenopsis*) with weak support (74%) based on the cpDNA data. Analyses of combined data showed that *P. lowii* was nested within the subgenus *Aphyllae*. Only independent data of the ITS of nrDNA showed that *P. minus* formed a clade with the other members of the subgenus *Aphyllae*, but this was not very highly supported (80%). Furthermore, *P. minus* (syn. *Kingidium minus*) was traditionally placed in the separate genus, *Kingidium*, based on the pollinium number (of 4) and a conspicuous spur (Seidenfaden, 1988b). Christenson (2001) treated the genus *Kingidium* as a synonym of the genus *Phalaenopsis* and placed them in different parts of *Phalaenopsis*, namely the subgenus *Aphyllae* and the section *Delicioase* of the subgenus *Phalaenopsis*. Characters of *P. minus* differ from those of the other allies in having a falcate-triangular tooth rising up from the posterior margin of the lateral lobes of the lip, and a prominent pair of vertical column wings (Christenson, 2001). Therefore, *P. minus* is not very close to the other members of the subgenus *Aphyllae* based on morphological characters. Christenson (2001) also indicated that the status of *P. minus* is still uncertain (Christenson 2001). In fact, Gruss and Rollke (1997) had used *P. minus* as a type species to establish a monotypic section of *Kingidium*, i.e., *Conspicuum*. In short, molecular data support *Kingidium* being treated as a synonym of *Phalaenopsis*, but do not support *P. minus* being placed in the subgenus *Aphyllae*. According to the above discussion, *P. minus* should be raised to a separate monotypic subgenus. In contrast, data of both the ITSs of nrDNA and cpDNA support the other traditional *Kingidium* species, *P. braceana* (syn. *Kingidium braceana*), being placed in the subgenus *Aphyllae*.

The subgenus *Parishianae* is characterized by having deciduous leaves, four pollinia, a mobile lip midlobe, and prominent swellings at the base of the column, which diverge at the middle to form a U-shaped compound structure (Christenson, 2001). Based on the phylogenetic tree of the combined data of molecular evidence, the subgenus *Parishianae* was shown to be a monophyletic group. Furthermore, the independent data of the ITS of nrDNA and the combined data showed that the subgenus *Parishianae* had a close relationship with the subgenera *Aphyllae* and *Proboscidioides*, and formed a clade with the section *Deliciosae* of the subgenus *Phalaenopsis*. Analyses of cpDNA showed that the subgenus *Parishianae* formed a clade with the section *Deliciosae* of the subgenus *Phalaenopsis*, but was separated from the subgenera *Aphyllae* and *Proboscidioides*. Basically, the subgenera *Proboscidioides* and *Aphyllae*, and the section *Deliciosae* of the subgenus *Phalaenopsis* have a close relationship based on molecular evidence. In fact, these groups also share the same

characteristics in having four pollinia, a deciduous habit, and proximal distributions (Sweet, 1980; Christenson, 2001).

Subgenus *Phalaenopsis*—This subgenus was established by Christenson (2001) and is characterized by having a single callus (with the exception of the three species of the section *Deliciosae*) and smooth lateral lobes of the lip without the characteristic tooth-like ridge, which is found in other subgenera. This subgenus was subdivided into the four sections of *Phalaenopsis*, *Deliciosae*, *Esmeralda*, and *Stauroglottis* based on the systematics of Christenson (2001). Two species of *Kingidium*, namely *P. deliciosa* (syn. *Kingidium deliciosa*) and *P. chibae* (syn. *Kingidium chibae*), were treated as being in the section *Deliciosae* of the subgenus *Phalaenopsis* of the genus *Phalaenopsis*. Furthermore, species of *Doritis* were also treated as being in the section *Esmeralda* of the subgenus *Phalaenopsis* (Christenson, 2001). *Doritis* had alternatively been accepted as a separate genus from *Phalaenopsis* because of the long column foot, the long rostellum, the presence of linear 'appendages' toward the base of the lip, and its terrestrial habitat (cf. Christenson, 2001). Based on the physiological concept, hybrids between *P. pulcherrima* and members of the subgenus *Parishianae*, particularly *P. lobbii*, showed high fertility (cf. Christenson, 2001). Therefore, *Doritis* was treated as a synonym of *Phalaenopsis* (Christenson, 2001). Furthermore, Christenson (2001) placed sections *Deliciosae* and *Esmeralda* in the subgenus *Phalaenopsis* since these two sections have smooth lateral lobes of the lip as do members of sections *Phalaenopsis* and *Stauroglottis*. However, the monophyly of the subgenus *Phalaenopsis* was not supported based on the independent data of ITS and cpDNA individually, as well as the combined data. According to the previous discussion, the section *Deliciosae* of the subgenus *Phalaenopsis* had a close relationship with the subgenera *Proboscidioides*, *Aphyllae*, and *Parishianae*, which have four pollinia. *Phalaenopsis pulcherrima*, a species of the section *Esmeralda* of the subgenus *Phalaenopsis*, showed a sister relationship to the other *Phalaenopsis* species having four pollinia based on the independent data of the ITS of nrDNA and the combined data. Excluding sections *Deliciosae* and *Emeralda* of the subgenus *Phalaenopsis*, the remaining members of the subgenus *Phalaenopsis*, namely sections *Phalaenopsis* and *Stauroglottis*, were shown to be a monophyletic group based on the independent data of both ITS and cpDNA with moderate support (80% and 77%, respectively), and on the combined data with high support (99%). The results are in agreement with the analyses of RAPD (Chen et al., 1995) and the IGS sequence of 5S rDNA (Kao, 2001).

Members of the section *Phalaenopsis* bear flowers with broad petals, with the petals being much broader than the sepals. Additionally, they bear prominent, erect, somewhat-glossy calli (Christenson, 2001). The monophyly of the section *Phalaenopsis* was supported according to both the independent data of ITS and the combined data. Based on the morphological characteristics, 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. Within the section *Phalaenopsis*, two subclades were shown based on both the independent ITS data and the combined data. The first subclade included *P. schilleriana*, *P. stuartiana*, and *P. philippinensis*, which have marbling on the upper surface of the leaves. Another subclade included *P. amabilis*, *P. aphrodite*, and *P. sanderiana*, which do not have such markings (Sweet, 1980; Christenson, 2001). Members of the section *Phalaenopsis* were also separated from those of the section *Stauroglottis* based on the chromosome sizes and flowers which lack transverse barred patterns in the section *Phalaenopsis* (Christenson, 2001). However, the analysis using cpDNA did not support the monophyly of the section *Phalaenopsis* because members of the section *Stauroglottis* were nested within the section *Phalaenopsis*.

The section *Stauroglottis* can be separated from the section *Phalaenopsis* (Christenson, 2001) due to its members having some characters of small flowers with mostly subsimilar sepals and petals and an undivided lip apex of the flower. However, the monophyly of the section *Stauroglottis* was not supported based on the independent data of ITS and cpDNA, as well as the combined data. In analysis of the ITS of nrDNA, *P. celebensis* was shown to be a unique species and was separated from the remaining species of the section *Stauroglottis*, namely *P. lindenii* and *P. equestris*, which formed a clade with high support (97%). In contrast, *P. lindenii* was shown to be a unique species based on the analysis of cpDNA, and *P. celebensis* and *P. equestris* formed a clade with high support (94%). Therefore, the phylogenetic relationship inferred from ITS data is incongruent with that derived from cpDNA data within the section *Stauroglottis*. Based on the morphological data, flowers of *P. lindenii* are closely related to those of *P. equestris*. In contrast, the leaves of *P. lindenii* are closely related to those of *P. celebensis* (Christenson, 2001).

Subgenus *Polychilos*—The subgenus *Polychilos* bears fleshy, long-lasting flowers with two pairs of calli on the lip (biseriate), the lateral lobes of the lip producing a raised tooth along the leading edge, and there being two pollinia (Christenson, 2001). This large subgenus

was subdivided into the four sections of *Polychilos*, *Fuscatae*, *Amboinenses*, and *Zebrinae* based on the systematics of Christenson (2001). This subgenus was traditionally treated as a separate genus, *Polychilos* by Breda in 1864 (cf. Christenson 2001). Until 1969, this genus was treated as a synonym of *Phalaenopsis* by Sweet (1969). However, Shim (1982) did not accept Sweet's concept. Recently, Christenson (2001) accepted Sweet's concept (1969) and placed it in the subgenus *Polychilos* of *Phalaenopsis*. In this study, molecular data support treating the genus *Polychilos* as a synonym of *Phalaenopsis*. However, the monophyly of the subgenus *Polychilos* of *Phalaenopsis* was not supported based on both the independent data of ITS and the combined data. In contrast, the subgenus *Polychilos* was shown to be a monophyletic group with weak support (77%) based on independent data of cpDNA. Excluding the section *Fuscatae* and three species of the section *Amboinenses*, namely *P. gigantea*, *P. doweryensis*, and *P. maculata*, the remainder of the subgenus *Polychilos* was shown to be a monophyly based on the independent data of ITS DNA and cpDNA individually as well as the combined data. Basically, two major clades with high support were shown within the subgenus *Polychilos* according to the molecular data of this study.

Members of the section *Polychilos* were shown to be very similar based on morphological characters, particularly the flowers. This section is characterized by 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 midlobe 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). The only character of *P. mannii* separating it from the remainder of the section *Polychilos* is the rachis, as *P. mannii* does not bear a flattened rachis. Therefore, Sweet (1980) and Christenson (2001) treated them as a unique group (i.e., section *Polychilos*) and separated them from the remaining species of the subgenus *Polychilos*. Within the subgenus *Polychilos*, only the section *Polychilos* was shown to be a monophyletic group with moderate support (78%) based on the independent data of ITS. However, monophyly was not supported based on both the independent data of cpDNA and the combined data due to *P. mannii* being separated from the other members of the section *Polychilos*. Whether *P. mannii* underwent gene introgression during the evolutionary process is still uncertain, because it was not placed with the other species of the section *Polychilos* based on the tree of cpDNA. Moreover, the flowers of this section do not exhibit post-pollination chlorophyll of the perianth as compared to sections *Amboinenses* and *Zebrinae* described by Christenson (2001). However, I do not accept the aforementioned

description by Christenson (2001) according to my hand pollination of species of the section *Polychilos* in a greenhouse, since the section *Polychilos* also exhibits post-pollination chloroplasts of the perianth (data not shown).

The section *Fuscatae* of the subgenus *Polychilos* was traditionally treated as a natural group due to its having concave striped lips with a longitudinal keel, pale yellow flowers with a variable brown mark (with the exception of *P. cochlearis*), and strongly revolute sepals and petals (Sweet, 1980; Christenson, 2001). However, members of the section *Fuscatae* of the subgenus *Polychilos* were not shown to be a monophyletic group based on the independent data of ITS and cpDNA individually as well as the combined data. In contrast, the section *Fuscatae* and three species of the section *Amboinenses*, namely *P. gigantea*, *P. doweryensis*, and *P. maculata*, formed a unique clade with high support and were separated from the remaining species of the subgenus *Polychilos* based on the independent data of ITS and cpDNA as well as the combined data. Molecular data of the IGS of 5S rDNA also showed that these species form a clade with high support (Kao, 2001). When I inspected the morphological characters of the species of this unique clade inferred from molecular data, I found that those species share the same character of having striped lips with longitudinal keels. Although the character of *P. maculata* was not apparent, this species truly bears the character according to the lip drawing of *P. maculata* from type specimen by Sweet (1980). Furthermore, I also found that these species do not exhibit post-pollination chlorophyll of the perianth as do the sections *Amboinenses*, *Zebrinae*, and *Polychilos* (data not shown).

Excluding sections *Polychilos* and *Fuscatae*, the remaining sections of the subgenus *Polychilos*, namely *Amboinenses* and *Zebrinae*, were shown to be highly incongruent between the concepts of Sweet (1980) and Christenson (2001) (see Table 1). Sweet (1980) separated the section *Amboinenses* from the section *Zebrinae* based on the shape of the perianths and the midlobe of the lip. However, Christenson (2001) did not accept Sweet's concept (1980); he separated the section *Zebrinae* from the section *Amboinenses* by the hooded anther bed. Therefore, only five species were left in the section *Zebrinae*, namely *P. sumatrana*, *P. corningiana*, *P. tetraspis*, *P. inscriptiosinensis*, and *P. speciosa*. Actually, Christenson (2001) also mentioned that it is not easy to distinguish between the sections *Zebrinae* and *Amboinenses*. In this study, sections *Amboinenses* and *Zebrinae* could not be separated based on the independent data of ITS and cpDNA individually or on the combined data. The results indicate that the character of the hooded anther bed has been overemphasized in the systematics of the subgenus *Polychilos*. Although molecular data

cannot discriminate among species of the sections *Amboinenses* and *Zebrinae*, eight species distributed in the Philippines, namely *P. bastianii*, *P. pallens*, *P. hieroglyphica*, *P. reichenbachiana*, *P. lueddemanniana*, *P. fasciata*, *P. pulchra*, and *P. mariae*, formed a subclade with high support based on the combined data. Among these eight species of the subgenus *Polychilos*, phylogenetic trees were inferred from molecular data between ITS and cpDNA. *Phalaenopsis mariae* was excluded from this subclade based on the analysis of ITS data, but it still showed a sister relationship to the subclade. In contrast, *P. reichenbachiana* was excluded from this subclade based on the analysis of cpDNA data, but it also still had a sister relationship to the subclade. In short, the aforementioned eight species of the section *Amboinenses* basically have a close relationship based on the molecular data. In fact, six species of this subclade, namely *P. pallens*, *P. hieroglyphica*, *P. reichenbachiana*, *P. lueddemanniana*, *P. fasciata*, and *P. pulchra*, were traditionally treated as a highly variable species, namely *P. lueddemanniana*. Sweet (1968, 1969) fully resolved the species and treated it as different species, namely *P. pallens*, *P. hieroglyphica*, *P. lueddemanniana*, *P. pulchra*, *P. fasciata*, and *P. reichenbachiana*. Christenson (2001) accepted Sweet's concept (1968, 1969) and placed them in the section *Amboinenses* of the subgenus *Polychilos*.

In addition, *P. micholitzii* is a species of the section *Amboinenses* based on Christenson (2001). However, analyses of the independent data of cpDNA and the combined data supported this species forming a clade with the section *Polychilos*. In fact, trees from the independent data of cpDNA also supported this result (Figs. 12, 13, 15). Since characters of this species and its geographical distribution greatly diverge from those of members of the section *Polychilos* (Christenson, 2001), it was never placed in the section *Polychilos* based on both the systematics of Sweet (1980) and Christenson (2001). According to geography, *P. micholitzii* has a restricted distribution in Mindanao, the Philippines, and is separate from the section *Polychilos*, which is distributed in Borneo, India, Java, and South China (Christenson, 2001). Since analyses of ITS data did not show that other species of *Phalaenopsis* form a clade with *P. micholitzii*, reasons for the conflict between the molecular and morphological data for both *P. micholitzii* and the section *Polychilos* are uncertain at present.

Biogeography

The above-described research on the molecular phylogeny of the genus *Phalaenopsis* in this study provides insights into the distribution of this genus. Comparison of the phylogenetic tree with the geographical distribution of the genus *Phalaenopsis* shows that

species of the four-pollinium clade are distributed in South China, India, and Indochina. Two widespread species of the four-pollinium clade, namely *P. deliciosa* and *P. pulcherrima*, extend to Southeast Asia. In contrast, most of the two-pollinium species are distributed in Malaysia, Indonesia, and the Philippines. Three species of the two-pollinium clade, namely *P. cornu-cervi*, *P. sumatrana* and *P. kunstleri*, extend to Indochina. In addition, one species, *P. mannii*, has a restricted distribution in South China and Indochina. Two major clades within *Phalaenopsis* were shown based on the molecular data of this study, and these two clades were basically congruent with distributional ranges of the species (Christenson, 2001) (Fig. 20). The species diversity center of the subgenus *Phalaenopsis* is located in the Philippines (81.3% of the total), while Borneo was the species diversity center of the subgenus *Polychilos* (57.7% of the total) (Christenson, 2001). The results are in agreement with several reports, which introduced higher plant species diversity in tropical areas (Stebbins, 1974; Moritz et al., 2000). To the present, there are two suggestions for explaining the higher species diversity in tropical areas. In an early suggestion, a stable tropical climate allows species to accumulate over time, with low rates of extinction in the absence of major environmental perturbations (Whitmore and Prance, 1987). Recent discoveries have suggested that tropical climates were unstable over the past 2 my during the Pleistocene (Haffer, 1982). Cyclical glacial events led to periods of cooler and/or drier climates in which species in tropical areas may have withdrawn to small refugial pockets, resulting in speciation through allopatric differentiation of populations in separate refugia (e.g., Gentry, 1982). Furthermore, land bridges forming occasionally between lands/islands in glacial times might have speeded up or interrupted the dispersal of species, resulting in rising species diversity through hybridization/isolation (e.g., Burnham and Graham, 1999). Although most species of the subgenus *Polychilos* are distributed in Indonesia and Malaysia, there are still ten species distributed in the Philippines, namely *P. micholitzii*, *P. sumatrana*, *P. lueddemanniana*, *P. pallens*, *P. bastianii*, *P. fasicata*, *P. pulchra*, *P. hieroglyphica*, *P. reichenbachiana*, and *P. mariae*. In particular, species of the subgenus *Polychilos* in the Philippines (with two exceptions of *P. mariae* and *P. sumatrana*) are not found in the other regions of Southeast Asia (Christenson, 2001). Based on the phylogeny of *Phalaenopsis* inferred from the molecular data, species of the subgenus *Polychilos* in the Philippines, with two exceptions of *P. micholitzii* and *P. sumatrana*, had a close relationship. Furthermore, the sections *Esmeralda* and *Deliciosae* could be raised into separate subgenera from the subgenus *Phalaenopsis*. Excluding these two sections, most of the species of the subgenus

Phalaenopsis with the two exceptions of *P. amabilis* and *P. celebensis* have restricted distributions in the Philippines. One of the two exceptions, *P. amabilis*, is a widespread species distributed in the Philippines (Palawan), Malaysia, Indonesia, and Australia. The other one, *P. celebensis*, has a restricted distribution in Sulawesi, Indonesia (Sweet, 1980; Christenson, 2001).

Based on the biogeographical tree of the genus *Phalaenopsis* (Fig. 19), New Guinea and Australia formed a clade. The second clade included Sulawesi and the Molucca Is. The third included Taiwan and the Philippines; the fourth clade included the Malay Peninsula, Borneo, Sumatra, and Java; and the fifth clade included India/Sri Lanka, Indochina, and South China. Furthermore, Flores was shown to be an independent clade. This result is in agreement with biogeographic regions of the world (Pianka, 1994), since the first clade belonging to the Australian region was not nested within the clades of the Oriental region (Figs. 2, 19). Furthermore, the Malay Peninsula, Borneo, Sumatra, and Java formed a clade based on the biogeographical tree. The result is in agreement with the historical geology of these regions, which were interconnected and comprised the Sunda Shelf during Pleistocene times (about 0.01~1.8 Mya) (Van Oosterzee, 1997). This would have made crossings among these regions easy. According to floral and faunal records between both regions, Wallace's Line was introduced in the late 19th century (Van Oosterzee, 1997). However, the biogeography of the genus *Phalaenopsis* was incongruent with the natural barriers of Weber's Line between Sulawesi and the Molucca Is., since the biogeographical relationship of *Phalaenopsis* species distributed in both regions of Sulawesi and Molucca Is. was close (Fig. 19).

Since deep straits (e.g., the Makassar Strait) form natural barriers, only a few *Phalaenopsis* species, namely *P. celebensis, P. venosa, P. amboinensis, P. floresensis*, and two subspecies of *P. amabilis*, were able to reach the east side of Wallace's Line, namely Sulawesi, Molucca Is., Flores, and even to New Guinea and Australia. Of these, three species, namely *P. celebensis*, *P. venosa* and *P. amboinensis*, have restricted distributions in Sulawesi/the Molucca Is. In addition, *P. floresensis* has a restricted distribution in Flores. The two subspecies of *P. amabilis*, namely *P. amabilis* subsp. *moluccana* and *P. amabilis* subsp. *rosenstromii*, are distributed in Sulawesi/the Molucca Is. and New Guinea/Australia, respectively (Christenson, 2001). When the Makassar Strait between Borneo and Sulawesi formed about 50 Mya (Moss and Wilson, 1998), it would have prevented species crossing from Borneo to Sulawesi (Van Oosterzee, 1997) (Fig. 4). The divergence time would have allowed species distributed in Sulawesi to have become highly separate from species

distributed in Borneo. However, the divergence between *Phalaenopsis* species of Borneo (e.g., *P. bellina*) and those of Sulawesi (*P. amboinensis*) is not so great based on the molecular data of the genus *Phalaenopsis*. Therefore, I suggest that the dispersal and speciation of *Phalaenopsis* species in Sulawesi did not occur until some time after 50 Mya. This evolutionary event might be happened in more-recent times. In addition, I also found that the flora of Sulawesi might have relationships with that of the Philippines based on the biogeography of species of the genus *Phalaenopsis*. For instance, *P. deliciosa* is distributed in the Philippines and Sulawesi, but is not found in Borneo. Furthermore, in Sulawesi (*P. celebensis*) and the Philippines (*P. lindenii* and *P. equestris*), one can find species of the section *Stauroglottis*, but they are absent from Borneo. According to the present geology between Sulawesi and the Philippines, northeast Sulawesi extends toward Mindanao, the Philippines, and there are several archipelagoes between these lands. Perhaps, Sulawesi and the Philippines might have been interconnected in ancient times. Since most islands of the Philippines are of recent age (< 5 Mya) (Aurelio et al., 1991; Quebral et al., 1994), dispersal from the Philippines to Sulawesi might have occurred later than 5 Mya.

Evolutionary trends

The evolutionary phylogenetic tree inferred from the combined data of nrDNA and cpDNA was constructed. The tree was rooted by the putative origin group, the subgenus *Aphyllae*, and species of consistent subgenera and sections were compressed (Fig. 22). Since South China (the Himalayas) is the distribution center of the subgenus *Aphyllae*, I suggest that *Phalaenopsis* first developed in the Himalayas. I mapped the geographical distributions of *Phalaenopsis* with the rooted and compressed evolutionary phylogenetic tree (Fig. 23). According to this rooted and compressed evolutionary phylogenetic tree (Fig. 22), I found that the section *Esmeralda* was the most-recently evolved group among the four-pollinium groups. This result is in agreement with the morphological characteristics of the subgenus *Esmeralda*, which shows intermediate morphological characters, including plant size and flower size, between the four-pollinium and the two-pollinium group. Furthermore, the section *Esmeralda* shared the common ancestor with both the subgenus *Parishianae* and the section *Deliciosae*. This result is supported by the surprising fertility of hybrids obtained from hybridization between *P. pulcherrima* (section *Esmeralda*) and members of the subgenus *Parishianae*, in particular *P. lobbii* (Christenson, 2001). However, the level of fertility of hybrids between species of sections *Esmeralda* and *Deliciosae* has not been

described to the present.

In the present study, the two-pollinium clade was suggested to have been derived from the four-pollinium clade. Three evolutionary lineages descended from the four-pollinium group to the two-pollinium clade based on the molecular data. They include lineages of the subgenera *Phalaenopsis* (with the exception of the sections *Deliciosae* and *Esmeralda*) and *Polychilos* (with the exception of the section *Fuscatae* plus *P. gigantea*, *P. doweryensis*, and *P. maculata*), as well as the section *Fuscatae* plus *P. gigantea*, *P. doweryensis*, and *P. maculata*. In the development of the lineage of the subgenus *Phalaenopsis*, since most of the four-pollinium species have restricted distributions in South China, India, and Indochina, while most species of the subgenus *Phalaenopsis* are distributed in the Philippines, only one species, *P. amabilis*, extends to the other regions of Southeast Asia, even to Australia. Therefore, there are two available dispersal pathways for the development of the subgenus *Phalaenopsis*. (1) Plant taxa dispersed directly from Asia to the Philippines. According to historical geology, parts of the Philippines, namely Mindoro and Palawan, were located on the margin of Eurasian Plate. Before the present South China Sea began to form (at ~30 Mya), Mindoro and Palawan were close to South China and Indochina (Hall, 1996). This offered greater opportunities for the dispersal of *Phalaenopsis* from Indochina (or South China) to Mindoro/Palawan (older lands of the Philippines) using ancient land bridges. (2) Plant taxa dispersed from Asia using the Malay Peninsula, Borneo, and Palawan as steppingstones to the Philippines. To the present, no species of the subgenus *Phalaenopsis* has been found in continental Asia or the Malay Peninsula. Therefore, I prefer to suggest that the ancestors of the subgenus *Phalaenopsis* dispersed directly from Asia to the Philippines.

Excluding sections *Deliciosae* and *Esmeralda*, the subgenus *Phalaenopsis* includes two subgroups, namely sections *Stauroglottis* and *Phalaenopsis*. Based on the rooted evolutionary phylogenetic tree, the section *Stauroglottis* developed earlier than the section *Phalaenopsis*. The section *Stauroglottis* evolved in the Philippines, and then the section *Phalaenopsis* developed. The section *Stauroglottis* was nested within the section *Phalaenopsis*, which included the *P. amabilis* complex and the *P. schilleriana* complex based on analysis of cpDNA (maternal inheritance) (Fig. 26a). In addition, the section *Stauroglottis* was separated from the section *Phalaenopsis* in the analysis of the ITS of nrDNA (biparental inheritance) (Fig. 26b). Furthermore, the *P. schilleriana* complex also shows the characteristic of marbled markings on the upper leaf surface as do two species of the section *Stauroglottis*, namely *P. lindenii* and *P. celebensis* (Sweet, 1980; Christenson, 2001). Therefore, these two sections of

the subgenus *Phalaenopsis*, namely sections *Phalaenopsis* and *Stauroglottis*, might have experienced reticulated evolution.

In the development of the lineage of the section *Fuscatae* plus *P. gigantea*, *P. doweryensis*, and *P. maculata*, although this group traditionally had been considered to have a close relationship to the remaining species of the subgenus *Polychilos* (Shim, 1982; Christenson, 2001), individual analysis of nrDNA and cpDNA showed that they form a clade with high statistical support and showed that these species could be separated from the remainder of the subgenus *Polychilos*. In addition, this group might have developed earlier than the remainder of the subgenus *Polychilos* according to the evolutionary phylogenetic tree (Fig. 22). Besides, in the development of the lineage of the subgenus *Polychilos*, three groups were subdivided, namely sections *Polychilos*, *Amboinenses*/*Zebrinae* (with the exception of the *Phalaenopsis lueddemanniana* complex, namely *P. bastianii*, *P. pallens*, *P. hieroglyphica*, *P. reichenbachiana*, *P. lueddemanniana*, *P. fasciata*, *P. pulchra*, and *P. mariae*), and the *P. lueddemanniana* complex. Of these three groups, the section *Amboinenses*/*Zebrinae* (with exception of the *P. lueddemanniana* complex) and the *P. lueddemanniana* complex shared the most recent common ancestor. In contrast, the section *Polychilos* was shown to be unique and was separated from both the section *Amboinenses*/*Zebrinae* and the *P. lueddemanniana* complex. Nevertheless, both sections of *Amboinenses* and *Polychilos* might have experienced reticulated evolution as a result of one species of the section *Amboinenses*, *P. micholitzii*, being nested within the section *Polychilos*. The morphological characteristics of *P. micholitzii* are very divergent with those of the section *Polychilos*. In addition, results of analyses of both nrDNA and cpDNA for *P. micholitzii* were conflicting; therefore, *P. micholitzii* was still placed in the section *Amboinenses* instead of the section *Polychilos*. Furthermore, the *P. lueddemanniana* complex was the most-recently developed group and shared the latest common ancestor with the section *Amboinenses*/*Zebrinae*. In addition, species of the *P. lueddemanniana* complex are only distributed in the Philippines (Christenson, 2001). Species of this section were closely related based on both morphological characters (Sweet, 1980; Christenson, 2001) and the molecular evidence of this study. Therefore, I suggest that species of this section descended from the same common ancestor. According to the evolutionary phylogenetic subtree of the section *Amboinenses*/*Zebrinae* and the *P. lueddemanniana* complex (Fig. 27), I suggest that *P. mariae* is a basal species of the *P. lueddemanniana* complex. The result is congruent with the biogeographical distribution, since *P. mariae* distributes in both Palawan and Borneo

(Christenson, 2001). Since no species of the section *Amboinenses*/*Zebrinae,* with the exception of *P. micholitzii* (distributed in Mindanao, the Philippines) and *P. sumatrana* (syn. *P. zebrina*) (distributed in Palawan, the Philippines), were found in the Philippines, I suggest that species of the *P. lueddemanniana* complex in the Philippines which descended from species of the section *Amboinenses*/*Zebrinae* distributed in Borneo developed as a unique lineage. If species of the *P. lueddemanniana* complex had not evolved from the section *Amboinenses*/*Zebrinae*, it might have high divergence with the section *Amboinenses*/*Zebrinae* due to the long-term isolation (5~10 my) of both plates of the Philippines (older lands) and Borneo (Karig et al., 1986; Hall, 1996). In fact, the section *Amboinenses*/*Zebrinae* and the *P. lueddemanniana* complex were very close based on morphological characters (Sweet, 1980; Christenson, 2001) and molecular data in the study. This indicates that the dispersal and speciation of the *P. lueddemanniana* complex in the Philippines were recent events. Furthermore, *P. micholitzii* (section *Amboinenses*) and *P. sumatrana* (syn. *P. zebrina*)(section *Zebrinae*) distributed in the Philippines were divergent from the species of the *P. lueddemanniana* complex based on the molecular data. The result indicates that these two species of the section *Amboinenses*/*Zebrinae* distributed in the Philippines dispersed from Borneo through other dispersal events. Based on historical geology, the combination of the plates of the Philippines and Borneo is at approximately 5~10 Mya (Karig et al., 1986; Stephan et al., 1986; Hall, 1996); this means that lands of Borneo and Palawan (the Philippines) had many chances to be interconnected during glacial times. Furthermore, the Sulu Archipelago might have formed another land bridge between Borneo and the Philippines during glacial times (Fig. 4). Therefore, this evolutionary trend of the *P. lueddemanniana* complex of this study is reasonable based on the historical geology of both the Philippines and Borneo.

Substitution rates

Unequal evolutionary rates in plants have been detected using noncoding DNA regions, e.g., the *trn*L intron of cpDNA (Gielly and Taberlet, 1996) and the ITSs of nuclear ribosomal DNA (Wendel et al., 1995; Baldwin and Sanderson, 1998). In the present study, unequal evolutionary rates of cpDNA have been found at four species of the section *Amboinenses*/*Zebrinae*, namely *P. bellina*, *P. violacea*, *P. fimbriata*, and *P. tetraspis*, based on Tajima's relative rate test between the speices of *P. lueddemanniana* complex and the section *Amboinenses*/*Zebrinae* distributed on the Sunda Shelf. In contrast, ITS data between the

speices of *P. lueddemanniana* complex and the section *Amboinenses*/*Zebrinae* distributed on the Sunda Shelf bear equal evolutionary rates. On the other hand, unequal evolutionary rates have only been found in cpDNA data (i.e., a species of the section *Stauroglottis*, *P. lindenii*) between the species of *P. lueddemanniana* complex and the section *Stauroglottis*. The results representing evolutionary rates between both nuclear and chloroplast genomes from those species are not in concerted evolution. Since Tajima's neutrality test for DNA regions of cpDNA studied of the genus *Phalaenopsis* were shown to be neutral, unequal evolutionary rates of cpDNA in these species might not have been caused by some gene-specific factor (e.g., selection). In addition, the generation time of these species is similar to those of the remaining species of the genus *Phalaenopsis*. Therefore, the unequal evolutionary rates of cpDNA in these species might have been caused by the speciation rate, the efficiency of DNA replication or repair, or some other unclear reason.

In the present study, the substitution rates of ITS and cpDNA data from the genus *Phalaenopsis* were 2.4~4.7 x 10^{-9} and 3.9~7.8 x 10^{-10} substitutions/site/year, respectively. The substitution rates of ITS data of the genus *Phalaenopsis* were nearly five times those of cpDNA. The substitution rates of cpDNA from the genus *Phalaenopsis* are in agreement with the typical rates of cpDNA ranging from 2.7 to 9.1 x 10^{-10} (Bousquet et al. 1992). In addition, the substitution rates of ITS data of the genus *Phalaenopsis* are also similar to those of several annual/perennial herbs (1~2 years) at 1.72~5.69 x 10^{-9} substitutions/site/year and perennial shrubs (about 1~3 years) at 2.44~9.0 x 10^{-9} substitutions/site/year (summarized in Richardson et al., 2001). Based on the substitution rates of both the ITS and cpDNA data from the genus *Phalaenopsis*, the divergence times of specific groups/taxa of this genus could be deduced. Based on the substitution rates of the genus *Phalaenopsis*, the average divergence times among the species of the *P. lueddemanniana* complex with the two exceptions of *P. mariae* and *P. reichenbachiana* were 2.7 ± 0.63~1.4 ± 0.32 my calculated by the substitution rates of ITS data and 1.3 ± 0.56~0.65 ± 0.28 my by cpDNA data. The results are basically in agreement with the historical geology of the Philippines, in which most of the islands recently developed (< 5 Mya) with the exception of Palawan, Mindoro, Zamboanga, and parts of the western Philippines (Aurelio et al., 1991; Quebral et al., 1994). Furthermore, several glacial periods took place during the Pleistocene (0.01~1.8 Mya), and land bridges between islands of the Philippines formed during those periods. During periods of Pleistocene sea-level fluctuations, both vicariance and dispersal would have led to species complexes (Richardson et al., 2001). In the present study, divergence times among species of

the *P. lueddemanniana* complex with the two exceptions of *P. mariae* and *P. reichenbachiana*, fell within the Pleistocene. I suggest, thus, that these six species of the *P. lueddemanniana* complex, namely *P. pallens*, *P. hieroglyphica*, *P. fasciata*, *P. lueddemanniana*, *P. bastianii*, and *P. pulchra*, evolved during the Pleistocene. According to the poorly resolved phylogeny of each of the cpDNA and combined cpDNA from species of the *P. lueddemanniana* complex of this study, the diversity of the complex recently arose from the most recent common ancestor as described by Richardson et al. (2001).

Furthermore, the divergence times between the sections *Deliciosae* and *Stauroglottis* were calculated based on the substitution rates of both ITS and cpDNA data. The divergence times between the sections *Deliciosae* and *Stauroglottis* obtained from both ITS and cpDNA data were similar and ranged from 20.9 ± 2.1 to 10.7 ± 1.1 my and from 21.2 ± 2.1 to 10.6 ± 1.5 my, respectively. Based on the historical geology of the Philippines, the older lands of the Philippines, namely Mindoro and Palawan, were parts of the Eurasian Plate in ancient times (Hall, 1996). The historical geology of the Philippines shows that the older lands, namely Mindoro and Palawan, began to split from the Eurasian Plate and form the South China Sea at approximately 30 Mya. Based on the putative divergence times of these two specific groups of the genus *Phalaenopsis*, namely the sections *Deliciosae* and *Stauroglottis*, Mindoro and Palawan became isolated from the Eurasian Plate at approximately 21~10.5 Mya.

In addition, the divergence times among the *P. lueddemanniana* complex calculated from ITS data (2.7 ± 0.63~1.4 ± 0.32 my) were shown to be higher than those from cpDNA data (1.3 ± 0.56~0.65 ± 0.28 my). In contrast, the divergence times between the sections *Deliciosae* and *Stauroglottis* were shown to be nearly identical based on the substitution rates of both ITS and cpDNA data. Among species of the *P. lueddemaniana* complex, the speciation of the *P. lueddemanniana* complex might have partly been caused by natural hybridization. Weak genetic barriers among species of the genus *Phalaenopsis* have been shown (Sweet, 1980), and these could have offered great potential for sympatric hybridizations. Since the ITS of nrDNA represents biparental inheritance, the number of nucleotide substitutions from ITS data of closely related species is caused by both nucleotide mutations and the recombination of those of both parents. This will cause the divergence times among them be overestimated, as in this study for the estimation of the divergence time among the species of the *P. lueddemanniana* complex based on substitution rates of ITS data. In contrast, the accumulation of sequence divergences between two divergent groups is only caused by nucleotide mutations. Therefore, the divergence times between the sections

Deliciosae and *Stauroglottis* as calculated by the substitution rates of both ITS and cpDNA data to form two divergent groups were similar in this study.

Furthermore, “time of divergence test” developed by Sang and Zhong (2000) can be examined whether the incongruence of between the cpDNA tree (chloroplast genome) and the ITS tree (nuclear genome) is a result of hybridization or lineage sorting. Within the subgenus *Phalaenopsis*, for instance, the ITS tree (Fig. 26a) is consistent with the morphology, and most likely represents the species relationships, whether the incongruence of the cpDNA tree (Fig. 26b) with the species tree is a result of hybridization or lineage sorting. I applied the “time of divergence test” developed by Sang and Zhong (2000) to examine whether the incongruence of the cpDNA tree with the species tree is a result of hybridization or lineage sorting. If I assume that the divergent time of the *P. schilleriana* complex from species of the *P. amabilis* complex in the ITS tree, and of speices of the section *Stauroglottis* from species of the *P. schilleriana* complex in the cpDNA tree, are T_i and T_c, respectively. According to Sang and Zhong (2000), $T_c < T_i$ indicates hybridization, wherea $T_c > T_i$ suggests lineage sorting. Since species of the section *Phalaenopsis* of both complexes of *P. amabilis* and *P. schilleriana* were rejected the constancy of equal evolutionary rate in ITS data by Tajima’s relative rate test, a nonparametric rate smoothing method [program r8s (Sanderson, 1997)] was applied to estimate the divergence times of the *P. schilleriana* complex from species of the *P. amabilis* complex in the ITS tree, and of species of the section *Stauroglottis* from species of the *P. schilleriana* complex in the cpDNA tree. The divergent times for T_i and T_c were 5.8~11.7 Myr and 6.1~12.1 Myr, respectively. Thus, $T_c > T_i$ indicated that the *P. schilleriana* complex might be an origin of a lineage sorting.

Conclusions

In this study, I present evidence obtained by sequencing the internal transcribed spacer (ITS) of ribosomal DNA from nuclear DNA (nrDNA) and the *trn*L intron, *trn*L-*trn*F intergenic spacer (IGS), and *atp*B-*rbc*L IGS from chloroplast DNA (cpDNA). These molecular data can offer new perspectives on the phylogenetic relationships among species of *Phalaenopsis*. Both the ITSs of nrDNA and cpDNA appear to be adequate for resolving intrageneric relationships of the genus *Phalaenopsis*. Phylogenetic trees inferred from both ITS data and cpDNA showed that the monotypic subgenus, *P. lowii*, had a close relationship to the subgenus *Aphyllae*. In addition, the subgenera *Aphyllae* and *Proboscidioides* formed a clade with the subgenus *Parishianae* and the sections *Esmeralda* and *Deliciosae* of the

subgenus *Phalaenopsis*. Furthermore, this clade also shared the same morphological character of four pollinia as well as proximal geographical distributions. In short, the status of the subgenera *Proboscidioides, Aphyllae,* and *Parishianae,* and sections *Deliciosae* and *Esmeralda* of the subgenus *Phalaenopsis* showed very close relationships and were separated from the subgenus *Polychilos* and the others of the subgenus *Phalaenopsis* based on molecular data. Therefore, there are two available alternatives: (1) the species in the four-pollinium clade can be raised into a separate genus or (2) these species of the four-pollinium clade can be treated as the genus *Phalaenopsis*. Since *P. lowii* (subgenus *Proboscidioides*) and the *P. wilsonii* complex (parts of the subgenus *Aphyllae*) of the four-pollinium clade traditionally have been placed in the genus *Phalaenopsis*, I thus agree with the systematics of *Phalaenopsis* proposed by Christenson (2001) and support the four-pollinium clade being treated as the genus *Phalaenopsis*. The results from this study concur that the genera *Doritis* and *Kingidium* can be treated as synonyms of the genus *Phalaenopsis*. However, molecular data do not support the treatment of Christenson (2001), in which both *Doritis* and parts of *Kingidium* (*P. deliciosa* and *P. chibae*) were placed in the subgenus *Phalaenopsis*. According to the treatment of Christenson (2001), *Kingidium* was spilt into different parts and placed in the section *Deliciosae* of the subgenus *Phalaenopsis* and the subgenus *Aphyllae*. Based on molecular data, parts of *Kingidium*, namely *P. chibae* (syn. *Kingidium chibae*) and *P. deliciosa* (syn. *Kingidium deliciosa*) (section *Deliciosae* of the subgenus *Phalaenopsis*), have a close relationship to the subgenus *Parishianae* and are separated from the remaining species of the subgenus *Phalaenopsis*. Therefore, the section *Deliciosae* could be raised into a separate subgenus from the subgenus *Phalaenopsis* based on molecular data. Molecular data on the other species of *Kingidium*, namely *P. braceana* (syn. *Kingidium braceana*), were in agreement with the treatment of Christenson (2001) of being placed in the subgenus *Aphyllae*. Besides, it is not suitable to place *P. minus* (syn. *Kingidium minus*) in the subgenus *Aphyllae* based on molecular evidence. This unique species can be treated as a separate subgenus. On the other hand, the molecular evidence did not support *P. pulcherrima* (syn. *Doritis pulcherrima*) being placed in the subgenus *Phalaenopsis*. This species formed a clade with the four-pollinium species with moderate statistical support based on both ITS data and combined data. Therefore, the section *Esmeralda* of the subgenus *Phalaenopsis* could be raised into a separate subgenus based on this study. Furthermore, members of the section *Fuscatae* plus three species of the section *Amboinenses*, namely *P. maculata*, *P. gigantea*, and *P. doweryensis*, formed a clade and were

separated from the remaining species of the subgenus *Polychilos* based on this study. Therefore, these species can be treated as a separate subgenus. In addition, parts of the section *Amboinenses*, namely the *P. lueddemanniana* complex, could be treated as a separate section from the section *Amboinenses*. In short, the results of this study support the systematics of the genus *Phalaenopsis* based on the generic level as described by Christenson (2001), while several conflicts are shown on the subgeneric or sectional levels.

In the biogeography and evolutionary trends of the genus *Phalaenopsis*, South China was the origin center of the genus *Phalaenopsis*, and there were two evolutionary trends from the origin center to Southeast Asia. In one of them, *Phalaenopsis* species dispersed from South China to Southeast Asia using Indochina and older lands of the Philippines (Mindoro, Palawan, Zamboanga, etc.) as steppingstones, and the subgenus *Phalaenopsis* developed there. In the other one, *Phalaenopsis* species dispersed from South China to Southeast Asia using the Malay Peninsula as a steppingstone, and lineages of the subgenus *Polychilos* (with the exception of the section *Fuscatae* plus *P. gigantea*, *P. doweryensis*, and *P. maculata*) and the section *Fuscatae* plus *P. gigantea*, *P. doweryensis*, and *P. maculata* developed there. The evolutionary trends of the genus *Phalaenopsis* in the study are in agreement with the investigation of mountain flora by Van Steenis (1935, 1964). He suggested that Asian flora used two different pathways into the Malay archipelagoes. First, plant taxa dispersed from southeastern continental Asia to the islands of the Sunda Shelf (the Malay Peninsula, Borneo, Sumatra, Java, etc.). Second, plant taxa dispersed from Asia using Taiwan and the Philippines as steppingstones.

Furthermore, molecular data and geological dating were used to estimate the substitution rates of DNA from the genus *Phalaenopsis* based on the hypothesis of the molecular clock. The substitution rates of both ITS and cpDNA data from the genus *Phalaenopsis* were 2.4~4.7 x 10^{-9} and 3.9~7.8 x 10^{-10} substitutions/site/year, respectively. The substitution rates of ITS data of the genus *Phalaenopsis* are approximately six times those of cpDNA. Based on the substitution rates, the divergence time among most of the *P. lueddemanniana* complex was estimated to have been during the Pleistocene. The section *Deliciosae* separated from the section *Stauroglottis* at 21~10.5 Mya.

Literature Cited

Allaby, R. G. and T. A. Brown. 2001. Network analysis provides insights into evolution of 5S

rDNA arrays in *Triticum* and *Aegilops*. Genetics 157: 1331-1341.

Andreasen, K. and B. G. Baldwin. 2001. Unequal evolutionary rates between annual and perennial lineages of checker mallows (*Sidalcea*, Malvaceae): evidence from 18S-26S rDNA internal and external transcribed spacers. Mol. Biol. Evol. 18: 936-944.

Aoyama, M., K. Kojima, and M. Kobayashi. 1994. Morphology of microspore in *Phalaenopsis* hybrids. Kinki Chugoku Agric. Res. 88: 49-53.

Arends, J. C. 1970. Cytological observation on genome homology in eight interspecific hybrids of *Phalaenopsis*. Genetica 41: 88-100.

Aurelio, M. A., E. Barrier, C. Rangin, and C. Muller. 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.

Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed sequences of nuclear ribosomal DNA in plants: an example from the Compositae. Mol. Phylogenet. Evol. 1: 3-16.

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

Baldwin, B. G. and M. J. Sanderson. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). Proc. Natl. Acad. Sci. USA 95: 9402-9406.

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

Baltimore, D. 1981. Gene conversion: some implications for immunoglobulin genes. Cell 24: 592-594.

Barker, R. F., N. P. Harberd, M. G. Jarvis, and R. B. Flavell. 1988. Structure and evolution of the intergenic region in a ribosomal DNA repeat unit of wheat. J. Mol. Biol. 201: 1-17.

Bayer, R. J., C. F. Puttock, and S. A. Kelchner. 2000. Phylogeny of South African *Gnaphalieae* (Asteraceae) based on two noncoding chloroplast sequences. Am. J. Bot. 87: 259-272.

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

Bousquet, J., S. H. Strauss, A. H. Doerksen, and R. A. Price. 1992. Extensive variation in evolutionary rate of *rbc*L gene sequences among seed plants. Proc. Natl. Acad. Sci. USA 89: 7844-7848.

Britten, R. J. 1986. Rates of DNA sequence evidence differ between taxonomic groups. Science 231: 1393-1398.

Brouat, C., L. Gielly, and D. McKey. 2001. Phylogenetic relationships in the genus Leonardoxa (Leguminosae: Caesalpinioideae) inferred from chloroplast *trn*L intron and *trn*L-*trn*F intergenic spacer sequences. Am. J. Bot. 88:143-149.

Burnham, R. J. and A. Graham. 1999. The history of neotropical vegetation: new developments and status. Ann. Missouri Bot. Gard. 86: 546-589.

Chen, W. H., Y. M. Fu, R. M. Hsieh, W. T. Tsai, M. S. Chyou, C. C. Wu, and Y. S. Lin. 1995. Application of DNA amplification fingerprinting in the breeding of *Phalaenopsis* Orchid. *In* M. Terzi et al. (eds.). Current Issues in Plant Molecular and Cellular Biology, pp. 341-346.

Chiang, T. Y., Y. C. Chiang, Y. J. Chen, C. H. Chou, S. Havanond, T. N. Hong, and S. Huang. 2001. Phylogeography of Kandelia candel in East Asiatic mangroves based on nucleotide variation of chloroplast and mitochondrial DNAs. Mol. Ecol. 10: 2697-710.

Chiu, W. L. and B. B. Sears. 1985. Recombination between chloroplast DNAs does not occur in sexual crosses of *Oenothera*. Mol. Gen. Genet. 198: 525-528.

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

Christenson, E. A. 2001. *Phalaenopsis*. Timber Press, Portland, Oregon, 330p.

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

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

Derepas, A. and H. Dulieu. 1992. Inheritance of the capacity to transfer plastids by the pollen parent in *Petunia hybrida* Hort. J. Heredity 83: 6-10.

Doi, K., A. Kaga, N. Tomooka, and D. A. Vaughan. 2002. Molecular phylogeny of genus Vigna subgenus Ceratotropis based on rDNA ITS and *atp*B-*rbc*L intergenic spacer of cpDNA sequences. Genetica. 114: 129-45.

Doyle, J. and J. Doyle. 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.

Edwards, A. W. F. and L. L. Cavalli-Sforza. 1963. The reconstruction of evolution. Heredity 18: 553.

Espelund, M., E. Bekele, A. Holst-Jensen, K. S. Jakobsen, and I. Nordal. 2000. A molecular genetic analysis of Eragrostis tef (Zucc.) Trotter: non-coding regions of chloroplast DNA, 18S rDNA and the transcription factor VP1. Hereditas 132: 193-202.

Eyre-Walker, A. and B. S. Gaut. 1997. Correlated rates of synonymous site evolution across plant genomes. Mol. Biol. Evol. 14: 455-460.

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., F. Hamer, and E. S. Siegerist. 1995. Inquilina orchidacea: Orchidaceae plaerumque Levyanae. Lindleyana 10: 174-182.

Gaut, B. S., L. G. Clark, J. F. Wendel, and S. V. Muse. 1997. Comparisons of the molecular evolutionary process at *rbc*L and *ndh*F in the grass family (Poaceae). Mol. Biol. Evol. 14: 769-777.

Gentry, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? Ann. Missouri Bot. Gard. 69:557–593.

Gielly, L. and P. Taberlet. 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbc*L sequences. Mol. Biol. Evol. 11: 769-777.

Gielly, L. and P. Taberlet. 1996. A phylogeny of the European gentians inferred from chloroplast *trn*L (UAA) intron sequences. Bot. J. the Linnean Soc. 120: 57-75.

Goldblatt, P., V. Savolainen, O. Porteous, I. Sostaric, M. Powell, G. Reeves, J. C. Manning, T. G. Barraclough, and M. W. Chase. 2002. Radiation in the Cape flora and the phylogeny of Racock irises Moraea (Iridaceae) based on four plastid DNA regions. Mol. Phylogenet. Evol. 25: 341-360.

Golenberg, E. M., M.T. Clegg, M. L. Durbin, J. Doebley, and D. P. Ma. 1993. Evolution of a noncoding region of the chloroplast genome. Mol. Phylogenet. Evol. 2: 52-64.

Gruss, O. and L. Rollke. 1991. Eine weitere *Phalaenopsis* von den Philippinen-*P. bastianii*

Gruss and Rollke. Die Orchidee 42: 76-79.

Gruss, O. and L. Rollke. 1997. *Kingidium minus* Seidenfaden. Die Orchidee 48: 259-268.

Haffer, J. 1982. General Aspects of the Refuge Theory. *In* Prance, G.T. (ed.). *Biological Diversification in the Tropics*. Colombia University Press, New York, pp. 6-24.

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 Publicatioin. No. 106, pp. 153-184.

Hall, R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. *In* R. Hall and J. Holloway (eds.). Biogeography and Geological Evolution of SE Asia. Backhuys, Leiden, pp. 99-124.

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

Hillis, D. M. and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66: 411-453.

Hodkinson, T. R., M. W. Chase, M. D. Lledo, N. Salamin, and S. A. Renvoize. 2002. Phylogenetics of *Miscanthus, Saccharum* and related genera (*Saccharinate, Andropogoneae,* Poaceae) based on the DNA sequences from ITS nuclear ribosomal DNA and plastid *trn*L intron and *trn*L-F intergenic spacer. J. Plant Res. 115: 381-392.

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

Huang, C. Y., M. A. Ayliffe and J. N. Timmis. 2003. Direct measurement of the transfer rate of chloroplast DNA into the nucleus. Nature 422: 72-76.

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

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

Kavanagh, T. A. and J. N. Timmis. 1988. Structure of melon rDNA and nucleotide sequences of 17-25S spacer region. Theor. Appl. Genet. 76: 673-680.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

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

Kiss, T., M. Kis, and F. Solymosy. 1989b. Nucleotide sequence of a 25S rRNA gene from

tomato. Nucl. Acids Res. 17: 796.

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

Kuzoff, R. K., J.A. Sweere, D. E. Soltis, P.S. Soltis, and E. A. Zimmer. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. Mol. Biol. Evol. 15: 251-263.

Laroche, J., P. Li, L. Maggia, and J. Bousquet. 1997. Molecular evolution of angiosperm mitochondrial introns and exons. Proc. Natl. Acad. Sci. USA 94: 5722-5727.

Larson, A. 1994. The comparison of morphological and molecular data in phylogenetic systematics. *In* B. Schierwater, B. Streit, G. P. Wagner and R. DeSalle (eds.), Molecular Ecology and Evolution: approaches and applications. Birkhauser Verlag, Basel, pp. 371-390.

Li, W. H. 1997. Molecular evolution. Sinauer, Sunderland, MA.

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

Liu, Z. L., D. Zhang , X. Q. Wang , X. F. Ma, and X. R. Wang. 2003. Intragenomic and interspecific 5S rDNA sequence variation in five Asian pines. *American*. Am. J. Bot. 90:17-24.

Manen, J. F. and A. Natali. 1995. Comparison of the ribulose-1, 5-biphosphate carboxylase (*rbc*L) and *atp*B-*rbc*L noncoding spacer sequences in a recent plant group, the tribe *Rubieae* (Rubiaceae). J. Mol. Evol. 41: 920-927.

Maynard, S. 1989. Evolutionary Genetics. 325pp. Oxford.

McDade, L. A. and M. L. Moody. 1999. Phylogenetic relationships among Acanthaceae: evidence from noncoding *trn*L-*trn*F chloroplast DNA sequences. Am. J. Bot. 86: 70-80.

Moritz, C., J. L. Patton, C. J. Schneider, and T. B. Smith. 2000. Diversification of rainforest faunas: An integrated molecular approach.. Ann. Rev. Ecol. Syst. 31:533–563.

Moss, S. J., and M. E. J. Wilson. 1998. Biogeographic implications from the Tertiary Paleaeogeographic evolution of Sulawesi and Borneo. *In* Hall, R. and Holloway, J. D. (eds.). Biogeography and geological evolution of SE Asia. Backhuys, Leiden.

Muse, S. 2000. Examining raets and patterns of nucleotide substitution in plants. Plant Mol. Biol. 42: 25-43.

Nei, M. and S. Kumar. 2000. Molecular Evoultion and Phylogenetics. Oxford University Press, New York. pp. 33-36.

Palmer, J. D. 1987. Chloroplast DNA evolution and biosystematic uses of chloroplast DNA

variation. Amer. Natural. 130, Suppl., S26-S29.

Palmer, J. D., B. Osorio, and W. F. Thompson. 1988. Evolutionary significance of inversions in legume chloroplast DNAs. Curr. Genet. 14: 65-74.

Pianka, E. R. 1994. Biogeography and Historical Constraints. *In* Evolutionary Ecology. 5th edition. Harpercollins college Publisher, NY, pp. 15-40.

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

Ranker, T. A., J. M. Geiger, S. C. Kennedy, A. R. Smith, C. H. Haufler, and B. S. Parris. 2003. Molecular phylogenetics and evolution of the endemic Hawaiian genus *Adenophorus* (Grammitidaceae). Mol. Phylogenet. Evol. 26: 337-347.

Rathgeber, J. and I. Capesius. 1989. Nucleotide sequence of the 18S-25S spacer region from mustard DNA. Nucl. Acids Res. 17: 7522-7522.

Richardson, J. E., R. T. Pennington, T. D. Pennington, P. M. Hollingsworth. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. Science 293: 2242-2245.

Rossetto, M., B. R. Jackes, K. D. Scott, and R. J. Henry. 2001. Intergeneric relationships in the Australian Vitaceae: new evidence from cpDNA analysis. Genet. Res.Crop Evol. 48: 307-314.

Rzhetsky, A. and M. Nei. 1992. A simple method for estimating and testing minimum-evolution trees. Mol. Biol. Evol. 9: 945-967.

Rzhetsky, A. and M. Nei. 1993. Theoretical foundation of the minimum-evolution method of phylogenetic inference. Mol. Biol. Evol. 10: 1073-1095.

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

Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14: 1218-1231.

Sang, T. and Y. Zhong. 2000. Testing hybridization hypotheses based on incongruent gene trees. Syst. Biol. 49: 422-434.

Sang, T., D. J. Crawford, and M. O. Silva. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). Syst. Bot. 20: 55-64.

Sang, T., D. J. Crawford, S. C. Kim and T. F. Stuessy. 1994. Radiation of the endemic genus *Dendroseris* (Ateraceae) on the Juan Fernandez Islands: Evidence from sequences of ribosomal DNA. Ameriacan. Am. J. Bot. 81: 1494-1501.

Schaal, B. A., D. A. Hayworth, K. M. Olsen, J. T. Rauscher, and W. A. Smith. 1998. Phylogeographical studies in plants: problems and prespects. Mol. Ecol. 7: 465-474.

Schiebel, K. and Hemleben V. 1989. Nucleotide sequence of the 18S-25S spacer region from rDNA of mung bean. Nucl. Acids Res. 17: 2852-2852.

Schwarzbach, A. E. and R. E. Ricklefs. 2000. Systematic affinities of Rhizophoraceae and Anisophylleaceae, and intergeneric relationships within Rhizophoraceae, based on chloroplast DNA, nuclear ribosomal DNA, and morphology. Am. J. Bot. 87: 547-564.

Second, G., A. Dally, and S. H. Zhang. 1989. Occasional biparent inheritance of chloroplast DNA in rice. Rice Genetics Newletters 6: 150-152.

Seidenfaden, G. 1988a. *Doritis*. Opera Bot. 95: 31-34.

Seidenfaden, G. 1988b. *Kingidium*. Opera Bot. 95:182-189.

Seidenfaden, G. 1988c. *Phalaenopsis*. Opera Bot. 95:236-241.

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

Shim, P. S. and J.A. Fowlie. 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. and H. Kamemoto. 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.

Simovic, N., D. Wolyn, and G. Jelenkovic. 1992. Sequence analysis of 18S ribosomal RNA gene in *Fragaria* x *Ananassa* Duch. Cultivated octoploid strawberry. Plant Mol. Biol. 18: 1217-1220.

Sitnikova, T., Rzhetsky A., and M. Nei. 1995. Interior-branch and bootstrap tests of phylogenetic trees. Mol. Biol. Evol. 12: 319-333.

Slowinski, J. B. and R. D. M. Page. 1999. How should species phylogenies be inferred from sequence data? Syst. Biol. 48: 814-825.

Smedmark, J. E. E. and T. Eriksson. 2002. Phylogenetic relationship of *Geum* (Rosaceae) and relatives inferred from the nrITS and *trn*L-*trn*F regions. Syst. Bot. 27: 303-317.

Smith, G. P. 1976. Evolution of repeated DNA sequences by unequal crossover. Science 191: 528-535.

Snaderson, M. J. and J. A. Doyle. 2001. Source of error and confidence intervals in estimating the age of angiosperms from *rbc*L and 18S rDNA data. Am. J. Bot. 88:

1499-1516.

Stebbins, G. L. 1974. Flowering plants. Evolution above the species level. Cambridge/Mass.: The Belkamp Press of Harvard Univ. Press.

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

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

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

Sweet, H. R. 1968. Revision of the genus *Phalaenopsis*, pts. 1 and 2. Am. Orchid Soc. Bull. 37: 867-877, 1089-1104.

Sweet, H. R. 1969. Revision of the genus *Phalaenopsis*, pts. 3-8. Am. Orchid Soc. Bull. 38: 33-43, 225-239, 321-336, 505-519, 681-694, 888-901.

Sweet, H. R. 1980. The genus *Phalaenopsis*. The Orchid Digest, Pomona, California.

Swofford, D. L. 1991. When are phylogeny estimates from molecular and morphological data incongruent? *In* M. M. Miyamoto and J. Cracraft (eds.), Phylogenetic Analysis of DNA Sequences. Oxford, pp. 295-333.

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

Tajima, F. 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.

Tajima, F. 1993. Simple methods for testing molecular clock hypothesis. Genetics 135: 599-607.

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

Takaiwa, F., K. Oono, and M. Sugiura. 1985a. Nucleotide sequence of the 17-25S spacer region from rice rDNA. Plant Mol. Biol. 4: 355-364.

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

Tan, Y., S. T. Bishoff, and M. A. Riley. 1993. Ubiquitins revisited: further examples of

within-and between-locus concerted evolution. Mol. Phylogenet. Evol. 2: 351-360.

Tanaka, R. and K. Kamemoto. 1984. Chromosome in orchids: counting and numbers. *In* Arditti, J. (ed.), Orchid Biology: Reviews and Perspectives. Cornell Univ. Press, Ithaca, NY.

Tharp, A. G., J. A. Fowlie, and C. Z. Tang. 1987. *Phalaenopsis philippinensis* Golamco ex Fowl. and Tang, C. Z., A new species. 51: 92.

Tsai, C. C. and C. H. Chou. 2000. High heterogeneity of internal transcribed spacers of rDNAs in *I. cylindrica.* Taiwania 45: 249-262.

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

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

Van Raamsdonk, L. M., W. Ensink, A. W. Van Heusden, M. Vrielink-Van Ginkel, and C. Kik. 2003. Biodiversity assessment based on cpDNA and crossability analysis in selected species of *Allium* subgenus *Rhizirideum.* Theor. Appl. Genet. 107: 1048-1058.

Van Steenis, G. G. G. J. 1935. On the origin of the Malaysian mountain flora. Part 2. Altitudinal zones, general considerations and renewed statement of the problem. Bull. J. Bot. Buitenzorg Ser. III 13-3: 289-417.

Van Steenis, G. G. G. J. 1964. Plant geography of the mountain flora of Mt. Kinabalu. Proc. R. Soc. B 161: 7-38.

Verboom, G. A., H. P. Linder, and W. D. Stock. 2003, Phylogenetics of the grass genus *Ehrharta*: evidence for radiation in the summer-arid zone of the South African Cape. Evolution 57: 1008-1021.

Vinnersten, A. and K. Bremer. 2001. Age and biogeography of major clades in Liliales. Am. J. Bot. 88: 1695-1703.

Wendel, J. F., A. Schnabel, and T. Seelanan. 1995. An unusual ribosomal DNA sequence from *Gossypium gossypioides* reveals ancient, cryptic, intergenomic introgression. Mol. Phylogenet. Evol. 4: 298-313.

Whitmore, T. C. and G. T. Prance (eds.). 1987. Biogeography and Quaternary quaternary History history in Tropical tropical America. Clarendon Press, Oxford.

Woodard, J. W. 1951. Some chromosome numbers in *Phalaenopsis.* Am. Orchid Soc. Bull. 20: 356-358.

Wu, C. I. and W. H. Li. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. Proc. Natl. Acad. Sci. USA 82: 1741-1745.

Yang, Y. W., P. Y. Tai, and W. H. Li. 2002. A study of the phylogeny of *Brassica rapa*, *B. nigra*, *Raphanus sativus*, and their related genera using noncoding regions of chloroplast DNA. Mol. Phylogenet. Evol. 23: 268-275.

Yuan, Y. M., S. Wohlhauser, M. Moller, P. Chassot, G. Mansion, J. Grant, P. Kupfer, and J. Klackenberg. 2003. Monophyly and relationships of the tribe *Exaceae* (Gentianaceae) inferred from nuclear ribosomal and chloroplast DNA sequences. Mol. Phylogenet. Evol. 28: 500-517.

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

Zuckerkandl, E. and L. Pauling. 1965. Evolutionary divergence and convergence in proteins. *In* Evolving genes and proteins, *Edited by* V. Bryson and H. G. Vogel. Academic Press, New York. pp. 97-166.

Table 1. Comparison of the systematics of the genus *Phalaenopsis* between Sweet (1980) and Christenson (2001).

Sweet 1980	New species [a] or revised species [b]	Christenson 2001
Section *Proboscidioides*		**Subgenus *Proboscidioides***
Phalaenopsis lowii		*Phalaenopsis lowii*
Section *Aphyllae*		**Subgenus *Aphyllae***
	Phalaenopsis braceana (syn. *Kingidium braceana*) (Christenson, 1986)[b]	*Phalaenopsis braceana*
	Kingidium minus (Seidenfaden, 1988b)[a]	*Phalaenopsis minus*
Phalaenopsis wilsonii		*Phalaenopsis wilsonii*
	Phalaenopsis honghenensis (Liu, 1988)[a]	*Phalaenopsis honghenensis*
	Phalaenopsis taenialis (syn. *Kingidium taenialis*) (Christenson, 1986)[b]	*Phalaenopsis taenialis*
Phalaenopsis stobartiana (syn. *Phalaenopsis hainanensis*)		*Phalaenopsis stobartiana* *Phalaenopsis hainanensis*
Section *Parishianae*		**Subgenus *Parishianae***
Phalaenopsis mysorensis	ship to the section *Deliciosae*	
Phalaenopsis appendiculata		*Phalaenopsis appendiculata*
Phalaenopsis gibbosa		*Phalaenopsis gibbosa*
Phalaenopsis parishii		*Phalaenopsis parishii*
Phalaenopsis lobii		*Phalaenopsis lobii*
Section *Polychilos*		**Subgenus *Polychilos*** **Section *Polychilos***
Phalaenopsis mannii		*Phalaenopsis mannii*
Phalaenopsis cornu-cervi *Phalaenopsis lamelligera*		*Phalaenopsis cornu-cervi*
	Phalaenopsis borneensis (Garay et al., 1995)[a]	*Phalaenopsis borneensis*
Phalaenopsis pantherina		*Phalaenopsis pantherina*
Section *Fuscatae*		**Section *Fuscatae***
Phalaenopsis cochlearis		*Phalaenopsis cochlearis*
Phalaenopsis viridis		*Phalaenopsis viridis*
Phalaenopsis fuscata		*Phalaenopsis fuscata*
Phalaenopsis kunstleri		*Phalaenopsis kunstleri*
Section *Amboinenses*		**Section *Amboinenses***
Phalaenopsis micholitzii		*Phalaenopsis micholitzii*
Phalaenopsis gigantea		*Phalaenopsis gigantea*
Phalaenopsis javanica		*Phalaenopsis javanica*
Phalaenopsis amboinensis		*Phalaenopsis amboinensis*
Phalaenopsis robinsonii		*Phalaenopsis robinsonii*
	Phalaenopsis venosa (Shim and Fowlie, 1983)[a]	*Phalaenopsis venosa*
	Phalaenopsis doweryensis (Garay et al., 1995)[a]	*Phalaenopsis doweryensis*
Section *Zebrinae*		
Subsection *Lueddemanninae*		
Phalaenopsis pulchra		*Phalaenopsis pulchra*
Phalaenopsis reichenbachiana		*Phalaenopsis reichenbachiana*
Phalaenopsis fasciata		*Phalaenopsis fasciata*
Phalaenopsis fimbriata		*Phalaenopsis fimbriata*
Phalaenopsis hieroglyphica		*Phalaenopsis hieroglyphica*
Phalaenopsis lueddemanniana		*Phalaenopsis lueddemanniana*
Phalaenopsis violacea		*Phalaenopsis violacea*
Phalaenopsis ×gersenii (syn. *Phalaenopsis ×singuliflora*)		*Phalaenopsis ×gersenii* *Phalaenopsis ×singuliflora*
	Phalaenopsis bastianii (Gruss and Rollke , 1991)[a]	*Phalaenopsis bastianii*
	Phalaenopsis floresensis (Fowlie, 1993) [a]	*Phalaenopsis floresensis*
	Phalaenopsis bellina (Christenson and Whitten 1995)[b]	*Phalaenopsis bellina*
Subsection *Zebrinae*		**Section *Zebrinae***

Phalaenopsis speciosa		*Phalaenopsis speciosa*
Phalaenopsis tetraspis		*Phalaenopsis tetraspis*
Phalaenopsis corningiana		*Phalaenopsis corningiana*
Phalaenopsis sumatrana		*Phalaenopsis sumatrana*
	Phalaenopsis inscriptiosinensis (Fowlie, 1983)[a]	*Phalaenopsis inscriptiosinensis*
Subsection *Hirsutae*		
Phalaenopsis pallens		*Phalaenopsis pallens*
Phalaenopsis mariae		*Phalaenopsis mariae*
Subsection *Glabrae*		
Phalaenopsis modesta		*Phalaenopsis modesta*
Phalaenopsis maculata		*Phalaenopsis maculata*
		Subgenus *Phalaenopsis*
Section *Phalaenopsis*		**Section *Phalaenopsis***
	Phalaenopsis philippinensis (Tharp et al., 1987) [a]	*Phalaenopsis philippinensis*
Phalaenopsis amabilis		*Phalaenopsis amabilis*
Phalaenopsis aphrodite		*Phalaenopsis aphrodite*
Phalaenopsis sanderiana		*Phalaenopsis sanderiana*
Phalaenopsis schilleriana		*Phalaenopsis schilleriana*
Phalaenopsis stuartiana		*Phalaenopsis stuartiana*
Phalaenopsis ×amphitrite		*Phalaenopsis ×amphitrite*
Phalaenopsis ×intermedia		*Phalaenopsis ×intermedia*
Phalaenopsis ×leucorrhoda		*Phalaenopsis ×leucorrhoda*
Phalaenopsis ×veitchiana		*Phalaenopsis ×veitchiana*
Section *Stauroglottis*		**Section *Stauroglottis***
Phalaenopsis equestris		*Phalaenopsis equestris*
Phalaenopsis celebensis		*Phalaenopsis celebensis*
Phalaenopsis lindenii		*Phalaenopsis lindenii*
		Section *Deliciosae*
	Phalaenopsis chibae (Yukawa, 1996)[a]	*Phalaenopsis chibae*
	Kingidium deliciosa (Siegerist, 1989)[a]	*Phalaenopsis deliciosa*
Genus *Doritis*		**Section *Esmeralda***
Doritis pulcherrima		*Phalaenopsis pulcherrima*
Doritis regnieriana		*Phalaenopsis regnieriana*
Doritis buyssoniana		*Phalaenopsis buyssoniana*

Table 2. List of the 52 *Phalaenopsis* species of this study, their systematic classification, and geographical distributions.

Taxa and systematic classification[a]	Distribution (Christenson, 2001)	Source
Genus Phalaenopsis		
Subgenus *Proboscidioides*		
Phalaenopsis lowii	Myanmar, adjacent western Thailand	KDAIS[b]-KC87
Subgenus *Aphyllae*		
Phalaenopsis wilsonii	China (Yunnan) and eastern Tibet at 800-2200m in elevation	KDAIS-KC108
Phalaenopsis minus	endemic to Thailand	KDAIS-KC226
Phalaenopsis braceana	Bhutan and China at 1100~1700 m in elevation	KDAIS-KC218
Subgenus *Parishianae*		
Phalaenopsis gibbosa	Vietnam and Laos	KDAIS-KC51
Phalaenopsis lobbii	India, Bhutan, Myanmar, and Vietnam	KDAIS-KC104
Phalaenopsis parishii	eastern Himalayas, India, Myanmar, and Thailand	KDAIS-KC192
Subgenus *Polychilos*		
Section *Polychilos*		
Phalaenopsis mannii	northeastern India, Nepal, and China to Vietnam at 500~1400m in elevation	KDAIS-KC77
Phalaenopsis cornu-cervi	northeastern India, Thailand and the Nicobar Is. to Java and Borneo from sea level to 800m in elevation	KDAIS-KC23
Phalaenopsis borneensis	endemic to Borneo	KDAIS-KC109
Phalaenopsis pantherina	endemic to Borneo from sea level to 800m in elevation	KDAIS-KC56
Phalaenopsis lamelligera		KDAIS-KC114
Section *Fuscatae*		
Phalaenopsis cochlearis	Malaysia (Malay Peninsula) and Indonesia (Sarawak) at 500~700 m in elevation	KDAIS-KC182
Phalaenopsis viridis	endemic to Indonesia (Sumatra) at 1000m in elevation	KDAIS-KC41
Phalaenopsis fuscata	Malaysia (Malay Peninsula), Borneo (West Koetai)	KDAIS-KC115
Phalaenopsis kunstleri	Myanmar and the Malay Peninsula	KDAIS-KC139
Section *Amboinenses*		
Phalaenopsis pulchra	endemic to the Philippines (Luzon, Leyte) at 100~650 m in elevation	KDAIS-KC17
Phalaenopsis bellina	Malaysia (Malay Peninsula) and East Malaysia (Sarawak)	KDAIS-KC67
Phalaenopsis violacea	Indonesia (Sumatra) and Malaysia (Malay Peninsula)	KDAIS-KC152
Phalaenopsis micholitzii	the Philippines (Mindanao)	KDAIS-KC85
Phalaenopsis fimbriata	Indonesia (Java, Sarawak, and Sumatra)	KDAIS-KC62
Phalaenopsis floresensis	endemic to the island of Flores, 300~500 m	KDAIS-KC54
Phalaenopsis gigantea	endemic toSabah in East Malaysia and adjacent Kalimantan Timur, from sea level to 400 m in elevation	KDAIS-KC131
Phalaenopsis fasciata	endemic to the Philippines (Luzon, Bohol, Mindanao)	KDAIS-KC189
Phalaenopsis doweryensis	East Malaysia, Sabah, without a precise locality	KDAIS-KC138
Phalaenopsis modesta	endemic to the island of Borneo in East Malaysia (Sabah) and Indonesia (Kalimantan)	KDAIS-KC159
Phalaenopsis maculata	Malaysia (Pahang), East Malaysia (Sabah, Sarawak), Indonesia (Kalimantan Timur)	KDAIS-KC49
Phalaenopsis javanica	endemic to Indonesia (Java)	KDAIS-KC38
Phalaenopsis mariae	endemic to the Philippines and Indonesia (Kalimantan, Borneo)	KDAIS-KC30
Phalaenopsis amboinensis	Indonesia (Molucca Archipelago and Sulawesi)	KDAIS-KC43
Phalaenopsis lueddemanniana	endemic to the Philippines	KDAIS-KC8
Phalaenopsis venosa	endemic to Indonesia (Sulawesi)	KDAIS-KC14
Phalaenopsis pallens	endemic to the Philippines	KDAIS-KC117
Phalaenopsis bastianii	endemic to the Philippines	KDAIS-KC34

Phalaenopsis hieroglyphica	endemic to the Philippines	KDAIS-KC33
Section *Zebrinae*		
Phalaenopsis inscriptiosinensis	apparently endemic to Indonesia (Sumatra)	
Phalaenopsis tetraspis	India (Andaman and Nicobar Islands) and Indonesia (Sumatra)	KDAIS-KC40
Phalaenopsis corningiana	Borneo (Sarawak and elsewhere on the island)	KDAIS-KC29
Phalaenopsis sumatrana	widespread from Myanmar, Thailand, Vietnam, to Indonesi (Java, Sumatra), Malaysia (Perak, Johore), East Malaysia (Sabah), and the Philipines (Palawan)	KDAIS-KC32
Subgenus *Phalaenopsis*		
Section *Phalaenopsis*		
Phalaenopsis philippinensis	endemic to the Philippines	KDAIS-KC26
Phalaenopsis amabilis	widespread from Sumatra and Java to the southern Philippines, and east to New Guinea and Queensland, Australia	KDAIS-KC96
Phalaenopsis aphrodite	the northern Philippines and southeastern Taiwan	KDAIS-KC99
Phalaenopsis sanderiana	endemic to the Philippines	KDAIS-KC35
Phalaenopsis schilleriana	endemic to the Philippines	KDAIS-KC4
Phalaenopsis stuartiana	endemic to the island of Mindanao in the southern Philippines.	KDAIS-KC2
Section *Deliciosae*		
Phalaenopsis chibae	endemic to Vietnam at 400~600 m in elevation	KDAIS-KC27
Phalaenopsis deliciosa	widespread from Sri Lanka and India to the Philippines and Sulawesi	KDAIS-KC73
Section *Esmeralda*		
Phalaenopsis pulcherrima	widespread from northeast India and southern China throughout Indochina to Malaysia (Malay Peninsula), Indonesia (Sumatra), and East Malaysia (Sabah)	KDAIS-KC20
Section *Stauroglottis*		
Phalaenopsis equestris	the Philippines and Taiwan	KDAIS-KC59
Phalaenopsis celebensis	endemic to Indonesia (Sulawesi)	KDAIS-KC64
Phalaenopsis lindenii	endemic to the Philippines	KDAIS-KC118
Genus Paraphalaenopsis		
Par. laycockii	endemic to the island of Borneo	KDAIS-KC122
Par. labukensis	endemic to the island of Borneo	KDAIS-KC121
Par. serpentilingua	endemic to the island of Borneo	KDAIS-KC124
Genus Gastrochilus		
G. japonicus		KDAIS-KC69
Genus Tuberolabium		
T. kotoense		KDAIS-KC37

[a] The classification of *Phalaenopsis* is based on Christenson (2001).
[b] Kaohsiung District Agricultural Improvement Station.

Table 3. Lengths of internal transcribed spacer 1 (ITS1) and ITS2 and GenBank accession numbers from 52 *Phalaenopsis* species plus the five taxa of related genera.

Taxa [a]	ITS1		ITS2		GenBank accession no.
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	
Phalaenopsis amabilis	230	77.4	256	76.1	AY391516
Phalaenopsis amboinensis	238	72.3	263	71.3	AF537006
Phalaenopsis aphrodite	229	77.3	257	77.5	AY391539
Phalaenopsis bastianii	213	71.8	263	72.2	AF537001
Phalaenopsis bellina	243	72.0	263	77.9	AY390237
Phalaenopsis borneensis	241	75.1	269	75.0	AF537024
Phalaenopsis braceana	231	68.8	259	70.0	AY228495
Phalaenopsis celebensis	239	77.0	268	71.3	AF537014
Phalaenopsis chibae	239	71.6	259	70.0	AF536996
Phalaenopsis cochlearis	239	72.4	262	70.0	AF537035
Phalaenopsis corningiana	234	71.8	263	73.0	AY390247
Phalaenopsis cornu-cervi	238	73.5	268	73.4	AF536994
Phalaenopsis deliciosa	230	69.6	259	70.4	AF537016
Phalaenopsis doweryensis	238	71.4	262	67.5	AF537032
Phalaenopsis equestris	237	74.3	264	72.6	AF537012
Phalaenopsis fasciata	235	72.3	263	71.1	AF537036
Phalaenopsis fimbriata	234	70.1	263	69.4	AF537013
Phalaenopsis floresensis	234	70.1	263	69.7	AF537010
Phalaenopsis fuscata	234	63.7	261	62.8	AY228498
Phalaenopsis gibbosa	233	70.4	259	70.4	AF537009
Phalaenopsis gigantea	231	66.2	261	62.9	AF537031
Phalaenopsis hieroglyphica	255	74.1	263	71.1	AF537000
Phalaenopsis inscriptiosinensis	234	70.1	263	69.0	AF537007
Phalaenopsis javanica	231	68.8	263	69.7	AF537004
Phalaenopsis kunstleri	233	63.1	260	62.8	AY228499
Phalaenopsis lamelligera	234	75.2	269	74.1	AF537025
Phalaenopsis lindenii	237	77.0	264	71.9	AF537027
Phalaenopsis lobbii	234	70.1	265	71.0	AF537022
Phalaenopsis lowii	231	74.0	260	71.9	AF537019
Phalaenopsis lueddemanniana	236	72.5	263	71.1	AF536991
Phalaenopsis maculata	231	70.1	263	68.3	AF537008
Phalaenopsis mannii	235	71.1	264	71.4	AY228496
Phalaenopsis mariae	222	71.6	263	70.8	AF536998
Phalaenopsis micholitzii	239	73.6	263	72.2	AF537018
Phalaenopsis minus	232	69.8	259	69.0	AY228494
Phalaenopsis modesta	228	69.7	263	69.8	AF537034
Phalaenopsis pallens	234	73.1	263	71.5	AF537026
Phalaenopsis pantherina	235	74.5	269	73.5	AF537011
Phalaenopsis parishii	233	70.8	265	71.0	AF537037
Phalaenopsis philippinensis	238	77.3	270	75.3	AF536995
Phalaenopsis pulcherrima	231	75.8	259	71.1	AF536993
Phalaenopsis pulchra	235	71.9	263	71.1	AF536992
Phalaenopsis reichenbachiana	235	72.8	263	71.1	AY228502
Phalaenopsis sanderiana	230	76.9	257	77.1	AY391551
Phalaenopsis schilleriana	238	77.2	265	74.5	AF536990
Phalaenopsis stuartiana	238	78.2	265	75.9	AF536989
Phalaenopsis sumatrana	234	71.8	263	73.0	AY390239
Phalaenopsis tetraspis	234	71.8	263	70.4	AF537005
Phalaenopsis venosa	236	71.2	263	70.4	AY228500
Phalaenopsis violacea	243	72.0	263	77.9	AY390228
Phalaenopsis viridis	228	65.8	263	63.8	AY228497
Phalaenopsis wilsonii	231	68.8	259	69.3	AF537023
Paraphalaenopsis laycockii	230	65.7	260	67.3	AF537029
Paraphalaenopsis labukensis	232	65.5	261	67.4	AF537028
Paraphalaenopsis serpentilingua	229	66.4	260	66.9	AF537030
Gastrochilus japonicus	234	66.2	262	68.9	AY228503
Tuberolabium kotoense	230	72.2	261	68.4	AF537003

Table 4. Number of characters, variable sizes, and genetic distances of the two-parameter method of Kimura among the 52 *Phalaenopsis* species based on the analyses of different DNA fragments of this study.

DNA fragments	Informative size	Conserved size	Variable size	Ratio of variable size (%)	Genetic distance	
					Range	Average
ITS1+ITS2	539	236	303	56.2	0.000-0.225	0.109
Intron of *trn*L	710	536	174	24.5	0.000-0.087	0.028
IGS of *trn*L-*trn*F	426	376	50	11.7	0.000-0.034	0.012
Intron of *trn*L + IGS of *trn*L-*trn*F	1129	892	237	21.0	0.001-0.046	0.021
IGS of *atp*B-*rbc*L	742	614	128	17.3	0.000-0.067	0.022
Intron of *trn*L+ IGS of *trn*L-*trn*F+ IGS of *atp*B-*rbc*L	1864	1461	403	21.6	0.000-0.054	0.022

Table 5. Number of informative sizes, and genetic distances of the two-parameter method of Kimura among 52 *Phalaenopsis* species plus the four outgroups based on the analyses of different DNA fragments of this study.

DNA fragments	Informative size	Conserved sizes	Varable sizes	Ratio of variable sizes (%)	Genetic distances	
					Ranges	Average
ITS1+ITS2	541	211	330	60.1	0.000-0.242	0.117
Intron of *trn*L	753	470	283	37.6	0.000-0.311	0.040
IGS of *trn*L-*trn*F	427	348	79	18.5	0.000-0.074	0.017
Intron of *trn*L + IGS of *trn*L-*trn*F	1165	793	372	31.9	0.001-0.167	0.029
IGS of *atp*B-*rbc*L	759	587	172	22.7	0.000-0.091	0.025
Intron of *trn*L + IGS of *trn*L-*trn*F+ IGS of *atp*B-*rbc*L	1902	1344	558	29.3	0.004-0.130	0.028

Table 6. Genetic distances of the two-parameter method of Kimura method inferred from the ITS1+ITS2 of nrDNA among the 52 taxa of the genus *Phalaenopsis* plus the five related species.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57]
[1]*																																																											
[2]	0.152																																																										
[3]	0.015	0.147																																																								
[4]	0.145	0.062	0.140																																																								
[5]	0.169	0.048	0.158	0.057																																																							
[6]	0.140	0.099	0.127	0.090	0.103																																																						
[7]	0.161	0.112	0.153	0.121	0.132	0.148																																																						
[8]	0.133	0.099	0.126	0.106	0.124	0.133	0.111																																																					
[9]	0.169	0.108	0.155	0.113	0.124	0.155	0.044	0.112																																																				
[10]	0.138	0.094	0.133	0.098	0.117	0.127	0.099	0.092	0.105																																																			
[11]	0.145	0.050	0.140	0.053	0.059	0.093	0.115	0.097	0.113	0.082																																																	
[12]	0.148	0.105	0.135	0.093	0.114	0.033	0.157	0.138	0.161	0.132	0.096																																																
[13]	0.176	0.123	0.162	0.132	0.140	0.161	0.044	0.123	0.062	0.101	0.121	0.165																																															
[14]	0.141	0.082	0.133	0.081	0.100	0.120	0.080	0.085	0.088	0.039	0.071	0.122	0.087																																														
[15]	0.111	0.091	0.096	0.080	0.102	0.114	0.111	0.086	0.108	0.084	0.085	0.120	0.116	0.070																																														
[16]	0.145	0.055	0.140	0.017	0.059	0.088	0.121	0.100	0.118	0.092	0.046	0.087	0.129	0.076	0.082																																														
[17]	0.148	0.042	0.143	0.051	0.052	0.091	0.108	0.095	0.101	0.076	0.029	0.094	0.114	0.064	0.083	0.044																																													
[18]	0.145	0.046	0.140	0.048	0.057	0.093	0.111	0.097	0.103	0.080	0.033	0.096	0.118	0.069	0.082	0.046	0.012																																													
[19]	0.205	0.145	0.199	0.145	0.164	0.179	0.138	0.153	0.145	0.099	0.132	0.185	0.154	0.076	0.134	0.137	0.112	0.117																																													
[20]	0.162	0.131	0.159	0.130	0.146	0.145	0.060	0.132	0.060	0.112	0.126	0.160	0.062	0.104	0.132	0.129	0.117	0.119	0.158																																													
[21]	0.230	0.150	0.224	0.157	0.162	0.205	0.154	0.167	0.159	0.129	0.137	0.206	0.168	0.104	0.147	0.151	0.128	0.127	0.166	0.166																																													
[22]	0.140	0.050	0.135	0.015	0.054	0.083	0.113	0.090	0.110	0.087	0.046	0.084	0.121	0.071	0.080	0.012	0.044	0.046	0.132	0.121	0.145																																													
[23]	0.147	0.046	0.142	0.053	0.057	0.095	0.113	0.099	0.105	0.080	0.029	0.099	0.118	0.069	0.087	0.048	0.020	0.025	0.124	0.121	0.130	0.048																																												
[24]	0.179	0.064	0.174	0.072	0.079	0.120	0.131	0.125	0.121	0.104	0.055	0.119	0.134	0.092	0.111	0.066	0.033	0.029	0.138	0.140	0.151	0.066	0.046																																											
[25]	0.214	0.155	0.209	0.157	0.175	0.190	0.148	0.159	0.153	0.109	0.142	0.191	0.165	0.085	0.144	0.148	0.122	0.127	0.012	0.169	0.178	0.142	0.135	0.146																																											
[26]	0.139	0.084	0.129	0.076	0.096	0.085	0.139	0.126	0.142	0.116	0.071	0.089	0.148	0.102	0.104	0.071	0.071	0.076	0.164	0.148	0.170	0.071	0.076	0.095	0.175																																										
[27]	0.119	0.103	0.104	0.085	0.107	0.119	0.106	0.093	0.103	0.086	0.085	0.125	0.111	0.068	0.026	0.087	0.085	0.085	0.126	0.125	0.145	0.085	0.089	0.111	0.136	0.111																																										
[28]	0.179	0.115	0.171	0.121	0.124	0.150	0.056	0.124	0.058	0.106	0.111	0.156	0.065	0.089	0.109	0.119	0.101	0.106	0.141	0.047	0.154	0.111	0.106	0.124	0.152	0.137	0.111																																									
[29]	0.178	0.132	0.161	0.133	0.141	0.147	0.051	0.125	0.078	0.123	0.130	0.164	0.067	0.103	0.131	0.133	0.125	0.125	0.169	0.086	0.189	0.125	0.130	0.144	0.180	0.146	0.123	0.081																																									
[30]	0.145	0.050	0.140	0.015	0.054	0.086	0.116	0.097	0.113	0.090	0.042	0.085	0.124	0.073	0.080	0.010	0.040	0.042	0.135	0.124	0.145	0.010	0.044	0.062	0.145	0.066	0.085	0.113	0.130																																									
[31]	0.151	0.085	0.143	0.084	0.101	0.117	0.085	0.077	0.084	0.051	0.076	0.120	0.097	0.029	0.071	0.079	0.065	0.069	0.076	0.100	0.110	0.074	0.069	0.091	0.086	0.108	0.066	0.087	0.114	0.076																																									
[32]	0.154	0.086	0.146	0.088	0.097	0.099	0.136	0.128	0.117	0.105	0.084	0.107	0.139	0.096	0.115	0.087	0.075	0.080	0.154	0.139	0.174	0.079	0.079	0.103	0.165	0.086	0.112	0.128	0.145	0.084	0.102																																									
[33]	0.142	0.052	0.137	0.043	0.056	0.081	0.108	0.094	0.106	0.089	0.034	0.086	0.122	0.073	0.089	0.038	0.036	0.038	0.135	0.122	0.146	0.036	0.041	0.063	0.146	0.070	0.089	0.109	0.118	0.036	0.073	0.072																																									
[34]	0.133	0.061	0.129	0.048	0.068	0.097	0.115	0.087	0.120	0.087	0.052	0.108	0.120	0.076	0.087	0.050	0.048	0.048	0.142	0.137	0.151	0.050	0.052	0.075	0.148	0.085	0.089	0.121	0.132	0.048	0.076	0.086	0.047																																									
[35]	0.163	0.112	0.155	0.120	0.131	0.150	0.027	0.113	0.042	0.099	0.115	0.156	0.055	0.082	0.115	0.120	0.108	0.110	0.143	0.053	0.161	0.112	0.112	0.128	0.154	0.142	0.111	0.053	0.058	0.115	0.087	0.125	0.110	0.120																																									
[36]	0.151	0.044	0.146	0.048	0.051	0.089	0.109	0.097	0.107	0.078	0.025	0.092	0.117	0.067	0.085	0.042	0.019	0.023	0.127	0.120	0.133	0.040	0.023	0.044	0.138	0.072	0.085	0.102	0.123	0.038	0.072	0.078	0.034	0.051	0.108																																									
[37]	0.145	0.055	0.135	0.013	0.055	0.084	0.116	0.097	0.113	0.087	0.044	0.087	0.124	0.071	0.078	0.012	0.042	0.044	0.132	0.122	0.148	0.012	0.046	0.064	0.143	0.071	0.080	0.111	0.125	0.010	0.074	0.084	0.036	0.048	0.115	0.040																																									
[38]	0.138	0.100	0.130	0.095	0.109	0.024	0.146	0.128	0.151	0.125	0.089	0.031	0.160	0.118	0.120	0.089	0.087	0.089	0.179	0.144	0.200	0.084	0.091	0.116	0.191	0.080	0.120	0.146	0.151	0.087	0.115	0.095	0.075	0.094	0.146	0.085	0.087																																									
[39]	0.173	0.113	0.165	0.121	0.122	0.145	0.063	0.121	0.060	0.106	0.111	0.151	0.069	0.089	0.114	0.119	0.101	0.106	0.141	0.053	0.154	0.111	0.106	0.121	0.152	0.132	0.112	0.010	0.088	0.113	0.088	0.123	0.104	0.121	0.060	0.102	0.111	0.141																																									
[40]	0.083	0.152	0.072	0.131	0.157	0.132	0.148	0.119	0.152	0.140	0.141	0.143	0.162	0.130	0.089	0.134	0.145	0.142	0.194	0.154	0.222	0.126	0.144	0.172	0.197	0.135	0.094	0.165	0.149	0.131	0.129	0.164	0.134	0.131	0.155	0.142	0.123	0.131	0.160																																									
[41]	0.169	0.132	0.155	0.136	0.151	0.150	0.092	0.115	0.078	0.098	0.132	0.164	0.099	0.099	0.120	0.133	0.125	0.125	0.156	0.090	0.183	0.124	0.130	0.151	0.166	0.141	0.120	0.095	0.102	0.130	0.106	0.135	0.128	0.132	0.082	0.128	0.128	0.146	0.102	0.144																																									
[42]	0.148	0.057	0.143	0.019	0.061	0.091	0.124	0.102	0.121	0.095	0.048	0.092	0.132	0.078	0.085	0.014	0.044	0.046	0.132	0.132	0.154	0.014	0.050	0.066	0.143	0.073	0.090	0.122	0.136	0.012	0.081	0.089	0.041	0.052	0.123	0.044	0.014	0.092	0.122	0.136	0.136																																									
[43]	0.142	0.052	0.137	0.015	0.057	0.086	0.115	0.094	0.113	0.087	0.044	0.084	0.124	0.071	0.080	0.012	0.042	0.044	0.132	0.124	0.145	0.004	0.044	0.064	0.142	0.069	0.084	0.113	0.127	0.010	0.074	0.082	0.036	0.050	0.115	0.040	0.012	0.087	0.113	0.131	0.127	0.014																																									
[44]	0.021	0.147	0.013	0.140	0.158	0.134	0.153	0.131	0.160	0.133	0.140	0.140	0.167	0.133	0.101	0.140	0.143	0.140	0.199	0.162	0.224	0.135	0.142	0.174	0.208	0.139	0.109	0.173	0.166	0.140	0.143	0.149	0.140	0.131	0.155	0.146	0.134	0.135	0.167	0.083	0.163	0.145	0.137																																									
[45]	0.088	0.154	0.076	0.136	0.160	0.143	0.145	0.122	0.155	0.145	0.144	0.149	0.159	0.133	0.092	0.139	0.148	0.145	0.197	0.160	0.225	0.131	0.147	0.175	0.200	0.140	0.096	0.165	0.147	0.136	0.132	0.169	0.137	0.136	0.158	0.145	0.129	0.141	0.159	0.014	0.149	0.142	0.136	0.086																																									
[46]	0.084	0.152	0.072	0.134	0.157	0.140	0.148	0.121	0.152	0.138	0.142	0.149	0.157	0.125	0.087	0.136	0.145	0.142	0.189	0.157	0.222	0.128	0.150	0.172	0.192	0.142	0.092	0.157	0.147	0.134	0.129	0.167	0.134	0.133	0.155	0.142	0.126	0.139	0.157	0.024	0.141	0.139	0.133	0.084	0.022																																									
[47]	0.145	0.050	0.140	0.053	0.059	0.093	0.115	0.097	0.113	0.082	0.000	0.096	0.121	0.071	0.085	0.046	0.029	0.033	0.132	0.126	0.137	0.046	0.029	0.055	0.142	0.071	0.085	0.111	0.130	0.042	0.076	0.084	0.034	0.052	0.115	0.025	0.044	0.089	0.111	0.141	0.132	0.048	0.044	0.140	0.144	0.142																																									
[48]	0.142	0.046	0.137	0.048	0.054	0.088	0.110	0.092	0.108	0.078	0.004	0.091	0.116	0.067	0.080	0.042	0.025	0.029	0.127	0.121	0.132	0.041	0.025	0.050	0.137	0.066	0.080	0.106	0.125	0.037	0.072	0.079	0.034	0.048	0.110	0.021	0.040	0.084	0.106	0.139	0.127	0.044	0.039	0.137	0.141	0.139	0.004																																									
[49]	0.148	0.048	0.143	0.055	0.061	0.095	0.113	0.099	0.110	0.085	0.010	0.094	0.118	0.073	0.087	0.048	0.031	0.035	0.134	0.124	0.140	0.048	0.031	0.052	0.145	0.073	0.087	0.108	0.127	0.044	0.078	0.086	0.041	0.055	0.112	0.027	0.046	0.091	0.108	0.147	0.135	0.050	0.046	0.143	0.149	0.147	0.010	0.006																																									
[50]	0.169	0.048	0.158	0.057	0.000	0.103	0.132	0.124	0.124	0.117	0.059	0.114	0.140	0.100	0.102	0.059	0.052	0.057	0.164	0.146	0.162	0.054	0.057	0.079	0.175	0.096	0.107	0.124	0.141	0.054	0.101	0.097	0.056	0.068	0.131	0.051	0.055	0.109	0.122	0.157	0.151	0.061	0.057	0.158	0.160	0.157	0.059	0.054	0.061																																									
[51]	0.210	0.148	0.204	0.146	0.158	0.197	0.148	0.155	0.151	0.105	0.138	0.191	0.162	0.091	0.138	0.143	0.125	0.125	0.141	0.160	0.047	0.138	0.127	0.151	0.152	0.165	0.135	0.149	0.180	0.140	0.096	0.166	0.138	0.143	0.148	0.133	0.141	0.189	0.149	0.212	0.169	0.146	0.137	0.204	0.215	0.206	0.138	0.133	0.140	0.158																																									
[52]	0.170	0.120	0.162	0.129	0.139	0.156	0.006	0.118	0.051	0.106	0.120	0.165	0.049	0.087	0.118	0.128	0.116	0.118	0.146	0.067	0.156	0.120	0.118	0.134	0.156	0.147	0.114	0.063	0.056	0.123	0.092	0.143	0.116	0.123	0.033	0.114	0.123	0.154	0.069	0.156	0.099	0.131	0.123	0.162	0.153	0.153	0.120	0.115	0.118	0.139	0.156																																									
[53]	0.206	0.158	0.192	0.158	0.176	0.176	0.129	0.157	0.124	0.131	0.161	0.186	0.132	0.120	0.143	0.162	0.149	0.151	0.158	0.130	0.195	0.153	0.153	0.160	0.169	0.177	0.141	0.132	0.149	0.156	0.112	0.166	0.153	0.167	0.134	0.152	0.156	0.180	0.138	0.179	0.155	0.165	0.156	0.201	0.182	0.179	0.161	0.156	0.158	0.176	0.189	0.137																																								
[54]	0.225	0.157	0.210	0.143	0.176	0.178	0.133	0.159	0.123	0.132	0.155	0.185	0.138	0.119	0.143	0.156	0.142	0.145	0.155	0.139	0.186	0.152	0.147	0.151	0.166	0.170	0.140	0.136	0.148	0.150	0.108	0.163	0.143	0.158	0.137	0.146	0.150	0.183	0.142	0.199	0.156	0.159	0.150	0.216	0.202	0.200	0.155	0.150	0.152	0.176	0.186	0.141	0.027																																								
[55]	0.212	0.147	0.197	0.143	0.165	0.164	0.138	0.154	0.128	0.130	0.147	0.171	0.133	0.117	0.143	0.148	0.134	0.137	0.165	0.139	0.180	0.144	0.139	0.143	0.171	0.162	0.140	0.136	0.148	0.142	0.113	0.155	0.132	0.144	0.135	0.138	0.142	0.169	0.137	0.190	0.156	0.151	0.142	0.203	0.196	0.194	0.147	0.142	0.144	0.165	0.177	0.146	0.029	0.023																																							
[56]	0.190	0.146	0.181	0.143	0.162	0.172	0.128	0.146	0.125	0.124	0.133	0.173	0.128	0.111	0.123	0.137	0.121	0.126	0.163	0.128	0.157	0.139	0.123	0.145	0.174	0.161	0.123	0.118	0.150	0.137	0.102	0.146	0.132	0.138	0.132	0.125	0.137	0.165	0.125	0.172	0.161	0.145	0.136	0.187	0.172	0.175	0.133	0.128	0.136	0.162	0.163	0.135	0.088	0.086	0.086																																						
[57]	0.242	0.196	0.236	0.188	0.213	0.220	0.170	0.194	0.198	0.188	0.198	0.228	0.176	0.183	0.188	0.196	0.196	0.193	0.250	0.196	0.255	0.190	0.198	0.214	0.256	0.223	0.196	0.193	0.183	0.193	0.174	0.224	0.190	0.186	0.177	0.195	0.185	0.217	0.194	0.213	0.194	0.200	0.193	0.233	0.216	0.210	0.198	0.198	0.207	0.213	0.230	0.179	0.183	0.187	0.182	0.199	

*[1] : P.amabilis, [2] : P.ambionensis, [3] : P.aphrodite, [4] : P.bastianii, [5] : P.bellina, [6] : P.borneensis, [7] : P.braceana, [8] : P.celebensis, [9] : P.chibae, [10] : P.cochlearis, [11] : P.corningiana, [12] : P.cornu-cervi, [13] : P.deliciosa, [14] : P.doweryensis, [15] : P.equestris, [16] : P.fasciata, [17] : P.fimbriata, [18] : P.floresensis, [19] : P.fuscata, [20] : P.gibbosa, [21] : P.gigantea, [22] : P.hieroglyphica, [23] : P.inscriptiosinensis, [24] : P.javanica, [25] : P.kunstleri, [26] : P.lamelligera, [27] : P.lindenii, [28] : P.lobbii, [29] : P.lowii, [30] : P.lueddemanniana, [31] : P.maculata, [32] : P.mannii, [33] : P.mariae, [34] : P.micholitzii, [35] : P.minus, [36] : P.modesta, [37] : P.pallens, [38] : P.pantherina, [39] : P.parishii, [40] : P.philippinensis, [41] : P.pulcherrima, [42] : P.pulchra, [43] : P.reichenbachiana, [44] : P.sanderiana, [45] : P.schilleriana, [46] : P.stuartiana, [47] : P.sumatrana, [48] : P.tetraspis, [49] : P.venosa, [50] : P.violacea, [51] : P.viridis, [52] : P.wilsonii, [53] : Par.labukensis, [54] : Par.laycockii, [55] : Par.serpentilingua, [56] : G.japonicus, [57] : T.kotoense

Table 7. The lengths and accession numbers of the *trn*L-*trn*F IGS, the *trn*L intron, and IGS of *atp*B-*rbc*L from 52 *Phalaenopsis* species plus the five taxa of related genus.

Taxa	Intron of *trn*L			IGS of *trn*L-*trn*F			IGS of *atp*B-*rbc*L		
	Length (bp)	G+C (%)	Accession no.	Length (bp)	G+C (%)	Accession no.	Length (bp)	G+C (%)	Accession no.
Phalaenopsis amabilis	562	26.7	AY265742	412	30.6	AF533472	688	22.8	AY389440
Phalaenopsis amboinensis	569	25.8	AY265743	394	30.7	AF533458	680	23.1	AY389422
Phalaenopsis aphrodite	561	26.4	AY265744	402	31.1	AF533473	688	22.5	AY389441
Phalaenopsis bastianii	602	24.6	AY265745	394	30.7	AF533452	695	22.9	AY389416
Phalaenopsis bellina	562	25.8	AY265746	394	30.5	AF533467	689	22.6	AY389433
Phalaenopsis borneensis	568	25.5	AY265747	394	30.2	AF533476	669	23.5	AY389386
Phalaenopsis braceana	554	25.8	AY265748	394	30.7	AY266119	670	23.3	AY389405
Phalaenopsis celebensis	574	24.9	AY265799	407	30.5	AF533466	680	22.2	AY389432
Phalaenopsis chibae	554	25.8	AY265800	395	29.6	AF533447	700	23.0	AY389412
Phalaenopsis cochlearis	542	27.1	AY265749	394	30.7	AF533489	669	22.9	AY389400
Phalaenopsis corningiana	573	25.3	AY265750	395	30.6	AF533448	681	22.8	AY389413
Phalaenopsis cornu-cervi	568	25.9	AY265751	393	30.5	AF533445	669	23.5	AY389408
Phalaenopsis deliciosa	555	25.5	AY265752	402	30.7	AF533468	705	22.7	AY389434
Phalaenopsis doweryensis	548	27.0	AY265753	395	30.2	AF533485	669	23.1	AY389395
Phalaenopsis equestris	539	26.4	AY265754	395	30.4	AF533464	686	22.5	AY389430
Phalaenopsis fasciata	602	24.6	AY265755	394	30.7	AF533490	681	23.2	AY389401
Phalaenopsis fimbriata	641	25.6	AY265756	394	30.7	AF533465	682	22.9	AY389431
Phalaenopsis floresensis	566	25.1	AY265797	394	30.7	AF533462	697	22.7	AY389428
Phalaenopsis fuscata	550	26.7	AY265757	394	30.7	AF533478	651	23.0	AY389388
Phalaenopsis gibbosa	555	25.4	AY265758	394	30.0	AF533461	675	23.3	AY389427
Phalaenopsis gigantea	567	26.5	AY265759	394	30.2	AF533484	659	23.2	AY389394
Phalaenopsis hieroglyphica	609	24.3	AY265760	394	30.7	AF533451	681	23.1	AY389443
Phalaenopsis inscriptiosinensis	565	25.7	AY265761	394	30.5	AF533459	681	22.8	AY389423
Phalaenopsis javanica	530	26.6	AY265763	394	30.5	AF533455	681	23.0	AY389424
Phalaenopsis kunstleri	550	26.7	AY265764	394	30.2	AF533486	669	22.9	AY389396
Phalaenopsis lamelligera	652	23.8	AY265765	394	30.7	AF533477	663	23.4	AY389387
Phalaenopsis lindenii	606	25.0	AY265766	394	30.2	AF533480	696	20.7	AY389390
Phalaenopsis lobbii	558	25.5	AY265767	403	29.5	AF533474	664	23.2	AY389442

Phalaenopsis lowii	554	28.2	AY265795	395	29.9	AY266117	662	23.1	AY389439
Phalaenopsis lueddemanniana	558	24.7	AY265768	394	30.7	AF533444	681	23.1	AY389436
Phalaenopsis maculata	568	26.2	AY265798	394	30.5	AF533460	662	22.8	AY389426
Phalaenopsis mannii	590	25.6	AY265769	396	30.3	AF533469	721	22.2	AY389435
Phalaenopsis mariae	586	24.4	AY265770	394	30.7	AF533449	681	22.9	AY389414
Phalaenopsis micholitzii	572	25.9	AY265771	394	30.2	AF533471	678	23.3	AY389438
Phalaenopsis minus	525	27.6	AY265772	395	30.4	AY266118	674	24.3	AY389407
Phalaenopsis modesta	588	24.8	AY265793	394	30.7	AF533488	681	23.0	AY389398
Phalaenopsis pallens	580	25.0	AY265773	394	30.7	AF533479	681	23.1	AY389389
Phalaenopsis pantherina	568	25.7	AY265775	393	30.5	AF533463	669	23.5	AY389429
Phalaenopsis parishii	557	25.9	AY265774	403	29.5	AF533491	664	23.2	AY389402
Phalaenopsis philippinensis	578	25.6	AY265776	395	30.4	AF533446	687	22.1	AY389411
Phalaenopsis pulcherrima	537	26.4	AY265777	408	30.4	AF533495	627	24.1	AY389404
Phalaenopsis pulchra	603	24.2	AY265778	404	30.7	AF533494	681	22.9	AY389399
Phalaenopsis reichenbachiana	569	26.0	AY265779	394	30.7	AY266120	681	23.1	AY389410
Phalaenopsis sanderiana	561	26.2	AY265780	402	31.1	AF533453	688	22.9	AY389417
Phalaenopsis schilleriana	578	25.6	AY265781	394	30.2	AF533443	687	22.1	AY389425
Phalaenopsis stuartiana	573	25.5	AY265782	394	30.2	AF533492	687	22.2	AY389403
Phalaenopsis sumatrana	535	26.4	AY265783	395	30.6	AF533450	681	23.0	AY389415
Phalaenopsis tetraspis	561	25.5	AY265784	395	30.6	AF533456	681	22.8	AY389419
Phalaenopsis venosa	569	25.8	AY265785	394	30.7	AF533493	680	23.3	AY389406
Phalaenopsis violacea	555	25.6	AY265796	394	30.2	AF533487	689	22.6	AY389397
Phalaenopsis viridis	635	25.0	AY265786	395	30.6	AF533457	668	22.9	AY389420
Phalaenopsis wilsonii	554	26.0	AY265787	394	30.7	AF533475	670	22.5	AY389385
Paraphalaenopsis laycockii	681	23.8	AY265790	393	29.8	AF533482	675	22.5	AY389392
Paraphalaenopsis labukensis	574	25.1	AY265789	381	28.4	AF533481	714	21.3	AY389391
Paraphalaenopsis serpentilingua	679	23.6	AY265791	391	29.4	AF533483	662	22.6	AY389393
Gastrochilus japonicus	546	25.5	AY265794	396	29.8	AY266122			
Tuberolabium kotoense	365	29.1	AY265792	395	29.9	AF533454	707	22.5	AY389418

Table 8. Genetic distances of the two-parameter method of Kimura method inferred from the *trn*L intron among the 52 taxa of the genus *Phalaenopsis* plus the five related species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
[1]																																																										
[2]	0.033																																																										
[3]	0.004	0.033																																																								
[4]	0.038	0.009	0.038																																																							
[5]	0.029	0.004	0.029	0.005																																																							
[6]	0.042	0.017	0.042	0.022	0.015																																																						
[7]	0.023	0.022	0.023	0.034	0.024	0.035																																																					
[8]	0.022	0.038	0.022	0.044	0.034	0.044	0.024																																																				
[9]	0.020	0.027	0.020	0.030	0.023	0.039	0.019	0.024																																																			
[10]	0.029	0.025	0.031	0.035	0.023	0.039	0.032	0.041	0.038																																																		
[11]	0.031	0.011	0.031	0.022	0.008	0.017	0.031	0.034	0.029	0.022																																																	
[12]	0.044	0.015	0.044	0.022	0.013	0.011	0.037	0.042	0.041	0.039	0.017																																														
[13]	0.017	0.025	0.017	0.028	0.021	0.039	0.015	0.020	0.011	0.036	0.027	0.041																																														
[14]	0.025	0.017	0.025	0.027	0.017	0.033	0.025	0.031	0.029	0.000	0.016	0.031	0.029																																													
[15]	0.010	0.022	0.010	0.029	0.020	0.031	0.010	0.007	0.012	0.028	0.020	0.035	0.010	0.022																																												
[16]	0.038	0.009	0.038	0.003	0.005	0.022	0.034	0.044	0.032	0.035	0.022	0.022	0.030	0.027	0.029																																												
[17]	0.056	0.041	0.056	0.049	0.037	0.043	0.054	0.070	0.054	0.051	0.032	0.043	0.052	0.043	0.051	0.049																																											
[18]	0.032	0.006	0.032	0.015	0.004	0.018	0.027	0.034	0.029	0.018	0.011	0.018	0.029	0.013	0.022	0.015	0.030																																											
[19]	0.029	0.021	0.029	0.031	0.019	0.037	0.030	0.039	0.035	0.004	0.020	0.037	0.033	0.000	0.026	0.031	0.047	0.017																																										
[20]	0.028	0.025	0.028	0.042	0.025	0.041	0.012	0.026	0.019	0.030	0.039	0.043	0.013	0.025	0.015	0.042	0.060	0.037	0.028																																										
[21]	0.028	0.021	0.028	0.030	0.019	0.036	0.029	0.037	0.034	0.004	0.019	0.036	0.032	0.000	0.025	0.030	0.045	0.017	0.000	0.027																																										
[22]	0.036	0.007	0.036	0.002	0.004	0.020	0.033	0.042	0.030	0.033	0.020	0.020	0.028	0.025	0.027	0.002	0.048	0.013	0.029	0.040	0.028																																										
[23]	0.031	0.009	0.031	0.017	0.006	0.015	0.029	0.034	0.029	0.020	0.013	0.015	0.027	0.016	0.020	0.017	0.029	0.006	0.018	0.033	0.017	0.015																																										
[24]	0.028	0.004	0.027	0.010	0.004	0.015	0.018	0.031	0.024	0.012	0.006	0.013	0.024	0.012	0.018	0.010	0.025	0.004	0.012	0.024	0.011	0.008	0.006																																									
[25]	0.029	0.021	0.029	0.031	0.019	0.037	0.030	0.039	0.035	0.004	0.020	0.037	0.033	0.000	0.026	0.031	0.047	0.017	0.000	0.028	0.000	0.029	0.018	0.012																																									
[26]	0.049	0.040	0.049	0.046	0.040	0.002	0.045	0.058	0.048	0.041	0.031	0.009	0.048	0.031	0.033	0.046	0.078	0.016	0.040	0.042	0.039	0.044	0.037	0.013	0.040																																									
[27]	0.016	0.032	0.016	0.050	0.030	0.040	0.018	0.022	0.022	0.041	0.039	0.042	0.018	0.029	0.004	0.050	0.065	0.041	0.039	0.022	0.038	0.048	0.039	0.027	0.039	0.050																																									
[28]	0.032	0.031	0.032	0.048	0.031	0.048	0.019	0.034	0.026	0.033	0.042	0.048	0.021	0.027	0.019	0.048	0.069	0.042	0.031	0.009	0.030	0.046	0.037	0.025	0.031	0.049	0.029																																									
[29]	0.020	0.023	0.022	0.023	0.023	0.030	0.007	0.017	0.017	0.024	0.028	0.033	0.015	0.025	0.012	0.025	0.054	0.023	0.024	0.017	0.024	0.023	0.028	0.020	0.024	0.030	0.015	0.020																																								
[30]	0.036	0.007	0.036	0.002	0.004	0.020	0.033	0.042	0.030	0.033	0.018	0.020	0.028	0.025	0.027	0.002	0.046	0.013	0.029	0.040	0.028	0.000	0.013	0.008	0.029	0.044	0.046	0.046	0.023																																								
[31]	0.030	0.017	0.030	0.026	0.015	0.030	0.021	0.033	0.030	0.011	0.015	0.030	0.028	0.005	0.021	0.026	0.041	0.013	0.007	0.021	0.007	0.024	0.013	0.008	0.007	0.035	0.030	0.024	0.020	0.024																																							
[32]	0.044	0.006	0.044	0.024	0.006	0.021	0.034	0.045	0.038	0.023	0.018	0.023	0.038	0.023	0.032	0.024	0.039	0.015	0.027	0.037	0.026	0.022	0.014	0.008	0.027	0.045	0.042	0.046	0.025	0.020	0.022																																							
[33]	0.042	0.011	0.042	0.014	0.007	0.029	0.035	0.048	0.036	0.037	0.024	0.029	0.030	0.029	0.033	0.014	0.054	0.015	0.033	0.046	0.032	0.012	0.013	0.011	0.033	0.055	0.056	0.051	0.023	0.012	0.028	0.024																																							
[34]	0.048	0.019	0.048	0.029	0.021	0.016	0.035	0.053	0.042	0.045	0.026	0.016	0.042	0.029	0.041	0.029	0.050	0.024	0.043	0.049	0.041	0.027	0.023	0.015	0.043	0.018	0.053	0.054	0.033	0.027	0.037	0.030	0.033																																							
[35]	0.048	0.057	0.050	0.066	0.057	0.065	0.039	0.052	0.051	0.062	0.062	0.065	0.042	0.054	0.036	0.066	0.087	0.056	0.060	0.043	0.060	0.063	0.060	0.051	0.060	0.077	0.041	0.049	0.032	0.064	0.051	0.070	0.062	0.065																																							
[36]	0.035	0.017	0.035	0.027	0.013	0.017	0.025	0.040	0.031	0.026	0.018	0.017	0.029	0.014	0.024	0.027	0.037	0.015	0.024	0.043	0.023	0.025	0.016	0.006	0.024	0.048	0.039	0.046	0.023	0.024	0.017	0.020	0.031	0.019	0.055																																							
[37]	0.033	0.007	0.033	0.002	0.004	0.019	0.029	0.040	0.027	0.027	0.015	0.017	0.025	0.021	0.026	0.002	0.044	0.008	0.023	0.031	0.023	0.000	0.013	0.008	0.023	0.044	0.040	0.033	0.023	0.000	0.019	0.013	0.004	0.024	0.061	0.020																																							
[38]	0.032	0.031	0.032	0.051	0.031	0.048	0.019	0.034	0.026	0.033	0.042	0.048	0.021	0.027	0.019	0.051	0.069	0.042	0.031	0.009	0.030	0.049	0.037	0.025	0.031	0.049	0.029	0.007	0.022	0.049	0.024	0.046	0.055	0.054	0.049	0.046	0.037																																							
[39]	0.040	0.015	0.040	0.020	0.013	0.002	0.033	0.042	0.037	0.037	0.015	0.009	0.037	0.031	0.029	0.020	0.041	0.017	0.035	0.039	0.034	0.018	0.013	0.013	0.035	0.000	0.038	0.046	0.030	0.018	0.028	0.019	0.027	0.014	0.063	0.015	0.017	0.046																																							
[40]	0.015	0.032	0.015	0.035	0.028	0.038	0.019	0.023	0.015	0.039	0.028	0.040	0.011	0.031	0.009	0.037	0.057	0.028	0.035	0.024	0.034	0.035	0.027	0.023	0.035	0.051	0.018	0.028	0.015	0.035	0.030	0.041	0.041	0.046	0.050	0.030	0.032	0.028	0.036																																							
[41]	0.027	0.034	0.027	0.039	0.032	0.039	0.022	0.031	0.025	0.038	0.036	0.039	0.025	0.033	0.020	0.039	0.056	0.033	0.038	0.027	0.037	0.037	0.033	0.030	0.038	0.037	0.025	0.031	0.017	0.037	0.031	0.035	0.041	0.043	0.046	0.037	0.036	0.031	0.037	0.027																																							
[42]	0.040	0.009	0.040	0.003	0.005	0.022	0.036	0.046	0.034	0.037	0.022	0.022	0.028	0.029	0.031	0.003	0.049	0.015	0.033	0.044	0.032	0.002	0.017	0.010	0.033	0.046	0.051	0.049	0.025	0.002	0.028	0.024	0.010	0.029	0.063	0.027	0.002	0.053	0.020	0.039	0.041																																							
[43]	0.033	0.002	0.033	0.011	0.005	0.019	0.023	0.040	0.027	0.027	0.013	0.017	0.025	0.019	0.024	0.011	0.042	0.007	0.023	0.027	0.022	0.009	0.011	0.006	0.023	0.042	0.034	0.033	0.023	0.009	0.019	0.008	0.013	0.021	0.059	0.019	0.009	0.033	0.017	0.034	0.034	0.011																																							
[44]	0.005	0.035	0.002	0.040	0.031	0.044	0.025	0.024	0.022	0.033	0.033	0.046	0.018	0.027	0.011	0.040	0.058	0.034	0.031	0.030	0.030	0.038	0.033	0.030	0.031	0.051	0.018	0.034	0.024	0.038	0.032	0.046	0.044	0.050	0.053	0.036	0.035	0.034	0.042	0.016	0.029	0.042	0.035																																							
[45]	0.015	0.032	0.015	0.035	0.028	0.038	0.019	0.023	0.015	0.039	0.028	0.040	0.011	0.031	0.009	0.037	0.057	0.028	0.035	0.024	0.034	0.035	0.027	0.023	0.035	0.051	0.018	0.028	0.015	0.035	0.030	0.041	0.041	0.046	0.050	0.030	0.032	0.028	0.036	0.000	0.027	0.039	0.034	0.016																																							
[46]	0.016	0.034	0.016	0.037	0.030	0.040	0.021	0.025	0.016	0.041	0.030	0.042	0.009	0.033	0.011	0.039	0.059	0.030	0.037	0.023	0.036	0.037	0.028	0.025	0.037	0.052	0.020	0.026	0.017	0.037	0.032	0.043	0.043	0.048	0.052	0.032	0.034	0.026	0.038	0.002	0.029	0.041	0.036	0.018	0.002																																						
[47]	0.025	0.008	0.025	0.011	0.006	0.017	0.018	0.031	0.023	0.016	0.008	0.015	0.023	0.013	0.020	0.011	0.027	0.006	0.016	0.027	0.015	0.009	0.008	0.002	0.016	0.019	0.029	0.029	0.020	0.009	0.009	0.010	0.013	0.023	0.053	0.011	0.009	0.029	0.015	0.025	0.030	0.011	0.008	0.027	0.025	0.027																																						
[48]	0.031	0.004	0.031	0.017	0.002	0.015	0.027	0.034	0.029	0.018	0.009	0.015	0.027	0.010	0.020	0.017	0.026	0.006	0.016	0.037	0.015	0.015	0.004	0.002	0.016	0.028	0.037	0.040	0.023	0.013	0.011	0.014	0.015	0.021	0.058	0.013	0.009	0.040	0.013	0.026	0.031	0.017	0.006	0.033	0.026	0.028	0.004																																						
[49]	0.033	0.000	0.033	0.009	0.004	0.017	0.022	0.038	0.027	0.025	0.011	0.015	0.025	0.017	0.022	0.009	0.041	0.006	0.021	0.025	0.021	0.007	0.009	0.004	0.021	0.040	0.032	0.031	0.023	0.007	0.017	0.006	0.011	0.019	0.057	0.017	0.007	0.031	0.015	0.032	0.034	0.009	0.002	0.035	0.032	0.034	0.008	0.004																																						
[50]	0.029	0.002	0.029	0.013	0.000	0.013	0.023	0.028	0.023	0.020	0.005	0.013	0.021	0.014	0.018	0.013	0.026	0.006	0.018	0.033	0.017	0.011	0.007	0.004	0.018	0.024	0.032	0.040	0.023	0.009	0.013	0.007	0.013	0.019	0.056	0.011	0.007	0.040	0.011	0.024	0.033	0.013	0.004	0.031	0.024	0.026	0.006	0.007	0.002																																						
[51]	0.030	0.041	0.030	0.048	0.037	0.038	0.036	0.046	0.036	0.006	0.026	0.038	0.034	0.002	0.031	0.048	0.082	0.019	0.002	0.032	0.002	0.046	0.022	0.013	0.002	0.078	0.045	0.036	0.027	0.046	0.009	0.036	0.049	0.043	0.068	0.042	0.041	0.036	0.036	0.039	0.039	0.049	0.042	0.032	0.039	0.041	0.017	0.017	0.041	0.021																																						
[52]	0.021	0.023	0.021	0.036	0.025	0.033	0.005	0.026	0.021	0.030	0.029	0.035	0.017	0.023	0.008	0.036	0.052	0.025	0.028	0.014	0.027	0.034	0.027	0.016	0.028	0.043	0.020	0.017	0.007	0.035	0.019	0.036	0.037	0.033	0.043	0.023	0.031	0.017	0.031	0.017	0.024	0.038	0.025	0.023	0.017	0.019	0.016	0.025	0.023	0.025	0.035																																					
[53]	0.040	0.046	0.040	0.060	0.044	0.057	0.027	0.040	0.030	0.044	0.057	0.057	0.028	0.047	0.031	0.064	0.102	0.054	0.050	0.042	0.050	0.063	0.049	0.035	0.050	0.075	0.049	0.046	0.020	0.061	0.044	0.058	0.064	0.068	0.056	0.078	0.051	0.046	0.057	0.032	0.037	0.064	0.048	0.042	0.032	0.034	0.037	0.047	0.046	0.051	0.064	0.027																																					
[54]	0.059	0.062	0.059	0.078	0.059	0.077	0.056	0.065	0.053	0.059	0.073	0.079	0.051	0.058	0.049	0.082	0.140	0.069	0.062	0.054	0.062	0.080	0.066	0.050	0.062	0.125	0.081	0.067	0.028	0.079	0.062	0.081	0.081	0.085	0.087	0.095	0.067	0.067	0.075	0.058	0.053	0.081	0.064	0.061	0.058	0.060	0.052	0.063	0.062	0.062	0.090	0.058	0.009																																					
[55]	0.067	0.064	0.067	0.079	0.060	0.076	0.063	0.064	0.057	0.062	0.075	0.078	0.055	0.064	0.050	0.083	0.132	0.072	0.065	0.057	0.065	0.084	0.070	0.054	0.065	0.112	0.103	0.070	0.032	0.082	0.065	0.077	0.083	0.084	0.090	0.097	0.068	0.070	0.074	0.061	0.053	0.083	0.066	0.069	0.061	0.063	0.055	0.065	0.064	0.067	0.084	0.065	0.011	0.024																																					
[56]	0.030	0.035	0.030	0.039	0.035	0.045	0.020	0.022	0.018	0.040	0.041	0.043	0.022	0.039	0.021	0.041	0.084	0.036	0.042	0.030	0.040	0.040	0.039	0.035	0.042	0.059	0.024	0.036	0.022	0.040	0.034	0.038	0.047	0.045	0.043	0.052	0.037	0.032	0.045	0.022	0.033	0.043	0.035	0.032	0.022	0.024	0.035	0.039	0.035	0.033	0.045	0.022	0.033	0.038	0.043																																					
[57]	0.259	0.283	0.263	0.283	0.276	0.292	0.269	0.263	0.256	0.270	0.282	0.284	0.248	0.272	0.257	0.283	0.311	0.275	0.273	0.256	0.272	0.283	0.284	0.283	0.273	0.292	0.261	0.262	0.238	0.280	0.268	0.276	0.280	0.288	0.300	0.284	0.283	0.259	0.292	0.255	0.271	0.290	0.288	0.267	0.255	0.251	0.284	0.277	0.283	0.277	0.275	0.265	0.272	0.294	0.282	0.272

[1], P. amabilis; [2], P. amboinensis; [3], P. aphrodite; [4], P. bastianii; [5], P. bellina; [6], P. borneensis; [7], P. braceana; [8], P. celebensis; [9], P. chibae; [10], P. cochlearis; [11], P. corningiana; [12], P. cornu-cervi; [13], P. deliciosa; [14], P. doweryensis; [15], P. equestris; [16], P. fasciata; [17], P. fimbriata; [18], P. floresensis; [19], P. fuscata; [20], P. gibbosa; [21], P. gigantea; [22], P. hieroglyphica; [23], P. inscriptiosinensis; [24], P. javanica; [25], P. kunstleri; [26], P. lamelligera; [27], P. lindenii; [28], P. lobbii; [29], P._lowii; [30], P. lueddemanniana; [31], P. maculata; [32], P. mannii; [33], P. mariae; [34], P. micholitzii; [35], P. minus; [36], P. modesta; [37], P. pallens; [38], P. parishii; [39], P. patherina; [40], P. philippinensis; [41], P. pulcherrima; [42], P. pulchra; [43], P._reichenbachiana; [44], P. sanderiana; [45], P. schilleriana; [46], P. stuartiana; [47], P. sumatrana; [48], P. tetraspis; [49], P._venosa; [50], P. violacea; [51], P. viridis; [52], P. wilsonii; [53], Par. labukensis; [54], Par. laycockii; [55], Par. serpentilingua; [56], G. japonicus; [57], T. kotoense.

Table 9. Genetic distances of the two-parameter method of Kimura method inferred from the *trn*L – *trn*F intergenic spacer among the 52 taxa of the genus *Phalaenopsis* plus the five related species.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56]
[1]																																																										
[2]	0.013																																																									
[3]	0.000	0.013																																																							
[4]	0.016	0.003	0.016																																																					
[5]	0.016	0.003	0.016	0.005																																																				
[6]	0.018	0.010	0.018	0.013	0.013																																																				
[7]	0.013	0.005	0.013	0.008	0.008	0.016																																																		
[8]	0.005	0.018	0.005	0.021	0.021	0.018	0.018																																																	
[9]	0.016	0.013	0.016	0.015	0.015	0.024	0.013	0.021																																																
[10]	0.018	0.005	0.018	0.008	0.008	0.016	0.010	0.023	0.018																																														
[11]	0.016	0.003	0.016	0.005	0.005	0.013	0.008	0.021	0.015	0.008																																												
[12]	0.015	0.008	0.015	0.010	0.010	0.003	0.013	0.015	0.021	0.013	0.010																																											
[13]	0.021	0.013	0.021	0.015	0.015	0.024	0.013	0.026	0.010	0.018	0.015	0.021																																										
[14]	0.018	0.005	0.018	0.008	0.008	0.016	0.010	0.023	0.018	0.005	0.008	0.013	0.018																																										
[15]	0.005	0.013	0.005	0.016	0.016	0.018	0.013	0.005	0.016	0.018	0.016	0.015	0.021	0.018																																									
[16]	0.016	0.003	0.016	0.000	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016																																									
[17]	0.016	0.003	0.016	0.005	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.005																																									
[18]	0.013	0.000	0.013	0.003	0.003	0.010	0.005	0.018	0.013	0.005	0.003	0.008	0.013	0.005	0.013	0.003	0.003																																								
[19]	0.018	0.005	0.018	0.008	0.008	0.016	0.010	0.023	0.018	0.000	0.008	0.013	0.018	0.005	0.018	0.008	0.008	0.005																																							
[20]	0.018	0.010	0.018	0.013	0.013	0.021	0.010	0.023	0.013	0.015	0.013	0.018	0.013	0.010	0.018	0.013	0.013	0.010	0.015																																							
[21]	0.018	0.005	0.018	0.008	0.008	0.016	0.010	0.023	0.018	0.005	0.008	0.013	0.018	0.000	0.018	0.008	0.008	0.005	0.005	0.010																																						
[22]	0.016	0.003	0.016	0.000	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.000	0.005	0.003	0.008	0.013	0.008																																					
[23]	0.016	0.003	0.016	0.005	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.005	0.005	0.003	0.008	0.013	0.008	0.005																																				
[24]	0.016	0.003	0.016	0.005	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.005	0.005	0.003	0.008	0.013	0.008	0.005	0.005																																			
[25]	0.018	0.005	0.018	0.008	0.008	0.016	0.010	0.023	0.018	0.005	0.008	0.013	0.018	0.000	0.018	0.008	0.008	0.005	0.005	0.010	0.000	0.008	0.008	0.008																																		
[26]	0.018	0.010	0.015	0.013	0.013	0.005	0.016	0.018	0.024	0.016	0.013	0.003	0.024	0.016	0.018	0.013	0.013	0.010	0.016	0.021	0.016	0.013	0.013	0.013	0.016																																	
[27]	0.003	0.010	0.003	0.013	0.013	0.015	0.010	0.003	0.013	0.016	0.013	0.013	0.018	0.016	0.003	0.013	0.013	0.010	0.016	0.016	0.016	0.013	0.013	0.013	0.016	0.015																																	
[28]	0.021	0.013	0.021	0.015	0.015	0.024	0.013	0.026	0.015	0.018	0.015	0.021	0.016	0.013	0.021	0.015	0.015	0.013	0.018	0.003	0.013	0.015	0.015	0.015	0.013	0.024	0.018																															
[29]	0.018	0.010	0.018	0.013	0.013	0.021	0.010	0.023	0.018	0.015	0.008	0.018	0.018	0.015	0.018	0.013	0.013	0.010	0.015	0.015	0.015	0.013	0.013	0.013	0.015	0.021	0.016	0.018																															
[30]	0.016	0.003	0.016	0.000	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.000	0.005	0.003	0.008	0.013	0.008	0.000	0.005	0.005	0.008	0.013	0.013	0.015	0.013																															
[31]	0.016	0.003	0.016	0.005	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.005	0.005	0.003	0.008	0.013	0.008	0.005	0.000	0.005	0.008	0.013	0.013	0.015	0.013	0.005																														
[32]	0.021	0.008	0.021	0.010	0.010	0.018	0.013	0.026	0.021	0.013	0.005	0.016	0.021	0.013	0.021	0.010	0.010	0.008	0.013	0.018	0.013	0.010	0.010	0.010	0.013	0.018	0.018	0.021	0.013	0.010	0.010																													
[33]	0.013	0.000	0.013	0.003	0.003	0.010	0.005	0.018	0.013	0.005	0.003	0.008	0.013	0.005	0.013	0.003	0.003	0.000	0.005	0.010	0.005	0.003	0.003	0.003	0.005	0.010	0.010	0.013	0.010	0.003	0.003	0.008																													
[34]	0.018	0.010	0.018	0.013	0.013	0.005	0.016	0.018	0.024	0.016	0.013	0.003	0.024	0.016	0.018	0.013	0.013	0.010	0.016	0.021	0.016	0.013	0.013	0.013	0.016	0.005	0.015	0.024	0.021	0.013	0.013	0.018	0.010																											
[35]	0.018	0.010	0.018	0.013	0.013	0.021	0.010	0.023	0.018	0.015	0.008	0.018	0.018	0.015	0.018	0.013	0.013	0.010	0.015	0.015	0.015	0.013	0.013	0.013	0.015	0.021	0.016	0.018	0.010	0.013	0.013	0.013	0.010	0.021																											
[36]	0.013	0.000	0.013	0.003	0.003	0.010	0.005	0.018	0.013	0.005	0.003	0.008	0.013	0.005	0.013	0.003	0.003	0.000	0.005	0.010	0.005	0.003	0.003	0.003	0.005	0.010	0.010	0.013	0.010	0.003	0.003	0.008	0.000	0.010	0.010																											
[37]	0.016	0.003	0.016	0.000	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.000	0.005	0.003	0.008	0.013	0.008	0.000	0.005	0.005	0.008	0.013	0.013	0.015	0.013	0.000	0.005	0.010	0.003	0.013	0.013	0.003																											
[38]	0.015	0.008	0.015	0.010	0.010	0.003	0.013	0.015	0.021	0.013	0.010	0.000	0.021	0.013	0.015	0.010	0.010	0.008	0.013	0.018	0.013	0.010	0.010	0.010	0.013	0.003	0.013	0.021	0.018	0.010	0.010	0.016	0.008	0.003	0.018	0.008	0.010																											
[39]	0.021	0.013	0.021	0.015	0.015	0.024	0.013	0.026	0.015	0.018	0.015	0.021	0.016	0.013	0.021	0.015	0.015	0.013	0.018	0.003	0.013	0.015	0.015	0.015	0.013	0.024	0.018	0.000	0.018	0.015	0.015	0.021	0.013	0.024	0.018	0.013	0.015	0.021																											
[40]	0.005	0.013	0.003	0.016	0.016	0.018	0.013	0.005	0.016	0.018	0.016	0.015	0.021	0.018	0.005	0.016	0.016	0.013	0.018	0.018	0.018	0.016	0.016	0.016	0.018	0.013	0.003	0.021	0.018	0.016	0.016	0.021	0.013	0.018	0.018	0.013	0.016	0.015	0.021																											
[41]	0.021	0.013	0.021	0.015	0.015	0.024	0.013	0.026	0.021	0.018	0.010	0.021	0.021	0.018	0.021	0.015	0.015	0.013	0.018	0.018	0.018	0.015	0.015	0.015	0.018	0.024	0.018	0.021	0.013	0.015	0.015	0.015	0.013	0.024	0.013	0.013	0.015	0.021	0.021	0.021																											
[42]	0.015	0.003	0.015	0.000	0.005	0.013	0.008	0.030	0.015	0.008	0.005	0.010	0.015	0.008	0.015	0.000	0.005	0.003	0.008	0.013	0.008	0.000	0.005	0.005	0.008	0.013	0.013	0.015	0.013	0.000	0.005	0.010	0.003	0.013	0.013	0.003	0.000	0.010	0.015	0.015	0.015																											
[43]	0.016	0.003	0.016	0.000	0.005	0.013	0.008	0.021	0.015	0.008	0.005	0.010	0.015	0.008	0.016	0.000	0.005	0.003	0.008	0.013	0.008	0.000	0.005	0.005	0.008	0.013	0.013	0.015	0.013	0.000	0.005	0.010	0.003	0.013	0.013	0.003	0.000	0.010	0.015	0.016	0.015	0.000																											
[44]	0.000	0.013	0.000	0.016	0.016	0.018	0.013	0.005	0.016	0.018	0.016	0.015	0.021	0.018	0.005	0.016	0.016	0.013	0.018	0.018	0.018	0.016	0.016	0.016	0.018	0.015	0.003	0.021	0.018	0.016	0.016	0.021	0.013	0.018	0.018	0.013	0.016	0.015	0.021	0.003	0.021	0.015	0.016																											
[45]	0.005	0.013	0.005	0.016	0.016	0.018	0.013	0.005	0.016	0.018	0.016	0.015	0.021	0.018	0.005	0.016	0.016	0.013	0.018	0.018	0.018	0.016	0.016	0.016	0.018	0.018	0.003	0.021	0.018	0.016	0.016	0.021	0.013	0.018	0.018	0.013	0.016	0.015	0.021	0.005	0.021	0.015	0.016	0.005																											
[46]	0.003	0.010	0.003	0.013	0.013	0.015	0.010	0.003	0.013	0.016	0.013	0.013	0.018	0.016	0.003	0.013	0.013	0.010	0.016	0.016	0.016	0.013	0.013	0.013	0.016	0.015	0.000	0.018	0.016	0.013	0.013	0.018	0.010	0.015	0.016	0.010	0.013	0.013	0.018	0.003	0.018	0.013	0.013	0.003	0.003																											
[47]	0.016	0.003	0.016	0.005	0.005	0.013	0.008	0.021	0.015	0.008	0.000	0.010	0.015	0.008	0.016	0.005	0.005	0.003	0.008	0.013	0.008	0.005	0.005	0.005	0.008	0.013	0.013	0.015	0.008	0.005	0.005	0.005	0.003	0.013	0.008	0.003	0.005	0.010	0.015	0.016	0.010	0.005	0.005	0.016	0.016	0.013																											
[48]	0.016	0.003	0.016	0.005	0.005	0.013	0.008	0.021	0.015	0.008	0.000	0.010	0.015	0.008	0.016	0.005	0.005	0.003	0.008	0.013	0.008	0.005	0.005	0.005	0.008	0.013	0.013	0.015	0.008	0.005	0.005	0.005	0.003	0.013	0.008	0.003	0.005	0.010	0.015	0.016	0.010	0.005	0.005	0.016	0.016	0.013	0.000																											
[49]	0.013	0.000	0.013	0.003	0.003	0.010	0.005	0.018	0.013	0.005	0.003	0.008	0.013	0.005	0.013	0.003	0.003	0.000	0.005	0.010	0.005	0.003	0.003	0.003	0.005	0.010	0.010	0.013	0.010	0.003	0.003	0.008	0.000	0.010	0.010	0.000	0.003	0.008	0.013	0.013	0.013	0.003	0.003	0.013	0.013	0.010	0.003	0.003																											
[50]	0.018	0.005	0.018	0.008	0.003	0.016	0.010	0.023	0.018	0.010	0.008	0.013	0.018	0.010	0.018	0.008	0.008	0.005	0.010	0.015	0.010	0.008	0.008	0.008	0.008	0.010	0.016	0.016	0.015	0.015	0.008	0.008	0.013	0.005	0.016	0.015	0.005	0.008	0.013	0.015	0.018	0.018	0.008	0.008	0.018	0.018	0.016	0.008	0.008	0.005																											
[51]	0.029	0.015	0.029	0.018	0.018	0.026	0.021	0.034	0.028	0.015	0.018	0.024	0.029	0.015	0.029	0.018	0.018	0.015	0.015	0.026	0.015	0.018	0.018	0.018	0.015	0.026	0.026	0.029	0.026	0.018	0.018	0.023	0.015	0.026	0.026	0.015	0.018	0.024	0.029	0.029	0.029	0.018	0.018	0.029	0.029	0.026	0.018	0.018	0.015	0.021																											
[52]	0.013	0.005	0.013	0.008	0.008	0.016	0.000	0.018	0.013	0.010	0.008	0.013	0.013	0.010	0.013	0.008	0.008	0.005	0.010	0.010	0.010	0.008	0.008	0.008	0.008	0.010	0.016	0.010	0.013	0.010	0.008	0.008	0.013	0.005	0.016	0.010	0.005	0.008	0.013	0.013	0.013	0.013	0.008	0.008	0.013	0.013	0.010	0.008	0.008	0.005	0.010	0.021																											
[53]	0.038	0.035	0.038	0.038	0.038	0.044	0.035	0.046	0.040	0.040	0.038	0.044	0.043	0.040	0.041	0.038	0.038	0.038	0.035	0.040	0.038	0.038	0.038	0.038	0.040	0.047	0.038	0.041	0.041	0.038	0.038	0.038	0.043	0.035	0.047	0.040	0.035	0.038	0.044	0.041	0.041	0.043	0.038	0.038	0.038	0.041	0.038	0.038	0.038	0.035	0.040	0.052	0.035																										
[54]	0.037	0.037	0.037	0.039	0.039	0.048	0.037	0.045	0.039	0.037	0.039	0.046	0.045	0.042	0.040	0.039	0.039	0.037	0.037	0.042	0.042	0.039	0.039	0.039	0.042	0.048	0.037	0.045	0.042	0.039	0.039	0.045	0.037	0.048	0.042	0.037	0.039	0.046	0.045	0.040	0.045	0.039	0.039	0.037	0.040	0.037	0.039	0.039	0.037	0.042	0.053	0.037	0.008																										
[55]	0.037	0.037	0.037	0.039	0.039	0.048	0.037	0.045	0.039	0.037	0.039	0.046	0.045	0.042	0.040	0.039	0.039	0.037	0.037	0.042	0.042	0.039	0.039	0.039	0.042	0.048	0.037	0.045	0.042	0.039	0.039	0.045	0.037	0.048	0.042	0.037	0.039	0.046	0.045	0.040	0.045	0.039	0.039	0.037	0.040	0.037	0.039	0.039	0.037	0.042	0.053	0.037	0.008	0.000																									
[56]	0.032	0.023	0.032	0.026	0.026	0.034	0.023	0.039	0.031	0.029	0.026	0.032	0.031	0.029	0.032	0.026	0.026	0.023	0.029	0.029	0.029	0.026	0.026	0.026	0.029	0.034	0.029	0.031	0.023	0.026	0.026	0.031	0.023	0.034	0.029	0.023	0.026	0.032	0.031	0.032	0.031	0.026	0.026	0.032	0.032	0.029	0.026	0.026	0.023	0.029	0.039	0.023	0.043	0.042	0.042																									
[57]	0.066	0.057	0.066	0.060	0.060	0.069	0.054	0.071	0.060	0.062	0.060	0.066	0.066	0.062	0.066	0.060	0.060	0.057	0.062	0.062	0.062	0.060	0.060	0.060	0.062	0.069	0.063	0.064	0.062	0.060	0.060	0.065	0.057	0.069	0.062	0.057	0.060	0.066	0.064	0.066	0.065	0.060	0.060	0.066	0.066	0.063	0.060	0.060	0.057	0.062	0.074	0.054	0.056	0.063	0.063	0.051	

[1], P. amabilis; [2], P. amboinensis; [3], P. aphrodite; [4], P. bastianii; [5], P. bellina; [6], P. borneensis; [7], P. braceana; [8], P. celebensis; [9], P. chibae; [10], P. cochlearis; [11], P. corningiana; [12], P. cornu-cervi; [13], P. deliciosa; [14], P. doweryensis; [15], P. equestris; [16], P. fasciata; [17], P. fimbriata; [18], P. floresensis; [19], P. fuscata; [20], P. gibbosa; [21], P. gigantea; [22], P. hieroglyphica; [23], P. inscriptiosinensis; [24], P. javanica; [25], P. kunstleri; [26], P. lamelligera; [27], P. lindenii; [28], P. lobbii; [29], P._lowii; [30], P. lueddemanniana; [31], P. maculata; [32], P. mannii; [33], P. mariae; [34], P. micholitzii; [35], P. minus; [36], P. modesta; [37], P. pallens; [38], P. parishii; [39], P. patherina; [40], P. philippinensis; [41], P. pulcherrima; [42], P. pulchra; [43], P._reichenbachiana; [44], P. sanderiana; [45], P. schilleriana; [46], P. stuartiana; [47], P. sumatrana; [48], P. tetraspis; [49], P._venosa; [50], P. violacea; [51], P. viridis; [52], P. wilsonii; [53], Par. labukensis; [54], Par. laycockii; [55], Par. serpentilingua; [56], G. japonicus; [57], T. kotoense.

Table 10. Genetic distances of the two-parameter method of Kimura method inferred from combined the *trn*L intron with the *trn*L-*trn*F IGS among the 52 taxa of the genus *Phalaenopsis* and the five related species.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56]
[1]*
[2]	0.002
[3]	0.033	0.033
[4]	0.027	0.027	0.005
[5]	0.034	0.034	0.019	0.016
[6]	0.026	0.027	0.017	0.015	0.026
[7]	0.017	0.017	0.031	0.026	0.030	0.020
[8]	0.024	0.024	0.024	0.021	0.032	0.017	0.017
[9]	0.022	0.023	0.024	0.019	0.028	0.017	0.025	0.025
[10]	0.029	0.029	0.016	0.006	0.015	0.021	0.026	0.022	0.016
[11]	0.033	0.033	0.018	0.015	0.007	0.024	0.027	0.031	0.027	0.015
[12]	0.024	0.024	0.024	0.018	0.030	0.017	0.017	0.011	0.023	0.021	0.028
[13]	0.028	0.028	0.029	0.021	0.030	0.022	0.027	0.029	0.008	0.018	0.029	0.026
[14]	0.012	0.012	0.023	0.020	0.024	0.016	0.006	0.014	0.020	0.017	0.024	0.014	0.020
[15]	0.032	0.032	0.002	0.005	0.019	0.018	0.031	0.026	0.024	0.017	0.018	0.024	0.029	0.023
[16]	0.041	0.041	0.031	0.020	0.032	0.040	0.043	0.037	0.032	0.018	0.031	0.035	0.033	0.034	0.031
[17]	0.026	0.026	0.008	0.005	0.013	0.016	0.024	0.022	0.014	0.004	0.012	0.020	0.018	0.017	0.008	0.020
[18]	0.023	0.023	0.023	0.018	0.026	0.021	0.023	0.025	0.001	0.016	0.025	0.023	0.006	0.018	0.023	0.031	0.014
[19]	0.029	0.029	0.024	0.020	0.030	0.016	0.024	0.018	0.027	0.022	0.030	0.014	0.027	0.020	0.024	0.034	0.021	0.028
[20]	0.023	0.023	0.023	0.017	0.026	0.022	0.023	0.025	0.003	0.015	0.025	0.023	0.004	0.018	0.023	0.030	0.014	0.002	0.025
[21]	0.032	0.032	0.001	0.004	0.018	0.021	0.030	0.025	0.025	0.016	0.017	0.023	0.033	0.022	0.001	0.030	0.007	0.027	0.023	0.027
[22]	0.030	0.031	0.011	0.005	0.016	0.020	0.030	0.026	0.021	0.010	0.015	0.024	0.023	0.019	0.011	0.024	0.006	0.020	0.023	0.019	0.015
[23]	0.024	0.024	0.007	0.004	0.013	0.015	0.020	0.019	0.011	0.004	0.012	0.018	0.012	0.015	0.008	0.016	0.003	0.011	0.016	0.011	0.007	0.005
[24]	0.023	0.023	0.023	0.018	0.026	0.021	0.023	0.025	0.003	0.016	0.025	0.023	0.004	0.018	0.023	0.031	0.014	0.002	0.025	0.000	0.027	0.020	0.011
[25]	0.039	0.039	0.019	0.016	0.003	0.037	0.037	0.031	0.027	0.025	0.006	0.028	0.029	0.024	0.019	0.055	0.012	0.025	0.028	0.025	0.018	0.016	0.012	0.025
[26]	0.015	0.015	0.035	0.026	0.033	0.019	0.017	0.015	0.024	0.025	0.031	0.015	0.027	0.008	0.035	0.044	0.022	0.024	0.023	0.023	0.038	0.029	0.017	0.024	0.036
[27]	0.031	0.031	0.028	0.024	0.033	0.020	0.028	0.023	0.029	0.025	0.032	0.018	0.030	0.022	0.028	0.040	0.023	0.030	0.006	0.027	0.027	0.027	0.018	0.027	0.032	0.027
[28]	0.024	0.024	0.014	0.014	0.023	0.008	0.016	0.016	0.016	0.014	0.022	0.015	0.016	0.014	0.015	0.029	0.013	0.016	0.015	0.016	0.014	0.017	0.013	0.016	0.023	0.015	0.018
[29]	0.032	0.032	0.001	0.004	0.018	0.021	0.030	0.025	0.025	0.014	0.017	0.023	0.033	0.022	0.001	0.028	0.007	0.027	0.023	0.027	0.000	0.015	0.007	0.027	0.018	0.034	0.027	0.014
[30]	0.020	0.020	0.017	0.012	0.020	0.018	0.018	0.019	0.009	0.010	0.019	0.017	0.012	0.013	0.017	0.025	0.009	0.007	0.023	0.007	0.021	0.012	0.007	0.007	0.019	0.018	0.025	0.013	0.021
[31]	0.034	0.034	0.018	0.006	0.020	0.019	0.032	0.027	0.023	0.020	0.019	0.025	0.026	0.023	0.018	0.034	0.010	0.022	0.023	0.021	0.022	0.012	0.007	0.022	0.020	0.033	0.029	0.016	0.022	0.018
[32]	0.034	0.035	0.010	0.005	0.019	0.020	0.034	0.028	0.025	0.019	0.018	0.024	0.029	0.024	0.010	0.035	0.009	0.024	0.025	0.024	0.014	0.009	0.008	0.024	0.019	0.033	0.029	0.013	0.014	0.018	0.011
[33]	0.032	0.032	0.014	0.011	0.009	0.024	0.032	0.027	0.025	0.014	0.008	0.025	0.028	0.024	0.014	0.028	0.013	0.024	0.026	0.024	0.013	0.012	0.011	0.024	0.009	0.032	0.028	0.023	0.013	0.018	0.015	0.014
[34]	0.037	0.038	0.040	0.035	0.046	0.024	0.031	0.032	0.033	0.037	0.043	0.028	0.036	0.030	0.040	0.050	0.035	0.033	0.036	0.034	0.038	0.040	0.031	0.033	0.044	0.029	0.039	0.020	0.038	0.028	0.042	0.040	0.043
[35]	0.022	0.022	0.014	0.004	0.012	0.023	0.021	0.015	0.011	0.003	0.011	0.015	0.014	0.010	0.015	0.017	0.002	0.011	0.016	0.011	0.014	0.008	0.002	0.011	0.030	0.021	0.019	0.011	0.012	0.005	0.017	0.017	0.012	0.030
[36]	0.030	0.030	0.001	0.004	0.018	0.017	0.028	0.023	0.021	0.011	0.017	0.021	0.023	0.022	0.001	0.024	0.007	0.020	0.022	0.019	0.000	0.007	0.007	0.020	0.018	0.032	0.024	0.014	0.000	0.014	0.008	0.003	0.013	0.036	0.009
[37]	0.031	0.031	0.030	0.024	0.033	0.020	0.028	0.023	0.029	0.025	0.032	0.018	0.030	0.022	0.030	0.040	0.023	0.030	0.006	0.027	0.029	0.027	0.018	0.027	0.032	0.027	0.004	0.019	0.029	0.025	0.029	0.032	0.028	0.039	0.019	0.026
[38]	0.032	0.032	0.017	0.014	0.002	0.024	0.028	0.030	0.025	0.013	0.005	0.027	0.028	0.022	0.017	0.030	0.011	0.024	0.027	0.024	0.016	0.014	0.011	0.024	0.001	0.030	0.031	0.022	0.016	0.018	0.018	0.017	0.007	0.043	0.010	0.016	0.031
[39]	0.017	0.017	0.033	0.027	0.032	0.018	0.018	0.021	0.024	0.023	0.032	0.023	0.026	0.009	0.034	0.043	0.024	0.022	0.028	0.021	0.033	0.031	0.018	0.022	0.028	0.014	0.030	0.015	0.033	0.016	0.038	0.038	0.031	0.035	0.019	0.029	0.030	0.029
[40]	0.027	0.027	0.021	0.018	0.024	0.016	0.024	0.024	0.023	0.018	0.023	0.022	0.024	0.019	0.021	0.030	0.017	0.022	0.021	0.022	0.020	0.019	0.017	0.022	0.023	0.021	0.023	0.014	0.020	0.018	0.021	0.021	0.022	0.033	0.016	0.020	0.023	0.022	0.023
[41]	0.034	0.034	0.002	0.005	0.019	0.019	0.032	0.027	0.025	0.017	0.018	0.023	0.030	0.024	0.002	0.031	0.008	0.024	0.025	0.024	0.001	0.011	0.008	0.024	0.019	0.036	0.029	0.015	0.001	0.018	0.018	0.008	0.014	0.038	0.015	0.001	0.032	0.017	0.035	0.022
[42]	0.030	0.030	0.006	0.005	0.019	0.015	0.029	0.024	0.020	0.010	0.018	0.022	0.022	0.023	0.006	0.023	0.008	0.019	0.022	0.018	0.005	0.009	0.007	0.019	0.019	0.029	0.026	0.014	0.005	0.015	0.007	0.008	0.014	0.039	0.008	0.005	0.026	0.017	0.030	0.019	0.006
[43]	0.003	0.001	0.034	0.028	0.035	0.028	0.018	0.025	0.024	0.030	0.034	0.025	0.029	0.013	0.033	0.042	0.027	0.024	0.031	0.024	0.033	0.032	0.026	0.024	0.040	0.016	0.032	0.025	0.033	0.022	0.035	0.036	0.033	0.039	0.023	0.031	0.032	0.033	0.018	0.028	0.035	0.031
[44]	0.017	0.017	0.033	0.027	0.032	0.018	0.018	0.021	0.024	0.023	0.032	0.023	0.026	0.009	0.034	0.043	0.024	0.022	0.028	0.021	0.033	0.031	0.018	0.022	0.030	0.014	0.030	0.015	0.033	0.016	0.038	0.038	0.031	0.035	0.019	0.029	0.030	0.029	0.002	0.023	0.035	0.030	0.018
[45]	0.017	0.017	0.033	0.027	0.032	0.018	0.018	0.021	0.024	0.023	0.032	0.020	0.026	0.009	0.034	0.043	0.024	0.022	0.026	0.021	0.033	0.031	0.018	0.022	0.030	0.014	0.028	0.015	0.033	0.016	0.038	0.038	0.031	0.035	0.019	0.029	0.028	0.029	0.002	0.023	0.035	0.030	0.018	0.002
[46]	0.023	0.023	0.006	0.004	0.013	0.014	0.021	0.020	0.012	0.004	0.012	0.019	0.013	0.016	0.008	0.016	0.003	0.012	0.018	0.012	0.006	0.005	0.002	0.012	0.012	0.018	0.020	0.011	0.006	0.008	0.008	0.008	0.011	0.032	0.002	0.006	0.020	0.011	0.018	0.015	0.008	0.005	0.024	0.018	0.018
[47]	0.025	0.025	0.015	0.003	0.011	0.019	0.021	0.019	0.011	0.005	0.011	0.018	0.011	0.012	0.016	0.016	0.001	0.011	0.015	0.011	0.015	0.008	0.002	0.011	0.021	0.021	0.018	0.011	0.013	0.007	0.014	0.017	0.011	0.031	0.003	0.008	0.018	0.009	0.019	0.015	0.016	0.005	0.026	0.019	0.019	0.002
[48]	0.028	0.028	0.006	0.003	0.017	0.014	0.027	0.022	0.018	0.008	0.016	0.020	0.020	0.021	0.006	0.021	0.006	0.016	0.020	0.016	0.005	0.006	0.004	0.016	0.017	0.027	0.024	0.013	0.005	0.013	0.005	0.006	0.012	0.036	0.005	0.005	0.024	0.015	0.028	0.018	0.006	0.002	0.030	0.028	0.028	0.004	0.003
[49]	0.027	0.027	0.008	0.003	0.014	0.016	0.022	0.022	0.020	0.008	0.013	0.020	0.022	0.019	0.008	0.024	0.005	0.019	0.019	0.018	0.007	0.008	0.005	0.019	0.013	0.024	0.022	0.015	0.007	0.013	0.010	0.009	0.014	0.034	0.005	0.007	0.022	0.012	0.026	0.019	0.008	0.008	0.029	0.026	0.026	0.005	0.004	0.006
[50]	0.028	0.028	0.035	0.030	0.037	0.033	0.034	0.030	0.010	0.029	0.036	0.028	0.012	0.025	0.035	0.063	0.022	0.007	0.033	0.007	0.039	0.031	0.016	0.007	0.055	0.032	0.035	0.023	0.039	0.013	0.034	0.037	0.038	0.040	0.036	0.032	0.035	0.035	0.030	0.028	0.036	0.031	0.029	0.030	0.030	0.017	0.024	0.029	0.028
[51]	0.025	0.026	0.018	0.016	0.025	0.003	0.021	0.018	0.016	0.019	0.023	0.018	0.021	0.015	0.019	0.038	0.015	0.019	0.017	0.021	0.023	0.019	0.014	0.019	0.035	0.020	0.019	0.009	0.023	0.017	0.020	0.022	0.023	0.027	0.021	0.018	0.019	0.023	0.017	0.017	0.020	0.016	0.027	0.017	0.017	0.012	0.018	0.015	0.017	0.032
[52]	0.028	0.028	0.006	0.003	0.017	0.014	0.027	0.022	0.018	0.008	0.016	0.020	0.020	0.021	0.006	0.021	0.006	0.016	0.020	0.016	0.005	0.006	0.004	0.016	0.017	0.027	0.024	0.013	0.005	0.013	0.005	0.006	0.012	0.036	0.005	0.005	0.024	0.015	0.028	0.018	0.006	0.002	0.030	0.028	0.028	0.004	0.003	0.000	0.006	0.029	0.015
[53]	0.054	0.054	0.048	0.046	0.060	0.038	0.048	0.042	0.046	0.048	0.060	0.044	0.054	0.044	0.051	0.075	0.047	0.048	0.048	0.051	0.054	0.050	0.042	0.050	0.084	0.055	0.052	0.036	0.053	0.047	0.050	0.050	0.061	0.056	0.054	0.050	0.052	0.057	0.050	0.047	0.051	0.048	0.055	0.050	0.050	0.042	0.046	0.046	0.046	0.072	0.039	0.046
[54]	0.060	0.060	0.059	0.050	0.064	0.038	0.056	0.050	0.055	0.059	0.065	0.051	0.061	0.050	0.062	0.087	0.050	0.056	0.050	0.058	0.065	0.057	0.042	0.058	0.090	0.061	0.055	0.037	0.064	0.056	0.062	0.064	0.065	0.064	0.064	0.053	0.055	0.062	0.063	0.049	0.062	0.051	0.062	0.063	0.063	0.043	0.056	0.049	0.050	0.081	0.039	0.049	0.010
[55]	0.059	0.061	0.055	0.046	0.051	0.043	0.056	0.051	0.059	0.052	0.053	0.051	0.064	0.048	0.058	0.074	0.044	0.061	0.051	0.061	0.062	0.051	0.041	0.061	0.051	0.060	0.057	0.032	0.061	0.056	0.054	0.049	0.054	0.059	0.052	0.049	0.057	0.049	0.060	0.046	0.058	0.049	0.062	0.060	0.060	0.041	0.049	0.047	0.045	0.070	0.043	0.047	0.030	0.043
[56]	0.161	0.163	0.155	0.155	0.165	0.148	0.157	0.154	0.156	0.155	0.162	0.153	0.156	0.157	0.156	0.167	0.151	0.156	0.151	0.156	0.155	0.158	0.153	0.156	0.163	0.156	0.152	0.141	0.155	0.151	0.156	0.155	0.161	0.165	0.153	0.155	0.154	0.162	0.156	0.156	0.158	0.155	0.164	0.156	0.152	0.151	0.153	0.153	0.156	0.164	0.146	0.153	0.157	0.159	0.154
[57]	0.041	0.041	0.029	0.028	0.037	0.033	0.034	0.032	0.040	0.028	0.036	0.033	0.043	0.033	0.030	0.043	0.029	0.040	0.034	0.040	0.034	0.034	0.030	0.040	0.036	0.035	0.039	0.023	0.034	0.034	0.035	0.033	0.037	0.045	0.027	0.029	0.036	0.034	0.034	0.031	0.031	0.028	0.042	0.034	0.034	0.029	0.027	0.027	0.029	0.045	0.034	0.027	0.044	0.048	0.045	0.149

[1], P. amabilis; [2], P. amboinensis; [3], P. aphrodite; [4], P. bastianii; [5], P. bellina; [6], P. borneensis; [7], P. braceana; [8], P. celebensis; [9], P. chibae; [10], P. cochlearis; [11], P. corningiana; [12], P. cornu-cervi; [13], P. deliciosa; [14], P. doweryensis; [15], P. equestris; [16], P. fasciata; [17], P. fimbriata; [18], P. floresensis; [19], P. fuscata; [20], P. gibbosa; [21], P. gigantea; [22], P. hieroglyphica; [23], P. inscriptiosinensis; [24], P. javanica; [25], P. kunstleri; [26], P. lamelligera; [27], P. lindenii; [28], P. lobbii; [29], P._lowii; [30], P. lueddemanniana; [31], P. maculata; [32], P. mannii; [33], P. mariae; [34], P. micholitzii; [35], P. minus; [36], P. modesta; [37], P. pallens; [38], P. parishii; [39], P. patherina; [40], P. philippinensis; [41], P. pulcherrima; [42], P. pulchra; [43], P._reichenbachiana; [44], P. sanderiana; [45], P. schilleriana; [46], P. stuartiana; [47], P. sumatrana; [48], P. tetraspis; [49], P._venosa; [50], P. violacea; [51], P. viridis; [52], P. wilsonii; [53], Par. labukensis; [54], Par. laycockii; [55], Par. serpentilingua; [56], G. japonicus; [57], T. kotoense.

Table 11. Genetic distances of the two-parameter method of Kimura method inferred from the *atp*B-*rbc*L IGS among the 52 taxa of the genus *Phalaenopsis* and four related species.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55]

```
[1]
[2]     0.023
[3]     0.001 0.021
[4]     0.026 0.003 0.024
[5]     0.027 0.006 0.026 0.006
[6]     0.028 0.009 0.026 0.009 0.011
[7]     0.032 0.023 0.031 0.026 0.028 0.030
[8]     0.020 0.017 0.018 0.020 0.021 0.020 0.025
[9]     0.025 0.020 0.024 0.022 0.023 0.023 0.025 0.021
[10]    0.033 0.025 0.031 0.025 0.027 0.032 0.035 0.031 0.026
[11]    0.031 0.012 0.029 0.012 0.014 0.003 0.033 0.023 0.026 0.034
[12]    0.024 0.003 0.022 0.003 0.004 0.009 0.026 0.020 0.022 0.025 0.012
[13]    0.027 0.015 0.026 0.018 0.018 0.022 0.028 0.018 0.006 0.025 0.025 0.018
[14]    0.031 0.027 0.030 0.027 0.029 0.027 0.034 0.028 0.028 0.005 0.029 0.027 0.026
[15]    0.024 0.018 0.022 0.021 0.023 0.021 0.029 0.010 0.025 0.034 0.024 0.021 0.023 0.032
[16]    0.024 0.004 0.022 0.001 0.006 0.011 0.025 0.018 0.021 0.025 0.014 0.004 0.017 0.027 0.019
[17]    0.024 0.001 0.023 0.001 0.004 0.008 0.025 0.018 0.021 0.024 0.011 0.001 0.017 0.025 0.020 0.003
[18]    0.030 0.007 0.029 0.009 0.001 0.012 0.031 0.024 0.026 0.028 0.015 0.007 0.021 0.030 0.026 0.009 0.006
[19]    0.034 0.026 0.032 0.026 0.027 0.031 0.034 0.032 0.026 0.000 0.033 0.026 0.026 0.005 0.035 0.026 0.024 0.029
[20]    0.029 0.017 0.028 0.020 0.022 0.023 0.028 0.017 0.014 0.028 0.026 0.020 0.008 0.027 0.023 0.018 0.018 0.023 0.029
[21]    0.033 0.026 0.032 0.025 0.027 0.029 0.036 0.030 0.025 0.005 0.031 0.025 0.027 0.006 0.033 0.025 0.024 0.029 0.005 0.027
[22]    0.026 0.003 0.024 0.000 0.004 0.009 0.026 0.020 0.022 0.025 0.012 0.003 0.018 0.027 0.021 0.001 0.001 0.007 0.026 0.020 0.025
[23]    0.026 0.003 0.024 0.003 0.006 0.009 0.026 0.020 0.023 0.025 0.012 0.003 0.018 0.027 0.021 0.004 0.001 0.007 0.026 0.020 0.025 0.003
[24]    0.025 0.004 0.024 0.004 0.006 0.011 0.028 0.021 0.024 0.027 0.014 0.001 0.020 0.028 0.022 0.006 0.003 0.009 0.027 0.021 0.027 0.004 0.004
[25]    0.033 0.025 0.031 0.025 0.027 0.032 0.035 0.031 0.026 0.000 0.034 0.025 0.025 0.005 0.034 0.025 0.024 0.028 0.000 0.028 0.005 0.025 0.025 0.027
[26]    0.025 0.009 0.023 0.009 0.011 0.002 0.031 0.020 0.025 0.029 0.005 0.009 0.022 0.024 0.022 0.011 0.008 0.012 0.029 0.024 0.026 0.009 0.009 0.011 0.029
[27]    0.044 0.043 0.042 0.046 0.048 0.045 0.053 0.038 0.050 0.058 0.045 0.046 0.044 0.054 0.038 0.044 0.044 0.051 0.060 0.049 0.057 0.046 0.046 0.047 0.058 0.044
[28]    0.025 0.014 0.023 0.017 0.019 0.020 0.027 0.014 0.015 0.024 0.024 0.017 0.012 0.025 0.022 0.016 0.016 0.020 0.023 0.008 0.025 0.017 0.017 0.019 0.024 0.021 0.047
[29]    0.034 0.030 0.032 0.033 0.031 0.030 0.023 0.034 0.034 0.044 0.033 0.032 0.038 0.043 0.032 0.031 0.031 0.034 0.043 0.028 0.044 0.033 0.033 0.031 0.044 0.028 0.053 0.037
[30]    0.026 0.003 0.024 0.000 0.004 0.009 0.026 0.020 0.022 0.025 0.012 0.003 0.018 0.027 0.021 0.001 0.001 0.007 0.026 0.020 0.025 0.000 0.003 0.004 0.025 0.009 0.046 0.017 0.033
[31]    0.030 0.022 0.028 0.022 0.024 0.026 0.032 0.028 0.021 0.019 0.029 0.022 0.024 0.018 0.031 0.023 0.020 0.027 0.020 0.024 0.016 0.022 0.022 0.022 0.019 0.023 0.054 0.023 0.037 0.022
[32]    0.025 0.004 0.024 0.004 0.006 0.008 0.026 0.018 0.030 0.023 0.011 0.004 0.017 0.022 0.019 0.006 0.003 0.009 0.024 0.018 0.020 0.004 0.004 0.006 0.023 0.008 0.042 0.015 0.033 0.004 0.017
[33]    0.024 0.001 0.022 0.001 0.003 0.008 0.025 0.018 0.021 0.023 0.011 0.001 0.017 0.025 0.019 0.003 0.000 0.006 0.024 0.018 0.024 0.001 0.001 0.003 0.023 0.008 0.044 0.016 0.031 0.001 0.020 0.003
[34]    0.026 0.006 0.024 0.006 0.009 0.003 0.028 0.020 0.023 0.029 0.006 0.006 0.021 0.027 0.021 0.007 0.004 0.010 0.028 0.023 0.029 0.006 0.006 0.007 0.029 0.005 0.046 0.020 0.031 0.006 0.025 0.007 0.004
[35]    0.042 0.030 0.041 0.033 0.035 0.040 0.035 0.038 0.033 0.031 0.042 0.033 0.034 0.033 0.041 0.033 0.031 0.036 0.032 0.038 0.033 0.033 0.033 0.034 0.031 0.040 0.069 0.036 0.050 0.033 0.043 0.034 0.031 0.037
[36]    0.026 0.003 0.024 0.003 0.006 0.009 0.026 0.020 0.023 0.025 0.012 0.003 0.018 0.027 0.021 0.004 0.001 0.007 0.026 0.020 0.025 0.003 0.003 0.004 0.025 0.009 0.046 0.017 0.033 0.003 0.022 0.004 0.001 0.006 0.033
[37]    0.026 0.003 0.024 0.000 0.004 0.009 0.026 0.020 0.022 0.025 0.012 0.003 0.018 0.027 0.021 0.001 0.001 0.007 0.026 0.020 0.025 0.000 0.003 0.004 0.025 0.009 0.046 0.017 0.033 0.000 0.022 0.004 0.001 0.006 0.033 0.003
[38]    0.028 0.009 0.026 0.009 0.011 0.000 0.030 0.020 0.023 0.032 0.003 0.009 0.022 0.027 0.021 0.011 0.008 0.012 0.031 0.023 0.029 0.009 0.009 0.011 0.032 0.002 0.045 0.020 0.030 0.009 0.026 0.008 0.008 0.003 0.040 0.009 0.009
[39]    0.025 0.014 0.023 0.017 0.019 0.020 0.027 0.014 0.015 0.024 0.024 0.017 0.012 0.025 0.022 0.016 0.016 0.020 0.023 0.008 0.025 0.017 0.017 0.019 0.024 0.021 0.047 0.000 0.037 0.017 0.023 0.015 0.016 0.020 0.036 0.017 0.017 0.020
[40]    0.019 0.020 0.018 0.022 0.024 0.024 0.021 0.015 0.025 0.033 0.024 0.022 0.027 0.032 0.019 0.021 0.021 0.027 0.034 0.028 0.033 0.022 0.023 0.024 0.033 0.025 0.041 0.023 0.029 0.022 0.031 0.022 0.021 0.023 0.039 0.023 0.022 0.024 0.023
[41]    0.048 0.037 0.046 0.041 0.038 0.044 0.048 0.038 0.045 0.052 0.047 0.038 0.039 0.054 0.039 0.040 0.038 0.040 0.053 0.039 0.052 0.041 0.040 0.039 0.052 0.044 0.067 0.037 0.058 0.041 0.050 0.039 0.040 0.044 0.055 0.040 0.041 0.044 0.037 0.039
[42]    0.027 0.004 0.026 0.001 0.006 0.011 0.028 0.021 0.024 0.027 0.014 0.004 0.020 0.028 0.022 0.003 0.003 0.009 0.027 0.022 0.027 0.001 0.004 0.006 0.027 0.011 0.047 0.019 0.034 0.001 0.023 0.006 0.003 0.007 0.035 0.004 0.001 0.011 0.019 0.024 0.043
[43]    0.026 0.003 0.024 0.000 0.004 0.009 0.026 0.020 0.022 0.025 0.012 0.003 0.018 0.027 0.021 0.001 0.001 0.007 0.026 0.020 0.025 0.000 0.003 0.004 0.025 0.009 0.046 0.017 0.033 0.000 0.022 0.004 0.001 0.006 0.033 0.003 0.000 0.009 0.017 0.022 0.041 0.001
[44]    0.001 0.021 0.000 0.024 0.026 0.026 0.031 0.018 0.024 0.031 0.029 0.022 0.026 0.030 0.022 0.022 0.023 0.029 0.032 0.028 0.032 0.024 0.024 0.024 0.031 0.023 0.042 0.023 0.032 0.024 0.028 0.024 0.022 0.024 0.041 0.024 0.024 0.026 0.023 0.018 0.046 0.026 0.024
[45]    0.019 0.020 0.018 0.022 0.024 0.024 0.021 0.015 0.025 0.033 0.024 0.022 0.027 0.032 0.019 0.021 0.021 0.027 0.034 0.028 0.033 0.022 0.023 0.024 0.033 0.025 0.041 0.023 0.029 0.022 0.031 0.022 0.021 0.023 0.039 0.023 0.022 0.024 0.023 0.000 0.039 0.024 0.022 0.018
[46]    0.022 0.023 0.021 0.026 0.027 0.028 0.024 0.018 0.025 0.034 0.028 0.025 0.027 0.033 0.022 0.024 0.024 0.030 0.035 0.028 0.035 0.026 0.026 0.027 0.034 0.028 0.044 0.023 0.032 0.026 0.031 0.025 0.024 0.026 0.041 0.026 0.026 0.028 0.023 0.003 0.043 0.027 0.026 0.021 0.003
[47]    0.025 0.004 0.024 0.004 0.006 0.011 0.028 0.021 0.024 0.027 0.014 0.001 0.020 0.028 0.022 0.006 0.003 0.009 0.027 0.021 0.027 0.004 0.004 0.000 0.027 0.011 0.047 0.019 0.031 0.004 0.022 0.006 0.003 0.007 0.034 0.004 0.004 0.011 0.019 0.024 0.039 0.006 0.004 0.024 0.024 0.027
[48]    0.024 0.003 0.022 0.003 0.004 0.009 0.026 0.020 0.022 0.025 0.012 0.000 0.018 0.027 0.021 0.004 0.001 0.007 0.026 0.020 0.025 0.003 0.003 0.001 0.025 0.009 0.046 0.017 0.032 0.003 0.022 0.004 0.001 0.006 0.033 0.003 0.003 0.009 0.017 0.022 0.038 0.004 0.003 0.022 0.022 0.025 0.001
[49]    0.026 0.003 0.024 0.003 0.006 0.009 0.026 0.020 0.023 0.025 0.012 0.003 0.017 0.026 0.021 0.004 0.001 0.007 0.026 0.019 0.024 0.003 0.003 0.004 0.025 0.009 0.046 0.016 0.033 0.003 0.022 0.004 0.001 0.006 0.032 0.003 0.003 0.009 0.016 0.023 0.038 0.004 0.003 0.024 0.023 0.026 0.004 0.003
[50]    0.027 0.006 0.026 0.006 0.000 0.011 0.028 0.021 0.023 0.027 0.014 0.004 0.018 0.029 0.023 0.006 0.004 0.001 0.027 0.022 0.027 0.004 0.006 0.006 0.027 0.011 0.048 0.019 0.031 0.004 0.024 0.006 0.003 0.009 0.035 0.006 0.004 0.011 0.019 0.024 0.038 0.006 0.004 0.026 0.024 0.027 0.006 0.004 0.006
[51]    0.033 0.025 0.031 0.025 0.027 0.032 0.035 0.031 0.027 0.000 0.034 0.025 0.025 0.005 0.034 0.025 0.024 0.028 0.000 0.028 0.005 0.025 0.025 0.027 0.000 0.029 0.058 0.024 0.044 0.025 0.019 0.023 0.023 0.029 0.031 0.025 0.025 0.032 0.024 0.033 0.052 0.027 0.025 0.031 0.033 0.034 0.027 0.025 0.025 0.027
[52]    0.026 0.020 0.025 0.023 0.025 0.026 0.012 0.021 0.025 0.035 0.030 0.023 0.028 0.037 0.026 0.022 0.022 0.028 0.033 0.028 0.036 0.023 0.023 0.025 0.035 0.028 0.050 0.020 0.023 0.023 0.032 0.023 0.022 0.025 0.042 0.023 0.023 0.026 0.020 0.015 0.044 0.025 0.023 0.025 0.015 0.018 0.025 0.023 0.023 0.025 0.035
[53]    0.035 0.034 0.033 0.038 0.038 0.037 0.041 0.029 0.032 0.039 0.041 0.034 0.032 0.036 0.033 0.037 0.035 0.043 0.040 0.028 0.040 0.038 0.037 0.032 0.039 0.038 0.056 0.023 0.041 0.038 0.037 0.038 0.037 0.037 0.050 0.037 0.038 0.037 0.023 0.032 0.052 0.040 0.038 0.033 0.032 0.032 0.032 0.034 0.037 0.038 0.039 0.034
[54]    0.037 0.028 0.036 0.033 0.033 0.038 0.042 0.036 0.034 0.043 0.042 0.033 0.033 0.045 0.040 0.031 0.030 0.038 0.042 0.032 0.043 0.033 0.031 0.031 0.043 0.039 0.065 0.026 0.043 0.033 0.041 0.034 0.031 0.035 0.047 0.031 0.033 0.038 0.026 0.036 0.055 0.034 0.033 0.036 0.036 0.031 0.033 0.032 0.033 0.043 0.035 0.015
[55]    0.039 0.032 0.038 0.036 0.036 0.039 0.044 0.036 0.033 0.044 0.042 0.036 0.032 0.043 0.041 0.035 0.033 0.040 0.044 0.033 0.042 0.036 0.035 0.035 0.044 0.039 0.066 0.027 0.049 0.036 0.038 0.035 0.035 0.038 0.051 0.035 0.036 0.039 0.027 0.038 0.054 0.038 0.036 0.038 0.038 0.038 0.035 0.036 0.035 0.036 0.045 0.037 0.020 0.012
[56]    0.079 0.058 0.077 0.060 0.059 0.060 0.072 0.065 0.079 0.071 0.060 0.060 0.069 0.082 0.063 0.062 0.057 0.063 0.068 0.070 0.075 0.060 0.059 0.062 0.071 0.062 0.091 0.068 0.080 0.060 0.082 0.061 0.059 0.058 0.077 0.059 0.060 0.060 0.068 0.067 0.067 0.062 0.060 0.077 0.067 0.070 0.062 0.060 0.058 0.059 0.071 0.068 0.088 0.080 0.083
```

[1], P. amabilis; [2], P. amboinensis; [3], P. aphrodite; [4], P. bastianii; [5], P. bellina; [6], P. borneensis; [7], P. braceana; [8], P. celebensis; [9], P. chibae; [10], P. cochlearis; [11], P. corningiana; [12], P. cornu-cervi; [13], P. deliciosa; [14], P. doweryensis; [15], P. equestris; [16], P. fasciata; [17], P. fimbriata; [18], P. floresensis; [19], P. fuscata; [20], P. gibbosa; [21], P. gigantea; [22], P. hieroglyphica; [23], P. inscriptiosinensis; [24], P. javanica; [25], P. kunstleri; [26], P. lamelligera; [27], P. lindenii; [28], P. lobbii; [29], P._lowii; [30], P. lueddemanniana; [31], P. maculata; [32], P. mannii; [33], P. mariae; [34], P. micholitzii; [35], P. minus; [36], P. modesta; [37], P. pallens; [38], P. parishii; [39], P. patherina; [40], P. philippinensis; [41], P. pulcherrima; [42], P. pulchra; [43], P._reichenbachiana; [44], P. sanderiana; [45], P. schilleriana; [46], P. stuartiana; [47], P. sumatrana; [48], P. tetraspis; [49], P._venosa; [50], P. violacea; [51], P. viridis; [52], P. wilsonii; [53], Par. labukensis; [54], Par. laycockii; [55], Par. serpentilingua; [56], T. kotoense.

Table 12. Genetic distances of the two-parameter method of Kimura method inferred from combined data of sequences of the intron *trn*L, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS among the 52 taxa of the genus *Phalaenopsis* and four related species.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55]
[1]*																																																									
[2]	0.117																																																							
[3]	0.002	0.117																																																						
[4]	0.030	0.102	0.029																																																				
[5]	0.032	0.110	0.031	0.012																																																			
[6]	0.033	0.108	0.032	0.016	0.020																																																			
[7]	0.026	0.105	0.025	0.025	0.029	0.030																																																		
[8]	0.025	0.107	0.024	0.025	0.030	0.030	0.025																																																	
[9]	0.028	0.107	0.028	0.026	0.028	0.032	0.020	0.018																																																
[10]	0.024	0.106	0.024	0.022	0.026	0.028	0.023	0.028	0.018																																															
[11]	0.025	0.102	0.025	0.008	0.013	0.011	0.022	0.022	0.020	0.017																																													
[12]	0.034	0.106	0.033	0.016	0.021	0.006	0.031	0.029	0.032	0.028	0.012																																												
[13]	0.030	0.106	0.030	0.026	0.027	0.031	0.020	0.018	0.007	0.019	0.019	0.031																																											
[14]	0.024	0.111	0.024	0.022	0.026	0.025	0.021	0.026	0.021	0.003	0.018	0.025	0.021																																										
[15]	0.020	0.106	0.019	0.020	0.024	0.024	0.021	0.008	0.017	0.022	0.016	0.024	0.017	0.021																																									
[16]	0.029	0.103	0.028	0.002	0.012	0.016	0.024	0.024	0.024	0.022	0.009	0.017	0.023	0.022	0.020																																									
[17]	0.037	0.107	0.037	0.022	0.022	0.022	0.033	0.033	0.032	0.028	0.011	0.023	0.031	0.027	0.026	0.023																																									
[18]	0.030	0.109	0.030	0.017	0.007	0.020	0.027	0.027	0.030	0.024	0.011	0.021	0.028	0.025	0.022	0.017	0.021																																								
[19]	0.024	0.106	0.023	0.021	0.025	0.026	0.022	0.028	0.018	0.001	0.017	0.026	0.019	0.003	0.022	0.021	0.029	0.023																																							
[20]	0.028	0.105	0.028	0.020	0.025	0.028	0.017	0.016	0.010	0.020	0.017	0.028	0.008	0.018	0.015	0.019	0.026	0.026	0.020																																							
[21]	0.025	0.108	0.025	0.022	0.027	0.027	0.022	0.030	0.021	0.004	0.018	0.027	0.021	0.003	0.023	0.022	0.030	0.025	0.003	0.018																																						
[22]	0.029	0.103	0.028	0.001	0.011	0.015	0.024	0.024	0.027	0.021	0.007	0.016	0.026	0.025	0.020	0.001	0.021	0.015	0.024	0.022	0.025																																				
[23]	0.026	0.104	0.025	0.009	0.014	0.013	0.023	0.025	0.021	0.017	0.007	0.013	0.020	0.019	0.017	0.010	0.012	0.014	0.018	0.018	0.019	0.009																																				
[24]	0.024	0.102	0.023	0.006	0.011	0.012	0.019	0.021	0.021	0.014	0.003	0.012	0.019	0.017	0.016	0.007	0.011	0.011	0.014	0.018	0.016	0.006	0.005																																				
[25]	0.024	0.107	0.023	0.020	0.025	0.027	0.022	0.028	0.018	0.002	0.017	0.027	0.019	0.002	0.022	0.020	0.028	0.023	0.001	0.019	0.002	0.024	0.017	0.014																																				
[26]	0.035	0.109	0.034	0.018	0.020	0.002	0.030	0.034	0.037	0.026	0.015	0.006	0.036	0.023	0.025	0.019	0.034	0.021	0.025	0.032	0.025	0.017	0.017	0.014	0.025																																				
[27]	0.038	0.119	0.038	0.036	0.040	0.040	0.037	0.026	0.031	0.039	0.032	0.039	0.030	0.041	0.024	0.035	0.047	0.040	0.042	0.032	0.042	0.039	0.034	0.034	0.041	0.045																																				
[28]	0.029	0.105	0.029	0.021	0.027	0.030	0.020	0.017	0.014	0.018	0.019	0.030	0.012	0.019	0.017	0.020	0.029	0.026	0.018	0.007	0.018	0.023	0.020	0.020	0.016	0.038	0.031																																				
[29]	0.029	0.109	0.028	0.024	0.029	0.027	0.015	0.023	0.024	0.026	0.025	0.028	0.025	0.026	0.021	0.024	0.032	0.029	0.026	0.024	0.027	0.024	0.026	0.023	0.026	0.027	0.037	0.026																																				
[30]	0.029	0.103	0.028	0.001	0.011	0.015	0.024	0.024	0.027	0.021	0.007	0.016	0.026	0.025	0.020	0.001	0.021	0.015	0.024	0.022	0.025	0.000	0.009	0.006	0.024	0.017	0.039	0.023	0.024																																				
[31]	0.025	0.113	0.025	0.020	0.027	0.025	0.021	0.030	0.021	0.011	0.017	0.026	0.021	0.010	0.023	0.021	0.028	0.025	0.010	0.019	0.010	0.023	0.016	0.014	0.010	0.023	0.043	0.021	0.027	0.023																																		
[32]	0.032	0.106	0.032	0.013	0.017	0.016	0.025	0.027	0.023	0.021	0.014	0.017	0.022	0.020	0.019	0.014	0.025	0.018	0.020	0.019	0.020	0.015	0.015	0.008	0.020	0.023	0.039	0.021	0.025	0.015	0.020																																		
[33]	0.030	0.101	0.029	0.006	0.014	0.016	0.025	0.025	0.024	0.021	0.008	0.016	0.022	0.021	0.020	0.007	0.022	0.015	0.020	0.019	0.022	0.005	0.009	0.006	0.020	0.018	0.037	0.021	0.023	0.005	0.020	0.014																																	
[34]	0.030	0.105	0.030	0.014	0.018	0.007	0.029	0.030	0.029	0.025	0.012	0.008	0.028	0.024	0.023	0.014	0.023	0.020	0.024	0.025	0.026	0.013	0.011	0.009	0.024	0.008	0.039	0.027	0.029	0.013	0.024	0.017	0.014																																	
[35]	0.034	0.114	0.034	0.035	0.039	0.042	0.025	0.036	0.031	0.033	0.031	0.042	0.030	0.033	0.034	0.035	0.045	0.039	0.032	0.028	0.032	0.035	0.032	0.030	0.032	0.042	0.049	0.031	0.030	0.035	0.030	0.036	0.034	0.039																																	
[36]	0.024	0.101	0.023	0.008	0.010	0.010	0.021	0.022	0.020	0.015	0.003	0.011	0.019	0.017	0.015	0.009	0.010	0.011	0.016	0.016	0.017	0.007	0.006	0.003	0.015	0.018	0.032	0.018	0.024	0.007	0.015	0.012	0.009	0.008	0.031																																	
[37]	0.028	0.102	0.027	0.001	0.010	0.013	0.023	0.023	0.024	0.019	0.005	0.013	0.023	0.020	0.019	0.001	0.019	0.013	0.018	0.019	0.020	0.000	0.007	0.006	0.018	0.015	0.036	0.021	0.024	0.000	0.019	0.008	0.002	0.010	0.033	0.007																																	
[38]	0.029	0.106	0.029	0.023	0.027	0.030	0.020	0.017	0.014	0.018	0.019	0.030	0.012	0.019	0.017	0.021	0.029	0.026	0.018	0.007	0.018	0.025	0.020	0.020	0.016	0.038	0.031	0.002	0.027	0.025	0.021	0.021	0.022	0.027	0.031	0.018	0.022																																	
[39]	0.032	0.107	0.031	0.014	0.019	0.001	0.029	0.029	0.030	0.027	0.010	0.004	0.029	0.024	0.023	0.015	0.021	0.019	0.025	0.027	0.026	0.014	0.011	0.011	0.025	0.001	0.039	0.029	0.027	0.014	0.024	0.015	0.014	0.006	0.041	0.009	0.011	0.029																																	
[40]	0.023	0.107	0.023	0.023	0.027	0.028	0.024	0.015	0.021	0.027	0.020	0.028	0.023	0.026	0.010	0.023	0.031	0.028	0.027	0.021	0.029	0.023	0.022	0.021	0.027	0.030	0.026	0.025	0.023	0.023	0.029	0.025	0.024	0.027	0.036	0.019	0.023	0.025	0.027																																	
[41]	0.049	0.124	0.049	0.043	0.047	0.051	0.041	0.040	0.040	0.036	0.038	0.049	0.038	0.040	0.038	0.042	0.049	0.045	0.036	0.037	0.039	0.045	0.040	0.040	0.036	0.054	0.050	0.038	0.044	0.045	0.043	0.042	0.042	0.050	0.046	0.038	0.042	0.038	0.049	0.042																																	
[42]	0.031	0.104	0.030	0.002	0.012	0.016	0.026	0.026	0.026	0.023	0.009	0.017	0.024	0.023	0.022	0.002	0.023	0.017	0.022	0.021	0.023	0.001	0.010	0.007	0.022	0.019	0.037	0.023	0.026	0.001	0.022	0.014	0.005	0.014	0.035	0.009	0.001	0.024	0.015	0.025	0.044																																	
[43]	0.028	0.102	0.028	0.006	0.011	0.016	0.025	0.026	0.022	0.020	0.009	0.017	0.021	0.021	0.020	0.006	0.019	0.017	0.019	0.021	0.021	0.005	0.011	0.006	0.019	0.016	0.036	0.022	0.024	0.005	0.020	0.012	0.009	0.014	0.034	0.006	0.003	0.022	0.015	0.023	0.042	0.006																																	
[44]	0.002	0.118	0.001	0.030	0.032	0.033	0.026	0.025	0.029	0.024	0.025	0.034	0.030	0.024	0.020	0.029	0.037	0.030	0.024	0.029	0.025	0.029	0.026	0.024	0.024	0.035	0.038	0.030	0.029	0.029	0.025	0.032	0.030	0.030	0.035	0.024	0.028	0.030	0.032	0.023	0.050	0.031	0.028																																	
[45]	0.023	0.107	0.023	0.023	0.027	0.028	0.024	0.015	0.021	0.027	0.020	0.028	0.023	0.026	0.010	0.023	0.031	0.028	0.027	0.021	0.029	0.023	0.022	0.021	0.027	0.032	0.026	0.025	0.023	0.023	0.029	0.025	0.024	0.027	0.036	0.019	0.023	0.025	0.027	0.001	0.042	0.025	0.023	0.023																																	
[46]	0.024	0.107	0.024	0.025	0.029	0.030	0.025	0.016	0.021	0.028	0.022	0.029	0.022	0.027	0.011	0.025	0.032	0.029	0.028	0.020	0.030	0.025	0.023	0.022	0.028	0.033	0.027	0.024	0.024	0.025	0.029	0.027	0.025	0.028	0.037	0.020	0.025	0.024	0.029	0.002	0.042	0.027	0.025	0.024	0.002																																	
[47]	0.025	0.102	0.024	0.006	0.011	0.012	0.022	0.022	0.021	0.017	0.003	0.013	0.020	0.019	0.017	0.007	0.011	0.011	0.017	0.018	0.019	0.006	0.005	0.001	0.017	0.011	0.034	0.020	0.022	0.006	0.017	0.007	0.006	0.009	0.032	0.003	0.006	0.020	0.011	0.021	0.039	0.007	0.006	0.025	0.021	0.022																																	
[48]	0.025	0.102	0.025	0.008	0.013	0.012	0.022	0.022	0.019	0.017	0.003	0.013	0.018	0.016	0.015	0.009	0.010	0.011	0.017	0.016	0.018	0.007	0.006	0.002	0.017	0.014	0.034	0.018	0.024	0.007	0.017	0.012	0.009	0.011	0.031	0.003	0.004	0.018	0.011	0.021	0.038	0.009	0.009	0.025	0.021	0.022	0.003																																	
[49]	0.032	0.108	0.031	0.014	0.007	0.021	0.029	0.032	0.028	0.025	0.016	0.021	0.028	0.026	0.026	0.015	0.025	0.012	0.023	0.028	0.025	0.014	0.017	0.012	0.024	0.021	0.043	0.029	0.029	0.014	0.025	0.019	0.015	0.019	0.039	0.012	0.012	0.029	0.019	0.029	0.050	0.015	0.010	0.032	0.029	0.030	0.013	0.016																																	
[50]	0.031	0.111	0.030	0.015	0.005	0.018	0.028	0.028	0.028	0.025	0.012	0.019	0.026	0.026	0.024	0.016	0.021	0.004	0.024	0.024	0.026	0.014	0.015	0.011	0.024	0.019	0.040	0.026	0.030	0.014	0.026	0.017	0.014	0.018	0.039	0.012	0.012	0.026	0.017	0.027	0.045	0.016	0.015	0.031	0.027	0.029	0.011	0.012	0.011																																	
[51]	0.027	0.111	0.026	0.028	0.030	0.029	0.025	0.034	0.024	0.006	0.021	0.029	0.024	0.006	0.026	0.028	0.037	0.027	0.004	0.025	0.006	0.031	0.023	0.017	0.004	0.031	0.047	0.023	0.029	0.031	0.013	0.029	0.027	0.030	0.036	0.021	0.023	0.023	0.028	0.032	0.042	0.029	0.025	0.027	0.032	0.033	0.020	0.020	0.030	0.028																																	
[52]	0.023	0.101	0.022	0.024	0.029	0.028	0.007	0.025	0.020	0.022	0.020	0.029	0.020	0.022	0.019	0.024	0.031	0.027	0.021	0.018	0.022	0.024	0.021	0.017	0.021	0.028	0.036	0.017	0.015	0.024	0.020	0.024	0.025	0.027	0.029	0.019	0.022	0.017	0.027	0.021	0.040	0.025	0.025	0.023	0.021	0.022	0.020	0.020	0.029	0.028	0.024																																	
[53]	0.060	0.130	0.059	0.053	0.058	0.059	0.052	0.053	0.047	0.058	0.049	0.061	0.048	0.057	0.051	0.053	0.061	0.059	0.057	0.051	0.059	0.053	0.052	0.049	0.057	0.061	0.063	0.046	0.045	0.053	0.057	0.057	0.054	0.060	0.064	0.049	0.053	0.046	0.058	0.050	0.065	0.055	0.056	0.060	0.050	0.050	0.048	0.051	0.060	0.056	0.062	0.050																																
[54]	0.055	0.114	0.054	0.052	0.052	0.053	0.047	0.044	0.034	0.046	0.045	0.054	0.037	0.047	0.042	0.052	0.071	0.049	0.046	0.038	0.050	0.055	0.049	0.041	0.047	0.073	0.054	0.040	0.043	0.055	0.050	0.058	0.053	0.055	0.057	0.053	0.050	0.040	0.052	0.043	0.058	0.053	0.047	0.055	0.043	0.043	0.040	0.045	0.052	0.050	0.062	0.045	0.030																														
[55]	0.055	0.116	0.054	0.052	0.052	0.053	0.045	0.043	0.036	0.044	0.044	0.055	0.037	0.048	0.042	0.052	0.065	0.050	0.045	0.039	0.048	0.055	0.048	0.043	0.047	0.070	0.056	0.041	0.046	0.055	0.048	0.052	0.051	0.053	0.056	0.052	0.053	0.041	0.052	0.044	0.058	0.054	0.046	0.055	0.044	0.044	0.042	0.044	0.052	0.051	0.058	0.043	0.028	0.011																													
[56]	0.030	0.108	0.030	0.014	0.007	0.021	0.028	0.031	0.026	0.025	0.016	0.021	0.027	0.026	0.025	0.015	0.025	0.012	0.023	0.027	0.026	0.014	0.017	0.012	0.024	0.021	0.041	0.027	0.028	0.014	0.025	0.019	0.015	0.019	0.038	0.012	0.012	0.027	0.019	0.027	0.049	0.015	0.010	0.030	0.027	0.029	0.013	0.016	0.001	0.011	0.030	0.027	0.059	0.051	0.050

[1], P. amabilis; [2], P. amboinensis; [3], P. aphrodite; [4], P. bastianii; [5], P. bellina; [6], P. borneensis; [7], P. braceana; [8], P. celebensis; [9], P. chibae; [10], P. cochlearis; [11], P. corningiana; [12], P. cornu-cervi; [13], P. deliciosa; [14], P. doweryensis; [15], P. equestris; [16], P. fasciata; [17], P. fimbriata; [18], P. floresensis; [19], P. fuscata; [20], P. gibbosa; [21], P. gigantea; [22], P. hieroglyphica; [23], P. inscriptiosinensis; [24], P. javanica; [25], P. kunstleri; [26], P. lamelligera; [27], P. lindenii; [28], P. lobbii; [29], P._lowii; [30], P. lueddemanniana; [31], P. maculata; [32], P. mannii; [33], P. mariae; [34], P. micholitzii; [35], P. minus; [36], P. modesta; [37], P. pallens; [38], P. parishii; [39], P. patherina; [40], P. philippinensis; [41], P. pulcherrima; [42], P. pulchra; [43], P._reichenbachiana; [44], P. sanderiana; [45], P. schilleriana; [46], P. stuartiana; [47], P. sumatrana; [48], P. tetraspis; [49], P._venosa; [50], P. violacea; [51], P. viridis; [52], P. wilsonii; [53], Par. labukensis; [54], Par. laycockii; [55], Par. serpentilingua; [56], T. kotoense.

Table 13. Genetic distances of the two-parameter method of Kimura method inferred from combined data of sequences of the ITS1 and ITS2 of nrDNA, the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L IGS among the 52 taxa of the genus *Phalaenopsis* and four related species.

```
[        1     2     3     4     5     6     7     8     9    10    11    12    13    14    15    16    17    18    19    20    21    22    23    24    25    26    27    28    29    30    31    32    33    34    35    36    37    38    39    40    41    42    43    44    45    46    47    48    49    50    51    52    53    54    55]
[1]*
[2]     0.044
[3]     0.004 0.043
[4]     0.048 0.017 0.046
[5]     0.048 0.014 0.046 0.015
[6]     0.051 0.031 0.048 0.032 0.030
[7]     0.051 0.038 0.049 0.045 0.042 0.053
[8]     0.037 0.036 0.036 0.042 0.040 0.051 0.041
[9]     0.049 0.035 0.045 0.040 0.038 0.054 0.026 0.038
[10]  0.043 0.030 0.042 0.036 0.034 0.047 0.039 0.039 0.037
[11]  0.047 0.015 0.046 0.019 0.015 0.030 0.043 0.039 0.040 0.030
[12]  0.053 0.033 0.051 0.033 0.033 0.012 0.056 0.052 0.056 0.049 0.032
[13]  0.050 0.038 0.047 0.044 0.041 0.055 0.027 0.041 0.020 0.039 0.041 0.058
[14]  0.045 0.028 0.043 0.032 0.030 0.044 0.035 0.036 0.034 0.015 0.027 0.045 0.035
[15]  0.031 0.032 0.028 0.034 0.033 0.044 0.040 0.025 0.036 0.036 0.033 0.046 0.038 0.030
[16]  0.048 0.017 0.047 0.006 0.015 0.032 0.044 0.040 0.041 0.035 0.019 0.033 0.043 0.032 0.034
[17]  0.054 0.021 0.053 0.028 0.022 0.039 0.049 0.050 0.046 0.039 0.015 0.041 0.047 0.035 0.043 0.027
[18]  0.046 0.015 0.045 0.016 0.014 0.030 0.041 0.038 0.038 0.030 0.010 0.031 0.040 0.027 0.032 0.016 0.012
[19]  0.060 0.046 0.059 0.050 0.049 0.064 0.050 0.054 0.051 0.029 0.044 0.066 0.053 0.026 0.049 0.050 0.050 0.043
[20]  0.046 0.037 0.045 0.040 0.039 0.049 0.027 0.040 0.020 0.036 0.038 0.052 0.020 0.033 0.038 0.040 0.043 0.036 0.050
[21]  0.062 0.044 0.060 0.049 0.047 0.065 0.052 0.054 0.048 0.033 0.042 0.065 0.052 0.028 0.048 0.049 0.050 0.041 0.044 0.046
[22]  0.049 0.015 0.048 0.004 0.014 0.031 0.044 0.040 0.041 0.035 0.018 0.032 0.042 0.033 0.034 0.004 0.027 0.016 0.052 0.038 0.050
[23]  0.048 0.015 0.047 0.018 0.016 0.030 0.044 0.040 0.039 0.031 0.011 0.032 0.041 0.028 0.034 0.018 0.015 0.011 0.045 0.039 0.042 0.017
[24]  0.050 0.017 0.048 0.020 0.020 0.034 0.042 0.041 0.038 0.033 0.013 0.034 0.040 0.030 0.036 0.019 0.016 0.010 0.044 0.039 0.044 0.019 0.014
[25]  0.061 0.047 0.059 0.051 0.050 0.066 0.054 0.054 0.051 0.031 0.045 0.067 0.054 0.026 0.050 0.051 0.051 0.044 0.004 0.051 0.044 0.053 0.046 0.045
[26]  0.048 0.031 0.045 0.032 0.031 0.020 0.050 0.052 0.051 0.044 0.030 0.024 0.053 0.040 0.041 0.032 0.045 0.027 0.061 0.049 0.057 0.031 0.031 0.030 0.063
[27]  0.039 0.044 0.036 0.044 0.044 0.056 0.046 0.036 0.043 0.045 0.043 0.057 0.044 0.040 0.020 0.044 0.055 0.043 0.057 0.045 0.056 0.047 0.045 0.046 0.058 0.054
[28]  0.052 0.039 0.050 0.041 0.039 0.053 0.030 0.042 0.025 0.037 0.038 0.055 0.025 0.032 0.038 0.041 0.044 0.037 0.048 0.017 0.047 0.040 0.039 0.039 0.048 0.050 0.047
[29]  0.058 0.046 0.054 0.048 0.047 0.052 0.022 0.048 0.038 0.049 0.046 0.055 0.037 0.044 0.047 0.048 0.052 0.045 0.061 0.039 0.064 0.047 0.048 0.048 0.065 0.049 0.054 0.041
[30]  0.049 0.015 0.048 0.005 0.014 0.031 0.044 0.041 0.041 0.035 0.017 0.032 0.043 0.033 0.034 0.004 0.026 0.015 0.051 0.040 0.050 0.004 0.016 0.018 0.052 0.030 0.047 0.041 0.048
[31]  0.046 0.028 0.044 0.031 0.031 0.045 0.036 0.034 0.032 0.020 0.027 0.046 0.035 0.015 0.030 0.032 0.035 0.026 0.030 0.033 0.032 0.033 0.026 0.028 0.031 0.042 0.039 0.034 0.047 0.033
[32]  0.047 0.024 0.045 0.027 0.026 0.033 0.046 0.041 0.040 0.034 0.027 0.036 0.043 0.032 0.036 0.027 0.034 0.025 0.050 0.041 0.048 0.028 0.025 0.026 0.051 0.035 0.045 0.044 0.053 0.029 0.031
[33]  0.044 0.016 0.043 0.015 0.016 0.030 0.040 0.036 0.037 0.031 0.016 0.033 0.038 0.028 0.032 0.014 0.024 0.015 0.046 0.037 0.044 0.015 0.016 0.018 0.047 0.032 0.041 0.038 0.044 0.014 0.027 0.021
[34]  0.048 0.022 0.046 0.020 0.021 0.026 0.049 0.042 0.046 0.039 0.020 0.029 0.046 0.036 0.038 0.021 0.029 0.019 0.055 0.045 0.055 0.021 0.020 0.024 0.056 0.025 0.049 0.045 0.051 0.021 0.036 0.030 0.022
[35]  0.054 0.043 0.052 0.050 0.047 0.061 0.027 0.047 0.030 0.043 0.047 0.063 0.032 0.040 0.045 0.049 0.054 0.046 0.057 0.030 0.057 0.048 0.047 0.046 0.058 0.060 0.052 0.034 0.037 0.049 0.039 0.050 0.043 0.053
[36]  0.047 0.015 0.045 0.017 0.015 0.028 0.041 0.039 0.038 0.029 0.007 0.029 0.039 0.025 0.032 0.017 0.013 0.008 0.043 0.036 0.041 0.016 0.010 0.012 0.044 0.030 0.043 0.036 0.045 0.015 0.025 0.025 0.015 0.018 0.045
[37]  0.045 0.015 0.042 0.004 0.014 0.029 0.040 0.036 0.037 0.031 0.016 0.030 0.039 0.027 0.030 0.004 0.024 0.015 0.044 0.037 0.045 0.004 0.016 0.018 0.045 0.030 0.040 0.038 0.047 0.003 0.026 0.023 0.010 0.020 0.045 0.015
[38]  0.049 0.030 0.047 0.032 0.030 0.007 0.052 0.049 0.052 0.046 0.028 0.011 0.054 0.043 0.044 0.031 0.038 0.028 0.064 0.047 0.063 0.030 0.029 0.033 0.065 0.019 0.055 0.051 0.052 0.030 0.043 0.032 0.029 0.024 0.060 0.026 0.029
[39]  0.051 0.038 0.048 0.042 0.038 0.052 0.031 0.041 0.025 0.037 0.038 0.053 0.026 0.031 0.038 0.042 0.044 0.037 0.050 0.017 0.046 0.040 0.039 0.038 0.050 0.049 0.047 0.005 0.043 0.041 0.033 0.042 0.037 0.044 0.035 0.035 0.038 0.050
[40]  0.026 0.046 0.024 0.047 0.047 0.052 0.047 0.036 0.046 0.047 0.048 0.055 0.049 0.045 0.029 0.047 0.059 0.046 0.062 0.045 0.063 0.048 0.049 0.050 0.062 0.052 0.037 0.050 0.052 0.048 0.044 0.047 0.044 0.051 0.055 0.047 0.042 0.051 0.049
[41]  0.051 0.046 0.048 0.050 0.048 0.056 0.041 0.045 0.038 0.044 0.048 0.057 0.040 0.045 0.043 0.049 0.054 0.045 0.063 0.037 0.062 0.049 0.049 0.049 0.063 0.053 0.050 0.040 0.048 0.050 0.045 0.047 0.046 0.053 0.043 0.047 0.046 0.054 0.040 0.049
[42]  0.050 0.017 0.048 0.006 0.016 0.033 0.047 0.045 0.043 0.037 0.019 0.034 0.044 0.033 0.036 0.006 0.027 0.016 0.050 0.042 0.050 0.005 0.018 0.019 0.051 0.032 0.046 0.043 0.050 0.005 0.032 0.028 0.014 0.022 0.050 0.017 0.005 0.032 0.044 0.049 0.051
[43]  0.044 0.014 0.042 0.006 0.014 0.029 0.040 0.036 0.037 0.030 0.016 0.029 0.039 0.027 0.031 0.006 0.024 0.014 0.045 0.035 0.044 0.004 0.015 0.018 0.046 0.029 0.040 0.037 0.047 0.005 0.026 0.022 0.012 0.019 0.044 0.014 0.005 0.028 0.037 0.043 0.045 0.007
[44]  0.005 0.043 0.003 0.046 0.046 0.049 0.049 0.036 0.047 0.042 0.046 0.051 0.048 0.043 0.029 0.047 0.053 0.045 0.059 0.045 0.060 0.048 0.047 0.048 0.059 0.047 0.037 0.050 0.055 0.048 0.044 0.045 0.043 0.047 0.053 0.045 0.042 0.048 0.049 0.026 0.049 0.049 0.042
[45]  0.026 0.045 0.024 0.047 0.047 0.053 0.045 0.036 0.046 0.047 0.047 0.055 0.048 0.045 0.029 0.047 0.058 0.046 0.062 0.045 0.062 0.048 0.048 0.049 0.061 0.053 0.036 0.049 0.051 0.048 0.044 0.047 0.044 0.051 0.055 0.046 0.042 0.052 0.047 0.006 0.049 0.049 0.043 0.025
[46]  0.026 0.045 0.023 0.046 0.046 0.053 0.046 0.036 0.045 0.045 0.047 0.055 0.045 0.043 0.028 0.047 0.058 0.046 0.060 0.043 0.061 0.047 0.049 0.049 0.060 0.053 0.036 0.046 0.051 0.048 0.042 0.047 0.043 0.051 0.053 0.046 0.041 0.052 0.045 0.008 0.047 0.049 0.042 0.025 0.007
[47]  0.043 0.013 0.041 0.015 0.015 0.028 0.038 0.035 0.036 0.028 0.002 0.029 0.037 0.025 0.030 0.014 0.014 0.010 0.042 0.035 0.040 0.014 0.009 0.012 0.043 0.025 0.040 0.035 0.044 0.013 0.024 0.022 0.012 0.018 0.043 0.007 0.013 0.026 0.035 0.043 0.044 0.015 0.012 0.041 0.043 0.043
[48]  0.045 0.016 0.043 0.017 0.016 0.029 0.041 0.037 0.038 0.029 0.002 0.031 0.039 0.026 0.030 0.016 0.014 0.011 0.044 0.037 0.042 0.016 0.010 0.012 0.045 0.027 0.041 0.037 0.045 0.015 0.026 0.024 0.014 0.019 0.045 0.007 0.013 0.028 0.037 0.045 0.045 0.017 0.012 0.043 0.045 0.045 0.001
[49]  0.044 0.011 0.042 0.016 0.016 0.030 0.038 0.037 0.036 0.029 0.006 0.030 0.037 0.026 0.031 0.015 0.018 0.013 0.044 0.035 0.041 0.015 0.011 0.014 0.045 0.028 0.041 0.037 0.045 0.014 0.026 0.024 0.013 0.020 0.043 0.011 0.013 0.028 0.037 0.045 0.047 0.016 0.012 0.042 0.045 0.044 0.004 0.007
[50]  0.048 0.015 0.046 0.016 0.001 0.031 0.042 0.040 0.038 0.034 0.015 0.034 0.041 0.030 0.034 0.016 0.021 0.014 0.049 0.039 0.047 0.015 0.016 0.020 0.050 0.031 0.044 0.038 0.047 0.015 0.031 0.026 0.016 0.022 0.047 0.014 0.015 0.031 0.037 0.047 0.048 0.017 0.015 0.046 0.047 0.046 0.015 0.015 0.017
[51]  0.059 0.046 0.057 0.050 0.049 0.065 0.052 0.053 0.048 0.029 0.045 0.065 0.052 0.027 0.048 0.050 0.053 0.041 0.038 0.048 0.017 0.052 0.045 0.044 0.039 0.062 0.056 0.048 0.064 0.052 0.031 0.051 0.045 0.054 0.056 0.043 0.046 0.063 0.048 0.062 0.061 0.052 0.045 0.057 0.061 0.060 0.041 0.043 0.044 0.048
[52]  0.050 0.039 0.048 0.045 0.043 0.053 0.007 0.041 0.027 0.041 0.042 0.055 0.027 0.036 0.039 0.045 0.049 0.040 0.051 0.028 0.051 0.044 0.043 0.041 0.055 0.050 0.047 0.029 0.023 0.045 0.036 0.047 0.040 0.049 0.031 0.040 0.041 0.051 0.030 0.046 0.042 0.047 0.041 0.049 0.045 0.045 0.037 0.040 0.038 0.043 0.052
[53]  0.067 0.057 0.064 0.062 0.062 0.071 0.056 0.059 0.051 0.056 0.059 0.074 0.055 0.053 0.055 0.062 0.071 0.059 0.068 0.050 0.071 0.064 0.059 0.057 0.069 0.075 0.065 0.054 0.062 0.065 0.052 0.064 0.057 0.067 0.060 0.061 0.058 0.071 0.054 0.062 0.063 0.064 0.059 0.066 0.062 0.061 0.055 0.058 0.056 0.062 0.072 0.055
[54]  0.073 0.064 0.070 0.067 0.068 0.078 0.059 0.067 0.054 0.061 0.067 0.082 0.059 0.059 0.063 0.068 0.084 0.063 0.070 0.059 0.073 0.072 0.066 0.060 0.072 0.086 0.070 0.060 0.067 0.071 0.056 0.068 0.062 0.074 0.065 0.068 0.064 0.077 0.060 0.074 0.068 0.070 0.065 0.071 0.073 0.072 0.059 0.064 0.062 0.067 0.079 0.059 0.017
[55]  0.071 0.062 0.068 0.065 0.065 0.075 0.062 0.065 0.054 0.061 0.063 0.078 0.058 0.059 0.062 0.066 0.077 0.061 0.071 0.059 0.071 0.069 0.063 0.059 0.073 0.081 0.071 0.060 0.068 0.069 0.056 0.065 0.059 0.069 0.063 0.064 0.062 0.074 0.059 0.070 0.066 0.068 0.063 0.069 0.070 0.069 0.058 0.062 0.060 0.065 0.075 0.061 0.016 0.015
[56]  0.127 0.114 0.126 0.111 0.114 0.121 0.115 0.115 0.117 0.114 0.113 0.123 0.114 0.114 0.113 0.114 0.117 0.112 0.135 0.115 0.132 0.112 0.114 0.116 0.136 0.123 0.124 0.118 0.121 0.113 0.113 0.119 0.110 0.112 0.118 0.112 0.111 0.121 0.118 0.120 0.118 0.115 0.112 0.126 0.120 0.117 0.112 0.112 0.115 0.115 0.128 0.115 0.123 0.122 0.122
```

[1], P. amabilis; [2], P. amboinensis; [3], P. aphrodite; [4], P. bastianii; [5], P. bellina; [6], P. borneensis; [7], P. braceana; [8], P. celebensis; [9], P. chibae; [10], P. cochlearis; [11], P. corningiana; [12], P. cornu-cervi; [13], P. deliciosa; [14], P. doweryensis; [15], P. equestris; [16], P. fasciata; [17], P. fimbriata; [18], P. floresensis; [19], P. fuscata; [20], P. gibbosa; [21], P. gigantea; [22], P. hieroglyphica; [23], P. inscriptiosinensis; [24], P. javanica; [25], P. kunstleri; [26], P. lamelligera; [27], P. lindenii; [28], P. lobbii; [29], P._lowii; [30], P. lueddemanniana; [31], P. maculata; [32], P. mannii; [33], P. mariae; [34], P. micholitzii; [35], P. minus; [36], P. modesta; [37], P. pallens; [38], P. parishii; [39], P. patherina; [40], P. philippinensis; [41], P. pulcherrima; [42], P. pulchra; [43], P._reichenbachiana; [44], P. sanderiana; [45], P. schilleriana; [46], P. stuartiana; [47], P. sumatrana; [48], P. tetraspis; [49], P._venosa; [50], P. violacea; [51], P. viridis; [52], P. wilsonii; [53], Par. labukensis; [54], Par. laycockii; [55], Par. serpentilingua; [56], T. kotoense.

Table 14. Genetic distances of the two-parameter method of Kimura derived from combined data of sequences of the internal transcribed spacer 1 (ITS1), ITS2, the *trn*L intron, the *trn*L-*trn*F intergenic spacer (IGS), and the *atp*B-*rbc*L IGS among six subgenera/sections of the four-pollinium *Phalaenopsis* divided according to the suggestions of the phylogenetic tree of this study.

	Subgenus *Aphyllae*	Section *Deliciosae*	Subgenus *Parishianae*	Subgenus *Proboscidioides*	Section *Conspicuum*	Section *Esmeralda*
Subgenus *Aphyllae*	-	0.027	0.031	0.023	0.025	0.041
Section *Deliciosae*	0.027	-	0.024	0.037	0.027	0.038
Subgenus *Parishianae*	0.031	0.024	-	0.041	0.029	0.039
Subgenus *Proboscidioides*	0.023	0.037	0.041	-	0.037	0.047
Section *Conspicuum*	0.025	0.027	0.029	0.037	-	0.041
Section *Esmeralda*	0.041	0.038	0.039	0.047	0.041	-
Sum	**0.147** [a]	0.153	0.164	0.185	0.159	0.206

[a] The value in boldface represents the smallest one.

Table 15. Tajima's neutrality tests of data of sequences of both ITS and chloroplast DNA obtained from the genus *Phalaenopsis* plus the outgroups of this study.

	Tajima's neutrality test							
	No. of sites	No. of segregated sites (S)	Segregated sites per total sites (pS)	Nucleotide diversity (π)	Theta (θ)	Difference	D	Significant difference between D and 0
ITS data	417	251	0.601918	0.099297	0.130526	-13.022865± 15.261857	-0.853295	—
cpDNA data	1243	284	0.228479	0.020427	0.049739	-36.434724± 17.274404	-2.109174	—

Table 16. Tajima's relative rate test for the ITS data set between species of the sections *Amboinenses* and *Zebrinae* distributed on the Sunda Shelf and species of the *Phalaenopsis lueddemanniana* complex, with *P. lobbii* (subgenus *Parishianae*) as the reference group.

	P. bellina	*P. corningiana*	*P. fimbriata*	*P. inscriptiosinensis*	*P. javanica*	*P. modesta*	*P. sumatrana*	*P. tetraspis*	*P. violacea*
P. mariae	0.18 (0.670)	0.00 (1.000)	1.00 (0.317)	0.22 (0.637)	0.93 (0.336)	0.60 (0.439)	0.00 (1.000)	0.29 (0.593)	0.18 (0.670)
P. pallens	0.39 (0.532)	0.05 (0.819)	1.32 (0.251)	0.43 (0.513)	0.57 (0.450)	0.89 (0.346)	0.05 (0.819)	0.53 (0.467)	0.39 (0.532)
P. hieroglyphica	0.73 (0.394)	0.00 (1.000)	0.80 (0.371)	0.18 (0.670)	0.86 (0.353)	0.22 (0.637)	0.05 (0.819)	0.06 (0.808)	0.73 (0.394)
P. fasciata	0.04 (0.841)	0.43 (0.513)	2.33 (0.127)	1.09 (0.297)	0.13 (0.715)	1.80 (0.180)	0.43 (0.513)	1.32 (0.251)	0.04 (0.841)
P. lueddemanniana	0.39 (0.532)	0.05 (0.819)	1.32 (0.251)	0.43 (0.513)	0.57 (0.450)	0.89 (0.346)	0.05 (0.819)	0.53 (0.467)	0.39 (0.532)
P. bastianii	0.17 (0.683)	0.73 (0.394)	2.91 (0.088)	2.13 (0.144)	0.00 (1.000)	2.33 (0.127)	0.73 (0.394)	1.80 (0.180)	0.17 (0.683)
P. pulchra	0.00 (1.000)	0.73 (0.394)	3.20 (0.074)	1.50 (0.221)	0.03 (0.853)	2.33 (0.127)	0.73 (0.394)	1.80 (0.180)	0.00 (1.000)
P. reichenbachiana	0.39 (0.532)	0.05 (0.819)	1.32 (0.251)	0.47 (0.491)	0.57 (0.450)	0.89 (0.346)	0.05 (0.819)	0.53 (0.467)	0.39 (0.532)

Note: Chi-square values were obtained from Tajima's relative rate test (Tajima, 1993). *p* values of the statistical significance test are shown in parentheses. For each cell, *p* values in bold are statistically significantly differ ($p < 0.05$).

Table 17. Tajima's relative rate test for the chloroplast DNA data set between species of the sections *Amboinenses* and *Zebrinae* distributed on the Sunda Shelf and species of the *Phalaenopsis lueddemanniana* complex, with *P. lobbii* (subgenus *Parishianae*) as the reference group.

	P. bellina	*P. corningiana*	*P. fimbriata*	*P. inscriptiosinensis*	*P. javanica*	*P. modesta*	*P. sumatrana*	*P. tetraspis*	*P. violacea*
P. mariae	5.40 (**0.020**)	0.82 (0.366)	6.55 (**0.011**)	0.33 (0.564)	0.33 (0.564)	0.67 (0.414)	0.33 (0.564)	2.78 (0.096)	5.40 (**0.020**)
P. pallens	5.40 (**0.020**)	0.82 (0.366)	6.55 (**0.011**)	0.33 (0.564)	0.33 (0.564)	0.67 (0.414)	0.33 (0.564)	2.78 (0.096)	5.40 (**0.020**)
P. hieroglyphica	5.40 (**0.020**)	0.82 (0.366)	6.55 (**0.011**)	0.33 (0.564)	0.33 (0.564)	0.67 (0.414)	0.33 (0.564)	2.78 (0.096)	5.40 (**0.020**)
P. fasciata	4.76 (**0.029**)	0.69 (0.405)	6.00 (**0.014**)	0.29 (0.593)	0.29 (0.593)	0.50 (0.480)	0.29 (0.593)	2.27 (0.132)	4.76 (**0.029**)
P. lueddemanniana	5.40 (**0.020**)	0.82 (0.366)	6.55 (**0.011**)	0.33 (0.564)	0.33 (0.564)	0.67 (0.414)	0.33 (0.564)	2.78 (0.096)	5.40 (**0.020**)
P. bastianii	4.00 (**0.046**)	1.33 (0.248)	5.26 (**0.022**)	0.69 (0.405)	0.69 (0.405)	1.29 (0.257)	0.69 (0.405)	3.60 (0.058)	4.00 (**0.046**)
P. pulchra	2.88 (0.090)	1.92 (1.666)	4.17 (**0.041**)	1.14 (0.285)	1.14 (0.285)	2.00 (0.157)	1.14 (0.285)	4.45 (**0.035**)	2.88 (0.090)
P. reichenbachiana	3.27 (0.071)	2.27 (0.132)	3.20 (0.074)	1.33 (0.248)	1.33 (0.248)	2.67 (0.102)	1.33 (0.248)	5.44 (**0.020**)	3.27 (0.071)

Note: Chi-square values were obtained from Tajima's relative rate test (Tajima, 1993). *p* values of the statistical significance test are shown in parentheses. For each cell, *p* values in bold statistically significantly differ ($p < 0.05$)

Table 18. Comparisons of internal transcribed spacer sequences of sections *Amboinenses* (with the exception of the *P. lueddemannina* complex) and *Zebrinae* distributed on the Sunda Shelf with species of the *P. lueddemanniana* complex distributed in the Philippines used to deduce the substitution rate of the genus *Phalaenopsis* between them based on the geological events of the combination of the Philippines and Borneo (5~10 Mya).

	Sections *Amboinenses* and *Zebrinae* [a]	Substitution rate (substitutions/site/year)
	No. of differences	
P. mariae	19.67	$2.1\sim4.2 \times 10^{-9}$
P. pallens	21.78	$2.3\sim4.6 \times 10^{-9}$
P. hieroglyphica	21.56	$2.3\sim4.6 \times 10^{-9}$
P. fasciata	23.00	$2.4\sim4.9 \times 10^{-9}$
P. lueddemanniana	21.00	$2.2\sim4.5 \times 10^{-9}$
P. bastianii	24.56	$2.6\sim5.2 \times 10^{-9}$
P. pulchra	23.78	$2.5\sim5.0 \times 10^{-9}$
P. reichenbachiana	21.89	$2.3\sim4.6 \times 10^{-9}$
Average	22.16	$2.4\sim4.7 \times 10^{-9}$

[a] Species of the sections *Amboinenses* and *Zebrinae* distributed on the Sunda Shelf and not reject by Tajima's relative rate test. These species include *P. bellina*, *P. corningiana*, *P. fimbriata*, *P. inscriptiosinensis*, *P. javanica*, *P. modesta*, *P. sumatrana*, *P. tetraspis*, and *P. violacea*.

Table 19. Comparisons of chloroplast DNA data sets of the group of the sections *Amboinenses* (with exception of the *Phalaenopsis lueddemannina* complex) and *Zebrinae* with that of species of the *P. lueddemanniana* complex to deduce the substitution rate of the genus *Phalaenopsis* between them based on the geological events of the combination of the Philippines and Borneo (5~10 Mya).

	Sections *Amboinenses* and *Zebrinae* [a]	Substitution rate (substitutions/site/year)
	No. of differences [b]	
P. mariae	11.8	$3.7\sim7.5 \times 10^{-10}$
P. pallens	11.8	$3.7\sim7.5 \times 10^{-10}$
P. hieroglyphica	11.8	$3.7\sim7.5 \times 10^{-10}$
P. fasciata	12.8	$4.1\sim8.1 \times 10^{-10}$
P. lueddemanniana	11.8	$3.7\sim7.5 \times 10^{-10}$
P. bastianii	12.8	$4.1\sim8.1 \times 10^{-10}$
P. pulchra	13.8	$4.4\sim8.8 \times 10^{-10}$
P. reichenbachiana	11.6	$3.7\sim7.4 \times 10^{-10}$
Average	12.3	$3.9\sim7.8 \times 10^{-10}$

[a] Species of the sections *Amboinenses* and *Zebrinae* distributed on the Sunda Shelf and not rejected by Tajima's relative rate test. These species included *P. corningiana*, *P. inscriptiosinensis*, *P. javanica*, *P. modesta*, and *P. sumatrana*.
[b] No. of sites = 1574.

Table 20. Tajima's relative rate test for the internal transcribed spacer data set among species of the *Phalaenopsis lueddemanniana* complex, with *P. lobbii* (subgenus *Parishianae*) as the reference group.

	P. mariae	*P. pallens*	*P. hieroglyphica*	*P. fasciata*	*P. lueddemanniana*	*P. bastianii*	*P. pulchra*
P. mariae							
P. pallens	0.07 (0.796)						
P. hieroglyphica	0.07 (0.796)	2.00 (0.157)					
P. fasciata	0.53 (0.467)	1.00 (0.317)	4.00 (**0.046**)				
P. lueddemanniana	0.07 (0.796)9	0.00 (1.000)	2.00 (0.157)	1.00 (0.317)			
P. bastianii	0.89 (0.346)	1.80 (0.180)	5.00 (**0.025**)	0.14 (0.705)	1.80 (0.180)		
P. pulchra	0.89 (0.346)	1.80 (0.180)	5.00 (**0.025**)	0.14 (0.705)	1.80 (0.180)	0.00 (1.000)	
P. reichenbachiana	0.07 (0.796)	0.00 (1.000)	2.00 (0.157)	1.00 (0.317)	0.00 (1.000)	1.80 (0.180)	1.80 (0.180)

Note: Below the diagonal are Chi-square values obtained from Tajima's relative rate test (Tajima, 1993). *p* values of the statistical significance test are shown in parentheses. For each cell, *p* values in bold are statistically significant differ ($p < 0.05$).

Table 21. Tajima's relative rate test for the chloroplast DNA data set among species of the *Phalaenopsis lueddemanniana* complex, *P. lobbii* (subgenus *Parishianae*) as the reference group.

	P. mariae	*P. pallens*	*P. hieroglyphica*	*P. fasciata*	*P. lueddemanniana*	*P. bastianii*	*P. pulchra*
P. pallens	0.00 (1.000)						
P. hieroglyphica	0.00 (1.000)	ND					
P. fasciata	0.00 (1.000)	0.00 (1.000)	0.00 (1.000)				
P. lueddemanniana	0.00 (1.000)	ND	ND	0.00 (1.000)			
P. bastianii	0.20 (0.655)	1.00 (0.317)	1.00 (0.317)	0.33 (0.564)	1.00 (0.317)		
P. pulchra	1.00 (0.317)	2.00 (0.157)	2.00 (0.157)	1.00 (0.317)	2.00 (0.157)	0.33 (0.564)	
P. reichenbachiana	0.50 (0.480)	1.00 (0.317)	1.00 (0.317)	0.67 (0.414)	1.00 (0.317)	0.20 (0.655)	0.00 (1.000)

Note: Below the diagonal: Chi-square values were obtained from Tajima's relative rate test (Tajima, 1993). *p* values of the statistical significance test are shown in parentheses. For each cell, *p* values in bold are statistically significantly ($p < 0.05$). ND = not determined.

Table 22. Number of differences of the internal transcribed spacer data set among species of the *Phalaenopsis lueddemanniana* complex with the exception of *P. hieroglyphica*.

	P. mariae	*P. pallens*	*P. fasciata*	*P. lueddemanniana*	*P. bastianii*	*P. pulchra*	*P. reichenbachiana*
P. mariae		4.05	4.16	4.05	4.38	4.27	4.05
P. pallens	17.00		2.43	2.22	2.43	2.63	2.22
P. fasciata	18.00	6.00		2.22	2.80	2.63	2.22
P. lueddemanniana	17.00	5.00	5.00		2.63	2.43	1.99
P. bastianii	20.00	6.00	8.00	7.00		2.97	2.63
P. pulchra	19.00	7.00	7.00	6.00	9.00		2.43
P. reichenbachiana	17.00	5.00	5.00	4.00	7.00	6.00	

Note: Below the diagonal are the numbers of differences. Above the diagonal are standard errors of the number of differences. Number of sites = 475.

Table 23. Number of differences of the chloroplast DNA data among species of the *Phalaenopsis lueddemanniana* complex.

	P. mariae	*P. pallens*	*P. hieroglyphica*	*P. fasciata*	*P. lueddemanniana*	*P. bastianii*	*P. pulchra*	*P. reichenbachiana*
P. mariae		1.91	1.91	2.19	1.91	1.95	2.02	3.06
P. pallens	4.00		0.00	1.52	0.00	0.97	1.31	2.28
P. hieroglyphica	4.00	0.00		1.52	0.00	0.97	1.31	2.28
P. fasciata	6.00	2.00	2.00		1.52	1.86	1.98	2.68
P. lueddemanniana	4.00	0.00	0.00	2.00		0.97	1.31	2.28
P. bastianii	5.00	1.00	1.00	3.00	1.00		1.57	2.45
P. pulchra	4.00	2.00	2.00	4.00	2.00	3.00		2.63
P. reichenbachiana	9.00	5.00	5.00	7.00	5.00	6.00	7.00	

Note: Below the diagonal are the numbers of differences. Above the diagonal are standard errors of the number of differences. Number of sites = 1614.

Table 24. Putative divergence times among species of the *Phalaenopsis lueddemanniana* complex with the exception of *P. hieroglyphica* calculated by the substitution rate of 2.4~4.7 x 10^{-9} substitutions/site/year of the internal transcribed spacer sequences of the genus *Phalaenopsis* obtained from this study.

	P. mariae	*P. pallens*	*P. fasciata*	*P. lueddemanniana*	*P. bastianii*	*P. pulchra*
P. pallens	7.5~3.8					
P. fasciata	7.9~4.0	2.6~1.3				
P. lueddemanniana	7.5~3.8	2.2~1.1	2.2~1.1			
P. bastianii	8.8~4.5	2.6~1.3	3.5~1.8	3.1~1.6		
P. pulchra	8.3~4.3	3.1~1.6	3.1~1.6	2.6~1.3	3.9~2.0	
P. reichenbachiana	7.5~3.8	2.2~1.1	2.2~1.1	1.8~0.90	3.1~1.6	2.6~1.3

Units: millions of years (my).

Table 25. Divergence times among species of the *Phalaenopsis lueddemanniana* complex calculated by the substitution rate of 3.9~7.8 x 10^{-10} substitutions/site/year of chloroplast DNA of the genus *Phalaenopsis* obtained from this study.

	P. mariae	*P. pallens*	*P. hieroglyphica*	*P. fasciata*	*P. lueddemanniana*	*P. bastianii*	*P. pulchra*
P. pallens	3.2~1.6						
P. hieroglyphica	3.2~1.6	< 0.40					
P. fasciata	4.8~2.4	1.6~0.79	1.6~0.79				
P. lueddemanniana	3.2~1.6	< 0.40	< 0.40	1.6~0.79			
P. bastianii	4.0~1.9	0.79~0.40	0.79~0.40	2.4~1.2	0.79~0.40		
P. pulchra	3.2~1.6	1.6~0.79	1.6~0.79	3.2~1.6	1.6~0.79	2.4~1.2	
P. reichenbachiana	7.1~3.6	4.0~1.9	4.0~1.9	5.6~2.8	4.0~1.9	4.8~2.4	5.6~2.8

Units: millions of years (my).

Table 26. Tajima's relative test between the section *Stauroglottis* and the *Phalaenopsis lueddemanniana* complex, with *P. lobii* (subgenus *Parishianae*) as the reference group.

	Internal transcribed spacer data set			Chloroplast DNA data set		
	P. celebensis	*P. equestris*	*P. lindenii*	*P. celebensis*	*P. equestris*	*P. lindenii*
P. celebensis						
P. equestris	0.13 (0.724)			0.08 (0.782)		
P. lindenii	0.47 (0.493)	0.40 (0.527)		11.31 (**0.001**)	15.11 (**0.000**)	
P. mariae	0.10 (0.752)	0.27 (0.602)	0.81 (0.369)	0.12 (0.732)	0.14 (0.705)	6.00 (**0.014**)
P. pallens	0.10 (0.752)	0.31 (0.577)	0.86 (0.353)	0.47 (0.493)	0.57 (0.450)	4.74 (**0.029**)
P. hieroglyphica	0.00 (1.000)	0.03 (0.853)	0.31 (0.577)	0.47 (0.493)	0.57 (0.450)	4.74 (**0.029**)
P. fasciata	0.38 (0.537)	0.81 (0.369)	1.58 (0.209)	0.47 (0.493)	0.57 (0.450)	4.92 (**0.027**)
P. lueddemanniana	0.10 (0.752)	0.31 (0.577)	0.86 (0.353)	0.47 (0.493)	0.57 (0.450)	4.74 (**0.029**)
P. bastianii	0.58 (0.446)	1.20 (0.273)	2.13 (0.144)	0.71 (0.398)	0.86 (0.353)	4.09 (**0.043**)
P. pulchra	0.58 (0.446)	1.13 (0.289)	2.00 (0.157)	0.68 (0.411)	0.81 (0.369)	3.95 (**0.047**)
P. reichenbachiana	0.10 (0.752)	0.31 (0.577)	0.86 (0.353)	0.47 (0.493)	0.57 (0.450)	5.45 (**0.020**)

Note: Chi-square values were obtained from Tajima's relative rate test (Tajima, 1993). *p* values of the statistical significance test are shown in parentheses. For each cell, *p* values in bold are statistically significant ($p < 0.05$).

Table 27. Number of differences and divergence times between species of the section *Deliciosae* and the section *Stauroglottis* (*Phalaenopsis lindenii* was excluded from the chloroplast DNA data set) obtained from substitution rates of both the internal transcribed spacer and cpDNA data sets.

	ITS data set	cpDNA data set
No. of differences (S.E.	48.17 (4.90)	26.50 (3.83)
No. of sites (in total)	480	1600
Divergent time (Mya)	20.9 ± 2.1~10.7 ± 1.1	21.2 ± 2.1~10.6 ± 1.5

Notes: The substitution rate of the ITS data set was $2.4\sim4.7 \times 10^{-9}$ substitutions/site/year. The substitution rate of the cpDNA data set was $3.9\sim7.8 \times 10^{-10}$ substitutions/site/year.

Fig. 1.

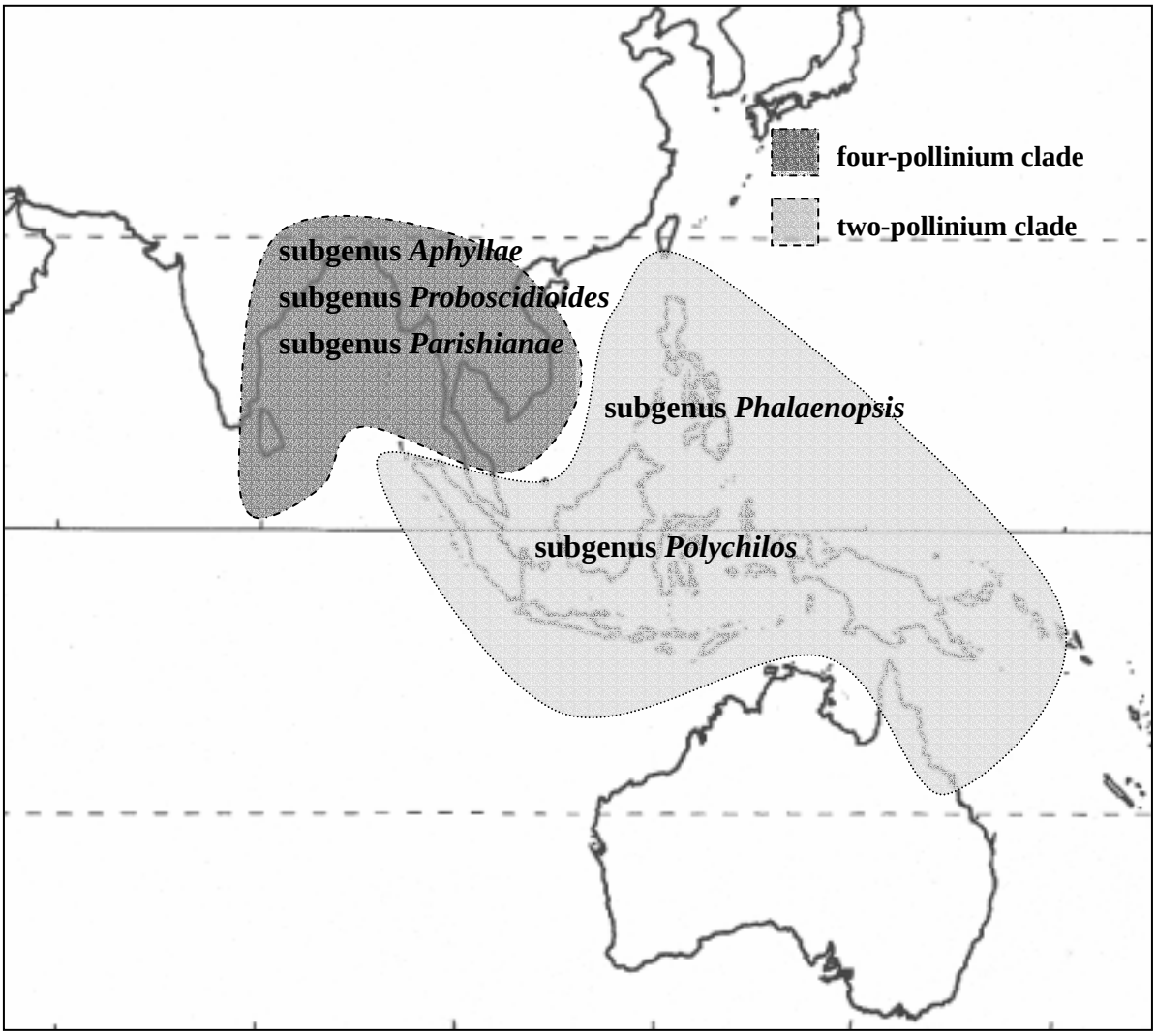


Fig. 1. Correlation between the distribution pattern and pollinia number of different subgenera of *Phalaenopsis*.

Fig. 2.

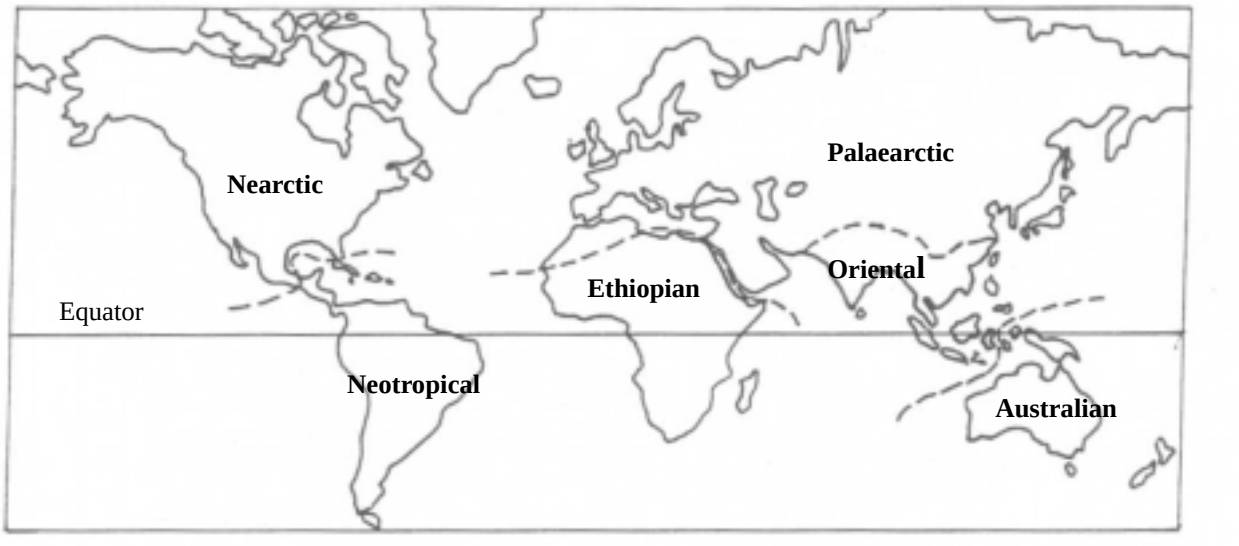


Fig. 2. The six major biogeographic regions of the world (redrawn from Pianke, 1994).

Fig. 3

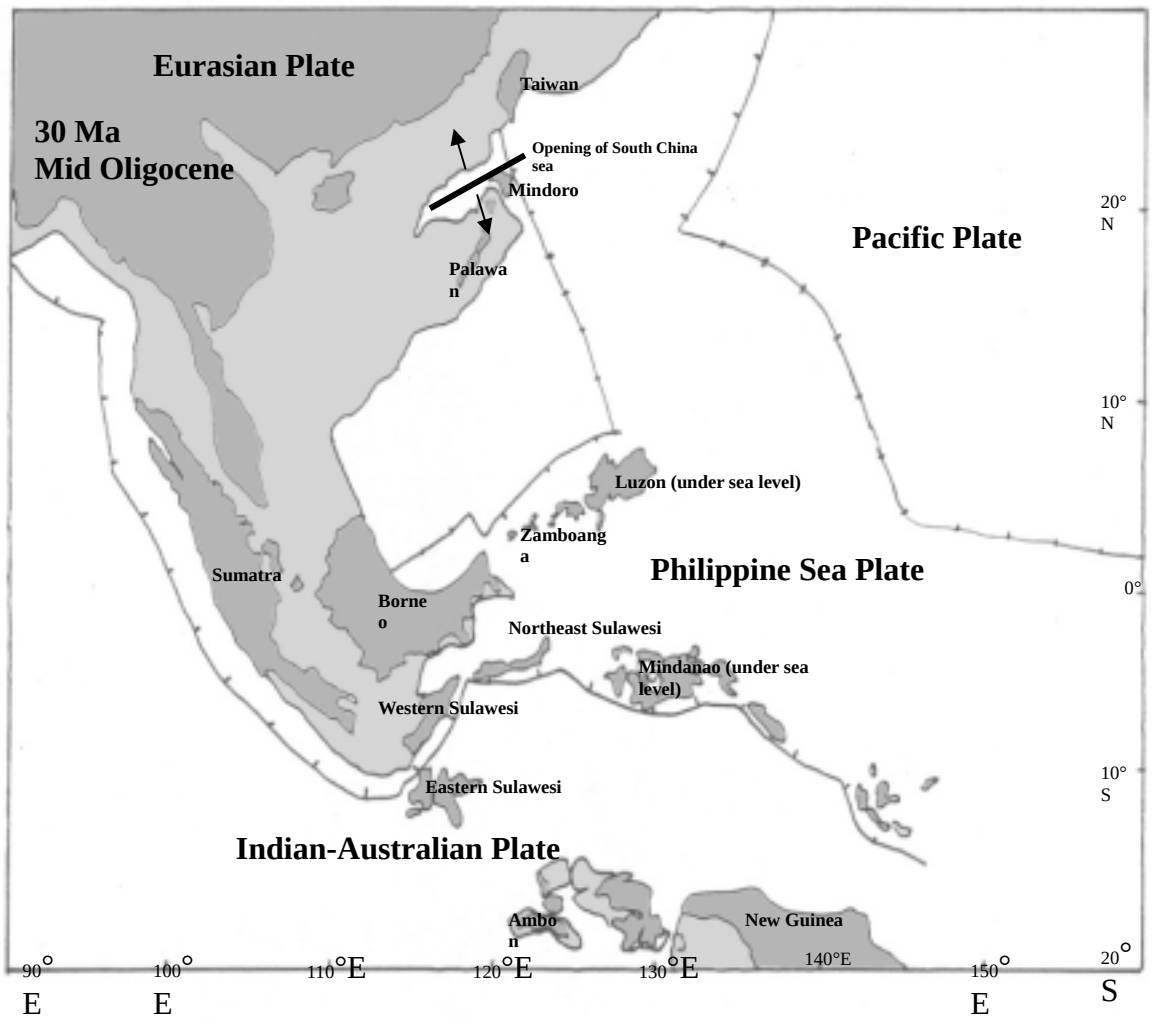


Fig. 3. Putative map of Southeast Asia 30 Mya (modified from Hall, 1996).

Fig. 4.

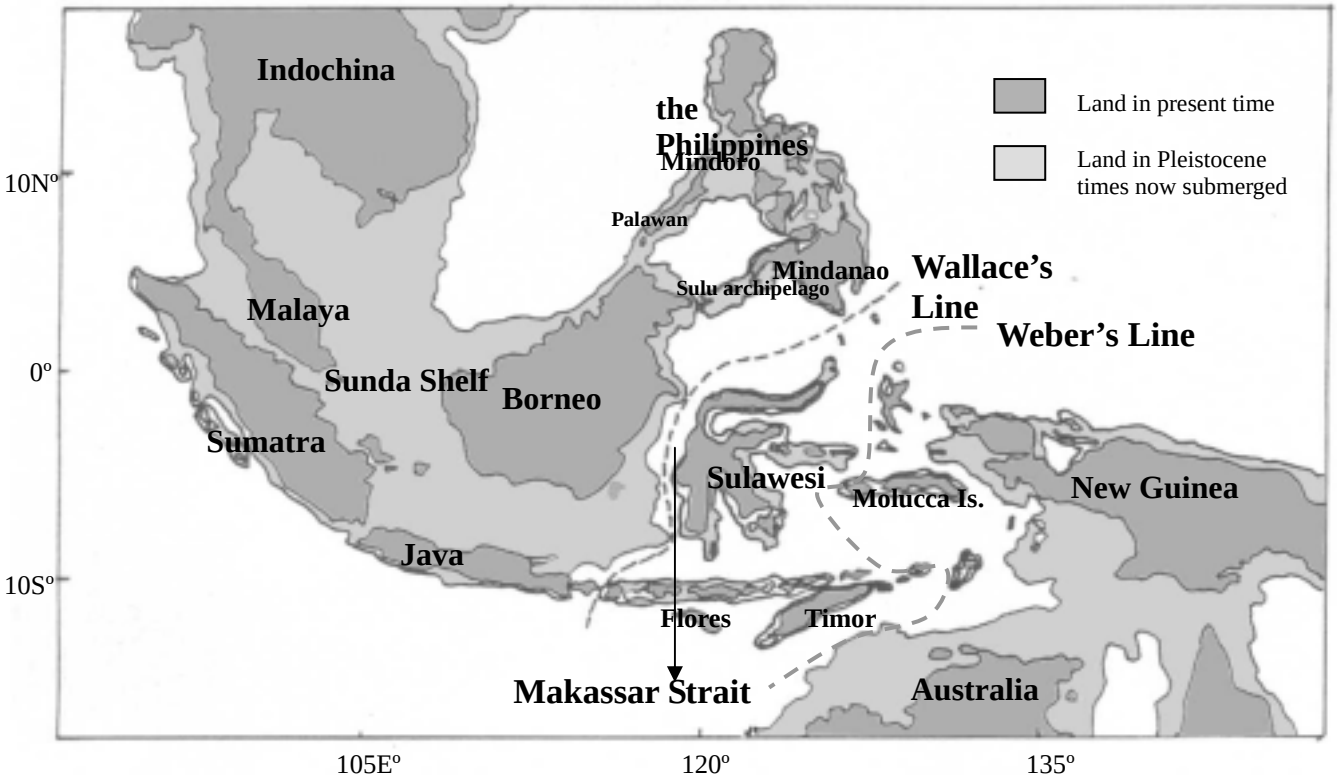
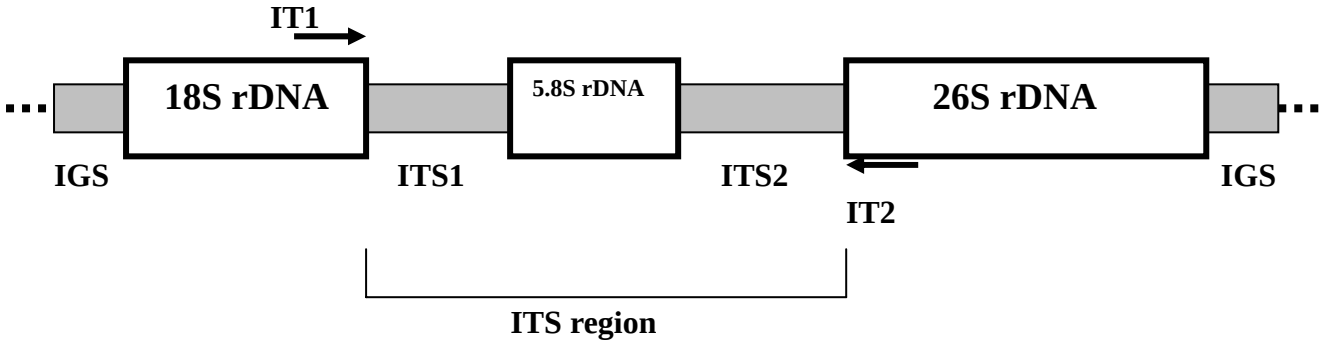


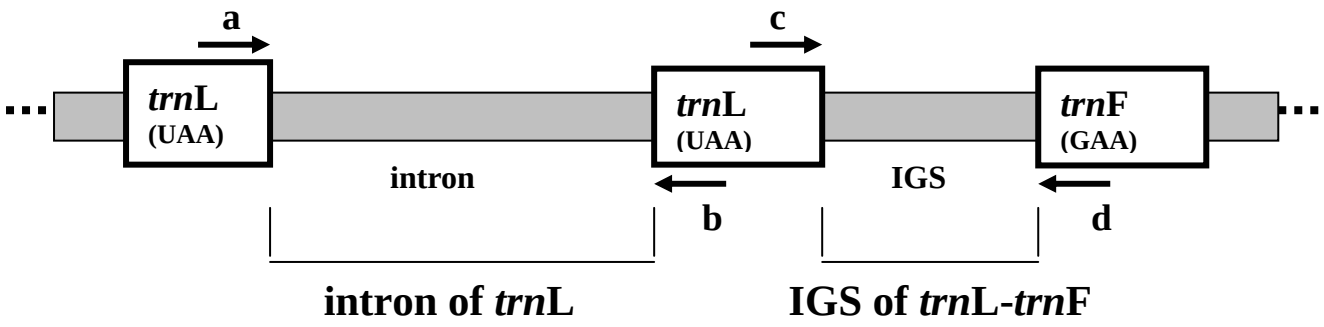
Fig. 4. Comparison of Southeast Asian lands between Pleistocene times and the present time. Indochina, Malaya, Sumatra, Java, Borneo, and the Philippines were interconnected and separated from Sulawesi by the Makassar Strait in Pleistocene times (modified from Van Oosterzee, 1997).

Fig. 5. Localities and sequences of primers for amplifying and sequencing the internal transcribed spacer 1 (ITS1) and ITS2 of nrDNA.



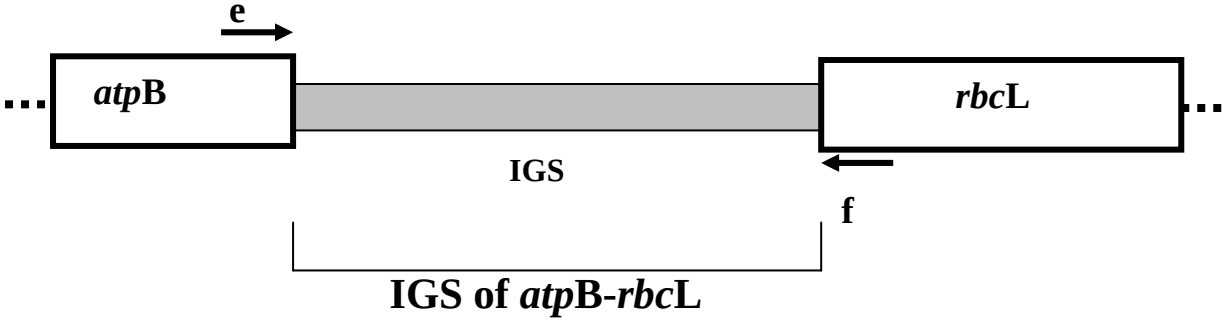
IT1: 5’ AGTCGTAACAAGGTTTCC 3' ; **IT2:** 5’ GTAAGTTTCTTCTCCTCC 3’

Fig. 6. Localities and sequences of primers for amplifying and sequencing the *trn*L intron (UAA) and the intergenic spacer (IGS) of *trn*L (UAA)-*trn*F (GAA).



a: 5’ CGAAATCGGTAGACGCTACG 3’; **b:** 5’ GGGGATAGAGGGACTTGAAC 3’
c: 5’ GGTTCAAGTCCCTCTATCCC 3’; **d:** 5’ ATTTGAACTGGTGACACGAG 3’

Fig. 7. Localities and sequences of primers for amplifying and sequencing the intergenic spacer (IGS) of *atp*B-*rbc*L.



e: 5’ CATCTAGGATTTACATATAC 3' ; **f:** 5’ GTCAATTTGTAATCTTTAAC 3’

Fig. 8

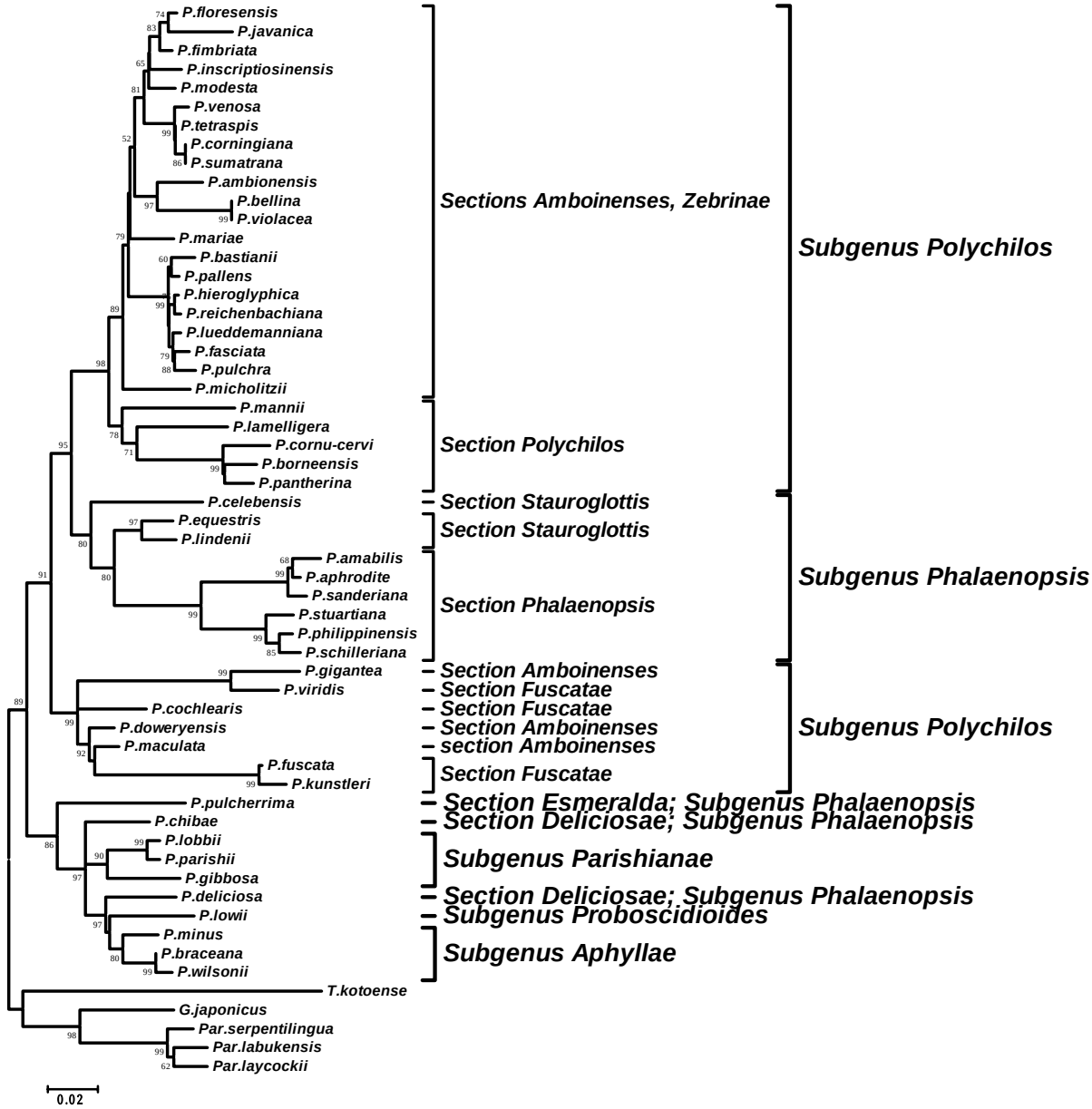


Fig. 8. Neighbor-joining tree of 52 *Phalaenopsis* species plus the five outgroups obtained from internal transcribed spacer 1 (ITS1) and ITS2 sequences. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. More than 50% interior branch test is shown on each branch. Branch lengths are proportional to the number of base changes along each branch.

Fig. 9. Sequence alignment of different lengths of the *atp*B-*rbc*L intergenic spacer of chloroplast DNA from an individual of *Phalaenopsis gibbosa*.

```
                              5          15         25         35         45         55
P._gibbosa-normal_type        TACAACATAT ATTACTGTCA AGAGAGGGGA CCGGGTCCTA TATTCTTTCT TTTTATTTCT
P._gibbosa-short_type         .........G .......... .......... .......... .......... ....C.....

                              65         75         85         95        105        115
P._gibbosa-normal_type        ATATTAGATA TTTCTATTTA CTATTTATTA TCTTTAATAT CTTTATTTTA TAATTGAAAT
P._gibbosa-short_type         .......... .......... .......... .......... .......... ----------

                              125        135        145        155       165        175
P._gibbosa-normal_type        TTCTTCATTC TAGAATTTCT GAATTCTAAT TTAGAATTCT ATTTCTATTC AATTTAATAT
P._gibbosa-short_type         ---------- ---------- ---------- ---------- ---------- ----------

                              185        195        205        215        225        235
P._gibbosa-normal_type        TTATCTATTT GAATTGAATT CTATTTAAAC TAGATTTCTG AATTGAAATG AAATCGAAAT
P._gibbosa-short_type         ---------- ---------- ---------- ---------- ---------- ----------

                              245        255        265        275        285        295
P._gibbosa-normal_type        TTTTCATTTT CTTTGATGTT TTTTTCTCTT TATTTTGATA TTCTTATTTC TTTATTTTTT
P._gibbosa-short_type         ---------- ---------- -----..... .C........ .......... ...T....C-

                              305        315        325        335        345        355
P._gibbosa-normal_type        TATATATTCA TATTCTATTA TCATATTCAT TATTTCATTA TTTATAAAAA AATATTAAGA
P._gibbosa-short_type         ---------- .........- .......... ..-------- .C------... ..........

                              365        375        385        395        405        415
P._gibbosa-normal_type        AGATGATAAA TTCCATTAGG AATAGAAATT TTCAAGAAGA TTGGGTTGCG CCATATATAT
P._gibbosa-short_type         ......G... .......... .......... .......... .......... ..........

                              425        435        445        455        465        475
P._gibbosa-normal_type        CAAAGAATAT AAAATAATGA TGTATTTGGT GAATCAAAGA AATGGTCCAA TAACGAACCC
P._gibbosa-short_type         ......G..G .......... .......... .......... .......... ..........

                              485        495        505        515        525        535
P._gibbosa-normal_type        TTTTCAAAAT TTCATTATTC ATTAGTTGAT AATATTAATT TCTAGTTTAG TTGAATCTTT
P._gibbosa-short_type         .......... .......... .......... .....G.... .......... ..........

                              545        555        565        575        585        595
P._gibbosa-normal_type        TTTGAATTGT AAATATTTTT GTCAAAGGTT TCATTCACGC TTAATTCATA TCGAGTAGAC
P._gibbosa-short_type         .......... C......... .......... .......... .......... ..........

                              605        615        625        635        645        655
P._gibbosa-normal_type        CTTGTTGTTG TGAGAATTCT TAATTCATGA GTTGTAGGGA GGGACTTATG TCACCACAAA
P._gibbosa-short_type         .......... .......... .......... .......... .......... ..........

                              665      675
P._gibbosa-normal_type        CAGAAACCTA AAGCA
P._gibbosa-short_type         .......TC. .GCA.
```

Fig. 10. Sequence alignment of different lengths of the *trn*L intron of chloroplast DNA from an individual of *Phalaenopsis lowii*.

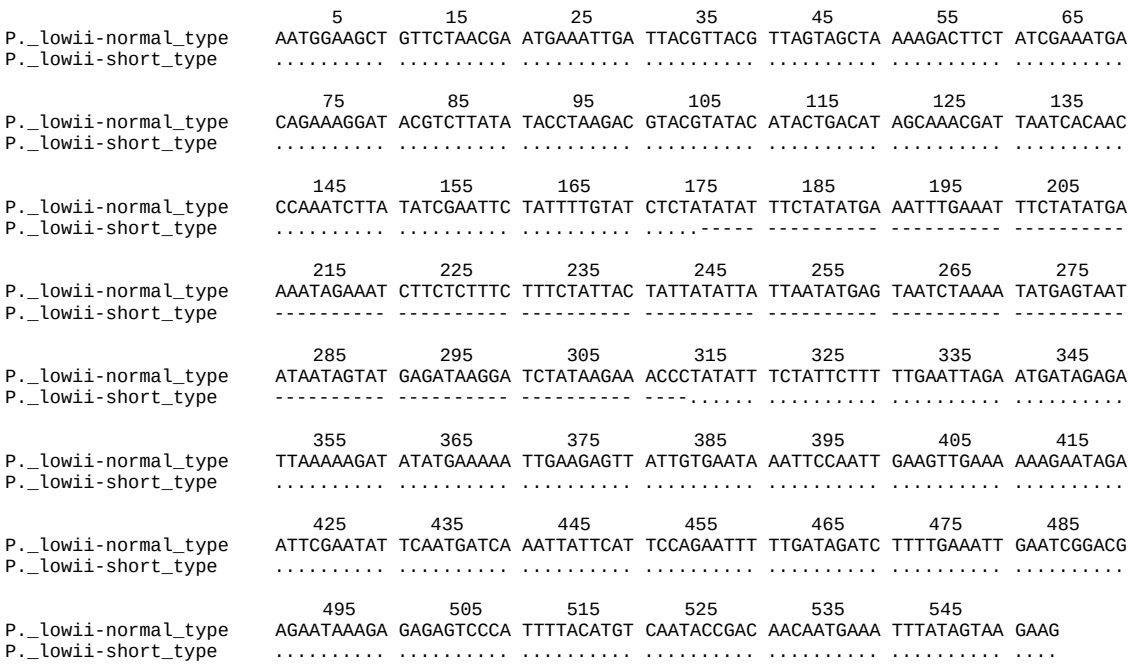


Fig. 11. Sequence alignment of different lengths of the intergenic spacer of *atp*B-*rbc*L of chloroplast DNA from an individual of *Phalaenopsis lowii*.

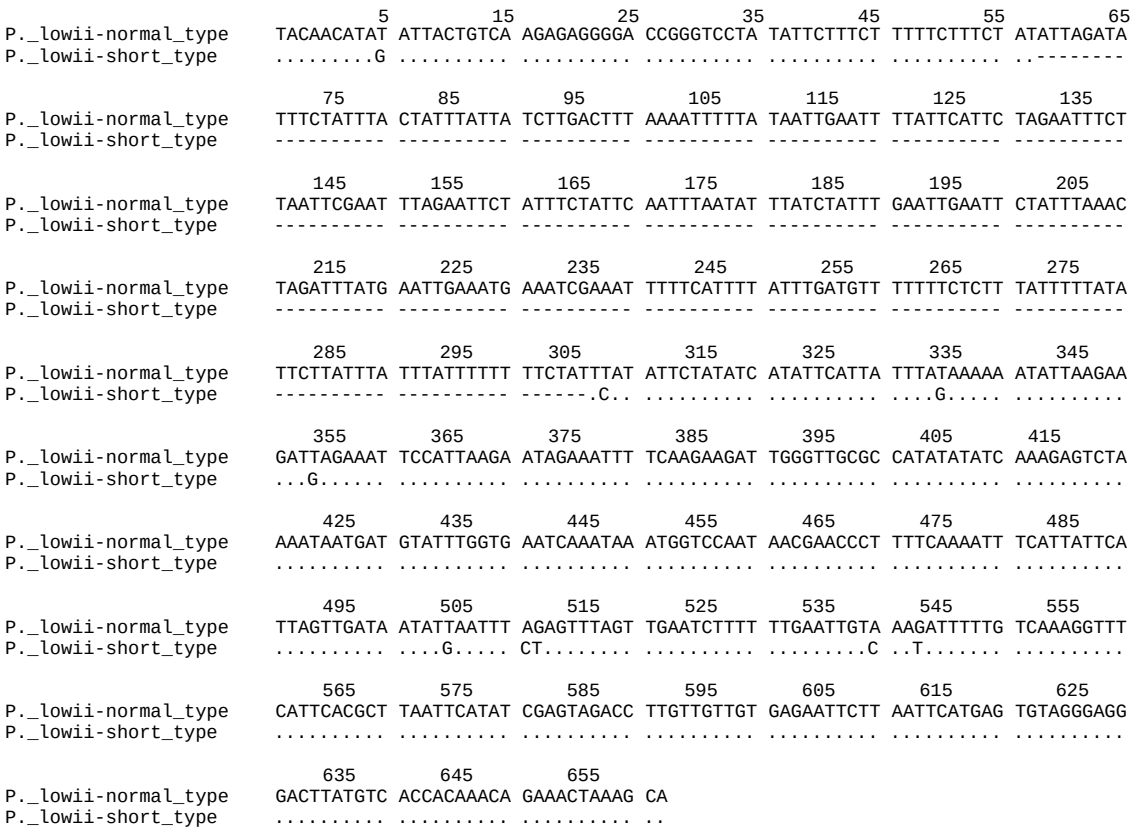


Fig. 12

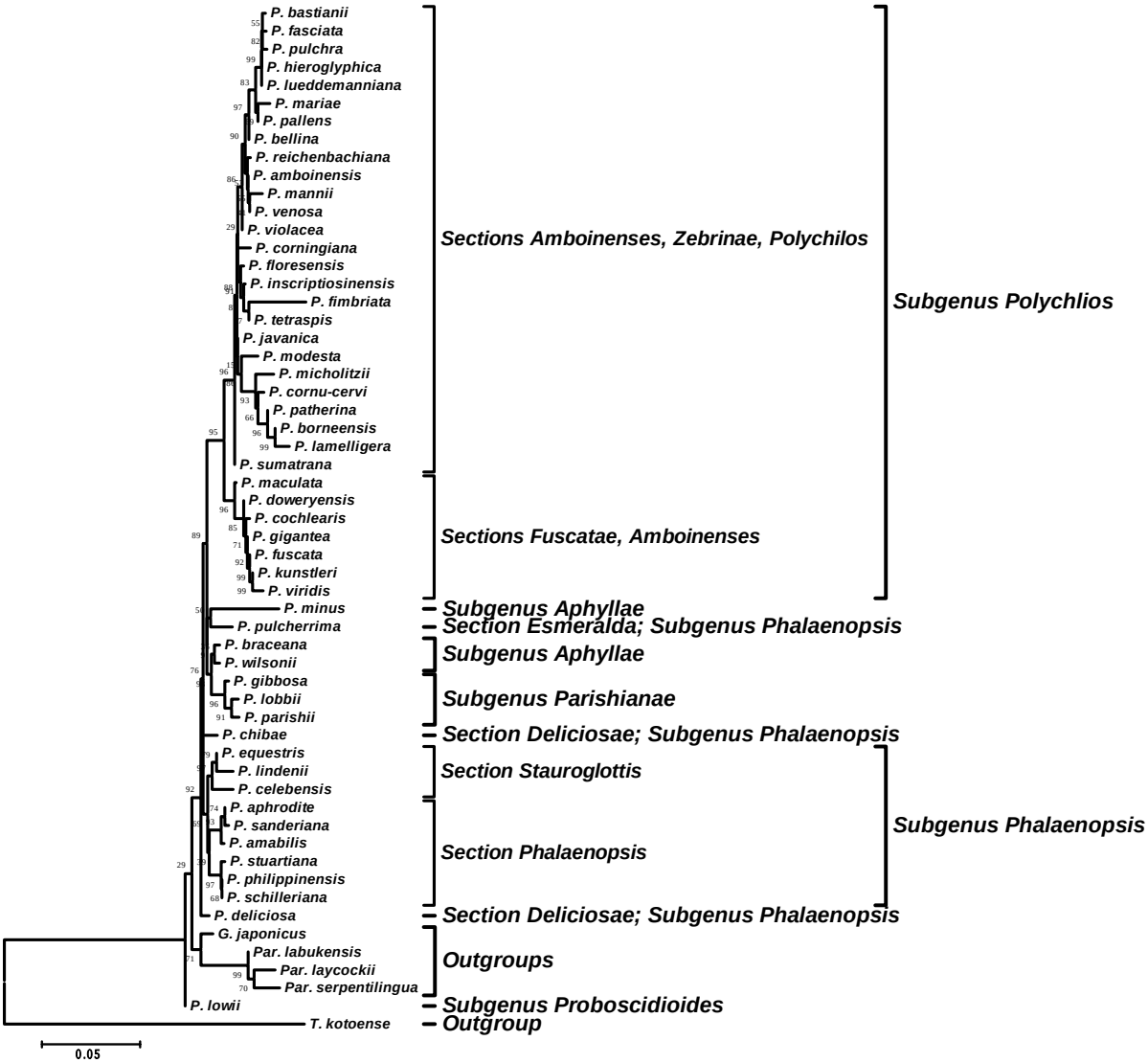


Fig. 12. Neighbor-joining tree of 52 *Phalaenopsis* species plus the five outgroups obtained from sequence comparison of the *trn*L intron. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch.

Fig. 13

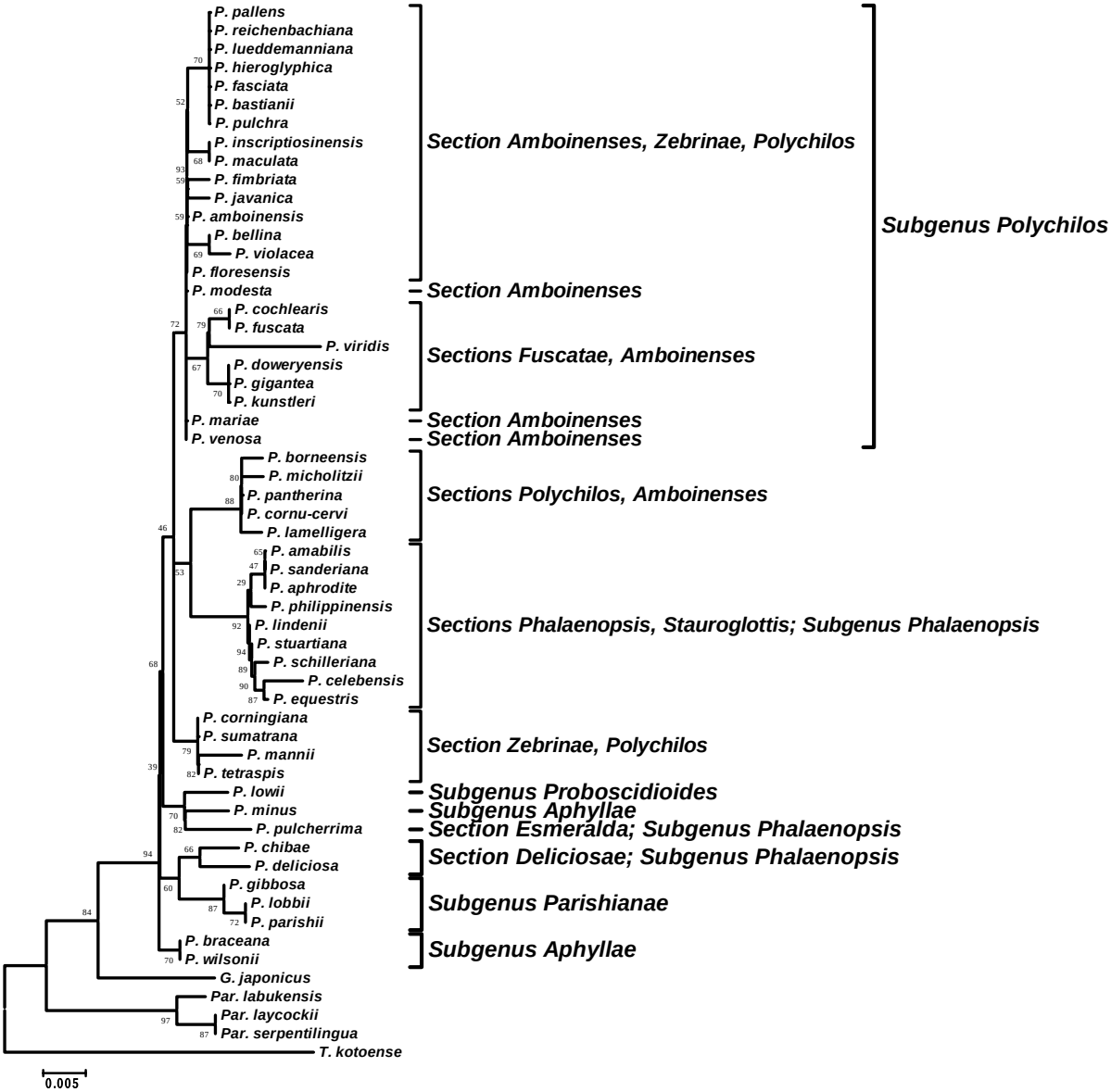


Fig. 13. Neighbor-joining tree of 52 *Phalaenopsis* species plus the five outgroups obtained from sequence comparisons of the *trn*L-*trn*F intergenic spacer. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch.

Fig. 14

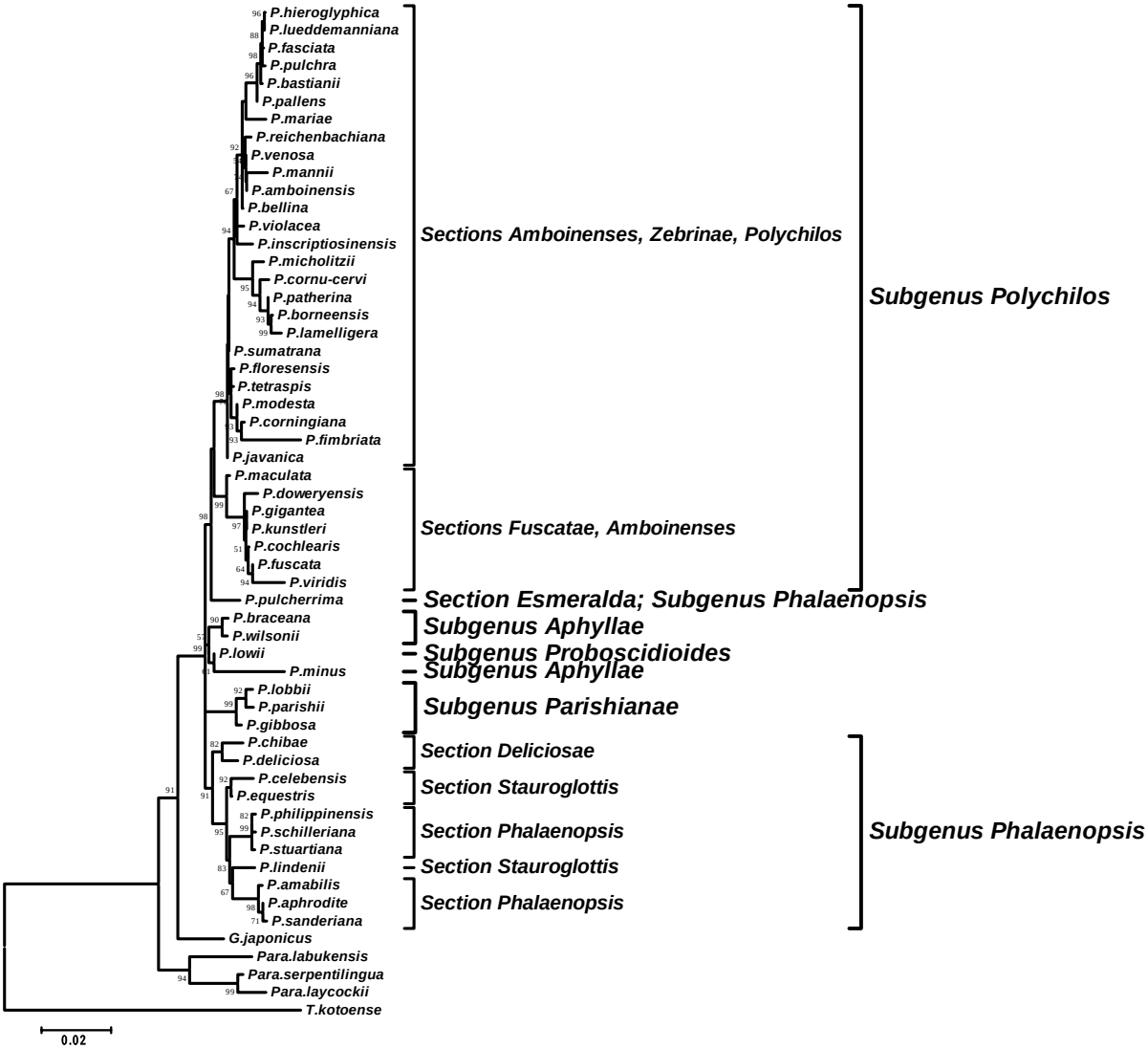


Fig. 14. Neighbor-joining tree of 52 *Phalaenopsis* species plus the five outgroups obtained from sequence comparisons of combined data of the *trn*L intron and the *trn*L-*trn*F intergenic spacer. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch.

Fig. 15

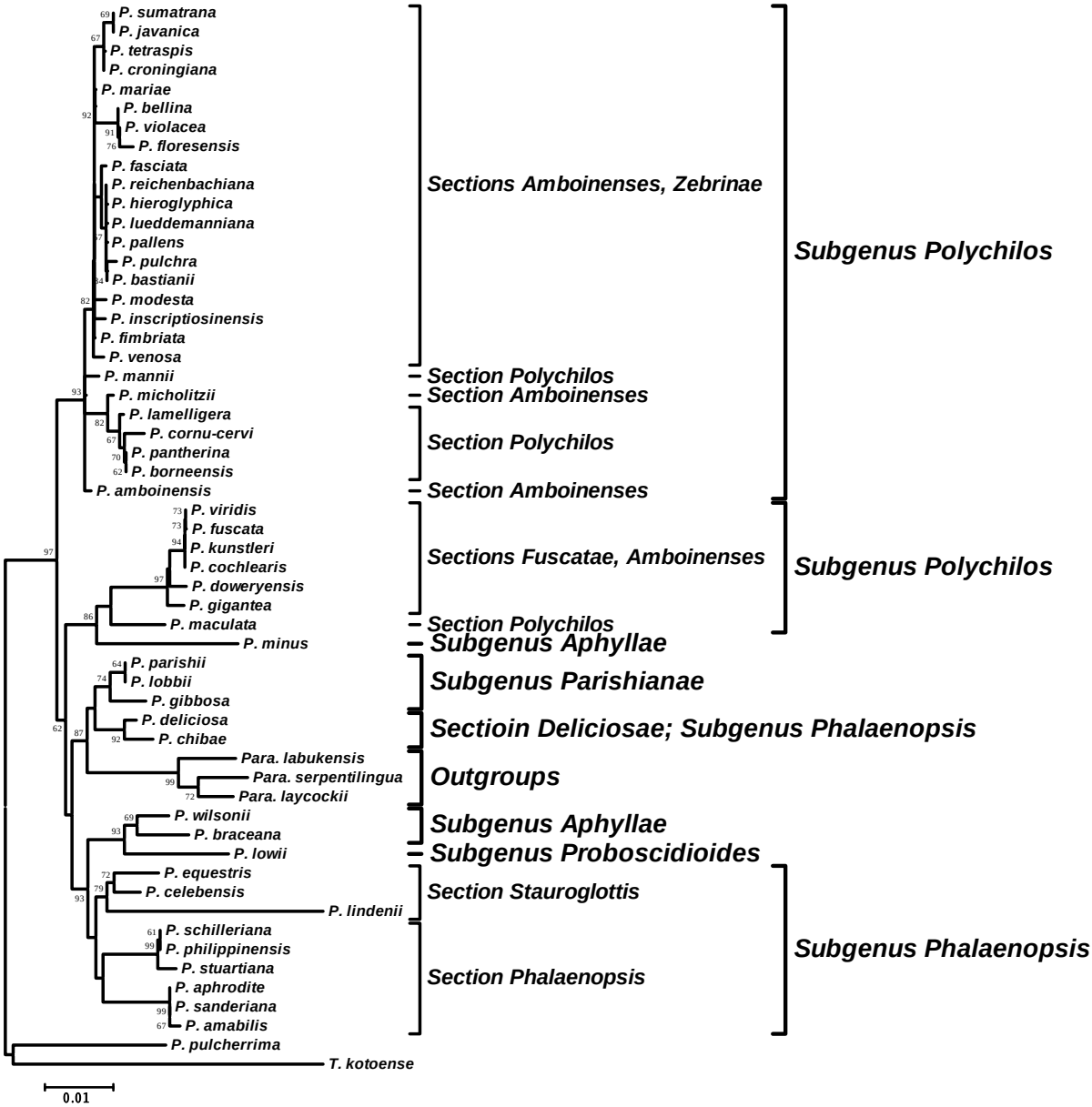


Fig. 15. Neighbor-joining tree of 52 *Phalaenopsis* species plus the four outgroups obtained from sequence comparisons of the *atp*B-*rbc*L intergenic spacer. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch.

Fig. 16

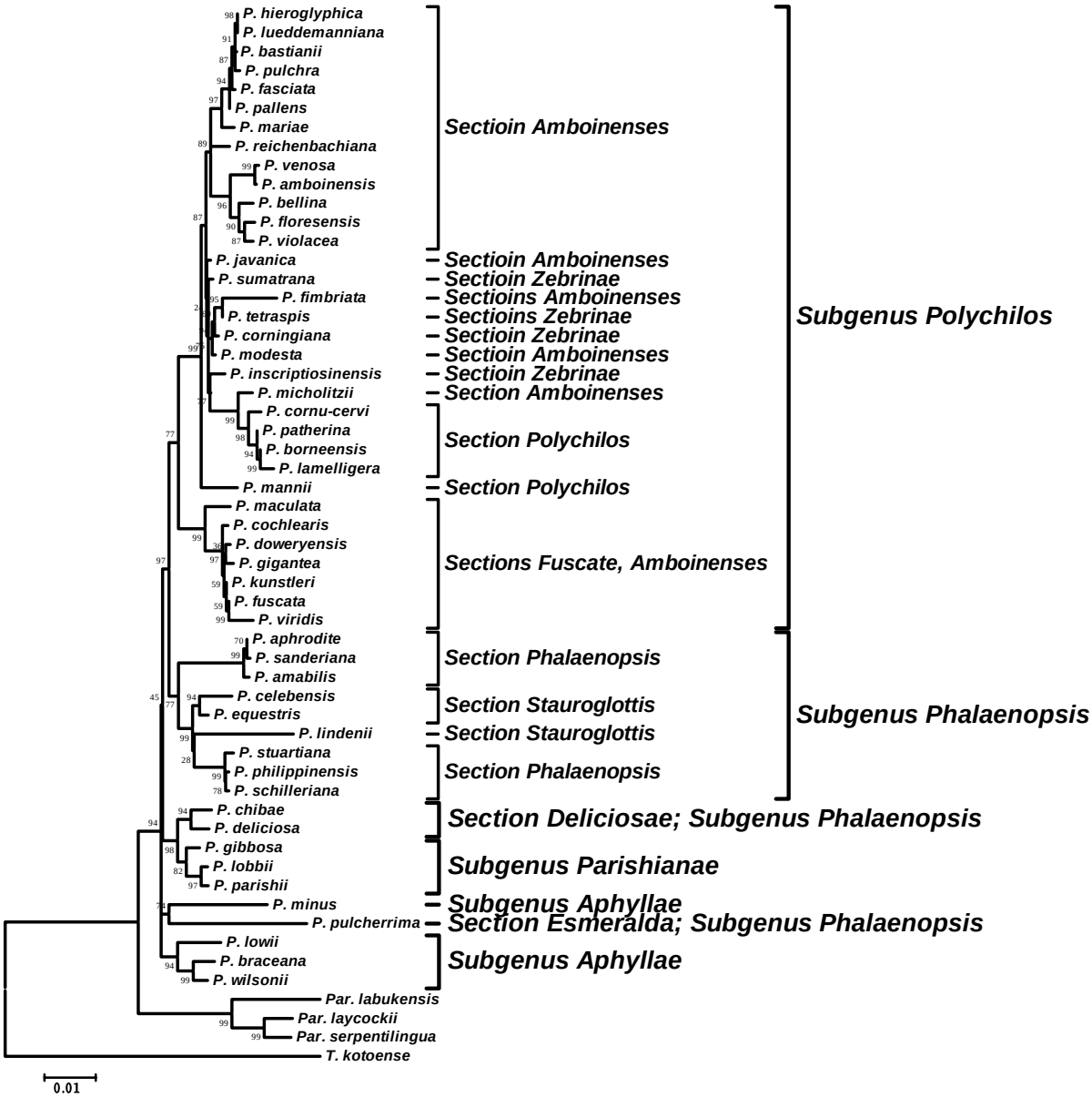


Fig. 16. Neighbor-joining tree of 52 *Phalaenopsis* species plus the four outgroups obtained from sequence comparisons of combined data of the *trn*L intron, the *trn*L-*trn*F IGS, and the *atp*B-*rbc*L intergenic spacer. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch.

Fig. 17

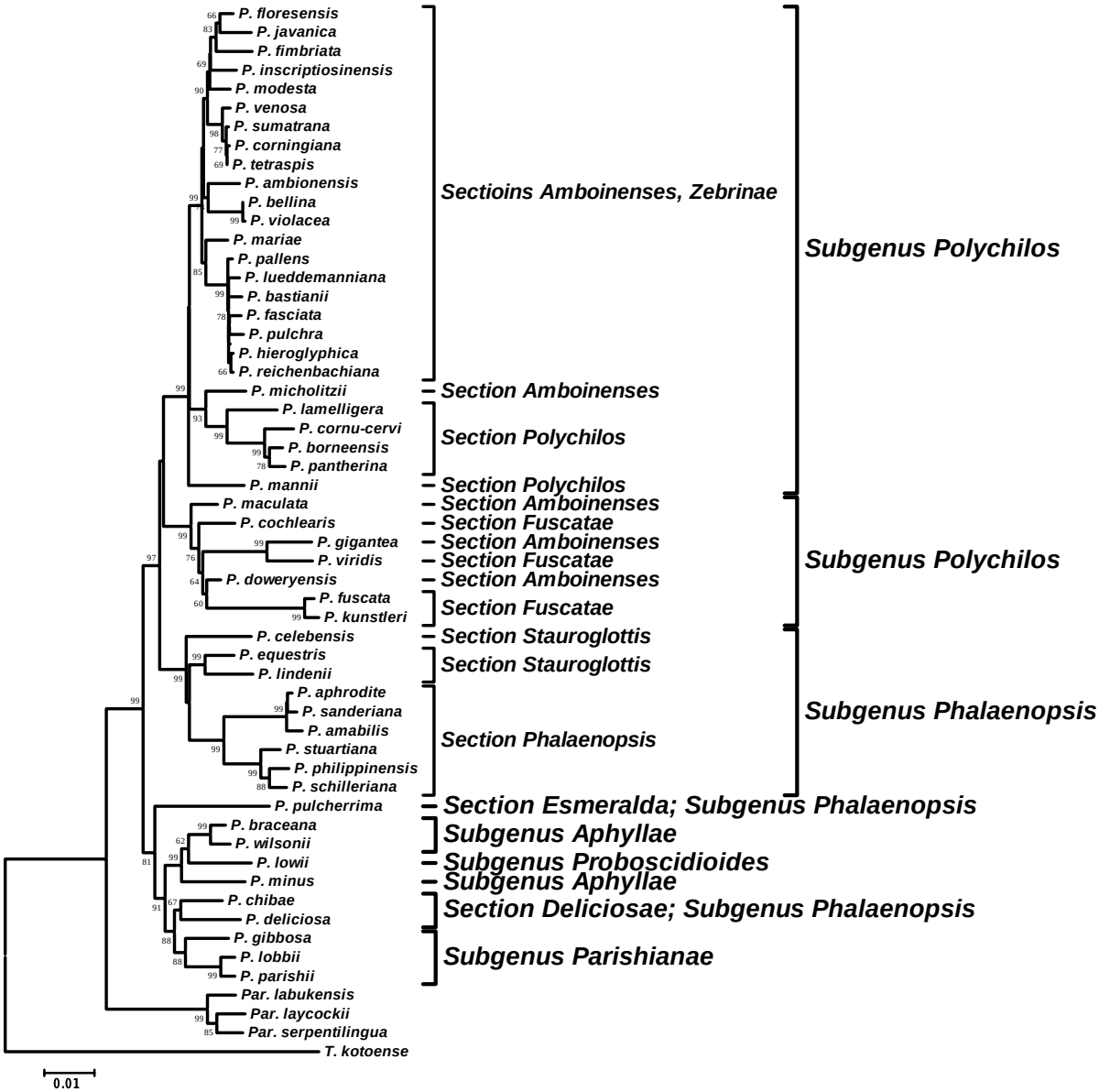


Fig. 17. Neighbor-joining tree of 52 *Phalaenopsis* species plus the four outgroups obtained from sequence comparison of combined data of internal transcribe spacer 1 (ITS1) and ITS2 of nuclear DNA, the *trn*L intron, the *trn*L-*trn*F intergenic spacer (IGS), and the *atp*B-*rbc*L IGS. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch.

Fig. 18. Matrix of geographical distributions of the genus *Phalaenopsis*.

	5	10	15	20	25	30	35	40	45	50	55	60	65*
India/Sri Lanka	00000	10000	11110	00000	00000	00000	00000	00000	00000	00000	01100	01000	00000
South China	01110	01100	11100	00000	00000	00000	00000	00000	00000	00000	00000	00000	00000
Indochina	10001	00001	11110	00000	10000	00000	00000	00000	00010	00000	11100	00011	00000
Malay Peninsula	00000	00010	00010	00101	10110	00000	01000	00000	00010	00000	00100	00000	00000
Borneo	00000	00000	00011	11101	00100	10101	11000	00000	00110	10000	00100	00000	00001
Sumatra	00000	00000	00000	00010	00010	10000	00000	00000	11010	10000	00100	00000	00010
Java	00000	00000	00010	00000	00000	10000	00100	00000	00010	10000	00000	00000	00000
Flores	00000	00000	00000	00000	00000	01000	00000	00000	00000	00000	00000	00000	00000
Sulawesi	00000	00000	00000	00000	00000	00000	00001	01000	00000	10000	01001	00000	00000
Molucca Is.	00000	00000	00000	00000	00000	00000	00001	00000	00000	10000	00000	00100	00000
New Guinea	00000	00000	00000	00000	00000	00000	00000	00000	00000	10000	00000	00000	00000
The Philippines	00000	00000	00000	00000	01001	00010	00010	10111	00011	11111	01010	10000	11100
Taiwan	00000	00000	00000	00000	00000	00000	00000	00000	00000	01000	00010	00000	00000
Australia	00000	00000	00000	00000	00000	00000	00000	00000	00000	10000	00000	00000	00000

* characters: 1, *P.lowii*; 2, *P. wilsonii*; 3, *P. honghenensis*; 4, *P. hainanensis*; 5, *P. minus*; 6, *P. taenialis*; 7, *P. braceana*; 8, *P. strobartiana*; 9, *P. appendiculata*; 10, *P. gibbosa*; 11, *P. lobbii*; 12, *P. parishii*; 13, *P. mannii*; 14, *P. cornu-cervi*; 15, *P. borneensis*; 16, *P. pantherina*; 17, *P. lamelligera*; 18, *P. cochlearis*; 19, *P. viridis*; 20, *P. fuscata*; 21, *P. kunstleri*; 22, *P. pulchra*; 23, *P. bellina*; 24, *P. violacea*; 25, *P. micholitzii*; 26, *P. fimbriata*; 27, *P. floresensis*; 28, *P. gigantea*; 29, *P. fasciata*; 30, *P.doweryensis*; 31, *P. modesta*; 32, *P. maculata*; 33, *P. javanica*; 34, *P. mariae*; 35, *P. amboinensis*; 36, *P. lueddemanniana*; 37, *P. venosa*; 38, *P. pallens*; 39, *P. bastianii*; 40, *P. hieroglyphica*; 41, *P. inscriptiosinensis*; 42, *P. tetraspis*; 43, *P. corningiana*; 44, *P. sumatrana*; 45, *P. philippinensis*; 46, *P. amabilis*; 47, *P. aphrodite*; 48, *P. sanderiana*; 49, *P. schilleriana*; 50, *P. stuartiana*; 51, *P. chibae*; 52, *P. deliciosa*; 53, *P. pulcherrima*; 54, *P. equestris*; 55, *P. celebensis*; 56, *P. lindenii*; 57, *P. mysorensis*; 58, *P. robinsonii*; 59, *P. regnieriana*; 60, *P. buyssoniana*; 61, *P. ×intermedia*; 62, *P. ×amphitrite*; 63, *P. ×veitchiana*; 64, *P. ×gersenii*; 65, *P. ×singuliflora*.

Fig. 19.

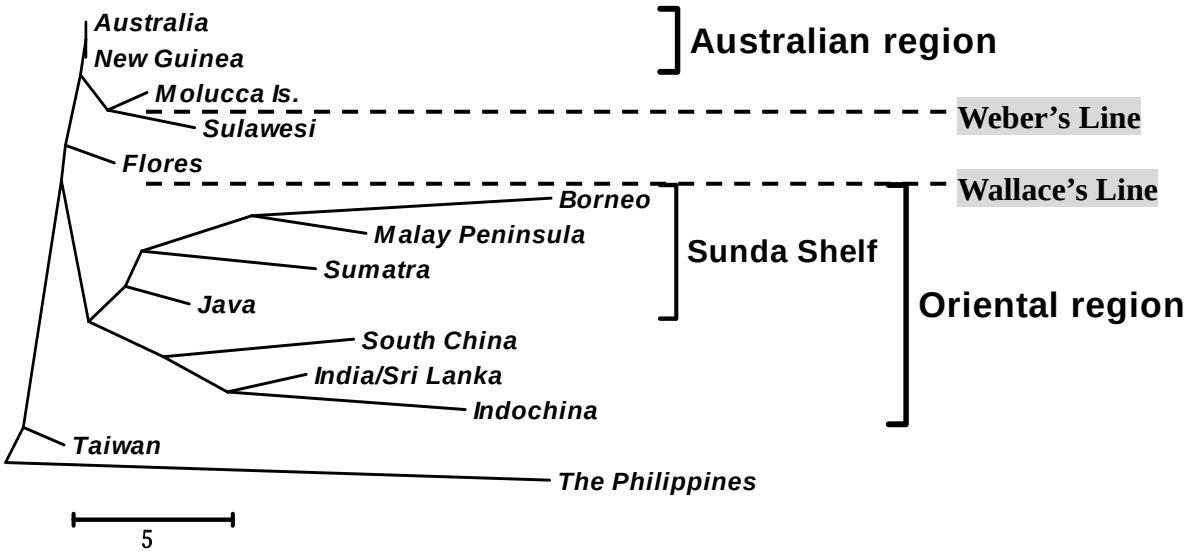


Fig. 19. Biogeographical tree of the genus *Phalaenopsis* constructed by the Neighbor-joining method.

Fig. 20.

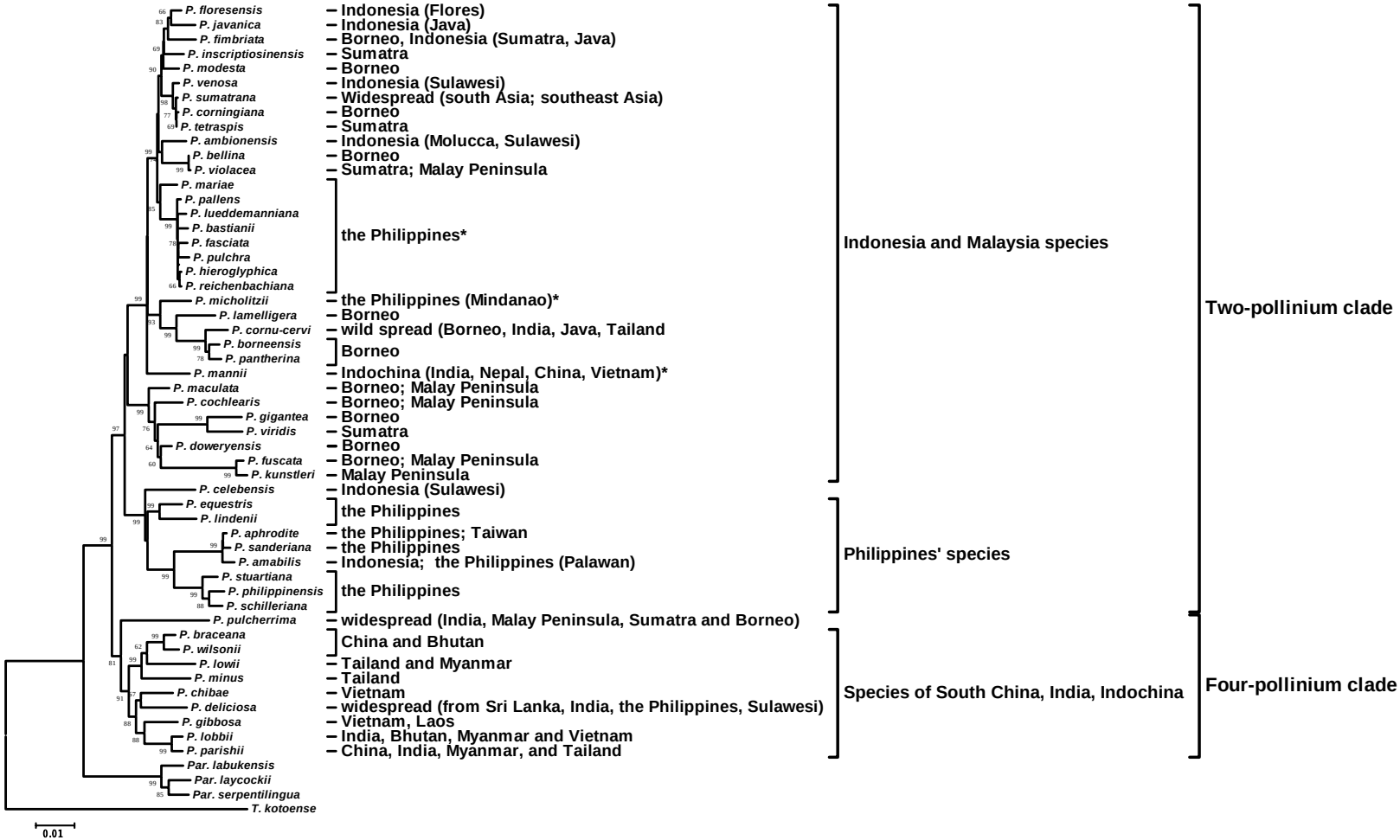


Fig. 20. Comparisons between phylogenetic relationships of the 52 *Phalaenopsis* species plus the four outgroups obtained from the combined data of nuclear and chloroplast DNA and the geographical distributions of the genus *Phalaenopsis*. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch. The distribution is stated to the right of the species name. An asterisk (*) indicates Philippine species within the subgenus *Polychilos*. The pollinium number is stated to the right of the distribution name (modified from Fig. 17).

Fig. 21

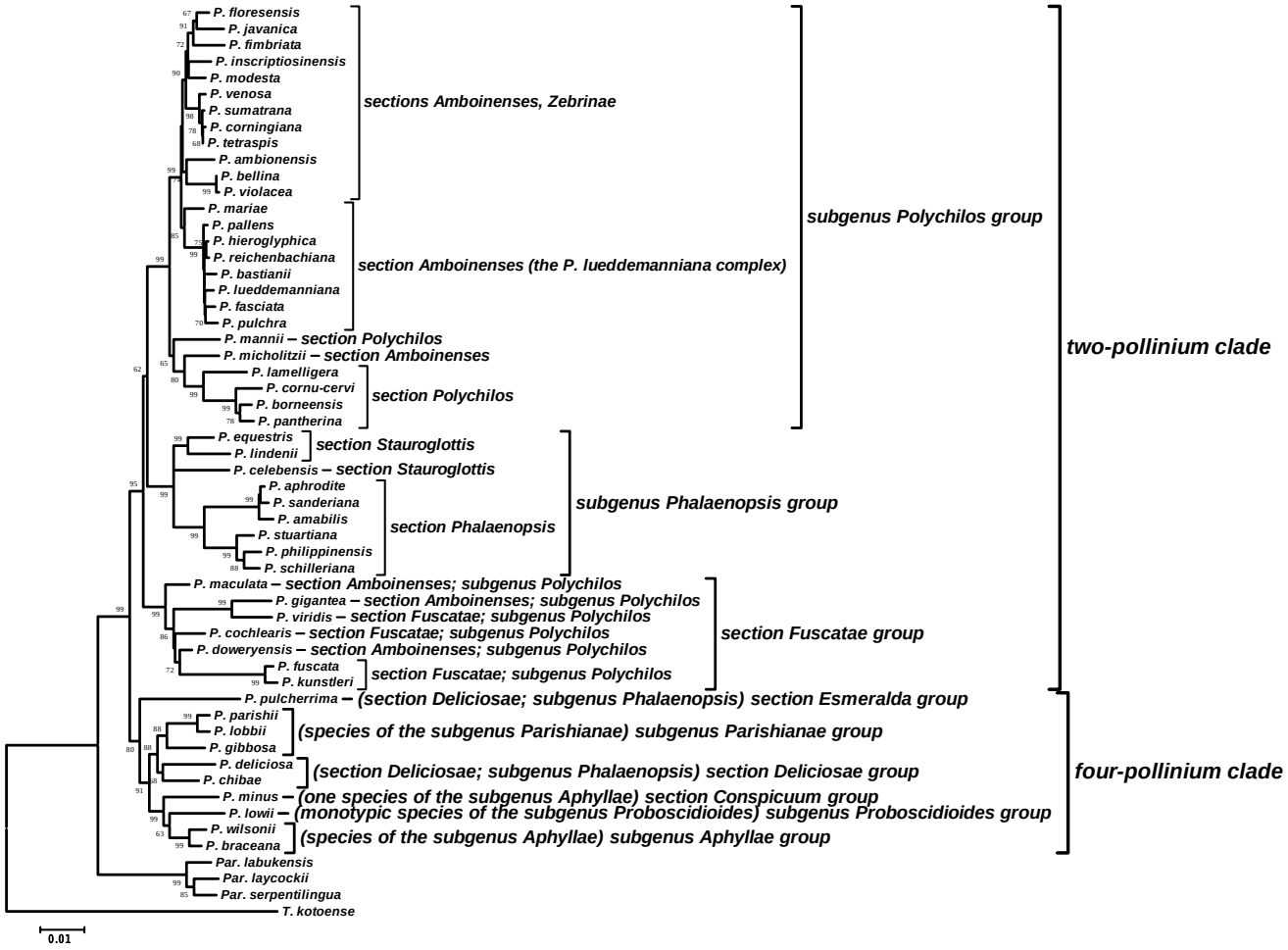


Fig. 21. Evolutionary phylogenetic tree of the genus *Phalaenopsis* inferred from the combined data of the internal transcribed spacer of nrDNA and chloroplast DNA reconstructed by minimum-evolution method. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch.

Fig. 22.

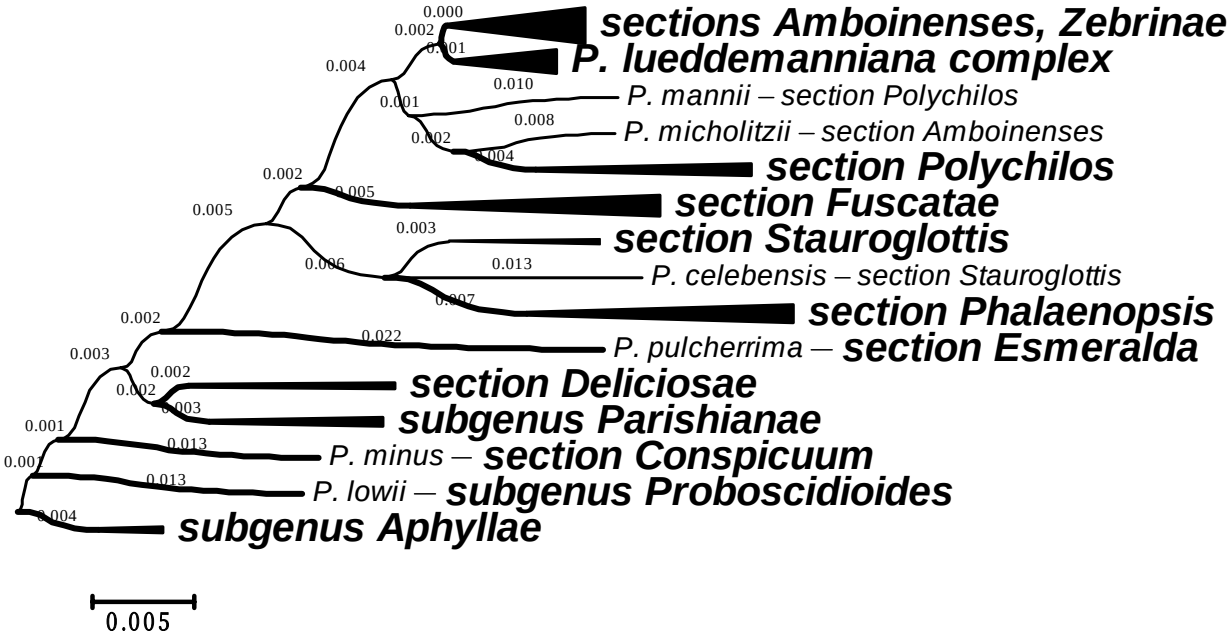


Fig. 22. Evolutionary phylogenetic tree of the genus *Phalaenopsis* inferred from the combined data of the internal transcribed spacer of nuclear DNA and chloroplast DNA reconstructed by the minimum-evolution method and rooted based on the subgenus *Aphyllae*. Bold branches show the species of consistent subgenera/sections according to the systematics of Christenson (2001). Values above the branches are the genetic distances of the Kimura two-parameter method.

Fig. 23.

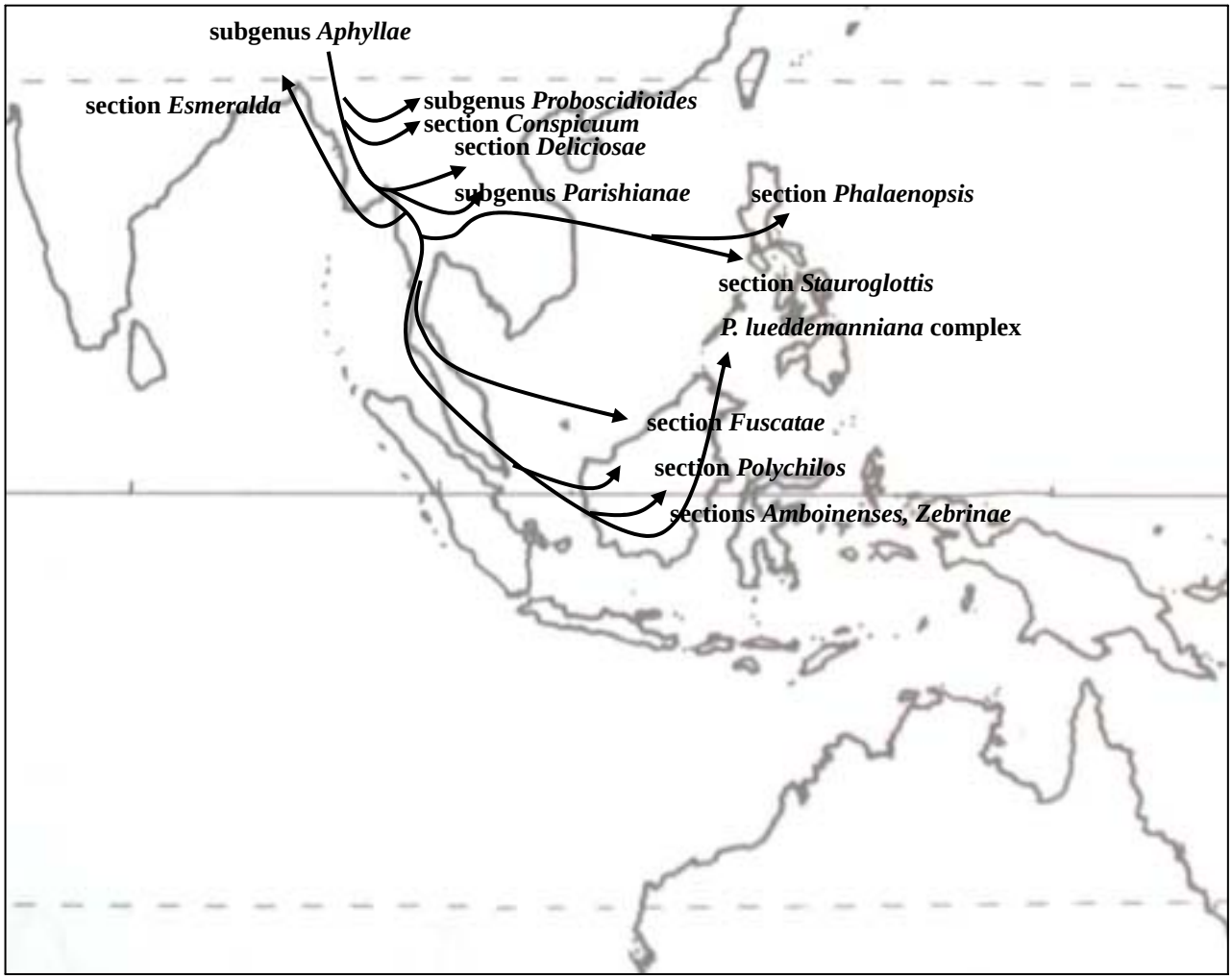


Fig. 23. Evolutionary trends of the genus *Phalaenopsis* obtained from this study plotted on a map of the geographical distribution of this genus.

Fig. 24. Sequence alignment of the ITS sequences from the five clones of *Phalaenopsis* × *intermedia* plus the species of the sections *Phalaenopsis* and *Stauroglottis*.

```
                                5          15         25         35         45         55
P._amabilis                     TCGAGACCGG AATCATACC- GAGCCAATCG GAGAACCCGT GAACCGAACG GCGGCGGCGG
P._aphrodite                    .......... .........- ....G..... .......... .......... ..........
P._sanderiana                   .........- .........C ....G.C... .......... .......... ..........
P._philippinensis               .......... ..CT...T.- ....G.T... .......... .......... ..........
P._schilleriana                 C......... ..CT...T.- ....G.T... .......... .......... ..........
P._stuartiana                   C......... ..C....T.- ....G.T... .......... .......... ..........
P._celebensis                   .........A ...T...T.- ....G.T... .......... ..G...GG.. .....C..C.
P._equestris                    .........A ...T...T.- ....G.T... .......... .......... ..........
P._lindenii                     .........A ...T.C.T.- ....G.T... .......... .......... ..........
P._intermedia-kc-82-clone5      .........A ...T...T.- ....G.C... .......... ........T. ..........
P._intermedia-kc-82-clone2      .......... ......G..- ....G..... .......... .......... ........--
P._intermedia-kc-82-clone3      .......... ......G..- ....G..... .......... .......... ..........
P._intermedia-kc-82-clone4      .......... ......G..- ....G..... .......... .......... ........--
P._intermedia-kc-82-clone1      .........A ...T...T.- ....G.C... .......... ........T. ..........

                                65         75         85         95         105        115
P._amabilis                     CCGCCGCCGC CGGACGGCCG CCCCCGCCGT CGCCCCCGCC CCCGTTCGGA GGGGGGGGGC
P._aphrodite                    .......... .......... .......... .......... .......... ......--..
P._sanderiana                   .......... .......... .......... .......... .......... ......A...
P._philippinensis               .G........ ..C....... .......... .......... .....C.... ......--..
P._schilleriana                 .G........ ..C....... .......... .......... .....CT... ......---.
P._stuartiana                   .......... ..C....... .......... .......... .....C.... ......--..
P._celebensis                   .G........ ..C....... .........C ..G....--. .T........ .......C..
P._equestris                    .G........ ..A....... .........C .......... .G.......- -T.....-..
P._lindenii                     .......... ..A....... .........C .A........ .A.......- -A.....-..
P._intermedia-kc-82-clone5      .......... ..A....... ........-- ---------- .G..C....- T......-..
P._intermedia-kc-82-clone2      -......... .......... .......... .......... .......... ......-...
P._intermedia-kc-82-clone3      .......... .......... .......... .......... .......... ......-...
P._intermedia-kc-82-clone4      -......... .......... .......... .......... .......... ......-...
P._intermedia-kc-82-clone1      .......... ..A....... ........-- ---------- .G..C....- T......-..

                                125        135        145        155        165        175
P._amabilis                     GCGGCGGGGG ACGGCCGGAA CCCCGAACCG GCGCGGATCG GCGCCAAGGG AACCCGTG-A
P._aphrodite                    .......... .......... .......... .......... .......... ....G...-.
P._sanderiana                   .......... .......... .......... .......... .......... ....G...-.
P._philippinensis               .......... .......A.. .......... ....A..... .......... ....G...-.
P._schilleriana                 .......... .......A.. .......... ....A..... .......... ....G...-.
P._stuartiana                   .......... .......A.. .......... ....A..... .......... ....G...-.
P._celebensis                   .......... ........G. .......... ....A..... .......... ....GT..G.
P._equestris                    ...A...... .......A.. .......... ....A..... .......... ....G...-.
P._lindenii                     ...A...... .......A.. .......... ....A..... .......... G...G...-.
P._intermedia-kc-82-clone5      ...A...... .......A.. .......A.. ....A..... .......... ....G...-.
P._intermedia-kc-82-clone2      .......... .......... .......... .......... .......... ....G...-.
P._intermedia-kc-82-clone3      .......... .......... .......... .......... .......... ....G...-.
P._intermedia-kc-82-clone4      .......... .......... .......... .......... .......... ....G...-.
P._intermedia-kc-82-clone1      ...A...... .......A.. .......A.. ....A..... .......... ....G...-.
```

```
                          185        195        205        215        225        235
P._amabilis               GAGACACGAG CCCGGCATCG GGCCCCCGTG GGGCGGAGCG ---------- CCTAACGTAC
P._aphrodite              .......... .......... .....T.... .......... ---------- ..C.......
P._sanderiana             .......... .......... .....T.... .......... ---------- ..........
P._philippinensis         ..A.....G. .......... .....T.... .......... GGGCTGCGCG ..GC......
P._schilleriana           ..A.....G. .......... .....T.... .......... GGGCTGCGCG ..GC......
P._stuartiana             ..A.....G. .......... .....T.... .......... GGGCTGCGCG ..GC......
P._celebensis             ..A....... .......... .....T.... .AA....... GCGCCGCGCG ..GC......
P._equestris              C......... .......... .....T.... .......... GTGCTGCGCA ..GC......
P._lindenii               A.A....... .......... .....T.... .......... GTGCTGCGCA ..GC.....A
P._intermedia-kc-82-clone5 A......... .......... .....T.... .......... GTGCCGCGCA ..GC...C..
P._intermedia-kc-82-clone2 .......... .......... .....T.... .......... ---------- ..C.......
P._intermedia-kc-82-clone3 .......... .......... .....T.... .......... ---------- ..C.......
P._intermedia-kc-82-clone4 .......... .......... .....T.... .......... ---------- ..C.......
P._intermedia-kc-82-clone1 A......... .......... .....T.... .......... GTGCCGCGCA ..GC...C..

                          245        255        265        275        285        295
P._amabilis               CGGTCGCGCC GCTCCGCGCC GAGTCCCCAT CCCCGCCGCG GCGGGGGTGC CGGGCGAGGA
P._aphrodite              .......... .......... ...C...... .......... .......... ..........
P._sanderiana             .......... .......... .C........ .......... .A........ ..........
P._philippinensis         .......... .......... ...C...... .......... .......... .........C
P._schilleriana           .......... .......... ...C...... .......T.. .......... .........C
P._stuartiana             .......... .......... ...C...... .......... .......... .........C
P._celebensis             ....T..... ......T... .......... .......... .T........ .........C
P._equestris              T...T..... ......T... ...C...... .......... .T........ .........C
P._lindenii               T...T..... ......T... ...C...... .......... .T........ .........C
P._intermedia-kc-82-clone5 ....T..... ......T... ...C...... ....A..... .T........ .........C
P._intermedia-kc-82-clone2 .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone3 .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone4 .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone1 ....T..... ......T... ...C...... .......... .T........ .........C

                          305        315        325        335        345        355
P._amabilis               CCGGACGTGC AGAGTGGCCC GTCGTGCCCG TCGGCGCGGC GGGCTGAAGA GCGGGCTGCC
P._aphrodite              .......... .......... .......... .......... .......... ..........
P._sanderiana             .......... .......... .......... .......... .......... ..........
P._philippinensis         .......... ..G....... .......... ........A. .......... ......C...
P._schilleriana           .......... ..G....... .......... .......... .......... ......C...
P._stuartiana             .......... ..G....... A......... C......... .......... ......C...
P._celebensis             .......... ..G....... ........G. .......... .......... .....T..T.
P._equestris              .......... ..G....... .......... .......... .......... .....T.AT.
P._lindenii               .......... .......... .......... .......... .......... .....TAAT.
P._intermedia-kc-82-clone5 .......... ..G....... ....C..... .......... .......... .....T.AT.
P._intermedia-kc-82-clone2 .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone3 .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone4 .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone1 .......... ..G....... .......... .......... .......... .....T.AT.

                          365        375        385        395        405        415
P._amabilis               GTCTCATCGG CCACGGACGA CGAGGGGTGG ATGAAAA--- --GAAGCCCT CGAG------
P._aphrodite              .......... .......... .......... .......--- --........ ....------
P._sanderiana             .......... .......... .......... .......--- --........ ....------
P._philippinensis         .......... ..G..A...G .......... .C.....--- --...A.... ....------
P._schilleriana           .......... ..G..A...G .......... .......--- --...A.... ....------
```

```
P._stuartiana                .......... ..G..A...G .......... ...G..G--- --...A.... ....------
P._celebensis                .......T.. ..G..A..A. .......... ......GAAA GGAGG..TGC ..C.GGCAGG
P._equestris                 .....G.... .....A..A. .......... ...G.TG--- -GAG....GC ..C.GGCGAG
P._lindenii                  .....G.... .....A..A. .......... .....TG--- -GAG....GC ..C.GGCGAG
P._intermedia-kc-82-clone5   .....G.... ..T..A..A. .......... .....TG--- -GAG....GC ..C.GGCGAG
P._intermedia-kc-82-clone2   .......... .......... .......... .......--- --........ ....------
P._intermedia-kc-82-clone3   .......... .......... .......... .......--- --........ ....------
P._intermedia-kc-82-clone4   .......... .......... .......... .......--- --........ ....------
P._intermedia-kc-82-clone1   .....G.... .....A..A. .......... .....TG--- -GAG....GC ..C.GGCGAG
```

```
                               425        435        445        455        465        475
P._amabilis                  ---CGCGTCG TCGCGTGCCG -CCGGAGAGG AGAGGAAACG GCCCTCCGCG CGATCCCATC
P._aphrodite                 ---....... .......... -......... .....G.... .......... ..........
P._sanderiana                ---....... .......... -......... .....G.... .......... .C........
P._philippinensis            ---....... ..T....... G......... ...C.G.... .....T..T. ..........
P._schilleriana              ---....... ..T....... G.......A. ...C.G.... .....T..T. ..........
P._stuartiana                ---....... ..T....... G......... ...C.G.... .....T..T. ..........
P._celebensis                GCC.....T. ..T....... G......... G..TC.C..- --...T.... .......G..
P._equestris                 GCC.....T. ..T....... G......... ...C.GC..- --......T. ..........
P._lindenii                  GCC.....T. ..T....... G......... ...C.GC..- --...T..T. ..........
P._intermedia-kc-82-clone5   GCC.....T. ..T....... G......... ...C.GC..- --...T..TC ..........
P._intermedia-kc-82-clone2   ---....... .......... -......... .....G.... .......... ..........
P._intermedia-kc-82-clone3   ---....... .......... -......... .....G.... .......... ..........
P._intermedia-kc-82-clone4   ---....... .......... -......... .....G.... .......... ..........
P._intermedia-kc-82-clone1   GCC.....T. ..T....... G......... ...C.GC..- --...T..TC ..........
```

```
                               485        495        505        515        525
P._amabilis                  CCGGGCGCCG CCCCTC---- ----GTGCGG -----CGGCT CGGAAC
P._aphrodite                 .......... ....C.C--- ---------- ----..... ......
P._sanderiana                .......... ....C.C--- ---------- ----..... ......
P._philippinensis            ...C...... ....C.ACGT CTGG.G.G.. GGGGG....C A....T
P._schilleriana              ...C...... ....C.ACGT CTGG.G.G.. ------....C T....T
P._stuartiana                ...C...... ....C.ACGT CTGG.G.... ------....A T....T
P._celebensis                ..AC...... ....C.---- ---------- ----..... T....T
P._equestris                 ..AC...... ....C.---- ---------- ----..... T....T
P._lindenii                  ..AC...... ....C.---- ---------- ----..... T....T
P._intermedia-kc-82-clone5   ..AC...... ....C.---- ---------- ------....C T....T
P._intermedia-kc-82-clone2   .......... ....C.C--- ---------- ----..... ......
P._intermedia-kc-82-clone3   .......... ....C.C--- ---------- ----..... ......
P._intermedia-kc-82-clone4   .......... ....C.C--- ---------- ----..... ......
P._intermedia-kc-82-clone1   ..AC...... ....C.---- ---------- ------....C T....T
```

Fig. 25

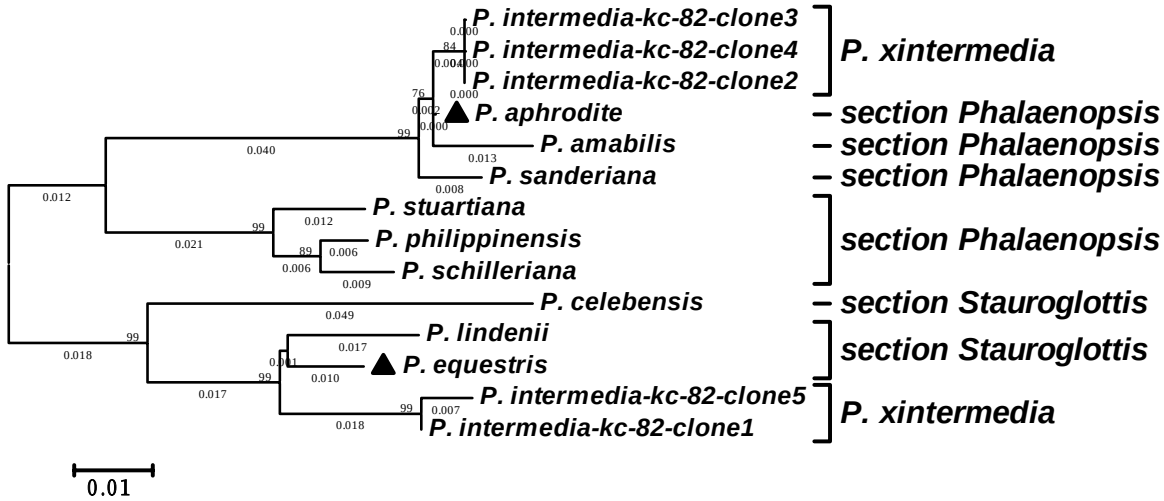
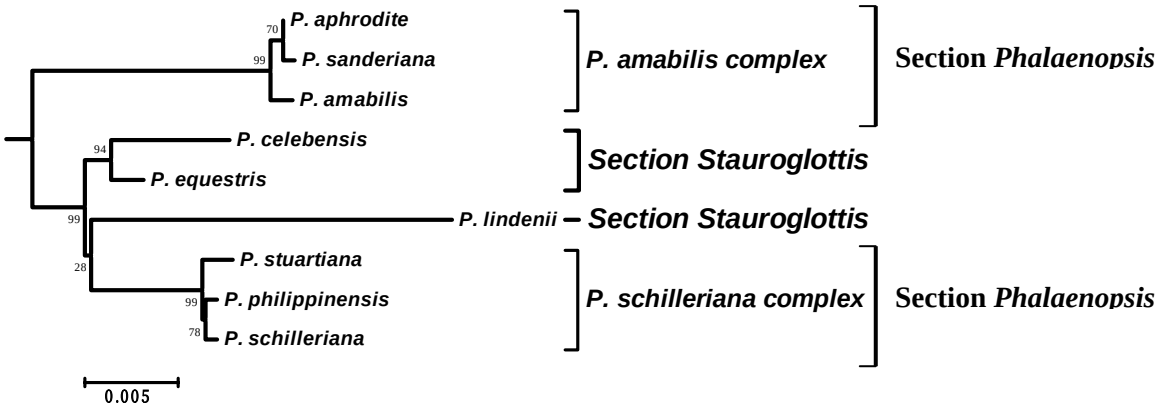


Fig. 25. The phylogenetic tree of five clones of *Phalaenopsis* ×*intermedia* plus species of the sections *Phalaenopsis* and *Stauroglottis* of the genus *Phalaenopsis* inferred from ITS data. Numbers above internodes indicate values of the interior branch tests from 1000 replicates. Branch lengths are proportional to the number of base changes along each branch. Solid bars (▲) indicate the putative parents of *P.* ×*intermedia*.

Fig. 26.

(a)



(b)

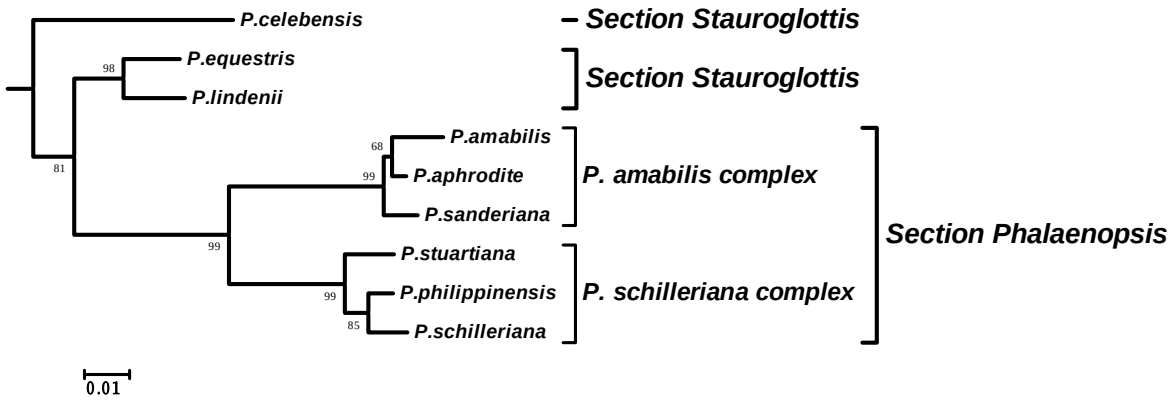


Fig. 26. (a) Phylogenetic subtree of the section *Phalaenopsis* obtained from combined data of the *trn*L intron, the *trn*L-*trn*F intergenic spacer (IGS), and the *atp*B-*rbc*L IGS of chloroplast DNA. (b) Phylogenetic subtree of the section *Phalaenopsis* obtained from internal transcribed spacer 1 (ITS1) and ITS2 of nuclear DNA.

Fig. 27.

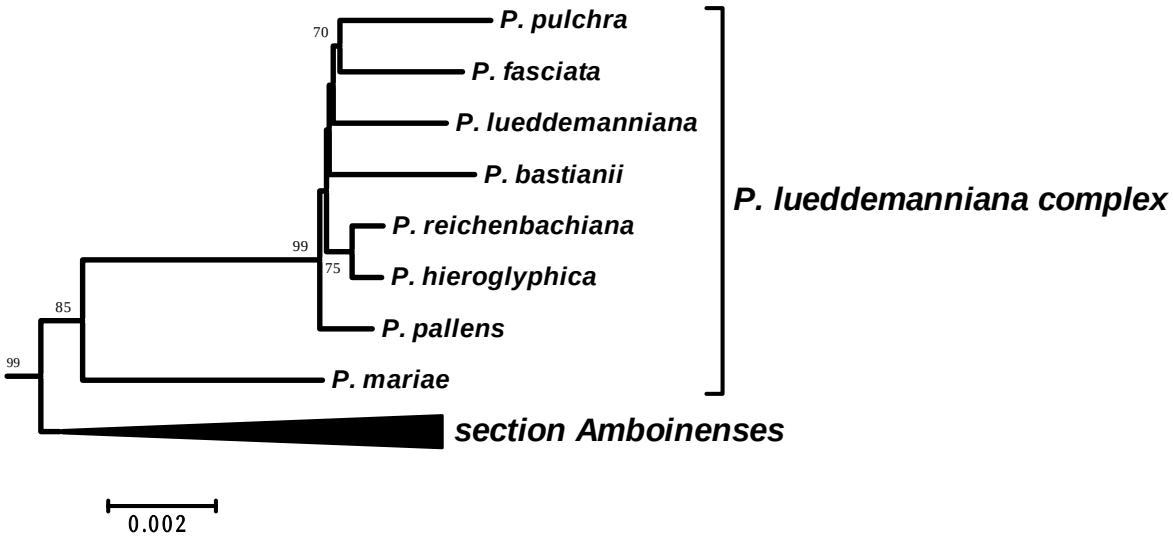


Fig. 27. Evolutionary phylogenetic subtree of both the section *Amboinenses* and the *P. lueddemanniana* complex inferred from the combined data of the internal transcribed spacer of nuclear DNA and chloroplast DNA data constructed using the minimum evolution method (redrawn from Fig. 21). Species of the section *Amboinenses* were compressed and are shown in bold branches.

Appendix 1.

Phalaenopsis amabilis

Phalaenopsis amboinensis

Phalaenopsis aphrodite

Phalaenopsis appendiculata

Phalaenopsis bastianii

Phalaenopsis bellina

Phalaenopsis borneensis

Phalaenopsis braceana

Phalaenopsis celebensis

Phalaenopsis chibae

Phalaenopsis cochleari
(Gower and Photographer: Hou-Tse Liu)

Phalaenopsis corningiana

Phalaenopsis cornu-cervi

Phalaenopsis deliciosa

Phalaenopsis doweryensis

Phalaenopsis equestris

Phalaenopsis fasciata

Phalaenopsis fimbriata
(Gower and Photographer: Hou-Tse Liu)

Phalaenopsis floresensis

Phalaenopsis fuscata

Phalaenopsis gibbosa
(Gower and Photographer: Hou-Tse Liu)

Phalaenopsis gigantea

Phalaenopsis hieroglyphica

Phalaenopsis honghenensis

Phalaenopsis inscriptiosinensis

Phalaenopsis intermedia

Phalaenopsis javanica

Phalaenopsis kunstleri

Phalaenopsis lamelligera

Phalaenopsis lindenii
(Gower and Photographer: Hou-Tse Liu)

Phalaenopsis lobbii

Phalaenopsis lowii
(Gower and Photographer: Hou-Tse Liu)

Phalaenopsis lueddemanniana

Phalaenopsis maculata

Phalaenopsis mannii

Phalaenopsis mariae

Phalaenopsis micholitzii
(Gower and Photographer: Hou-Tse Liu)

Phalaenopsis minus

Phalaenopsis modesta

Phalaenopsis pallens

Phalaenopsis pantherina
(Gower and Photographer: Hou-Tse Liu)

Phalaenopsis parishii

Phalaenopsis philippinensis

Phalaenopsis pulcherrima

Phalaenopsis reichenbachiana

Phalaenopsis sanderiana

Phalaenopsis schilleriana

Phalaenopsis stuartiana

Phalaenopsis sumatrana

Phalaenopsis tetraspis

Phalaenopsis venosa

Phalaenopsis violacea

Phalaenopsis viridis

Phalaenopsis wilsonii

Phalaenopsis zebrina

Chapter 2

Phylogenetics, Biogeography, and Evolutionary Trends of the *Phalaenopsis amabilis* Complex Inferred from ITS1 and ITS2 of Nuclear DNA

Abstract

The internal transcribed spacers 1 and 2 (ITS1+ITS2) region of nuclear ribosomal DNA (nrDNA) was applied to evaluate the phylogenetics of the *Phalaenopsis amabilis* complex, namely *P. amabilis*, *P. amabilis* subsp. *moluccana*, *P. amabilis* subsp. *rosenstromii*, *P. aphrodite*, *P. aphrodite* subsp. *formosana*, and *P. sanderiana*. The phylogenetic tree of the ITS of nrDNA for the *P. amabilis* complex was constructed following the Neighbor-joining method. Based on the tree, each of the species/subspecies from the *P. amabilis* complex with the exception of *P. aphrodite* and its subspecies could be separated from each other based on the phylogenetic tree. Within accessions of *P. amabilis* and its subspecies, different locations of *P. amabilis* and its subspecies formed different separated clades with the exception of accessions distributed in both Palawan and Borneo. *Phalaenopsis aphrodite* from different locations and its subspecies could not be separated from each other, but all of them were separable from different populations/subspecies of *P. amabilis*. 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*. Furthermore, the genetic relationship of *P. amabilis* distributed in Palawan was intermediate between *P. aphrodite* and *P. amabilis* distributed in Sabah based on the tree. This result is in agreement with the biogeography of these two groups. According to the phylogenetic tree derived from ITSs of nrDNA, *P. aphrodite* was suggested as being the origin group of the *P. amabilis* complex. *Phalaenopsis amabilis* and *P. sanderiana* descended from *P. aphrodite* (or their most recent common ancestor). In addition, the evolutionary trends of the *P. amabilis* complex included two different lineages corresponding to two different dispersal pathways. First, *P. aphrodite* dispersed into Palawan and *P. amabilis* evolved, thereafter further dispersing into Borneo. Second, *P. aphrodite* 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. *Phalaenopsis amabilis* subsp. *rosenstromii* in New Guinea further dispersed into Northern Australia and Timor.

Introduction

The *Phalaenopsis amabilis* complex includes *P. amabilis*, *P. aphrodite*, *P. sanderiana*, *P. amabilis* subsp. *moluccana*, *P. amabilis* subsp. *rosenstromii*, and *P. aphrodite* subsp. *formosana*. Those species and their subspecies were placed in the section *Phalaenopsis* (Sweet, 1980; Christenson, 2001). *Phalaenopsis amabilis* is a pure white, large-flowered *Phalaenopsis* species. The species is widespread and ranges from Mentawai Is. (Sumatra), Java, Borneo to the southern Philippines, and east to New Guinea and Queensland, Australia. Plants of *P. amabilis* from the eastern portion of the range are treated as two distinct subspecies (Christenson, 2001). The variation of this species is discontinuous because of isolation resulting from the well-established phytogeographic breaks (Wallace's Line) between Borneo and Sulawesi on the one hand and between Sulawesi and New Guinea on the other (Weber's Line) (Christenson, 2001). Therefore, two subspecies of *P. amabilis*, namely *P. amabilis* subsp. *moluccana* and *P. amabilis* subsp. *rosenstromii* have been introduced. *Phalaenopsis amabilis* subsp. *moluccana* is distributed in the Molucca Islands (Seram, Buru) and northern and southeastern Sulawesi. This subspecies is easily recognized by the shape of the midlobe of the lip that is merely linear-oblong with a very slight dilation toward the base instead of being cruciform (Sweet, 1980). Furthermore, the population of this subspecies native to Sulawesi with a grayish color on the upper surface of the leaves was once treated as *P. amabilis* var. *cinerascens* by J. J. Smith (cf. Sweet, 1969a). This variety, however, was not accepted by Sweet (1969a), which treated the aforementioned variety of *P. amabilis* as a synonym of *P. amabilis* var. *moluccana*. Until Christenson (2001), the plants of the *P. amabilis* complex native to both Molucca Is. and Sulawesi were treated as a subspecies of *P. amabilis*, namely *P. amabilis* subsp. *moluccana*. Furthermore, another subspecies of *P. amabilis*, namely *P. amabilis* subsp. *rosenstromii*, is distributed in New Guinea (Kaiser Wilhelms Land), New Ireland (Neumecklenburg), and Australia (northeastern Queensland). This subspecies differs from *P. amabilis* and *P. amabilis* subsp. *moluccana* in having a lip with a triangular midlobe. In other words, the shape of midlobe of this subspecies is very similar to that of *P. aphrodite*. Those two taxa, however, can be easily separated by the structure of the callus (cf. Sweet, 1980). Sweet (1969a) treated *P. amabilis* distributed in Queensland, Australia as a synonym of *P. amabilis* var. *papuana* distributed in New Guinea and New Ireland. Clement (1989), however, treated the plants of the *P. amabilis* complex native to Australia as a separate species, namely *P. rosenstromii*. Recently, Christenson (2001) did not accept the narrow species concept of Clement (1989) and treated it as a subspecies of *P. amabilis*,

namely *P. amabilis* subsp. *rosenstromii*.

Another large, white-flowered *Phalaenopsis* species, *P. aphrodite*, has a close relationship to *P. amabilis* based on morphology. *Phalaenopsis aphrodite* has been confused with *P. amabilis* for a long time. This taxon was treated as a variety of *P. amabilis*, namely *P. amabilis* var. *aphrodite* Rchb.f. after 1908 (cf. Sweet, 1969a). Up to Sweet (1969a), this taxon was separated from *P. amabilis* and was treated as a separate species, *P. aphrodite*, based on the characters of the callus and midlobe shape. *Phalaenopsis aphrodite* is distributed in the Philippines (including Bataan, Camiguin, Luzon, Mindoro, Bilirau, Leyte, Samar, Negros, and Babuyan Islands) and southeastern Taiwan and is separate from *P. amabilis*, which is distributed in Indonesia, New Guinea, and Australia (Sweet, 1969a). Now, these two species have been separated based on the callus structures and their distinct geographical distributions. In the callus structure, the posterior edge of the callus is divided into four teeth in *P. aphrodite* which is separated from those of *P. amabilis* having only two teeth (Christenson, 2001). Furthermore, the sagittate-hastate lip with its carmine markings of *P. aphrodite* also can separate it from each of the related species, namely *P. amabilis* and its two subspecies plus *P. sanderiana*. In addition, Sweet (1980) separated *P. amabilis* from *P. aphrodite* based on the undersurface color of the leaves. The undersurface color of leaves of *P. amabilis* usually show green, but there is a purplish suffusion in those of *P. aphrodite*. However, Christenson (2001) indicated that this characteristic had at least two exceptions, namely *P. aphrodite* from Taiwan and somewhere Philippines. He found that those populations of *P. aphrodite* lack the purple undersurface to the leaves. In fact, recent collections also did not show the purplish suffusion on the undersurface of leaves in *P. aphrodite*. In contrast, a related species, *P. sanderiana*, always shows the characteristics with the exception of the album form (data not shown). Accessions of the *P. amabilis* complex distributed in Taiwan were once treated as *P. amabilis* var. *formosana* by Shimadzu in 1921 (cf. Christenson 2001). Miwa (1941) once treated the plants native to Taiwan and Babuyan Islands by distinct specific names of *P. formosana* and *P. babuyana*, respectively. Sweet (1969a) treated *P. formosana* and *P. babuyana* as synonyms of *P. aphrodite* based on their four-toothed callus and triangular midlobe. Recently, Christenson (2001) treated the plants native to Taiwan as a subspecies of *P. aphrodite*, namely *P. aphrodite* subsp. *formosana*, since the plants bear apple-green leaves with no trace of anthocyanin pigments, somewhat smaller flowers, and much-branched panicles that typically produce side branches from even the most-basal nodes on the inflorescence.

In addition, another closely related species, namely *P. sanderiana*, has a restricted distribution in the southern Philippines (including Mindanao Island, Igat Island, and Balut and Saragani Islands) (Sweet, 1969b). This taxon was once treated as a variety of *P. aphrodite* by Reichenbach in 1941 or as a variety of *P. amabilis* by Davis in 1949 (cf. Sweet, 1969b). Based on DNA markers, *P. sanderiana* is very close to both *P. amabilis* and *P. aphrodite* as well (Tsai et al., 2003). *Phalaenopsis sanderiana* bears flowers of different degrees of pink. Plants of this species grown at higher elevations have a definitely deeper flower color than plants grown at sea level (Fowlie and Miller, 1974). The callus structure of this species, however, shows four teeth, as does *P. aphrodite*, but the teeth of *P. sanderiana* are highly unequal, and the two inner teeth are significantly taller than the outer two. The teeth of *P. aphrodite*, in contrast, are subequal, and the two outer teeth are slightly taller than the inner two (Christenson, 2001). Furthermore, *P. sanderiana* blooms in mid to late summer, which is separable from the other species of the section *Phalaenopsis*, which only bloom in spring (Christenson, 2001).

Basically, the structure of the callus is the only characteristic by which *P. amabilis*, *P. aphrodite*, and *P. sanderiana* can be identified. Wallbrunn (1971) indicated that plants of the *P. amabilis* complex are distributed over many lands as well as islands. Such a widespread distribution of this species results in a tremendous amount of variability from geographical isolation and selective forces. In addition, he discovered that the shape of the callus among the *P. amabilis* complex was not restricted to only three types. Moreover, he indicated that the callus morphology is not a congruent character according to the inspection of self-offspring of *P. schilleriana*. The results indicated that the callus structure is not a good marker for identifying plants of the *P. amabilis* complex. Therefore, he suggested that the three species of the *P. amabilis* complex be lumped together as one species (Wallbrunn, 1971). In fact, *P. aphrodite* having six-tooth callus, can be found in a restricted region of the Philippines (Liu, pers. comm.). Undoubtedly, *P. amabilis*, *P. aphrodite*, and *P. sanderiana* descended from the same common ancestor, and *P. sanderiana* is distributed in the region where the other two species have never been found. Based on the topophysiography, *P. amabilis* and *P. aphrodite* are suggested to be of more-recent origin (Fowlie, 1981).

According to the historical geology of Southeast Asia, the older islands of the Philippines, namely Palawan, Mindoro, Zamboanga, etc., are on the margin of the Eurasian Plate and may have been moving away from the main land mass since the early Miocene (~20 Mya). Until 5~10 Mya, the crust of the older plate was combined with Borneo (Karig

et al., 1986; Stephan et al., 1986; Hall, 1996). Most of the other Philippine islands are young (< 5 Mya) (Aurelio et al., 1991; Quebral et al., 1994). Furthermore, the Malay Peninsula, Borneo, Sumatra, and Java comprise the Sunda Shelf, and when sea levels were low during glacial times, the Malay Peninsula, Borneo, Sumatra, Java, Bali, and the Philippines, would have been interconnected. This would have made crossings among these regions easy (Van Oosterzee, 1997). In addition, western Sulawesi was a part of the Sunda Shelf of ancient times; it slid away from the Sunda Shelf during the Paleogene (~50 Mya). The deep Makassar Straits formed and divided western Sulawesi from the Sunda Shelf. The new strait prevented any further dispersal of Bornean species to Sulawesi. This historical geology can explain why Sulawesi's terrestrial organisms are closely related to those of Borneo at the family level and higher. This also accounts for the high degree of endemism among Sulawesi's faunal and floral species (Moss and Wilson, 1998) (Fig. 2).

To the present, the genetic relationship of the *P. amabilis* complex is still confusing. The three species, namely *P. amabilis*, *P. aphrodite*, and *P. sanderiana*, and their different subspecies/populations have been selected for analysis of ITS sequences of nrDNA in order to reveal the genetic relationship in the present study. Furthermore, it was suggested that the section *Phalaenopsis* descended from the section *Stauroglottis* (or their most recent common ancestor) (Tsai, see chapter 1). When the species of the section *Phalaenopsis* developed, two different lineages descended, namely the *P. amabilis* complex and the *P. schilleriana* complex, which share the most recent common ancestor. Therefore, the *P. schilleriana* complex was placed as the outgroup to reconstruct the evolutionary phylogenetic tree of the *P. amabilis* complex in the study. Based on the tree, both the relative origin group and the evolutionary trends of the *P. amabilis* complex can be deduced.

Materials and Methods

Plant materials

The 39 accessions of the *P. amabilis* complex obtained from 13 different populations/subspecies/species plus the outgroup, the *P. schilleriana* complex, were analyzed to clarify the phylogenetic relationships and evolutionary trends of the *P. amabilis* complex (Table 1). The geographical distributions of species of this complex are shown in Fig. 1.

DNA extraction

Total DNA was extracted using the method of CTAB (cetyltrimethylammonium bromide) (Doyle and Doyle, 1987). The approximate DNA yields were then determined using a spectrophotometer (Hitachi U-2001).

PCR amplification and electrophoresis

Two primers designed for amplifying the internal transcribed spacer (ITS) of nrDNA of *Phalaenopsis* plants as well as the PCR condition are described in Tsai (see chapter 1). These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained using 0.5 μg/mL ethidium bromide, and finally photographed under UV light exposure.

DNA recovery and sequencing

PCR products of different DNA fragments from the plant material studied were recovered by glassmilk (BIO 101, California) and sequenced directly by the dideoxy chain-termination method using an ABI377 automated sequencer with a Bigdye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, California). Sequencing primers were the same as those used for PCR. These reactions were performed based on the recommendations of the manufacturers.

Data analyses

Boundaries of the ITS regions (including ITS1, 5.8S rDNA, and ITS2) in *Phalaenopsis* species were determined by comparison to several published sequences as described in Tsai (see chapter 1). The sequences were aligned using the program Clustal W Multiple alignment in BioEdit (Hall, 1999). Genetic relationships were performed using the program MEGA version 2.1 (Kumar et al., 2001). A genetic distance matrix was calculated by the two-parameter method of Kimura (1980), and was used to reconstruct a tree using the minimum evolution method (Edwards and Cavalli-Sforza, 1963; Rzhetsky and Nei, 1992, 1993) with interior branch tests of 1000 replicates (Sitnikova et al., 1995). Since the *P. amabilis* complex and the *P. schilleriana* complex (the outgroup) shared the most recent common ancestor, the basal species of the phylogenetic tree was considered the relative origin group of the *P. amabilis* complex. The above phylogenetic tree is rooted

based on the relative origin group in order to evaluate the evolutionary trends of the *P. amabilis* complex.

Results and Discussion

Sequence characteristics

The accession numbers of the ITSs of nrDNA from the 39 accessions of the *P. amabilis* complex are shown in Table 2. The sequence lengths of ITS1 ranged from 228 to 233 bp and those of ITS2 were from 256 to 258 bp. Percentages of the G+C content across the ITS1 region of the *Phalaenopsis amabilis* complex varied from 76.5% to 77.6%. On the other hand, percentages of G+C content across the ITS2 region varied from 76.1% to 77.9% (Table 2). By combining the ITS1 and ITS2 sequences, those sequences of the 39 accessions of the *P. amabilis* complex were aligned and resulted in 494 characters. Eight gap sites and 16 variable sites (of three singleton sites) were found in the alignment sequence of the 39 accessions of the *P. amabilis* complex (Fig. 3).

Genetic distances among accessions of the P. amabilis complex

The genetic distances among the 39 accessions of the *P. amabilis* complex were in the range of from 0.000 to 0.017 with an average of 0.0058 using the two-parameter method of Kimura (1980). Within accessions of *Phalaenopsis amabilis*, the ranges of genetic distances were from 0.000 to 0.013 with an average of 0.00472. Within the accessions of *P. aphrodite*, the ranges of genetic distances were from 0.000 to 0.004 with an average of 0.00055. Within the accessions of *P. sanderiana*, the genetic distances ranged from 0.000 to 0.004 with an average of 0.00275 (Table 3). The average of genetic distances between species of *P. amabilis* and *P. aphrodite* was 0.00714. Based on the genetic distances of ITS sequences, *P. amabilis* was closer to *P. sanderiana* (at 0.00780 between both species) than to *P. aphrodite* (at 0.01065 between *P. aphrodite* and *P. sanderiana*). In addition, the average differentiation coefficient between species of *P. amabilis* and *P. aphrodite* was 0.4995. That between species of *P. amabilis* and *P. sanderiana* as well as that between the species of *P. aphrodite* and *P. sanderiana* were 0.3082 and 0.5366, respectively. Furthermore, the genetic distances among the 13 different populations/subspecies/species of the *P. amabilis* complex are shown in Table 4. Within the 13 different populations/subspecies/species of the *P. amabilis* complex, *P. sanderiana*

and *P. amabilis* subsp. *rosenstromii* (or *P. amabilis* Timor population) had a close relationship (with 0.0055 for the average of genetic distances) according to the genetic distance. This result is partially supported based on the morphological characters, in which *P. sanderiana* and *P. amabilis* subsp. *rosenstromii* share similar morphologies in having a lip with a triangular midlobe, which is not shown in other subspecies/populations of *P. amabilis* (cf. Sweet, 1980). However, the results are incongruent with the geographical relationship between *P. sanderiana* and *P. amabilis* subsp. *rosenstromii*, since they are far away from each other. In contrast, *P. sanderiana* was more divergent with *P. aphrodite* from Calayan due to having a higher genetic distance (0.0157) than between it and each of the remaining members of the *P. amabilis* complex. *Phalaenopsis aphrodite* subsp. *formosana* was shown to be more divergent to plants of *P. amabilis* native to Java due to having a higher genetic distance (0.0146) than between it and each of the remaining members of the *P. amabilis* complex. In addition, both *P. amabilis* subsp. *moluccana* and *P. amabilis* subsp. *rosenstromii* had a close relationship to plants of *P. amabilis* native to Timor. In contrast, those two subspecies of *P. amabilis* were more divergent from *P. amabilis* native to Java.

Phylogenetic reconstruction

The phylogenetic tree constructed following the minimum evolution method is shown in Fig. 4. Based on the phylogenetic tree, three species of the *P. amabilis* complex were very close. Neither of the species of *P. aphrodite* and *P. sanderiana* could be separated from *P. amabilis* based on ITS sequences. In addition, the 15 accessions of *P. aphrodite* collected from different locations were very close to each other. In addition, *P. aphrodite* subsp. *formosana* also could not be separated from *P. aphrodite* based on ITS sequences. As for *P. sanderiana*, three accessions of this species formed a clade, but it was nested within the species of *P. amabilis*. Furthermore, accessions of *P. amabilis* showed high genetic variations based on genetic distances of ITS sequences, which ranged from 0.000 to 0.013. Different subspecies/populations of *P. amabilis*, with two exceptions of both the Palawan and Sabah populations, examined in the present study could be separated from each other. In addition, three accessions of *P. amabilis* subsp. *moluccana* formed a clade and were separated from the other accessions of *P. amabilis*. Furthermore, each of the subspecies/species of *P. amabilis* subsp. *rosenstromii* and *P. amabilis* collected from Mentawai Is. formed different separate clades. Neither population of *P. amabilis* collected from Palawan and Sabah could be separated from each other based on ITS sequences.

Furthermore, I found that plants of *P. amabilis* native to Palawan, the Philippines were closer to *P. aphrodite* based on the phylogenetic tree. Specially, the ITS sequence of *P. amabilis* distributed in Timor was identical to that of *P. amabilis* subsp. *rosenstromii*. This result indicates that plants of *P. amabilis* from Timor have close relationship to *P. amabilis* subsp. *rosenstromii*. Furthermore, accessions of *P. amabilis* collected from Palawan had a sister relationship to the accessions of both *P. aphrodite* and its subspecies. In addition, *P. amabilis* subsp. *moluccana* and *P. amabilis* subsp. *rosenstromii* were not quite divergent to the other populations of *P. amabilis*. In contrast, the Java population of *P. amabilis* was the most singular population among the seven groups of *P. amabilis*.

Based on an inspection of the morphology, the undersurface color of the leaves did not show the purplish suffusion in *P. aphrodite*. This result is in agreement with the inspection of both species of *P. amabilis* and *P. aphrodite* by Christenson (2001). Therefore, the concept of Sweet (1980), who had described the difference of the undersurface color of leaves between *P. amabilis* and *P. aphrodite*, is not supported. In contrast, I discovered that *P. amabilis* collected from Timor showed a light purplish suffusion over the leaves, and *P. sanderiana* showed a heavy purplish suffusion on the undersurface of leaves (data not shown). Therefore, the characteristic showing the purplish/green undersurface of leaves is not a good marker to separate *P. aphrodite* from *P. amabilis*. Although *P. aphrodite* from Taiwan has been treated as a subspecies of *P. aphrodite*, namely *P. aphrodite* subsp. *formosana*, based on having apple-green leaves with no trace of anthocyanin pigments, somewhat smaller flowers, and much-branched panicles that typically produce side branches from even the most basal nodes on the inflorescence (Christenson, 2001). However, *Phalaenopsis aphrodite* and its subspecies (the Taiwanese population) also could not be separated from each other based on ITS sequences. This result reveals two available alternatives: (1) the molecular markers from ITS sequences are not a good marker to separate *P. aphrodite* from its subspecies; or (2) *P. aphrodite* subsp. *formosana* should be treated as a synonym of *P. aphrodite*. For understanding the relationship between *P. aphrodite* and its subspecies, therefore, *P. aphrodite* subsp. *formosana*, other molecular markers should be used in the future.

Within the 39 accessions of the *P. amabilis* complex, the Java population of *P. amabilis* was shown to be a group unique from the remaining members of this complex in this study. Nevertheless, I cannot suggest that the Java population should be treated as a separate variety/subspecies of *P. amabilis*, since the type species of *P. amabilis* was collected from Java as described by Linnaeus in 1753 (cf. Christenson 2001). In addition,

P. sanderiana was very close to *P. amabilis* in the present study. This result does not support *P. sanderiana* being treated as a separate species. In contrast, this study supported the treatment of Davis in 1949, which treated *P. sanderiana* as a variety of *P. amabilis*, namely *P. amabilis* var. *sanderiana* (cf. Sweet, 1969b). In addition, the average genetic distances between the accessions of *P. amabilis* and *P. aphrodite* (of 0.00714) fell into those ranges (from 0.000 to 0.013) among the accessions of *P. amabilis*. Thus, this study does not support *P. aphrodite* being treated as a separate species from *P. amabilis* as done by Sweet (1969a, 1980) and Christenson (2001). Like the above discussion, there are two available alternatives: (1) a broad species concept in which *P. aphrodite* and *P. sanderiana* should be treated as different varieties/subspecies of *P. amabilis*; or (2) a narrow species concept in which *P. aphrodite* and *P. sanderiana* are retained as separate species from *P. amabilis*, while different locations of *P. amabilis* (with the exception of the Java population) will have to be raised to separate species from *P. amabilis*. Since the distributions and genetic variations of *P. amabilis* are so variable, I therefore prefer to accept that *P. aphrodite* and *P. sanderiana* should be treated as different varieties/subspecies of *P. amabilis*.

Biogeography and evolutionary trends

The evolutionary phylogenetic tree of the *P. amabilis* complex plus three outgroups, namely the *P. schilleriana* complex, was constructed and is shown in Fig. 4. According to this tree, *P. aphrodite* is located at the basal branch of the *P. amabilis* complex. Therefore, I suggest that *P. aphrodite* is the relative origin group of the *P. amabilis* complex. In order to discuss the evolutionary trend of the *P. amabilis* complex, the evolutionary phylogenetic tree was further compressed using accessions of consistent subspecies/locations and was rooted based on the relative origin group, *P. aphrodite* plus its subspecies. This compressed and rooted tree is shown in Fig. 5. Plants of *P. amabilis* native to Palawan were shown to be intermediate between plants of *P. aphrodite* and *P. amabilis* distributed in Sabah. Based on the genetic distances among 13 different populations/subspecies/species of the *P. amabilis* complex studied, *P. amabilis* distributed in Sabah (a part of Borneo) has a very close relationship to that of Palawan, since they have a genetic distance of 0.0014. Furthermore, the genetic distance between *P. aphrodite* and *P. amabilis* from Palawan is smaller than that between *P. aphrodite* and the remaining *P. amabilis*. This result indicates that Palawan was a steppingstone when species of the *P. amabilis* complex dispersed from the Philippines to Borneo. In addition, both species of *P.*

amabilis and *P. sanderiana* were descended from *P. aphrodite* (or their most recent common ancestor) according to the rooted evolutionary phylogenetic tree (Fig. 5).

Based on the distribution of the section *Phalaenopsis* of the present time (Christenson, 2001), the historical geology (Karig et al., 1986; Stephan et al., 1986; Hall, 1996; Aurelio et al., 1991; Quebral et al., 1994), and the evolutionary trend of genus *Phalaenopsis* (Tsai, see chapter 1), the Philippines was suggested to be the origin center of this section of *Phalaenopsis* (Tsai, see chapter 1). At the present time, *P. aphrodite* is distributed throughout the Philippines with the exceptions of Palawan and southern Mindanao. Therefore, *P. aphrodite* could have dispersed over most islands of the Philippines, since those islands were interconnected during glacial periods (Van Oosterzee, 1997) (Fig. 2). According to the evolutionary phylogenetic tree (Fig. 5), the plants of the *P. amabilis* complex native to Palawan/Borneo descended from *P. aphrodite* (or their most recent common ancestor). When *P. aphrodite* dispersed into Palawan, *P. amabilis* evolved and developed. Since the land bridge between Palawan and Borneo might have formed during the historical geology of Pleistocene times (about 0.01~1.8 Mya) (Van Oosterzee, 1997), *P. amabilis* distributed in Palawan had a chance to disperse into Borneo. Therefore, *P. amabilis* might have dispersed into Borneo during 0.01~1.8 Mya. To the present, *P. amabilis* distributed in Palawan still has a close relationship to *P. amabilis* distributed in Borneo based on molecular data. Thus, the movement of *P. amabilis* from Palawan to Borneo was a recent dispersal event.

Based on the evolutionary phylogenetic tree (Fig. 5), *P. amabilis* subsp. *moluccana*, *P. amabilis* subsp. *rosenstromii*, and *P. sanderiana* share the most recent common ancestor. *Phalaenopsis amabilis* subsp. *moluccana* might have evolved from *P. amabilis* subsp. *rosenstromii* or *P. sanderiana* (or their most recent common ancestor). The results indicate that *P. amabilis* subsp. *moluccana* did not descend from *P. amabilis* distributed in Borneo. In particular, *P. sanderiana* is distributed in southern Mindanao, the Philippines, while *P. amabilis* subsp. *moluccana* is restricted to regions of Sulawesi and Molucca Is. Although *P. sanderiana* and *P. amabilis* subsp. *moluccana* are distributed in two distinct regions, they have a close relationship. This result is in agreement with previous research, which discovered that *Phalaenopsis* species distributed in the Philippines have close relationships to those of Sulawesi (Tsai, see chapter 1). Based on historical geology, most lands of the Philippines with the exception of older lands (namely Mindoro, Palawan, Zamboanga, etc.) and the northeastern part of Sulawesi are located at the southern edge of the Philippine Sea Plate. When the Indian-Australian plate collided northward with both the Philippine Sea

Plate and Eurasian Plate, those submerged islands of the Philippines migrated northward, while the northern part of Sulawesi moved toward western Sulawesi (Hall, 1996). The cessation of this migration processing was recent (< 5 Mya) (Aurelio et al., 1991; Quebral et al., 1994), after young lands of the Philippines began developing. In addition, several archipelagoes in the northern part of Sulawesi have been found to extend towards Mindanao, the Philippines based on the present geology. Therefore, the archipelagoes might have formed a land bridge and offered opportunities for species to cross from the Philippines to Sulawesi during glacial times.

According to the biogeography of Southeast Asia from a former report, species of both flora and fauna show much divergence between western and eastern regions of Wallace's Line (Van Oosterzee, 1997). *Phalaenopsis amabilis* is distributed in the area west of Wallace's Line (e.g., Palawan and Borneo), while *P. amabilis* subsp. *moluccana* and *P. amabilis* subsp. *rosenstromii* are distributed in the area east of that (Sulawesi, Molucca Is., and New Guinea). Biogeographical differences between the two distribution regions of both subspecies, namely Sulawesi (*P. amabilis* subsp. *moluccana)* and New Guinea (*P. amabilis* subsp. *rosenstromii*), have been shown. The result is in agreement with the phytogeographical break described by Weber (cf. Pianka, 1994). However, another distribution area of *P. amabilis* subsp. *moluccana* is Molucca Is., which is located in the area east of Weber's Line. Therefore, the biogeographical distribution of *P. amabilis* subsp. *moluccana* on Molucca Is. is not in agreement with the phytogeographical break based on the concept of Weber. Since Sulawesi and Molucca Is. also share the same distribution of *P. amboinensis* based on a previous study of biogeographies of the genus *Phalaenopsis* (Tsai, see chapter 1), therefore, the biogeographical relationship of Sulawesi and Molucca Is. is shown to be close based on biogeographical data of the genus *Phalaenopsis*. Nevertheless, whether Weber's Line should be shifted toward the east to exclude the Molucca Is. is uncertain.

In particular, *P. amabilis* from Timor has a close relationship to *P. amabilis* subsp. *rosenstromii* (distributed in New Guinea and Australia). Plants of the *P. amabilis* complex distributed in both regions might be the same taxon based on the molecular data. *Phalaenopsis amabilis* subsp. *rosenstromii* might have crossed from New Guinea to Timor using the archipelagoes between both regions during glacial times. Based on the geographical distributions, plants of the *P. amabilis* complex native to Mentawai Is. (a few islands of western Sumatra) are quite different, since plants of this complex have not been found on Sumatra in present times (Sweet, 1980; Christenson, 2001). In addition, plants

native to both Java and Borneo are far from Mentawai Is. (Fig. 1). Therefore, the origin of plants of the *P. amabilis* complex distributed in Mentawai Is. is curious. Since *P. amabilis* distributed on Mentawai Is. had a close genetic relationship to that on Borneo based on molecular data (Table 4; Fig. 4), I therefore suggest that *P. amabilis* might have dispersed into Sumatra from Borneo in ancient times (perhaps in Pleistocene times). An unknown reason, for instance, a natural impact (e.g., a volcanic eruption), climate change, geology, illness, etc. caused the disappearance of plants of *P. amabilis* distributed in Sumatra. In addition, it is amazing to me that plants of the *P. amabilis* complex native to Java might have descended from *P. amabilis* subsp. *moluccana*/*P. amabilis* subsp. *rosenstromii* (or their most recent common ancestor) according to the evolutionary phylogenetic tree. This result is incongruent with the historical geology of Java, which was a part of the Sunda Shelf (Van Oosterzee, 1997), as well as biogeographies of the genus *Phalaenopsis* (Tsai, see chapter 1). Based on the callus structure and the shape of the midlobe of the lips, the plants of *P. amabilis* native to Java are similar to those in Borneo. Therefore, there are two available alternatives: (1) *P. amabilis* native to Java evolved from *P. amabilis* subsp. *moluccana*/*P. amabilis* subsp. *rosenstromii*, and convergent evolution occurred between the population of *P. amabilis* distributed in Borneo and that on Java; or (2) the population of *P. amabilis* distributed in Java might have been derived from reticulated evolution between both lineages, namely *P. amabilis* from Palawan/Borneo and *P. amabilis* subsp. *moluccana*/*P. amabilis* subsp. *rosenstromii*.

Conclusions

This study supports *P. amabilis*, *P. aphrodite*, and *P. sanderiana* having descended from the most recent common ancestor, as was suggested by Fowlie (1981). *Phalaenopsis sanderiana* is closer to the subspecies of *P. amabilis*, namely *P. amabilis* subsp. *rosenstromii*, instead of *P. aphrodite*. In addition, neither *P. sanderiana* nor *P. aphrodite* shows much divergence with *P. amabilis*. Therefore, I prefer to treat *P. sanderiana* and *P. aphrodite* as different varieties/subspecies of *P. amabilis*. *Phalaenopsis aphrodite* was the relative origin group of the *P. amabilis* complex and developed in the Philippines. It is suggested that *P. amabilis* and *P. sanderiana* descended from *P. aphrodite* (or their most recent common ancestor). The evolutionary trend of the *P. amabilis* complex included two different lineages corresponding to different dispersal pathways. First, *P. aphrodite* dispersed into Palawan and evolved into *P. amabilis* there, thereafter further dispersing

into Borneo. Second, *P. aphrodite* 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* respectively developed. *Phalaenopsis amabilis* subsp. *rosenstromii* further dispersed into Northern Australia and Timor. Therefore, *P. sanderiana*, *P. amabilis* subsp. *moluccana*, and *P. amabilis* subsp. *rosenstromii*, sharing the same lineage, are separated from *P. amabilis* distributed in Palawan/Borneo, etc. Finally, the evolutionary trend of the *P. amabilis* complex was redrawn on the map of biogeographical distribution and is shown in Fig. 6.

Literature Cited

Aurelio, M. A., E. Barrier, C. Rangin, and C. Muller. 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.

Christenson, E. A. 2001. *Phalaenopsis*. Timber Press, Portland, Oregon. 330p.

Clements, M. A. 1989. Catalogue of Australian Orchidaceae. Austral. Orch. Res. 1: 1-160.

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

Edwards, A. W. F. and L. L. Cavalli-Sforza. 1963. The reconstruction of evolution. Heredity 18: 553.

Fowlie, J. A. 1981. Speciation amongst the Orchidaceae as a function of climate change and topophysiography. Orch. Dig. 45: 44-49.

Fowlie, J. A. and J. Miller. 1974. Notes on the distribution of *Phalaenopsis* in the Philippines – with a useful climate summary Part III. Orch. Dig. 38: 219-221.

Hall, R. 1996. Reconstructing Cenozoic SE Asia. In: Hall, R. and Blundell, D. J. (eds.) Tectonic Evolution 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.

Karig, D. E., D. R. Sarewitz, and G. D. Iiaeck. 1986. Role of strike-slip faulting in the evolution of allochthonous terraces in the Philippines. Geology 14: 852-855.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution

through comparative studies of nucleotide sequences. Mol. Evol. 16: 111-120.

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

Miwa, A. 1941. *Phalaenopsis* of Formosa. Pract. Hort. 27: 113-120.

Moss, S. J., and M. E. J. Wilson. 1998. Biogeographic implications from the Tertiary Paleaeogeographic evolution of Sulawesi and Borneo. *In* Hall, R. and Holloway, J. D. (eds.). Biogeography and geological evolution of SE Asia. Backhuys, Leiden.

Pianka, E. R. 1994. Biogeography and Historical Constraints. *In* Evolutionary Ecology. 5th edition. HarperCollins College Publisher, NY, pp. 15-40.

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

Rzhetsky, A. and M. Nei. 1992. A simple method for estimating and testing minimum-evolution trees. Mol. Biol. Evol. 9: 945-967.

Rzhetsky, A. and M. Nei. 1993. Theoretical foundation of the minimum-evolution method of phylogenetic inference. Mol. Biol. Evol. 10: 1073-1095.

Sitnikova, T., A. Rzhetsky, M. Nei. 1995. Interior-branch and bootstrap tests of phylogenetic trees. Mol. Biol. Evol. 12: 319-333.

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

Sweet, H. R. 1969a. A revision of the genus *Phalaenopsis*-VII. Amer. Orchid Soc. Bull. 38: 681-694.

Sweet, H. R. 1969b. A revision of the genus *Phalaenopsis*-VIII. Am. Orchid Soc. Bull. 38: 888-901.

Sweet, H. R. 1980. The genus *Phalaenopsis*. The Orchid Digest, Pomona, California.

Tsai, C. C., S. C. Huang, P. L. Huang, and C. H. Chou. 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: 867-879.

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

Wallbrunn, H. M. 1971. A second look at the “Revision of the genus *Phalaenopsis*.” Am. Orchid Soc. Bull. 40: 223-231.

Table 1. A list of the 39 accessions of the *Phalaenopsis amabilis* complex, namely *P. amabilis*, *P. aphrodite*, and *P. sanderiana*, and their different geographical distributions.

Taxa and systematic classification [a]	Distribution	Source [b]
P. amabilis 'Java'	Java, Indonesia	KDAIS-KC66
P. amabilis 'Java'	Java, Indonesia	KDAIS-KC96
P. amabilis 'Java'	Java, Indonesia	KDAIS-KC97
P. amabilis 'Mentawai Is.'	Mentawai Is., Indonesia	KDAIS-KC238
P. amabilis 'Mentawai Is.'	Mentawai Is., Indonesia	KDAIS-KC239
P. amabilis 'Mentawai Is.'	Mentawai Is., Indonesia	KDAIS-KC240
P. amabilis 'Palawan'	Palawan, the Philippines	KDAIS-KC91
P. amabilis 'Palawan'	Palawan, the Philippines	KDAIS-KC92
P. amabilis 'Palawan'	Palawan, the Philippines	KDAIS-KC93
P. amabilis 'Sabah'	Sabah, Indonesia	KDAIS-KC327
P. amabilis 'Sabah'	Sabah, Indonesia	KDAIS-KC342
P. amabilis 'Sabah'	Sabah, Indonesia	KDAIS-KC444
P. amabilis 'Timor'	East Timor	KDAIS-KC254
P. amabilis 'Timor'	East Timor	KDAIS-KC343
P. amabilis subsp. *moluccana*	Molucca Is., Indonesia	KDAIS-KC248
P. amabilis subsp. *moluccana*	Molucca Is., Indonesia	KDAIS-KC249
P. amabilis subsp. *moluccana*	Molucca Is., Indonesia	KDAIS-KC319
P. amabilis subsp. *rosenstromii*	New Guinea	KDAIS-KC94
P. amabilis subsp. *rosenstromii*	New Guinea	KDAIS-KC95
P. amabilis subsp. *rosenstromii*	New Guinea	KDAIS-KC260
P. amabilis subsp. *rosenstromii*	New Guinea	KDAIS-KC329
P. aphrodite	Mindanao, the Philippines	KDAIS-KC172
P. aphrodite	Mindanao, the Philippines	KDAIS-KC173
P. aphrodite	Mindanao, the Philippines	KDAIS-KC174
P. aphrodite	Luzon, the Philippines	KDAIS-KC419
P. aphrodite	Luzon, the Philippines	KDAIS-KC420
P. aphrodite	Luzon, the Philippines	KDAIS-KC421
P. aphrodite ''Fuga Is.'	Fuga Is., the Philippines	KDAIS-KC171
P. aphrodite 'Calayan Is.'	Calayan Is., the Philippines	KDAIS-KC169
P. aphrodite 'Calayan Is.'	Calayan Is., the Philippines	KDAIS-KC181
P. aphrodite subsp. *formosana*	Taiwan	KDAIS-KC179
P. aphrodite subsp. *formosana*	Taiwan	KDAIS-KC180
P. aphrodite subsp. *formosana*	Taiwan	KDAIS-KC198
P. aphrodite subsp. *formosana*	Taiwan	KDAIS-KC199
P. aphrodite subsp. *formosana*	Taiwan	KDAIS-KC202
P. aphrodite subsp. *formosana*	Taiwan	KDAIS-KC253
P. sanderiana	the Philippines	KDAIS-KC35
P. sanderiana	the Philippines	KDAIS-KC175
P. sanderiana	the Philippines	KDAIS-KC176

[a] The classification of *Phalaenopsis* is based on Christenson (2001).
[b] Kaohsiung District Agricultural Improvement Station.

Table 2. Lengths of ITS1 and ITS2 and GenBank accession nos. of the 39 accessions of the *Phalaenopsis amabilis* complex.

Taxa [a]	ITS1		ITS2		GenBank accession no.
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	
P. amabilis-Java-kc-66	230	77.4	256	76.1	AY391515
P. amabilis-Java-kc-96	230	77.4	256	76.1	AY391516
P. amabilis-Java-kc-97	230	77.4	256	76.1	AY391517
P. amabilis-Mentawai Is.-kc-238	230	77.0	257	77.1	AY391518
P. amabilis-Mentawai Is.-kc-239	230	77.0	257	77.1	AY391519
P. amabilis-Mentawai Is.-kc-240	229	76.9	257	77.1	AY391520
P. amabilis-Palawan-kc-91	229	76.8	257	77.5	AY391521
P. amabilis-Palawan-kc-92	229	77.3	257	77.1	AY391522
P. amabilis-Palawan-kc-93	229	76.8	257	77.1	AY391523
P. amabilis-Sabah-kc-327	229	76.8	257	77.1	AY391524
P. amabilis-Sabah-kc-342	230	77.0	257	77.1	AY391525
P. amabilis-Sabah-kc-444	229	76.8	257	77.1	AY391526
P. amabilis-Timor-kc-254	232	77.2	257	77.1	AY391527
P. amabilis-Timor-kc-343	232	77.2	257	77.1	AY391528
P. amabilis subsp. *moluccana*-kc-248	233	77.2	257	77.1	AY391529
P. amabilis subsp. *moluccana*-kc-249	233	77.2	257	77.1	AY391530
P. amabilis subsp. *moluccana*-kc-319	230	77.4	257	77.1	AY391531
P. amabilis subsp. *rosenstromii*-kc-94	229	76.9	258	77.1	AY391532
P. amabilis subsp. *rosenstromii*-kc-95	229	76.9	258	77.1	AY391533
P. amabilis subsp. *rosenstromii*-kc-260	229	76.9	258	77.1	AY391534
P. amabilis subsp. *rosenstromii*-kc-329	229	76.9	258	77.1	AY391535
P. aphrodite-Mindanao-kc-172	229	77.3	257	77.5	AY391536
P. aphrodite-Mindanao-kc-173	229	77.3	257	77.9	AY391537
P. aphrodite-Mindanao-kc-174	229	77.3	257	77.5	AY391538
P. aphrodite-Luzon-kc-419	229	77.3	257	77.5	AY391539
P. aphrodite-Luzon-kc-420	229	77.3	257	77.5	AY391540
P. aphrodite-Luzon-kc-421	229	77.3	257	77.5	AY391541
P. aphrodite-Fuga Is.-kc-171	229	77.3	257	77.5	AY391542
P. aphrodite-Calayan Is.-kc-169	231	77.0	257	77.5	AY391543
P. aphrodite-Calayan Is.-kc-181	228	77.2	257	77.1	AY391544
P. aphrodite subsp. *formosana*-kc-179	229	77.3	257	77.5	AY391545
P. aphrodite subsp. *formosana*-kc-180	229	77.3	257	77.5	AY391546
P. aphrodite subsp. *formosana*-kc-198	231	77.5	257	77.5	AY391547
P. aphrodite subsp. *formosana*-kc-199	232	77.6	257	77.5	AY391548
P. aphrodite subsp. *formosana*-kc-202	229	77.3	258	77.5	AY391549
P. aphrodite subsp. *formosana*-kc-253	230	77.4	257	77.5	AY391550
P. sanderiana-kc-35	230	76.9	257	77.1	AY391551
P. sanderiana-kc-175	231	76.7	257	77.1	AY391552
P. sanderiana-kc-176	230	76.5	257	77.1	AY391553

Table 3. The genetic distance matrix of the Kimura two-parameter method among the 39 accessions of the *Phalaenopsis amabilis* complex based on ITS1 and ITS2 of nrDNA.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39]
[1]*																																							
[2]	0.000																																						
[3]	0.000	0.000																																					
[4]	0.012	0.012	0.012																																				
[5]	0.012	0.012	0.012	0.000																																			
[6]	0.012	0.012	0.012	0.000	0.000																																		
[7]	0.012	0.012	0.012	0.004	0.004	0.004																																	
[8]	0.012	0.012	0.012	0.004	0.004	0.004	0.004																																
[9]	0.010	0.010	0.010	0.002	0.002	0.002	0.002	0.002																															
[10]	0.010	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.000																														
[11]	0.010	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.000	0.000																													
[12]	0.010	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000																												
[13]	0.008	0.008	0.008	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.002	0.002																											
[14]	0.008	0.008	0.008	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.002	0.002	0.000																										
[15]	0.012	0.012	0.012	0.006	0.006	0.006	0.006	0.006	0.004	0.004	0.004	0.004	0.002	0.002																									
[16]	0.012	0.012	0.012	0.006	0.006	0.006	0.006	0.006	0.004	0.004	0.004	0.004	0.002	0.002	0.000																								
[17]	0.010	0.010	0.010	0.006	0.006	0.006	0.006	0.006	0.004	0.004	0.004	0.004	0.002	0.002	0.000	0.000																							
[18]	0.008	0.008	0.008	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.002	0.002	0.000	0.000	0.002	0.002	0.002																						
[19]	0.008	0.008	0.008	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.002	0.002	0.000	0.000	0.002	0.002	0.002	0.000																					
[20]	0.008	0.008	0.008	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.002	0.002	0.000	0.000	0.002	0.002	0.002	0.000	0.000																				
[21]	0.008	0.008	0.008	0.004	0.004	0.004	0.004	0.004	0.002	0.002	0.002	0.002	0.000	0.000	0.002	0.002	0.002	0.000	0.000	0.000																			
[22]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006																		
[23]	0.017	0.017	0.017	0.008	0.008	0.008	0.004	0.004	0.006	0.006	0.006	0.006	0.008	0.008	0.010	0.010	0.010	0.008	0.008	0.008	0.008	0.002																	
[24]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002																
[25]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000															
[26]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000														
[27]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000													
[28]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000												
[29]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.006	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000	0.000											
[30]	0.017	0.017	0.017	0.008	0.008	0.008	0.004	0.004	0.006	0.006	0.006	0.006	0.008	0.008	0.010	0.010	0.010	0.008	0.008	0.008	0.008	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.002									
[31]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002								
[32]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000							
[33]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000						
[34]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000					
[35]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000				
[36]	0.015	0.015	0.015	0.006	0.006	0.006	0.002	0.002	0.004	0.004	0.004	0.004	0.006	0.006	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000			
[37]	0.017	0.017	0.017	0.008	0.008	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.008	0.008	0.010	0.010	0.010	0.008	0.008	0.008	0.008	0.010	0.012	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.013	0.010	0.010	0.010	0.010	0.010	0.010		
[38]	0.012	0.012	0.012	0.008	0.008	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.004	0.004	0.006	0.006	0.006	0.004	0.004	0.004	0.004	0.010	0.012	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.010	0.010	0.010	0.010	0.010	0.010	0.004	
[39]	0.012	0.012	0.012	0.008	0.008	0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.004	0.004	0.006	0.006	0.006	0.004	0.004	0.004	0.004	0.010	0.012	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.010	0.010	0.010	0.010	0.010	0.010	0.004	0.000

*[1], P. amabilis-Java-kc-66; [2], P. amabilis-Java-kc-96; [3], P. amabilis-Java-kc-97; [4], P. amabilis-Mentawai Is.-kc-238; [5], P. amabilis-Mentawai_Is.-kc-239; [6], P. amabilis-Mentawai_Is.-kc-240; [7], P. amabilis-Palawan-kc-91; [8], P. amabilis-Palawan-kc-92; [9], P. amabilis-Palawan-kc-93; [10], P. amabilis-Sabah-kc-327; [11], P. amabilis-Sabah-kc-342; [12], P. amabilis-Sabah-kc-444; [13], P. amabilis-Timor-kc-254; [14], P. amabilis-Timor-kc-343; [15], P. amabilis subsp. moluccana-kc-248; [16], P. amabilis subsp. moluccana-kc-249; [17], P. amabilis subsp. moluccana-kc-319; [18], P. amabilis subsp. rosenstromii-kc-94; [19], P. amabilis subsp. rosenstromii-kc-95; [20], P. amabilis subsp. rosenstromii-kc-260; [21], P. amabilis subsp. rosenstromii-kc-329; [22], P. aphrodite-Mindanao-kc-172; [23], P. aphrodite-Mindanao-kc-173; [24], P. aphrodite-Mindanao-kc-174; [25], P. aphrodite-Luzon-kc-419; [26], P. aphrodite-Luzon-kc-420; [27], P. aphrodite-Luzon-kc-421; [28], P. aphrodite-Fuga_Is.-kc-171; [29], P. aphrodite-Calayan_Is.-kc-169; [30], P. aphrodite-Calayan_Is.-kc-181; [31], P. aphrodite subsp. formosana-kc-179; [32], P. aphrodite subsp. formosana-kc-180; [33], P. aphrodite subsp. formosana-kc-198; [34], P. aphrodite subsp. formosana-kc-199; [35], P. aphrodite subsp. formosana-kc-202; [36], P. aphrodite subsp. formosana-kc-253; [37], P. sanderiana-kc-35; [38], P. sanderiana-kc-175; [39], P. sanderiana-kc-176.

Table 4. Genetic distances among 13 inter-populations/subspecies/species of the *Phalaenopsis amabilis* complex based on ITS1 and ITS2 of nrDNA.

	P. amabilis -Java	*P. amabilis* -Mentawai Is.	*P. amabilis* -Palawan	*P. amabilis* -Sabah	*P. amabilis* -Timor	*P. amabilis* subsp. *moluccana*	*P. amabilis* subsp. *rosenstromii*	*P. aphrodite* -Mindanao	*P. aphrodite* -Luzon	*P. aphrodite* -Fuga Is.	*P. aphrodite* -Calayan Is.	*P. aphrodite* subsp. *formosana*
P. amabilis -Java												
P. amabilis -Mentawai Is.	0.0125											
P. amabilis -Palawan	0.0118	0.0034										
P. amabilis -Sabah	0.0104	0.0021	0.0014									
P. amabilis -Timor	0.0083	0.0041	0.0034	0.0021								
P. amabilis subsp. *moluccana*	0.0118	0.0062	0.0055	0.0041	0.0021							
P. amabilis subsp. *rosenstromii*	0.0083	0.0041	0.0034	0.0021	0.0000	0.0021						
P. aphrodite -Mindanao	0.0153	0.0069	0.0034	0.0048	0.0069	0.0090	0.0069					
P. aphrodite -Luzon	0.0146	0.0062	0.0028	0.0041	0.0062	0.0083	0.0062	0.0007				
P. aphrodite -Fuga Is.	0.0146	0.0062	0.0028	0.0041	0.0062	0.0083	0.0062	0.0007	0.0000			
P. aphrodite -Calayan Is.	0.0157	0.0072	0.0038	0.0055	0.0072	0.0093	0.0073	0.0017	0.0010	0.0010		
P. aphrodite subsp. *formosana*	0.0146	0.0062	0.0028	0.0041	0.0062	0.0083	0.0062	0.0007	0.0000	0.0000	0.0010	
P. sanderiana	0.0139	0.0083	0.0076	0.0062	0.0055	0.0076	0.0055	0.0111	0.0104	0.0104	0.0114	0.0104

Fig. 1

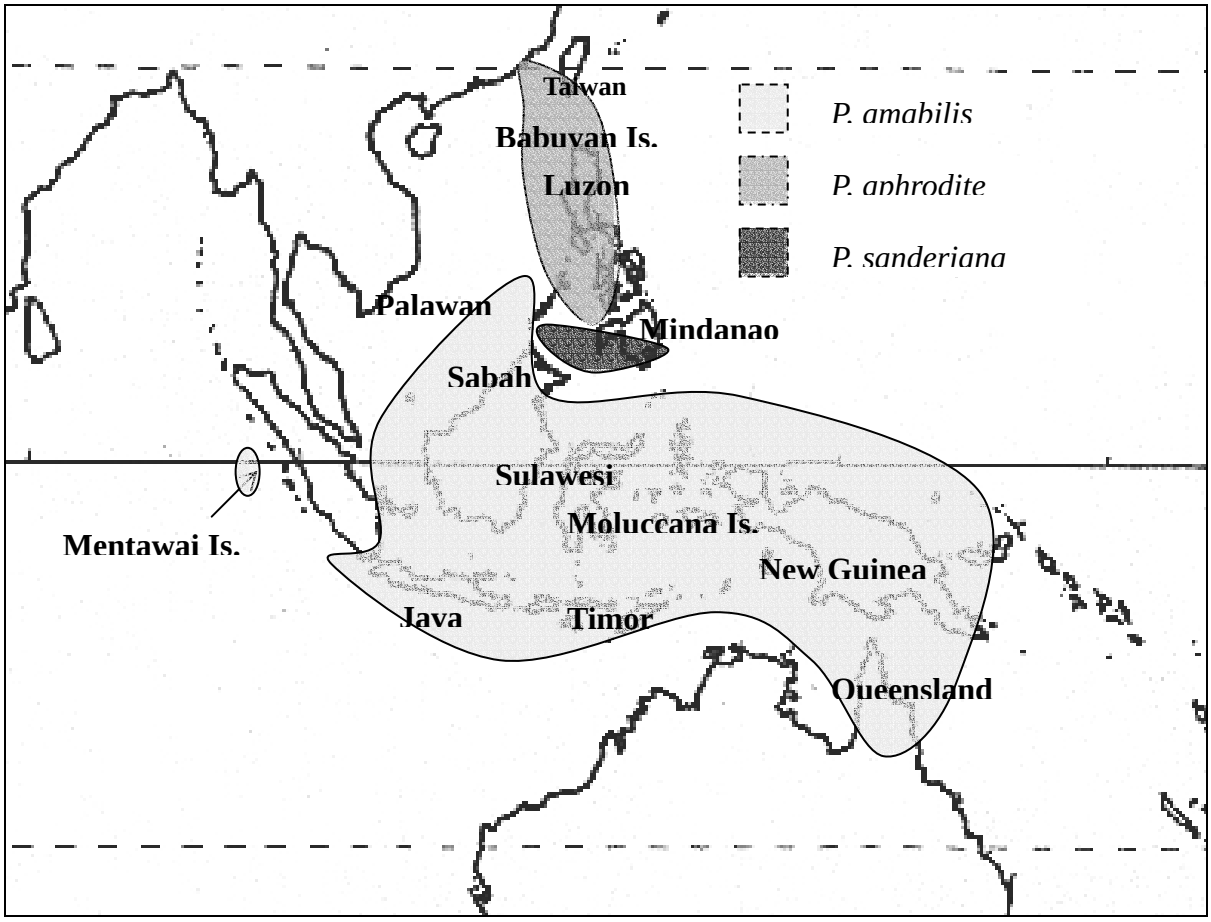


Fig. 1. Geographical distributions of *Phalaenopsis amabilis*, *P. aphrodite*, and *P. sanderiana*.

Fig. 2.

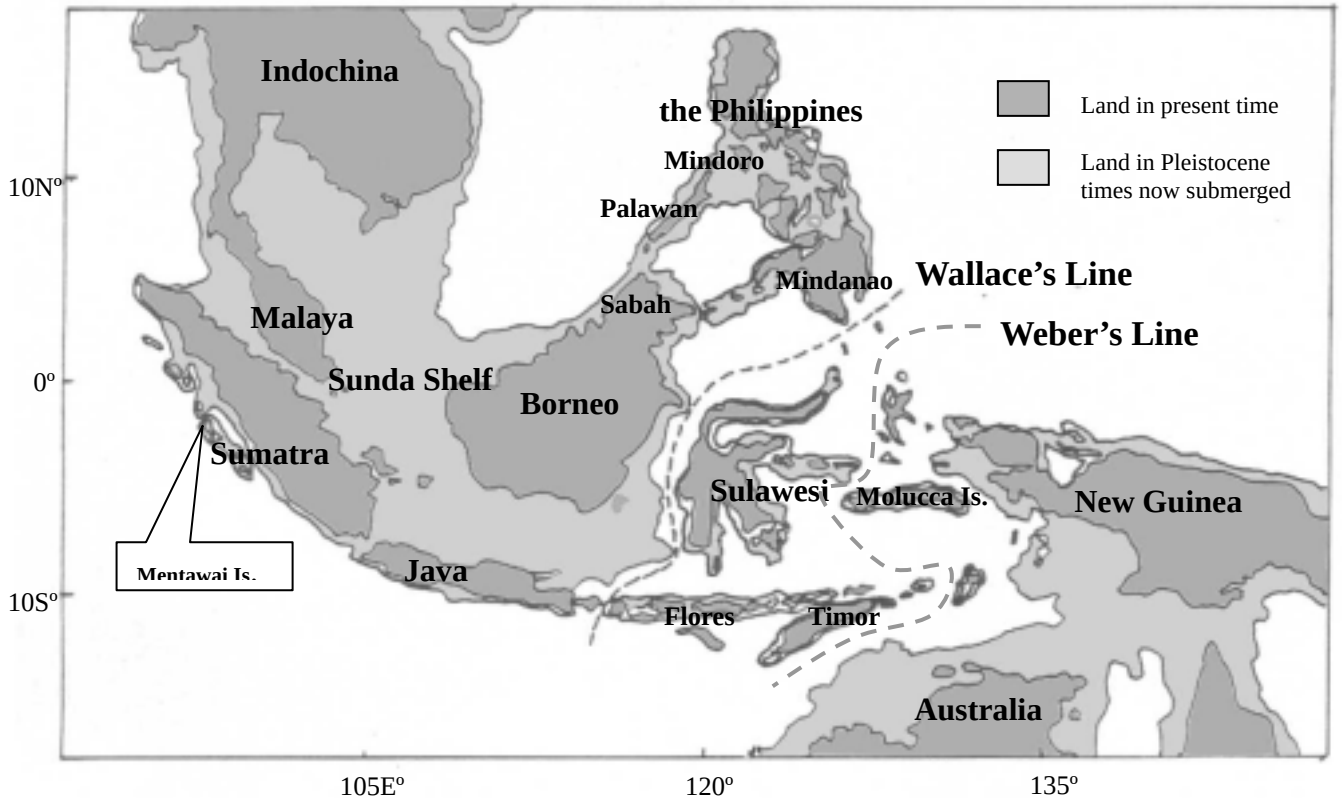
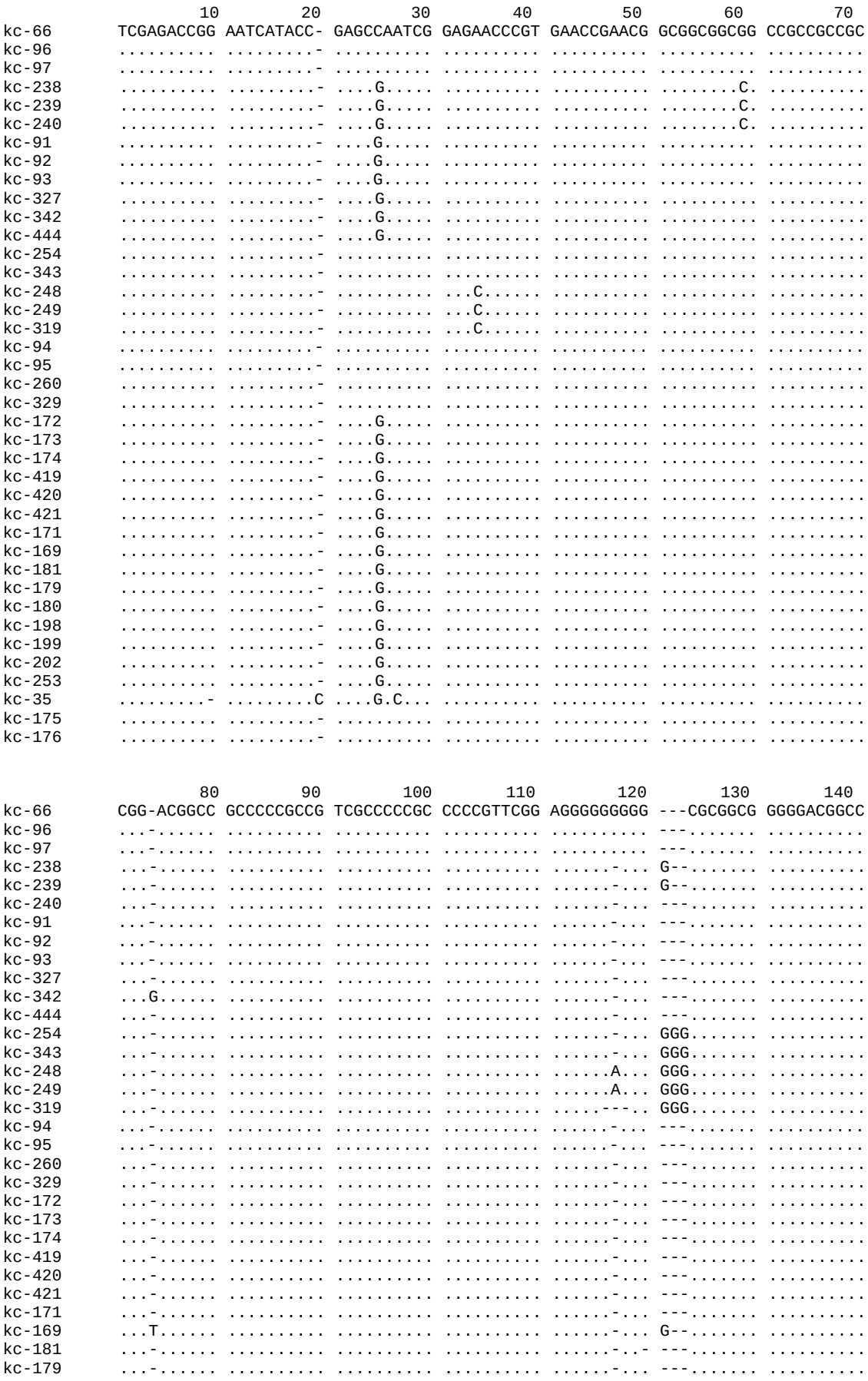


Fig. 2. Comparison of Southeast Asia lands between Pleistocene times and the present time. Indochina, Malaya, Sumatra, Java, Borneo, and the Philippines were interconnected and separated from Sulawesi by the Makassar Strait in Pleistocene times. (modified from Van Oosterzee, 1997)

Fig. 3. Sequence alignment of ITS1 and ITS2 of nrDNA from the 39 accessions of the *Phalaenopsis amabilis* complex.



```
kc-180      ...-...... .......... .......... .......... ......-... ---....... ..........
kc-198      ...-...... .......... .......... .......... ......-... GG-....... ..........
kc-199      ...-...... .......... .......... .......... ......-... GGG....... ..........
kc-202      ...-...... .......... .......... .......... ......-... ---....... ..........
kc-253      ...-...... .......... .......... .......... ......-... G--....... ..........
kc-35       ...-...... .......... .......... .......... ......-.A. G--....... ..........
kc-175      ...-...... .......... .......... .......... ......-.A. G--....... ..........
kc-176      ...-...... .......... .......... .......... ......-.A. G--....... ..........


                     150        160        170        180        190        200        210
kc-66       GGAACCCC-G AACCGGCGCG GATCGGCGCC AAGGGAACCC GTGAGAGACA CGAGCCCGGC ATCGGGCCCC
kc-96       .........- .......... .......... .......... .......... .......... ..........
kc-97       .........- .......... .......... .......... .......... .......... ..........
kc-238      .........- .......... .......... .........G .......... .......... .........T
kc-239      .........- .......... .......... .........G .......... .......... .........T
kc-240      .........- .......... .......... .........G .......... .......... .........T
kc-91       .........- .......... .......... .........G .......... .......... .........T
kc-92       .........- .......... .......... .........G .......... .......... .........T
kc-93       .........- .......... .......... .........G .......... .......... .........T
kc-327      .........- .......... .......... .........G .......... .......... .........T
kc-342      .........- .......... .......... .........G .......... .......... .........T
kc-444      .........- .......... .......... .........G .......... .......... .........T
kc-254      .........- .......... .......... .........G .......... .......... .........T
kc-343      .........- .......... .......... .........G .......... .......... .........T
kc-248      .........- .......... .......... .........G .......... .......... .........T
kc-249      .........- .......... .......... .........G .......... .......... .........T
kc-319      .........- .......... .......... .........G .......... .......... .........T
kc-94       .........- .......... .......... .........G .......... .......... .........T
kc-95       .........- .......... .......... .........G .......... .......... .........T
kc-260      .........- .......... .......... .........G .......... .......... .........T
kc-329      .........- .......... .......... .........G .......... .......... .........T
kc-172      .........- .......... .......... .........G .......... .......... .........T
kc-173      .........- .......... .......... .........G .......... .......... .........T
kc-174      .........- .......... .......... .........G .......... .......... .........T
kc-419      .........- .......... .......... .........G .......... .......... .........T
kc-420      .........- .......... .......... .........G .......... .......... .........T
kc-421      .........- .......... .......... .........G .......... .......... .........T
kc-171      .........- .......... .......... .........G .......... .......... .........T
kc-169      .........- .......... .......... .........G .......... .......... .........T
kc-181      .........- .......... .......... .........G .......... .......... .........T
kc-179      .........- .......... .......... .........G .......... .......... .........T
kc-180      .........- .......... .......... .........G .......... .......... .........T
kc-198      .........- .......... .......... .........G .......... .......... .........T
kc-199      .........- .......... .......... .........G .......... .......... .........T
kc-202      .........- .......... .......... .........G .......... .......... .........T
kc-253      .........- .......... .......... .........G .......... .......... .........T
kc-35       .........- .......... .......... .........G .......... .......... .........T
kc-175      ........C. .......... .......... .........G .......... .......... .........T
kc-176      .........- .......... .......... .........G .......... .......... .........T


                     220        230        240        250        260        270        280
kc-66       CGTGGGGCGG AGCGCCTAAC GTACCGGTCG CGCCGCTCCG CGCCGAGTCC CCATCCCCGC CGCGGCGGGG
kc-96       .......... .......... .......... .......... .......... .......... ..........
kc-97       .......... .......... .......... .......... .......... .......... ..........
kc-238      .......... .......... .......... .......... .......... .......... ..........
kc-239      .......... .......... .......... .......... .......... .......... ..........
kc-240      .......... .......... .......... .......... .......... .......... ..........
kc-91       .......... .......... .......... .......... .......C.. .......... ..........
kc-92       .......... ......C... .......... .......... .......... .......... ..........
kc-93       .......... .......... .......... .......... .......... .......... ..........
kc-327      .......... .......... .......... .......... .......... .......... ..........
kc-342      .......... .......... .......... .......... .......... .......... ..........
kc-444      .......... .......... .......... .......... .......... .......... ..........
kc-254      .......... .......... .......... .......... .......... .......... ..........
kc-343      .......... .......... .......... .......... .......... .......... ..........
kc-248      .......... .......... .......... .......... .......... .......... ..........
kc-249      .......... .......... .......... .......... .......... .......... ..........
kc-319      .......... .......... .......... .......... .......... .......... ..........
kc-94       .......... .......... .......... .......... .......... .......... ..........
kc-95       .......... .......... .......... .......... .......... .......... ..........
kc-260      .......... .......... .......... .......... .......... .......... ..........
kc-329      .......... .......... .......... .......... .......... .......... ..........
kc-172      .......... ......C... .......... .......... .......C.. .......... ..........
kc-173      .......... ......C... .......... .......... .......C.. .......... ..........
kc-174      .......... ......C... .......... .......... .......C.. .......... ..........
kc-419      .......... ......C... .......... .......... .......C.. .......... ..........
kc-420      .......... ......C... .......... .......... .......C.. .......... ..........
```

```
kc-421      .......... ......C... .......... .......... .......C.. .......... ..........
kc-171      .......... ......C... .......... .......... .......C.. .......... ..........
kc-169      .......... ......C... .......... .......... .......C.. .......... ..........
kc-181      .......... ......C... .......... .......... .......C.. .......... ......A...
kc-179      .......... ......C... .......... .......... .......C.. .......... ..........
kc-180      .......... ......C... .......... .......... .......C.. .......... ..........
kc-198      .......... ......C... .......... .......... .......C.. .......... ..........
kc-199      .......... ......C... .......... .......... .......C.. .......... ..........
kc-202      .......... ......C... .......... .......... .......C.. .......... ..........
kc-253      .......... ......C... .......... .......... .......C.. .......... ..........
kc-35       .......... .......... .......... .......... .......... .......... ..........
kc-175      .......... .......... .......... .......... .......... .......... ..........
kc-176      .......... .......... .......... .......... .......... .......... ..........

                     290        300        310        320        330        340        350
kc-66       GTGCCGGGCG AGGACCGGAC GTGCAGAGTG GCCCGTCGTG CCCGTCGGCG CGGCGGGCTG AAGAGCGGGC
kc-96       .......... .......... .......... .......... .......... .......... ..........
kc-97       .......... .......... .......... .......... .......... .......... ..........
kc-238      .......... .......... .......... .......... .......... .......... ..........
kc-239      .......... .......... .......... .......... .......... .......... ..........
kc-240      .......... .......... .......... .......... .......... .......... ..........
kc-91       .......... .......... .......... .......... .......... .......... ..........
kc-92       .......... .......... .......... .......... .......... .......... ..........
kc-93       .......... .......... .......... .......... .......... .......... ..........
kc-327      .......... .......... .......... .......... .......... .......... ..........
kc-342      .......... .......... .......... .......... .......... .......... ..........
kc-444      .......... .......... .......... .......... .......... .......... ..........
kc-254      .......... .......... .......... .......... .......... .......... ..........
kc-343      .......... .......... .......... .......... .......... .......... ..........
kc-248      .......... .......... .......... .......... .......... .......... ..........
kc-249      .......... .......... .......... .......... .......... .......... ..........
kc-319      .......... .......... .......... .......... .......... .......... ..........
kc-94       .......... .......... .......... .......... .......... .......... ..........
kc-95       .......... .......... .......... .......... .......... .......... ..........
kc-260      .......... .......... .......... .......... .......... .......... ..........
kc-329      .......... .......... .......... .......... .......... .......... ..........
kc-172      .......... .......... .......... .......... .......... .......... ..........
kc-173      .......... .......... ......G... .......... .......... .......... ..........
kc-174      .......... .......... .......... .......... .......... .......... ..........
kc-419      .......... .......... .......... .......... .......... .......... ..........
kc-420      .......... .......... .......... .......... .......... .......... ..........
kc-421      .......... .......... .......... .......... .......... .......... ..........
kc-171      .......... .......... .......... .......... .......... .......... ..........
kc-169      .......... .......... .......... .......... .......... .......... ..........
kc-181      .......... .......... .......... .......... .......... .......... ..........
kc-179      .......... .......... .......... .......... .......... .......... ..........
kc-180      .......... .......... .......... .......... .......... .......... ..........
kc-198      .......... .......... .......... .......... .......... .......... ..........
kc-199      .......... .......... .......... .......... .......... .......... ..........
kc-202      .......... .......... .......... .......... .......... .......... ..........
kc-253      .......... .......... .......... .......... .......... .......... ..........
kc-35       .......... .......... .......... .......... .......... .......... ..........
kc-175      .......... .......... .......... .......... .......... .......... ..........
kc-176      .......... .......... .......... .......... .......... .......... ..........

                     360        370        380        390        400        410        420
kc-66       TGCCGTCTCA TCGGCCACGG ACGACGAGGG GTGGATGAAA AGAAGCCCTC GAGCGCGTCG TCGCGTGCCG
kc-96       .......... .......... .......... .......... .......... .......... ..........
kc-97       .......... .......... .......... .......... .......... .......... ..........
kc-238      .......... .......... .......... .......... .......... .......... ..........
kc-239      .......... .......... .......... .......... .......... .......... ..........
kc-240      .......... .......... .......... .......... .......... .......... ..........
kc-91       .......... .......... .......... .......... .......... .......... ..........
kc-92       .......... .......... .......... .......... .......... .......... ..........
kc-93       .......... .......... .......... .......... .......... .......... ..........
kc-327      .......... .......... .......... .......... .......... .......... ..........
kc-342      .......... .......... .......... .......... .......... .......... ..........
kc-444      .......... .......... .......... .......... .......... .......... ..........
kc-254      .......... .......... .......... .......... .......... .......... ..........
kc-343      .......... .......... .......... .......... .......... .......... ..........
kc-248      .......... .......... .......... .......... .......... .......... ..........
kc-249      .......... .......... .......... .......... .......... .......... ..........
kc-319      .......... .......... .......... .......... .......... .......... ..........
kc-94       .......... .......... .......... .......... .......... .......... ..........
kc-95       .......... .......... .......... .......... .......... .......... ..........
kc-260      .......... .......... .......... .......... .......... .......... ..........
kc-329      .......... .......... .......... .......... .......... .......... ..........
```

```
kc-172      .......... .......... .......... .......... .......... .......... ..........
kc-173      .......... .......... .......... .......... .......... .......... ..........
kc-174      .......... .......... .......... .......... .......... .......... ..........
kc-419      .......... .......... .......... .......... .......... .......... ..........
kc-420      .......... .......... .......... .......... .......... .......... ..........
kc-421      .......... .......... .......... .......... .......... .......... ..........
kc-171      .......... .......... .......... .......... .......... .......... ..........
kc-169      .......... .......... .......... .......... .......... .......... ..........
kc-181      .......... .......... .......... .......... .......... .......... ..........
kc-179      .......... .......... .......... .......... .......... .......... ..........
kc-180      .......... .......... .......... .......... .......... .......... ..........
kc-198      .......... .......... .......... .......... .......... .......... ..........
kc-199      .......... .......... .......... .......... .......... .......... ..........
kc-202      .......... .......... .......... .......... .......... .......... ..........
kc-253      .......... .......... .......... .......... .......... .......... ..........
kc-35       .......... .......... .......... .......... .......... .......... ..........
kc-175      .......... .......... .......... .......... .......... .......... ..........
kc-176      .......... .......... .......... .......... .......... .......... ..........


                     430        440        450        460        470        480        490
kc-66       CCGGAGAGGA GAGGAAACGG CCCTCCGCGC GATCCCATCC CGGGCGCCGC CCCTC--GTG CGGCGGCTCG
kc-96       .......... .......... .......... .......... .......... .....--... ..........
kc-97       .......... .......... .......... .......... .......... .....--... ..........
kc-238      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-239      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-240      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-91       .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-92       .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-93       .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-327      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-342      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-444      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-254      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-343      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-248      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-249      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-319      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-94       .......... ....G..... .......... .......... .......... ...C.CC... ..........
kc-95       .......... ....G..... .......... .......... .......... ...C.CC... ..........
kc-260      .......... ....G..... .......... .......... .......... ...C.CC... ..........
kc-329      .......... ....G..... .......... .......... .......... ...C.CC... ..........
kc-172      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-173      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-174      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-419      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-420      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-421      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-171      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-169      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-181      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-179      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-180      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-198      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-199      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-202      .......... ....G..... .......... .......... .......... ...C.CC... ..........
kc-253      .......... ....G..... .......... .......... .......... ...C.C-... ..........
kc-35       .......... ....G..... .......... C......... .......... ...C.C-... ..........
kc-175      .......... ....G..... .......... C......... .......... ...C.C-... ..........
kc-176      .......... ....G..... .......... C......... .......... ...C.C-... ..........


                 494
kc-66       GAAC
kc-96       ....
kc-97       ....
kc-238      ....
kc-239      ....
kc-240      ....
kc-91       ....
kc-92       ....
kc-93       ....
kc-327      ....
kc-342      ....
kc-444      ....
kc-254      ....
kc-343      ....
kc-248      ....
kc-249      ....
```

```
kc-319      ....
kc-94       ....
kc-95       ....
kc-260      ....
kc-329      ....
kc-172      ....
kc-173      ....
kc-174      ....
kc-419      ....
kc-420      ....
kc-421      ....
kc-171      ....
kc-169      ....
kc-181      ....
kc-179      ....
kc-180      ....
kc-198      ....
kc-199      ....
kc-202      ....
kc-253      ....
kc-35       ....
kc-175      ....
kc-176      ....
```

Fig. 4.

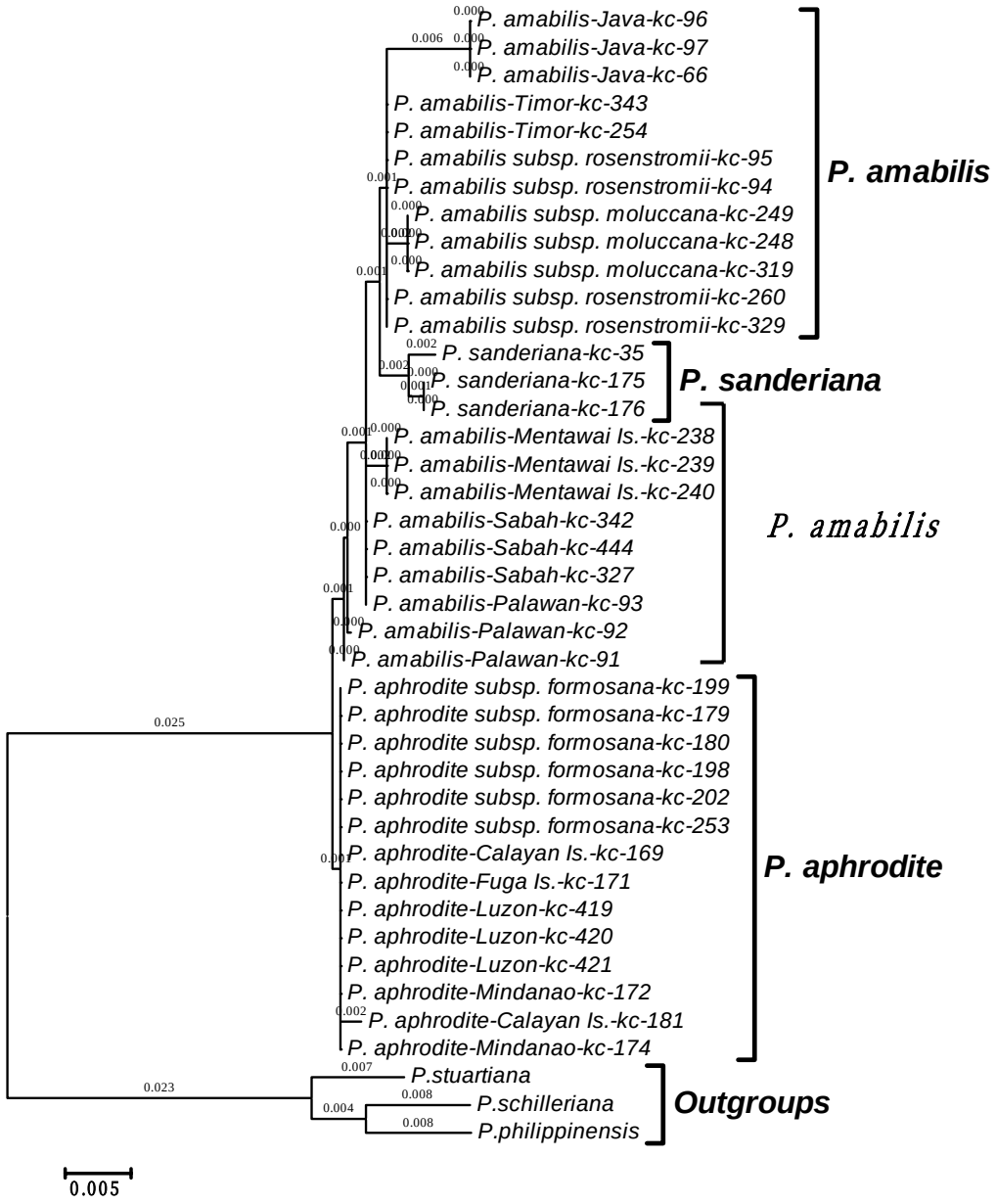


Fig. 4. Minimum evolution tree of 39 accessions from three closely *Phalaenopsis* species and their subspecies, namely *Phalaenopsis amabilis*, *P. aphrodite*, *P. sanderiana*, *P. amabilis* subsp. *moluccana*, *P. amabilis* subsp. *rosenstromii*, *P. aphrodite* subsp. *formosana*, plus three groups, namely *P. stuartiana*, *P. schilleriana*, and *P. philippinensis*, obtained from sequence comparisons of the ITS region of rDNA. Numbers above internodes indicate values of the interior branch test from 1000 replicates. More than 50% of interior branch test is shown on each branch. Branch lengths are proportional to the number of base changes along each branch.

Fig. 5.

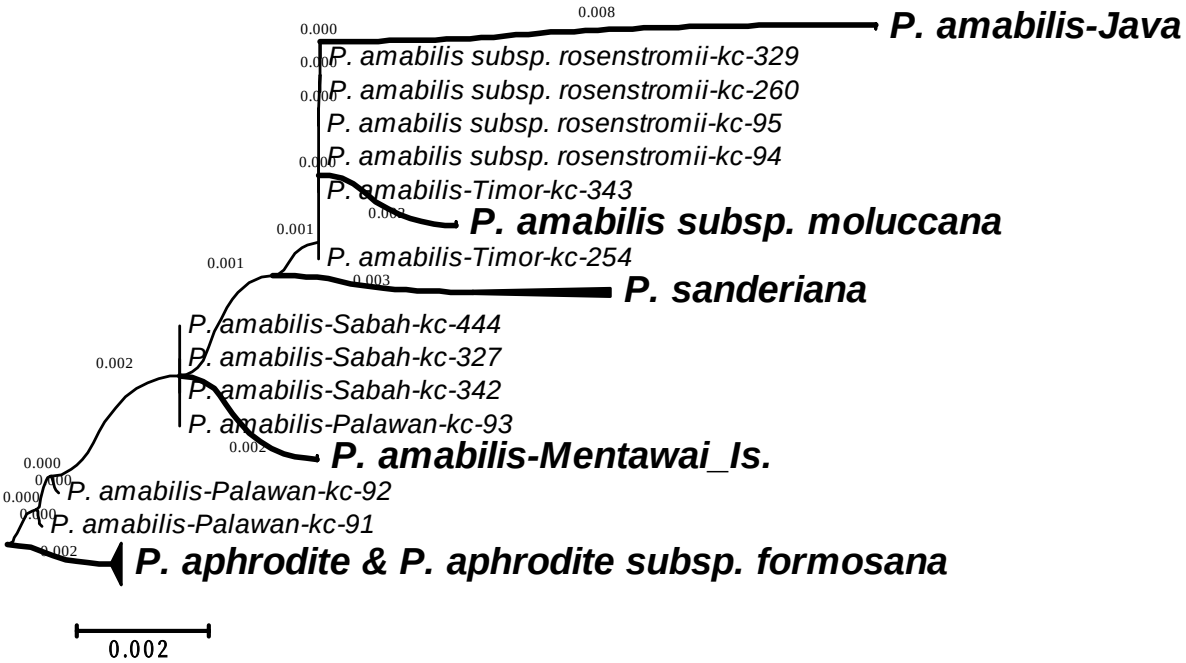


Fig. 5. Evolutionary phylogenetic tree of minimum evolution rooted based on *Phalaenopsis aphrodite*, the origin group of the *P. amabilis* complex suggested by this study. Bold branches show that the group forms a clade. Values above the branches show the genetic distance of the Kimura two-parameter method.

Fig. 6.

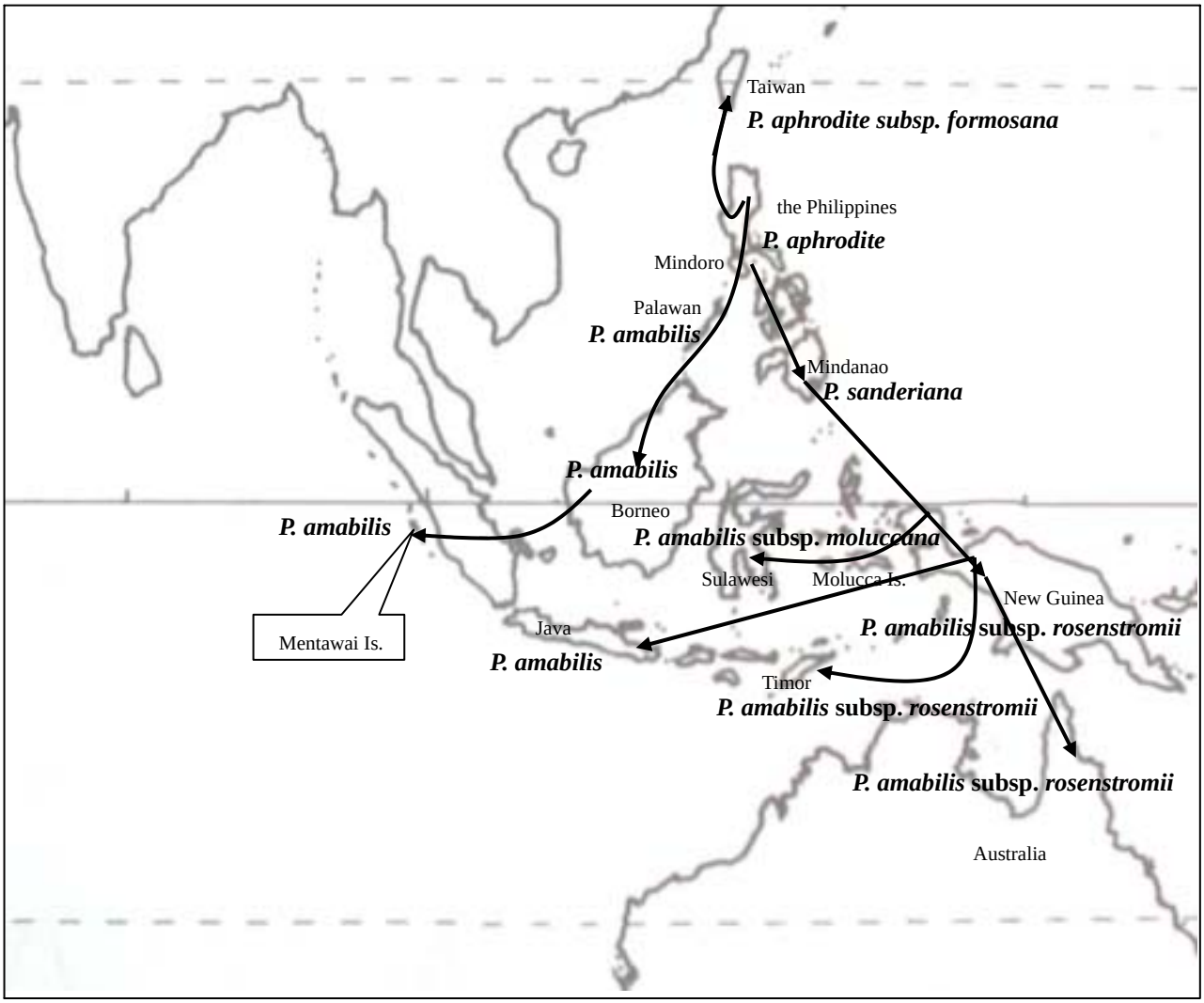


Fig. 6. To map eolutionary trends of the *Phalaenopsis amabilis* complex obtained from this study on the distribution of this complex.

Chapter 3

Phylogenetics, Biogeography, and Evolutionary Trends of the *Phalaenopsis sumatrana* Complex Inferred from Nuclear DNA and Chloroplast DNA

Abstract

Phylogenetic trees inferred from the internal transcribed spacers 1 and 2 (ITS1+ITS2) region of nuclear ribosomal DNA (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*.

Introduction

The *Phalaenopsis sumatrana* complex includes *P. sumatrana* and *P. corningiana* plus one questionable species, *P. zebrina*. Actually, the morphological characters of these three species of this complex are not easily differentiated even to the present. *Phalaenopsis 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 to represent plants with narrower brown markings on a white or yellowish ground color in horticulture to the present (Masaaki, 2002). Furthermore, Sweet (1968), Fowlie (1969), and Christenson (2001) have the congruent concept of *P. sumatrana* and *P. corningiana* each having species level status, being separate from each other, and being treated as two separate species.

In addition, confusion has also surrounded plants of *P. corningiana* and various darker-colored plants of *P. sumatrana*. Plants of *P. corningiana* can be separated from those of *P. sumatrana* based on callus morphology and the marking pattern on the petals and sepals. The callus morphology of plants of *P. corningiana* apparently shows it to be uniseriate, sulcate, bifid, and at the base continuous with a structure analogous to a posterior keel forming a sunken pit. The callus of *P. sumatrana*, on the other hand, shows it to be biseriate, with the posterior callus being four lobulate at the apex and granular at the base, and the anterior callus being longer, sulcate, and bifid. In the marking pattern on the petals, the markings of *P. corningiana* show longitudinal stripes toward the apex of the sepals and petals. In contrast, those of *P. sumatrana* always show transverse stripes from side to side (Sweet, 1968, 1980; Christenson, 2001). However, these different characteristics between *P. corningiana* and *P. sumatrana* are still obscured in some special clones. For example, a solid red clone, *P. sumatrana* var. *sanguinea* has been treated as a synonym of *P. corningiana* (Sweet, 1980). In addition, the length of the floral inflorescences of *P. corningiana* (much shorter than the leaves) is considered to be shorter than that in *P. sumatrana* (Sweet 1968). Wallbrunn (1971), however, did not accept this concept according to inspections of several living plants of these two species. Furthermore, Christenson (2001) considered that the floral fragrance is the best character for separating these two sister species. Plants of *P. corningiana* bear a wonderful scent of spicy candy. Those of *P. sumatrana*, on the other hand, bear a mildy acrid fragrance.

Doubtlessly, species of the *P. sumatrana* complex, namely *P. zebrina*, *P. sumatrana*, and *P. corningiana*, are closely related. To evaluate the phylogenetics and evolutionary trends of this complex, sequences of the ITS of nrDNA, the intron of *trn*L, and the IGS of *atp*B-*rbc*L of chloroplast DNA were analyzed in this study.

Materials and Methods

Plant materials

Materials of 14 accessions of the *P. sumatrana* complex were selected and used for this study (Table 1). The geographical distribution of each species of this complex is shown in Fig. 1. Furthermore, one species of the section *Fuscatae*, *P. fuscata*, as well as one species of the section *Polychilos*, *P. cornu-cervi*, were placed in the outgroup of the *P. sumatrana* complex to clarify the evolutionary trend of this complex due to a common ancestor being shared by this complex (section *Amboinenses*) and sections *Fuscatae* and *Polychilos* (Tsai, see chapter 1).

DNA extraction

Total DNA was extracted using the CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle, 1987). The approximate DNA yields were then determined using a spectrophotometer (Hitachi U-2001).

PCR amplification and electrophoresis

Primer sets designed for amplifying the ITS of nrDNA, the intron of *trn*L, and the IGS of *atp*B-*rbc*L of chloroplast DNA (cpDNA) from *Phalaenopsis* plants as well as PCR conditions are described in Tsai (see chapter 1). These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained by 0.5 μg/mL of ethidium bromide, and finally photographed under UV light exposure.

DNA recovery and sequencing

PCR products of different DNA fragments from the plant material studied were recovered by glassmilk (BIO 101, California) and sequenced directly by the dideoxy chain-termination method using an ABI377 automated sequencer with a Bigdye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, California). Sequencing primers were the same as those used for PCR. These reactions were performed as recommended by the manufacturers.

Data analyses

Boundaries of the ITS regions (including ITS1, 5.8S rDNA, and ITS2), the intron of *trn*L, and the IGS of *atp*B-*rbc*L in species of the *P. sumatrana* complex were determined

by comparison to several published sequences as described in Tsai (see chapter 1). Sequences were aligned using the program Clustal W Multiple alignment in BioEdit (Hall, 1999). Genetic relationships were determined using the program MEGA version 2.1 (Kumar et al., 2001). Genetic distances were calculated by the two-parameter method of Kimura (1980), and were used to reconstruct a tree using the Neighbor-joining method (NJ) (Saitou and Nei, 1987) with interior branch tests of 1000 replicates (Sitnikova et al., 1995).

Results and Discussion

Sequence characteristics

Accession numbers of the 14 accessions of the *Phalaenopsis sumatrana* complex are shown in Table 2. Sequence lengths of ITS1 among the 14 accessions of the *P. sumatrana* complex were the same at 234 bp. Among accessions of this complex, sequence lengths of the ITS2 region were the same at 263 bp. Percentages of the G+C content across the ITS1 region of the *P. sumatrana* complex varied from 71.3% to 71.8%. On the other hand, percentages of G+C content across the ITS2 region varied from 73.0% to 73.4% (Table 2). When combining the ITS1 and ITS2 regions, those sequences of the 14 accessions of the *P. sumatrana* complex were aligned and resulted in 497 characters. Eleven variable sites were found in the alignment sequence of the 14 accessions of the *P. sumatrana* complex. Of them, six sites were singleton. In addition, no gap site was found in the alignment sequence of this complex (Fig. 2).

The sequence lengths of the intron of *trn*L from the 14 accessions of the *P. sumatrana* complex ranged from 535 to 599 bp. Percentages of G+C content across this region of *P. sumatrana* complex varied from 24.5% to 26.6% (Table 3). Those sequences were aligned and resulted in 599 characters. Six variable sites were found in the alignment sequence of the 14 accessions of the *P. sumatrana* complex, and four of the variable sites were singleton (Fig. 3). Within sequences of the *trn*L intron from the *P. sumatrana* complex, a mutational hot spot of length variation was found (Fig. 4). In fact, several mutational hot spot regions had previously been located in the noncoding region of chloroplast DNA (Morton and Clegg, 1993; Ogihara et al., 1991). These mutational hot spot regions appear to be confined to specific sectors. In sequences of the intron of *trn*L of this study, this mutational hot spot bore a length variation in a specific region among different *Phalaenopsis* species, even among closely related species. The length variation of the mutational region in the intron of *trn*L within the *P. sumatrana* complex offered no

valuable information for identifying species of the complex because no consistent deletion/insertion existed within species of the *P. sumatrana* complex. This result indicates that the mutational hot spot of length variation within the *P. sumatrana* complex has a relatively random and rapid evolutionary rate.

Sequence lengths of the IGS of *atp*B-*rbc*L from the 14 accessions of the *P. sumatrana* complex were the same at 681 bp. Percentages of G+C content across this region of the *P. sumatrana* complex varied from 22.8% to 23.0% (Table 3). Alignment of those sequences resulted in 682 characters. Two variable sites were found in the alignment sequence of the 14 accessions of the *P. sumatrana* complex, and neither was a singleton (Fig. 5).

Genetic distances between accessions/species

Genetic distances among the 14 accessions of the *Phalaenopsis sumatrana* complex, based on an analysis of ITS1 and ITS2 of nrDNA, were in the range of from 0.000 to 0.018 with an average of 0.005 using the two-parameter method of Kimura (1980). Within the accessions of *P. sumatrana*, the ranges of genetic distances were from 0.000 to 0.002 with an average of 0.001. Within the accessions of *P. corningiana*, the genetic distances were the same as 0.000. Within the accessions of *P. zebrina*, the genetic distances ranged from 0.006 to 0.012 with an average of 0.008 (Table 4). Furthermore, the average genetic distance between species of *P. sumatrana* and *P. corningiana* was shown to be 0.000. That between species of *P. sumatrana* and *P. zebrina* was shown to be 0.013, as between *P. corningiana* and *P. zebrina*.

In analysis of sequences of the *trn*L intron, genetic distances among the 14 accessions of the *P. sumatrana* complex were in the range of from 0.000 to 0.006 with an average of 0.002 using the two-parameter method of Kimura (1980). Within accessions of *P. sumatrana*, ranges of genetic distances were also from 0.000 to 0.006 with an average of 0.002. Within accessions of *P. corningiana*, genetic distances ranged from 0.000 to 0.002 with an average of 0.001. Within accessions of *P. zebrina*, genetic distances ranged from 0.002 to 0.004 with an average of 0.002 (Table 5). The average genetic distance between species of *P. sumatrana* and *P. corningiana* was 0.0023. Those between species of *P. sumatrana* and *P. zebrina* and between species of *P. corningiana* and *P. zebrina* were 0.0032 and 0.0016, respectively.

In analysis of sequences of the IGS *atp*B-*rbc*L, genetic distances among the 14

accessions of the *P. sumatrana* complex were in the range from 0.000 to 0.003 with an average of 0.001 using the two-parameter method of Kimura (1980). Within accessions of *P. sumatrana*, ranges of genetic distances were from 0.000 to 0.001 with an average of 0.0005. Within accessions of *P. corningiana*, ranges of genetic distances were from 0.000 to 0.001 with an average of 0.0009. No genetic distance was found within accessions of *P. zebrina* (Table 6). The average genetic distance between species of *P. sumatrana* and *P. corningiana* was 0.0018. Those between species of *P. sumatrana* and *P. zebrina* and between species of *P. corningiana* and *P. zebrina* were 0.0027 and 0.0009, respectively.

Phylogenetic reconstructions

The phylogenetic tree inferred from combined data of sequences of ITS1 and ITS2 of nrDNA from 14 accessions of the *P. sumatrana* complex plus outgroups, namely *P. cornu-cervi* and *P. fuscata*, was reconstructed following the NJ method and is shown in Fig. 6. Based on the phylogenetic tree, accessions of both *P. sumatrana* and *P. corningiana* formed a clade with 97% support by the interior branch test. Accessions of the *P. sumatrana* complex formed a clade with moderate support (of 87%) by the interior branch test. In addition, accessions of *P. zebrina* showed the basal group within the *P. sumatrana* complex. In analysis of sequences of the intron of *trn*L, the phylogenetic tree of 14 accessions of the *P. sumatrana* complex plus the outgroup, *P. fuscata*, was reconstructed following the NJ method and is shown in Fig. 7. Based on the phylogenetic tree, species of *P. sumatrana*, *P. corningiana*, and *P. zebrina* cannot be separated from one other. In analysis of the IGS of *atp*B-*rbc*L, the phylogenetic tree of 14 accessions of the *P. sumatrana* complex plus the outgroup, *P. fuscata*, was reconstructed following the NJ method and is shown in Fig. 8. Based on the phylogenetic tree, species of *P. sumatrana*, *P. corningiana*, and *P. zebrina* can apparently not be separated from one other as well. Of all accessions of the *P. sumatrana* complex, accessions of *P. zebrina* were closer to two accessions of *P. corningiana*, namely *P. corningiana*-kc-330 and *P. corningiana*-kc-345, and separated from the remaining accessions of both species of *P. corningiana* and *P. sumatrana*. In addition, one accession of *P. sumatrana*, namely *P. sumatrana*-kc-405, was closer to three accessions of *P. corningiana*, namely *P. corningiana*-kc-346, *P. corningiana*-kc-383, and *P. corningiana*-kc-384. The results indicate that species of the *P. sumatrana* complex could not be identified based on the IGS of *atp*B-*rbc*L. Therefore, of the three analyses of DNA fragments, only the analysis of ITS1 and ITS2 of nrDNA offered valuable information for identifying parts of accessions of the *P. sumatrana*

complex, namely accessions of *P. zebrina*.

Based on the ITS sequences of nrDNA, *P. sumatrana* and *P. corningiana* cannot be separated from each other. The result does not support *P. corningiana* and *P. sumatrana* being treated as two separate species as described by Sweet (1968), Fowlie (1969), and Christenson (2001). Although characteristics of the callus morphology and marking pattern on the petals could be identified between *P. sumatrana* and *P. corningiana as* described by Sweet (1980) and Christenson (2001), I found that these two characteristics of the separation between *P. sumatrana* and *P. corningiana* were not consistent based on samples of this study. In my inspection, some accessions of *P. corningiana* showed a marking pattern with longitudinal stripes toward the apex of sepals and petals, but the callus morphology was similar to that of *P. sumatrana*, which was biseriate without forming a sunken pit at the base of the callus (data not shown). Furthermore, differentiation between *P. corningiana* and *P. sumatrana* was also proposed based on the floral fragrance (Christenson, 2001).

Accessions of *P. zebrina* in this complex can be separated from both *P. sumatrana* and *P. corningiana* based on the ITS sequence of nrDNA. In analyses of sequences of both the intron of *trn*L and the IGS of *atp*B-*rbc*L, neither species of this complex could be separated from the other. Therefore, 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 (1982). However, the floral ground color of *P. zebrina* is not a characteristic of this species as described by Fowlie (1982). According to my inspection of plants of *P. zebrina*, both white and yellowish ground color in the flower of this species were apparent, as described by Masaaki (2002). Based on my inspection of plants of the *P. sumatrana* complex for this study, the characters of *P. zebrina* of having a special floral fragrance as that of *P. floresensis*, which smells like a “roach/insect”. Therefore, it is easy to separate plants of *P. zebrina* from the other two species of this complex based on floral fragrances. Furthermore, all sepals and petals of these three species are retained after pollination (Christenson, 2001). I 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 suitable to treat *P. zebrina* as a synonym of *P. sumatrana* as described by Sweet (1968, 1980) and Christenson (2001).

Biogeography and evolutionary trends

As to the biogeography of the *P. sumatrana* complex, the distributions of these three species overlap in Borneo (Fig. 1). This allows a much greater chance for these three species to hybridize in their natural environment. This makes these three species difficult to discriminate. In fact, some naturally hybridized plants between species of *P. sumatrana* and *P. corningiana* have been collected (Christenson, 2001). Apparently, these three species share a common ancestor. According to the phylogenetic tree inferred from the ITS of nrDNA (Fig. 6), 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. The suggestion is partly supported by analysis of sequences of the IGS of *atp*B-*rbc*L of cpDNA (Fig. 8). Based on the evolutionary trend derived from molecular data, the dispersal pathway of the *P. sumatrana* complex was deduced and is shown in Fig. 9. According to the research of Tsai (see chapter 1), species of the subgenus *Polychilos* in the Philippines were dispersed from Borneo. Thereafter, *P. zebrina* developed in Borneo and dispersed into Palawan, the Philippines. *P. corningiana* and *P. sumatrana* might have evolved from *P. zebrina* in Borneo. Since then, *P. sumatrana* dispersed into Sumatra, Malay Peninsula, and the Andaman Is. based on land bridges in glacial times, and it has been shown to be a widespread species. The land bridges among Sumatra, the Malay Peninsula, and Borneo could have been formed, since Borneo, Sumatra, the Malay Peninsula, and Java comprised the Sunda Shelf (Van Oosterzee, 1997). However, *P. sumatrana* have not been found in Java at present. 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, see chapter 1). Furthermore, Andaman Is. might have been interconnected to Sumatra in ancient times based on the evidences of both biogeography and evolutionary trends of the *P. sumatrana* complex.

Literature Cited

Christenson, E. A. 2001. *Phalaenopsis*. Timber Press, Portland, Oregon. 330p.

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

Fowlie, J. A. 1969. A note on *Phalaenopsis sumatrana* and *Phalaenopsis corningiana*.

Orch. Dig. 33: 301.

Fowlie, J. A. 1982. Malaya revised, pt. 21: *Phalaenopsis corningiana* on saplings and streamside trees near waterfalls. Orchid Dig. 46: 138-142.

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.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. Mol. Evol. 16: 111-120.

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

Masaaki, C. 2002. *Phalaenopsis* species. Chiba Masaaki *Phalaenopsis* Species Publishing Society, Saitama-ken, Japan.

Morton, B. R. and M. T. Clegg. 1993. A chloroplast DNA mutational hotspot and gene conversion in a noncoding region near *rbc*L in the grass family (Poaceae). Curr. Genet. 24: 357-365.

Ogihara, Y., T. Terachi, and T. Sasakuma. 1991. Molecular analysis of the hot spot region related to length mutations in wheat chloroplast DNAs. I. Nucleotide divergence of genes and intergenic spacer regions located in the hot spot region. Genetics 129: 873-884.

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

Sitnikova, T., A. Rzhetsky, and M. Nei. 1995. Interior-branch and bootstrap tests of phylogenetic trees. Mol. Biol. Evol. 12: 319-333.

Sweet, H. R. 1968. A revision of the genus *Phalaenopsis*-II. Am. Orchid Soc. Bull. 37: 1091.

Sweet, H. R. 1980. The genus *Phalaenopsis*. The Orchid Digest, Pomona, California.

Van Oosterzee, P. 1997. Where worlds collide: the Wallace Line. Cornell Univ. Press, Ithaca, NY.

Wallbrunn, H. M. 1971. A second look at the “Revision of the genus *Phalaenopsis*.” Am. Orchid Soc. Bull. 40: 223-231.

Table 1. A list of 16 accessions from three closely *Phalaenopsis* species of *P. sumatrana*, *P. corningiana* and *P. zebrina*, and their different geographical distributions.

Taxa and systematic classification [a]	Distribution	Accession nos.	Source [b]
P. sumatrana	Sumatra, Indonesia	AY390239	KDAIS-KC32
P. sumatrana	Sumatra, Indonesia	AY390240	KDAIS-KC160
P. sumatrana	Sumatra, Indonesia	AY390241	KDAIS-KC161
P. sumatrana	Sumatra, Indonesia	AY390242	KDAIS-KC403
P. sumatrana	Sumatra, Indonesia	AY390243	KDAIS-KC404
P. sumatrana	Sumatra, Indonesia	AY390244	KDAIS-KC405
P. corningiana	Borneo	AY390245	KDAIS-KC330
P. corningiana	Borneo	AY390246	KDAIS-KC345
P. corningiana	Borneo	AY390247	KDAIS-KC346
P. corningiana	Borneo	AY390248	KDAIS-KC383
P. corningiana	Borneo	AY390249	KDAIS-KC384
P. zebrina	Borneo	AY390250	KDAIS-KC57
P. zebrina	Borneo	AY390251	KDAIS-KC257
P. zebrina	Borneo	AY390252	KDAIS-KC231

[a] The classification of *Phalaenopsis* is based on Christenson (2001).
[b] Kaohsiung District Agricultural Improvement Station.

Table 2. Lengths of ITS1 and ITS2 and GenBank accession nos. of the 14 accessions of the *Phalaenopsis sumatrana* complex.

Taxa [a]	ITS1		ITS2		GenBank accession no.
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	
P. sumatrana-kc-32	234	71.8	263	73.0	AY390239
P. sumatrana-kc-160	234	71.8	263	73.0	AY390240
P. sumatrana-kc-161	234	71.8	263	73.0	AY390241
P. sumatrana-kc-403	234	71.8	263	73.0	AY390242
P. sumatrana-kc-404	234	71.8	263	73.0	AY390243
P. sumatrana-kc-405	234	71.8	263	73.0	AY390244
P. corningiana-kc-330	234	71.8	263	73.0	AY390245
P. corningiana-kc-345	234	71.8	263	73.0	AY390246
P. corningiana-kc-346	234	71.8	263	73.0	AY390247
P. corningiana-kc-383	234	71.8	263	73.0	AY390248
P. corningiana-kc-384	234	71.8	263	73.0	AY390249
P. zebrina-kc-57	229	71.3	263	73.0	AY390250
P. zebrina-kc-257	232	71.4	263	73.4	AY390251
P. zebrina-kc-231	232	71.8	263	73.4	AY390252

Table 3. Lengths and G+C contents of the *trn*L intron and IGS of *atp*B-*rbc*L among the 14 accessions of the *Phalaenopsis sumatrana* complex.

Taxa [a]	*trn*L intron		IGS of *atp*B-*rbc*L		GenBank accession no.
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	
P. sumatrana-kc-32	26.4	535	681	23.0	
P. sumatrana-kc-160	26.4	535	681	23.0	
P. sumatrana-kc-161	26.4	535	681	23.0	
P. sumatrana-kc-403	25.2	579	681	23.0	
P. sumatrana-kc-404	26.6	530	681	23.0	
P. sumatrana-kc-405	25.1	579	681	22.8	
P. corningiana-kc-330	24.5	599	681	22.9	
P. corningiana-kc-345	24.5	599	681	22.9	
P. corningiana-kc-346	25.8	551	681	22.8	
P. corningiana-kc-383	24.5	599	681	22.8	
P. corningiana-kc-384	25.9	551	681	22.8	
P. zebrina-kc-57	26.0	546	682	22.9	
P. zebrina-kc-231	25.6	551	681	22.9	
P. zebrina-kc-257	25.7	554	681	22.9	

Table 4. Genetic distance matrix among the 14 accessions of the *Phalaenopsis sumatrana* complex based on the ITS1 and ITS2 of nrDNA.

[13]	1	2	3	4	5	6	7	8	9	10	11	12	
[1]*													
[2]	0.000												
[3]	0.000	0.000											
[4]	0.000	0.000	0.000										
[5]	0.000	0.000	0.000	0.000									
[6]	0.000	0.000	0.000	0.000	0.000								
[7]	0.000	0.000	0.000	0.000	0.000	0.000							
[8]	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
[9]	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002					
[10]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002				
[11]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000			
[12]	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.014	0.012	0.012		
[13]	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.018	0.016	0.016	0.012	
[14]	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.010	0.010	0.006	0.006

* [1], P. corningiana-kc-330; [2], P. corningiana-kc-345; [3], P. corningiana-kc-346; [4], P. corningiana-kc-383; [5], P. corningiana-kc-384; [6], P. sumatrana-kc-160; [7], P. sumatrana-kc-161; [8], P. sumatrana-kc-32; [9], P. sumatrana-kc-403; [10], P. sumatrana-kc-404; [11], P. sumatrana-kc-405; [12], P. zebrina-kc-231; [13], P. zebrina-kc-257; [14], P. zebrina-kc-57.

Table 5. Genetic distance matrix of the intron of *trn*L among the 14 accessions of the *Phalaenopsis sumatrana* complex.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	]
[1]														
[2]	0.002													
[3]	0.002	0.000												
[4]	0.004	0.002	0.002											
[5]	0.002	0.000	0.000	0.002										
[6]	0.006	0.004	0.004	0.002	0.004									
[7]	0.004	0.002	0.002	0.000	0.002	0.002								
[8]	0.004	0.002	0.002	0.000	0.002	0.002	0.000							
[9]	0.004	0.002	0.002	0.000	0.002	0.002	0.000	0.000						
[10]	0.004	0.002	0.002	0.000	0.002	0.002	0.000	0.000	0.000					
[11]	0.006	0.004	0.004	0.002	0.004	0.004	0.002	0.002	0.002	0.002				
[12]	0.006	0.004	0.004	0.002	0.004	0.004	0.002	0.002	0.002	0.002	0.004			
[13]	0.006	0.004	0.004	0.002	0.004	0.004	0.002	0.002	0.002	0.002	0.004	0.004		
[14]	0.004	0.002	0.002	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.002	0.002	0.002	

[1], P. sumatrana-kc-32; [2], P. sumatrana-kc-160; [3], P. sumatrana-kc-161; [4], P. sumatrana-kc-403; [5], P. sumatrana-kc-404; [6], P. sumatrana-kc-405; [7], P. corningiana-kc-330; [8], P. corningiana-kc-345; [9], P. corningiana-kc-346; [10], P. corningiana-kc-383; [11], P. corningiana-kc-384; [12], P. zebrina-kc-57; [13], P. zebrina-kc-231; [14], P. zebrina-kc-257.

Table 6. Genetic distance matrix among 14 accessions of the *Phalaenopsis sumatrana* complex based on analysis of the IGS of *atp*B-*rbc*L.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	]
[1]														
[2]	0.000													
[3]	0.000	0.000												
[4]	0.000	0.000	0.000											
[5]	0.000	0.000	0.000	0.000										
[6]	0.001	0.001	0.001	0.001	0.001									
[7]	0.003	0.003	0.003	0.003	0.003	0.001								
[8]	0.003	0.003	0.003	0.003	0.003	0.001	0.000							
[9]	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001						
[10]	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.000					
[11]	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.000	0.000				
[12]	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.001	0.001	0.001			
[13]	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.001	0.001	0.001	0.000		
[14]	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.001	0.001	0.001	0.000	0.000	

[1], P. sumatrana-kc-32; [2], P. sumatrana-kc-160; [3], P. sumatrana-kc-161; [4], P. sumatrana-kc-403; [5], P. sumatrana-kc-404; [6, P. sumatrana-kc-405; [7], P. corningiana-kc-330; [8], P. corningiana-kc-345; [9], P. corningiana-kc-346; [10], P. corningiana-kc-383; [11], P. corningiana-kc-384; [12], P. zebrina-kc-57; [13], P. zebrina-kc-231; [14], P. zebrina-kc-257.

Fig. 1.

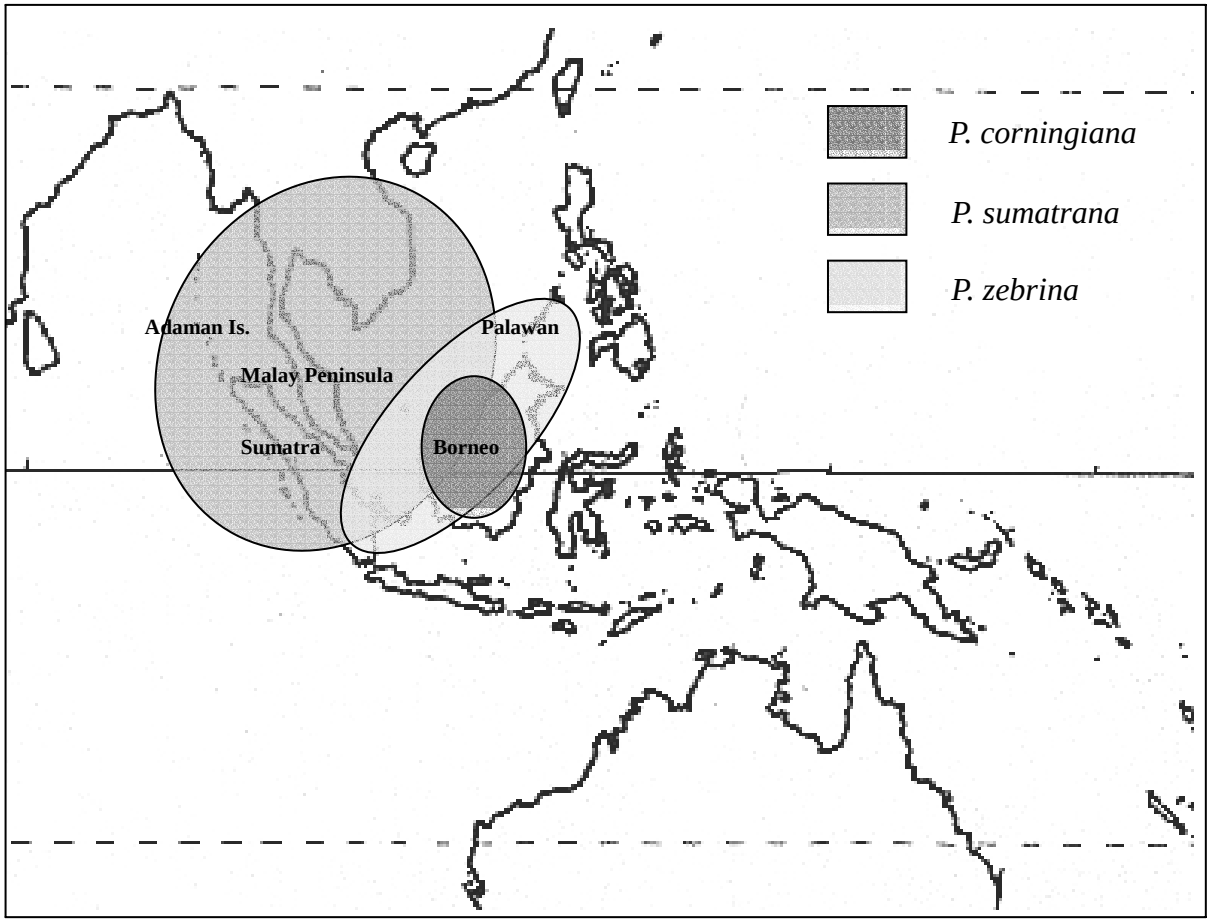


Fig. 1. Geographical distributions of *P. sumatrana*, *P. corningiana*, and *P. zebrina*.

Fig. 2. The sequence alignment of ITS1 and ITS2 of rDNA from the14 accessions of the *P. sumatrana* complex.

```
                          10         20         30         40         50         60         70
P._corningiana-kc-33      TCGAGACCGA AATTATATCG AGCGATTCGG AGAACCCGTG AACCAGGCGG CGGCGGCCGC CGCCGCCGCC
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-32        .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .........G ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ........A.
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... ..........

                          80         90         100        110        120        130        140
P._corningiana-kc-33      GAACGGCCGC CCCCGCCGTC GGCCCCTCTC GTTCGGAGGG GGCGCGACGG GGGACGGCCG AAACCCCGAA
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-32        .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... .......AG.
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... .......A..

                          150        160        170        180        190        200        210
P._corningiana-kc-33      CCGGCGCAGA TCGGCGCCAA GGGAACCGGT GAAAAACACG AGCCCGGCAT CGGGCCTTCG TGGGGCGGAG
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-32        .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... ..........

                          220        230        240        250        260        270        280
P._corningiana-kc-33      CGGTGCTGCG CACCGCACGT ACTGGTTGCG CCGCTCCGTG CCGAGTCCCC ATCCCCGCCG CGGTGGGGGT
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-34      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-38      .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-32        .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........
```

```
P._zebrina-kc-57        ..........  ..........  ..........  ..........  ..........  ..........  ..........

                                290         300         310         320         330         340         350
P._corningiana-kc-33    GCCGGGCGAG  GCCCGGATGC  GCGGAGTGGC  CCGTCGTGCC  CGTCGGTGCG  GCGGGCTGAA  GAGCGGGTGA
P._corningiana-kc-34    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-34    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-38    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-38    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-160     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-161     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-32      ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-403     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-404     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-405     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._zebrina-kc-231       ..........  ..........  ..........  ..........  ..........  ..........  ........C.
P._zebrina-kc-257       ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._zebrina-kc-57        ..........  ..........  ..........  ..........  ..........  ..........  ..........

                                360         370         380         390         400         410         420
P._corningiana-kc-33    TCGTCTCATT  GCCCACGAAC  AACGAGGGGT  GATTGAAAGA  AGGCTGCCGC  GGGCAGGGCC  CGCGTTGTCA
P._corningiana-kc-34    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-34    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-38    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-38    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-160     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-161     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-32      ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-403     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-404     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-405     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._zebrina-kc-231       ........G.  ..........  ..........  .GA.......  ..........  ..........  .........T
P._zebrina-kc-257       ..........  ..........  ..........  .GA.......  ..........  ..........  .........T
P._zebrina-kc-57        ..........  ..........  ..........  .GA.......  ..........  ..........  .........T

                                430         440         450         460         470         480         490
P._corningiana-kc-33    CGTGCCGGCC  GGAGAGGAGA  CGACGCCCTC  GGTGCGATCC  CATCGCGAGC  GCCGCCCCCC  GTGCGGCGGC
P._corningiana-kc-34    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-34    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-38    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._corningiana-kc-38    ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-160     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-161     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-32      ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-403     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-404     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sumatrana-kc-405     ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._zebrina-kc-231       ..........  ..........  ..........  A.........  ..........  ..........  ..........
P._zebrina-kc-257       ..........  ..........  ........C.  A.........  ..........  ..........  ..........
P._zebrina-kc-57        ..........  ..........  ..........  A.........  ..........  ..........  ..........

                            497
P._corningiana-kc-33    TTGGAAT
P._corningiana-kc-34    .......
P._corningiana-kc-34    .......
P._corningiana-kc-38    .......
P._corningiana-kc-38    .......
P._sumatrana-kc-160     .......
P._sumatrana-kc-161     .......
P._sumatrana-kc-32      .......
P._sumatrana-kc-403     .......
P._sumatrana-kc-404     .......
P._sumatrana-kc-405     .......
P._zebrina-kc-231       .......
P._zebrina-kc-257       .......
P._zebrina-kc-57        .......
```

Fig. 3. The sequences alignment of the intron of *trn*L of chloroplast DNA from the 14 accessions of the *P. sumatrana* complex.

```
                          5         15         25         35         45         55         65
P._sumatrana-kc-32        AATGGAAGCT GTTCTAACGA ATGAAATTGA TTACGTTACG TTAGTAGCTA AAAGACTTCT ATCGAAATGA
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330     .......... .......... .......... .......... .......... .......... ..........
P._Corningiana-kc-345     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384     .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........

                          75         85         95         105        115        125        135
P._sumatrana-kc-32        CAGAAAGGAT ACGTCTTATA CGTACGTATA CATACTGACA TAGCAAACGA TTAATCACAA CCCAAATCTT
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330     .......... .......... .......... .......... .......... .......... ..........
P._Corningiana-kc-345     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384     .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... .......G..
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........

                          145        155        165        175        185        195        205
P._sumatrana-kc-32        ATATCGAATT CTATTTTGTA TCTCTATATA TTTAGATATG AAATTTGAAA TTTCTATATG AAAATAGAAA
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... ...T...... ..........
P._corningiana-kc-330     .......... .......... .......... .......... .......... .......... ..........
P._Corningiana-kc-345     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384     .......... .......... ......G... .......... .......... .......... ..........
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........

                          215        225        235        245        255        265        275
P._sumatrana-kc-32        TCTTCTCTTT CTTTCTA--- ---------- ------ATAA TATTATATTA ---------- ----------
P._sumatrana-kc-160       .......... .......--- ---------- ------T... .......... ---------- ----------
P._sumatrana-kc-161       .......... .......--- ---------- ------T... .......... ---------- ----------
P._sumatrana-kc-403       .......... .......--- ----T-TTTC TTTCTAT... .......... TCTTTCTATT A----ATATT
P._sumatrana-kc-404       .......... .......--- ---------- ------T... .....----- ---------- ----------
P._sumatrana-kc-405       .......... .......--- ----T-TTTC TTTCTAT... .......... TCTTTCTATT A----ATATT
P._corningiana-kc-330     .......... .......AAT CTTCTCTTTC TTTCTAT... .......... AATTATATTA ATATTATATT
P._Corningiana-kc-345     .......... .......AAT CTTCTCTTTC TTTCTAT... .......... AATTATATTA ATATTATATT
P._corningiana-kc-346     .......... .......--- ---------- ------T... .......... ---------- ----------
P._corningiana-kc-383     .......... .......AAT CTTCTCTTTC TTTCTAT... .......... AATTATATTA ATATTATATT
P._corningiana-kc-384     .......... .......--- ---------- ------T... .......... ---------- ----------
P._zebrina-kc-57          .......... .......--- ---------- ------T... .......... ---------- ----------
P._zebrina-kc-231         .......... .......--- ---------- ------T... .......... ---------- ----------
P._zebrina-kc-257         .......... .......--- ---------- ------T... .......... ---------- ----------

                          285        295        305        315        325        335        345
```

```
P._sumatrana-kc-32      ---------- -------TTA ATAT------ --GAGTAATA TAATAGTATG AGATAAGGAT CTATAAGAAA
P._sumatrana-kc-160     ---------- -------... ...------ --........ .......... .......... ..........
P._sumatrana-kc-161     ---------- -------... ...------ --........ .......... .......... ..........
P._sumatrana-kc-403     ATATTAATAT TATATTA... ...------ --........ .......... .......... ..........
P._sumatrana-kc-404     ---------- -------... ...------ --........ .......... .......... ..........
P._sumatrana-kc-405     ATATTAATAT TATATTA... ...------ --........ .......... .......... ..........
P._corningiana-kc-330   ATATTAATAT TATATTA... ....ATTAAT AT........ .......... .......... ..........
P._Corningiana-kc-345   ATATTAATAT TATATTA... ....ATTAAT AT........ .......... .......... ..........
P._corningiana-kc-346   -TATTAATAT TATATTA... ...------ --........ .......... .......... ..........
P._corningiana-kc-383   ATATTAATAT TATATTA... ....ATTAAT AT........ .......... .......... ..........
P._corningiana-kc-384   -TATTAATAT TATATTA... ...------ --........ .......... .......... ..........
P._zebrina-kc-57        ------ATAT TATATTA... ...------ --........ .......... .......... ..........
P._zebrina-kc-231       -TATTAATAT TATATTA... ...------ --........ .......... .......... ..........
P._zebrina-kc-257       ------ATAT TATATTA... ....ATTAAT AT........ .......... .......... ..........

                           355        365        375        385        395        405        415
P._sumatrana-kc-32      CCCTATATTT CTATTCTTTT TTAATTAGAA TGATAATGAT AGAGATCAAA AAGAGATATG AAAAATTGAA
P._sumatrana-kc-160     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330   .......... .......... .......... .......... .......... .......... ..........
P._Corningiana-kc-345   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384   .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57        .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231       .......... A......... .......... .......... .......... .......... ..........
P._zebrina-kc-257       .......... .......... .......... .......... .......... .......... ..........

                           425        435        445        455        465        475        485
P._sumatrana-kc-32      GAGTTATTGT GAATAAATTC CAATTGAAGT TGAAAAAAGA ATAGAATTCG AATATTCAAT GATCAAATTA
P._sumatrana-kc-160     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403     .......... ....C..... .......... .......... .......... .......... ..........
P._sumatrana-kc-404     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405     .......... ....C..... .......... .......... .......... .......... ..........
P._corningiana-kc-330   .......... ....C..... .......... .......... .......... .......... ..........
P._Corningiana-kc-345   .......... ....C..... .......... .......... .......... .......... ..........
P._corningiana-kc-346   .......... ....C..... .......... .......... .......... .......... ..........
P._corningiana-kc-383   .......... ....C..... .......... .......... .......... .......... ..........
P._corningiana-kc-384   .......... ....C..... .......... .......... .......... .......... ..........
P._zebrina-kc-57        .......... ....C..... .......... .......... .......... .......... ..........
P._zebrina-kc-231       .......... ....C..... .......... .......... .......... .......... ..........
P._zebrina-kc-257       .......... ....C..... .......... .......... .......... .......... ..........

                           495        505        515        525        535        545        555
P._sumatrana-kc-32      TTCATTCCAG AATTTTTGAT AGATCCTTTG AAATTGAATC GGACGAGAAT AAAGAGAGAG TCCCATTTTA
P._sumatrana-kc-160     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330   .......... .......... .......... .......... .......... .......... ..........
P._Corningiana-kc-345   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384   .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57        .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257       .......... .......... .......... .......... .......... .......... ..........

                           565        575        585        595
P._sumatrana-kc-32      CATGTCAATA CCGACAACAA TGAAATTTAT AGTAAGAGG
P._sumatrana-kc-160     .......... .......... .......... .........
P._sumatrana-kc-161     .......... .......... .......... .........
P._sumatrana-kc-403     .......... .......... .......... .........
```

P._sumatrana-kc-404 ....
P._sumatrana-kc-405 ....
P._corningiana-kc-330 ....
P._Corningiana-kc-345 ....
P._corningiana-kc-346 ....
P._corningiana-kc-383 ....
P._corningiana-kc-384 ....
P._zebrina-kc-57 ....
P._zebrina-kc-231 ....
P._zebrina-kc-257 ....

Fig. 4.

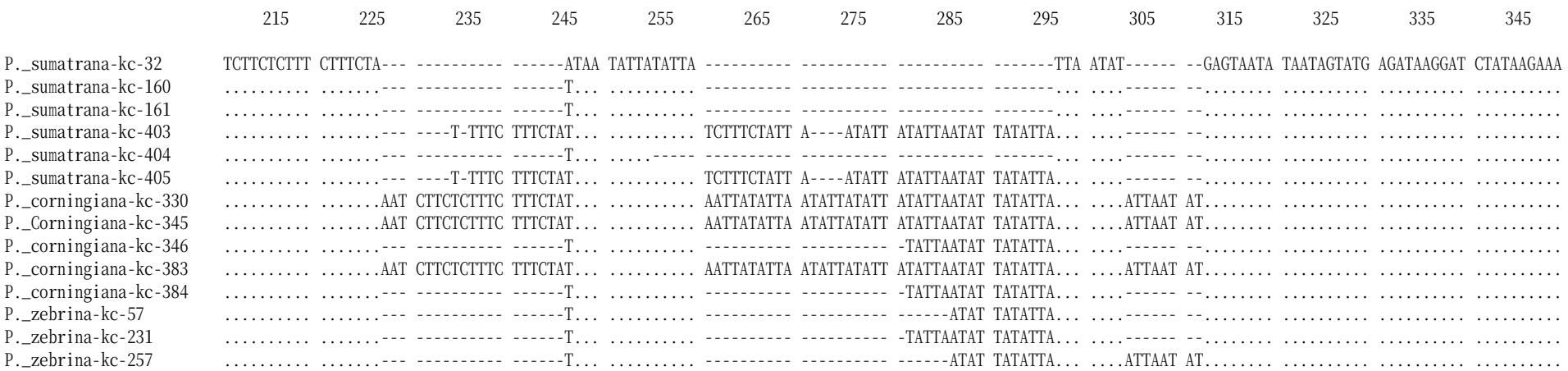


Fig. 4. The mutational hot spot of length variations within the intron of *trn*L of chloroplast DNA from the *P. sumatrana* complex.

Fig. 5. The sequences alignment of the IGS of *atp*B-*rbc*L of chloroplast DNA from the14 accessions of the *P. sumatrana* complex.

```
                          5         15         25         35         45         55         65
P._sumatrana-kc-32        TACAACATAT ATTACTGTCA AGAGAGGGGA CCGGGTCCTA TATTCTTTCT TTTTATTTCT ATATTAGATA
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-345     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384     .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........

                          75         85         95         105        115        125        135
P._sumatrana-kc-32        TTTCTATTTA CTATTTATTA TCTTTAATAT CTTTATTATC TTTACTTTAA AATTTTTATA ATTGAAATTT
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-345     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384     .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........

                          145        155        165        175        185        195        205
P._sumatrana-kc-32        ATTCATTCTA TAATTTCTTA ATTCTAATTT AGAATTCTAT TTCTATTCAA TTTAATATTT ATCTATTTGA
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-345     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384     .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57          .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231         .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257         .......... .......... .......... .......... .......... .......... ..........

                          215        225        235        245        255        265        275
P._sumatrana-kc-32        ATTGAATTCT ATTTAAACTA GATTTCTGAA TTGAAATGAA CTCGAAATTT TTCATTTTCT TTGATGTTTT
P._sumatrana-kc-160       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404       .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405       .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-345     .......... .......... .......... .......... .......... .......... ..........
```

```
P._corningiana-kc-346  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384  .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231      .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257      .......... .......... .......... .......... .......... .......... ..........

                            285        295        305        315        325        335        345
P._sumatrana-kc-32     TTTCTCTTTA TTTTGATATT CTTA-TTTCT TTCTTTTTTT TATATTCATA TTTTATATCA TATTCATTCT
P._sumatrana-kc-160    .......... .......... ....-..... .......... .......... .......... ..........
P._sumatrana-kc-161    .......... .......... ....-..... .......... .......... .......... ..........
P._sumatrana-kc-403    .......... .......... ....-..... .......... .......... .......... ..........
P._sumatrana-kc-404    .......... .......... ....-..... .......... .......... .......... ..........
P._sumatrana-kc-405    .......... .......... ....-..... .......... .......... .......... ..........
P._corningiana-kc-330  .......... .......... ....-..... .......... .......... ..C....... ..........
P._corningiana-kc-345  .......... .......... ....-..... .......... .......... ..C....... ..........
P._corningiana-kc-346  .......... .......... ....-..... .......... .......... .......... ..........
P._corningiana-kc-383  .......... .......... ....-..... .......... .......... .......... ..........
P._corningiana-kc-384  .......... .......... ....-..... .......... .......... .......... ..........
P._zebrina-kc-57       .......... .......... ....A..... .......... .......... ..C....... ..........
P._zebrina-kc-231      .......... .......... ....-..... .......... .......... ..C....... ..........
P._zebrina-kc-257      .......... .......... ....-..... .......... .......... ..C....... ..........

                            355        365        375        385        395        405        415
P._sumatrana-kc-32     TTATAAAAAA TATTAAGAAG ATGATAAATT CCATTAGGAA TAGAAATTTT CAAGAAGATT GGGTTGCGCC
P._sumatrana-kc-160    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405    .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-345  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384  .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231      .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257      .......... .......... .......... .......... .......... .......... ..........

                            425        435        445        455        465        475        485
P._sumatrana-kc-32     ATATATATCA AAGAGTATAA AATAATGATG TATTTGGTGA ATCAAATAAA TGGTCCAATA ACGAACCCTT
P._sumatrana-kc-160    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405    .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-345  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383  .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384  .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231      .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257      .......... .......... .......... .......... .......... .......... ..........

                            495        505        515        525        535        545        555
P._sumatrana-kc-32     TTCAAATTTT CATTATTCAT TAGTTGATAA TATTAATTTA GAGTTTAGTT GAATCTTTTT TGAATTGTAA
P._sumatrana-kc-160    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161    .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403    .......... .......... .......... .......... .......... .......... ..........
```

```
P._sumatrana-kc-404     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405     .......... .......... .......... .......... T......... .......... ..........
P._corningiana-kc-330   .......... .......... .......... .......... T......... .......... ..........
P._corningiana-kc-345   .......... .......... .......... .......... T......... .......... ..........
P._corningiana-kc-346   .......... .......... .......... .......... T......... .......... ..........
P._corningiana-kc-383   .......... .......... .......... .......... T......... .......... ..........
P._corningiana-kc-384   .......... .......... .......... .......... T......... .......... ..........
P._zebrina-kc-57        .......... .......... .......... .......... T......... .......... ..........
P._zebrina-kc-231       .......... .......... .......... .......... T......... .......... ..........
P._zebrina-kc-257       .......... .......... .......... .......... T......... .......... ..........

                          565        575        585        595        605        615        625
P._sumatrana-kc-32      ATATTTTTGT CAAAGGTTTC ATTCACGCTT AATTCATATC GAGTAGACCT TGTTGTTGTG AGAATTCTTA
P._sumatrana-kc-160     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-161     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-403     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-404     .......... .......... .......... .......... .......... .......... ..........
P._sumatrana-kc-405     .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-330   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-345   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-346   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-383   .......... .......... .......... .......... .......... .......... ..........
P._corningiana-kc-384   .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-57        .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-231       .......... .......... .......... .......... .......... .......... ..........
P._zebrina-kc-257       .......... .......... .......... .......... .......... .......... ..........

                          635        645        655        665        675
P._sumatrana-kc-32      ATTCATGAGT TGTAGGGAGG GACTTATGTC ACCACAAACA GAAACTAAAG CA
P._sumatrana-kc-160     .......... .......... .......... .......... .......... ..
P._sumatrana-kc-161     .......... .......... .......... .......... .......... ..
P._sumatrana-kc-403     .......... .......... .......... .......... .......... ..
P._sumatrana-kc-404     .......... .......... .......... .......... .......... ..
P._sumatrana-kc-405     .......... .......... .......... .......... .......... ..
P._corningiana-kc-330   .......... .......... .......... .......... .......... ..
P._corningiana-kc-345   .......... .......... .......... .......... .......... ..
P._corningiana-kc-346   .......... .......... .......... .......... .......... ..
P._corningiana-kc-383   .......... .......... .......... .......... .......... ..
P._corningiana-kc-384   .......... .......... .......... .......... .......... ..
P._zebrina-kc-57        .......... .......... .......... .......... .......... ..
P._zebrina-kc-231       .......... .......... .......... .......... .......... ..
P._zebrina-kc-257       .......... .......... .......... .......... .......... ..
```

Fig. 6.

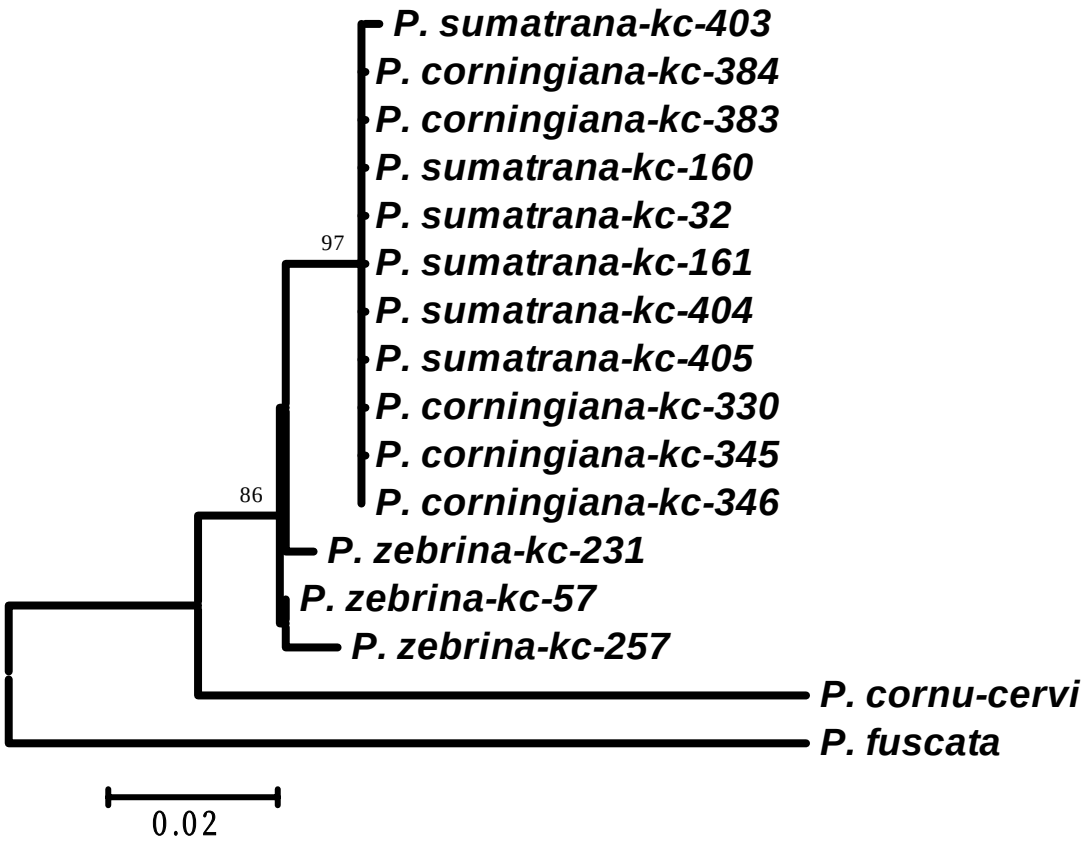


Fig. 6. The Neighbor-joining tree of the 14 accessions from *Phalaenopsis sumatrana* complex plus outgroups, namely *Phalaenopsis cornu-cervi* and *P. fuscata*, obtained from sequence comparisons of the ITS region of rDNA. Numbers above internodes indicate values of the interior branch test from 1000 replicates. More than 50% of interior branch test is shown on each branch. Branch lengths are proportional to the number of base changes along each branch.

Fig. 7.

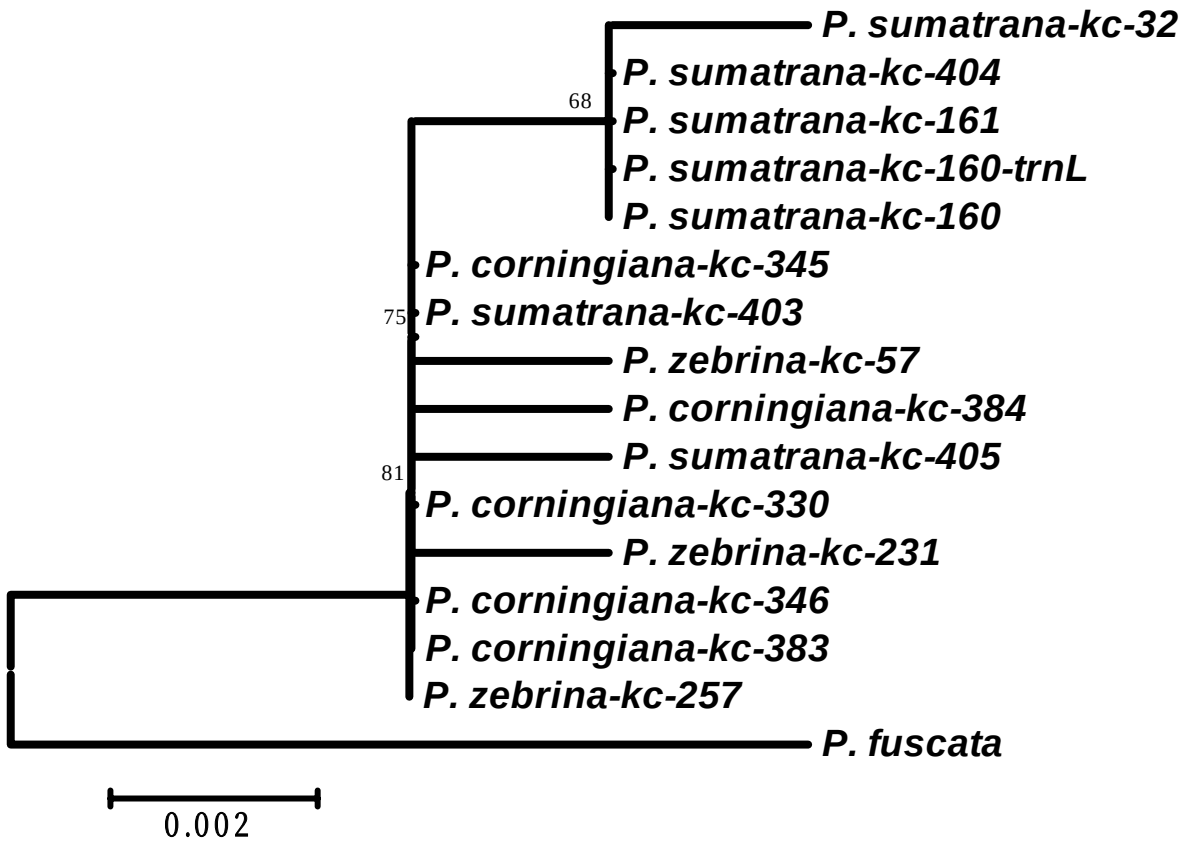


Fig. 7. The Neighbor-joining tree of the 14 accessions from *Phalaenopsis sumatrana* complex plus one outgroup, namely *P. fuscata*, obtained from sequence comparisons of the intron of *trn*L of chloroplast DNA. Numbers above internodes indicate values of the interior branch test from 1000 replicates. More than 50% of interior branch test is shown on each branch. Branch lengths are proportional to the number of base changes along each branch.

Fig. 8.

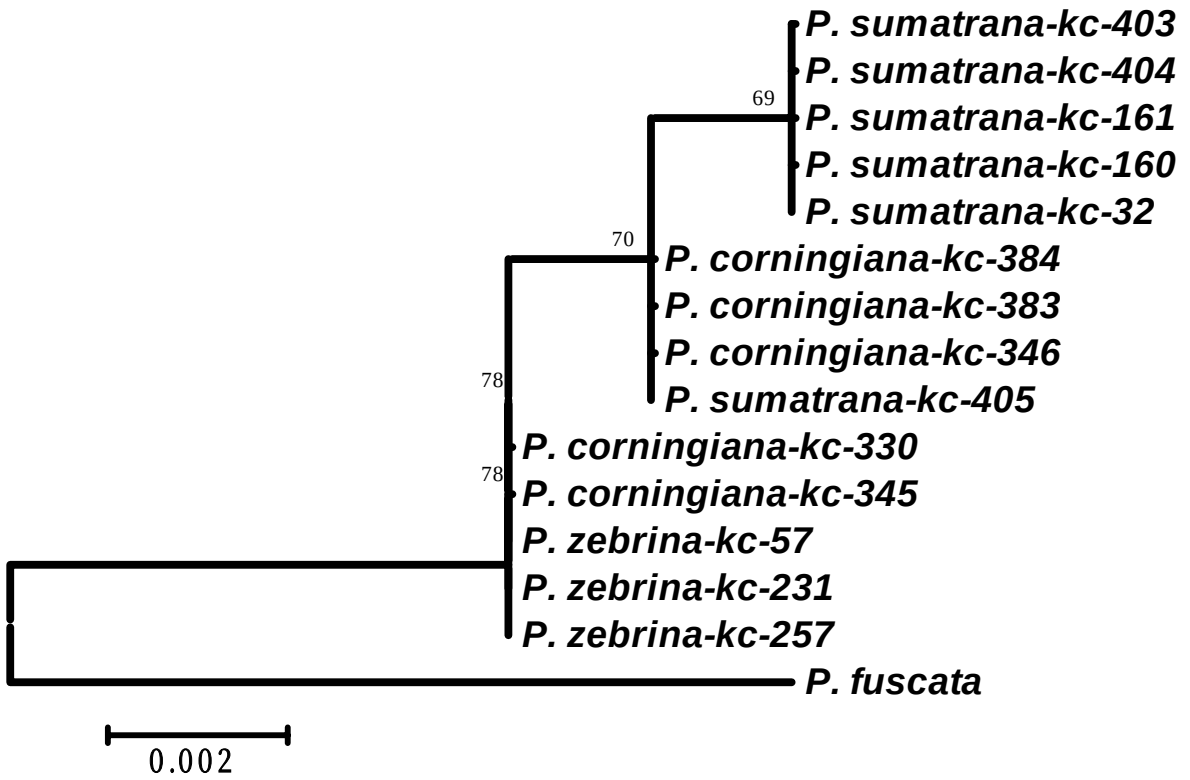


Fig. 8. The Neighbor-joining tree of the 14 accessions from *Phalaenopsis sumatrana* complex plus one outgroup, namely *P. fuscata*, obtained from sequence comparisons of the IGS of *atp*B-*rbc*L of chloroplast DNA. Numbers above internodes indicate values of the interior branch test from 1000 replicates. More than 50% of interior branch test is shown on each branch. Branch lengths are proportional to the number of base changes along each branch.

Fig. 9.

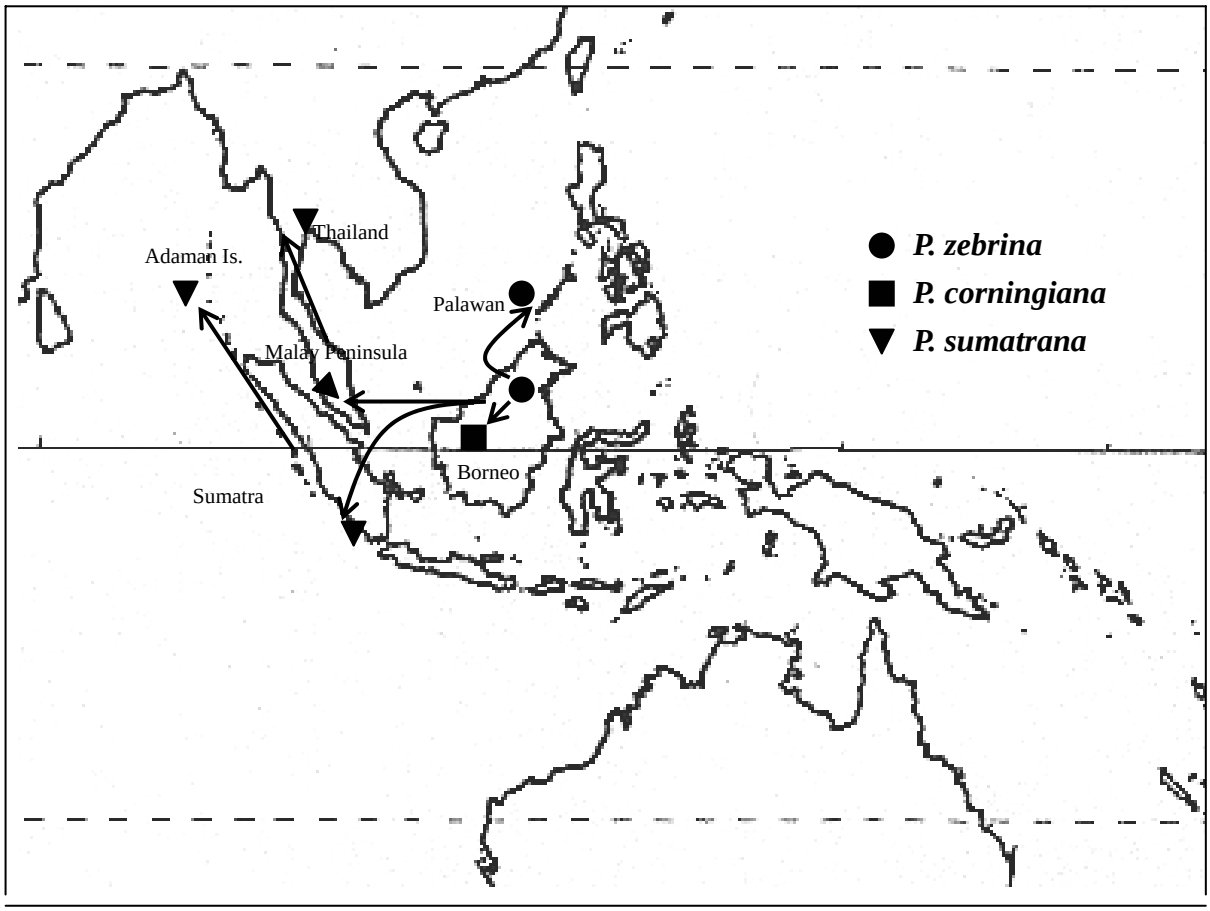


Fig. 9. Evolutionary trend of *P. sumatrana* complex based on the phylogenetic tree.

Chapter 4
Phylogenetics, Biogeography, and Evolutionary Trends of the *P. violacea* Complex Inferred from Nuclear DNA and Chloroplast DNA

Abstract

The phylogenetic trees inferred from the internal transcribed spacer 1 and 2 (ITS1+ITS2) region of nuclear ribosomal DNA (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, biogeography, and evolutionary trends of the *Phalaenopsis violacea* complex. The *P. violacea* complex includes the two species of *P. violacea* and *P. bellina* according to the concept of Christenson (2001). Based on the phylogenetic tree inferred from the ITS sequence, *P. bellina* cannot be separated from accessions of *P. violacea,* with the exception of the population distributed on Mentawai Islands, Indonesia. In addition, analyses of both sequences of the *trn*L intron and *atp*B-*rbc*L IGS of cpDNA apparently cannot discriminate these two species of the *P. violacea* complex. Based on morphological characters, *P. violacea* distributed on Mentawai Island has a long floral rachis and is separate from the other groups of the *P. violacea* complex. Therefore, the results in this study suggest treating the population of the *P. violacea* complex on Mentawai Islands as a separate species from *P. violacea*. As to the evolutionary trends of the *P. violacea* complex, Mentawai plants of this complex evolved from plants of Sumatra/the Malay Peninsula according to phylogenetic analysis and biogeography.

Introduction

The characteristic of the species *P. bellina* distributed in Borneo is that it bears purplish inside the base of the lateral sepals, which separates if from those of *P. violacea* of Sumatra and the Malay Peninsula with rose-pink color over the entire surface of the sepals and petals (known as the Malaysian type). Plants of *P. bellina* were traditionally treated as the ‘Borneo form’ of *P. violacea* based on their similar morphology of the lip and calli (Kuhn and Kuhn, 1965). Until an examination of floral fragrances and a review of other morphological differences by Christenson and Whitten (1995), plants of the *P. violacea* complex distributed in Borneo were treated as *P. bellina* and were separated from the other plants of this complex. Plants of *P. bellina* bear greenish-white flowers with a purple suffusion restricted to the lip and column, the lateral sepals, and the basal parts of the petals and dorsal sepal. In contrast, plants of *P. violacea* bear a purple suffusion throughout the flowers with the exception of a greenish color on the tips of the sepals and petals. Furthermore, the leaf shape of *P. bellina* is broader (generally more than 10 cm wide) than that of *P. violacea* (generally less than 8 cm wide) (Christenson and Whitten, 1995). In addition, the lateral sepals of *P. bellina* are ‘bow-legged’ and the apices of the three sepals form an isosceles triangle. In contrast, the lateral sepals of *P. violacea* are not ‘bow-legged’, and the apices of the three sepals form an equilateral

triangle (Christenson and Whitten, 1995). As to the geographical distribution, *P. bellina* is only distributed in Borneo (Sarawak), while *P. violacea* is distributed in Sumatra and the Malay Peninsula (Christenson, 2001). In addition, plants of *P. bellina* possess a lemony fragrance, while plants of *P. violacea* have a spicy scent (Kaiser, 1993; Christenson and Whitten, 1995).

Although plants of the *P. violacea* complex distributed in Borneo have been raised to a separate species, *P. bellina*, this species is undoubtedly closely related to its sister species, *P. violacea*. Furthermore, plants of *P. violacea* are divided into two different forms, namely the 'Sumatra form' and 'Malay form' based on their geographical distribution (Masaaki, 2002). Masaaki identified these two forms of *P. violacea* based on differences in the flower shape and floral color pattern. In addition, Masaaki (2002) also suggested that the 'Sumatra form' of *P. violacea* came from the natural hybridization between *P. bellina* and the 'Malay form' of *P. violacea*. Besides, a distinct population of *P. violacea* can be found on Mentawai Islands (off the west coast of Sumatra). Plants of the *P. violacea* complex on Mentawai have much longer inflorescences (to ca. 50 cm) and lack the somewhat flattened instead of a fleshy rachis. In contrast, both the 'Sumatra form' and the 'Malay form' of *P. violacea* as well as plants of *P. bellina* bear shorter (shorter than the leaves) and much-flatter inflorescences. In addition, flowers of Mentawai plants are larger, fuller in shape, and a quite-brilliant color compared to the other plants of *P. violacea* (Christenson, 2001).

Plants of the *P. violacea* complex have caused a lot of confusion to the present. In order to evaluate the phylogenetics, biogeography, and evolutionary trends of the *P. violacea* complex, its two species of *P. bellina* and *P. violacea* originating from different locations were collected for examination based on analyses of sequences of the ITS1 and ITS2 of nrDNA, the intron of *trn*L, and the IGS of *atp*B-*rbc*L of cpDNA.

Materials and Methods

Plant materials

Materials of the 14 accessions of the *P. violacea* complex, namely *P. violacea* and *P. bellina*, were used for this study (Table 1). The geographical distribution of this complex is shown in Fig. 1.

DNA extraction

Total DNA was extracted using the CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle, 1987). The approximate DNA yields were then determined using a spectrophotometer (Hitachi U-2001).

PCR amplification and electrophoresis

Primer sets designed for amplifying the ITS of nrDNA, the intron of *trn*L, and the IGS of *atp*B-*rbc*L of chloroplast DNA (cpDNA) from *Phalaenopsis* plants as well as PCR conditions were referenced from Tsai (see chapter 1). These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained by 0.5 μg/mL ethidium bromide, and finally photographed under UV light exposure.

DNA recovery and sequencing

PCR products of different DNA fragments from the plant material studied were recovered by glassmilk (BIO 101, California) and directly sequenced using the dideoxy chain-termination method with an ABI377 automated sequencer and a Bigdye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, California). Sequencing primers were the same as those used for PCR. These reactions were performed as recommended by the manufacturers.

Data analyses

Boundaries of the ITS regions (including ITS1, 5.8S rDNA, and ITS2), the intron of *trn*L, and the IGS of *atp*B-*rbc*L in accessions of the *P. violacea* complex, were determined by comparison to several published sequences as described by Tsai (see chapter 1). Sequences were aligned using the program Clustal W Multiple alignment in BioEdit (Hall, 1999). Genetic relationships were determined using the program MEGA version 2.1 (Kumar et al., 2001). A genetic distance matrix was calculated by the two-parameter method of Kimura (1980), and was used to construct a tree by the Neighbor-joining method (NJ) (Saitou and Nei, 1987) with interior branch tests of 1000 replicates (Sitnikova et al., 1995).

Results and Discussion

Sequence characteristics

The accession numbers of the 14 accessions of the *P. violacea* complex are shown in Table 2. The sequence lengths of ITS1 from each accession were all 243 bp, and those of ITS2 were all 263 bp. Percentages of the G+C content across the ITS1 region of the *P. violacea* complex varied from 72.0% to 72.4%. On the other hand, percentages of the G+C content across the ITS2 region from different accessions in the complex were all 77.9%. Combining ITS1 and ITS2, those sequences of the 14 accessions of the *P. violacea* complex were aligned and resulted in 506 characters. No gap sites and two variable sites were found in the alignment sequence of the 14 accessions of the *P. violacea* complex (Fig. 2).

There were no base substitutions in the sequences of the *trn*L intron within the 14 accessions of the *P. violacea* complex. Different lengths of deletions/insertions could be found across the alignment sequence of the *P. violacea* complex. The sequence lengths of the *trn*L intron from the 14 accessions of the *P. violacea* complex ranged from 555 to 588 bp. Percentages of the G+C content across this region of the *P. violacea* complex varied from 24.9% to 25.8% (Table 3). Those sequences were aligned and resulted in 588 characters. However, no base substitutions were found in this region. However, the mutational hot spot of length variations was found in sequences of the *trn*L intron from the *P. violacea* complex, as it was in those of the *P. sumatrana* complex (Fig. 3). In the hot spot region of the *trn*L intron, accessions of both *P. bellina* and *P. violacea* originating from Mentawai Islands possessed the same DNA length. Accessions of *P. violacea* from Sumatra had the same DNA length. In contrast, each of the accessions of *P. violacea* from Malay possessed different DNA lengths (Fig. 4). The results indicate that there is no constituent rule in the hot spot region of sequences of the *trn*L intron of cpDNA among species/forms of the *P. violacea* complex.

Sequence lengths and base pairs of sequences of *atp*B-*rbc*L IGS obtained from the 14 accessions of the *P. violacea* complex were shown to be identical. The sequence lengths and the percentages of G+C content of the *P. violacea* complex were 689 bp and 22.6%, respectively (Table 3, Fig. 5).

Genetic distances between accessions/species of the P. violacea complex

Genetic distances of ITS1 and ITS2 among the 14 accessions of the *P. violacea* complex were in the range of from 0.000 to 0.004 with an average of 0.002 using the two-parameter method of Kimura (1980). Within accessions of *P. violacea*, ranges of genetic distances were also from 0.000 to 0.004 with an average of 0.002. No genetic distance was observed among accessions of *P. bellina*. Furthermore, genetic distances among accessions of *P. violacea* collected from the Malay Peninsula, *P. violacea* collected from Sumatra, and *P. bellina* were shown to be 0.000. Of accessions of the *P. violacea* complex, only accessions of *P. violacea* collected from Mentawai Is. showed a genetic distance of 0.003 with the remainder of this complex studied (Table 4).

In the analysis of sequences of the *trn*L intron, no genetic distance was detected among the 14 accessions of the *P. violacea* complex due to there being no substitutions among those sequences. In the analysis of sequences of *atp*B-*rbc*L IGS, no genetic distance was found among the 14 accessions of the *P. violacea* complex due to those sequences being identical (Fig. 5). Therefore, ITS sequences of nuclear DNA were shown to be more variable than sequences of both the *trn*L intron and *atp*B-*rbc*L IGS of cpDNA in the analyses of the *P. violacea* complex. These results are congruent with analyses of various other species of the genus *Phalaenopsis* (Tsai, see chapter 1).

Phylogenetic reconstructions

Molecular data of ITS1 and ITS2 of nrDNA, the *trn*L intron, and *atp*B-*rbc*L IGS were examined among the *P. violacea* complex. Only the ITS1 and ITS2 of nrDNA offered valuable information for identifying accessions of the *P. violacea* complex. The phylogenetic tree inferred from ITS1 and ITS2 of nrDNA and reconstructed following the NJ method is shown in Fig. 6. Based on the phylogenetic tree, accessions of *P. bellina* formed a clade with accessions of *P. violacea* with the exceptions of Mentawai plants of *P. violacea*. Accessions of *P. violacea* collected from Mentawai Is. formed a clade and were separated from the other accessions of the *P. violacea* complex.

All sequences of the IGS of *atp*B-*rbc*L from the *P. violacea* complex were identical. Therefore, sequences of the IGS of *atp*B-*rbc*L contributed nothing to the reconstruction of the phylogenetics of the *P. violacea* complex. In addition, although the mutational hot spot of length variation of the intron of *trn*L from the *P. violacea* complex was highly variable, base substitutions in the regions among accessions of the *P. violacea* complex were not found. Therefore, the intron of *trn*L also contributed nothing to reconstruct the phylogenetic tree of the *P. violacea* complex. In addition, these length variations also offer no valuable information for identifying accessions of the *P. violacea* complex. The viewpoint is that consistent deletions/insertions do not exist within the population/species of the *P. violacea* complex, in particular within accessions of *P. violacea* distributed on the Malay Peninsula but they each have different lengths of deletions/insertions (Fig. 4).

Although differences exist in the floral fragrance and leaf shape between *P. violacea* and *P. bellina* (Christenson and Whitten 1995), these two species still have a close relationship based on the morphological characters as described by Kuhn and Kuhn (1965). Therefore, *P. violacea* and *P. bellina* were suggested to be of more-recent origin (Kuhn and Kuhn, 1965). Molecular data of this study also cannot discriminate these two species. This result supports both species of *P. bellina* and *P. violacea* having a close relationship. Within accessions of *P. violacea*, plants of the “Sumatra form” and “Malay form” also could not be separated based on the ITS sequence of nrDNA. The result does not support the separation of these two forms of *P. violacea* as described by Masaaki (2002). In fact, Christenson (2001) also did not accept the separation of these two forms of *P. violacea*. Actually, it is not easy to separate the *P. violacea* “Sumatra form” from the “Malay form” according to my inspection of the samples studied.

To the present, plants of the *P. violacea* complex distributed on Mentawai Is. are treated as species of *P. violacea* (Christenson, 2001). However, this population was unique and separate from the other accessions of this complex according to the ITS sequence of nrDNA in this study. In addition, based on an inspection of the Mentawai plants of the *P. violacea* complex studied, these plants bear the much-longer inflorescences and roundish rachis described by Christenson (2001). Furthermore, the midlobe shape of the lip and leaf shape of the *P. violacea* complex distributed on

Mentawai Is. are thinner than those of plants of the *P. violacea* complex distributed in Sumatra/the Malay Peninsula as well as those of *P. bellina* (data not shown). Therefore, Mentawai plants of the *P. violacea* complex show unique characters from the other plants of the *P. violacea* complex based on the molecular and morphological data. The results indicated that *P. violacea* distributed on Mentawai Is. shows a higher rate of evolution than plants of the *P. violacea* complex distributed elsewhere, namely Sumatra, the Malay Peninsula, and Borneo. The founder effect and the bottleneck effect more easily occur on small isolated islands, resulting in plants of islands more readily evolving into unique types (Tamarin and Leavitt, 1991). This possibly explains why *P. violacea* distributed on Mentawai Is. was shown to be unique. In short, the results of this study suggest treating the population of Mentawai Is. of the *P. violacea* complex as a separate species from *P. violacea*.

Biogeography and evolutionary trends

According to the geographical distribution of the *P. violacea* complex, Mentawai Is. is closer to Sumatra than to the Malay Peninsula/Borneo. This has offered a greater chance in the past for plants of *P. violacea* to have dispersed between Mentawai Is. and Sumatra. Based on the phylogenetic tree of the *P. violacea* complex inferred from ITS sequences of nrDNA, Mentawai plants of *P. violacea* are the derived population. Therefore, it is suggested that Mentawai plants of the *P. violacea* complex were derived from Sumatra based on the phylogenetic tree and biogeography. In addition, Sumatra/the Malay Peninsula/Borneo are relative origin regions of plants of the *P. violacea* complex according to the phylogenetic tree. However, which region is the relative original distributional site of the *P. violacea* complex cannot be ascertained by this study. Nevertheless, plants within the geographical distribution of the *P. violacea* complex could have crossed over in ancient times, since land bridges had formed during glacial periods (Van Oosterzee, 1997). The *P. violacea* complex is distributed all over the lands of the Sunda Shelf with the exception of Java. This indicates that Java has been more isolated from the other lands of the Sunda Shelf. This result is in agreement with the biogeography of the genus *Phalaenopsis*, in that *Phalaenopsis* species found on Java differ from those of the other lands of the Sunda Shelf (Christenson, 2001; Tsai, see chapter 1). Furthermore, plants of the *P. amabilis* complex in Java are separate from those of Borneo according to the analysis of ITS sequences of nrDNA (Tsai, see chapter 2). In addition, plants of the *P. sumatrana* complex are also distributed over the Sunda Shelf with the exception of Java (Tsai, see chapter 3). Those results show that Java is a relatively isolated region among the lands of the Sunda Shelf according to research on *Phalaenopsis* species.

Literature Cited

Christenson, E. A. 2001. *Phalaenopsis*. Timber Press, Portland, Oregon. 330p.

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

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

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.

Kaiser, R. 1993. The scent of orchids--olfactory and chemical investigations. Elsevier, Amsterdam.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Mol. Evol. 16: 111-120.

Kuhn, J. and L. Kuhn. 1965. *Phalaenopsis violaceae*. Am. Orchid Soc. Bull. 34: 204-208.

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

Masaaki, C. 2002. *Phalaenopsis* species. Chiba Masaaki *Phalaenopsis* species Publishing Society, Saitama-ken, Japan.

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

Sitnikova, T., A. Rzhetsky, and M. Nei. 1995. Interior-branch and bootstrap tests of phylogenetic trees. Mol. Biol. Evol. 12: 319-333.

Tamarin, R. and R. W. Leavitt. 1991. Principles of genetics. 3rd ed. Wm. C. Brown Publishers, Dubuque, IA.

Tsai, C. C., S. C. Huang, P. L. Huang, and C. H. Chou. 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. Biotechnol. 78: 879-887.

Van Oosterzee, P. 1997. Where worlds collide: the Wallace line. Ithaca, NY: Cornell University Press.

Table 1. A list of 15 accessions from the two closely *Phalaenopsis* species of *P. bellina* and *P. violacea* and their different geographical distributions.

Taxa and systematic classification [a]	Distribution	Accession no.	Source
P. violacea	Malay Peninsula	AY390227	KDAIS-KC423
P. violacea	Malay Peninsula	AY390228	KDAIS-KC152
P. violacea	Malay Peninsula	AY390229	KDAIS-KC153
P. violacea	Sumatra	AY390230	KDAIS-KC349
P. violacea	Sumatra	AY390231	KDAIS-KC365
P. violacea	Sumatra	AY390232	KDAIS-KC366
P. violacea	Mentawai Island	AY390233	KDAIS-KC361
P. violacea	Mentawai Island	AY390234	KDAIS-KC367
P. violacea	Mentawai Island	AY390235	KDAIS-KC422
P. violacea	Mentawai Island	AY390236	KDAIS-KC439
P. bellina	Borneo	AY390237	KDAIS-KC67
P. bellina	Borneo	AY390238	KDAIS-KC351
P. bellina	Borneo		KDAIS-KC106
P. bellina	Borneo		KDAIS-KC107

[a] The classification of *Phalaenopsis* is based on Christenson (2001).
[b] Kaohsiung District Agricultural Improvement Station.

Table 2. Lengths of ITS1 and ITS2 and GenBank accession numbers of the 14 accessions of the *Phalaenopsis violacea* complex.

Taxa [a]	ITS1		ITS2		GenBank accession no.
	Length (bp)	G+C (%)	Length (bp)	G+C (%)	
P. violacea-Malay-kc-423	243	72.0	263	77.9	AY390227
P. violacea-Malay-kc-152	243	72.0	263	77.9	AY390228
P. violacea-Malay-kc-153	243	72.0	263	77.9	AY390229
P. violacea-Sumatra-kc-349	243	72.0	263	77.9	AY390230
P. violacea-Sumatra-kc-365	243	72.0	263	77.9	AY390231
P. violacea-Sumatra-kc-366	243	72.0	263	77.9	AY390232
P. violacea-Mentawai_Is.-kc-361	243	72.1	263	77.9	AY390233
P. violacea-Mentawai_Is.-kc-367	243	72.4	263	77.9	AY390234
P. violacea-Mentawai_Is.-kc-422	243	72.4	263	77.9	AY390235
P. violacea-Mentawai_Is.-kc-439	243	72.4	263	77.9	AY390236
P. bellina-kc-67	243	72.0	263	77.9	AY390237
P. bellina-kc-351	243	72.0	263	77.9	AY390238
P. bellina-kc-106	243	72.0	263	77.9	
P. bellina-kc-107	243	72.0	263	77.9	
Average	243	72.1	263	77.9	

Table 3. Lengths and G+C contents of the *trn*L intron and the IGS of *atp*B-*rbc*L among the 14 accessions of the *Phalaenopsis violacea* complex.

Taxa [a]	*trn*L intron		IGS of *atp*B-*rbc*L	
	Length (bp)	G+C (%)	Length (bp)	G+C (%)
P. violacea-Malay-kc-423	555	25.6	689	22.6
P. violacea-Malay-kc-152	574	25.3	689	22.6
P. violacea-Malay-kc-153	588	24.9	689	22.6
P. violacea-Sumatra-kc-349	574	25.3	689	22.6
P. violacea-Sumatra-kc-365	574	25.3	689	22.6
P. violacea-Sumatra-kc-366	574	25.3	689	22.6
P. violacea-Mentawai_Is.-kc-361	562	25.8	689	22.6
P. violacea-Mentawai_Is.-kc-367	562	25.8	689	22.6
P. violacea-Mentawai_Is.-kc-422	562	25.8	689	22.6
P. violacea-Mentawai_Is.-kc-439	562	25.8	689	22.6
P. bellina-kc-67	562	25.8	689	22.6
P. bellina-kc-351	562	25.8	689	22.6
P. bellina-kc-106	562	25.8	689	22.6
P. bellina-kc-107	562	25.8	689	22.6

Table 4. Genetic distance matrix of ITS1 and ITS2 of nrDNA among the 14 accessions of the *Phalaenopsis violacea* complex.

[	1	2	3	4	5	6	7	8	9	10	11	12	13]
[1]*													
[2]	0.000												
[3]	0.000	0.000											
[4]	0.000	0.000	0.000										
[5]	0.000	0.000	0.000	0.000									
[6]	0.000	0.000	0.000	0.000	0.000								
[7]	0.002	0.002	0.002	0.002	0.002	0.002							
[8]	0.004	0.004	0.004	0.004	0.004	0.004	0.002						
[9]	0.004	0.004	0.004	0.004	0.004	0.004	0.002	0.000					
[10]	0.004	0.004	0.004	0.004	0.004	0.004	0.002	0.000	0.000				
[11]	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.004	0.004			
[12]	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.004	0.004	0.000		
[13]	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.004	0.004	0.000	0.000	
[14]	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.004	0.004	0.000	0.000	0.000

*[1], P. violacea-Malay-kc-152; [2], P. violacea-Malay-kc-153; [3], P. violacea-Malay-kc-423; [4], P. violacea-Sumatra-kc-349; [5], P. violacea-Sumatra-kc-365; [6], P. violacea-Sumatra-kc-366; [7], P. violacea-Mentaiwai_Is.-kc-361; [8], P. violacea-Mentaiwai_Is.-kc-367; [9], P. violacea-Mentaiwai_Is.-kc-422; [10], P. violacea-Mentaiwai_Is.-kc-439; [11], P. bellina-kc-67; [12], P. bellina-kc-106; [13], P. bellina-kc-107, [14] : P. bellina-kc-351.

Fig. 1.

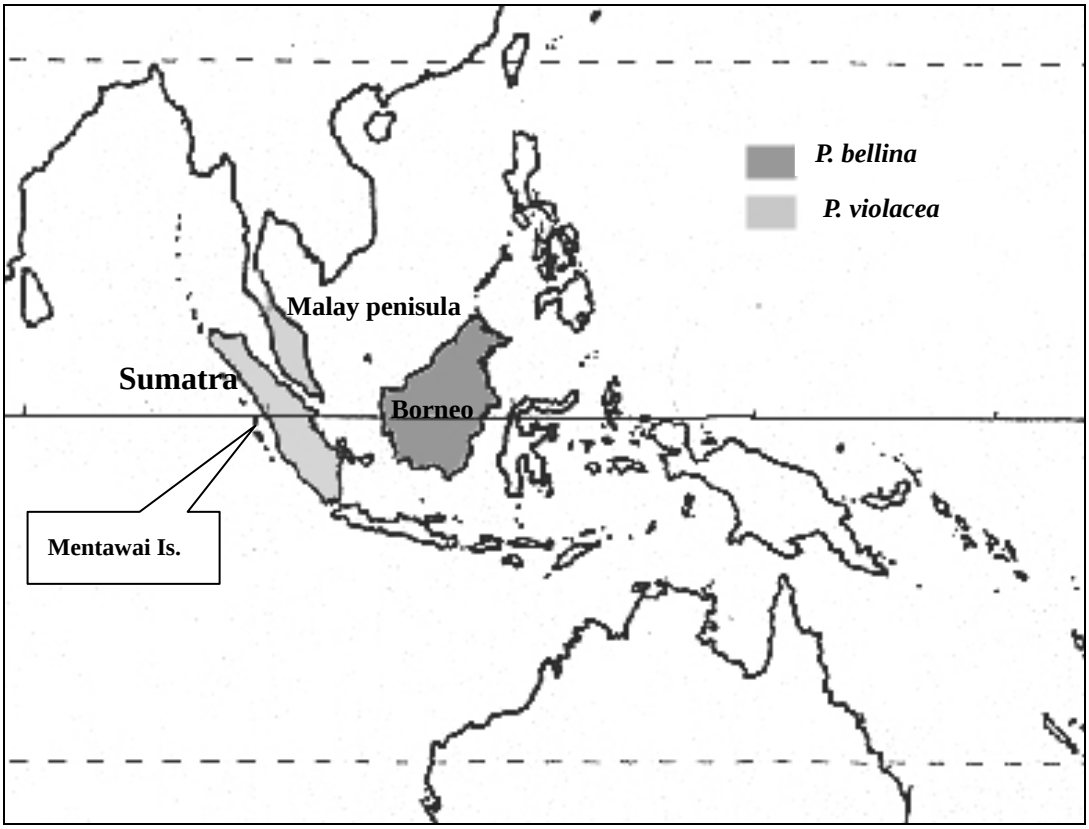
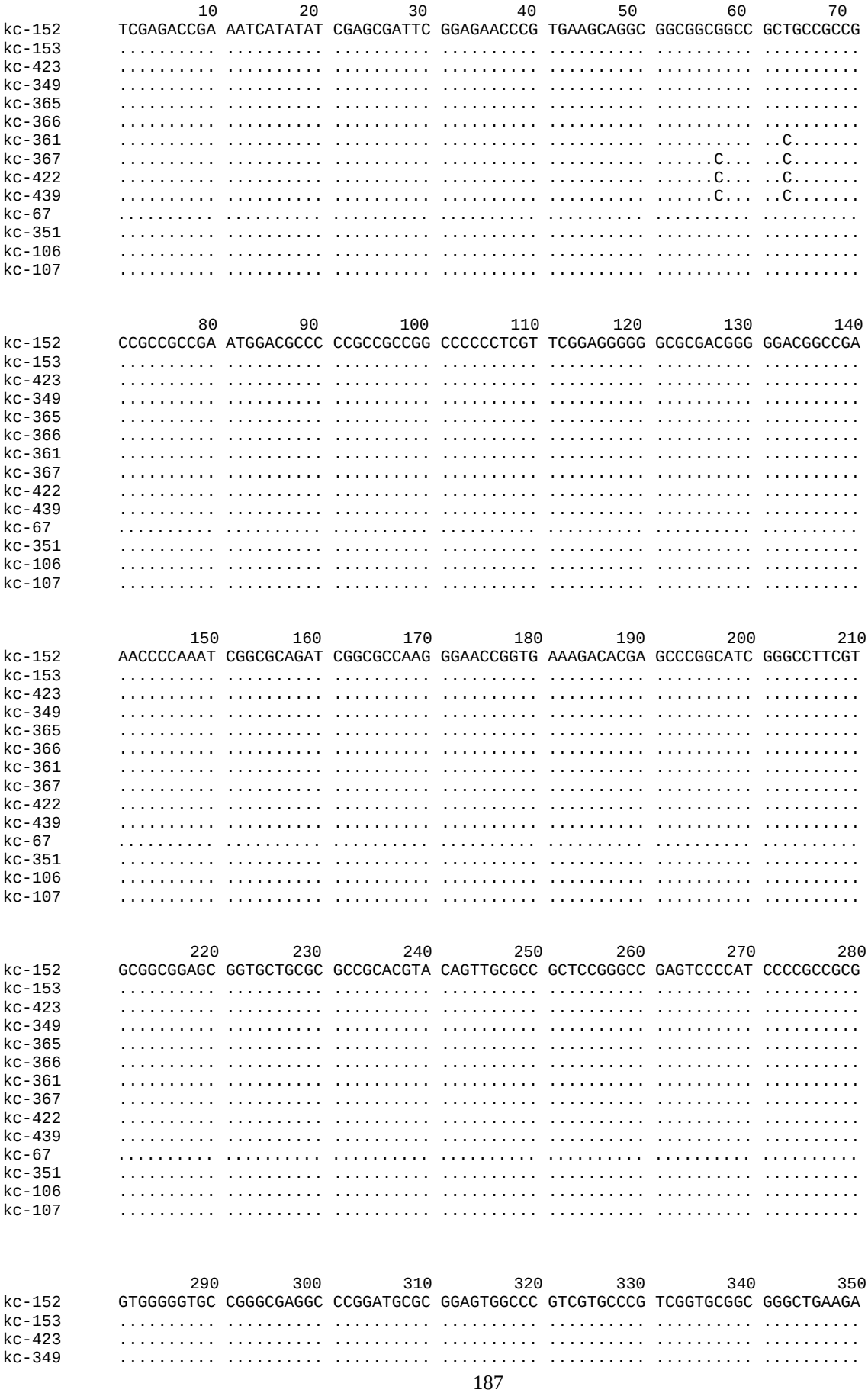


Fig. 1. Geographical distributions of *Phalaenopsis bellina* and *P. violacea*.

Fig. 2. Sequence alignment of ITS1 and ITS2 of rDNA from the14 accessions of the *Phalaenopsis violacea* complex.



```
kc-365      .......... .......... .......... .......... .......... .......... ..........
kc-366      .......... .......... .......... .......... .......... .......... ..........
kc-361      .......... .......... .......... .......... .......... .......... ..........
kc-367      .......... .......... .......... .......... .......... .......... ..........
kc-422      .......... .......... .......... .......... .......... .......... ..........
kc-439      .......... .......... .......... .......... .......... .......... ..........
kc-67       .......... .......... .......... .......... .......... .......... ..........
kc-351      .......... .......... .......... .......... .......... .......... ..........
kc-106      .......... .......... .......... .......... .......... .......... ..........
kc-107      .......... .......... .......... .......... .......... .......... ..........


                   360        370        380        390        400        410        420
kc-152      GCGGGGCATC GTCTCAGTGC CTACGAACAA CGAGGGGTGG ATGAGAGAAG GCCGCCGCGG GCAGGGCCCG
kc-153      .......... .......... .......... .......... .......... .......... ..........
kc-423      .......... .......... .......... .......... .......... .......... ..........
kc-349      .......... .......... .......... .......... .......... .......... ..........
kc-365      .......... .......... .......... .......... .......... .......... ..........
kc-366      .......... .......... .......... .......... .......... .......... ..........
kc-361      .......... .......... .......... .......... .......... .......... ..........
kc-367      .......... .......... .......... .......... .......... .......... ..........
kc-422      .......... .......... .......... .......... .......... .......... ..........
kc-439      .......... .......... .......... .......... .......... .......... ..........
kc-67       .......... .......... .......... .......... .......... .......... ..........
kc-351      .......... .......... .......... .......... .......... .......... ..........
kc-106      .......... .......... .......... .......... .......... .......... ..........
kc-107      .......... .......... .......... .......... .......... .......... ..........


                   430        440        450        460        470        480        490
kc-152      CGTTGTCTCG TGCCGGCCGG AGAGGAGACG GCGCCCTCAG TGCGATCCCA TCCCGAGCGC CGCCCCCCGT
kc-153      .......... .......... .......... .......... .......... .......... ..........
kc-423      .......... .......... .......... .......... .......... .......... ..........
kc-349      .......... .......... .......... .......... .......... .......... ..........
kc-365      .......... .......... .......... .......... .......... .......... ..........
kc-366      .......... .......... .......... .......... .......... .......... ..........
kc-361      .......... .......... .......... .......... .......... .......... ..........
kc-367      .......... .......... .......... .......... .......... .......... ..........
kc-422      .......... .......... .......... .......... .......... .......... ..........
kc-439      .......... .......... .......... .......... .......... .......... ..........
kc-67       .......... .......... .......... .......... .......... .......... ..........
kc-351      .......... .......... .......... .......... .......... .......... ..........
kc-106      .......... .......... .......... .......... .......... .......... ..........
kc-107      .......... .......... .......... .......... .......... .......... ..........


                   500   505
kc-152      GCGGCGGCTT GGAAT
kc-153      .......... .....
kc-423      .......... .....
kc-349      .......... .....
kc-365      .......... .....
kc-366      .......... .....
kc-361      .......... .....
kc-367      .......... .....
kc-422      .......... .....
kc-439      .......... .....
kc-67       .......... .....
kc-351      .......... .....
kc-106      .......... .....
kc-107      .......... .....
```

Fig. 3. Sequences alignment of the intron of *trn*L of chloroplast DNA from the 14 accessions of the *Phalaenopsis violacea* complex.

```
                                5          15         25         35         45         55
P._violacea-Malay-kc-152        AATGGAAGCT GTTCTAACGA ATGAAATTGA TTACGTTACG TTAGTAGCTA AAAGACTTCT
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439 .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                65         75         85         95         105        115
P._violacea-Malay-kc-152        ATCGAAATGA CAGAAAGGAT ACGTCTTATA CGTACGTATA CATACTGACA TAGCAAACGA
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439 .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                125        135        145        155        165        175
P._violacea-Malay-kc-152        TTAATCACAA CCCAAATCTT ATATCGAATT CTATTTTGTA TCTCTATATA TTTAGATATG
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439 .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                185        195        205        215        225        235
P._violacea-Malay-kc-152        AAATTTGAAA TTTATATATG AAAATAGAAA TCTTCTCTTT CTTTCTATTA ATCTATTAAT
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422 .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439 .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........
```

```
                                 245         255         265         275         285         295
P._violacea-Malay-kc-152         ATTAT----- ---------- ---------- --------AT TTAATATTAT ATTATTAATA
P._violacea-Malay-kc-153         .....CTCTT TCTATTAATA TTAT------ --------.. .......... ..........
P._violacea-Malay-kc-423         .....CTCTT TCTATTAATA TTATTCTATT AATATTAT.. .......... ..........
P._violacea-Sumatra-kc-349       .....CTCTT TCTATTAATA TTAT------ --------.. .......... ..........
P._violacea-Sumatra-kc-365       .....CTCTT TCTATTAATA TTAT------ --------.. .......... ..........
P._violacea-Sumatra-kc-366       .....CTCTT TCTATTAATA TTAT------ --------.. .......... ..........
P._violacea-Mentawai_Is.-kc-361  .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......
P._violacea-Mentawai_Is.-kc-367  .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......
P._violacea-Mentawai_Is.-kc-422  .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......
P._violacea-Mentawai_Is.-kc-439  .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......
P._bellina-kc-67                 .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......
P._bellina-kc-107                .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......
P._bellina-kc-106                .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......
P._bellina-kc-351                .....CTCTT TCTATTAATA TTAT------ --------.. .---------- ---.......

                                 305         315         325         335         345         355
P._violacea-Malay-kc-152         TGAGTAATAT AATAGTATGA GATAAGGATC TATAAGAAAC CCTATATTTC TATTCTTTTT
P._violacea-Malay-kc-153         .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423         .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349       .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365       .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366       .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439  .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                 .......... .......... .......... .......... .......... ..........
P._bellina-kc-107                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106                .......... .......... .......... .......... .......... ..........
P._bellina-kc-351                .......... .......... .......... .......... .......... ..........

                                 365         375         385         395         405         415
P._violacea-Malay-kc-152         TAATTAGAAT GATAATGATA GAGATCAAAA AGAGATATGA AAAATTGAAG AGTTATTGTG
P._violacea-Malay-kc-153         .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423         .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349       .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365       .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366       .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439  .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                 .......... .......... .......... .......... .......... ..........
P._bellina-kc-107                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106                .......... .......... .......... .......... .......... ..........
P._bellina-kc-351                .......... .......... .......... .......... .......... ..........

                                 425         435         445         455         465         475
P._violacea-Malay-kc-152         AATCAATTCC AATTGAAGTT GAAAAAAGAA TAGAATTCGA ATATTCAATG ATCAAATTAT
P._violacea-Malay-kc-153         .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423         .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349       .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365       .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366       .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439  .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                 .......... .......... .......... .......... .......... ..........
P._bellina-kc-107                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106                .......... .......... .......... .......... .......... ..........
P._bellina-kc-351                .......... .......... .......... .......... .......... ..........

                                 485         495         505         515         525         535
```

```
P._violacea-Malay-kc-152          TCATTCCAGA ATTTTTGATA GATCCTTTGA AATTGAATCG GACGAGAATA AAGAGAGAGT
P._violacea-Malay-kc-153          .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423          .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349        .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365        .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-361   .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-367   .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-422   .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-439   .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                  .......... .......... .......... .......... .......... ..........
P._bellina-kc-107                 .......... .......... .......... .......... .......... ..........
P._bellina-kc-106                 .......... .......... .......... .......... .......... ..........
P._bellina-kc-351                 .......... .......... .......... .......... .......... ..........

                                    545        555        565        575        585
P._violacea-Malay-kc-152          CCCATTTTAC ATGTCAATAC CGACAACAAT GAAATTTATA GTAAGAGG
P._violacea-Malay-kc-153          .......... .......... .......... .......... ........
P._violacea-Malay-kc-423          .......... .......... .......... .......... ........
P._violacea-Sumatra-kc-349        .......... .......... .......... .......... ........
P._violacea-Sumatra-kc-365        .......... .......... .......... .......... ........
P._violacea-Sumatra-kc-366        .......... .......... .......... .......... ........
P._violacea-Mentawai_Is.-kc-361   .......... .......... .......... .......... ........
P._violacea-Mentawai_Is.-kc-367   .......... .......... .......... .......... ........
P._violacea-Mentawai_Is.-kc-422   .......... .......... .......... .......... ........
P._violacea-Mentawai_Is.-kc-439   .......... .......... .......... .......... ........
P._bellina-kc-67                  .......... .......... .......... .......... ........
P._bellina-kc-107                 .......... .......... .......... .......... ........
P._bellina-kc-106                 .......... .......... .......... .......... ........
P._bellina-kc-351                 .......... .......... .......... .......... ........
```

Fig. 4.

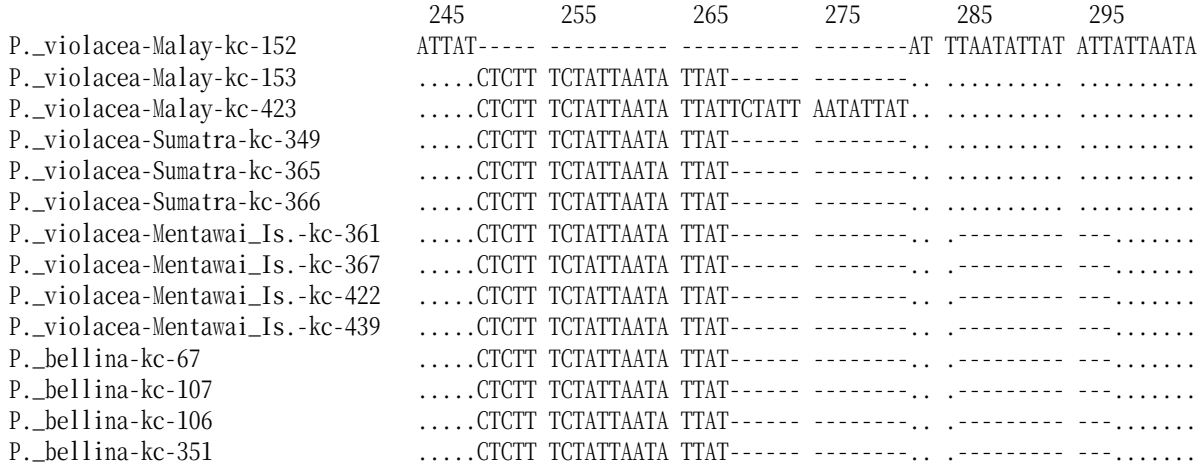


Fig. 4. Mutational hot spot of length variations within the intron of *trn*L of chloroplast DNA from the *Phalaenopsis violacea* complex.

Fig. 5. Sequence alignment of the IGS of *atp*B-*rbc*L of chloroplast DNA from the 14 accessions of the *Phalaenopsis violacea* complex.

```
                                5          15         25         35         45         55
P._violacea-Malay-kc-152        TACAACATAT ATTACTGTCA AGAGAGGGGA CCGGGTCCTA TATTCTTTCT TTTTATTTCT
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                65         75         85         95         105        115
P._violacea-Malay-kc-152        ATATTTTTAT ATTAGATATT TCTATTTACT ATTTATTATC TTTAATATCT TTATTATCTT
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                125        135        145        155        165        175
P._violacea-Malay-kc-152        TACTTGAAAA TTTTTATAAT TGAAATTTAT TCATTCTATA ATTTCTTAAT TCTAATTTAG
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                185        195        205        215        225        235
P._violacea-Malay-kc-152        AATTCTATTT CTATTCAATT TAATATTTAT CTATTTGAAT TGAATTCTAT TTAAACTAGA
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
```

```
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                      245        255        265        275        285        295
P._violacea-Malay-kc-152        TTTCTGAATT GAAATGAACT CGAAATTTTT CATTTTCTTT GATGTTTTTT TCTCTTTATT
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                      305        315        325        335        345        355
P._violacea-Malay-kc-152        TTGATATTCT TATTTCTTTC TTTTTTTTAT ATTTATATTC TATATCATAT TCATTCTTTA
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                      365        375        385        395        405        415
P._violacea-Malay-kc-152        TAAAAAATAT TAAGAAGATG ATAAATTCCA TTAGGAATAG AAATTTTCAA GAAGATTGGG
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                      425        435        445        455        465        475
P._violacea-Malay-kc-152        TTGCGCCATA TATATCAAAG AGTATAAAAT AATGATGTAT TTGGTGAATC AAATAAATGG
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
```

```
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                     485        495        505        515        525        535
P._violacea-Malay-kc-152        TCCAATAACG AACCCTTTTC AAATTTTCAT TATTCATTAG TTGATAATAT TAATTTATAG
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                     545        555        565        575        585        595
P._violacea-Malay-kc-152        TTTAGTTGAA TCTTTTTTGA ATTGTAAATA TTTTTGTCAA AGGTTTCATT CACGCTTAAT
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                     605        615        625        635        645        655
P._violacea-Malay-kc-152        TCATATCGAG TAGACCTTGT TGTTGTGAGA ATTCTTAATT CATGAGTTGT AGGGAGGGAC
P._violacea-Malay-kc-153        .......... .......... .......... .......... .......... ..........
P._violacea-Malay-kc-423        .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-36  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-42  .......... .......... .......... .......... .......... ..........
P._violacea-Mentawai_Is.-kc-43  .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-349      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-365      .......... .......... .......... .......... .......... ..........
P._violacea-Sumatra-kc-366      .......... .......... .......... .......... .......... ..........
P._bellina-kc-67                .......... .......... .......... .......... .......... ..........
P._bellina-kc-106               .......... .......... .......... .......... .......... ..........
P._bellina-kc-107               .......... .......... .......... .......... .......... ..........
P._bellina-kc-351               .......... .......... .......... .......... .......... ..........

                                665        675        685
```

```
P._violacea-Malay-kc-152          TTATGTCACC ACAAACAGAA ACTAAAGCA
P._violacea-Malay-kc-153          .......... .......... .........
P._violacea-Malay-kc-423          .......... .......... .........
P._violacea-Mentawai_Is.-kc-36    .......... .......... .........
P._violacea-Mentawai_Is.-kc-36    .......... .......... .........
P._violacea-Mentawai_Is.-kc-42    .......... .......... .........
P._violacea-Mentawai_Is.-kc-43    .......... .......... .........
P._violacea-Sumatra-kc-349        .......... .......... .........
P._violacea-Sumatra-kc-365        .......... .......... .........
P._violacea-Sumatra-kc-366        .......... .......... .........
P._bellina-kc-67                  .......... .......... .........
P._bellina-kc-106                 .......... .......... .........
P._bellina-kc-107                 .......... .......... .........
P._bellina-kc-351                 .......... .......... .........
```

Fig. 6.

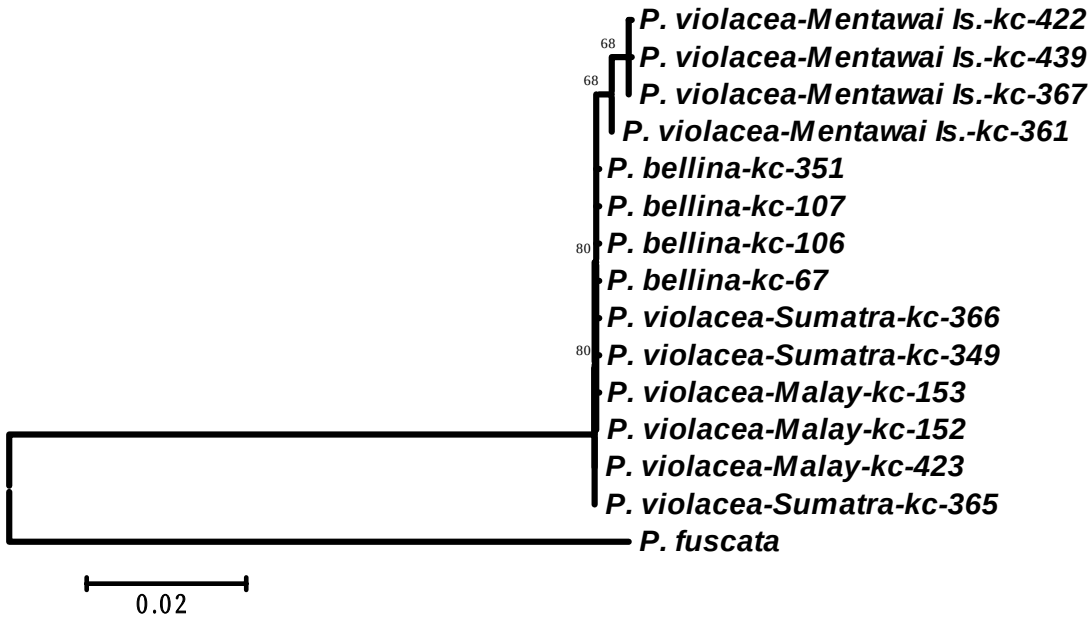


Fig. 6. The neighbor joining tree of the 14 accessions of the *Phalaenopsis violacea* complex plus the outgroup of *P. fuscata* obtained from sequence comparisons of the ITS region of rDNA. Numbers above the internodes indicate values of the interior branch test from 1000 replicates. The > 50% interior branch test is shown on each branch. Branch lengths are proportional to the number of base changes along each branch.

博碩士論文授權書

（國科會科學技術資料中心版本 92.2.17）

本授權書所授權之論文為本人在 國 立 中 山 大學(學院) 生物科學 系所 (空白) 組 九十二 學年度第 一 學期取得 博 士學位之論文。

論文名稱：蝴蝶蘭屬植物之分子親緣、生物地理及演化趨勢之研究

☑同意 ☐不同意 （政府機關重製上網）

本人具有著作財產權之論文全文資料，授予行政院國家科學委員會科學技術資料中心、國家圖書館及本人畢業學校圖書館，得不限地域、時間與次數以微縮、光碟或數位化等各種方式重製後散布發行或上載網路。

本論文為本人向經濟部智慧財產局申請專利(未申請者本條款請不予理會)的附件之一，申請文號為：_____，註明文號者請將全文資料延後半年再公開。

☑同意 ☐不同意 （圖書館影印）

本人具有著作財產權之論文全文資料，授予教育部指定送繳之圖書館及本人畢業學校圖書館，為學術研究之目的以各種方法重製，或為上述目的再授權他人以各種方法重製，不限地域與時間，惟每人以一份為限。

上述授權內容均無須訂立讓與及授權契約書。依本授權之發行權為非專屬性發行權利。依本授權所為之收錄、重製、發行及學術研發利用均為無償。上述同意與不同意之欄位若未鉤選，本人同意視同授權。

指導教授姓名：周昌弘 博士

研究生簽名：蔡奇助 學號:d8921803

（親筆正楷） （務必填寫）

日期:民國 93 年 2 月 16 日

---

1. 本授權書（得自http://sticnet.stic.gov.tw/sticweb/html/theses/authorize.html 下載或至http://www.stic.gov.tw首頁右下方下載）請以黑筆撰寫並影印裝訂於書名頁之次頁。
2. 授權第一項者，請確認學校是否代收，若無者，請個別再寄論文一本至台北市(106-36)和平東路二段 106 號 1702 室 國科會科學技術資料中心 王淑貞。(本授權書諮詢電話:02-27377746)
3. 本授權書於民國 85 年 4 月 10 日送請內政部著作權委員會(現為經濟部智慧財產局)修正定稿，89.11.21 部份修正。
4. 本案依據教育部國家圖書館 85.4.19 台(85)圖編字第 712 號函辦理。