天然雜交種蝴蝶蘭 *Phalaenopsis* × *intermedia* Lindl. 之起源的分子證據

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摘 要

為探討 *Phalaenopsis* × *intermedia* 之起源，本研究分析屬於雙親遺傳的核糖體核酸(ribosomal DNA, rDNA)內轉錄間隔區(internal transcribed spacer, ITS)，以及母系遺傳的葉綠體 DNA 的 *trn*L 基因之內隱子(intron), *trn*L-*trn*F 之基因間隔區 (intergenic spacer, IGS) 與 *atp*B-*rbc*L 之基因間隔區。由 ITS 序列得知，*P.* × *intermedia* 之單株個體所含之 ITS 序列具異質性(heterogeneity)，主要可以分成兩大類，經群叢分析及比對樣本兩兩間之遺傳距離發現，雜交種 ITS 序列的兩大類分別與 *P. aphrodite* 及 *P. equestris* 之 ITS 序列相近，因此推斷 *P. aphrodite* 及 *P. equestris* 為 *P.* × *intermedia* 之親本。另外，由葉綠體 DNA 得知 *P.* × *intermedia* 與 *P. aphrodite* 最相相近，基於葉綠體 DNA 為母系遺傳之故，因此推斷 *P. aphrodite* 為 *P.* × *intermedia* 之母本，所以 *P. equestris* 則為父本。

關鍵語: *Phalaenopsis* × *intermedia*、天然雜交種、分子證據

前 言

蘭科 (Orchidaceae) 植物分佈遍及世界各地，可能是被子植物中種類最多的一科，分成 500 多個屬(genus)，據估計有 17,000~35,000 種 (7)。主要分佈在熱帶及亞熱帶地區，溫帶及寒帶地區也可發現其蹤影，甚至極地環境也能生存。蝴蝶蘭屬 (*Phalaenopsis*) 是為蘭科中的一個極具經濟價值的類群，屬於樹蘭亞科 (subfamily Epidendroideae)、萬代蘭族 (tribe Vandeae)、仙人指甲蘭亞族 (subtribe Aeridinae) (7)。全球的蝴蝶蘭屬植物約有 65 種左右，其中有 5 種僅有一次採集記錄。主要分佈在東南亞地區，北界為東印度、中國大陸雲南及貴州至台灣，南界為澳洲北部，東至新幾內亞，往西延伸至印度東緣，其中印尼、菲律賓及馬來西亞為主要產地(5)。台灣地區可以找到兩種，分別為台灣白花蝴蝶蘭 (*Phalaenopsis aphrodite* subsp. *formosana*) 及姬蝴蝶蘭 (*P. equestris*)，分佈於台東、恆春及蘭嶼等地區(25)。依據 Christenson (2001) (5) 的系統分類，將蝴蝶蘭屬分成五個亞屬，分別為 *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos* 及 *Phalaenopsis*，其中

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Polychilos 亞屬內又區分四個節，分別為 *Polychilos* (Breda) Rchb. F., *Fuscatae* Sweet, *Amboinenses* Sweet, *Zebrinae* Pfitz.；*Phalaenopsis* 亞屬內也區分四個節，分別為 *Phalaenopsis* , *Deliciosae* E. A. Christ., *Esmeralda* Rchb.f.及 *Stauroglottis* (Schauer) Benth。

Phalaenopsis × *intermedia* 是一種天然雜交種，因其具有鮮紅的唇瓣而被廣泛應用於蝴蝶蘭的育種上。Christenson (2001)[5] 將此天然雜交種處理為蝴蝶蘭亞屬(subgenus *Phalaenopsis*)、蝴蝶蘭節(section *Phalaenopsis*)的成員之一。在此節之內還有 *P. amabilis*、*P. aphrodite*、*P. sanderiana*、*P. philippinensis* 、*P. schilleriana* 、*P. stuartiana* 等六種。*Phalaenopsis* × *intermedia* 的植株大小及葉的型態特徵與 *P. aphrodite* 及 *P. amabilis* 相近。此外，花的唇瓣外形也與 *P. aphrodite* 特別相近，不過唇瓣的顏色與 *P. equestris* 相近[26,27,5]。因此，自從 1853 年命名此種以來， *P.* × *intermedia* 就一直被視為 *P. aphrodite* 及 *P. equestris* 的天然雜交種。此外，在 1886 年 Seden 也以人工雜交試驗支持 *P.* × *intermedia* 是上述兩物種的天然雜交種[26]。而地理分佈的資料亦支持 *P. aphrodite* 及 *P. equestris* 可能為 *P.* × *intermedia* 之親本[26,27,5]。雖然 *P. equestris* 也屬於蝴蝶蘭亞屬，不過並非是蝴蝶蘭節之成員，而是屬於 *Stauroglottis* 節，本節另外還有 *P. celebensis* 及 *P. lindenii* 兩種。不過分子證據顯示蝴蝶蘭節及 *Stauroglottis* 節的親緣關係甚為相近[13,30]。

核糖體核酸 (ribosomal DNA, rDNA) 是目前廣被應用於探討演化及親緣關係的 DNA 區域，它普遍存在於真核細胞中，是一群成縱線排列 (tandem array) 的重複性基因族 (repeated gene families)，重複次數約數百至數萬次，位於染色體的核仁組成中心 (nucleolar organizer region)[1]，由於受到不等重組 (unequal crossing over) [23]和偏斜的基因轉變 (biased gene conversion) [11]的調控，使每個重複單位間能逐漸趨於一致，為一協同演化 (concerted evolution) 的 DNA 區域。rDNA 之 ITS 區域廣泛被應用於建構低分類階層的類群之親緣關係[32,2]。就目前在高等植物的 ITS 研究中發現，經雜交後 ITS 重複序列遺傳至後代有三種形式，第一種是雜交後代保有雙親的 ITS 重複序列，如 Krigia [14], *Arabidopsis suecica* [19] 及 Cardamine[8]，第二種是後代僅保留雙親其中一種 ITS 重複序列，如 Gossypium [35] 及 Cardamine [8]，第三種是後代 ITS 有產生均質化但序列中依然可以發現雙親特有的少數序列分散其中，如 *Gossypium gossypioides*[34], Paenoia [22] 及 Microthlaspi [18]。

與大部分高等植物一樣，蝴蝶蘭葉綠體 DNA (chloroplast DNA) 屬於母系遺傳(maternal inheritance) [3]。葉綠體 DNA 中也有一些 DNA 片段常被應

用於族群、演化及品種鑑定等分析[20]。其中基因間隔區 (intergenic spacer, IGS) 及內隱子 (intron) 由於非屬基因區，因此可以有較快的演化速率。Taberlet *et al.* (1991) [28] 設計許多葉綠體 DNA 的廣用引子，其中 *trn*L 基因的 intron 區域、*trn*L-*trn*F 之 IGS 區域已被廣泛應用屬內物種間之親緣關係研究，如 *Miscanthus* 和 *Saccharum* (Poaceae)[12], *Moraea* (Iridaceae)[9], *Allium* (Liliaceae) [31]。此外，*atp*B-*rbc*L 之 IGS 也曾被應用於建構種內的親緣關係，如水筆仔 (*Kandelia candel*) [4]。若針對較低分類階層的研究上，通常基因間隔區 (IGS)，如 *trn*L-*trn*F 之 IGS 區域; 或內隱子 (intron)，如 *trn*L 基因的 intron 區域。這些 DNA 序列在演化過程中變異也較快，因此也適合用來進行屬內的 DNA 分析，以獲取各種原的分子標誌。

由於核 DNA 與葉綠體 DNA 對種間雜交種的基因組具有不同的遺傳特性，因此普遍被用於鑑別雜交種之親本[36, 17,33]。檢視 *P.* × *intermedia* 雜交種的分佈及外部特徵 (morphological characters)[27,5]，推測蝴蝶蘭節及 *Stauroglottis* 節之成員為其可能的親本。本研究藉由分析核 DNA 的 ITS 區域及三段葉綠體 DNA 片段以獲取 *P.* × *intermedia* 天然雜交種的親本分子證據，並進一步可以得知其母本及父本。

材料與方法

一、材料

分析材料除了三株 *Phalaenopsis* × *intermedia* 外，亦包含蝴蝶蘭節及 *Stauroglottis* 節的成員，其中蝴蝶蘭節成員有 *P. amabilis*、*P. aphrodi*te、*P. sanderiana*、*P. philippinensis*、*P. schilleriana*、*P. stuartiana*，另外，*Stauroglottis* 節成員有 *P. equestris*、*P. celebensis* 及 *P. lindenii*。此外，以一株蝴蝶蘭亞屬 *Deliciosae* 節成員 *P. pulcherrima* 及一株 *Polychilos* 亞屬 *Amboinenses* 節成員 *P. reichenbachiana* 為外群 (outgroups) (表 1)。

二、方法

(一)總 DNA 抽取、PCR 反應及電泳分析

依 Doyle 和 Doyle (1987) [6] 的方法抽取植物幼葉之總 DNA，以分光光度計 (Hitachi U-2001 Spectrophotometer) 測定波長 260 nm 紫外光吸光度，定量 DNA 後，存於 -20℃ 中備用。核糖體核酸(rDNA)內轉錄間隔區(ITS)及 *trn*L 之內隱子、*trn*L-*trn*F 之基因間隔區、*atp*B-*rbc*L 之基因間隔區等 DNA 片段的 PCR 反應所使用的引子、反應濃度及溫度條件是參考 Taberlet *et al.* (1991) [28]及蔡 (2003) [29]的文章。最後將 PCR 複製產物，於 0.8 % 瓊膠 (agarose) 以 TBE 緩衝液中電泳分離。經 EtBr (ethidium bromide, 0.5 μg/ml) 染色後，在紫外燈下觀察、照相。

表 1. 本研究的試驗材料名稱、系統分類、分佈及來源

Table 1. List of *Phalaenopsis* species, systematic classification, and geographical distributions examined in this study

Taxa and systematic classification[a]	Distribution (Christenson, 2001) [5]	Source[b]
Subgenus *Phalaenopsis*		
Section *Phalaenopsis* Benth.		
Phalaenopsis amabilis (L.) Blume	Widespread from Sumatra and Java to the southern Philippines, and east to New Guinea and Queensland, Australia	KDAIS KC-327
Phalaenopsis aphrodite Rchb.f.	Northern Philippines and southeastern Taiwan	KDAIS KC-171
Phalaenopsis sanderiana Rchb.f.	Endemic to the Philippines (Mindanao Island, Igat Island, and Balut and Saragani Islands)	KDAIS KC-175
Phalaenopsis schilleriana Rchb.f.	Endemic to the Philippines	KDAIS KC-429
Phalaenopsis stuartiana Rchb.f.	Endemic to the island of Mindanao in the southern Philippines.	KDAIS KC-528
Phalaenopsis philippinensis Golamco ex Fowlie & Tang	Endemic to the Philippines	KDAIS KC-534
Phalaenopsis × *intermedia* Lindl.	Endemic to the Philippines	KDAIS KC-82
Phalaenopsis × *intermedia* Lindl.	Endemic to the Philippines	KDAIS KC-83
Phalaenopsis × *intermedia* Lindl.	Endemic to the Philippines	KDAIS KC-84
Section *Stauroglottis* (Schauer) Rchb.f.		
Phalaenopsis equestris (Schauer) Rchb.f.	Endemic to the Philippines and Taiwan	KDAIS KC-75
Phalaenopsis celebensis Sweet	Endemic to Indonesia (Sulawesi)	KDAIS KC-482
Phalaenopsis lindenii Loher	Endemic to the Philippines	KDAIS KC-119
Section *Deliciosae*		
Phalaenopsis pulcherrima (Lindl.) J. J. Sm.	Widespread from northeast India and southern China throughout Indochina to Malaysia (Malay Peninsula), Indonesia (Sumatra), and East Malaysia (Sabah)	KDAIS KC-256
Subgenus *Polychilos* (Breda) E. A. Christ.		
Section *Amboinenses* Sweet		
Phalaenopsis reichenbachiana Rchb.f. & Sander	Endemic to the Philippines	KDAIS KC-389

[a] The classification of *Phalaenopsis* is based on Christenson (2001) [5]

[b] Living plants are cultivated at Kaohsiung District Agricultural Research and Extension Station

(二)回收 DNA 與序列分析

將 PCR 複製產物以瓊脂膠體電泳分離，經 EtBr 染色後，在 UV 燈箱使用長波長 (365 nm) 之 UV 光觀察 DNA 條帶 (band)，用解剖刀將上述 DNA 條帶切下，置入 1.5 ml 微量離心管中，每 100 mg 的膠塊加入 500 µl NaI solution (6 M sodium iodide)，置於 50℃ 水浴中，時常將微量離心管翻轉，至膠塊完全溶解後，取出並加入適當量的 Glass milk (Geneclean Kit Ⅱ,

BIO 101)(DNA 量在 5 μg 以下，加入 5 μl 的 Glass milk，每增加 1μg，須增加 1μl Glass milk)，翻轉數次，使之均勻混合，並置於冰中 5 分鐘，使 DNA 得以與 Glass milk 結合，瞬間離心，使 Glass milk 沈澱，倒掉上層液，以 500-700 μl 的 New wash solution (NaCl /ethanol /water) 清洗沈澱物 3 次，最後將沈澱物以適量無菌水懸浮，於 10,000 rpm 離心 5 分鐘，取上層液至一新的微量離心管，即取得回收之 DNA。

(三)序列分析

回收的 DNA 以 PCR 所用的引子為讀序引子，經由 dideoxy chain-termination 法，以 ABI377 自動讀序儀進行非放射線標定讀序。每一樣本經由兩次或三次的重複確認序列。上述序列反應委託廠商代為行之，讀序反應的步驟依據藥品商所建議的標準步驟行之。不過 三株 *P.* × *intermedia* 的 ITS 序列無法利用 PCR 產物進行直接讀序，所以利用 T-vector 為基礎的選殖法進行序列分析，每一個樣本隨機選取 5 或 7 個轉型系進行序列分析，其步驟亦依據藥品商所建議的標準步驟行之。

(四)資料分析

分別處理 ITS 及葉綠體 DNA 之序列。處理後之序列利用 BioEdit 軟體[10] 中之 Clustal W multiple alignment 程式進行編排比對後，以 Kimura 2-parameters [15]進行換算樣本兩兩間的遺傳距離 (genetic distance)。再經由 MEGA version 2.1 套裝軟體[16]，Neighbor-joining 法[21]完成樹狀圖，並進行 interior branch tests 重複檢定[24]。

結果與討論

分析三株 *P.* × *intermedia* 及蝴蝶蘭節及 *Stauroglottis* 節的所有物種與兩個外群物種的核糖體核酸內轉錄間隔區之序列，其結果發現，除了三株 *P.* × *intermedia* 的樣本無法由聚合酵素連鎖反應的產物直接讀序外，其它的樣本皆可以直接進行讀序，此顯示 *P.* × *intermedia* 植株之 ITS 重複序列皆非同質性 (homogeneity)，此現象也出現在芍藥 (*Paeonia*) 的雜交種上[22]、四倍體棉花[35]、dwarf dandelions [14]。因此本研究之 *P.* × *intermedia* 植物之 ITS 序列利用 T-vector 為基礎的選殖方法進行序列分析，其中樣本 KC-82、KC-83、KC-84 分別隨機挑選七、七及五個菌落進行讀序，分析其序列發現，*P.* × *intermedia* 之 ITS 序列中的 ITS1 之序列長度介於 226~229 bp 間，ITS2 之序列長度介於 257~264 bp 間，而 5.8S rRNA 基因之長度皆為 163 bp，此與本研究其它蝴蝶蘭一樣(表 2)。

為獲得 *P.* × *intermedia* 天然雜交種的分子證據，將其 ITS 序列與本研究的其它蝴蝶蘭之 ITS 序列進行序列比對與編排 (圖 1)，隨後完成樣本兩兩間的遺傳距離矩陣 (genetic distance matrix)(data not shown)，然後完成親緣關係樹狀圖(phylogenetic tree) (圖 2)，除了 KC-82 選殖系 6 之外，*P.* × *intermedia* 的其它 18 個選殖系分成二大群，分別稱為 A 群及 B 群。因此進一步換算 A 群及 B 群與 9 個候選親本及外群兩兩間之平均遺傳距離 (表 3)，其中 A 群與 *P. aphrodite* 最相近，其平均遺傳距離為 0.006，另外，B 群與 *P. equestris* 最相近，其平均遺傳距離為 0.022，此分子證據顯示，*P. aphrodite* 及 *P. equestris* 為天然雜交種 *P.* × *intermedia* 的親本。

此外，經由分析葉綠體 DNA 之 *trn*L 基因內隱子、*trn*L-*trn*F 之基因間隔區及 *atp*B-*rbc*L 基因間隔區之序列發現，本研究所有的分析樣本皆可以直接讀取聚合酵素連鎖反應之產物 (表 2)，定序的引子與聚合酵素連鎖反應的引子一樣即可。將三段葉綠體 DNA 組合起來一樣分析，經適當比對編排後 (data not shown)，隨後換算進行樣本兩兩間遺傳距離 (表 4)，並進一步完成親緣關係樹狀圖 (圖 3)。由葉綠體 DNA 所換算的遺傳距離顯示，*P.* × *intermedia* 與 *P. aphrodite* 最為相近，其遺傳距離為 0.018。基於蝴蝶蘭葉綠體 DNA 的母系遺傳 (maternal inheritance) 之特性[3]，可以判斷 *P. aphrodite* 為天然雜交種 *P.* × *intermedia* 之母本。搭配上述核 DNA 之 ITS 分析，可以得知 *P. equestris* 為父本。此外，由 *P. aphrodite* 與 *P. equestris* 在花期與分佈的重疊性亦支持 *P.* × *intermedia* 是上述兩種蝴蝶蘭的雜交親本。

此雜交種之 ITS 重複序列並無發生協同演化現象 (concerted evolution)，此結果也曾經在 *Krigia* [14], *Arabidopsis suecica*[19], and *Cardamine*[8] 被報導。檢視 *P.* × *intermedia* 的 19 個 ITS 序列，大部分的核苷酸不是來自父親的 ITS，就是母親的 ITS 核苷酸，並無發現 *P.* × *intermedia* 之 ITS 核苷酸鹼基有其它候選親本之基因滲入 (introgression) 現象 (圖 1)。此外也發現 *P.* × *intermedia* 之 ITS 序列出現新的核苷酸鹼基，亦即有許多鹼基是既非來自父親，也非來自母親，此奇特的現象也在研究台北秋海棠 (*Begonia* × *taipeiensis*) 的天然雜交或人工雜交中之 ITS 重複序列中發現[4]。我們推測這些特異的鹼基是來自雙親的兩個不同之 ITS 序列，在配對過程中產生重組或突變所造成。

由台北秋海棠 (*Begonia* × *taipeiensis*) 的天然雜交或人工雜交中及 *P.* × *intermedia* 天然雜交種的分子證據顯示，不同形式的 ITS 重複序列存在有性生殖過程中，可能會造成 ITS 重複序列的不穩定，因而加速序列的演化。此與我們所認知的重複序列在有性生殖過程中會經由不等重組 (unequal

crossing over)[23]與偏斜的基因轉變 (biased gene conversion)[11]等機制讓重複序列具同質性。因此，未來這方面的研究還有兩個重要的問題待釐清，首先是啟動重複序列同質性的關鍵因子為何，確實有些例子可以發現不等重組與偏斜的基因轉變在有性生殖過程中沒有作用，如台北秋海棠 (*Begonia* × *taipeiensis*) [4] 天然雜交種與第一代人工雜交、*Paeonia* hybrids[22]、四倍體棉花[35]，以及本研究的 *P.* × *intermedia* 雜交種，此結果顯示有另一個更上游的調控機制會決定是否要啟動不等重組與偏斜的基因轉變等機制，以造成重複序列是否能變成同質性或維持異質性。另一個值得探討的是從第一代人工雜交後代 (F_1 progeny) 亦能發現新的核苷酸鹼基顯示，維持異質性的 ITS 重複序列中的序列差異並非來自隨年代的自然突變累積 (natural mutation accumulation)，而是有另一機制會造成重複序列的不穩定而突變。

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                                5         15        25        35        45        55        65        75
P._amabilis                     TCGAGACCGG AATCATACCG AGCCAATCGG AGAACCCGTG AACCGAACGG CGGCGGCGGC CGCCGCCGCC GGACGGCCGC
P._aphrodite                    .......... .......... ...G...... .......... .......... .......... .......... ..........
P._philippinensis               CG........ ...T.C.T.. ...G.T.... .......... .......... ........T. .......... ..........
P._sanderiana                   .......... .......... .......... .......... .......... .......... .......... ..........
P._schilleriana                 C......... ..CT...T.. ...G.T.... .......... .......... .......... G......... .C........
P._stuartiana                   C......... ..C....T.. ...G.T.... .......... .......... .......T.. .......... .C........
P._intermedia-kc-82-clone-1     .........A ...T...T.. ...G.C.... .......... .......T.. .......... .......... .A........
P._intermedia-kc-82-clone-2     .......... ......G... ...G...... .......... .......... ..---..... .......... ..........
P._intermedia-kc-82-clone-3     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone-4     .......... ......G... ...G...... .......... .......... ..---..... .......... ..........
P._intermedia-kc-82-clone-5     .........A ...T...T.. ...G.C.... .......... .......T.. .......... .......... .A........
P._intermedia-kc-82-clone-6     .........A ...T...T.. ...G.C.... .......... .......T.. .......... .......... .A........
P._intermedia-kc-82-clone-7     .........A ...T...T.. ...G.C.... .......... .......T.. .......... .......... .A........
P._intermedia-kc-83-clone-1     .......... ...T..G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-2     .......... ......G... ...G...... .......... ...T...... .......... .......... ..........
P._intermedia-kc-83-clone-3     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-4     .......... ......G... GA.G...... .......... .......... ..A....... .......... ..........
P._intermedia-kc-83-clone-5     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone 6     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-7     .......... ......G... ...G...... .......... ...T...... .......... .......... ..........
P._intermedia-kc-84-clone-1     .........A ...T...T.. ...G.C.... .......... .......T.. .......... .......... .A........
P._intermedia-kc-84-clone-2     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-3     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-4     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-5     .......... ......G... ...G...... .......... .......... .......... .......... ..........
P._celebensis                   .........A ...T...T.. ...G.T.... .......... .G...GG... ....C..C.. G......... .C........
P._equestris                    .........A ...T...T.. ...G.T.... .......... .......... .......... G......... .A........
P._lindenii                     .........A ...T.C.T.. ...G.T.... .......... .......--- .......... .......... .A.....TT.
P._pulcherrima                  .........A .......T.. ...G.TC... .......... ......G... .......C.T .....---.. .A........
P._reichenbachiana              .........A ...T...T.. ...G.T.... .......... ......G... .......C.. .....T.... .A........

                                85        95        105       115       125       135       145       155
P._amabilis                     CCCCGCCGTC GCCCCCGCCC CCGTTCGGAG GGGGGGGGCG CGGCGGGGGA CGGCCGGAAC CCC-GAACCG GCGCGGATCG
P._aphrodite                    .......... .......... .......... .....-.... .......... .......... ...-...... ..........
P._philippinensis               .......... .......... ....C..... .....--... .........G ......A... ...-...... ....A.....
P._sanderiana                   .......... .......... .......... .....A.... .......... .......... ...C...... ..........
P._schilleriana                 .......... .......... ....C..... .....--... .......... ......A... ...-...... ....A.....
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P._stuartiana                ..........  ..........  ....C.....  .....--...  ..........  ......A...  ...-......  ....A.....
P._intermedia-kc-82-clone-1  .......---  ----------  G..C....T.  ......--..  ..A.......  ......A...  ...-...A..  ....A.....
P._intermedia-kc-82-clone-2  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-82-clone-3  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-82-clone-4  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-82-clone-5  .......---  ----------  G..C....T.  ......--..  ..A.......  ......A...  ...-...A..  ....A.....
P._intermedia-kc-82-clone-6  .......---  ----------  G..C....T.  .....--...  ..A.......  ......A...  ...-...A..  ....A.....
P._intermedia-kc-82-clone-7  .......---  ----------  G..C....T.  ......--..  ..A.......  ......A...  ...-...A..  ....A.....
P._intermedia-kc-83-clone-1  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-83-clone-2  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-83-clone-3  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-83-clone-4  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  .....A....
P._intermedia-kc-83-clone-5  .......---  ----------  G..C....T.  ......--..  ..A.......  ......A...  ...-...A..  ....A.....
P._intermedia-kc-83-clone-6  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-83-clone-7  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-84-clone-1  .......---  ----------  G..C....T.  ......--..  ..A.......  ......A...  ...-...A..  ....A.....
P._intermedia-kc-84-clone-2  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-84-clone-3  ..........  ..........  ..........  .....-....  ..........  ..........  ...-......  ..........
P._intermedia-kc-84-clone-4  ..........  ..........  ..........  .....-....  ..........  ..A.......  ...-......  ..........
P._intermedia-kc-84-clone-5  ..........  ..........  ..........  .....-....  ..........  ..A.......  ...-......  ..........
P._celebensis                ........C.  .G....--..  T.........  ......C...  ..........  .......G..  ...-......  ....A.....
P._equestris                 ........C.  ..........  G.......T.  .....---..  ..A.......  ......A...  ...-......  ....A.....
P._lindenii                  ........C.  .T........  A.........  .....---..  ..A.......  ......A...  ...-......  ....A.....
P._pulcherrima               ..........  .A....--.-  T..CC.....  ....C---..  ......A...  ......AG..  ...-AG....  ....A..C..
P._reichenbachiana           ........C.  .G....--..  T.........  .....---..  ..A.......  ......A...  ...-......  ....A.....
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                             165         175         185         195         205         215         225         235
P._amabilis                  GCGCCAAGGG  AACCCGTGAG  -AGACACGAG  CCCGGCATCG  GGCCCCCGTG  GGGCGGAGCG  ----------  CCTAACGTAC
P._aphrodite                 ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._philippinensis            ..........  ....G.....  -.A.....G.  ..........  .....T....  ..........  GGGCTGCGCG  ..GC......
P._sanderiana                ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..........
P._schilleriana              ..........  ....G.....  -.A.....G.  ..........  .....T....  ..........  GGGCTGCGCG  ..GC......
P._stuartiana                ..........  ....G.....  -.A.....G.  ..........  .....T....  ..........  GGGCTGCGCG  ..GC......
P._intermedia-kc-82-clone-1  ..........  ....G....A  -.........  ..........  .....T....  ..........  GTGCCGCGCA  ..GC...C..
P._intermedia-kc-82-clone-2  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-82-clone-3  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-82-clone-4  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-82-clone-5  ..........  ....G....A  -.........  ..........  .....T....  ..........  GTGCCGCGCA  ..GC...C..
P._intermedia-kc-82-clone-6  ..........  ....G....A  -.........  ..........  .....T....  ..........  GTGCCGCGCA  ..GC...C..
P._intermedia-kc-82-clone-7  ..........  ....G....A  -.........  ..........  .....T....  ..........  GTGCCGCGCA  ..GC...C..
P._intermedia-kc-83-clone-1  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-83-clone-2  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-83-clone-3  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-83-clone-4  ..........  ....G.....  -.........  ...A......  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-83-clone-5  ..........  ....G....A  -.........  ..........  .....T....  ..........  GTGCCGCGCA  ..GC...C..
P._intermedia-kc-83-clone-6  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-83-clone-7  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-84-clone-1  ..........  ....G....A  -.........  ..........  .....T....  ..........  GTGCCGCGCA  ..GC...C..
P._intermedia-kc-84-clone-2  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-84-clone-3  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-84-clone-4  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._intermedia-kc-84-clone-5  ..........  ....G.....  -.........  ..........  .....T....  ..........  ----------  ..C.......
P._celebensis                ..........  ....GT..GA  G.A.......  ..........  .....T....  .AA.......  GCGCCGCGCG  ..GC......
P._equestris                 ..........  ....G....C  -.........  ..........  .....T....  ..........  GTGCTGCGCA  ..GC......
P._lindenii                  ..........  ....G....A  -.A.......  ....A.G...  .....T....  ..........  GTGCTGCGCA  ..GC.....A
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P._pulcherrima            ..........  ....G.A..A -....G....  ..........  ....TT....  ..........  GCGCTGCGCG  ..GC...CGG
P._reichenbachiana        ..........  ....G....A -.........  ..........  ....TT....  ..........  GTGCTGCGCA  ..GC......
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                                245         255         265         275         285         295         305         315
P._amabilis               CGACACGACT  CTCGACAATG  GATATCTCGG  CTCTCGCATC  GATGAAGAGC  GCAGCGAAAT  GCGATACGTG  GTGCGAATTG
P._aphrodite              ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._philippinensis         ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._sanderiana             ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._schilleriana           ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._stuartiana             ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-1 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-2 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-3 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-4 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-5 ..........  ....G.....  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-6 T.........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-7 T.........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-1 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-2 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-3 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-4 ..........  .A........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-5 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-6 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-7 ..........  ..........  ......C...  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-84-clone-1 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-84-clone-2 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-84-clone-3 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-84-clone-4 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-84-clone-5 ..........  .A........  ..........  ..........  ..........  ..........  ..........  ..........
P._celebensis             ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._equestris              T.........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._lindenii               T.........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._pulcherrima            T.........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._reichenbachiana        T.........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
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                                325         335         345         355         365         375         385         395
P._amabilis               CAGAATCCCG  CGAACCATCG  AGTCTTTGAA  CGCAAGTTGC  GCCCGAGGCC  AATCGGCCGA  GGGCACGCCC  GCCTGGGCGT
P._aphrodite              ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._philippinensis         ..........  ..........  ..........  ..........  ..........  ......T...  .......T..  ..........
P._sanderiana             ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._schilleriana           ..........  ..........  ..........  ..........  ..........  ..........  .......T..  ..........
P._stuartiana             ..........  ..........  ..........  ..........  ..........  ..........  .......T..  ..........
P._intermedia-kc-82-clone-1 ..........  ..........  ..........  ..........  ..........  ......T...  .......T..  ..........
P._intermedia-kc-82-clone-2 ..........  ......G...  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-3 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-4 ..........  ......G...  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-5 ..........  ..........  ..........  ..........  ..........  ......T...  .......T..  ..........
P._intermedia-kc-82-clone-6 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-82-clone-7 ..........  ..........  ..........  ..........  ..........  ......T...  .......T..  ..........
P._intermedia-kc-83-clone-1 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-2 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-3 ..........  ..........  ..........  ..........  ..........  ..........  ..........  .....A....
P._intermedia-kc-83-clone-4 ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
P._intermedia-kc-83-clone-5 ..........  ..........  ..........  ..........  ..........  ......T...  .......T..  ..........
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P._intermedia-kc-83-clone-6 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-7 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-1 .......... .......... .......... .......... .......... ......T... .......T.. ..........
P._intermedia-kc-84-clone-2 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-3 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-4 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-5 .......... .......... .......... .......... .......... .......... .......... ..........
P._celebensis               .......... .......... .......... .......... .......... ......T... .......T.. ..........
P._equestris                .......... .......... .......... .......... .......... ......T... .......T.. ..........
P._lindenii                 .......... .......... .......... .......... .......... ......T... .......T.. ..........
P._pulcherrima              .......... .......... .......... .......... .......... .......... .......T.. ..........
P._reichenbachiana          .......... .......... .......... .......... .......... ......T... .......T.. ..........

                              405        415        425        435        445        455        465        475
P._amabilis                 CGAGCGTCGC GCCGCTCCGC GCCGAGTCCC CATCCCCGCC GCGGCGGGGG TGCCGGGCGA GGACCGGACG TGCAGAGTGG
P._aphrodite                .......... .......... ......C... .......... .......... .......... .......... ..........
P._philippinensis           .......T.. .......... .......... .......... ....T..... .......... ..C....... .....G....
P._sanderiana               .......... .......... .......... .......... .......... .......... .......... ..........
P._schilleriana             .......... .......... ......C... .......... .......... .......... ..C....... .....G....
P._stuartiana               .......... .......... ......C... .......... .......... .......... ..C....... .....G....
P._intermedia-kc-82-clone-1 .......T.. .........T ......C... .......... ....T..... .......... ..C....... .....G....
P._intermedia-kc-82-clone-2 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone-3 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone-4 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone-5 .......T.. .........T ......C... .......A.. ....T..... .......... ..C....... .....G....
P._intermedia-kc-82-clone-6 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone-7 .......T.. .........T ......C... .......... ....T..... .......... ..C....... .....G....
P._intermedia-kc-83-clone-1 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-2 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-3 ..........A......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-4 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-5 .......T.. .........T ......C... .......... ....T..... .......... ..C....... .....G....
P._intermedia-kc-83-clone-6 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-7 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-1 .......T.. .........T ......C... .......... ....T..... .......... ..C....... .....G....
P._intermedia-kc-84-clone-2 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-3 .......... .......... .......... .......... .......... ..T....... .......... ..........
P._intermedia-kc-84-clone-4 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-5 .......... .......... .......... .......... .......... .......... .......... ..........
P._celebensis               .......T.. .........T .......... .......... ....T..... .......... ..C....... .....G....
P._equestris                .......T.. .........T ......C... .......... ....T..... .......... ..C....... .....G....
P._lindenii                 .......T.. .........T ......C... .......... ....T..... .......... ..C....... ..........
P._pulcherrima              .......T.. .........T ......C... .G........ .......... .C........ ..CG...... .....G....
P._reichenbachiana          .A.....T.. .........T .......... .......... ....T..... .......... ..C.....T. C..G......
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                              485        495        505        515        525        535        545        555
P._amabilis                 CCCGTCGTGC CCGTCGGCGC GGCGGGCTGA AGAGCGGGCT GCCGTCTCAT CGGCCACGGA CGACGAGGGG TGGATGAAAA
P._aphrodite                .......... .......... .......... .......... .......... .......... .......... ..........
P._philippinensis           .......... .......... .......... .........C .......... .....G..A. ..G....... ......G...
P._sanderiana               .......... .......... .......... .......... .......... .......... .......... ..........
P._schilleriana             .......... .......... .......... .........C .......... .....G..A. ..G....... ..........
P._stuartiana               .......... .......... .......... .........C .......... .....G..A. ..G....... ......G...
P._intermedia-kc-82-clone-1 .......... .......... .......... ........T. AT......G. ........A. .A........ ........TG
P._intermedia-kc-82-clone-2 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone-3 .......... .......... .......... .......... .......... .......... .......... ..........
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P._intermedia-kc-82-clone-4 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-82-clone-5 .......C.. .......... .......... ........T. AT......G. .....T..A. .A........ ........TG
P._intermedia-kc-82-clone-6 .......... .......... .......... .......... .......... .......... .......... .........G
P._intermedia-kc-82-clone-7 .......... .......... .......... ........T. AT......G. ........A. .A........ ........TG
P._intermedia-kc-83-clone-1 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-2 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-3 .......... ......A... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-4 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-5 .......... .......... .......... ........T. AT......G. ........A. .A........ ........TG
P._intermedia-kc-83-clone-6 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-83-clone-7 .......... .........A. .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-1 .......... .......... .......... ........T. AT......G. ........A. .A........ ........TG
P._intermedia-kc-84-clone-2 .......... .......... .......... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-3 .....T.... .......... .......... .......... .......... T......... .......... ..........
P._intermedia-kc-84-clone-4 .......... .......... ....A..... .......... .......... .......... .......... ..........
P._intermedia-kc-84-clone-5 .......... .......... .......... .......... .......... .......... .......... ..........
P._celebensis               .......... .G........ .......... ........T. .T........ T....G..A. .A........ .........G
P._equestris                .......... .......... .......... ........T. AT......G. ........A. .A........ ........TG
P._lindenii                 .......... .......... .......... ........T. AT......G. ........A. .A........ ........TG
P._pulcherrima              .......... ....A..T.. .......... ........T. .T........ T.......A. .A........ .......G.G
P._reichenbachiana          .......... .......T.. .T........ ........TC .T.......C T.C.....A. .AG....... .........G

                                565        575        585        595        605        615        625        635
P._amabilis                 GA-------- ---AGCCCTC GAG---CGCG TCGTCGCGTG CCG-CCGGAG AGGAGAGGAA ACGGCCCTCC GCGCGATCCC
P._aphrodite                ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._philippinensis           ..-------- ---..... ...---.... .....T.... ...G...... ......C.G. .........T. .T........
P._sanderiana               ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ....C.....
P._schilleriana             ..-------- ---.A... ...---.... .....T.... ...G...... ......C.G. .........T. .T........
P._stuartiana               ..-------- ---.A... ...---.... .....T.... ...G...... ......C.G. .........T. .T........
P._intermedia-kc-82-clone-1 ..----GAGC CGCC..GGG. ...GCC.... .T...T.... ...G...... ......C.GC ---A....T. .TC.......
P._intermedia-kc-82-clone-2 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-82-clone-3 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-82-clone-4 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-82-clone-5 ..----GAGC CGCC..GGG. ...GCC.... .T...T.... ...G...... ......C.GC ---A....T. .TC.......
P._intermedia-kc-82-clone-6 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-82-clone-7 ..----GAGC CGCC..GGG. ...GCC.... .T...T.... ...G...... G.....C.GC ---A....T. .TC.......
P._intermedia-kc-83-clone-1 ..-------- ---..... ...---.... .A........ ...-...... ........G. .......... ..........
P._intermedia-kc-83-clone-2 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-83-clone-3 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-83-clone-4 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-83-clone-5 ..----GAGC CGCC..GGG. ...GCC.... .T...T.... ...G...... ......C.GC ---A....T. .TC.......
P._intermedia-kc-83-clone-6 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-83-clone-7 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-84-clone-1 ..----GAGC CGCC..GGG. ...GCC.... .T...T.... ...G...... ......C.GC ---A....T. .TC.......
P._intermedia-kc-84-clone-2 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-84-clone-3 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._intermedia-kc-84-clone-4 ..-------- ---..... ...---.... .......... ...-...... ........G. .....T.... ..........
P._intermedia-kc-84-clone-5 ..-------- ---..... ...---.... .......... ...-...... ........G. .......... ..........
P._celebensis               A.AGGAGGGC TGCC..GGG. AG.GCC.... .T...T.... ...G...... ...G..TC.C ---A....T. ..........
P._equestris                ..----GAGC CGCC..GGG. ...GCC.... .T...T.... ...G...... ......C.GC ---A....T. .T........
P._lindenii                 ..----GAGC CGCC..GGG. ...GCC.... .T...T.... ...G...... ......C.GC ---A....T. .T........
P._pulcherrima              ---------C TGC...GGG. AG.GCC.... .T...T.... ...G...... ..AG..T... ---.....T. .A........
P._reichenbachiana          A.-----GGC TGCC..GGG. AG.GCC.... .T...T.... ...G...... ......A..C ---......A .T........
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(continued)

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                              645        655        665        675
P._amabilis                   ATCCCGGGCG CCGCCCCTC- -GT------G CGGCGGCTCG GAAC
P._aphrodite                  .......... .......C.- C..------. .......... ....
P._philippinensis             ......C... .......C.G C..C-GGGG. .......CT. ...T
P._sanderiana                 .......... .......C.- C..------. .......... ....
P._schilleriana               ......C... .......C.A C..CTGGGG. G......CT. ...T
P._stuartiana                 ......C... .......C.A C..CTGGGG. .......AT. ...T
P._intermedia-kc-82-clone-1   .....AC... .......C.- -..------. .......CT. ...T
P._intermedia-kc-82-clone-2   .......... .......C.- C..------. .......... ....
P._intermedia-kc-82-clone-3   .......... .......C.- C..------. .......... ....
P._intermedia-kc-82-clone-4   .......... .......C.- C..------. .......... ....
P._intermedia-kc-82-clone-5   .....AC... .......C.- -..------. .......CT. ...T
P._intermedia-kc-82-clone-6   .......... .......C.- C..------. .......... ....
P._intermedia-kc-82-clone-7   .....AC... .......C-- -..------. .......CT. ...T
P._intermedia-kc-83-clone-1   .......... .......C.- C..------. .......... ....
P._intermedia-kc-83-clone-2   .......... .......C.- C..------. .......... ....
P._intermedia-kc-83-clone-3   .......... .......C.- C..------. .......... ....
P._intermedia-kc-83-clone-4   .......... .......C.- C..------. .......... ....
P._intermedia-kc-83-clone-5   .....AC... .......C.- -..------. .......CT. A..T
P._intermedia-kc-83-clone-6   .......... .......C.- C..------. .......... ....
P._intermedia-kc-83-clone-7   .......... .......C.- C..------. .......... ....
P._intermedia-kc-84-clone-1   .....AC... .......C.- -..------. .......CT. ...T
P._intermedia-kc-84-clone-2   .......... .......C.- C..------. .......... ....
P._intermedia-kc-84-clone-3   .......... .......C.- C..------. .......... ....
P._intermedia-kc-84-clone-4   .......... .......C.- C..------. .......... ....
P._intermedia-kc-84-clone-5   .......... .......C.- C..------. .......... ....
P._celebensis                 G....AC... .......C.- -..------. ........T. ...T
P._equestris                  .....AC... .......C.- -.C------. ........T. ...T
P._lindenii                   .....AC... .......C.- -..------. ........T. ...T
P._pulcherrima                .....AC... .......CA- -..------. .......... ...T
P._reichenbachiana            ......A... .......C.- -.C------. ....C...T. ...T
```

圖 1. 19 個 *Phalaenopsis* × *intermedia* ITS 序列選殖系與其它 9 個候選親本及兩個外群編排結果，其中顯示粗體且網底之鹼基為新的特徵。

Fig 1. Sequence comparison of the ITS of rDNA from 19 clones of *Phalaenopsis* × *intermedia* Lindl., 11 species of the sections *Phalaenopsis* and *Stauroglottis* as well as two outgroups. Dots (**...**) indicate the same nucleotides, and gaps (---) are introduced to maximize homology. Nucleotides in bold and shaded are “new” characters in the ITS repeat sequences of this natural hybrid.

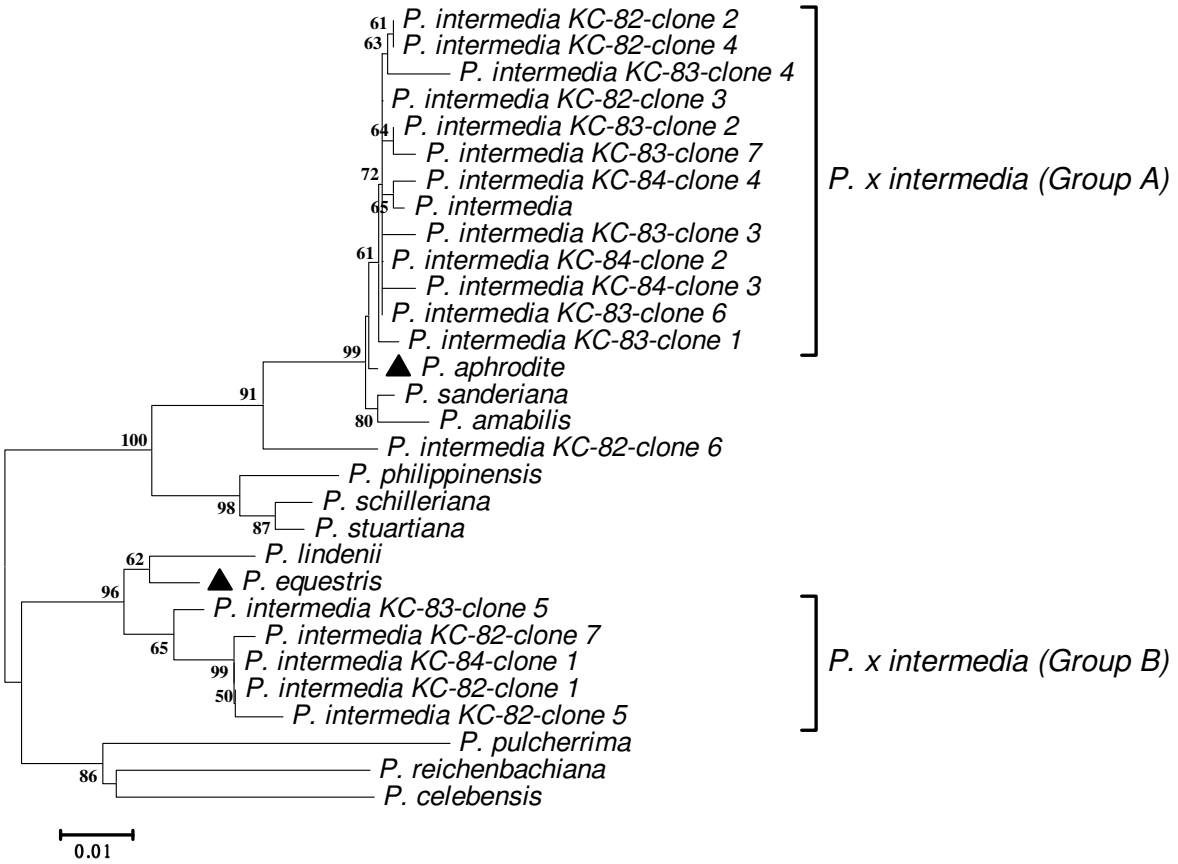

圖 2. 19 個 *Phalaenopsis* × *intermedia* ITS 序列選殖系與其它 9 個候選親本及兩個外群所建構出之親緣關係樹狀圖，其中標記(▲)者為可能之親本。

Fig 2. The phylogenetic tree of 19 clones of *Phalaenopsis* × *intermedia* Lindl., 11 species of the sections *Phalaenopsis* and *Stauroglottis* and two groups inferred from ITS data. The value of the interior branch tests > 50% is shown on each branch. Branch lengths are proportional to the number of base changes along each branch. Solid triangles (▲) indicate the putative parents of *P.* × *intermedia* Lindl.

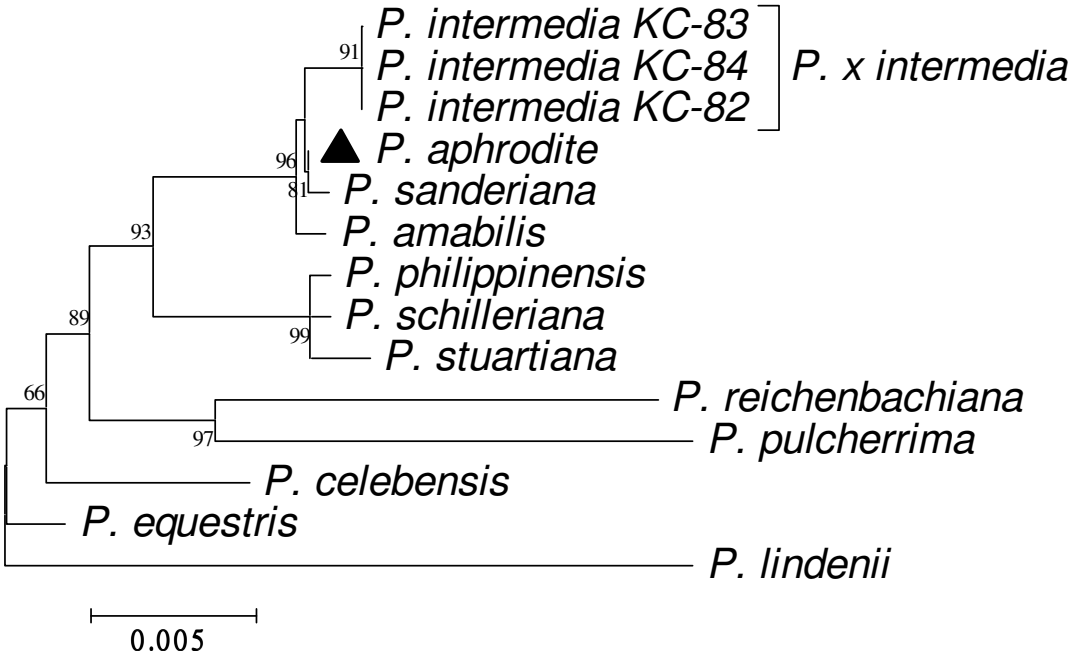

圖 3. *Phalaenopsis* × *intermedia* 與其它 9 個候選親本及兩個外群由葉綠體 DNA 所建構出之親緣關係樹狀圖，其中標記(▲)者為本雜交種之母本。

Fig 3. The phylogenetic tree of three individuals of *Phalaenopsis* × *intermedia* Lindl., 11 species of the sections *Phalaenopsis* and *Stauroglottis* and two groups inferred from chloroplast data. The value of the interior branch tests > 50% is shown on each branch. Branch lengths are proportional to the number of base changes along each branch. The solid triangle (▲) indicates the maternal parent of *P.* × *intermedia* Lindl.

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Molecular evidence for the natural hybrid origin of *Phalaenopsis* × *intermedia* Lindl.

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Abstract

In order to confirm the hybrid origin of *Phalaenopsis* × *intermedia* Lindl., the internal transcribed spacers (ITSs) of ribosomal DNAs (rDNAs) and three fragments of chloroplast DNAs (cpDNAs) were studied. Nineteen clones of ITS sequences from three individuals of *P.* × *intermedia* Lindl. were aligned with 9 species of sections *Phalaenopsis* and *Stauroglottis* that are candidate parents. The phylogenetic tree derived from ITS data was constructed by the Neighbor-joining (NJ) method. Two major groups were shown for 19 clones of ITS sequences of *P.* × *intermedia* based on phylogenetic tree. The average genetic distance between aforementioned two groups and candidate parents was calculated based on Kimura 2-parameter method. One group has the lowest genetic distance referring to ITS repeat sequences of *P. aphrodite* Rchb.f., and *P. equestris* (Schauer) Rchb.f. is that of another. The results indicated that both *P. aphrodite* Rchb.f. and *P. equestris* (Schauer) Rchb.f. are parents of *P.* × *intermedia* based on ITS data. In addition, analysis of three fragments of cpDNAs, namely *trn*L intron, *trn*L-*trn*F intergenic spacer (IGS), and *atp*B-*rbc*L IGS, showed that *P.* × *intermedia* Lindl. has a lowest genetic distance with *P. aphrodite* Rchb.f. From both ITSs and cpDNAs data, as well as referring the property of maternal inheritance of cpDNAs, it suggested that *P. aphrodite* Rchb.f. is the maternal parent and *P. equestris* (Schauer) Rchb.f. is the paternal parent of *P.* × *intermedia* Lindl. Therefore, from these molecular evidence we support *P.* × *intermedia* Lindl. is a natural hybrid derived from *P. aphrodite* Rchb.f. and *P. equestris* (Schauer) Rchb.f.

Key words: *Phalaenopsis* × *intermedia*, natural hybrid, molecular evidence

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