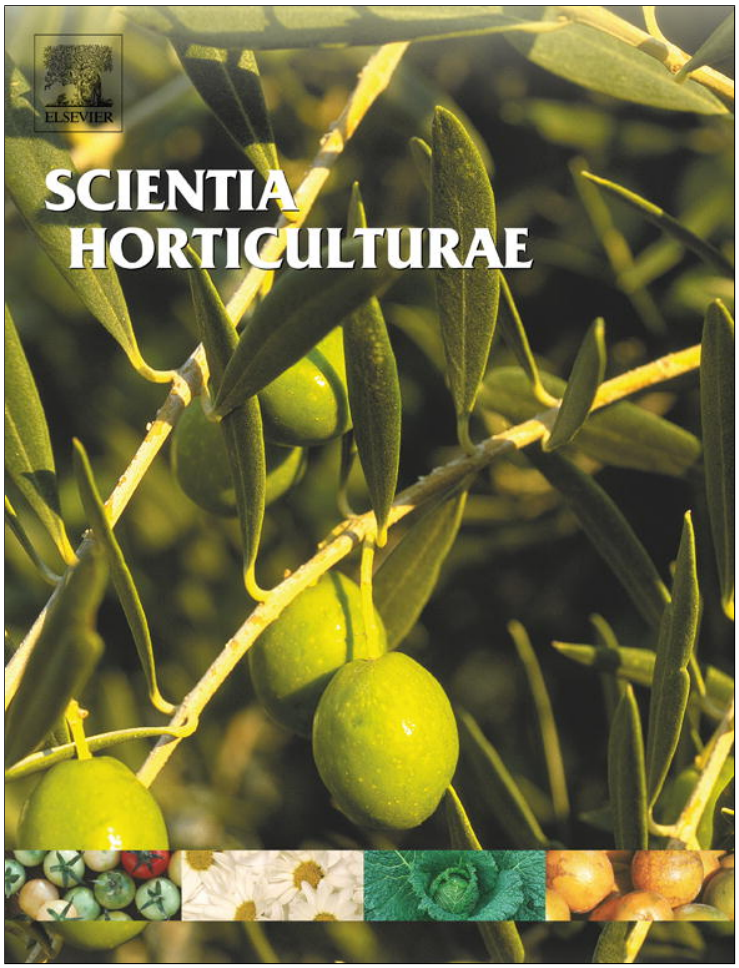

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Plastid *trn*L intron polymorphisms among *Phalaenopsis* species used for identifying the plastid genome type of *Phalaenopsis* hybrids

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ABSTRACT

The *trn*L intron sequences of plastid DNA for over 95% of the living native species of *Phalaenopsis* were determined in this study, and nearly all *Phalaenopsis* species were found to bear unique *trn*L intron sequences resulting from mutations, insertions/deletions, or both. These *trn*L intron sequences have been deposited into GenBank database for further identifying the plastid genome type of *Phalaenopsis* hybrids. Molecular evidence has demonstrated that maternal inheritance of the plastid genome occurs during interspecific hybridization of *Phalaenopsis* species. Therefore, the plastid genome type of *Phalaenopsis* hybrids can be determined by comparing the *trn*L intron sequences of the hybrids to GenBank database. The plastid genome type of the hybrids that is revealed through this analysis can be used to re-evaluate their genealogies because plastid DNA is maternally inherited. We examined *trn*L intron sequences from three *Phalaenopsis* hybrids including *P.* Yungho Gelb Canary, *P.* Timonthy Christopher, and *P.* Rainbow Chip to re-evaluate their genealogies from the recording of the Sander's List of Orchid Hybrids. No heterogeneous *trn*L intron sequences were found for any of the *Phalaenopsis* hybrids examined. After sequence comparing to GenBank database, the plastid genome types of the hybrids are determined. The conflict of genealogy and the plastid genome type in two hybrids *P.* Timonthy Christopher and *P.* Rainbow Chip can be found. This conflict results from their female parent *P.* Cassandra with wrong registration in Sander's List of Orchid Hybrids at Royal Horticultural Society (RHS).

1. Introduction

Moth orchids (*Phalaenopsis* spp.) are some of the most beautiful and popular plants. They consist of approximately 66 native species worldwide, 56 of which are extant (Christenson, 2001). Based on the classification of Christenson (2001), the *Phalaenopsis* genus is divided into five subgenera, namely *Proboscidioides*, *Aphyllae*, *Parishianae*, *Polychilos*, and *Phalaenopsis*, which were determined mainly by plant size and floral morphology (including callus, lip structure, pollinium number, etc.). The subgenus *Polychilos* was further subdivided into four sections, including *Polychilos*, *Fuscatae*, *Amboinenses*, and *Zebrinae*. In addition, subgenus *Phalaenopsis* was also subdivided into four sections, namely, *Phalaenopsis*, *Deliciosae*, *Esmeralda*, and *Stauroglottis*. Species of *Phalaenopsis* are found throughout tropical Asia and the larger islands of the Pacific Ocean. All *Phalaenopsis* species, excluding the natural tetraploid species *Phalaenopsis buyssoniana* Rchb.f., have 38 ($2n = 38$) chromosomes (Tanaka and Kamemoto, 1984; Christenson, 2001). Recently, the plastid genome of *Phalaenopsis aphrodite* have been completely sequenced (Chang et al., 2006), and molecular phylogenies of *Phalaenopsis* species also have been conducted based on the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) and plastid DNA (Tsai et al., 2006a, 2009, 2010a,b). In addition, molecular data was applied to determine the inheritance of the natural hybrid, *Phalaenopsis x intermedia*, showing *P. aphrodite* was the maternal parent and *Phalaenopsis equestris* was the paternal parent (Tsai et al., 2006b).

Most plastid genomes are multicopy circular molecules (120–160 kbp) that retain highly conserved structures among vascular plants, mosses, and algae (Palmer, 1985). The majority of angiosperm species undergo uniparental maternal plastid genome inheritance (Kuroiwa, 1991; Mogensen, 1996), and recombination of genes between plastids is rare (Chiu and Sears, 1985). The degeneration time of pollen plastid progeny has been suggested to be the interval of time between pollination and fertilization (Chiu and Sears, 1993). Electron microscopy suggested that the plastids were excluded from the early generative cell during the

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first pollen mitosis in *Syringa oblata* (Liu et al., 2004). In fact, the most common mechanism for maternal plastid inheritance is the exclusion of plastids during the first pollen mitosis via unequal plastid distribution (Lycopersicon type) or during generative or sperm cell development via plastid degeneration (Solanum type) (Hageman and Schroder, 1989; Mogensen, 1996). In addition, the maternal inheritance of plastid DNA for both interspecific hybrids and intergeneric hybrids between *Phalaenopsis* and *Doritis* has been determined based on specific DNA markers (Chang et al., 2000).

Universal primers for the *trn*L intron and *trn*L-*trn*F spacer were developed by Taberlet et al. (1991) and have been used successfully to identify DNA sequences that are useful for phylogenetic markers at the intrageneric level, such as within *Miscanthus*, *Saccharum* (Poaceae; Hodkinson et al., 2002), *Moraea* (Iridaceae; Goldblatt et al., 2002), and *Allium* (Liliaceae; Van Raamsdonk et al., 2003). Furthermore, because organellar genomes are often uniparentally inherited, plastid and mitochondrial DNA polymorphisms have become molecular markers for investigating sex-biased dispersal and the directionality of introgression (Wills et al., 2005).

In this study, the plastid *trn*L intron sequence was determined for 54 native *Phalaenopsis* species. The inheritance of the plastid genome of three interspecific hybridizations of *Phalaenopsis* species was determined based on inspection of the *trn*L intron sequence. In addition, the native *trn*L sequences were used to identify the plastid genome type of various *Phalaenopsis* hybrids.

2. Materials and methods

2.1. Plant materials

In this study, 54 native *Phalaenopsis* species, and three *Phalaenopsis* hybrids including *P.* Yungho Gelb Canary, *P.* Timonthy Christopher, *P.* Rainbow Chip were examined (Table 1). In all cases, fresh leaves were taken from living plants grown in greenhouses at the Kaohsiung District Agricultural Research and Extension Station (KDARES) in Pingtung, Taiwan.

2.2. DNA extraction, PCR amplification, and electrophoresis

Total DNA of samples studied was extracted using a cetyltrimethylammonium bromide (CTAB) method that has been previously described (Doyle and Doyle, 1987), and approximate DNA yields were determined using a spectrophotometer (Hitachi U-2001, Tokyo, Japan). Primer sets were then used to amplify the *trn*L intron region of the chloroplast DNA (cpDNA) of all of the *Phalaenopsis* plants described in Taberlet et al. (1991), using polymerase chain reaction (PCR) conditions that have been previously described (Tsai, 2003). PCR products were separated by agarose gel electrophoresis [0.8% (w/v)] in 1× TBE buffer, stained with 0.5 $\mu g\,ml^{-1}$ ethidium bromide and photographed under UV light.

2.3. DNA recovery and sequencing

PCR products were separated on 0.8% agarose gel, and the DNA was subjected to purify and quantify prior to sequencing. PCR products were sequenced on an ABI 3700 sequencer (Applied Biosystems Inc., Foster City, CA, USA) using the dideoxy chain termination method. Sequencing was performed using the Big Dye Terminator labeling mix following the manufacturer's instructions.

2.4. BLAST searching

The *trn*L intron sequences from the 54 native *Phalaenopsis* species were deposited into GenBank, whereby these sequences were made publically available through various NCBI databases. To determine which *trn*L intron sequence was present in each *Phalaenopsis* hybrid, an optimized sequence comparison algorithm was used to search NCBI databases to identify optimal local alignments to a query sequence based on the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). Inspection of the BLAST results identified the native *Phalaenopsis trn*L intron sequence that represented the corresponding plastid genome type for each of the *Phalaenopsis* hybrids.

3. Results and discussion

3.1. Plastid trnL intron polymorphisms in the genus Phalaenopsis

PCR-amplified DNA sequencing was used to determine the *trnL* intron genotypes of 54 *Phalaenopsis* species, representing over 95% of the living species diversity within this genus, and these sequences were submitted to GenBank (accession numbers: AY265742–48, AY265750–61, AY265763–87, AY265793, AY265795–800, DQ194981–82, DQ195040). The variation in length for the *trn*L intron sequences of the *Phalaenopsis* species ranged from 627 bp in *Phalaenopsis pulcherrima* to 721 bp in *Phalaenopsis mannii*. Nearly all of the *Phalaenopsis* species had a unique *trn*L intron sequence resulting from mutations, insertions/deletions (indels), or both. Within the subgenus *Phalaenopsis*, 117 indels and 28 polymorphic sites were identified by multiple sequence alignment of the *trn*L intron sequences of 12 species of this subgenus. Each species of the subgenus *Phalaenopsis* encoded a unique *trn*L intron sequence with the exception of *Phalaenopsis schilleriana* and *Phalaenopsis philippinensis*, which had identical *trn*L intron sequences. These two species belong to the section *Phalaenopsis* (Fig. 1). *P. philippinensis* had been treated as *Phalaenopsis x leucorrhoda*, a natural hybrid between *P. aphrodite* and *P. schilleriana*, until this was reassessed by Tharp et al. (1987). An artificial hybridization between *P. aphrodite* (♀) and *P. schilleriana* (♂) was conducted by Dr. Robert J. Griesebach to determine the morphology between the hybrids and *P. x leucorrhoda* (see Fowlie, 1991). This result did not support the previous observation that *P. x leucorrhoda* was a natural hybrid of *P. aphrodite* (♀) and *P. schilleriana* (♂). However, from the comparison of *trn*L intron between *P. schilleriana* and *P. philippinensis*, it revealed that *P. philippinensis* may be a recent natural hybrid between *P. schilleriana* as the maternal parent and *P. aphrodite* as the paternal parent.

Within the subgenus *Polychilos*, 290 indels and 42 polymorphic sites were found among the sequences in the multiple sequence alignment of the *trn*L intron sequences of 34 species of this subgenus. One variable length polymorphism was found within the *trn*L intron sequence of the various species of the subgenus *Polychilos*, and this variation occurred within a known hot spot region. This hot spot is highly enriched with A and T nucleotides and contains AAT/ATT/AT repeat sequences. The A+T rich nature of the hot spot region of the plastid DNA is well known and has also been reported elsewhere (Ogihara et al., 1991, 1992). The A+T content within the hot spot region ranges from 83.0% to 100.0%, which is higher than that observed for the entire *trn*L intron ranging from 71.8% to 76.2%. Moreover, variable length polymorphisms that occur within hot spot regions of plastid DNA have been described in several reports (Tassopulu and Kung, 1984; Ogihara and Tsunewaki, 1988; Ogihara et al., 1991; Guo and Terachi, 2005). Two mechanisms, slipped-strand mispairing and molecular recombination, are thought to account for indels in the noncoding regions of the plastid genome during evolution (Kelchner, 2000). Each species of the subgenus *Polychilos* had a unique *trn*L intron sequence with the exception of *Phalaenopsis fuscata* and *Phalaenopsis kunstleri*, which had the same sequence. These two species belong to the section *Fuscatae* (Fig. 2). Actually, these two species were confused for each other

Table 1
Taxon names, geographical distributions of the species, source information, and GenBank accession numbers for the samples used in this study.

Taxa and systematic classification[a]	Geographical distribution	Source[b]	Accession number
Genus *Phalaenopsis*			
Subgenus *Proboscidioides* (Rolfe) E.A. Christ.			
Phalaenopsis lowii Rchb.f.[b]	Myanmar, and adjacent western Thailand	KDAIS KC-87	AY265795
Subgenus *Aphyllae* (Sweet) E.A. Christ.			
Phalaenopsis wilsonii Rolfe	China (Sichuan, Yunnan, and eastern Tibet)	KDAIS KC-108	AY265787
Phalaenopsis minus (Seidenf.) E.A. Christ.	Endemic to Thailand	KDAIS KC-226	AY265772
Phalaenopsis braceana (J.D. Hook.) E.A. Christ.	Bhutan and China	KDAIS KC-218	AY265748
Phalaenopsis honghenensis F.Y. Liu	China (Yunnan)	KDAIS KC-301	DQ195040
Subgenus *Parishianae* (Sweet) E.A. Christ.			
Phalaenopsis gibbosa Sweet	Vietnam and Laos	KDAIS KC-51	AY265758
Phalaenopsis lobbii (Rchb.f.) Sweet	India, Bhutan, Myanmar, and Vietnam	KDAIS KC-104	AY265767
Phalaenopsis parishii Rchb.f.	Eastern Himalayas, India, Myanmar, and Thailand	KDAIS KC-192	AY265774
Phalaenopsis appendiculata C.E. Carr	Endemic to Malaysia (Malay Peninsula)	KDAIS KC-411	DQ194981
Subgenus *Polychilos* (Breda) E.A. Christ.			
Section *Polychilos* (Breda) Rchb.f.			
Phalaenopsis mannii Rchb.f.	Northeast India, Nepal, and China to Vietnam	KDAIS KC-77	AY265769
Phalaenopsis cornu-cervi (Breda) Bl. & Rchb.f.	Northeast India and the Nicobar Islands to Java and Borneo	KDAIS KC-23	AY265751
Phalaenopsis borneensis Garay	Endemic to Borneo	KDAIS KC-109	AY265747
Phalaenopsis pantherina Rchb.f.	Endemic to Borneo	KDAIS KC-56	AY265775
Phalaenopsis lamelligera Sweet	Endemic to Borneo	KDAIS KC-114	AY265765
Section *Fuscatae* Sweet			
Phalaenopsis cochlearis Holtt.	Malaysia (Malay Peninsula) and Indonesia (Sarawak)	KDAIS KC-484	DQ194982
Phalaenopsis viridis J.J. Sm.	Endemic to Indonesia (Sumatra)	KDAIS KC-41	AY265786
Phalaenopsis fusca Rchb.f.	Malaysia (Malay Peninsula), Boeneo (West Koetai)	KDAIS KC-115	AY265757
Phalaenopsis kunstleri J.D. Hook.	Myanmar and Malay Peninsula	KDAIS KC-139	AY265764
Section *Amboinenses* Sweet			
Phalaenopsis pulchra (Rchb.f.) Sweet	Endemic to the Philippines (Luzon and Leyte)	KDAIS KC-17	AY265778
Phalaenopsis bellina (Rchb.f.) E.A. Christ.	Malaysia (Malay Peninsula) and East Malaysia (Sarawak)	KDAIS KC-67	AY265746
Phalaenopsis violacea Witte	Indonesia (Sumatra) and Malaysia (Malay Peninsula)	KDAIS KC-152	AY265796
Phalaenopsis micholitzii Rolfe	Philippines (Mindanao)	KDAIS KC-85	AY265771
Phalaenopsis fimbriata J.J. Sm.	Indonesia (Java, Sarawak, and Sumatra)	KDAIS KC-62	AY265756
Phalaenopsis floresensis Fowlie	Endemic to the island of Flores	KDAIS KC-54	AY265797
Phalaenopsis gigantea J.J. Sm.	Endemic to Sabah in Esat Malaysia and adjacent Kalimantan Timur, from sea level to 400 m in elevation	KDAIS KC-131	AY265759
Phalaenopsis fasciata Rchb.f.	Endemic to the Philippines (Luzon, Bohol, and Mindanao)	KDAIS KC-189	AY265755
Phalaenopsis doweryensis Garay & E.A. Christ.	East Malaysia, Sabah, without a precise locality	KDAIS KC-138	AY265753
Phalaenopsis modesta J.J. Sm.	Endemic to the island of Borneo in East Malaysia (Sabah) and Indonesia (Kalimantan)	KDAIS KC-159	AY265793
Phalaenopsis maculata Rchb.f.	Malaysia (Pahang), East Malaysia (Sabah and Sarawak), and Indonesia (Kalimantan Timur)	KDAIS KC-49	AY265798
Phalaenopsis javanica J.J. Sm.	Endemic to Indonesia (Java)	KDAIS KC-38	AY265763
Phalaenopsis mariae Burb. ex Warn. & B. S. Wms.	Endemic to the Philippines and Indonesia (Kalimantan and Borneo)	KDAIS KC-30	AY265770
Phalaenopsis amboinensis J.J. Sm.	Indonesia (Molucca Archipelago and Sulawesi)	KDAIS KC-43	AY265743
Phalaenopsis lueddemanniana Rchb.f.	Endemic to the Philippines	KDAIS KC-8	AY265768
Phalaenopsis venosa Shim & Fowlie	Endemic to Indonesia (Sulawesi)	KDAIS KC-14	AY265785
Phalaenopsis pallens (Lindl.) Rchb.f.	Endemic to the Philippines	KDAIS KC-117	AY265773
Phalaenopsis bastianii Gruss & Rollke	Endemic to the Philippines	KDAIS KC-34	AY265745
Phalaenopsis hieroglyphica (Rchb.f.) Sweet	Endemic to the Philippines	KDAIS KC-33	AY265760
Phalaenopsis reichenbachiana Rchb.f. & Sander	Endemic to the Philippines	KDAIS KC-235	AY265779
Section *Zebrinae* Pfitz.			
Phalaenopsis inscriptiosinensis Fowlie	Endemic to Indonesia (Sumatra)	KDAIS KC-48	AY265761
Phalaenopsis tetraspis Rchb.f.	India (Andaman and Nicobar Islands) and Indonesia (Sumatra)	KDAIS KC-40	AY265784
Phalaenopsis corningiana Rchb.f.	Borneo (Sarawak and elsewhere on the island)	KDAIS KC-29	AY265750
Phalaenopsis sumatrana Korth. & Rchb.f.	Widespread from Myanmar, Thailand, Vietnam, to Indonesia (Java and Sumatra), Malaysia (Perak and Johore), East Malaysia (Sabah), and the Philippines (Palauan)	KDAIS KC-32	AY265783
Subgenus *Phalaenopsis*			
Section *Phalaenopsis* Benth			
Phalaenopsis philippinensis Golamco ex Fowlie & Tang	Endemic to the Philippines	KDAIS KC-26	AY265776
Phalaenopsis amabilis (L.) Blume	Widespread from Sumatra and Java to the southern Philippines, and east to New Guinea and Queensland, Australia	KDAIS KC-96	AY265742
Phalaenopsis aphrodite Rchb.f.	Northern Philippines and southeastern Taiwan	KDAIS KC-99	AY265744
Phalaenopsis sanderiana Rchb.f.	Endemic to the Philippines	KDAIS KC-35	AY265780
Phalaenopsis schilleriana Rchb.f.	Endemic to the Philippines	KDAIS KC-4	AY265781
Phalaenopsis stuartiana Rchb.f.	Endemic to the island of Mindanao in the southern Philippines	KDAIS KC-2	AY265782

Table 1 (*Continued*)

Taxa and systematic classification[a]	Geographical distribution	Source[b]	Accession number
Section *Deliciosae* E.A. Christ.			
Phalaenopsis chibae Yukawa	Endemic to Vietnam	KDAIS KC-27	AY265800
Phalaenopsis deliciosa Rchb.f.	Widespread from Sri Lanka and India to the Philippines and Sulawesi	KDAIS KC-73	AY265752
Section *Esmeralda* Rchb.f.			
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-20	AY265777
Section *Stauroglottis* (Schauer) Benth.			
Phalaenopsis equestris (Schauer) Rchb.f.	Philippines and Taiwan	KDAIS KC-59	AY265754
Phalaenopsis celebensis Sweet	Endemic to Indonesia (Sulawesi)	KDAIS KC-64	AY265799
Phalaenopsis lindenii Loher	Endemic to the Philippines	KDAIS KC-118	AY265766
***Phalaenopsis* hybrids**			
Phalaenopsis Yungho Gelb Canary		KDAIS Ph-114	FJ705059
Phalaenopsis Timonthy Christopher		KDAIS Ph-94	FJ472585
Phalaenopsis Rainbow Chip		KDAIS Ph-115	FJ472586

[a] The systematics of *Phalaenopsis* are based on Christenson (2001).
[b] Plant materials were cultivated at the Kaohsiung District Agricultural Research and Extension Station, Taiwan.

based on morphology (Holttum, 1957), and Sweet (1969) separated them into distinct species based on column morphology (slender, cylindrical column in *P. fuscata*; short, squatty column in *P. kunstleri*). However, these two species cannot be distinguished based on *trn*L intron sequence.

Within the subgenus *Parishianae*, two indels and 11 polymorphic sites were found among the *trn*L intron sequences compared by multiple sequence alignment for four species of this subgenus. Each species of this subgenus had a unique *trn*L intron sequence (Fig. 3). Within the subgenus *Aphyllae*, two indels and 23 polymorphic sites were found among the *trn*L intron sequences from four species of this subgenus. Each species in this subgenus had a unique *trn*L intron sequence with the exception of *P. wilsonii* and *P. honghensensis*, which had the same sequence (Fig. 4). Actually, these two species are still frequently confused for one another based on the morphological parameters that are currently used to distinguish these two species. Based on the results obtained above, it was determined that the plastid DNA region containing the *trn*L intron sequence could be used as a marker for determining the plastid genome type.

3.2. Identification of plastid genome types of Phalaenopsis hybrids

The PCR products amplified from the *trn*L intron of three *Phalaenopsis* hybrids were directly sequenced. The nucleotide substitutions and length variation within the *trn*L intron region were useful characters for identifying the plastid genome type of the hybrids based on BLAST searching in GenBank. The genealogy for each registered *Phalaenopsis* hybrid could be found in the Wildcatt Orchids Database, which is based on the Sander's List of Orchid Hybrids (Moir, 1995). The *trn*L intron sequence from *P.* Yungho Gelb Canary (accession number: FJ705059) was the same as that of *P. amboinensis*, indicating that the plastid genome type for the hybrid was inherited from *P. amboinensis*. This result was in agreement with the genealogy of *P.* Yungho Gelb Canary as reported in the Wildcatt Database (Fig. 5a). The *trn*L intron sequence from *P.* Timonthy Christopher (accession number: FJ472585) showed that the plastid genome type for this hybrid was inherited from *P. stuartiana*. This result was not in agreement with the Wildcatt Database, which indicated that the plastid genome type of this hybrid was inherited from *P. equestris* (Moir, 1995). According to the genealogy of *P.* Timonthy Christopher acquired from the Wildcatt Database, *P.* Cassandra was the maternal parent and *P. amabilis* was the paternal parent, according to the registration by Sandrik in 1982. This observation suggested that the registration for *P.* Cassandra was incorrect. To verify this, the maternal parent, *P.* Cassandra, was further examined. *P.* Cassandra was reportedly derived via hybridization between *P. equestris* as the maternal parent and *P. stuartiana* as the paternal parent, with the genealogy being registered

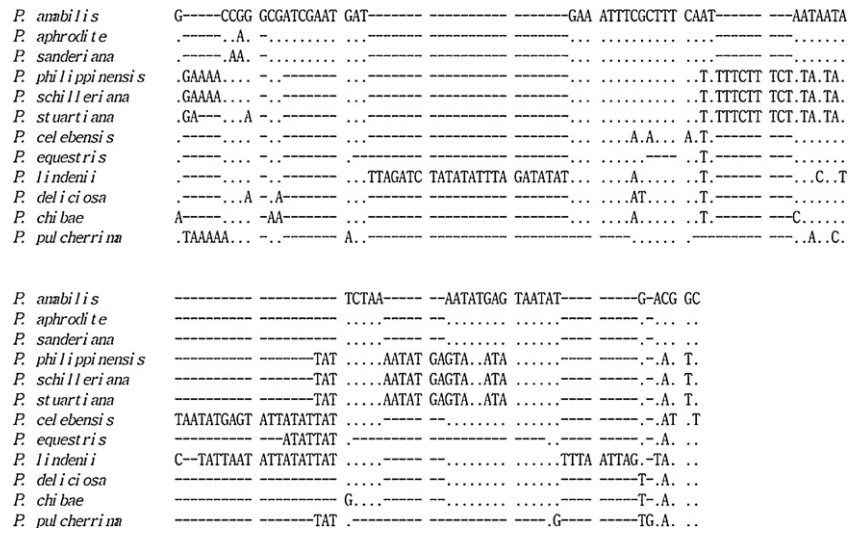

Fig. 1. The polymorphic sites identified in the multiple sequence alignment for 12 species of the subgenus *Phalaenopsis*. Dots (···) indicate identical nucleotides, and dashes (– – –) indicate insertions or deletions.

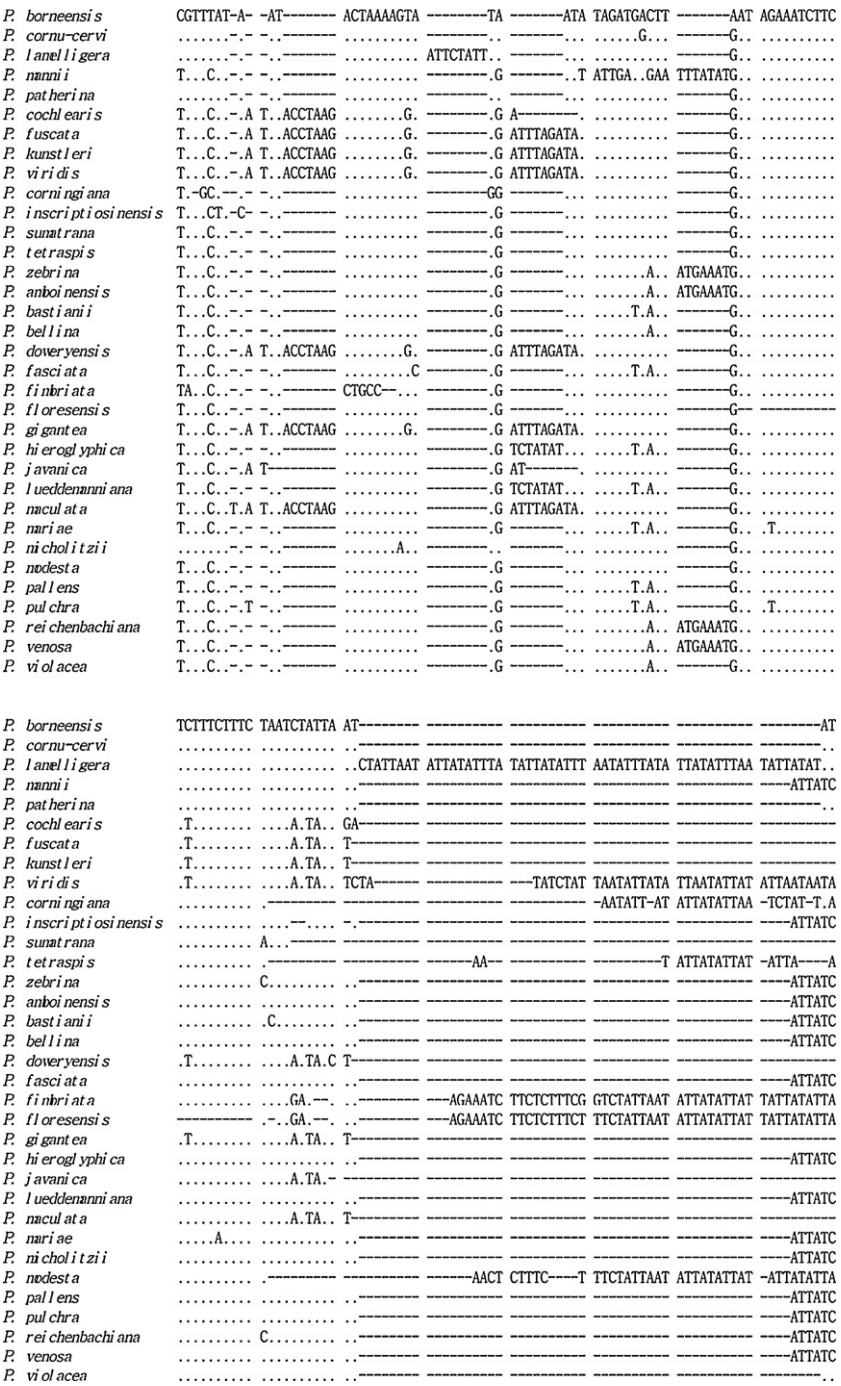

Fig. 2. The polymorphic sites identified in the multiple sequence alignment for 34 species of the subgenus *Polychilos*. Dots (···) indicate identical nucleotides, and dashes (– – –) indicate insertions or deletions.

by Veitch in 1896 (see Moir, 1995) (Fig. 5b). However, the plastid genome type of the *P.* Cassandra was determined based on its *trn*L intron sequence (accession number: JQ613334), and the results showed that the plastid genome type for *P.* Cassandra was inherited from *P. stuartiana*, indicating that *P. stuartiana* is the maternal parent instead of *P. equestris*. These results conclude that the genealogy of *P.* Cassandra registered was incorrect. The erroneous genealogy of *P.* Cassandra further affects the genealogy of next generation hybrid, *P.* Timonthy Christopher. For another hybrid, *P.* Rainbow Chip, the *trn*L intron sequence (accession number: FJ472586) indicated that the plastid genome type of the hybrid was inherited from *P. stuartiana*. This result was not in agreement with the genealogy of the hybrid according to the Wildcatt Database, which indicated that the plastid genome type would be inherited from *P. equestris* (Moir, 1995) (Fig. 5c). The analysis of the genealogy of this hybrid also revealed that *P.* Cassandra was registered as the maternal parent for *P.* Rainbow Chip. Therefore, the erroneous genealogy of *P.* Rainbow Chip also resulted from the wrong registration of *P.* Cassandra as with the previous examination of *P.* Timonthy Christopher.

For further confirming the result based on the *trn*L intron sequence, another plastid DNA fragment *atp*B-*rbc*L intergenic

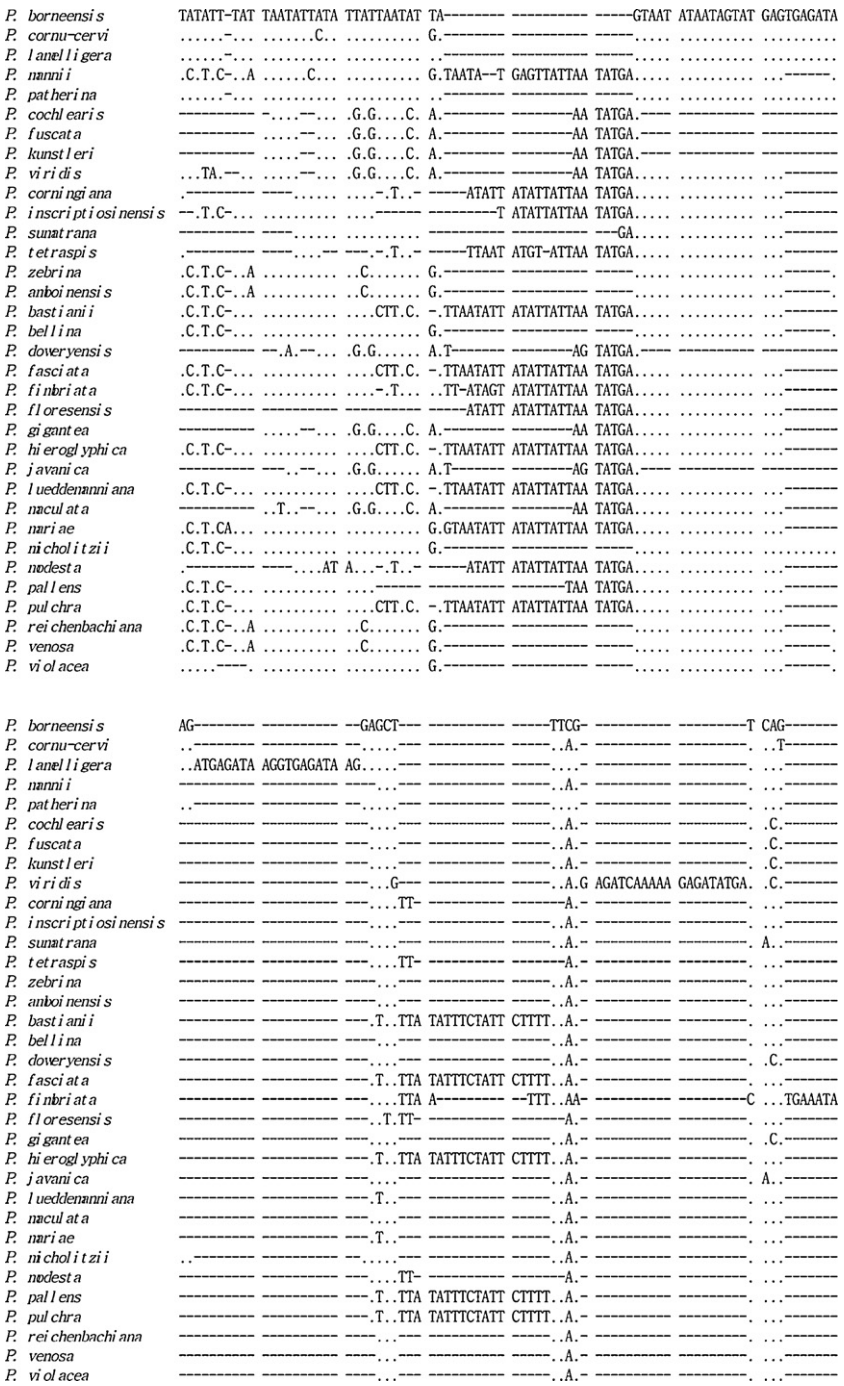

Fig. 2. (*continued*)

spacer (IGS) was included to analyze the genealogies of the three *Phalaenopsis* hybrids. The *atp*B-*rbc*L IGS sequence from *P.* Yungho Gelb Canary (accession number: JQ956409) was the same as that of *P. amboinensis*. The result is agreement with the analysis of *trn*L intron for the genealogy of the hybrid. The *atp*B-*rbc*L IGS sequence from *P.* Timonthy Christopher (accession number: JQ956410) showed that the plastid genome type for this hybrid was inherited from *P. stuartiana*. The result supports the analysis based on the *trn*L intron sequence for the genealogy of the hybrid, and showing not in agreement with the Wildcatt Database, which indicated that the plastid genome type of this hybrid was inherited from *P. equestris* (Moir, 1995). For another hybrid, *P.* Rainbow Chip, the *atp*B-*rbc*L IGS sequence (accession number: JQ956411) indicated that the plastid genome type of the hybrid was inherited from *P. stuartiana*. The result supports the analysis based on the *trn*L intron sequence for the genealogy of the hybrid, and showing not

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P. borneensis          -------AAA A
P. cornu-cervi         -------... .
P. lanelligera         -------... .
P. mannii              -------... C
P. patherina           -------... .
P. cochlearis          -------... .
P. fuscata             -------... .
P. kunstleri           -------... .
P. viridis             -------... .
P. corningiana         -------... .
P. inscriptiosinensis  -------... .
P. sumatrana           -------... .
P. tetraspis           -------... .
P. zebrina             -------... .
P. amboinensis         -------... .
P. bastianii           -------... .
P. bellina             -------... .
P. doweryensis         -------... .
P. fasciata            -------... .
P. fimbriata           GATCCTTGGG C
P. floresensis         -------... .
P. gigantea            -------... .
P. hieroglyphica       -------... .
P. javanica            -------... .
P. lueddemanniana      -------... .
P. maculata            -------... .
P. mariae              -------... .
P. micholitzii         -------... .
P. modesta             -------... .
P. pallens             -------... .
P. pulchra             -------... .
P. reichenbachiana     -------... .
P. venosa              -------... .
P. violacea            -------... .
```

Fig. 2. (*continued*).

```
P. appendiculata   GCC------- ------CT-- ---------- -------GA GAGG
P. parishii        A..------- CTATTAAAGA GTAATCTAAA ATATGAA.C .CCA
P. gibbosa         A.AATTTATA CTATTAAAGA GTAATC---- -----AA.C A...
P. lobbii          AA.------- CTATTAAAGA GTAATCTAAA ATATGAATC ..C.
```

Fig. 3. The polymorphic sites identified in the multiple sequence alignment for four species of the subgenus *Parishianae*. Dots (···) indicate identical nucleotides, and dashes (– – –) indicate insertions or deletions.

```
P. braceana        TTACGTAATT TCTATATGAA ATTTGAAATT TATATAAGAC TTCAAAGACG G
P. wilsonii        .G..... ..... ..... .C..... .....A... .
P. honghenensis    .G..... ..... ..... .C..... .....A... .
P. minus           C.C-ACCC-- ---------- ---------- ------CTCA GGACCC.CAT T
```

Fig. 4. The polymorphic sites identified in the multiple sequence alignment for four species of the subgenus *Aphyllae*. Dots (···) indicate identical nucleotides, and dashes (– – –) indicate insertions or deletions.

in agreement with the Wildcatt Database, which indicated that the plastid genome type of this hybrid was inherited from *P. equestris* (Moir, 1995).

4. Conclusions

The *trn*L intron sequences of plastid DNA for over 95% of living native species of *Phalaenopsis* were determined and submitted to GenBank. No heterogeneous *trn*L intron sequences were found for any of the samples examined, including native species and hybrids. Molecular evidence had previously demonstrated that maternal inheritance occurs during interspecific hybridization of *Phalaenopsis* species; therefore, the plastid genome type of *Phalaenopsis* hybrids will be maternally inherited from one of the native *Phalaenopsis* species. In this study, we demonstrated that the *trn*L intron sequences of different *Phalaenopsis* species are unique, and this DNA sequence can be used as an indicator of plastid genome type for *Phalaenopsis* hybrids. To verify this, three *Phalaenopsis* hybrids registered in Sander's List of Orchid Hybrids at Royal Horticultural Society (RHS) were examined. The *trn*L intron for each *Phalaenopsis* hybrid was amplified via PCR and sequenced. The identity of each *trn*L intron sequence for each examined hybrid was determined by searching NCBI databases via the BLAST program, which provided matches to the *trn*L intron sequences of native *Phalaenopsis* species in GenBank. Using this approach, the plastid genome types for each of the hybrids were determined and the conflict of genealogy and the plastid genome type in two hybrids *P.* Timonthy Christopher and *P.* Rainbow Chip can be found. The conflict has been shown to be caused by their female parent *P.* Cassandra with inverted registration between its female parent and male parent in Sander's List of Orchid Hybrids at RHS.

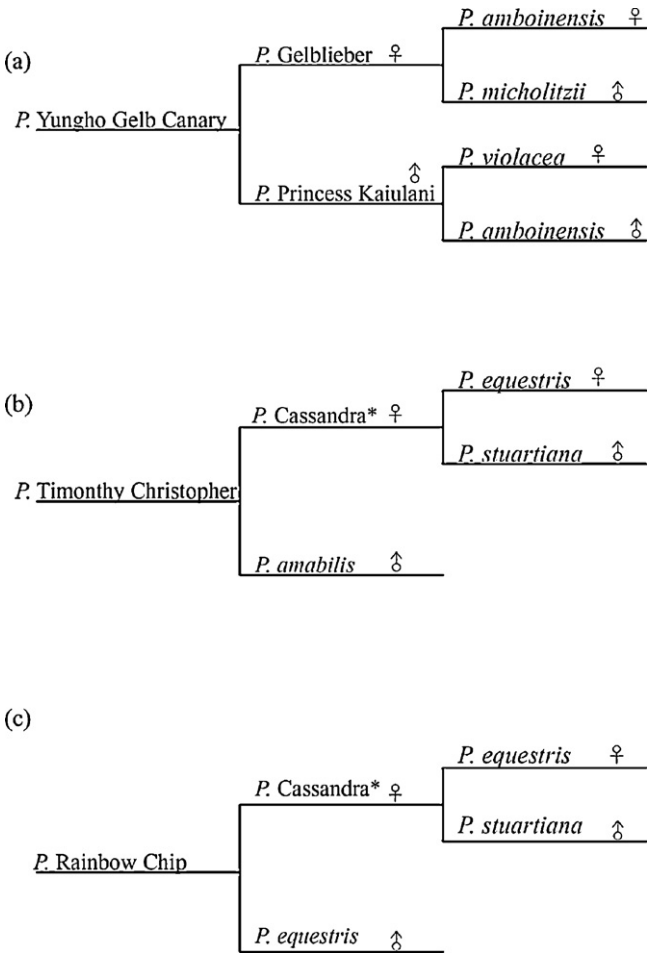

Fig. 5. The genealogies of *Phalaenopsis* Yungho Gelb Canary (A), *P.* Timonthy Christopher (B), and *P.* Rainbow Chip (C). These genealogies were redrawn based on the information from the Wildcatt Database. The asterisk represents the hybrid with the wrong genealogy resulting from the inverted submission between female parent and male parent.

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