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

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ABSTRACT

The trnL 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 trnL intron sequences resulting from mutations, insertions/deletions, or both. These trnL 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 trnL 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 trnL 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).

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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, Fuscatae, Amboinenses,* and *Zebrinae.* In addition, subgenus *Phalaenopsis, Deliciosae, Esmeralda,* and *Stauroglottis.* Species of *Phalaenopsis* are found throughout tropical Asia and the larger islands of the Pacific Ocean.

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All *Phalaenopsis* species, excluding the natural tetraploid species *Phalaenopsis buyssoniana* Rchb.f., have 38 (2*n* = 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 *trnL* intron and *trnL-trnF* 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 *trnL* 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 *trnL* intron sequence. In addition, the native *trnL* 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 *trnL* 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 µg 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 *trnL* 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 *trnL* 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 trnL* 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 trnL 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 trnL 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 trnL intron sequences of 12 species of this subgenus. Each species of the subgenus Phalaenopsis encoded a unique trnL intron sequence with the exception of Phalaenopsis schilleriana and Phalaenopsis philippinensis, which had identical trnL 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* (φ) and *P. schilleriana* (σ) 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* (φ) and *P. schilleriana* (σ). However, from the comparison of trnL 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 trnL intron sequences of 34 species of this subgenus. One variable length polymorphism was found within the trnL 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 trnL 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, slippedstrand 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 trnL 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

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Table 1

Taxa and systematic classification ^a	Geographical distribution	Source ^b	Accession numbe	
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 (Seideni,) E.A. Christ,	Endemic to Inaliand Phytop and China	KDAIS KC-226	AY265772	
Phulaenopsis Diaceana (J.D. HOOK.) E.A.	Dilutari and Cilina	KDAIS KC-216	A1203746	
Phalaenonsis honghenensis FV Liu	China (Yunnan)	KDAIS KC-301	DO195040	
Subgenus Parishianae (Sweet) E.A. Christ.	cinita (Tainan)	RD/IIB RC 501	50155010	
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	
halaenopsis appendiculata C.E. Carr	Endemic to Malaysia (Malay Peninsula)	KDAIS KC-411	DQ194981	
ubgenus Polychilos (Breda) E.A. Christ.				
Section Polychilos (Breda) Rchb.f.	North cost India Namel and Chine to Wetware		42265760	
Phalaenopsis mannii Kchb.f.	Northeast India, Nepal, and China to Vietnam	KDAIS KC-77	AY265769	
chb f	Northeast mula and the Nicobal Islands to Java and Borneo	KDAIS KC-25	A1203/31	
Phalaenonsis horneensis Carav	Endemic to Borneo	KDAIS KC-109	AV265747	
Phalaenopsis porticerisis Garay	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 (RCnD.I.) E.A. Christ.	Malaysia (Malay Peninsula) and East Malaysia (Sarawak)	KDAIS KC-67	AY265746	
Phalaenopsis micholitzii Rolfe	Philippines (Mindanao)	KDAIS KC-152	A1203790 AV265771	
Phalaenopsis fimbriata LL 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	KDAIS KC-131	AY265759	
1 00 55	Kalimantan Timur, from sea level to 400 m in elevation			
Phalaenopsis fasciata Rchb.f.	Endemic to the Philippines (Luzon, Bohol, and Mindanao)	KDAIS KC-189	AY265755	
Phalaenopsis doweryensis Garay & E.A.	East Malaysia, Sabah, without a precise locality	KDAIS KC-138	AY265753	
hrist.				
Phalaenopsis modesta J.J. Sm.	Endemic to the island of Borneo in East Malaysia (Sabah)	KDAIS KC-159	AY265793	
Dhalaan anais maaulata Dahh f	and Indonesia (Kalimantan)	KDAIC KC 40	42205700	
Phalaenopsis maculata RChD.1.	Malaysia (Palialig), East Malaysia (Sabali aliu Sarawak),	KDAIS KC-49	A1205/98	
Phalaenonsis javanica LL Sm	Endemic to Indonesia (Iava)	KDAIS KC-38	AY265763	
Phalaenopsis mariae Burb. ex Warn. & B. S.	Endemic to the Philippines and Indonesia (Kalimantan and	KDAIS KC-30	AY265770	
Vms.	Borneo)			
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 reichenbachlana KChD.I. &	Endemic to the Philippines	KDAIS KC-235	AY265779	
Section Zehringe Dfitz				
Phalaenonsis inscriptiosinensis Fowlie	Endemic to Indonesia (Sumatra)	KDAIS KC-48	AV265761	
Phalaenonsis tetrasnis Rohh f	India (Andaman and Nicobar Islands) and Indonesia	KDAIS KC-40	AY265784	
r haldenopsis terraspis kensil.	(Sumatra)	Refile Re To	111203701	
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	KDAIS KC-32	AY265783	
	Indonesia (Java and Sumatra), Malaysia (Perak and Johore),			
	East Malaysia (Sabah), and the Philippines (Palauan)			
ubgenus Phalaenopsis				
Section Phalaenopsis Benth			11/20057720	
Phalaenopsis philippinensis Golamco ex	Endemic to the Philippines	KDAIS KC-26	AY265776	
Owne & Lang	Wideepread from Sumatra and Java to the couthern	VDAIS VC OF	AV265742	
rnaidenopsis anabilis (L.) Blume	Philippines and east to New Cuines and Oueensland	NDAIS KC-90	A1203/42	
	Australia			
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	KDAIS KC-2	AY265782	
-	Philippines			

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Table I (Continueu

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 *trnL* intron sequence.

Within the subgenus *Parishianae*, two indels and 11 polymorphic sites were found among the *trnL* intron sequences compared by multiple sequence alignment for four species of this subgenus. Each species of this subgenus had a unique *trnL* intron sequence (Fig. 3). Within the subgenus *Aphyllae*, two indels and 23 polymorphic sites were found among the *trnL* intron sequences from four species of this subgenus. Each species in this subgenus had a unique *trnL* intron sequences from four species of this subgenus. Each species in this subgenus had a unique *trnL* 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 *trnL* 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 *trnL* intron of three *Phalaenopsis* hybrids were directly sequenced. The nucleotide

substitutions and length variation within the *trnL* 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 trnL 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 trnL 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

Р.	amabilis	GCCGG	GCGATCGAAT	GAT		GAA	ATTTCGCTTT	CAAT	AATAATA
Р.	aphrodi te	A.							
Ρ.	sanderi ana	AA.							
Р.	phi l i ppi nensi s	.GAAAA						T.TTTTCTT	TCT.TA.TA.
Р.	schilleri ana	.GAAAA						T.TTTTCTT	TCT.TA.TA.
Р.	stuart i ana	.GAA						T.TTTTCTT	TCT.TA.TA.
Р.	cel ebensi s						A.A	A.T	
Р.	equestris							T	
Р.	l i ndeni i			TTAGATC	TATATATTTA	GATATAT	A	T	CT
Р.	del i ci osa	A	A				AT	T	
Р.	chi bae	A	-AA				A	T	C
Р.	pul cherri na	. TAAAAA		A					AC.
Р.	anabi l i s			TCTAA	AATATGAG	TAATAT	G-ACG	GC	
Р.	aphrodite								
Р.	sanderi ana								
Ρ.	phi l i ppi nensi s		TAT	AATAT	GAGTAATA		A.	Τ.	
Р.	schill eri ana		TAT	AATAT	GAGTAATA		A.	Τ.	
Р.	stuarti ana		TAT	AATAT	GAGTAATA		A.	Τ.	
Р.	cel ebensi s	TAATATGAGT	ATTATATTAT				AT	.T	
Р.	equest ris		ATATTAT				A.		
Р.	l i ndeni i	CTATTAAT	ATTATATTAT			TTTA	ATTAGTA.		
Р.	del i ci osa						TA.		
Р.	chi bae			G			TA.		
P.	oul cherri na		TAT			G	TG A		

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.

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Ρ.	borneensi s	CGTTTAT-A-	-AT	ACTAAAAGTA	TA	ATA	TAGATGACTT	AAT	AGAAATCTTC
P	cornu-cervi						G	G	
D	Longligoro				ATTOTATT			C	
r.	I allel I I gel a				ALICIALL.			0	
Р.	nanni i	TC			G	T	ATIGA GAA	TITATATG	
Ρ.	pat heri na							G	
P	cochlearis	T C - A	T ACCTAAG	G	G	A		G	
	function of the second s	T C A	T ACCTAAC			ATTTACATA		G	
Ρ.	Tuscata	1CA	1ACCIAAG	G.	6	ATTIAGATA.			
Ρ.	kunst l er i	TCA	TACCTAAG	G.	G	ATTTAGATA.		G	
Ρ.	viridis	TCA	T. ACCTAAG	G.	G	ATTTAGATA.		G	
P	corpi nai ana	T -CC			66			6	
<i>r</i> .	Contra tigi ana	100						u	
Р.	i nscri pti osi nensi s	TCIC-			G			G	
Ρ.	sunatrana	TC			G			G	
P.	t et raspi s	TC			G			G	
D	a chui no	т. С. – –						ATCALATC	
<i>P</i> .	zeorina	1			6			AIGAAAIG	
Р.	anboi nensi s	TC			G		A	ATGAAATG	
Ρ.	bast i ani i	TC			G		T.A	G	
P	belling	T C			6		٨	C	
1.	Jerrina .	T					· · · · · · · · · · · · · · · · · · ·	u	
Р.	dower yensi s	TCA	T. ACCTAAG	G.	G	ATTTAGATA.		G	
Ρ.	f asci at a	TC		C	G		T.A	G	
P	finbriata	TA C		CTGCC	G			G	
D.	fl ana ana i a	т. с.		01000				G	
r.	i or esensi s	1		•••••••••••••••••••••••••••••••••••••••					
Р.	gi gant ea	TCA	T. ACCTAAG	G.	G	ATTTAGATA.		G	
Ρ.	hi erogl yphi ca	TC			G	TCTATAT	T.A	G	
P	i avani ca	T. C - A	Т		G	AT		G	
1.	Javan Ca	TCA	1					u	
Р.	l ueddenanni ana	TC			G	ICIAIAT	T.A	G	
Ρ.	nacul at a	TCT.A	T. ACCTAAG		G	ATTTAGATA.		G	
P	nariae	т с – –			G		ТА	G	т
n.	ni shali taki	1						u C	
Ρ.				A				G	
Ρ.	nødest a	TC			G			G	
Ρ.	pallens	TC			G		T.A	G	
P	pulchra	T C - T			6		ТΑ	G	т
r.		1			.0		·····		.1
Р.	rei chenbachi ana	TC			G		A	AIGAAAIG	
Ρ.	venosa	TC			G		A	ATGAAATG	
P	vi ol acea	T C			G		A	G	
P	borneensis	TETTETTE	TAATCTATTA	AT					AT
<i>P</i> .	borneensi s	тстттстттс	ТААТСТАТТА	AT					AT
Р. Р.	borneensi s cornu-cervi	тстттстттс	TAATCTATTA	AT					AT
P. P. P.	borneensi s cornu-cervi I anæl I i gera	тстттстттс	TAATCTATTA	AT CTATTAAT	ATTATATTTA	 TATTATATTT	 AATATTTATA	 TTATATTTAA	AT TATTATAT
P. P. P. P. P.	borneensis cornu-cervi lanelligera nannii	тстттстттс		AT CTATTAAT	ATTATATTTA	TATTATATTT	AATATTTATA	TTATATTTAA	AT TATTATAT ATTATC
P. P. P. P.	borneensi s cornu-cervi I anel I i gera nanni i sat hari na	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT	ATTATATTTA	TATTATATTT	AATATTTATA		AT
P. P. P. P. P.	borneensi s cornu-cervi I anel I i gera nanni i pat heri na	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT 	 ATTATATTTA 	 TATTATATTT	AATATTTATA	 TTATATTTAA	AT AT TATTATAT ATTATC
P. P. P. P. P.	borneensi s cornu-cervi I anel I i gera manni i pat heri na cochl eari s	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT GA		 TATTATATTT 	AATATTTATA		AT AT TATTATAT ATTATC ATTATC
P. P. P. P. P. P.	borneensi s cornu-cervi lanelligera nanni i patheri na cochl eari s fuscata	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT GA T	ATTATATTTA			 TTATATTTAA 	AT AT TATTATAT ATTATC
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi I anell i gera nanni i patheri na cochl eari s fuscata kunst I eri	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT 	ATTATATTTA		AATATTTATA	 TTATATTTAA 	AT TATTATAT ATTATC ATTATC
P. P. P. P. P. P. P. P.	borneensi s cornu-cervi I anel I i gera nanni i pat heri na cochl eari s f uscat a kunst I eri	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT GA T TCTA	ATTATATTTA			 TTATATTTAA 	AT TATTATAT ATTATC
P. P	borneensi s cornu-cervi I anell i gera manni i patheri na cochi eari s fuscata kunstl eri viri di s	TCTTTCTTTC 	TAATCTATTA	AT 	ATTATATTTA		AATATTTATA 		AT TATTATAT ATTATC
P.	borneensi s cornu-cervi I anel I i gera nanni i patheri na cochl eari s fuscata kunst I eri vi ri di s corni ngi ana	TCTTTCTTTC 	TAATCTATTA	AT 	ATTATATTTA	TATTATATTT			AT TATTATAT ATTATC
P.	borneensi s cornu-cervi lamel li gera nanni i patheri na cochl eari s fuscata kunst l eri vi ri di s corni ngi ana i nscri pti osi nensi s	TCTTTCTTTC 	TAATCTATTA	AT CTATTAAT GA T TCTA 	ATTATATTTA 			TTATATTTAA	AT TATTATAT ATTATC ATTATC
P. P	borneensi s cornu-cervi l anell i gera nanni i patheri na cochl eari s fuscata kunstl eri vi ri di s corni ngi ana i nscri pti osi nensi s sumat rana	TCTTTCTTTC 	TAATCTATTA 	AT CTATTAAT 	ATTATATTTA 	TATTATATTT TATCTAT	AATATTTATA 		AT TATTATAT ATTATC ATTATC
P. P	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunstl eri vi ri di s corni ngi ana i nscri pt i osi nensi s sunat rana	TCTTTCTTTC 	TAATCTATTA	AT 	ATTATATTTA	TATTATATTT	AATATTTATA AATATTTATA TAATATTATA AATATTAT	TTATATTTAA 	AT TATTATAT ATTATC
P. P	borneensi s cornu-cervi lanelli gera nanni i patheri na cochi eari s fuscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumatrana tet raspi s	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT 	ATTATATTTA	TATCTAT	T	TTATATTTAA TTATATTTAA TTAATATTAT ATTATATATTAA ATTATATTAT	AT TATTATAT ATTATC ATTATC
P. P	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscat a kunstl eri vi ri di s corni ngi ana i nscri pt i osi nensi s sunat rana tet raspi s zebri na	TCTTTCTTTC 	TAATCTATTA	AT CTATTAAT 	ATTATATTTA 	TATTATATTT	AATATTTATA 	TTATATTTAA TTATATTTAA TTAATATTAT ATTATATTAT	AT TATTATAT ATTATC
P. P	borneensi s cornu-cervi lanel li gera nanni i patherina cochl eari s fuscata kunst l eri viri di s corni ngi ana i nscri pti osi nensi s sunat rana tet raspi s zebri na anboi nensi s	TCTTTCTTTC 	TAATCTATTA	AT CTATTAAT 	ATTATATTTA 	TATTATATTT TATCTAT TATCTAT	AATATTTATA AATATTTATA TAATATTATA AATATTAT		ATTATTAT ATTATC ATTATC
P. P	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pt i osi nensi s sunat rana tet raspi s zebri na anboi nensi s basti ani i	TCTTTCTTTC	TAATCTATTA	AT CTATTAAT GA GA TCTA TCTA 	ATTATATTTA	TATCTAT	AATATTTATA AATATTTATA TAATATTATA -AATATT-AT 	TTATATTTAA TTAATATTAT TTAATATTAT ATTATATTAT	AT TATTATAT ATTATC
<i>P. P. P</i>	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunst l eri vi ri di s corni ngi ana i nscri pti osi nensi s sumat rana tet raspi s zebri na anboi nensi s basti ani i bell i no	TCTTTCTTTC 	TAATCTATTA	AT CTATTAAT CT	ATTATATTTA	TATTATATTT TATCTAT TATCTAT	ААТАТТТАТА ААТАТТТАТА ТААТАТТАТА 	TTATATTTAA TTATATATTAA TTAATATTAT ATTATATATTAT	ATTATATAT.
P. P	borneensi s cornu-cervi lanelli gera manni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumatrana tetraspi s zebri na anboi nensi s basti ani i belli na	TCTTTCTTTC 	TAATCTATTA	AT	ATTATATTTA	TATCTAT	AATATTTATA AATATTTATA TAATAATTATA -AATATTATA -AATATT-AT	TTATATTTAA TTAATATTAT ATTAATATTAT ATTATATTAT	AT TATTATAT ATTATC
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanell i gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pt i osi nensi s sunat rana t et raspi s zebri na anboi nensi s basti ani i bell i na doveryensi s	TCTTTCTTTC 	TAATCTATTA	AT CTATTAAT CT	ATTATATTTA 	TATTATATTT TATCTAT TATCTAT		TTATATTTAA TTATATTTAA TTAATATTAT ATTATATTAT	AT ATTATC. ATTATC
<i>₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.</i> ₽.₽.₽.₽.	borneensi s cornu-cervi lanelli gera manni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sumatrana tetraspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci ata	TCTTTCTTTC 	TAATCTATTA	AT	ATTATATTTA	TATCTAT	AATATTTATA 	TTATATTTAA TTATATATTAT TTAATATTAT ATTATATTAT	ATTATAT TATTATAT
<i>₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.₽.</i>	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pt i osi nensi s sunat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci at a	TCTTTCTTTC	TAATCTATTA 	AT CTATTAAT C	ATTATATTTA 	TATCTAT TATCTAT 		TTATATTTAA TTATATATTAA TTAATATTAT ATTAATATTAT	ATTATATAT.
<i>P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.</i>	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunst l eri vi ri di s corni ngi ana i nscri pti osi nensi s sunat rana tet raspi s zebri na anboi nensi s bast i ani i bel li na doveryensi s fasci at a fi ubri at a	TCTTTCTTTC 	TAATCTATTA 	AT CTATTAAT CTATTAAT 	ATTATATTTA 	TATCTAT TATCTAT TATCTAT TATCTAT 	ААТАТТТАТА ААТАТТТАТА ТААТАТТАТА 		ATTATATAT.
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscat a kunstl eri viri di s corni ngi ana inscri pt i osi nensi s sunat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci at a fi nbri at a fl oresensi s	TCTTTCTTTC	TAATCTATTA	AT	ATTATATTTA ATTATATTTA	TATCTAT TATCTAT TATCTAT TATCTAT TATCTAT TATCTTTCC	AATATTTATA AATATTTATA TAATATTATA -AATATTATA -AATATTATA -AATATT-AT 	TTATATTTAA TTATATTATTAA TTAATATTAT ATTAATATTAT	AT TATTATAT TATTATATC ATTATC
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunst l eri vi ri di s corni ngi ana i nscri pti osi nensi s sumat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci at a fi nbri at a fi orensi s gi gante a	TCTTTCTTTC 	TAATCTATTA 	AT CTATTAAT CTATTAAT 	ATTATATTTA AGAAATC AGAAATC	TATCTAT TATCTAT TATCTAT TATCTAT 	Алтатттата Алтатттата Талтаттата 		ATTATATAT.
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi l anell i gera manni i patheri na cochl eari s f uscata kunstl eri vi ri di s corni ngi ana i nscri pti osi nensi s sumatrana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s f asci at a fi nbri at a fl oresensi s gi gantea hi erogl yphi ca	TCTTTCTTTC 	TAATCTATTA	AT	ATTATATTTA	TATCTAT TATCTAT TATCTAT TATCTAT TATCTAT TATCTAT 	AATATTTATA AATATTTATA TAATATTATA AATATT-AT AATATT-AT 	TTATATTTAA TTATATATTAT TTAATATTAT ATTATATTAT	AT TATTATAT ATTATC
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelligera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci ata fi nbri ata fi lo resensi s gi gantea hi erogl yphi ca i avani ca	TCTTTCTTTC 	TAATCTATTA 	AT CTATTAAT C	ATTATATTTA 	TATCTAT TATCTAT TATCTAT TATCTAT TATCTAT 			ATTATATAT.
<i>P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.</i>	borneensi s cornu-cervi lanelli gera manni i patherina cochl eari s fuscata kunstl eri viridi s corningi ana inscri pti osi nensi s sumat rana tet raspi s zebri na anboi nensi s basti ani i bel I ina doveryensi s fasci at a fi nbri at a fl oresensi s gi gante a hi erogl yphi ca j avani ca	TCTTTCTTTC 	TAATCTATTA 	AT CTATTAAT GA T TCTA 	ATTATATTTA	TATCTATTATCTATTATCTATTATCTATTATCTAT	ААТАТТТАТА 	TTATATTTAA TTATATATTAT ATTATATTAT ATTATATTAT	ATTATAT TATTATAT
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunstl eri vi ri di s corni ngi ana i nscri pt i osi nensi s sumat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci ata fi nbri ata fi lo resensi s gi gant ea hi erogl yphi ca j avani ca l ueddenanni ana	TCTTTCTTTC 	TAATCTATTA	AT CTATTAAT C	ATTATATTTA 	TATCTAT TATCTAT TATCTAT TATCTAT 		TTATATTTAA TTATATTTAA TTAATATTAT ATTATATTAT ATTATATTAT ATTATATTAT ATTATATTAT ATTATATTAT ATTATATTAT	AT
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lamel li gera nanni i patheri na cochl eari s fuscata kunst l eri vi ri di s corni ngi ana i nscri pti osi nensi s sumat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci at a fi nbri at a fl oresensi s gi gant ea hi er ogl yphi ca j avani ca l ueddenanni ana macul at a	TCTTTCTTTC 	TAATCTATTA 	AT	ATTATATTTA	TATTATATTT TATCTAT TATCTAT TATCTAT TATCTAT 	AATATTTATA AATATTTATA TAATATTATA -AATATTATA -AATATT-AT 		ATTATATAT.
<i>P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.</i>	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscat a kunstl eri viri di s corni ngi ana i nscri pt i osi nensi s sunat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci at a fi nbri at a fl oresensi s gi gant ea hi erogi yphi ca j avani ca l ueddenanni ana macul at a mari ae	TCTTTCTTTC 	TAATCTATTA 	AT CTATTAAT CTATTAAT CTATTAAT T TCTA CTATTAAT CTATTA	ATTATATTTA ATTATATTTA 	TATCTAT TATCTAT TATCTAT TATCTAT 	AATATTTATA AATATTTATA 	TTATATTTAA TTATATATTAT ATTAATATTAT ATTATATTAT	ATTATATAT.
R R R R R R R R R R R R R R R R R R R	borneensi s cornu-cervi l anell i gera nanni i patheri na cochl eari s f uscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumat rana tetraspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci ata f i nbri ata f l oresensi s gi gant ea hi erogl yphi ca j avani ca l ueddenanni ana nacul at a nari ae ni choli i zi i	TCTTTCTTTC 	TAATCTATTA A.TA A.TA A C C 	AT	ATTATATTTA	TATCTAT TATCTAT TATCTAT 	Алтатттата Алтатттата Талататтата 		AT
R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pt i osi nensi s sunat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci ata fi nbri at a fl oresensi s gi gantea hi erogi yphi ca j avani ca l ueddenanni ana nacul at a mari ae ni chol it zi i	TCTTTCTTTC 	TAATCTATTA	AT	ATTATATTTA	TATCTATTATCTATTATCTATTATCTATTATCTAT	ААТАТТТАТА 	TTATATTTAA TTATATTTTAA TTAATATTAT ATTATATTAT	AT TATTATAT
RRRRRRRRRRRRRRRRRRRRRRRRRRRRRR	borneensi s cornu-cervi l anell i gera nanni i patheri na cochl eari s f uscata kunstl eri viri di s corni ngi ana i nscri pt i osi nensi s sunat rana t etraspi s zebri na anboi nensi s basti ani i bel l i na doveryensi s f asci ata f i nbri ata f i nbri ata f i norensi s gi gant ea hi erogl yphi ca j avani ca l ueddenanni ana nacul ata mari ae ni chol i tzi i nodesta	TCTTTCTTTC 	TAATCTATTA A.TAA.TAA.TAA.TA CCCC	AT	ATTATATTTA ATTATATTTA 	TATCTAT TATCTAT TATCTAT TATCTAT 	AATATTTATA AATATTTATA AATATATTATA AATAAT		ATTATATAT.
R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.	borneensi s cornu-cervi lanel li gera manni i patheri na cochl eari s fuscata kunst l eri vi ri di s corni ngi ana i nscri pti osi nensi s sumat rana tet raspi s zebri na anboi nensi s bast i ani i bel li na doveryensi s fasci at a fi nbri at a fl oresensi s gi gant ea hi er ogl yphi ca j avani ca lueddenanni ana macul at a mari ae ni choi li zi i nodesta pal lens	TCTTTCTTTC 	TAATCTATTA 	AT	ATTATATTTA	TATTATATTT TATCTAT TATCTAT TATCTAT 	AATATTTATA AATATTTATA 		ATTATATAT TATTATAT TATTATAT ATTAATAATA ATTAATAATA -TCTAT-T.A -TCTAT-T.A -TTATATATA -TTATATATA -ATTATC
R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumatrana tetraspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci ata fi nbri ata fi lo resensi s gi gantea hi erogi yphi ca j avani ca l ueddenanni ana nacul ata nari ae ni chol i tzi i nødesta pal lens pul chra	TCTTTCTTTC 	TAATCTATTA 	AT	ATTATATTTA ATTATATTTA 	TATCTAT TATCTAT TATCTAT TATCTAT 		TTATATTTAA TTATATTTAA TTAATATTAT ATTATATTAT ATTATATTAT	
<i>P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.</i>	borneensi s cornu-cervi l anel li gera nanni i patheri na cochl eari s f uscata kunst l eri vi ri di s corni ngi ana i nscri pti osi nensi s sunat rana tet raspi s zebri na amboi nensi s basti ani i bel li na doveryensi s f asci at a fi nbri at a fl oresensi s gi gant ea hi er ogl yphi ca j avani ca l ueddenanni ana macul at a nari ae ni cha z pal l ens pul chra rea chebochi ano	TCTTTCTTTC 	TAATCTATTA 	AT	ATTATATTTA	TATTATATTT TATCTAT TATCTAT 	AATATTTATA AATATTTATA TAATATTATA -AATATT-AT 		ATTATATAT.
<u>PPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPP</u>	borneensi s cornu-cervi lanel li gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pt i osi nensi s sunat rana tet raspi s zebri na anboi nensi s basti ani i bel li na doveryensi s fasci ata fi nbri ata fi loresensi s gi gantea hi erogi yphi ca j avani ca l ueddenanni ana nacul ata nari ae ni chol i t zi i nodesta pal lens pul chra rei chenbachi ana	TCTTTCTTTC 	TAATCTATTA	AT	ATTATATTTA ATTATATTTA 	TATCTAT TATCTAT TATCTAT TATCTAT 	AATATTTATA AATATTTATA 	TTATATTITAA TTATATTITAA TTAATATTAT ATTATATTAT ATTATATTAT	
PPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPP	borneensi s cornu-cervi l anel li gera nanni i patheri na cochl eari s f uscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumat rana t etraspi s zebri na anboi nensi s basti ani i bel li na doveryensi s f asci at a f i nbri at a fi nbri at a fi nbri at a fi oresensi s gi gant ea hi erogl yphi ca j avani ca l ueddenanni ana nacul at a nari ae ni chol i tzi i nodest a pal l ens pul chra rei chenbachi ana venosa	TCTTTCTTTC 	TAATCTATTA A.TAA.TAA.TAA.TA 	AT	ATTATATTTA	TATTATATTT TATTATATTT TATTATATTT TATTATATTT TATTAT	AATATTTATA AATATTTATA 		ATTATATAT.

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 *trnL* 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 *trnL* 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 *trnL* intron sequence, another plastid DNA fragment *atpB-rbcL* intergenic

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Р.	borneensi s	TATATT-TAT	TAATATTATA	TTATTAATAT	ТА		GTAAT	ATAATAGTAT	GAGTGAGATA
Ρ.	cornu-cervi		C		G				
Ρ.	lanelligera								
Р.	nanni i	.C.T.CA	C		G.TAATAT	GAGTTATTAA	TATGA		
Р.	pat heri na								
Р.	cochl eari s			.G.GC.	A	AA	TATGA		
Ρ.	fuscata			.G.GC.	A	AA	TATGA		
Р.	kunst l eri			.G.GC.	A	AA	TATGA		
Ρ.	viridis	TA		.G.GC.	A	AA	TATGA		
<i>P</i> .	corni ngi ana			T	ATATT	ATATTATTAA	TATGA		
<i>P</i> .	i nscri pti osi nensi s	T.C			T	ATATTATTAA	TATGA		
<i>P</i> .	sunatrana			·····			GA		
<i>P</i> .	tetraspis			1	TTAAT	AIGT-ATTAA	TAIGA		
<i>P</i> .	zebrina	.C.T.CA			G				
Р.	anboi nensi s	.C.T.CA			G				
P.	Dast i ani i				ITAATATT	AIAIIAIIAA	1A1GA		
P.	Del I I na	.0.1.0			G	10	TATCA		
P.	dower yensi s		A	.6.6	A. 1	ATATTATTA	TAIGA		
P.	Tasci at a			tir.c.	IIAAIAII	ATATTATTA	TAIGA		
<i>P</i> .	finbriata			1	II-AIAGI	ATATTATTA	TAIGA		
<i>P</i> .	I l oresensi s				AIAII	AIAIIAIIAA	TAIGA		
P.	gi gant ea	0. 70. 0		.6.6	A	A	TAIGA		
P.	hi erogi ypni ca				TIAAIAII	ATATIATIAA	TAIGA		
<i>P</i> .	javani ca			.6.6	A. 1	AG	TAIGA		
<i>P</i> .	l ueddenanni ana	.C.T.C			TTAATATT	ATATTATTAA	TAIGA		
Ρ.	macul at a			.6.6	A	AA	TAIGA		
Р.	mariae	.C.T.CA			G.GIAATATT	ATATTATTAA	TAIGA		
Ρ.	m chol i t zi i	.C.T.C			G				
Ρ.	nodesta		AT	AT	ATATT	ATATTATTAA	TAIGA		
<i>P</i> .	pallens	.C.T.C				TAA	TATGA		
Ρ.	pulchra	.C.T.C		CIT.C.	TTAATATT	ATATTATTAA	TATGA		
Р.	rei chenbachi ana	.C.T.CA			G				
Р.	venosa	.C.T.CA		c	G				
Ρ.	VA OL ACEA				G				
	vi or acca								
	vi or acca								
D	borneenaia	AC						Τ	CAC
P.	borneensi s	AG		GAGCT		TTCG-		T	CAG
P. P.	borneensi s cornu-cervi	AG		GAGCT		TTCG-		T 	CAG
P. P. P.	borneensi s cornu-cervi I anel I i gera	AG ATGAGATA	AGGTGAGATA	GAGCT AG		TTCG- A		T 	CAG T
P. P	borneensi s cornu-cervi lanel li gera nanni i	AG ATGAGATA	AGGTGAGATA	GAGCT AG		TTCG- A A		T T 	CAG T
P. P	borneensi s cornu-cervi lanel li gera nanni i patheri na	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A		T T 	CAG
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi I anel I i gera manni i pat heri na cochi eari s	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A		T	CAG T
P. P	borneensi s cornu-cervi I anel I i gera mani i pat heri na cochl eari s fuscata	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A A		T 	CAG .C .C
P. P	borneensi s cornu-cervi lanelligera nanni i patheri na cochl eari s fuscata kunstl eri	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A		T	CAG T
P. P	borneensi s cornu-cervi I anel I i gera nanni i patheri na cochi eari s fuscata kunst I eri vi ri di s	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A A A.G		T 	CAG .C .C .C
P. P	borneensi s cornu-cervi I anel I i gera nanni i patheri na cochl eari s fuscata kunst I eri vi ri di s corni ngi ana inacrini ci cinanzi i	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A A A. G A	 	T 	CAG T
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelli gera nanni i patheri na cochl eari s fuscata kunstl eri vi ri di s corni ngi ana inscri pti osi nensi s	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A A A A	 AGATCAAAAA	T 	CAG T
P. P	borneensi s cornu-cervi lanelligera nunni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sunut rana	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A A. G A A A	 AGATCAAAAA	T 	CAG T
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelligera nanni i patheri na cochl eari s fuscata kunstleri viri di s corni ngi ana inscri pti osi nensi s sumatrana tetraspi s	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A A. G A. G A A		T	CAG T
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelli gera mani i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetranpi s zebri na	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG- A A A A. G A. G A. G A A A	AGATCAAAAA	T	CAG .c
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelli gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pt i osi nensi s sumat rana tetraspi s zebri na anboi nensi s borti oni j	AG ATGAGATA 	AGGTGAGATA	GAGCT AG 		TTCG A A A A A A A A A A	AGATCAAAAA	T	CAG .C .C .C .A
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelligera nunni i patheri na coch eari s fuscata kunstleri viri di s corni ngi ana inscri pti osi nensi s sumatrana tetraspi s zebri na anboi nensi s basti ani i belli eo	AG	AGGTGAGATA	GAGCT AG 	TATTTCTATT	TTCG	AGATCAAAAA	T 	CAG T
P.	borneensi s cornu-cervi lanelli gera mani i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumat rana tetraspi s zebri na anboi nensi s basti ani i belli na	AG	AGGTGAGATA	GAGCT AG 		TTCG- A A A A.G A.G A A A CTTT .A A		T	CAG .c
P.	borneensi s cornu-cervi lanelli gera nanni i patheri na cochl eari s fuscata kunstleri viri di s corni ngi ana inscri pti osi nensi s sumat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryeensi s	AG ATGAGATA	AGGTGAGATA	GAGCT AG 		TTCG A A A A A.G A.G A A A CTTTT. A A CTTTT. A	AGATCAAAAA	T	CAG T
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelligera nanni i patheri na cochi eari s fuscata kunstleri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci ata	AG ATGAGATA	AGGTGAGATA	GAGCT AG 		TTCG	AGATCAAAAA	T	CAG T
R.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P	borneensi s cornu-cervi lanelligera nanni i patheri na cochl eari s fuscata kuustl eri viri di s corni ngi ana i nscri pti osi nensi s sunat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci ata fi nbri ata	AG	AGGTGAGATA	GAGCT AG AG 		TTCG	AGATCAAAAA	T	CAG T .C .C .C TGAAATA
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelli gera mani i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri ata	AG	AGGTGAGATA	GAGCT AG 		TTCG- A A A A A A A A A CTTT. A TTT. A A	AGATCAAAAA	T	CAG T
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornurcervi lanelli gera nanni i patheri na cochl eari s fuscata kunstleri viri di s corni ngi ana inscri pti osi nensi s sunat rana tet ranşi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi oresensi s gi gantea	AG	AGGTGAGATA	GAGCT AG 		TTCG	AGATCAAAAA	T 	CAG .C .C .C .C .C
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelligera manni i patheri na cochi eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetraspi s zebri na amboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri ata fi lo resensi s gi gantea bi erogi yphi ca iorani ca	AG	AGGTGAGATA	GAGCT AG 		TTCG	AGATCAAAAA	 GAGATATGA GAGATATGA 	CAG
P.	borneensi s cornurcervi lanelli gera mani i patheri na cochl eari s fuscata kunstleri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri at a fl oresensi s gi gantea hi erogi yphi ca j avani ca	AG	AGGTGAGATA	GAGCT AG AG 		TTCG- A A A A A A A A CTTT .A A A A CTTT .A A CTTT .A A	AGATCAAAAA	T	CAG .C .C .C A A A
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornu-cervi lanelli gera nanni i patheri na cochl eari s fuscata kunstleri viri di s corni ngi ana inscri pti osi nensi s sumatrana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri at a floresensi s gi gantea hi erogl yphi ca j avani ca	AG	AGGTCAGATA	GAGCT AG 		TTCG A A A A A A A A CTTT. A A CTTT. A A CTTT. A A CTTT. A	AGATCAAAAA	T	CAG T .C .C .C .C TGAAATA TGAAATA TGAAATA TGAAATA
R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.	borneensi s cornu-cervi lanelligera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pt i osi nensi s sunat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri at a fi lo resensi s gi gant ea hi erogl yphi ca j avani ca lueddemanni ana nucul at a	AG	AGGTGAGATA	GAGCT AG 		TTCG	AGATCAAAAA		CAG
<i>RRPRPPPPPPPPPPPPP</i>	borneensi s cornurcervi lanelli gera mani i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana i nscri pti osi nensi s sumat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri at a fl oresensi s gi gante a hi erogl yphi ca j avani ca lueddemani ana macul at a marine	AG		GAGCT AG AG 		TICG- 	AGATCAAAAA	T	CAG .C .C .C A A A A A
<i>RREPREREPREREPREREPREREPREREPREREPRERE</i>	borneensi s cornurcervi lanelli gera manii patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri ata floresensi s gi gantea hi erogl yphi ca j avani ca lueddemanni ana macul at a mari ae ni choli t zi i	AG	AGGTGAGATA	GAGCT AG 		TTCG- 	AGATCAAAAA	T	CAG T .c .c .c .c A A A A A A A A A A A A
<i>RREPERERPEREREPERER</i>	borneensi s cornurcervi lanelli gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetranspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri at a fi oresensi s gi gantea hi erogl yphi ca j avani ca lueddenanni ana mari ae mi choli tzi i nodest a	AG		GAGCT AG AG 		TTCG A A A A A A A A CTTT. A A CTTT. A A CTTT. A A CTTT. A A	AGATCAAAAA	T	CAG
<i>RREPEREREPEREREPEREREPEREREPERE</i>	borneensi s cornu-cervi lanelligera nanni i patheri na cochi eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetraspi s zebri na amboi nensi s basti ani i belli na doveryensi s fasci ata fi nbri ata fi lo resensi s gi gantea hi erogl yphi ca j avani ca lueddemanni ana macul ata mari ne ni choli tzi i nodest a pal lens	AG		GAGCT AG AG 		TICG	AGATCAAAAA	T	CAG .C .C .C A A A
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornurcervi lanelli gera manii patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fl oresensi s gi gantea hi erogl yphi ca j avani ca l ueddemanni ana macul at a mari ae ni choi i tzi i nødesta pal lens pul chra	AG	AGGTGAGATA	GAGCT AG AG 		TTCG- 	AGATCAAAAA	T	CAG .C .C .C A A A A A A A A A A
P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.P.	borneensi s cornurcervi lanelli gera manni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sumat rana tetraspi s zebri na anboi nensi s basti ani i belli na doveryensi s fasci at a fi nbri at a fl oresensi s gi gant ea hi er ogl yphi ca j avani ca lueddenanni ana maru at a mari ae ni chol i tzi i nodest a pal lens pul chra rei chenbachi ana	AG	AGGTCAGATA	GAGCT AG 		TTCG	AGATCAAAAA	T	CAG T .c .c .c .c .c .c TGAAATA TGAAATA TGAAATA TGAAATA
PPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPP	borneensi s cornurcervi lanelli gera nanni i patheri na cochl eari s fuscata kunstl eri viri di s corni ngi ana inscri pti osi nensi s sunat rana tet raspi s zebri na doveryensi s fasci ata fi nbri ata fi lo resensi s gi gantea hi erogi yphi ca j avani ca lueddenanni ana nacul ata nari ae ni chol i tzi i nodest a pal chen a pul chra rei chenbachi ana venosa	AG		GAGCT AG 		TTCG A A A A A A A A A CTTT. A A CTTT. A A CTTT. A A CTTT. A A CTTT. A A CTTT. A A A A A A	AGATCAAAAA	T	CAG

Fig. 2. (continued)

spacer (IGS) was included to analyze the genealogies of the three *Phalaenopsis* hybrids. The *atpB-rbcL* 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 *trnL* intron for the genealogy of the hybrid. The *atpB-rbcL* 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 *trnL* 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 *atpB-rbcL* 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 *trnL* intron sequence for the genealogy of the hybrid, and showing not

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Р.	borneensi s	AAA A
Ρ.	cornu-cervi	
Ρ.	l anel l i gera	
Ρ.	nanni i	C
Ρ.	pat heri na	
Ρ.	cochl eari s	
Ρ.	fuscata	
Ρ.	kunst l eri	
Ρ.	viridis	
Ρ.	corni ngi ana	
Ρ.	i nscri pti osi nensi s	
Ρ.	sunatrana	
Ρ.	t et raspi s	
Ρ.	zebrina	
Ρ.	anboi nensi s	
Ρ.	bast i ani i	
Ρ.	bellina	
Ρ.	dower yensi s	
Ρ.	f asci at a	
Ρ.	finbriata	GATCCTTGGG C
Ρ.	floresensi s	
Ρ.	gi gant ea	
Ρ.	hi erogl yphi ca	
Ρ.	j avani ca	
Ρ.	l ueddenanni ana	
Ρ.	nacul at a	
Ρ.	nari ae	
Р.	mi chol i t zi i	
Ρ.	nødest a	
Ρ.	pal l ens	
Р.	pul chra	
Ρ.	rei chenbachi ana	
Ρ.	venosa	
Р.	vi ol acea	

Fig. 2. (continued).

Р.	appendi cul at a	GCC	CT		GA GAGG
Р.	pari shi i	A	CTATTAAAGA	GTAATCTAAA	ATATGAA.C .CCA
Ρ.	gi bbosa	A.AATTTATA	CTATTAAAGA	GTAATC	AA.C A
Ρ.	l obbi i	AA	CTATTAAAGA	GTAATCTAAA	ATATGAATCC.

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.

Р.	braceana	TTACGTAATT	TCTATATGAA	ATTTGAAATT	TATATAAGAC	TTCAAAGACG	6
Р.	wilsonii	.G			.C	A	
Р.	honghenensi s	.G			.C	A	
Р.	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 *trnL* intron sequences of plastid DNA for over 95% of living native species of *Phalaenopsis* were determined and submitted to GenBank. No heterogeneous *trnL* 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 *trnL* 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



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.

Horticultural Society (RHS) were examined. The *trnL* intron for each *Phalaenopsis* hybrid was amplified via PCR and sequenced. The identity of each *trnL* intron sequence for each examined hybrid was determined by searching NCBI databases via the BLAST program, which provided matches to the *trnL* 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.

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