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ITS Sequences and Speciation on Far Eastern *Indigofera* (Leguminosae)

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The internal transcribed spacer (ITS) regions of 18-26S nuclear ribosomal DNA was sequenced to address phylogenetic relationships and to measure the extent of differentiation among six species of the Far Eastern *Indigofera*. ITS 1 had 230-240 base pairs (bp) long while ITS 2 had 211-213 bp long. The 5.8S rRNA coding region was 161 bp long. Sequence divergence calculated by Kimura's two parameter method between species ranged from 0.00 to 13.49%. A single most parsimonious tree was produced from 77 variable nucleotide sites, which had a consistency index of 0.97 and a retention index of 0.83. ITS sequence data suggested that the continental species of *I. kirilowii* (2n=16) is diverged from the common ancestor of other species at first, and then the island species of *I. decora* (2n=48) and *I. venulosa* and the Korean Peninsular species of *I. grandiflora* (2n=16) and *I. koreana* (2n=32) are diverged from the ancestor. The molecular data supports that the speciation in the Far Eastern *Indigofera* occurred with polyploidization from continental ancestor to peripheral peninsular and island species.

Key words: Far East — *Indigofera* — Internal transcribed spacer sequences — Leguminosae — Nuclear ribosomal DNA — Speciation

The genus *Indigofera* comprises about 720-730 species which are mostly distributed throughout the tropics and subtropics. The infrageneric classification of the genus was recently revised by Schrire (1995), but the phylogeny of the Asian section *Psiloceratiae* (Gillette) Shrire has remained obscure (Schrire 1995). The eastern Asiatic species of *Indigofera* were reviewed by Craib (1913) and recently by Fang and Zheng (1989). Five species of the genus *Indigofera* have been recorded in NE China, Korea and Japan, and all these species are included in the section *Psiloceratiae*. Among them, except *I. pseudotinctoria* Matsum. which is included in the subsection *Pseudotinctoria*, other four species are classified into the subsection *Psiloceratiae* (=subsection *Decorae* Fang and Zheng) (Fang and Zheng 1989, Choi 1996): *I. decora* Lindl., *I. grandiflora* B. Choi et S. Cho, *I. kirilowii* Maxim. ex Palib., and *I. koreana* Ohwi. These species of the subsection *Psiloceratiae* are restricted to the Far East, being the northeastern border of its distributional range of the genus, and have a distinct distribution pattern from each other, that is, isopatric species: *I. kirilowii* in from NE China to E & N Korea extending to Tsushima Island, *I. koreana* in SW Korea, *I. grandiflora* restricted to Mt. Kaya in S Korea and *I. decora* in from S Japan to SE China including Taiwan. Moreover, *I. venulosa* Champ. ex Benth. (subsection *Psiloceratiae*) in SE China including Taiwan was taxonomically confused with Korean species (Palibin 1898) and is also very similar to *I. decora* in appearance. However, the phylogenetic relationships among the Far Eastern *Indigofera* species have not been studied yet.

The basic chromosome number of the tribe Indigoferae is x=8 (Goldblatt 1981, Schrire 1995). The Far Eastern species show variation in chromosome number: 2n=16 in *I. kirilowii* (Kawakami 1930), *I. grandiflora* (Choi 1996) and *I. pseudotinctoria* (Kawakami 1930, Sugiura 1931, Kodama 1967), 2n=32 in *I. koreana* (Cho *et al.* 1997) and 2n=48 in *I. decora* (Tschechow 1930 & 1935 cited from Fedorov 1969). Thus, it is likely that *Indigofera* may be a good example by which to study the speciation process in the Far East in relation to polyploidization.

Molecular data from chloroplast and nuclear ribosomal (nr) DNA are useful in analyses of polyploidy in plants (reviewed Soltis *et al.* 1992). The nrDNA also has proven to be a useful phylogenetic tool because it is ubiquitous in all organisms and is present as repeated units in high copy numbers (Hamby and Zimmer 1992). The nrDNA units, separated in numerous replications by intergenic spacers, consist of the 18S, 5.8S and 26S coding regions in plants. Internal transcribed spacers (ITS) sequences of nrDNA have been shown to provide good phylogenetic resolution at the recently diverging lineages, because the sequences of spacer regions evolve more rapidly than coding regions (reviewed in Baldwin *et al.* 1995).

In this study, ITS 1, ITS 2 and 5.8S regions of nrDNA were sequenced in order to resolve the phylogenetic relationships and speciation process among the Far Eastern *Indigofera* species comparing to the variations of the chromosomal numbers and the patterns of geographical distribution.

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Materials and Methods

Plant material

The collection data for the plant materials used in this study are given in Table 1. Voucher specimens are deposited in IUI and TUS. All plant materials were kept at ca. −4 C in the field and stored at −70 C in the laboratory until use.

DNA exraction and purification

Total genomic DNA was extracted from freshly frozen leaf tissue pulverized in liquid nitrogen using the CTAB extraction buffer technique of Doyle and Doyle (1987). We added 2% PVP-40 in the extraction buffer to eliminate phenolic compounds. The extracted DNA were purified through Geneclean kit (Bio 101 Inc., CA, USA) and Chelex 100 (Bio-Rad Lab., CA, USA). DNA samples were stored at −20 C until use.

Amplification of ITS region

Double-stranded DNA of the complete ITS region (including 5.8S coding region) in each genomic DNA was amplified by 35 cycles of symmetric polymerase chain reaction (PCR) using the primers ITS 4 and ITS 5 (White *et al.* 1990). Amplifications were performed in 100 μl reactions containing 10-50 ng DNA, 200 μM deoxyribonucleotide triphosphates (equimolar), 1.5 units AmpliTaq DNA polymerase (Perkin & Elmer, Cetus), 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, and primers at 0.5 to 1.0 μM. Before the symmetric PCR cycles, the PCR mixture was predenatured at 92 C for 3 min. The PCR cycle consisted of 1 min at 92 C for denaturation, 1 min at 52 C for annealing, and 3 min at 72 C for extension. After 35 cycles the PCR reactions were incubated at 72 C for 7 min to complete final extension. Finally, amplified double-stranded PCR products were purified by Geneclean kit (Bio 101 Inc., CA, USA) and stored −20 C until use.

Sequencing of ITS region

Purified double-stranded PCR products were directly sequenced using the Sequenase DNA Polymerase (USB 70170) dideoxy chain-termination method following the protocols specified by the manufacturers with ^{35}S-dATP as the labelling reagent. Three forward (ITS 5, ITS 1 and ITS 3) and two reverse (ITS 2 and ITS 4) primers (White *et al.* 1990) were employed as the sequencing primers in the corresponding reactions. Sequences were resolved in 6% acrylamide-8 M urea glycerol tolerant gels (USB 70170). The gels were fixed in 5% methanol/20% glacial acetic acid for 15 min, transfered to Whatmann 3 MM paper, vacuum dried at 80 C for 2 hr, and exposed to Agfa Curix X-ray film for 12-72 hr.

Sequence alignment of ITS region

The sequence boundaries of two ITS regions and three coding regions (18S, 5.8S and 26S) of nrDNA were determined by comparing with published sequences (Yokota *et al.* 1989, Baldwin 1992, Suh *et al.* 1993, Kim and Jansen 1994). Sequences were aligned using Clustal V program with gap adjustments and were eventually determined by naked eye.

Phylogenetic analysis

Indigofera pseudotinctoria (subsection *Pseudotinctoria*) was selected as an outgroup, because it is the only Far Eastern species included in the same section, *Psiloceratiae*, of the genus *Indigofera* as the ingroup taxa (subsection *Psiloceratiae*). Parsimony analyses were performed with Wagner parsimony using PAUP (ver. 3.1, Swofford 1992) on a Macintosh II FX. Gaps were not included in the analyses. The shortest tree was found using Branch-and-Bound method. For character-state optimization, ACCTRAN was used in tree description. Bootstrap analysis (Felsenstein 1985) was carried out with 100 replicates using the Branch and Bound algorithm. Sequence divergence values between species were calculated by the Kimura's two-parameter method (1980) for 462 aligned sites of the ITS 1 and ITS 2 regions using the DNADIST program of PHYLIP (ver 3.5s, Felsenstein 1993) on IBM compatible PC 486 DXII. The Kimura's two-parameter method with the transition-transversion ratio setting at 1 : 2 was selected as the correction method.

Results

ITS sequence, size, variation, and base composition

The complete sequences of the ITS region (including 5.8S coding region) were determined for eight individuals of six *Indigofera* species in the Far East, including one outgroup species, *I. pseudotinctoria* (Table 1). Aligned sequences of ITS 1, the 5.8S coding region, and ITS 2 from this study are presented in Appendix 1. The characteristics of these sequences are summarized in Tables 2 and 3. The ITS region including the 5.8S coding region varied in length from 603 bp to 614 bp. In all species, the length of ITS 1 was longer than that of ITS 2. The length of ITS 1 ranges from 230 bp to 240 bp, and ITS 2 from 211 bp to 213 bp (Tables 2, 3). The 5.8S coding region was commonly 161 bp long in all species examined.

Sequence alignments require 15 (6.07%) and 10 (4.50%) independent insertion/deletion mutations in ITS 1 and ITS 2, respectively. Also, there were one two-bp insertions/deletions (50-51) and one three-bp insertions/deletions (270-272) in ITS 1 (Appendix 1). The G+C content was near the 50% level with a narrow range of vaiation in ITS 1 (50.8-54.7%), ITS 2 (44.6-48.4%), and the 5.8S coding region (51.5%). Transitions were 15, with 10 (66.7%) in ITS 1 and 5 (33.3%) in ITS 2. Transversions were 44, with 25 (56.8%) in ITS 1 and 19 (43.2%) in ITS 2 (Table 2). The ratio of transition/transversion in ITS 1 (0.40) was approximately two times higher than in ITS 2 (0.26).

Sequence divergence and phylogenetic analyses

Aligned sequences between the two amplifying primers (White *et al.* 1990, ITS 4 and ITS 5) included 741 positions.

Table 1. Chromosome numbers and collection data of the Far Eastern *Indigofera* species used in this study. Vouchers are deposited in IUI and TUS

Species	Chromosome numbers (2n)	Symbols	Voucher	Collection locality
I. kirilowii	16	KIR1	Cho 369	Mt. Kyeryong, Prov. Chungnam, Korea
		KIR2	Cho 253	Yeongjong Isl., Incheon City, Korea
I. Koreana	32	KOR1	Choi *et al.* 9113	Pyeonsan, Prov. Chunbuk, Korea
		KOR2	Choi *et al.* 8031	Mt. Mudeung, Prov. Chunnam, Korea
I. grandiflora	16	GRAN	Park 151	Mt. Kaya, Prov. Kyongbuk, Korea
I. decora	48	DECO	Ohashi 20679	Sendai-shi, Pref. Miyagi, Japan
I. venulosa	—	VENU	Endo 2018	Chingshan to Malun, Taichung, Taiwan
I. pseudotinctoria	16	PSEU	Cho 380	Tonneko, Prov. Cheju, Korea

Table 2. Sequence characteristics of ITS region sequenced from the Far Eastern *Indigofera* species

	ITS 1	5.8S	ITS 2
Length range (bp)	230-240	161	211-213
Number of indels	17	0	10
GC content	50.83-54.74%	51.55%	44.60-48.36%
Ambiguous sites	3	0	1
Transitions (Ts)	10	0	5
Transversions (Tv)	25	0	19
Ts./Tv.	0.40	0.00	0.26

Table 3. Size and G+C contents of ITS 1, ITS 2 and 5.8S coding regions of nuclear ribosomal DNA from Far Eastern *Indigofera* species

Species	Length, bp (GC%)		
	ITS 1	5.8S	ITS 2
Indigofera kirilowii	232 (54.74)	161 (51.55)	212 (48.11)
I. koreana	232 (53.02)	161 (51.55)	212 (46.11)
I. grandiflora	232 (52.59)	161 (51.55)	213 (48.36)
I. decora	230 (53.48)	161 (51.55)	212 (47.64)
I. venulosa	233 (54.08)	161 (51.55)	211 (48.34)
I. pseudotinctoria	240 (50.83)	161 (51.55)	213 (44.60)

No sequence variation was observed for the initial 50 bp (the 3′ end of 18S coding region) and the last 40 bp (the 5′ end of 26S coding region). These regions, the 5.8S coding region and all ambiguous gap sites were removed prior to the calculation of sequence divergence and phylogenetic analysis. Sequence divergences calculated by the Kimura's two-parameter method (1980) from the ITS region (combined ITS 1 and ITS 2) are presented in Table 4.

ITS sequence divergences between species within the subsection *Psilioceratiae* ranged from 0.00 to 1.15%, while the values between ingroup and outgroup ranged from 13.18 to 13.49%. Also, there were no differences between individuals of the same species collected from different localities (KIR1 and KIR2, KOR1 and KOR2; Table 4).

Using the aligned sequences, the total number of variable sites was 77 (18.9%), with 52 (21.3%) in ITS 1 and 35 (16.1%) in ITS 2. The number of variable sites between ingroup and outgroup was 62 (13.4%), with 35 (14.3%) in ITS 1 and 27 (12.4%) in ITS 2. Of the variable sites, moreover, the cladistically informative ones excepting the outgroup were 7 (2.9%; positions 90, 96, 120, 263, 269, 275 and 281) in ITS 1 and 2 (0.9%; positions 556 and 631) in ITS 2. On the basis of the analysis of combined ITS 1 and ITS 2 sequences, a single most parsimonious tree was obtained by using unweighted Wagner parsimony, with a consitency index of 0.967 and a retention index of 0.833 (Fig. 1). The bootstrap value and the number of informative sites for each branch are given in Fig. 1.

Discussion

The ITS regions of nrDNA are shown to be very heterogeneous both in size and nucleotide sequences among various angiosperms (reviewed in Baldwin *et al.* 1995). In this study, the length of ITS 1 (230-240 bp) is longer than the length of ITS 2 (211-213 bp), similar to the most cases of other flowering plants (Baldwin 1992, Suh *et al.* 1993, Kim and Jansen 1994, Sang *et al.* 1995). The G+C content of ITS 1 (50.83-54.74%) in the present study is shown to be similar to those in others (approximately over 50 to 60%), but it is interesting that the G+C content of ITS 2 (44.60-48.36%) is lower than those of most other angiosperms examined (approximately 50-70% in Baldwin *et al.* 1995). The length of the 5.8S coding region (161 bp) is similar to that of other angiosperms (161-164 bp), and the G+C content of the region (51.55%) is also similar to that in others (approximately over 50 to 60%).

There are different evolutionary rates between ITS 1 and ITS 2. ITS 1 changes about one and a half times faster than ITS 2. The sequence divergence values between ingroup taxa (avg. 0.85%) are very low in comparison with those between ingroup and outgroup taxa (avg. 13.35%). The results of ITS sequences show the possibility that ITS sequence data could be applied to study the phylogenetic

Table 4. Sequnce divergences of ITS region (ITS 1 and ITS 2) from the Far Eastern *Indigofera* species derived by Kimura's two-parameter method

	KIR1	KIR2	KOR1	KOR2	GRAN	DECO	VENU	PSEU
KIR1	—	0.000	0.0114	0.0114	0.0115	0.0116	0.0115	0.1342
KIR2	0	—	0.0114	0.0114	0.0115	0.0116	0.0115	0.1342
KOR1	5	5	—	0.0000	0.0000	0.0069	0.0115	0.1339
KOR2	5	5	0	—	0.0000	0.0069	0.0115	0.1339
GRAN	5	5	0	0	—	0.0069	0.0115	0.1318
DECO	6	6	4	4	4	—	0.0023	0.1328
VENU	5	5	5	5	5	2	—	0.1349
PSEU	53	53	53	53	52	53	53	—

Calculated sequence divergences are given above the diagonal and observed numbers of nucleotide differences below the diagonal.

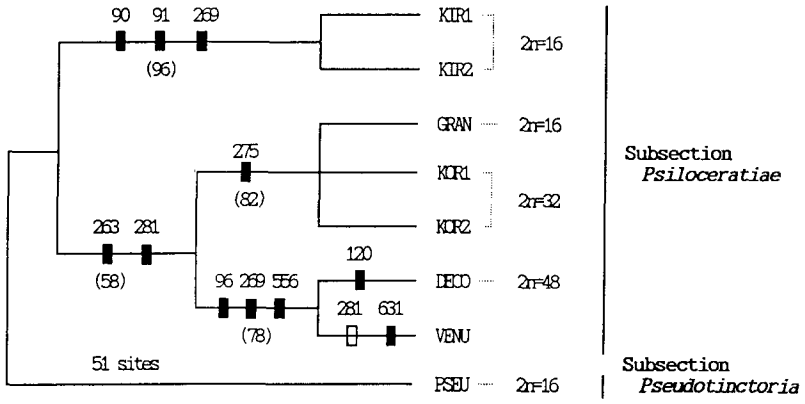

Fig. 1. Single most parsimonious tree of Far Eastern *Indigofera* species based on the sequence of ITS region (nrDNA). Numbers above heavy bars and open bars indicate the positions of nucleotide substitutions and those in parentheses give bootstrap percentages from 100 replications. Consistency Index (CI)= 0.967 and 0.833 excluding uninformative characters, and Retention Index (RI)=0.833.

relationships among the infrageneric groups of *Indigofera*.

The most parsimonious tree from the ITS sequences shows that the subsection *Psiloceratiae* is grouped as a monophyletic group distinctly (Fig. 1) in spite of showing the low resolution owing to the low sequence divergences (Table 4). Firstly, *I. kirilowii* is diverged into showing an independent evolutionary route through 3 site variations of nucleotides from the node of primitive ancestor. And then, the island species of *I. decora* and *I. venulosa* are separated from the Korean Peninsular species of *I. koreana* and *I. grandiflora*. On the other hand, the outgroup taxon, *I. pseudotinctoria* of the subsection *Pseudotinctoria*, showed clearly distinct from the ingroup ones in sequence divergence (Table 4). Moreover, *I. pseudotinctoria* is clearly distinguished from those species of the subsection Psiloceratiae by the distinct morphological characteristics such as having small flowers and inflorescence features. ITS sequences, therefore, support the morphological distinctness of the species from other Far Eastern species (Fig. 1).

I. koreana was described from Korea by Ohwi (1936), and then treated as a synonym of *I. kirilowii* with a question mark by the same author (Ohwi 1965). Although the species certainly resembles *I. kirilowii* in external morphology, it was shown to be clearly distinguished from *I. kirilowii* by flower length and hairiness of the leaflets on the basis of the recent survey in Korea (Cho *et al.* 1997). Furthermore, the distributional range of the species restricted to the southwestern Korea is clearly separated from that of *I. kirilowii* which is distributed in the north and southeastern parts of Korea. ITS sequences support the separation of *I. koreana* from *I. kirilowii* (Fig. 1). *I. grandiflora* has distinct characteristics in having larger flowers and hairiness of the leaflets (Choi 1996). The species is related in external morphology to both species of *I. kirilowii* and *I. koreana* and also resembles *I. decora* in appearance (Choi 1996). However, the species is completely similar to *I. koreana* in the ITS sequences (Table 4). The molecular data suggests that *I. koreana* having tetraploidy diverged from *I. grandiflora* having diploidy chromosome in the southern part of Korea most recently.

The cytological data based on somatic chromosome numbers indicate a distinction among Far Eastern *Indigofera* species. The somatic chromosome numbers are

2n=16 (*I. kirilowii, I. grandiflora* and *I. pseudotinctoria*), 2n=32 (*I. koreana*), and 2n=48 (*I. decora*), and all taxa are euploidy. The results of ITS region sequences support an evolutionary trend from diploidy to polyploidy. We can, therefore, establish a hypothesis on the speciation process among them as follows: The peripheral island hexaploidy and Korean peninsular tetraploidy evolved from the primitive continental diploidy ancestor. In Japan higher polyploid levels have also been recorded on some plant groups which have diploids in other continental regions (reviewed by Hara 1984, 1985). It appears that polyploidization also played an important role on the speciation of the Far Eastern *Indigofera* species.

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Appendix 1. Aligned nucleotide sequences of internal transcribed spacers (ITS1 and ITS2) and 5.8S coding regions on nuclear ribosomal DNA of Far Eastern *Indigofera* species. Abbreviation: DECO=*I. decora*; GRAN=*I.* grandiflora; KIR1, KIR2=*I. Kirilowii*; KOR1, KOR2=*I. Koreana*; PSEU=*I. pseudotinctoria*; VENU=*I. venulosa*; R=A or G; X=non detected site; Y=C or T.

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                                               18S    ITS1
       1                                                             60
KIR1   ACAAGGTTTC CGTAGGTGAA CCTGCGGAAG GATCATTGTT CGATGCCTCA --AGCAATCC
KIR2   .......... .......... .......... .......... .......... --........
KOR1   .......... .......... .......... .......... .......... --........
KOR2   .......... .......... .......... .......... .......... --........
GRAN   .......... .......... .......... .......... .......... --........
DECO   XXXX...... .......... .......... .......... .......... --........
VENU   .......... .......... .......... .......... .......... --........
PSEU   .......... .......... .......... .......... .......... CA.......T

                                                                    120
KIR1   GACTCGTGAA CATGTTTGCT TACTTGGGGC TGGTTTGGCG TGTGAAAACA CGACTGCCTC
KIR2   .......... .......... .......... .......... .......... ..........
KOR1   .......... .......... .........T .......... .......... ..........
KOR2   .......... .......... .........T .......... .......... ..........
GRAN   .......... .......... .........T .......... .......... ..........
DECO   .......... .-........ .........T .....C.... .......... .........R
VENU   .......... .......... .........T .....C.... .......... ..........
PSEU   .......... .T........ ...A....-T ........G. .......... ..C.......

                                                                    180
KIR1   CCCC-GGCGC A-GGAGGCGG CCA-TGCTTC A-TGGCTGTC TCTT-GCCTT TAACAAACCC
KIR2   ....-..... .-........ ...-...... .-........ ....-..... ..........
KOR1   ....-..... .-........ ...-...... .-........ ....-..... ..........
KOR2   ....-..... .-........ ...-...... .-........ ....-..... ..........
GRAN   ....-..... .-.....R.. ...-...... .-........ ....-..... ..........
DECO   ...-T..... .-........ ...-...... .-........ ....-..... ..........
VENU   ...-T..... .-........ ...-...... .-........ ....-..... ..........
PSEU   ....TAAG.. .T.......A ...CAC...G TG...T.... ....A..... ..........

                                                                    240
KIR1   ACGGCGCTGC ATGCGCCAAG GAAATCTAAT TCATTCAATG AGCTC-TTGC CAGCCCGGGA
KIR2   .......... .......... .......... .......... .....-.... ..........
KOR1   .......... ......-... .......... .......... .....-.... ........-.
KOR2   .......... ......-... .......... .......... .....-.... ........-.
GRAN   .......... ......-... .......... .......... .....-Y... ........-.
DECO   .......... ......-... .......... .......... .....C-... ........-.
VENU   .......... .......... .......... .......... .....C-... ........-.
PSEU   C......A.. ......-... .......... .T....G... C.T..CC-CT ...T....-.

                                                ITS1    5.8S
                                                                    300
KIR1   AACGGTGCTG CGCGGGTGGC TTCGTGATC- --TATTAT-C AAAATGACTC TCGGCAACGG
KIR2   .......... .......... .........- --......-. .......... ..........
KOR1   .......... .......... ..T.....A- --..C...T. T......... ..........
KOR2   .......... .......... ..T.....A- --..C...T. T......... ..........
GRAN   .......... .......... ..T.....A- --..C...T. T......... ..........
DECO   .......... .......... ..T......- --..-...T. T......... ..........
VENU   .......... .......... ..T......- --......T. G......... ..........
PSEU   .......... T.......A. G.......AC AT......T. G......... ..........

                                                                    360
KIR1   ATATCTTCGC TCTTGCATCG ATGAAGAACG TAGCGAAATG CGATACTTGG TGTGAATTGC
KIR2   .......... .......... .......... .......... .......... ..........
KOR1   .......... .......... .......... .......... .......... ..........
KOR2   .......... .......... .......... .......... .......... ..........
GRAN   .......... .......... .......... .......... .......... ..........
DECO   .......... .......... .......... .......... .......... ..........
VENU   .......... .......... .......... .......... .......... ..........
PSEU   .......... .......... .......... .......... .......... ..........
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Appendix 1. (Continued)

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                                                                                                  420
KIR1        AGAATCCCGT GAACCATCGA GTCTTTGAAC GCAAGTTGCG CCCAAAGCCA TTAGGTTGAG
KIR2        .......... .......... .......... .......... .......... ..........
KOR1        .......... .......... .......... .......... .......... ..........
KOR2        .......... .......... .......... .......... .......... ..........
GRAN        .......... .......... .......... .......... .......... ..........
DECO        .......... .......... .......... .......... .......... ..........
VENU        .......... .......... .......... .......... .......... ..........
PSEU        .......... .......... .......... .......... .......... ..........

                                  5.8S      ITS2
                                  ----------                                                      480
KIR1        GGCACGCCTG CCTGGGTGTC ACACATCGTT GCTCCAATGC CAATGTCTCC TTTTGGGGTC
KIR2        .......... .......... .......... .......... .......... ..........
KOR1        .......... .......... .......... .......... .......... ..........
KOR2        .......... .......... .......... .......... .......... ..........
GRAN        .......... .......... .......... .......... .......... ..........
DECO        .......... .......... .......... .......... .......... ..........
VENU        .......... .......... .......... .......... .......... ..........
PSEU        .......... .......... .......... A.....ACA .........T .......T..

                                                                                                  540
KIR1        GTTGGGGAGT GTATG-TTGG CTTCCCATGA GCTT-CGTCT CATGGTTGGT TGAAAATCAA
KIR2        .......... .....-.... .......... ....-..... .......... ..........
KOR1        .......... .....-.... .......... ....-..... .......... ..........
KOR2        .......... .....-.... .......... ....-..... .......... ..........
GRAN        .......... .....C.... .......... ....-..... .......... ..........
DECO        .......... .....C.-.. .......... ....-..... .......... ..........
VENU        .......... .....C.-.. .......... ....-..... .......... ..........
PSEU        A......... .A...-.... .......... ....TT.... .......... ..........

                                                                                                  600
KIR1        GTCTGTGGTG GGAGTGCACC GCGATTATTT GGTGGTTGAG TAAAAGCTCG AAACCAATCG
KIR2        .......... .......... .......... .......... .......... ..........
KOR1        .......... .......... .......... .......... .......... ..........
KOR2        .......... .......... .......... .......... .......... ..........
GRAN        .......... .......... .......... .......... .......... ..........
DECO        .......... .....A.... .......... .......... .......... ..........
VENU        .......... .....A.... .......... .......... .......... ..........
PSEU        .......... .-........ ......G..- .......... .........G ...C....

                                                                                                  660
KIR1        TGCGTGGCTC TTGC-AGG-T TTCAGACTTT GTGACCCA-T ATGCATCCTT GATGCTCATA
KIR2        .......... ....-...-. .......... ........-. .......... ..........
KOR1        .......... ....-...-. .......... ........-. .......... ..........
KOR2        .......... ....-...-. .......... ........-. .......... ..........
GRAN        .......... ....-...-. .......... ........-. .......... ..........
DECO        .......... ....-...-. .......... R.......-. .......... ..........
VENU        .......... ....-...-. .......... ........T. -........- ..........
PSEU        ..T...A... ..C.T.A.T. ..TG.....C A.......-. .......TA. .........G

            ITS2   26S
            ----------
                                                                                                  741
KIR1        ACGAGACCCA GGTCAGGCGG GGCTACCGCT GAGTTAAGAC T
KIR2        .......... .......... .......... .......... .
KOR1        .......... .......... .......... .......... .
KOR2        .......... .......... .......... .......... .
GRAN        .......... .......... .......... .......... .
DECO        .......... .......... .......... ......XXXX X
VENU        .......... .......... .......... .......... .
PSEU        G......... .......... .......... .......... .
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