Journal of Plant Biology, January 2008, 51(1) : 20-24

Reappraisal of *Diplolabellum coreanum* (Orchidaceae) as Inferred from Molecular Data

Sang Mi Eum[1], Tomohisa Yukawa[2], Yibo Luo[3], John V. Freudenstein[4], and Nam Sook Lee[5]*

[1,5]Division of Eco Science, Ewha Womans University, Seoul, 120-750, Korea
[2]Tsukuba Botanical Garden, National Science Museum, Amakubo, Tsukuba, 305-0005, Japan
[3]Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China
[4]Department of Evolution, Ecology and Organismal Biology, Ohio State University Herbarium, Columbus, Ohio, USA 43212

The taxonomic treatment of a monotypic genus, *Diplolabellum*, has been disputed by various authors. Maekawa (1935) described the genus on the basis of *Oreorchis coreana* Finet. Because distribution of its plants is very limited, i.e., to Jeju Island of the South Korean Peninsula, it has not been well studied. To reappraise the phylogenetic relationship of *Diplolabellum coreanum* Finet, we obtained ITS, *matK*, *trnT-trnL*, and *trnL-trnF* sequences from several species of *Oreorchis* and related genera. Sequence analysis showed that *D. coreanum* is closely related to one group of *Oreorchis* that consists of *O. patens* Lindle and *O. fargesii* Finet. Therefore, our molecular data support treating the species as *O. coreana* rather than as *D. coreanum*, even though the latter genus is distinct from *Oreorchis* in morphological characters such as callus, pedicel, column, and caudicle.

Keywords: *Diplolabellum coreanum*, ITS, *matK*, phylogenetic relationship, *trnL-trnF*, *trnT-trnL*

Oreorchis coreana (Orchidaceae) was reported as a new species by Finet in 1908. In 1935, however, Maekawa described a new genus, *Diplolabellum*, based on that species, because it lacked the caudicle that is a diagnostic character in *Oreorchis*. Maekawa also distinguished *D. coreanum* from members of *Oreorchis* because plants of the former possess a 3-lobed lip with V-shaped lamellae (rather than two parallel-shaped ones), as well as compressed, rounded pollinia. Since Maekawa's treatment, this species has been reported by some authors as endemic to Korea (WT Lee 1969, 1996; TB Lee, 1984; Paik 1994, 1999), while YN Lee (1996, 2006) has either retained it as a species or treated it as a subspecies of *Oreorchis*. This species is classified as VU (vulnerable) in the IUCN Red List categories (Lee and Choi, 2006). Although Dressler (1993) has listed the genus *Diplolabellum* in the tribe Calypsoeae, he has marked it as questionable. Moreover, Pearce and Cribb (1997) have treated it as an uncertain genus because of the limited study of its morphological characters and the paucity of research materials. Therefore, it is necessary to examine the phylogenetic status of *Diplolabellum* based on other taxonomic characters and by utilizing more specimens.

Nuclear DNA data provide valuable information toward the phylogenetic study of plants. For example, the internal transcribed spacer (ITS) region of nuclear ribosomal DNA is evidence that helps to resolve phylogenetic relationships at different taxonomic levels, especially intraspecific, because of the relatively rapid evolutionary rates of the ITS fragment (Sun et al., 2002). This technique has been widely used for evolutionary research on Orchidaceae members, e.g., *Orchis* (Aceto et al., 1999), *Diseae* (Douzery et al., 1999), and *Dendrochilum acuiferum* (Barkman and Simpson, 2002). Likewise the genes of chloroplast DNA show high variability and good resolution among closely related species (Small et al., 1998; Schönenberger and Conti, 2003; Shaw et al., 2005). Since the *matK* region of chloroplastic DNA was first used in plant phylogenetic studies by Sang et al. (1997), it has also been applied to members of Orchidaceae (Gravendeel et al., 2001; Hidayat et al., 2005; Ponsie et al., 2007). The *trnT-trnL* region has an intergenic spacer between *trnT* (UGU) and the *trnL* (UAA) 5′ exon, while *trnL-trnF* consists of the *trnL* (UAA) intron and another intergenic spacer between the *trnL* (UAA) 3′ exon and *trnF* (GAA) (Taberlet et at., 1991). The *trnL-trnF* sequences are particularly rich in indels, and have been used to determine associations among closely related genera and tribes (Bayer and Starr, 1998). This region has been analyzed for the phylogeny of Orchidaceae (Koehler et al., 2002; Gravendeel et al., 2004) and other genera (Yang and Pak, 2006).

The objective of our study was to examine the phylogenetic status of *Diplolabellum coreanum* and the endemism of the genus *Diplolabellum*. Therefore, we conducted DNA sequence analysis of the ITS, *matK*, *trnT-trnL* spacer, and *trnL-trnF* spacer regions to examine relationships at the intraspecific level.

MATERIALS AND METHODS

Plant Material

Appendix 1 lists the taxa sampled, and includes authorities, vouchers, and GenBank accession numbers for DNA sequences from the nuclear ribosomal DNA ITS, as well as the chloroplast *matK*, *trnT-L* spacer, and *trnL-F* spacer region. *Cremastra aphylla*, *Corallorhiza trifida*, and *Aplectrum hyemale* served as the outgroup because they belong to the same tribe, Calypsoeae. Among the plant materials studied here, those of *Oreorchis* sp1 and 2 could not be exactly identified at the specific rank based on the dried specimens of their fruiting stage, but could be identified as distinct members of that genus.

*Corresponding author; fax +82-2-3277-2385
e-mail namsook@ewha.ac.kr

DNA Extraction and PCR Amplification

Total genomic DNA was extracted from fresh or silica-dried leaves using the DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The ITS region of the nuclear ribosomal DNA was amplified as a single fragment containing ITS1, the 5.8S gene, or ITS2 (primers AB101, AB102; Douzery et al., 1999). The *matK* region was amplified using three pairs of primer sets – OMAT1F-OMAT2R, OMAT396F-OMAT3R, and OMAT841F-*trn*K2R (Inoue and Yukawa, 2002). Finally, the *trnT-trnL* and *trnL-trnF* spacer plastid DNA regions were amplified as two parts - the *trnT-trnL* spacer and the *trnL* intron plus *trnL-trnF* spacer, using six primers described by Taberlet et al. (1991). Primer pair trn-a and trn-b was used for PCR-amplification and sequencing of the *trnT-trnL* region, while the entire *trnL* intron/*trnL-trnF* spacer region was amplified with trn-c, -d, -e, and -f primers. AccuPower PCR Premix (Bioneer Inc., Korea) was used for the PCR reactions, and those products were cleaned with the AccuPrep PCR Purification Kit (Bioneer Inc.) DNA sequences were produced with the BigDye™ Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and sequence reactions were run on a Base Station sequencer (MJ Research, USA). The primers for those sequencing reactions were the same as for the amplification process.

Data Analysis

Sequences were aligned by ClustalX software (Thompson et al., 1997), followed by manual adjustments to those alignments according to the principles of Kelchner and Clark (1997).

Phylogenetic analysis using maximum parsimony (MP) criteria was performed with PAUP* 4.0b10 (Swofford, 2002) Under MP criteria, heuristic searches were executed with 1000 random addition replicates and TBR branch-swapping, MulTrees option saving, and swapping to completion on all optimal trees found during each replicate. MP bootstrap proportions were determined from 1000 non-parametric bootstrap replicates performed in PAUP*, using the heuristic search option with 100 random addition sequence replicates per bootstrap replicate and TBR branch-swapping on all optimal trees (Felsenstein, 1985). Characters were equally weighted in the analysis. Finally, the consistency index (CI) and retention index (RI) were calculated.

RESULTS

ITS and Chloroplast Sequence Data

Table 1 provides a comparison of sequence attributes found here. Although the ITS2 region yielded the highest percentage (13.6%) of parsimony informative (PI) characters, the *trnL-trnF* spacer region entailed the largest total characters of PI (59). The ITS region was approximately three times more variable than the chloroplast regions, with 11.4% of the sites exhibiting parsimony informative variation, versus only 3.5% from the total chloroplast data. Among the three chloroplast regions, the percentage of PI in relation to the per-region number of characters included in our analysis was lowest (2.6%) for *matK*.

Table 1. Variation in sequences for the genomic regions and subregions for all exemplars. The data include the number of accessions for this study (N), the ranges in length of the generated sequences (total length), the length of the aligned sequences (aligned length), the number of constant characters (constant), the number of parsimony informative characters (PI), the percentage of PI in relation to the number of characters included in analysis per region (%PI), and the percentage of PI per region in relation to the total number of PI (PI / total PI).

Genomic regions	N	Total length	Aligned length	Constant	PI	%PI
Nuclear						
ITS1	22	323-325	326	273	43	13.2
ITS2	22	160-162	162	135	22	13.6
5.8S	22	158-159	159	143	11	6.9
Total	22	640-644	647	553	74	11.4
Chloroplast						
matK	16	1707-1725	1726	1615	45	2.6
trnT-trnL	20	461-545	655	583	42	6.4
trnL-trnF	16	1141-1341	1480	137	59	4.0
Total	16	3338-3593	3861	3487	135	3.5
Combined	16	3980-4233	4508	4042	182	4.0

Table 2. Summary of tree statistics for the parsimony analyses of the four genomic regions in independent and combined analyses. The data include the length of the most parsimonious tree (tree length), the number of most parsimonious trees found in the analyses (MPT), consistency index (CI), retention index (RI), and rescaled consistency index (RC).

	Tree length	MPT	CI	RI	RC
Nuclear					
ITS	107	2	0.94	0.97	0.92
Chloroplast					
matK	125	1	0.94	0.95	0.89
trnT-trnL	96	4	0.85	0.91	0.77
trnL-trnF	241	2	0.91	0.87	0.79
Total	461	1	0.90	0.89	0.80
Combined	566	1	0.91	0.90	0.82

Phylogeny Analyses

Tree statistics were developed for the most parsimonious reconstructions obtained from inferences based on individual partitions and the combined data set (Table 2). Among our analyses, the numbers of most parsimonious tree (MPT) ranged narrowly, from one to four.

The strict consensus trees (Fig. 1) based on ITS and the chloroplast region showed a similar topology, making the bootstrap values of the combined tree (Fig. 2) relatively higher than those previous trees (separate analyses of the three chloroplast regions are not shown). In the strict consensus tree based on combined data, the genus *Oreorchis* was divided into two groups, and *Diplolabellum coreanum* was a sister to *O. patens* and *O. fargesii*. Each group of *Oreorchis* and *Corallorhiza* was supported with 100% bootstrap

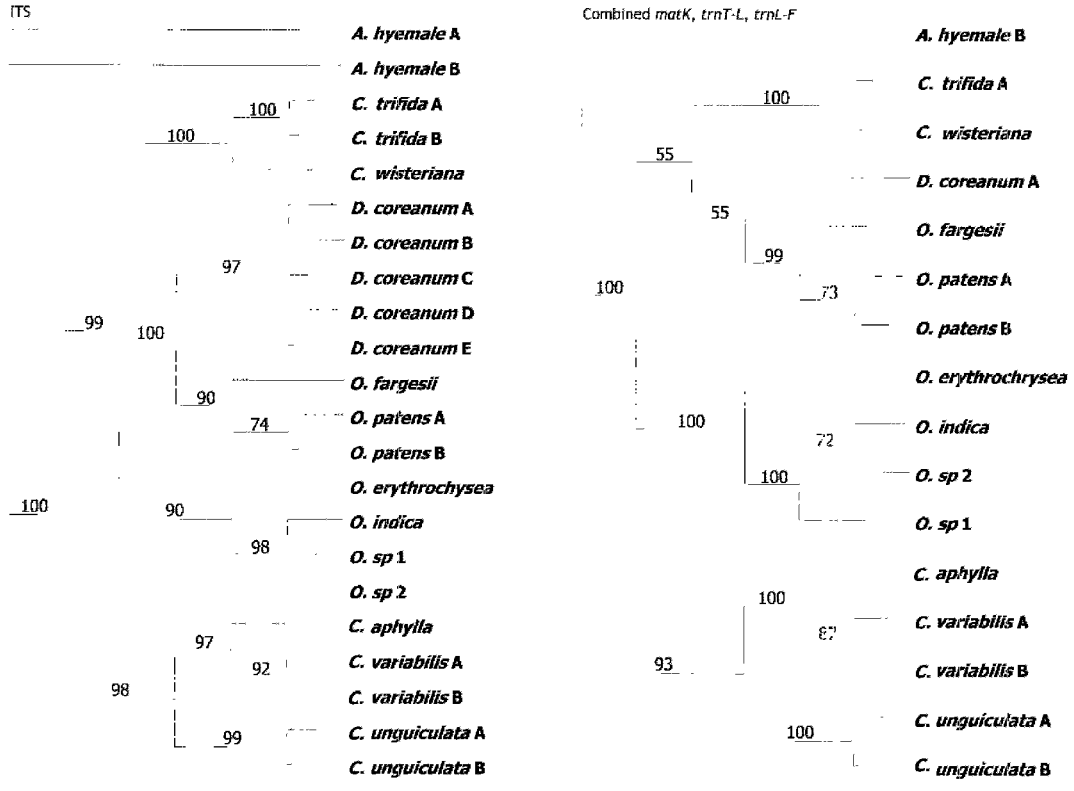

Figure 1. Strict consensus tree based on ITS and chloroplast region sequences. Numbers at branches indicate bootstrap values from 100 replicates of parsimony analysis.

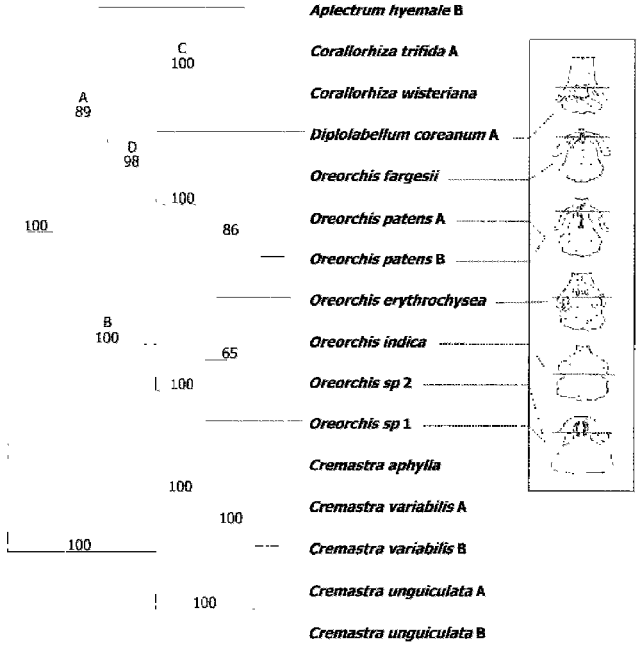

Figure 2. Strict consensus tree based on combined ITS, *matK*, *trnT-L*, and *trnL-F* sequence data. Numbers at branches indicate bootstrap values from 100 replicates of parsimony analysis. *Illustrations of callus position on lip are re-drawn from those of Pearce and Cribb (1997).

values. Inference from the combined data set demonstrated that Clades A and B were the monophyletic sister group. Within Clade A, the genus *Oreorchis* containing *D. coreanum* (D) and *Corallorhiza* (C) was monophyletic. Inferences from the position of the *D. coreanum* and *Oreorchis* species also supported *D. coreanum* as a member of *Oreorchis*.

DISCUSSION

Using phylogenetic inferences from the variation in sequences of one nuclear and three chloroplast loci, we have been able to resolve lineages for *Diplolabellum coreanum* that are congruent with those first reported by Finet (1908).

In our ITS sequence analysis, *D. coreanum* is sister to the *Oreorchis* groups, comprised of *O. patens* and *O. fargesii*, with 100% bootstrap values. That is, *O. patens* groups first with *O. fargesii*, and that clade then forms a clade with *D. coreanum*. This suggests that *D. coreanum* is distinct from *O. patens*, although the former had been treated as *O. patens* (Lindl.) Lindley var. *coreana* (Finet) Y. Lee & K. Lee (Lee, 2006). The remaining *Oreorchis* taxa -- *O. erythrochrysea*, *O. indica*, *O. sp1*, and *O. sp2* – fall into the other group of *Oreorchis*, with 90% bootstrap values. The ITS tree shows that the clade of *D. coreanum* - *O. patens* - *O. fargesii* is nested within the other clade of *O. erythrochrysea* - *O. indica* - *O. sp1* - *O. sp2*, which means that *D. coreanum* cannot be a genus distinct from *Oreorchis*.

The topology of the phylogenetic tree for *matK* and *trnT-L* is very similar to that of the ITS tree, but the *trnL-trnF* tree differs from the *matK*, *trnT-L*, and ITS trees (data not shown). Our *trnL-trnF* analysis demonstrates that *D. coreanum* forms a distinct clade from the other *Oreorchis* and *Corallorhiza* taxa. Although the *trnL-trnF* region does not represent the

lowest percentage of parsimony informative characters per region, the tree based on *trnL-trnF* data is of low resolution, having poor bootstrap values. The topology of the strict consensus tree based on the *trnL-trnF* region is not consistent with the tree topology based on the combined data. Likewise, in each of the ITS, *matK*, and *trnT-trnL* parsimonious analyses, *Oreorchis* is divided into two distinct groups; i.e., *D. coreanum* forms one clade with *O. fargesii* and *O. patens*, and this group then forms a clade with the other group of *O. erythrochrysea* - *O. indica* - *O. sp*1 - *O. sp*2. The phylogenetic trees do not support that *Diplolabellum* is a distinct genus from *Oreorchis*. Moreover, Clade C (Fig. 2) suggests that the genus *Corallorhiza* could be included in the genus *Oreorchis* along with *Diplolabellum*. Previous ITS data suggested that *Oreorchis* is more closely related with *Corallorhiza* rather than *Aplectrum* and *Cremastra* (Senyo et al., 2000). For the taxonomic rank of *Corallorhiza* needs to be more studied in the future.

In these sequence analyses, the species of *Oreorchis* always separate into two groups: one consisting of *O. patens* and *O. fargesii*; the other, of *O. erythrochrysea*, *O. indica*, *O. sp*1, and *O. sp*2. We note that the latter two could not be exactly identified here based on dried specimens because they were collected during their fruiting stage. Nonetheless, the *O. sp*1 and 2 accessions may, in fact, be *O. nana* and *O. indica*, respectively, based on their sequences and habitats.

The two groups within the genus *Oreorchis* also are supported by morphological and ecological characters. Positioning and morphology of the lamellae may be associated with geographical elevation. For example, *O. patens*, *O. fargesii* (Su, 2000), and *O. coreana* (=*D. coreanum*), all sequenced in this study, are distributed in damp habitats ranging from 650 m to 2800 m a.s.l. (Group 1). Their lamellae are elongated from the base of the lip to below the reinsertion point of the lateral lobe. In contrast, our accessions of *O. erythrochrysea*, *O. indica*, and *O. nana* (Group 2) are distributed from 2500 m to 4000 m in the alpine zones of China, Tibet, India, and Bhutan (Chen, 1999; Wu and Peter, 2002). They have no or reduced lamellae, which are not elongated below the reinsertion point of the lateral lobes. This relationship between callus position and elevation is a trend consistent with descriptions of the other species of *Oreorchis*. The first group corresponds to *O. patens* var. *gracilis*, *O. micrantha*, and *O. bilamellata* from China (Wu and Peter, 2002); the second, to *O. porphyranthes* from Nepal (Tuyama, 1975), and *O. parvula* and *O. oligantha* from China (Wu and Peter, 2002, Li et al., 2005)

Even though *Diplolabellum* was distinguished from *Oreorchis* by Maekawa (1935) based on the presence of a lip with a V-shaped lamella and compressed, rounded pollinia, but the absence of a caudicle and viscidium, *D. coreanum* is similar to *Oreorchis* in other morphological characters, such as the shape of its pseudobulbs, leaves, and inflorescence, and the number of pollinia. In their revision of *Oreorchis*, Pearce and Cribb (1997) mentioned that, although the usual form of the lamella on the lip in *Oreorchis* is bilamellate, there is considerable variation within the genus. That is, they did not consider the shape of the lamella to be a good diagnostic character for separating *D. coreanum* from other members of *Oreorchis*.

Sometimes taxonomic status based on morphological characters is not supported by molecular data. In the case of *Kitigorchis itoana* F. Maek., which has been placed in the monotypic genus *Kitigorchis* Maekawa, the molecular data suggest that it also belongs in *Oreorchis* and, in fact, is conspecific with *O. indica* (Yukawa et al., 2003).

As discussed above, our molecular data of the ITS and chloroplastic DNA regions do not support the endemism of the genus *Diplolabellum*. Instead, these data suggest that the placement of *D. coreanum* within the genus *Oreorchis* and the species status of *O. coreana* are deserved and valid.

ACKNOWLEDGEMENTS

This research was supported by grants (No. 052-061-025, No. 05002-0067-0) from the Core Environmental Technology Development Project for Next Generation funded by the Ministry of Environment of the Korean Government. We thank Jin Ohk Kim for lab work and Dr. Daniel J. Crawford of the University of Kansas for critical reading of this manuscript.

Received April 10, 2007; accepted November 10, 2007.

LITERATURE CITED

Aceto S, Caputo P, Cozzolino S, Gaudio L, Moretti A (1999) Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: Morphological gaps and molecular continuity. Mol Phylog Evol 13: 67-76

Bayer RJ, Starr JR (1998) Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the *trnL* intron and *trnL/trnF* intergenic space. Ann MO Bot Gard 85: 242-256

Barkman T, Simpson BB (2002) Hybrid origin and parentage of *Dendrochilum acuiferum* (Orchidaceae) inferred in a phylogenetic context using nuclear and plastid DNA sequence data. Syst Bot 272: 209-220

Chen SC (1999) Flora Reipublicae Popularis Sinicae, 18:160. Institutum Botanicum Academiae, Peijing. [In Chinese]

Dressler RL (1993) Phylogeny and Classification of the Orchid Family. Dioscorides Press, Portland, OO, USA

Douzery EJ, Pridgeon AM, Kores P, Linder HP, Kurzweil H, Chase MW (1999) Molecular phylogenetics of *Diseae* (Orchidaceae): A contribution from nuclear ribosomal ITS sequences. Amer J Bot 86: 887-899

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791

Finet EA (1908) *Oreorchis coreana* sp. nov. Bull Soc Bot Fr 55: 337

Gravendeel B, Chase MW, Vogel EF, Roos MC, Mes THM, Bachmann K (2001) Molecular phylogeny of *Coelogyne* (Epidendroideae; Orchidaceae) based on plastid RFLPS, *matK*, and nuclear ribosomal ITS sequences: Evidence for polyphyly. Amer J Bot 88: 1915-1927

Gravendeel B, Eurlings MCM, Berg CV, Crib P (2004) Phylogeny of Pleione (Orchidaceae) and parentage analysis of its wild hybrids based on plastid and nuclear ribosomal ITS sequences and morphological data. Syst Bot 29: 50-63

Hidayat T, Yukawa T, Ito M (2005) Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): Insights from plastid *matK* and nuclear ribosomal ITS sequences. J Plant Res 118: 271-284

Inoue K, Yukawa T (2002) A new species of *Yoania* (Orchidaceae) from southern Nagano, central Japan. Acta Phytotax Geobot 53: 107-114

Kelchner SA, Clark LG (1997) Molecular evolution and phylogenetic utility of the *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). Mol Phylog Evol 8: 385-397

Koehler S, Williams NH, Whitten WM, CE Amaral M (2002) Phylogeny of the *Bifrenaria* (Orchidaceae) complex based on morphology and sequence data from nuclear rDNA internal transcribed spacers (ITS) and chloroplast trnL-trnF region. Intl J Plant Sci 163: 1055-1066

Lee JS, Choi BH (2006) Distribution and red data of wild orchids in the Korean Peninsula. Kor J Plant Taxon 36: 335-360

Lee TB (1984) Outline of Korean endemic plants and their distribution. Kor J Plant Taxon 14: 21-32 [In Korean]

Lee WT (1969) A discussion on Korean endemic genera plants. Kor J Plant Taxon 1: 15-21 [In Korean]

Lee WT (1996) Lineamenta Florae Koreae. Academy Press, Seoul [In Korean]

Lee YN (1996) Flora of Korea. Gyohaksa, Seoul [In Korean]

Lee YN (2006) Flora of Korea. Gyohaksa, Seoul [In Korean]

Li P, Siyuan T, Li D, Yong K, Perner H, Luo Y (2005) Temperate paradise. Orchid Rev 113: 154-159

Maekawa F (1935) *Diplolabellum coreanum*. J Jpn Bot 11: 305 [In Japanese]

Paik WK (1994) Substance of the Korean endemic plants and investigation of their distribution. Bull KACN Ser 13: 5-84 [In Korean]

Paik WK (1999) The status of endemic plants in Korea and our tasks in the 21st century. Kor J Plant Taxon 29: 263-273 [In Korean]

Pearce N, Cribb PJ (1997) A revision of the genus *Oreorchis* (Orchidaceae). Edinburgh J Bot 54: 289-328

Ponsie M, Mitchell A, Edwards T, Johnson S (2007) Phylogeny of *Bonatea* (Orchidaceae: Habenariinae) based on molecular and morphological data. Plant Syst Evol 263:263-268.

Sang T, Crawford DJ, Stuessy TF (1997) Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). Amer J Bot 84: 1120-1136

Schönenberger J, Conti E (2003) Molecular phylogeny and floral evolution of Penaeaceae, Oliniaceae, Rhynchocalycaceae and Alzateaceae (Myrtales). Amer J Bot 90: 293-309

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Amer J Bot 92: 142-166

Small RL, Ryburn JA, Cronn RC, Seelanan T, Wendel JF (1998) The tortoise and the hare I: Choosing between noncoding plastome and nuclear Adh sequences for phylogenetic reconstruction in a recently diverged plant group. Amer J Bot 85: 1301-1315

Senyo DM, Freudenstein JV (2000) A molecular phylogeny of *Corallorhiza* (Orchidaceae) and related genera. 2000 Botany Conference, Portland, OR, USA, 6-10 August, 2000. (Abstract)

Su, HJ (2000) Flora of Taiwan, 2nd Ed, Vol 5. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan

Sun K, Chen X, Ma R, Li C, Wang Q, Ge S (2002) Molecular phylogenetics of *Hippophae* L. (Elaeagnaceae) based on the internal transcribed spacer (ITS) sequences of nrDNA. Plant Syst Evol 235: 121-134

Swofford DL (2002) PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. Sinauer, Sunderland, MA, USA

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17: 1104-1109

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876-4882

Tuyama T (1975) A new species of Orchidaceae, *Oreorchis porphyranthes*, from Nepal. J Jpn Bot 50: 69

Wu Z, Peter HR (2002) Flora of China. http://flora.huh.harvard.edu/china

Yang JY, Pak JH (2006) Phylogeny of Korean Rubus (Rosaceae) Based on ITS (nrDNA) and trnL/F Intergenic Region (cpDNA). J Plant Biology 49: 44-54

Yukawa T, Chung SW, Luo Y, Peng CI, Momohara A, Setoguchi H (2003) Reappraisal of *Kitigorchis* (Orchidaceae). Bot Bull Acad Sin 44: 345-351

Appendix 1. Species, voucher, and locality of samples used in this study. GenBank sequence accessions are ordered as ITS, *matK*, *trnT-L*, and *trnL-F*. Sequences obtained in this study are in boldface. The sequences of the species marked with asterisks were obtained from the previous work by Yukawa *et al*. (2003).

Aplectrum hyemale Torr. A, *Chase O-104 (K)*, AY008468 (ITS); *A. hyemale* Torr. B, EWU-Roh0301, USA-Michigan, **EU266404** (ITS), **EU266416** (*matK*), **EU266423** (*trnT-L*), **EU266434** (*trnL-F*).

Corallorhiza trifida Chatel. A*, Berdutenko s. n. (TNS), Rusia-Commander Isls.; *C. trifida* Chatel. B, EWU-UHAan0301, Korea-Mt. Baekdu, **EU266405** (ITS), **EU266424** (*trnT-L*); *C. wisteriana* Conrad.*, Carlsward s. n (TNS), USA-Florida.

Cremastra aphylla T. Yukawa*, Yukawa98-71 (TNS), Japan-Honshu; *C. unguiculata* (Finet) Finet A*, Kurashige s. n.(TNS), Japan-Honshu; *C. unguiculata* (Finet) Finet B, EWU-NSLee0406, Korea-Jeju Is., **EU266415** (ITS), **EU266422** (*matK*), **EU266433** (*trnT-L*), **EU266440** (*trnL-F*); *C. variabilis* Blume A, EWU-SMEum0302, Korea-MT. Naejang, **EU266414** (ITS), **EU266421** (*matK*), **EU266432** (*trnT-L*), **EU266439** (*trnL-F*); *C. variabilis* Blume B*, Tanaka s. n.(TNS), Japan-Kyushu.

Diplolabellum coreanum Finet A, EWU-NSLee0301, Korea-Jeju Is., **EU266406** (ITS), **EU266417** (*matK*), **EU266425** (*trnT-L*), **EU266435** (*trnL-F*); *D. coreanum* Finet B, EWU-NSLee0302, Korea-Jeju Is., **EU266407** (ITS); *D. coreanum* Finet C, EWU-NSLee0303, Korea-Jeju Is., **EU266408** (ITS), **EU266426** (*trnT-L*); *D. coreanum* Finet D, EWU-NSLee0304, Korea-Jeju Is., **EU266409** (ITS), **EU266427** (*trnT-L*); *D. coreanum* Finet E, EWU-NSLee0305, Korea-Jeju Is., **EU266410** (ITS), **EU266428** (*trnT-L*).

Oreorchis erythrochrysea Hand.-Mazz.* Luo & Sun 766 (PE), China-Yunnan; *O. fargesii* Finet*, Luo 735 (PE), China-Hunan; *O. indica* Hook. f.*, Yukawa01-1 (TNS), Japan-Honshu; *O. patens* (Lindl.) Lindl. A*, Kita s. n. (TNS), Japan-Honshu; *O. patens* (Lindl.) Lindl. B, EWU-SMEum0301, Korea-Mt. Sobaek, **EU266411** (ITS), **EU266418** (*matK*), **EU266429** (*trnT-L*), **EU266436** (*trnL-F*); *O. sp1*, Luo Yi-bo 721 (PE), China-Mt. Gonggashan, **EU266412** (ITS), **EU266419** (*matK*), **EU266430** (*trnT-L*), **EU266437** (*trnL-F*); *O. sp2*, Luo Yi-bo 665 (PE), China-Miyaluo, **EU266413** (ITS), **EU266420** (*matK*), **EU266431**(*trnT-L*), **EU266438** (*trnL-F*).