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ORIGINAL RESEARCH

Two New Species of the Genus *Liparis* (Orchidaceae) from Korea Based on Morphological and Molecular Data

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Abstract Two new species of *Liparis* Rich. (Orchidaceae) from Korea are described: *Liparis yongnoana* and *Liparis pterosepala*. *Liparis yongnoana* is similar to plants called as *L. japonica* and *L. makinoana* in having an anther cap with a beaked apex and a weakly reflexed labellum. However, *L. yongnoana* can be distinguished from them by a presence of a narrowly elliptic line on a labellum, a less emarginated apex of a more reflexed labellum, a short column, and a few flowers. *L. pterosepala* is similar to *Liparis kumokiri*, *Liparis koreojaponica*, and *Liparis fujisanensis* in having an anther cap with a mucronate apex and an excessively reflexed labellum. But *L. pterosepala* can be distinguished from the three similar taxa by its wide sepals and its early flowering time. Based on the molecular data using nuclear internal transcribed spacer (ITS) and cpDNA regions (*matK*, *trnS-trnG*, *trnL* with *trnL-trnF*), *L. yongnoana* has five autapomorphic substitutions in ITS region and four substitutions and one deletion in cpDNA. Another new taxon, *L. pterosepala*, has one autapomorphy in ITS and cpDNA regions, respectively. A molecular phylogeny also indicates that *L. yongnoana* is close to plants called as *L. japonica* and *L. makinoana*, and *L. pterosepala* is close to *L. kumokiri*, *L. koreojaponica*, and *L. fujisanensis*.

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Introduction

The genus *Liparis* Rich. (Orchidaceae) comprises 400 species that occur in both tropical and temperate regions around the world (Garay and Romero-Gonzalez 1999). Distinguishing characteristics of the genus are racemose inflorescence, a small bract subtending each flower, and similar shaped sepals and lateral petals, and also, usually regarded as having naked pollinia, a relatively long column and resupinate flowers, especially as compared to its related genus *Malaxis* (Li 1978; Satake et al. 1982; Ohwi 1984; Cribb and Govaerts 2005).

Eight species of *Liparis* were known to be in Korea: *L. auriculata* Blume ex Miq., *L. japonica* (Miq.) Maxim., *L. makinoana* Schltr., *L. kumokiri* F. Maek., *L. koreana* (Nakai) Nakai ex W.T. Lee (Lee 1996), *L. krameri* Franch. and Sav. and *L. nervosa* (Thunb.) Lindl. (Lee 2006), and *L. koreojaponica* Tsutsumi et al. (2008b), except *L. fujisanensis* F. Maekawa ex F. Konta and S. Matsumoto (Kim and Kim 1986), as which *L. koreojaponica* was misidentified (not published). Four out of the Korean *Liparis* taxa occur on Jeju Island. Jeju Island is located in the most southern part of the Korea, close to Kyushu, Japan, and has many endemic plants including 18 of 64 orchid taxa (Lee and Choi 2006). Based on the systematic of *Liparis* dividing 19 sections by Garay and Romero-Gonzalez (1999), *L. nervosa* belongs to the section *Elatae*, defined by a cane or stem like pseudobulb developed at anthesis and several ribbed leaves born in apical part of the pseudobulb. The others are in the section *Liparis*

characterized by the undeveloped pseudobulb at anthesis and two subfleshy non-ribbed leaves borne in the apical part of the pseudobulb.

Recently, samples of two undescribed taxa of section *Liparis* were collected from Jeju Island. One is similar to plants treated as *L. makinoana* and *L. japonica*, in having an anther cap with a beaked apex and a weakly reflexed labellum. And the other is similar to *L. kumokiri* and *L. koreojaponica* in having an anther cap with a mucronate apex and an excessively reflexed labellum. To clarify taxonomic treatments of the putative new taxa, a molecular phylogeny and a comparative morphology of closely related taxa with the new plants within the section *Liparis* in Korea and Japan (Tsutsumi et al. 2007, 2008a, b) were studied. Based on these results, the two new plants of *Liparis* on Jeju Island of Korea are herein described as new species.

Materials and Methods

Materials

The GenBank accession numbers and the sources of plant materials are listed in Table 1 and the Appendix. It is

Table 1 The sequence accessions from GenBank are ordered as ITS, *matK*, *trnS-trnG*, *trnL*, and *trnL-trnF* of section *Liparis* and related taxa studied

Taxa	DNA voucher number	GenBank accession number			
		ITS	*matK*	*trnS-trnG*	*trnL* and *trnL-trnF*
L. fujisanensis	J8	EU024936	EU024937	EU024938	EU024939
	L18	AB289461	AB289487	AB289511	AB289525
L. japonica	K2	EU017406	EU017429	EU017451	EU017473
	K3	EU017407	EU017430	EU017452	EU017474
L. koreojaponica	K14	EU017418	EU017440	EU017462	EU017485
	K15	EU017419	EU017441	EU017463	EU017486
	K16	EU017420	EU017442	EU017464	EU017487
	K17	EU017421	EU017443	EU017465	EU017488
	K18	EU017422	EU017444	EU017466	EU017489
	K19	EU017423	EU017445	EU017467	EU017490
	L3	AB289566	AB289492	AB299514	AB289528
	L4	AB289567	AB289493	AB299515	AB289529
L. kumokiri	K7	EU017411	Not available	Not available	EU017478
	K8	EU017412	EU017434	EU017456	EU017479
	K9	EU017413	EU017435	EU017457	EU017480
	K10	EU017414	EU017436	EU017458	EU017481
	K11	EU017415	EU017437	EU017459	EU017482
	K12	EU017416	EU017438	EU017460	EU017483
	K13	EU017417	EU017439	EU017461	EU017484
	Y99-60	AB289472	AB289499	AB289518	AB289532
	L16	AB289473	AB289498	AB28519	AB289533
L. pterosepala	K20	EU017424	EU017446	EU017468	EU017491
	K21	EU017425	EU017447	EU017469	EU017492
	K22	EU017426	EU017448	EU017470	EU017493
L. liliifolia	NA	EU017427	EU017449	EU017471	EU017494
L. makinoana	K1	EU017405	EU017428	EU017450	EU017472
L. purpureovittata	L24	AB289480	AB289506	AB289522	AB289536
	L27	AB289481	AB289507	AB289523	AB289537
L. Type 1	L6	EU017405	EU017428	EU017450	EU017472
L. Type 2	L2	AB289463	AB289489	AB289512	AB289526
	L7	AB289462	Not available	Not available	AB289488
L. yongnoana	K4	EU017408	EU017431	EU017453	EU017475
	K5	EU017409	EU017432	EU017454	EU017476
	K6	EU017410	EU017433	EU017455	EU017477

Table 2 Comparative morphological characters for two new species and six related taxa of the genus *Liparis*

		Japonica clade				Kumokiri clade				
		L. yongnoana (5)	*L. makinoana* (15)	*L. koreana* (1)	*L. japonica* (15)	*L. pterosepala* (6)	*L. kumokiri* (20)	*L. koreojaponica* (20)	*L .fujisanensis* (4)	*L. purpureovittata* (4)
Pseudobulb length (mm)		10.0–5.0	8.0–12.0	5.0–10.0	5.0–12.0	20.0	15.0–20.0	10.0–20.0	10.0–15.0	10–20.0
Leaf	Length (cm)	5.0–7.0	5.0–12.0	6.0–13.0	6.0–12.0	8.0–10.0	5.0–15.0	10.0–20.0	1.5–5.0	5.0–13.0
	Width (cm)	3.0–4.0	2.5–7.0	1.8–3.3	2.5–6.0	4.0–5.0	2.5–5.0	2.0–6.0	0.7–2.3	2.0–5.0
	Margin	almost undulate	almost entire	almost entire	almost entire	undulate	almost undulate	undulate	undulate	entire undulate
Scape length (cm)		9.0–10	15–30	10–18	12–50	12–14	15–30	15–35	3–10	10–25
Length ratio of floral portion/scape		1/3	1/2–2/3	1/2	1/2	1/4	1/5–1/4	1/3–1/4	1/2–2/3	1/3
Ratio of lateral sepal width/length		1/3	1/5	Ca. 1/4	1/4	1/2	1/3–1/4	1/3–1/4	1/3	1/2–1/3
Angle between the lateral sepals and labellum (°)		45–60	50–60	not available	40–55	85–95	70–100	70–90	90–100	45–60
Labellum	Color	green + dark purple at center	light green, light purple	greenish purple	light–dark purple	dark purple	light green, light purple	light green, light purple	dark–light purple	yellowish dull green + purplish or black at base
	Narrowly elliptic line	distinct	none	none	none	none	none	none	none	none
	Apex shape	emarginate + apiculate tip	cuspidate	cuspidate	cuspidate	emarginate + apiculate tip	emarginate	emarginate + apiculate tip	emarginate + apiculate tip	emarginate
	Length (mm)	9.0–10.0	12.0–18.0	8.0–11.0	6.0–8.0	6.5–8.0	8.2–12.0	9.0–11.0	10.0–12.00	8.0–9.0
	Width (mm)	7.0–8.0	8.0–15.0	5.0–8.0	4.0–5.0	4.0–6.0	6.0–7.0	6.0–8.0	5.0–7.0	6.0–7.0
	Reflex angle (°)[a]	80–90	70–80	Ca. 70	60–70	100–110	130–140	100–110	90–100	125–135
Column length (mm)		3.0–3.5	3.0–5.0	4.0–5.0	2.5–4.0	4.0–5.0	3.0–4.5	5.0–6.0	4.0–5.0	4.0–5.0
Apex of anther cap		beaked	beaked	beaked	beaked	mucronate	mucronate	mucronate	mucronate	mucronate
Flowering period		June–July	April–June	June–July	May–July	May–June	June–July	June–July	June–July	July

Taxa are listed by their specific epithets, and the figures in parenthesis are number of specimens examined

[a] The reflexed angles of labellum measured are shown in Figs. 1 and 2

supposed that misidentifications occur in plants widely called as *L. makinoana* or *L. japonica* (Tsutsumi and Yukawa 2008). Therefore, the plants have been called as *L. makinoana* and *L. japonica* in Korea (Chung 1957; Kim and Kim 1986; Lee 2006; Lee and Choi 2006) were treated as *L. makinoana* and *L. japonica* tentatively. *Liparis auriculata* and *L. krameri* that are phylogenetically distant from the other species (Tsutsumi et al. 2008a) were excluded. All voucher specimens were deposited at EWH or TNS.

Morphology

Fourteen morphological characters were recorded from herbarium specimens and living plants of the two undescribed entities from Jeju Island of Korea, and six presumably closely related taxa, *L. japonica*, *L. koreojaponica*, *L. kumokiri*, and *L. makinoana* from Korea and *L. fujisanensis* and *L. purpureovittata* from Japan. In *L. koreana*, which was recorded from northern Korea (Nakai 1931), the type of specimen and the description were included to the analysis (Table 2). The angle between the lateral sepals and labellum and the reflexed angle of the labellum are illustrated in Figs. 1 and 2.

DNA Extraction, PCR Amplification, and Sequencing

DNA sequences were analyzed from all taxa from Korea included in the morphological analysis except *L. koreana*, as well as the American species, *L. liliifolia* (L.) A. Rich. ex Lindl. of section *Liparis*. Leaf samples for DNA extraction were collected in the field. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) amplifications, purifications, and DNA sequencing strategies followed White et al. (1990). The amplification reaction mixture was prepared using TaKaRa Ex Taq® DNA polymerase (Takara Bio, Japan). The internal tran-

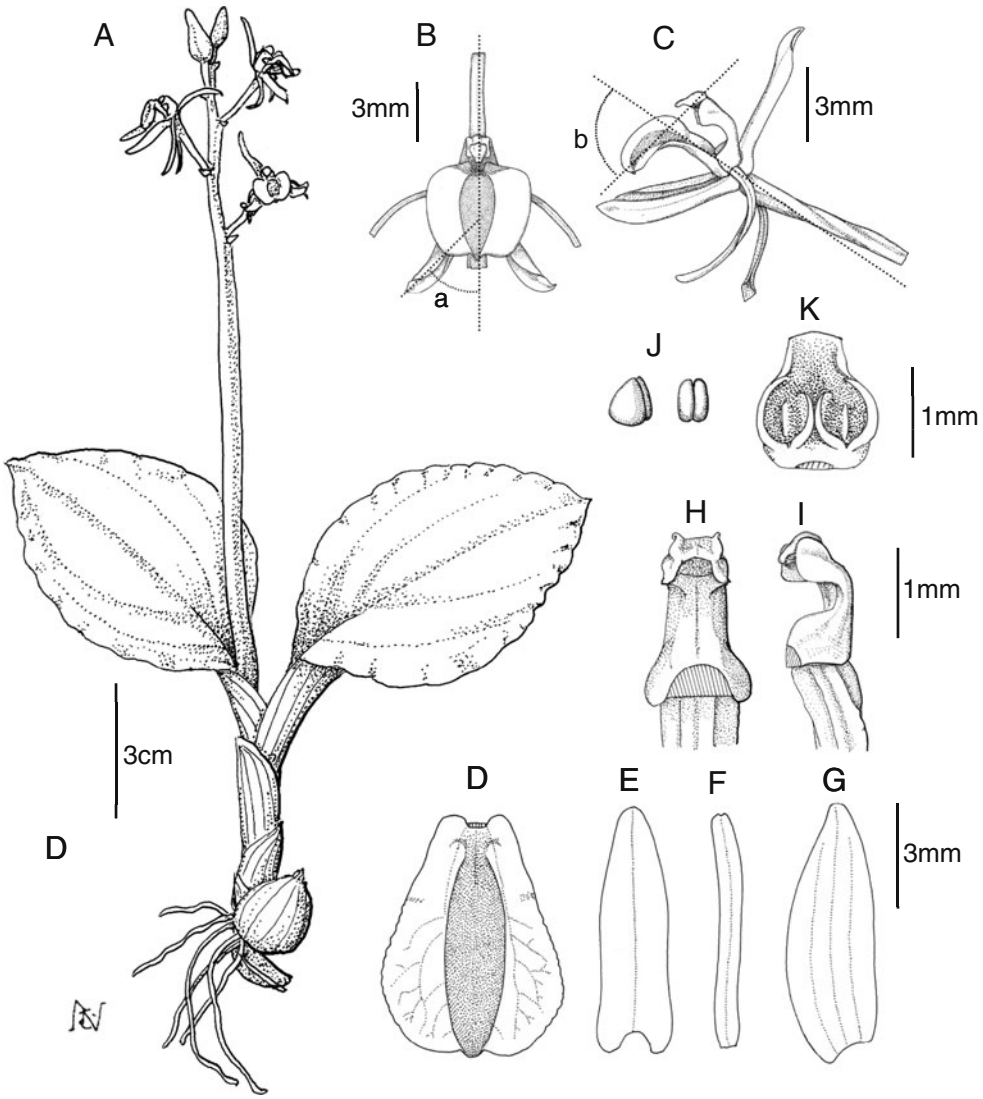

Fig. 1 *Liparis yongnoana* N.S. Lee, C.S. Lee and K.S. Lee (drawing the specimen of *N. S. Lee et al. D744*). *A* Habit, *B* flower (front view), *a* angle between lateral sepal and center of labellum, *C* flower (lateral view), *b* reflex angle of labellum, *D* labellum, *E* dorsal sepal, *F* lateral petal, *G* lateral sepal, *H* column, ventral view, *I* column, lateral view, *J* pollinia, *K* anther cap, anther removed

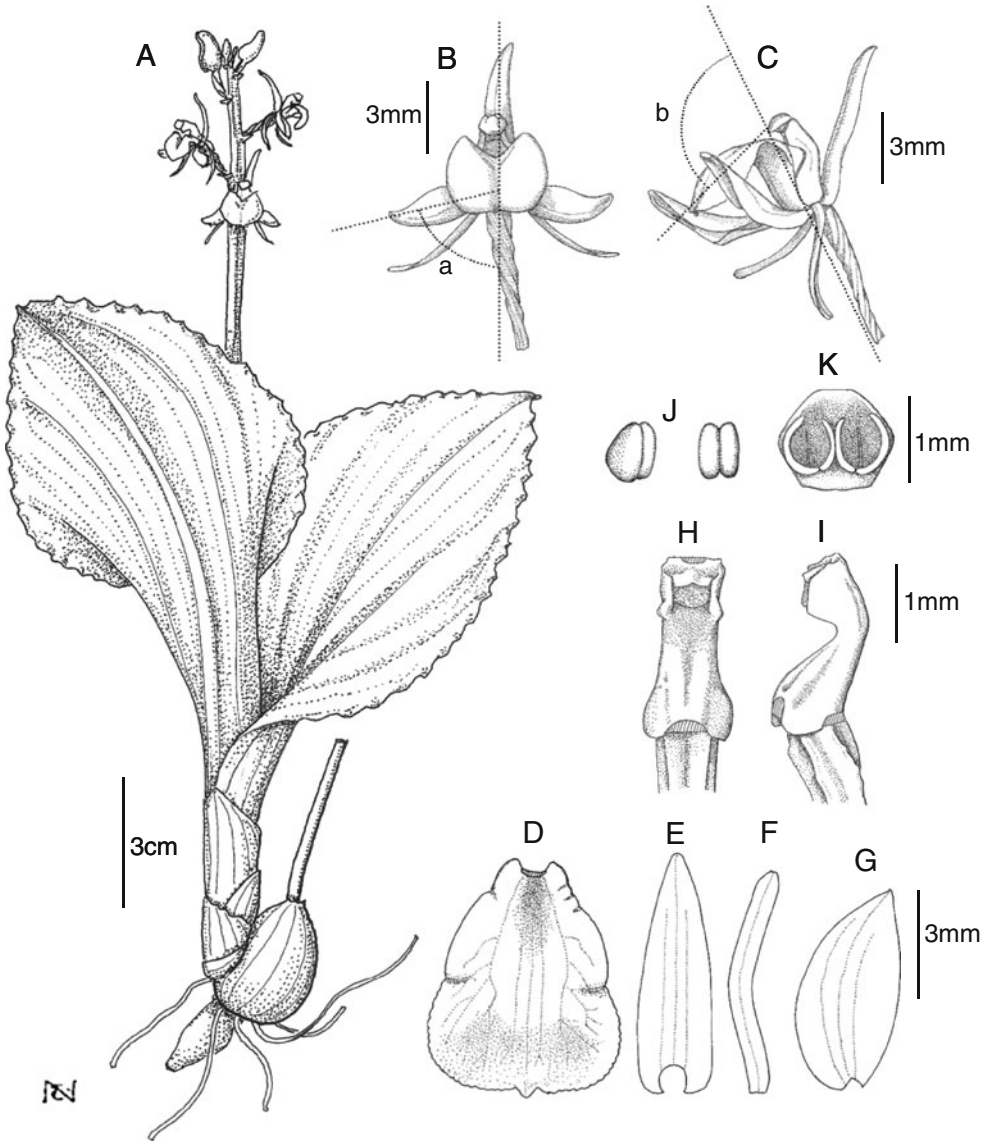

Fig. 2 *Liparis pterosepala* N.S. Lee, C.S. Lee and K.S. Lee (drawing the specimen of *N. S. Lee et al. D745*). *A* Habit, *B* flower (front view), *a* angle between lateral sepal and center of labellum, *C* flower (lateral view), *b* reflex angle of labellum, *D* labellum, *E* dorsal sepal, *F* lateral petal, *G* lateral sepal, *H* column, ventral view, *I* column, lateral view, *J* pollinia, *K* anther cap, anther removed

scribed spacer (ITS) of nuclear ribosomal DNA, which consists of two noncoding spacer regions flanking the 5.8S gene and the *matK*, *trnS-trnG*, *trnL* with *trnL-trnF* regions of chloroplast DNA (cpDNA) were analyzed. Primers used for the amplifications and sequencing were ITS4, ITS5, ITS2, and ITS3 for the ITS region (Baldwin et al. 1995), OMAT1F and trnK2R for *matK* (Hidayat et al. 2005), trnS (GCU) and trnG (UCC) for *trnS-trnG* spacer (Hamilton 1999), and c, d, e, and f for *trnL-trnF* (GAA) with *trnL* (UAA) intron and *trnL-trnF* spacer regions (Taberlet et al. 1991). Amplifications were conducted using a PTC-100 thermal cycler (MJ Research, USA) with the following temperature profile for all regions: a 32 to 37 cycle reaction with denaturalization at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 2 to 3 min. In addition, an initial denaturalization at 94°C for 2.5 min and a final extension at 72°C for 10 min were performed. PCR products were purified with AccuPrep PCR Purification Kit (Bioneer Inc, Korea). An automated sequencing analysis was performed using a sequencer (Base Station, MJ Research, USA).

Phylogenetic Analysis

The data previously reported in Tsutsumi et al. (2007) were also included to the analyses. Nucleotide sequences of the ITS and cpDNA regions were each aligned with gap adjustments, using the Clustal X program, followed by the manual adjustment (Gibson et al. 1994; Thompson et al. 1997).

The incongruence length difference (ILD) test of Farris et al. (1995) was implemented using the partition-homogeneity test of PAUP* version 4.0b10 (Swofford 2002) to examine the extent of conflict between the ITS

and cpDNA matrices for a comparable set of taxa. The test was conducted by heuristic search with 100 replicates, using 10 simple additions. There was no clear conflict among the four datasets examined by ILD test (P value=0.11), so all regions were combined.

Combined matrices of the ITS and three cpDNA regions were analyzed using maximum parsimony (MP) and maximum likelihood (ML) methods. For the ML method, Modeltest ver. 3.5 (Posada and Crandall 1998) was used to determine the optimal nucleotide substitution model. The GTR + I model (the alpha level of significance of 0.01 and proportion of invariable sites value of 0.8513) was selected based on hierarchical likelihood ratio test. The ML analysis was implemented using PAUP as heuristic search with 100 random addition sequence replicates, and gaps were treated as missing data. In MP method, gap states were treated by using the simple indel coding method as separate characters (Simmons and Ochoterena 2000). Characters were treated as unordered, and all character transformations were weighted equally. Heuristic MP searches were replicated 1,000 times with 100 random stepwise addition of taxa, tree-bisection-reconnection (TBR) branch swapping, and saving multiple trees. Bootstrap values (Felsenstein 1985) were calculated from 100 replicate analyses using TBR branch swapping and simple stepwise addition of taxa (MaxTrees were set to 100 for the ITS analysis). The consistency index (CI), retention index (RI), rescaled consistency index (RC), homoplasy index (HI), and uncorrected pairwise nucleotide distances were calculated by PAUP.

Results

Description of New Species

Liparis yongnoana N.S. Lee, C.S. Lee, and K.S. Lee, sp. nov.—Type: Korea, Jeju Island, Geoinoreum, 27 June 2006, *N. S. Lee et al. D744* (Holotype: EWH, Isotype: EWH). (Figs. 1 and 3a, Table 2).

Korean name: Gye-wu-ok-jahm-nan-cho

Ex affinitate *L. makinoanae* et specierum affinium distinguenda folio margine undulato, floribus paucis, labello in mediano purpureo, apice apiculato, et columna brevi.

Terrestrial herb. Pseudobulb ovoid, 10 to 15 mm long. Leaves 2, ovate-elliptic, obtuse or subacute, 5 to 7 cm long, 3 to 4 cm wide, conduplicate, glossy, glabrous, green, margin undulate; petiole 2 to 4 cm long, nearly as long as blade, winged. Inflorescence terminal, racemose, 9 to 12 cm long, bearing four to five greenish to purplish flowers, axis glabrous, ridged, green. Floral bract ovate, obtuse, 1 mm long, green; dorsal sepal linear-lanceolate, erect or somewhat reflexed, subacute, 8 to 9 mm long, 2 mm wide, greenish; lateral sepals lanceolate, revolute, extending headed to the apex of labellum, 7 to 8 mm long, 2.5 mm wide, green; lateral petals linear, strongly revolute, pendulous, 7 mm long, 0.5 mm wide, green; labellum oblong, clawed, reflexed, emarginated with apiculate tip, 9 mm long, 7 to 8 mm wide, an elliptic purple line at the central part; column incurved strongly, 3.0 to 3.5 mm long, winged at apex, wings round, broadly dilated at base, green; pollinia four in two pairs, waxy, yellow; anther cap ovoid, beaked, green; ovary pedicellate, clavate, twisted only at base, 7 to 8 mm long, slightly winged, green.

Fig. 3 Photographs of *Liparis yongnoana* N.S. Lee, C.S. Lee and K.S. Lee (**a**) and *Liparis pterosepala* N.S. Lee, C.S. Lee and K.S. Lee (**b**)

Phenology Flowering from June to July.

Additional specimens examined Korea. Jeju Island, Geoinoreum, 27 June 2006, *N. S. Lee et al. D163*, *D164*, *D342*, *L163*, *L163*, *L342*, *L343*, *A321*; Eorimok, 29 Aug. 2004, *N. S. Lee et al. A091*, *A092*; Nongoak, 26 Jun 2003, *N. S. Lee et al. L163*, *L164*, *A097* (EWH).

Etymology This new scientific name is named after the late Dr. Yong No Lee, a great Korean plant taxonomist to commemorate his accomplishments.

Distribution Korea, Jeju Island (Eorimok, Goeinoreum, Nongoak).

Habitat 800–900 m alt., moss rock in the forest near the valley stream, with *L. kumokiri*, *Galeola elata*, *Sasa guelpaertensis*, *Rubus crataegifolius*, *Carpinus laxiflora*, *Acer palmatum*, *Quercus serrata*, and *Styrax japonica* etc.

Table 3 Informative nucleotide sites in ITS for two new species and related taxa of *Liparis*. Species-specific bases were shown in italics

Taxa/nucleotide sites	80	125	126	426	502	532	586	649
L. yongnoana	*T*	*A*	*C*	*T*	C	C	T	*G*
L. makinoana	C	T	G	C	C	C	T	A
L. japonica	C	T	G	C	C	C	T	A
L. type 1	C	T	G	C	C	C	T	A
L. type 2	C	T	G	C	C	C	T	A
L. pterosepala	C	T	G	C	C	C	*A*	A
L. kumokiri	C	T	G	C	C	C	T	A
L. koreojaponica	C	T	G	C	*T*	*A*	T	A
L. fujisanensis	C	T	G	C	*T*	*A*	T	A
L. purpureovittata	C	T	G	C	C	C	T	A
L. liliifolia	C	T	G	C	C	C	T	A

Liparis pterosepala N.S. Lee, C.S. Lee, and K.S. Lee, sp. nov.—Type: Korea, Jeju Island, Nongoak, 6 June 2006. *N. S. Lee et al. D745* (Holotype: EWH; Isotype: EWH). (Figs. 2 and 3b, Table 2).

Korean name Nal-ge-ok-jahm-nan-cho

Differt a *L. kumokiri*, *L. koreojaponica* et *L. fujisanensi* sepalis lateralibus latioribus et florescentia praecoci.

Terrestrial herb. Pseudobulb ovoid, 2 cm long. Leaves 2, ovate-elliptic, obtuse or subacute, 8 to 10 cm long, 4 to 5 cm wide, conduplicate, glossy, glabrous, margin somewhat undulate, green; petiole 2 to 5 cm long, winged. Inflorescence terminal, racemose, glabrous, ridged, 12 to 15 cm long, bearing six to nine dark purplish flowers. Floral bract ovate, obtuse, 1 mm long, green; dorsal sepal linear-lanceolate, erect or somewhat reflexed, subacute, 10 to 11 mm long, 1.6 to 2.0 mm wide, purple; lateral sepals obliquely ovate-obliquely lanceolate, revolute, twisted distally, somewhat enfolding the labellum, 6 to 7 mm long, 3.0 to 3.3 mm wide, greenish purple; lateral petals linear, strongly revolute, pendulous, sometimes slightly twisted, 9.5 to 11 mm long, 0.6 to 0.8 mm wide, purple; labellum oblong, reflexed, sometimes gently concave distally, minutely erose, obtuse or apiculate at apex, apex broader than base, 6.5 to 8 mm long, 4 to 6 mm wide, dark purple;

Table 4 Informative nucleotide sites in cpDNA for new taxa and related *Liparis* taxa

Taxa/nucleotide sites	*matK*		*trnS-trnG*					*trnL* with *trnL-trnL-F*						
	919	1074	1545	1561	*1583*	1840	2336	2413	2555	*2817*	3056	3166	3300	3425
L. yongnoana	*T*	*C*	A	C	1	C	*C*	A	T	*1*	*A*	A	–	A
L. makinoana	C	A	A	C	1	C	G	A	T	0	–	A	–	A
L. japonica	C	A	A	C	1	C	G	A	T	0	–	A	–	A
L. type 1	C	A	A	C	1	C	G	A	T	0	–	A	–	A
L. type 2	C	A	A	C	1	C	G	A	T	0	–	A	–	A
L. pterosepala	C	A	T	C	1	C	G	A	T	0	G	A	*T*	A
L. kumokiri	C	A	A	C	1	C	G	A	T	0	G	A	–	A
L. koreojaponica	*C*	A	A	C	1	C	G	*G*	*C*	0	G	*C*	–	A
L. fujisanensis	C	A	A	*A*	*0*	*A*	G	A	T	0	G	A	–	*G*
L. purpureovittata	C	A	T	*C*	1	*C*	G	A	T	0	G	A	–	*A*
L. liliifolia	C	A	A	*C*	1	*C*	G	A	T	0	G	A	–	*A*

Gaps were treated as 1. The sites, 1583 and 2817, mean the gaps of 1,583rd to 1,612th base (ACTGGTTACCGGCCTGGTTAAGTATAAGT) and 2,817th to 2,821st base (ATCTA), respectively. Species-specific bases and gap were shown in italics

column incurved, 4 to 5 mm long, winged at apex, wings triangular, broadly dilated at base, green at upper part, purplish at base; pollinia four in two pairs, waxy, yellow; anther cap ovoid, mucronate, greenish purple; ovary pedicellate, clavate, twisted, 7 to 8 mm long, slightly winged, with purplish at base, green.

Phenology Flowering from May to June.

Additional specimens examined Jeju Island, Nongoak, 26 June 2006. *N. S. Lee et al. D746*, *D747*, *D1190*, *L335*, *L340*, *A096*; Goeinoreum, 22 Jul. 2004, *N. S. Lee et al. A25*, *A26*, *A105*, *A129* (EWH).

Etymology This new scientific name was named after the shape of lateral sepal

Distribution Korea, Jeju Island (Nongoak, Goeinoreum)

Habitat 700–800 m alt. ground in the forest near upper stream of valley, with *L. kumokiri*, *Platanthera ussuriensis*, *C. laxiflora*, and *A. palmatum*, etc.

Molecular Data Analysis

Molecular analyses also indicated that the two new species are distinct from the other described species. In the ITS region examined (689 bp), *L. yongnoana* has five autapomorphies and *L. pterosepala* has one autapomorphy in the sequences (Table 3). Both *L. yongnoana* and *L. pterosepala* do not show sequence variations among the populations of each taxon. In the *matK*, *trnS-trnG*, and *trnL* with *trnL-trnF* region of cpDNA [3,245 bp (*L. japonica*) to 3,500 bp (*L. fujisanensis*)], *L. yongnoana* has four autapomorphic substitutions and one species-specific indel character state, and *L. pterosepala* has one autapomorphy (Table 4).

The values of uncorrected pairwise sequence divergence in combined ITS and cpDNA regions are listed in Table 5. *L. yongnoana* shows the lowest values (0.26%) with *Liparis* Type 1, which was usually treated as *L. makinoana* Schltr. in Japan (Tsutsumi et al. 2008b). *L. yongnoana* shows the highest values (1.24% to 1.31%) with *L. purpureovittata* within ingroup. *L. pterosepala* has the lowest values (0.12% to 0.25%) with *L. kumokiri* and the highest values (1.03% to 1.06%) with *Liparis* Types 1 and 2.

The ML tree (−Ln=8112.71) of the combined ITS, *matK*, *trnS-trnG*, and *trnL* with *trnL-trnF* datasets for 31 individuals are shown in Fig. 4. The topology suggested by the ML tree is congruence with the two equally most parsimonious trees with 197 steps by MP method (CI, RI, RC, and HI values are 0.92, 0.95, 0.87, and 0.08, respectively). The Makinoana clades and the Kumokiri clades were clearly separated from North American *L. liliifolia* (Fig. 4). *Liparis yongnoana* is nested within the Makinoana clade together with plants widely called *L. japonica–L. makinoana*. *L. pterosepala* formed a clade with *L. koreojaponica* and then formed the

Table 5 Pairwise divergence percentages in the combined ITS, *matK*, *trnS-trnG*, *and trnL* with *trnL-trnF* regions between the two new taxa and related taxa within the genus *Liparis*. The numbers mean the taxa: *1, L. youngnoana*; *2, L. makinoana*; *3, L. japonica*; *4, L. Type 1; 5*, *L. Type 2; 6, L. pterosepala*; *7, L. kumokiri*; *8, L. koreojaponica*; *9, L. fujisanensis*; *10, L. purpureovittata*; *11, L. liliifolia*

Taxa	*1*	*2*	*3*	4	*5*	*6*	*7*	*8*	*9*	*10*	*11*
1	0.00–0.00										
2	0.39–0.39	0.00–0.00									
3	0.36–0.36	0.02–0.02	0.00–0.00								
4	0.26–0.26	0.24–0.24	0.24–0.24	0.00–0.00							
5	0.33–0.33	0.24–0.24	0.24–0.24	0.06–0.06	0.00–0.00						
6	0.75–0.81	0.75–0.77	0.75–0.77	1.03–1.06	1.03–1.06	0.00–0.00					
7	0.67–0.98	0.62–1.02	0.62–1.02	0.85–0.94	0.85–0.94	0.12–0.25	0.00–0.09				
8	0.88–1.18	0.83–1.23	0.83–1.23	1.06–1.19	1.06–1.19	0.16–0.20	0.20–0.44	0.02–0.09			
9	0.79–1.05	0.77–1.16	0.77–1.16	1.00–1.06	1.00–1.06	0.24–0.38	0.14–0.28	0.24–0.38	0.00–0.09		
10	1.24–1.31	1.29–1.29	1.29–1.29	1.25–1.25	1.25–1.25	0.78–0.81	0.62–0.75	0.81–0.84	0.75–0.85	0.00–0.00	
11	2.77–2.82	2.83–3.45	2.83–2.83	3.47–3.47	3.47–3.47	2.80–2.83	2.73–3.34	2.85–3.40	2.91–3.47	3.59–3.59	0.00–0.00

Taxa are listed by their specific epithets except types 1 and 2

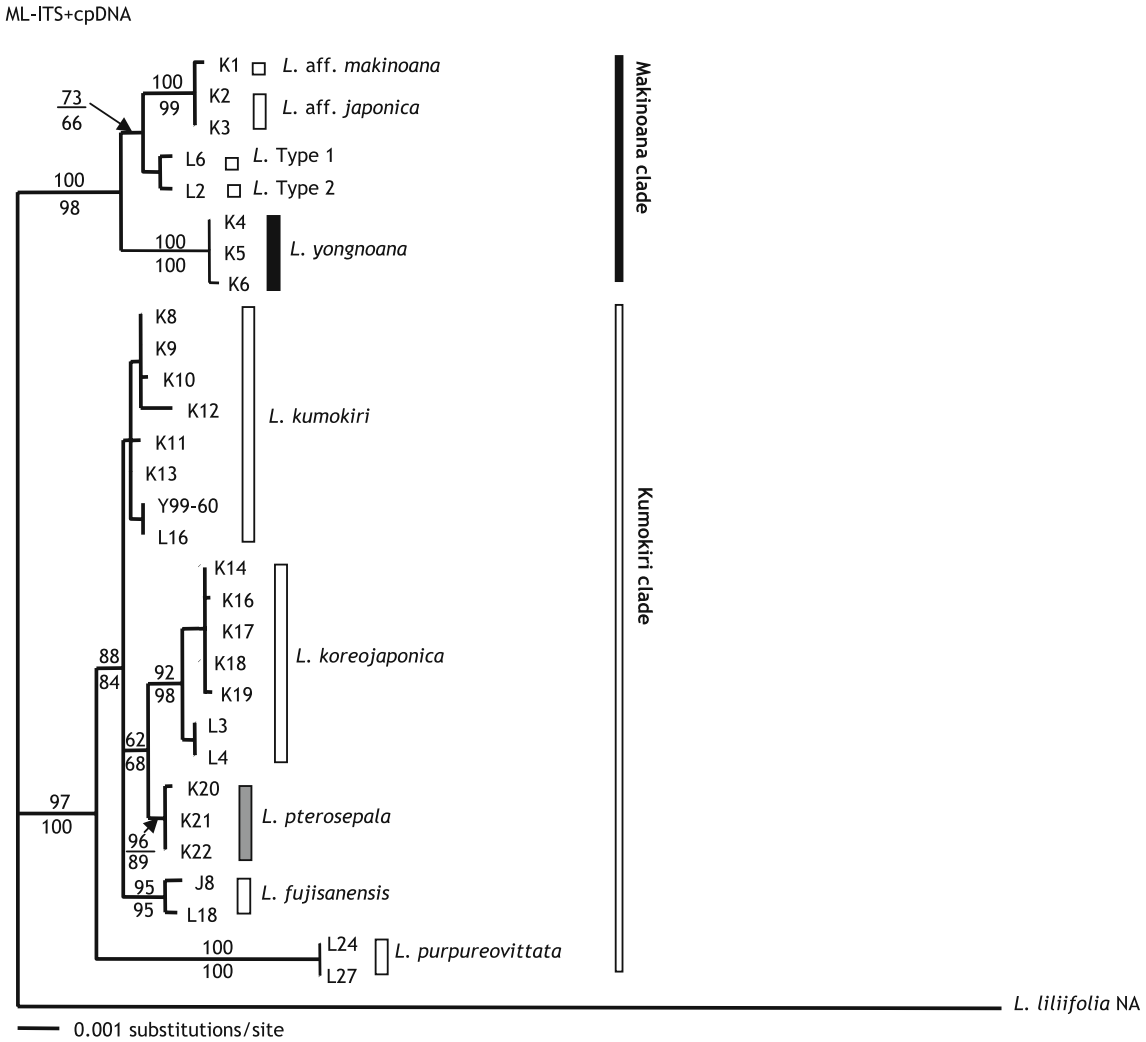

Fig. 4 ML tree of *Liparis* section *Liparis* and related taxa deduced from combined ITS and cpDNA (*matK*, *trnS-trnG*, and *trnL* with *trnL-trnF*) data. Bootstrap values (>50%) by MP method are shown above branches and those by ML method below them. *Liparis liliifolia* is used as outgroup. *Liparis* Types 1 and 2 were treated as *L. makinoana* and *L. japonica* in Tsutsumi et al. (2007)

Kumokiri clade with *L. kumokiri–L. fujisanensis–L. purpureovittata*.

Discussion

We recognized two new species of *Liparis* section *Liparis* from Jeju Island, Korea. Both of the morphological and the molecular data suggested that *L. yongnoana* is a member of the Makinoana clade having an anther cap with a beaked apex, and *L. pterosepala* is a member of the Kumokiri clade having a mucronate anther cap. Within each clade, each taxon is distinguishable from related taxa by some morphological characters (Table 2). However, our molecular data within the Makinoana clade showed that Korean *L. makinoana* and *L. japonica* are different from *Liparis* Type 1 and Type 2, which are widely called as *L. makinoana* and *L. japonica* in Japan (Tsutsumi and Yukawa 2008). Therefore, the taxonomic delimitation of *L. japonica* and *L. makinoana* from Japan and Korea should be studied in the future.

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Appendix

Collection data of the *Liparis* and related taxa studied in molecular analyses and morphological characters

Species, voucher number (herbarium)—collection information (collector, locality)

L. fujisanensis F. Maek. ex F. Konta and S. Matsumoto

DNA voucher: J8 (EWH)—C. Tsutsumi & H. Nakayama, Yamananshi, Japan; L18 (TNS)—C. Tsutsumi, cult. Tsukuba Bot. Gard, Japan. Morphological voucher: D499, L189 (EWH)—C. Tsutsumi & H. Nakayama, Yamananshi, Japan

L. japonica (Miq.) Maxim.

DNA voucher: K2 (EWH)—N. Lee et al., Imgye-myeon, Korea; K3 (EWH)—Y. Kim, Mt. Seorak, Korea. Morphological voucher: D435, L147 (EWH)—S. Eum & J. Kim, Mt. Sobaek, Korea; D736~7 (EWH)—N. Lee, Seorak, Korea; D773~4, L287~8 (EWH)—Kumdebong, Korea;; L148 (EWH)—N. Lee et al., Isl. Jeju, Korea

L. koreojaponica Tsutsumi et al.

DNA voucher: K14 (EWH)—C. Lee & J. Kim, Mt. Gwangdeok, Korea; K15 (EWH)—C. Lee & C. Tsutsumi, Isl. Jeju, Korea; K16 (EWH)—N. Lee et al., Mt. Yumyeong, Korea; K17 (EWH)—Y. Kim, Mt. Seorak, Korea; K18 (EWH)—Y. Kim, Mt. Taebaek, Korea; K19 (EWH)—N. Lee et al., Mt. Deogyu, Korea; L3 (TNS)—C. Tsutsumi, Hokkaido, Japan; L4 (TNS)—C. Tsutsumi, Hokkaido, Japan. Morphological voucher: D207 (EWH)—C. Lee & J. Kim, Mt. Gwangdeok, Korea; D491, L053~4, 157~9 (EWH)—N. Lee & Y. Kim, Mt. Seorak, Korea; L160 (EWH) –; N. Lee, Mt. Seorak, Korea; D208 (EWH)—N. Lee & Y. Kim, Mt. Taebaek, Korea; D732, L178, L210 (EWH)—Y. Kim, Mt. Taebaek, Korea; D436, 489~90, L153, L154~6 (EWH)—N. Lee & K. Lee, Mt. Deogyu, Korea

L. kumokiri F. Maek.

DNA voucher: K7 (EWH)—Y. Kim, Gangwon-do, Korea; K8 (EWH)—Y. Kim, Mt. Mudeung, Korea; K9 (EWH)—N. Lee et al., Mt. Minjuji, Korea; K10 (EWH)—N. Lee et al., Mt. Yumyeong, Korea; K11 (EWH)—Y. Kim, Mt. Seorak, Korea; K12 (EWH)—N. Lee et al., Isl. Jeju, Korea; K13 (EWH)—N. Lee et al., Isl. Jeju, Korea; Y99-60 (TNS)—T. Yugawa, Nara, Japan; L16 (TNS)—C. Tsutsumi, Hokkaido, Japan. Morphological voucher: D221~2,225, L042~3 (EWH)—Y. Kim, Mt. Jukyueop, Korea; D214 (EWH)—J. Kim, Mt. Cheonma, Korea; D482, 484, 83, L142 (EWH)—N. Lee et al., Mt. Yumyeong, Korea; D554~5, L174~5 (EWH)—Y. Kim, Mt. Meongji, Korea; D210 (EWH)—Mt. Mudeung, Korea; D725~6, D728 (EWH)—N. Lee & M. Cha– Mt. Chiri, Korea; D213, D218~20, L073 (EWH)—N. Lee et al., Isl. Jeju, Korea; D486~8, (EWH)—C. Lee & B. Jang, Isl. Jeju, Korea; L141(EWH)—Y. Kim, Mt. Taebaek, Korea; D769~71, D857 (EWH)—C. Lee & S. Um, Mt. Deokhang, Korea

L. pterosepala N. Lee et al.

DNA voucher number: K20 (EWH)—N. Lee et al., Isl. Jeju, Korea; K21 (EWH)—N. Lee et al., Isl. Jeju, Korea; K22 (EWH)—N. Lee et al., Isl. Jeju, Korea

Morphological voucher: D745, D746~7, D1190, L335 (EWH)—N. Lee et al., Isl. Jeju, Korea

L. liliifolia (L.) A.Rich. ex Lindl.

DNA voucher: NA (EWH)—M. Gross, NJ, USA

L. makinoana Schltr.

DNA voucher: K1 (EWH)—K. Lee, Isl. Jeju, Korea. Morphological voucher: D226, D227~8, D426, D733, L208, L222 (EWH)—N. Lee et al., Isl. Jeju, Korea

L. type 1

DNA voucher: L6 (TNS)—C. Tsutsumi & H. Nakayama, Kanagawa, Japan

L. type 2

DNA voucher: L2 (TNS)—C. Tsutsumi, Hokkaido, Japan; L7 (TNS)—C. Tsutsumi, cult. Tsukuba Bot. Gard., Japan

References

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell DS, Donoghue MJ (1995) The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann Mo Bot Gard 82:247–277

Chung TH (1957) Korean flora. Shinzisa, Seoul, In Korean

Cribb P, Govaerts R (2005) Just how many orchids are there? In: Raynal-Roques A, Roguenant A, Prat D (eds) Proceedings of the 18th World Orchid Conference. France Orchidees, Dijon, pp 161–172

Farris JS, Kallersjo M, Kluge AG, Bult C (1995) Testing significance of incongruence. Cladistics 10:315–319

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

Garay LA, Romero-Gonzalez GA (1999) Schedulae Orchidum II. Harvard Pap Bot 4:475–488

Gibson T, Higgins D, Thompson J (1994) Clustal X Program. EMBL, Heidelberg

Hamilton MD (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol Ecol 8:521–523

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

Kim SH, Kim YS (1986) Morphological and cytological study on genus *Liparis* in Korea. Kor J Plant Tax 16:59–88, In Korean

Lee WC (1996) Lineamenta Florae Koreae. Academy, Seoul, In Korean

Lee YN (2006) New Flora of Korea. Kyo-Hak, Seoul, In Korean

Lee JS, Choi BH (2006) Distributions and red data of wild orchids in the Korean peninsula. Korean J Pl Taxon 36:335–360, In Korean

Li HL (1978) Flora of Taiwan, vol 5. Epoch, Taipei

Nakai T (1931) Notulae and plantas Japoniae & Korea XL. Bot Mag Tokyo 45:107–108

Ohwi J (1984) Flora of Japan, 2nd edn. Smithsonian Inst, Washington

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Satake Y, Ohwi S, Kitamura S, Watari S, Tominari T (1982) Wild flowers of Japan. Herbaceous plants, vol 1. Heibonsha, Tokyo, In Japanese

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analysis. Syst Bot 49:369–381

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.01b10. Sinauer, Sunderland

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl Mol Biol 17:105–1109

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. Nucl Acids Res 25:4876–4882

Tsutsumi C, Yukawa T (2008) Taxonomic status of *L. japonica* and *L. makinoana* (Orchidaceae): a preliminary report. Bull Natl Mus Nat Sci Ser B 34:89–94

Tsutsumi C, Yukawa T, Lee NS, Lee CS, Kato M (2007) Phylogeny and comparative seed morphology of epiphytic and terrestrial species of *Liparis* (Orchidaceae) in Japan. J Plant Res 120:405–412

Tsutsumi C, Yukawa T, Kato M (2008a) *Liparis purpureovittata* (Orchidaceae)—a new species from Japan. Acta Phytotax et Geobot 59:73–77

Tsutsumi C, Yukawa T, Lee NS, Lee CS, Kato M (2008b) A new species of *Liparis* from Japan and Korea. Acta Phytotax et Geobot 59:211–218

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322