A New Variety of Liparis koreojaponica (Orchidaceae) from Nara Prefecture, Japan

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Liparis koreojaponica Tsutsumi, T. Yukawa, N. S. Lee, C. S. Lee & M. Kato var. kiiensis Suetsugu & Tsutsumi (Orchidaceae) from Nara Prefecture, Japan is described as a new variety. It is similar to L. koreojaponica var. koreojaponica, but differs in spacing of the flowers (ca. 1.5 cm apart in the former vs. ca. 2 cm apart in L. koreojaponica var. koreojaponica), length ratio of rachis/inflorescence (1/3–1/2 vs. 1/4–1/3 in L. koreojaponica var. koreojaponica) and length of the labellum (13–15 mm long vs. 9–12 mm long in L. koreojaponica var. koreojaponica).

Keywords: Japan, Liparis, molecular phylogeny, new locality, taxonomy, Orchidaceae

Liparis Rich. (Malaxideae, Epidendroideae, Orchidaceae), a relatively large, cosmopolitan genus of approximately 350 species, is mostly tropical and subtropical, with some representatives extending to temperate regions (Pridgeon et al. 2005, Li & Yan 2013). The species of Liparis are terrestrial, lithophytic or epiphytic with small to prominent pseudobulbs, one to several (rarely none) conduplicate to plicate leaves, a terminal inflorescence of one to many, usually resupinate yellow, green, orange or purple flowers. The lateral sepals that are often wider and shorter than the dorsal sepal. The labellum is firmly attached to a footless, often arched, column and the incumbent anther bearing four pollinia grouped in two pairs usually lacks caudicles or a stipe but has a minute viscidium (Lee et al. 2010, Li & Yan 2013, Damian & Ormerod 2016).

In Japan, 18 species of Liparis have been recognized (Tsutsumi et al. 2019), but continued discovery of new species and range extensions suggest that the distribution and diversity of Japanese Liparis remain uncertain (Tsutsumi et al. 2008a, b, 2019). As anticipated, some unknown plants of Liparis were discovered in Nara Prefecture, Japan during a field survey and herbarium investigations. To identify these plants, we compared them morphologically and by molecular phylogenetic analysis with similar species. On completion of our comparisons, we concluded that the unknown plants represent an undescribed variety of Liparis koreojaponica Tsutsumi, T. Yukawa, N. S. Lee, C. S. Lee & M. Kato, which we propose to name L. koreojaponica var. kiiensis Suetsugu & Tsutsumi. Its phylogenetic position is also discussed.

Materials and Methods

Morphological observations

To compare the morphology of the unknown plants of Liparis, we thoroughly reviewed the literature, collected samples from throughout Japan and consulted specimens in the herbaria KYO, OSA, TI and TNS as well as online digital images of specimens on JSTOR Global Plants (http://plants.jstor.org/).

Molecular phylogeny

DNA was extracted from specimens collected on Inamuragatake (S. Nakamura KS744, holo-
type) and dried in silica gel with the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer’s instructions. The internal transcribed spacer regions of 18S-26S nuclear ribosomal DNA (ITS) and three chloroplast regions (part of matK, trnL with trnL-trnF spacer, trnS-trnG spacer) were analyzed. Procedures for amplification and sequencing followed those in Tsutsumi et al. (2007). The sequences analyzed in this study were registered in GenBank (Appendix 1). For phylogenetic analysis, sequences of related species analyzed in Lee et al. (2010) and Takayama et al. (2019) were used. A best-scoring maximum-likelihood (ML) tree search with a rapid bootstrap analysis was conducted using RAxML v8.2.12 (Stamatakis 2014). The analysis utilized a concatenated data set partitioned into four parts (ITS, part of matK, trnL with trnL-trnF spacer, and trnS-trnG spacer) with the model set to GTR with gamma-distributed rates among sites. Insertion and deletion mutations were not evaluated in the phylogenetic analysis. Bootstrap values were calculated from 1,000 replicates in the analysis. To assess tree incongruence between ITS and chloroplast datasets, the parsimony-based ILD test (Farris et al. 1995) was performed using the partition homogeneity test in PAUP 4.0a168 (Swofford 2002) with 10 random taxon addition, TBR branch swapping, and heuristic searches of 100 replications.

Results and Discussion

The plants of Liparis from Nara Prefecture were most similar to L. koreojaponica described from Hokkaido, Japan, and South Korea (Tsutsumi et al. 2008b), in having a terrestrial habit, relatively large flowers, mucronate anther cap and twisted lateral sepals protruding from both sides of the labellum in front view. Despite their similarities, the unknown Liparis and L. koreojaponica differed in the arrangement of flowers on rachis (ca. 1.5 cm apart vs. ca. 2 cm apart), length ratio of rachis/inflorescence (1/3–1/2 vs. 1/4–1/3) and length of the labellum (13–15 mm long vs. 9–12 mm long; Tsutsumi et al. 2008b, Lee et al. 2010). The aforementioned traits of L. koreojaponica (particularly floral size) are stable among the populations of Hokkaido and South Korea. As far as we know, none of plants from Hokkaido and South Korea bear flowers as large as those of the plants from Nara. Considering the small but obvious differences in the morphology of the newly discovered plants, we considered them to represent an undescribed variety of L. koreojaponica.

Although Liparis koreojaponica has been confused with L. koreana in taxonomic treatments in Korea and Japan (Tsutsumi et al. 2008b), L. koreana is distinguishable in its laxly arranged flowers [vs. densely arranged (usually less than 1 cm apart)], apex of the anther cap mucronate (vs. beaked) and twisted lateral sepals protruding from both sides of the labellum in front view (vs. extending to the apex of the labellum in front view) (Tsutsumi et al. 2008b). The flowers of our unknown plants have the spacing of the flowers somewhat similar to L. koreana, but the plants are distinguishable from L. koreana in having a mucronate anther cap and the twisted lateral sepals protruding from both sides of the labellum. Notably, all species of the Makinoana clade sensu Lee et al. (2010) have an anther cap with a beaked apex (Lee et al. 2010, Takayama et al. 2019). Therefore, L. koreana must be phylogenetically distant from our newly discovered plants and from L. koreojaponica of the Kumokiri clade, which have a mucronate anther cap (Lee et al. 2010, Takayama et al. 2019). Our plants are also similar to L. fujisanensis F. Maek. ex Kanta & S. Matsumoto in floral morphology, but differ in the length of the inflorescence (15–35 cm vs. 3–18 cm in L. fujisanensis), spacing of the flowers (ca. 1.5 cm apart vs. less than 1 cm apart), length of the labellum (13–15 mm long vs. usually less than 10 mm long) and habit (on forest floor, on rocks or fallen tree trunks vs. on tree trunks; Tsutsumi et al. 2007, 2008b).

Molecular phylogenetic analysis of sect. Liparis using nuclear ribosomal ITS regions and three plastid regions (matK, trnL with trnL-trnF spacer, trnS-trnG spacer) supported the morphological data (Fig. 4). The results of the ILD test
suggest no inconsistencies between the ITS and chloroplast regions ($p = 0.36$). The phylogenetic analysis showed that our plants and *Liparis koreojaponica* form a clade with strong branching support (100%) that is sister to *L. fujisanensis*, although with low support (53%). In the DNA regions, the sequences from our plants were identical with those of *L. koreojaponica* collected in Korea (L41), except in the poly (T) length of the *trnL-trnF* spacer. Those were different from the Japanese *L. koreojaponica* by one substitution and from other Korean *L. koreojaponica* by another substitution in the *trnL-trnF* spacer. In contrast, our plants and *L. fujisanensis* were distinguishable by nine substitutions in the plastid regions, although they had identical sequences in the nuclear ITS region. Our phylogenetic interpretation leads us to believe that our plants should be treated as a variety *L. koreojaponica*, and are distinct from *L. fujisanensis* at the species level. We therefore describe them as *L. koreojaponica*, *L. koreojaponica* var. *kiiensis* from Nara Prefecture, Japan.

**Taxonomic Treatment**


*Liparis koreojaponica* var. *kiiensis* is most similar to *Liparis koreojaponica* var. *koreojaponica*, but is distinguishable by the flowers on the rachis (ca. 1.5 cm apart vs. ca. 2 cm apart in *L. koreojaponica* var. *koreojaponica*), length ratio of rachis/inflorescence (1/3–1/2 vs. 1/4–1/3 in *L. koreojaponica* var. *koreojaponica*) and labellum 13–15 mm long vs. 9–12 mm long in *L. koreojaponica* var. *koreojaponica*).

**Typus.** JAPAN. Honshu, Nara Prefecture, Yoshino-gun, Tenkawa-mura, Inamuragatake, alt. 1,350 m, 1 June 2020, S. Nakamura KS744 (holo- KYO, dried plant on an herbarium sheet and liquid preserved material in a bottle labeled as the same specimen).

Pseudobulb ovoid, 1–2 cm long. Leaves 2; petiole 4–9 cm long, winged, nearly as long as blade; blade ovate-elliptic, obtuse or subacute, 9–18 cm long, 2–5 cm wide, conduplicate, glossy, glabrous, margin undulate, green. Inflorescence terminal, racemose, 15–35 cm long, with 10–26 flowers; axis glabrous, ridged, green. Bract ovate, acute, 1–3 mm long, green. Ovary pedicellate, clavate, twisted, 13–18 mm long, purplish green or purple. Dorsal sepal linear-lanceolate, subacute, slightly revolute, erect or somewhat recurved, 12.5–13.5 mm long, ca. 2.8 mm wide when flattened, greenish purple or dull yellowish green. Lateral sepals obliquely ovate or obliquely lanceolate, subacute, somewhat revolute, distally twisted, protruding from both sides of labellum in front view, 12–13 mm long, ca. 3.2 mm wide when flattened, greenish purple. Petals falcate, linear, obtuse, strongly revolute, pendulous, sometimes slightly twisted, 12.8–13.8 mm long, ca. 1 mm wide when flattened, greenish purple. Labellum entire or minutely erose, gourd-shaped, strongly recurved at middle, margins sometimes slightly revolute, apex obtuse, 13–15 mm long,
Fig. 2. *Liparis koreojaponica* var. *kiiensis* from type locality. **A–C**, Flowers. **D–E**, Flower.
11–14 mm wide when flattened, greenish purple or dull yellowish green, base and central groove light to dark purple. Column terete, incurved, with rounded wings, dilated at base, with shallow groove at base on ventral surface, 5–6 mm long, green, pale green on ventral surface; pollinia 4, in 2 pairs, waxy, yellow, with prominent viscidium; anther cap ovoid, mucronate, green.

**Japanese name.** Kii-oh-fugaku-suzumushi, (nov.)

**Distribution and ecology.** Japan (endemic to Nara Prefecture): usually terrestrial in cool-temperate deciduous and/or coniferous forests, rarely on rocks or fallen tree trunks.

It is noteworthy that *Liparis koreojaponica* var. *kiiensis* is restricted to a limited area in southern Nara Prefecture, Japan (cool-temperate forests exceeding alt. 1,000 m), while *L. koreojaponica* var. *koreojaponica* occurs in Hokkaido, Japan, and South Korea (Tsutsumi *et al.* 2008b, Lee *et al.* 2010). Even though the two varieties exhibit some morphological differences, it is in-
triguing that *Liparis koreojaponica* exhibits a disjunct distribution. *Liparis koreojaponica* may be distributed more widely than documented. Further exploration is needed to elucidate the distribution and morphological variation of *L. koreojaponica*.

Additional specimens examined. JAPAN. Honshu, Nara Prefecture, Yoshino-gun: Tenkawa-mura, Inamuragatake, on the route from Renge-toge to Inamura-goya, alt. 1,600 m, 28 June 1978, *K. Ueda s.n.* (KYO); Kamikitayama-mura, Nishihara, Nihongatake to Mt. Wasamata, alt. 1,250m, 18 June 2005, *N. Morimoto 7505* (OSA).

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**References**


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Appendix 1. Materials used the molecular phylogenetic analysis. Information for each sample is shown in the order: taxon, isolate code (if any), locality, herbarium code and number of newly examined sample and GenBank accession numbers in order ITS, trnL with trnL-trnF spacer, trnS-trnG spacer, and part of matK.