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# Taxonomic Status of *Lipairs japonica* and *L. makinoana* (Orchidaceae): A Preliminary Report

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**Abstract** To clarify the taxonomic status of *Liparis japonica* and *L. makinoana*, we investigated molecular differences among three dubious entities, which have been assigned to *L. japonica* or *L. makinoana*. The three entities were distinct in nucleotide substitutions of the nuclear ribosomal ITS region. A morphological comparison of the three entities and the original descriptions of the two species indicates that the type of *L. japonica* is distinct from the plants currently called "*L. japonica*" and is identical with *Malaxis monophyllos*. Further, the entity widely called "*L. makinoana*" does not agree with the protologue of *L. makinoana*.
**Key words :** Japan, *Liparis japonica*, *Liparis makinoana*, molecular phylogeny, taxonomy.

## Introduction

*Liparis* Rich. (Orchidaceae), consisting of over 400 species (Cribb and Govaerts, 2005), is widely distributed in tropical and temperate regions of the world. It is characterized by one or more relatively soft, fleshy, green leaves, which sheathe a fleshy pseudobulb or corm. A terminal inflorescence has small, resupinate flowers with a long arching column and four pollinia without viscidia. Section *Liparis*, one of its 19 sections, is defined by the undeveloped pseudobulb at anthesis and two, subfleshy, non-ribbed leaves borne in the apical part of the pseudobulb (Garay and Gonzalez, 1999). In Japan, nine of 15 described *Liparis* species are assigned to sect. *Liparis*; *L. auriculata* Blume ex Miq., *L. fujisanensis* F. Maek. ex Konta et S. Matsumoto, *L. hostaefolia* (Koidz.) Koidz. ex Nakai, *L. japonica* (Miq.) Maxim., *L. krameri* Franch. et Sav., *L. kumokiri* F. Maek., *L. makinoana* Schltr., *L. purpureovittata* Tsutsumi, T. Yukawa et M. Kato, and *L. truncata* F. Maek. ex T. Hashim. Our previous molecular phylogenetic study using these species except *L. hostaefolia* revealed that sect. *Liparis* in Japan is separated into three clades; *L. auriculata*, the Krameri clade including *L. krameri* and *L. truncata*, and a clade comprising the other species. The last clade is subdivided into two groups: one comprises *L. purpureovittata* (labeled as *L.* sp. [24, 27] in Tsutsumi *et al*., 2007) and the Kumokiri clade (*L. fujisanensis*, *L.* sp. [treated as *L. koreana* in Tsutsumi *et al*., 2007] and *L. kumokiri*), and the other is the Makinoana clade consisting of *L. japonica* and *L. makinoana* (Tsutsumi *et al*., 2007).

Our molecular and morphological studies discovered a new and a putatively new species in the Kumokiri clade and related species. One is *L. purpureovittata*, which is vernacularly recognized as "Azumi-kumokiri", "Chikuma-jigabachi", "Fugaku-kumokiri" and "Nanbu-kumokiri" (Tsutsumi *et al*., 2008). The other putatively new species is *L.* sp., which has been misidentified as *L. makinoana* var. *koreana* Nakai or *L. koreana* (Nakai) Nakai ex W. T. Lee (Tsutsumi *et al*., unpubl. data). Previous insufficient recognition of the group is supposed to be caused by the lack of important diagnostic characters in herbarium specimens, such as the morphology of the anther cap and the three-dimensional structure of the perianth lobes. Another reason is insufficient descriptions of these taxa, in which such diagnostic characters were mostly

overlooked.

Much confusion exists in interpretations on *Liparis japonica* and *L. makinoana*. *L. japonica* and *L. makinoana* have been recorded from eastern Asia; China, Japan, Korea, Russia, and Taiwan for *L. japonica*, and Japan, Korea and Russia for *L. makinoana* (Komarov, 1968; Satomi, 1982; Chen *et al*., 1999; Su, 2000; Lee, 2002). Historically, key taxonomic references on Japanese flora interpreted that the two species are distinguishable in the length of inflorescence and the size of flower: *L. makinoana* has a shorter inflorescence and a larger flower than *L. japonica* (Maekawa, 1971; Ohwi, 1978; Satomi, 1982; Takahashi, 1985; Hashimoto, 1990; Hashimoto and Kanda, 1991). These diagnostic characters, however, do not separate the two species satisfactorily. Our preliminary observation on the basis of morphological characters indicated that this species complex may comprise three entities (Fig. 1).

To clarify the taxonomic status of these entities in the species complex, we performed preliminary macromoleular and morphological studies, using three entities assigned to *L. makinoana* and *L. japonica* (Fig. 1). The three entities are called Types 1–3 here to avoid further name confusion.

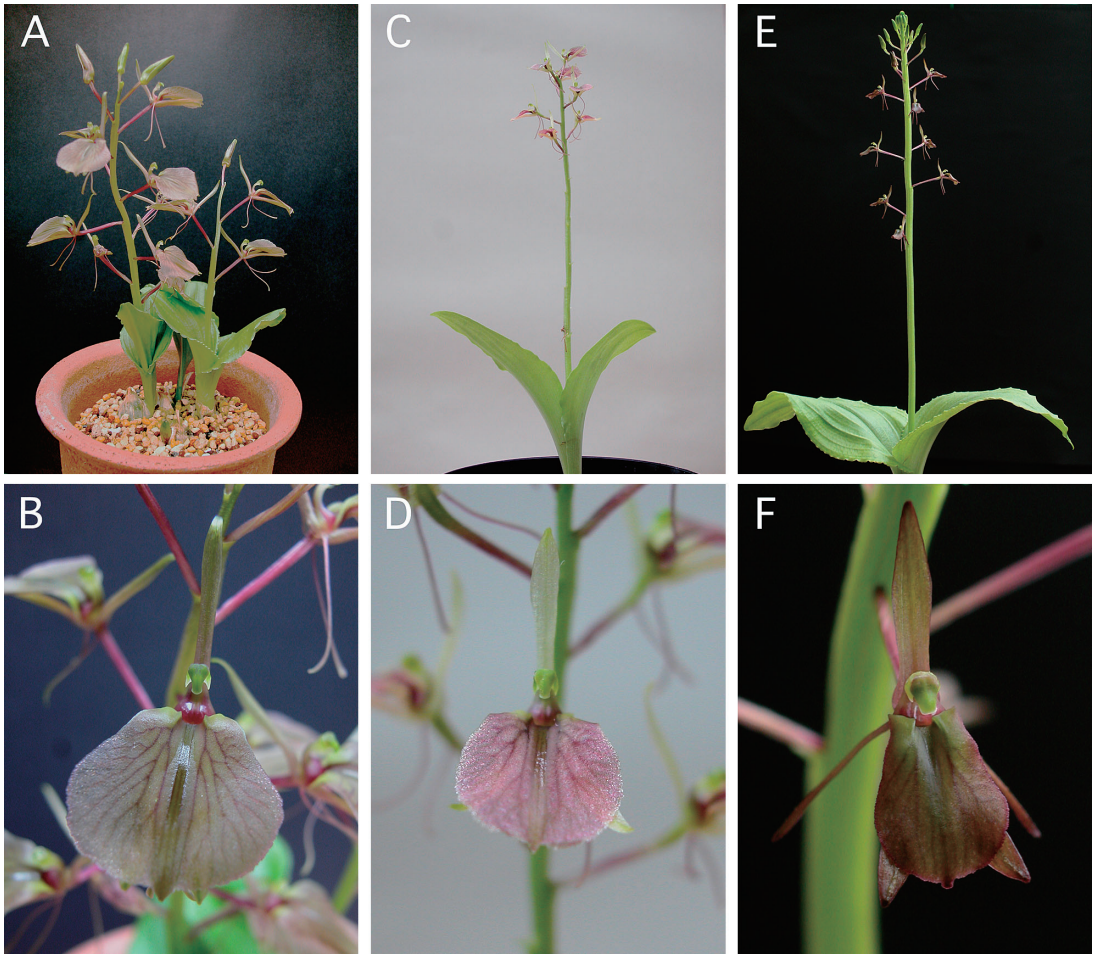


Fig. 1. Types 1–3 in *Liparis* cultivated at Tsukuba Botanical Garden, National Museum of Nature and Science. A–B, Type 1 (*C. Tsutsumi & H. Nakayama L6*); C–D, Type 2 (*C. Tsutsumi L7*); E–F, Type 3 (*C. Tsutsumi L8*). A, C, E, Flowering plant; B, D, F, Flower, front view. Photographs taken by C. Tsutsumi.

Table 1. Nucleotide variations of the three *Liparis* entities in the nuclear ribosomal ITS region.

| Entity | Source; voucher | Nuclear ribosomal ITS | | | | |
|---|---|---|---|---|---|---|
| | | 138 | 453 | 534 | 672 | 710 |
| Type 1 | Kanagawa, Japan; *C. Tsutsumi and H. Nakayama L6* | A | G | C | A | G |
| | Unknown; *C. Tsutsumi L20* | A | G | C | A | G |
| Type 2 | Hokkaido, Japan; *C. Tsutsumi*, *K. Watanabe and H. Hongo L2* | A | G | C | G | A |
| | Unknown; *C. Tsutsumi L7* | A | G | C | G | A |
| Type 3 | Unknown; *C. Tsutsumi L8* | G | C | T | A | G |

## Materials and Methods

Materials were collected in the field or from plants cultivated at Tsukuba Botanical Garden, National Museum of Nature and Science (Table 1). Vouchers are deposited in the Department of Botany Herbarium (TNS). Molecular analyses, procedures of extraction, amplification and sequencing were conducted, following Tsutsumi *et al*. (2007). Genetic regions examined were internal transcribed spacer regions of the 18S-26S nuclear ribosomal DNA (ITS) and three plastid regions, *trnL* and its flanking *trnL-trnF* spacer, *trnS-trnG* spacer and part of the maturase-encoding gene (*matK*). GenBank accession numbers of ITS, *trnL* and its flanking *trnL-trnF* spacer, *trnS-trnG* spacer and partial *matK* of Type 3 (*C. Tsutsumi L8*) are AB435655, AB435656, AB435657, AB435658, respectively. The others were shown in Tsutsumi *et al*. (2007), in which Types 1 and 2 were named as *L. makinoana* and *L. japonica*, respectively.

The phylogenetic relationships were deduced by the maximum likelihood (ML) method with PAUP* 4.0b10 (Swofford, 2002), following Tsutsumi *et al*. (2007). Modeltest 3.7 (Posada and Crandall, 1998) was used to determine the nucleotide substitution model. Bootstrap values were calculated with 1000 replicates by the maximum parsimony (MP) method. Bayesian inference of phylogeny was performed using MrBayes 3.1.2 to estimate posteriori support of clades in ML tree (Huelsenbeck and Ronquist, 2001) with the nucleotide model determined by MrModeltest 2.0 (Nylander, 2004). Bayesian searches were conducted by mcmc with four chains over one million generations, sampling every 100 generations. A quarter of trees obtained were discarded as burn-in trees.

## Results and Discussion

The molecular phylogenetic analysis showed that the three entities formed a monophyletic clade, although the phylogenetic relationship among the three was unclear (Fig. 2). In the ITS region of the three entities, Types 1 and 2 are distinguishable by two substitutions, and Type 3 differs from Type 1 by three substitutions and from Type 2 by five substitutions (Table 1). There is no substitution among the three types in the plastid regions. However, two to five substitutions in the ITS region among Types 1–3 endorsed independent status of each type.

A preliminary comparison of floral characters was made for the three entities, Types 1–3. The result is summarized in Table 2. The three entities are distinct in the combination of floral characters: Type 1 has the largest labellum and blooms earlier than the others. Type 2 is intermediate between Types 1 and 3 in the labellum size. Type 3 has the longest inflorescence, on which the flowers are sparser than those of the other two types. Type 3 is also characterized by a somewhat thickened, deep-colored apex of lateral sepal.

The present study revealed that the species complex currently interpreted as *Liparis japonica* and *L. makinoana* includes three entities, which are distinct by the molecular and the morphological characters. Types 1 and 2 were ap-

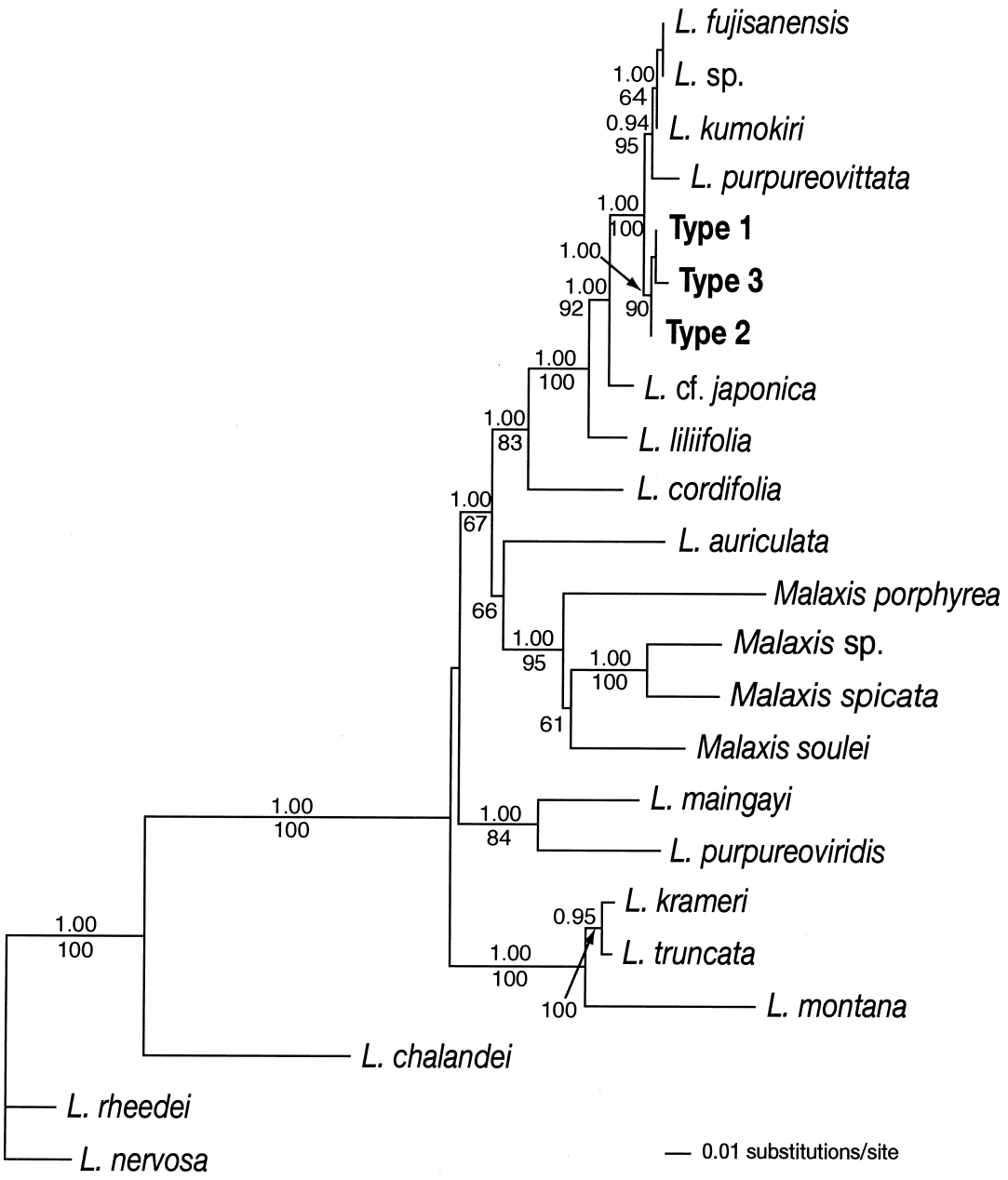


Fig. 2. Maximum Likelihood (ML) tree of *Liparis* section *Liparis* (−ln=4586.53) based on ITS sequences (825 bp.), revised by Tsutsumi *et al*. (2007). Figures above and below branches indicate posterior probabilities (>0.9) calculated by Bayesian analysis and bootstrap values (>50%) by maximum parsimony analysis, respectively. *L. nervosa* is chosen as an outgroup.

plied to be *L. makinoana* and *L. japonica*, respectively, in the pictures of Satomi (1982) and Hashimoto and Kanda (1991), and Type 3 was identified as *L. japonica* in the illustration of Iinuma (1913). To clarify this nomenclatural confusion, we need to examine protologues and type specimens of *L. japonica* and *L. makinoana*.

*Liparis japonica* was originally described as

*Microstylis japonica* by Miquel (1866) based on *Buerger s.n*. (a specimen with fruits collected in Japan). Although the type specimen has not been located (Ohba *et al*., 2005; A. Schuiteman, pers. commun.), morphological characters described in the protologue suggested that this species is synonymous with *Malaxis monophyllos* Lindl. Transfer of this entity to *Lparis* is inappropriate and identification of Type 2 or 3 to this name must be mistaken.

*Liparis makinoana* was described by Schlechter (1919), where he emphasized the differences from *L. liliifolia* of North America. The type specimen collected from Hokkaido, Japan was destroyed during the World War II and the duplicates were not located (Yukawa and Ohba, 1995). Furthermore, Schlechter (1919) did not mention any qualitative characters to distinguish the three entities. However, the size of labellum (12 mm long, 8 mm wide) in the description is identical to those of Type 2, rather than Type 1 currently interpreted as *L. makinoana*.

In conclusion, we demonstrated that Type 2, designated as "*Liparis japonica*" in most references available in Japan, is likely to represent *L. makinoana*, and Types 1 and 3 do not match with any published scientific names.

Table 2. Comparison of the three *Liparis* entities in floral characters and flowering season.

| | Type 1 | Type 2 | Type 3 |
|---|---|---|---|
| Flower stalk length | 10–25 cm | 10–30 cm | 15–40 cm |
| Flower number | 4–16 | 4–30 | 10–40 |
| Labellum length | 14–17 mm | 9–12 mm | 8–10 mm |
| Labellum width | 11–15 mm | 6–8 mm | 5–7 mm |
| Flowering season | May–Jun. | Jun.–Jul. | Jun.–Jul. |

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