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Abbildung Titelseite: *Liparis kumokiri* subsp. *nemoralis*, Italien, Friaul, Prato Carnico (UD),  
20.6.2011, fot. G. Perazza.



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## **Considerations on *Liparis loeselii* s.l. in Europe in relation to the East Asian *Liparis kumokiri* (Orchidaceae)**

### **Keywords**

*Orchidaceae*, *Liparis loeselii* s.l., *Liparis loeselii* subsp. *nemoralis*, *Liparis kumokiri*, *Liparis kumokiri* subsp. *nemoralis*, Flora of Japan, Italy, Friuli, Veneto, Trentino.

### **Summary**

Perazza, G. & C. Tsutsumi (2015): Considerations on *Liparis loeselii* s.l. in Europe in relation to the East Asian *Liparis kumokiri* (Orchidaceae).- J. Eur. Orch. 47 (2-4): 309-322.

Based on morphological traits and molecular analysis of the ITS region, *Liparis loeselii* subsp. *nemoralis*, recently described from Northeast Italy, showed a great similarity with the East-Asianic *Liparis kumokiri*. Its systematic position is revised. It is considered not as an ecological subspecies of *L. loeselii*, but as a geographically isolated taxon belonging to *L. kumokiri* and is recombined here as *Liparis kumokiri* subsp. *nemoralis*.

### **Zusammenfassung**

Perazza, G. & C. Tsutsumi (2015): Betrachtungen über *Liparis loeselii* s.l. (Orchidaceae) in Europa und ihre Beziehungen zur ostasiatischen *Liparis kumokiri*.- J. Eur. Orch. 47 (2-4): 309-322.

Weitere Untersuchungen der Morphologie und der ITS-Region der DNA zeigen auf, dass die kürzlich aus Nordost-Italien beschriebene *Liparis loeselii* subsp. *nemoralis* große Ähnlichkeit mit der ostasiatischen *Liparis kumokiri* besitzt. Ihre systematische Stellung wird deshalb revidiert. Danach wird die Sippe nicht mehr als ökologisch abgesetzte Unterart von *L. loeselii* bewertet, sondern als geographisch isoliertes Taxon zu *L. kumokiri* gestellt und hier zu *Liparis kumokiri* subsp. *nemoralis* umkombiniert.

## Riassunto

Perazza, G. & C. Tsutsumi (2015): Considerazioni su *Liparis loeselii* s.l. (*Orchidaceae*) in Europa in relazione all'est-asiatica *Liparis kumokiri*.- J. Eur. Orch. 47 (2-4): 309-322.

In base ai caratteri morfologici e all'analisi molecolare della regione ITS del DNA, *Liparis loeselii* subsp. *nemoralis*, recentemente descritta nel nord-est-Italia, si è rivelata alquanto simile all'est-asiatica *Liparis kumokiri*. La sua posizione tassonomica è qui riconsiderata: essa va valutata non più una sottospecie ecologica di *L. loeselii*, ma un taxon geograficamente isolato afferente a *L. kumokiri* e viene ricombinata come *Liparis kumokiri* subsp. *nemoralis*.

\* \* \*

## 1. Introduction

*Liparis* Rich. (1817) is a cosmopolitan genus with about 320 species, including some epiphytes and present on all continents, mainly in tropical and subtropical areas (PRIDGEON et al. 2005). Till recently a single species has been reported for the flora of Europe, *Liparis loeselii* (L.) Rich. 1817, a circumboreal entity, present in wetlands like fens or bogs with oligotrophic, neutral to alkaline drainage water from Europe to Siberia and North America (HULTÉN & FRIES 1986: 293, map 586). One variety, *L. loeselii* var. *ovata* Ridd. ex Godfery, is known from dune slacks near the Atlantic coast in France and Great Britain. Only very recently there have been added further taxa within *Liparis loeselii* s.l. In his study on the genus *Liparis* in Russia EFIMOV (2010) describes *L. loeselii* subsp. *orientalis* from Altai region (central southern Siberia) and combines *L. sachalinensis*, a “mysterious” endemic entity from southern Sakhalin Island, as *L. loeselii* subsp. *sachalinensis*.

Little later a new entity of *Liparis* has been detected in Northeast Italy and were described as *Liparis loeselii* subsp. *nemoralis* Perazza, Decarli, Filippin, Bruna & Regattin (PERAZZA et al. 2012). This detection raised interest for two reasons. Firstly, nobody expected a further entity of the genus *Liparis* in Italy and in Europe; secondarily, the entity is growing in a secondary wood, which differs from a typical habitat of *L. loeselii* (see above). The habitat of the most important (largest) population (locus classicus) has been developed from zero on the slopes above the artificial lake Vajont near Erto (PN) destroyed 50 years ago by a catastrophic tsunamilike wave caused by an enormous landslide from M. Toc into the lake, which lead to death and extinction in the whole valley.

Contemporary photographs show the total devastation of the original vegetation, where none of a blade of grass remained. After the wave only earth and gravel covered the slopes. During the spontaneous vegetational recolonization, under strong dynamics, several orchids invaded and developed stable groups, surprisingly some plants of *Liparis* too, which first have been attributed to *L. loeselii* (LASSEN 2007). This species is very rare in Italy and severely endangered (ORSENIGO et al. 2012), known only from flat bogs and mires, with very few recent sites in the northeastern regions of Italy including the lowlands of Friuli, where the species disappeared nearly everywhere (PERAZZA & LORENZ 2013). During the field research for mapping the orchids of Northeast Italy some further small groups of *Liparis* were found in woody habitats on well-drained soil covered with mosses, leaves and decaying wooden residues, characterized by elevated atmospheric humidity (with frequent falls of rain, persistent fog).

After repeated observations it became clear, that this *Liparis* “of woods” (just “*nemoralis*”) shows own and constant morphological characteristics. As these traits however were not very much different from typical *L. loeselii*, this entity has been classified as autonomous ecological subspecies and described as *Liparis loeselii* subsp. *nemoralis*. According to current knowledge it seems to be an endemic entity, restricted to the area of Friuli Venezia Giulia and Veneto. A historical site, today extinct, in Valsugana in eastern Trentino is known from the early 19th century. The specimens collected by rev. Ferdinando Paterno are kept in various herbaria, like FI, PAD, TR (PERAZZA et al. 2012).

Shortly after publication of *L. loeselii* subsp. *nemoralis* there raised evidence that it shows more morphological affinities to east-Asian *Liparis kumokiri* F. Maek. 1936 than to *L. loeselii* s.str. Therefore we further studied the morphology and the molecular phylogeny and revised its systematic position.

## 2. Materials and Methods

Apical leaf samples were collected from four sites at 2013 for molecular analysis: *Liparis loeselii* subsp. *loeselii* from Passo Durone (Trentino); *L. loeselii* subsp. *nemoralis* from Val Canzoi (Veneto), Erto (locus classicus, Friuli) and Prato Carnico (Friuli). A sample of *L. loeselii* subsp. *loeselii* from Leningrad Region, Pozharische village was also examined in Russia (collected by P. EFIMOV).





Fig. 1: *Liparis loeselii* subsp. *loeselii*, Levico, Trento, Italy, 10.06.1990, UTM<sub>WGS84</sub> 32T PR 79.86 (phot. G. Perazza). a: Plants, b: Inflorescence, c: Flower, d: Fruits.

Total DNA was extracted from silica-gel-dried materials with the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions. Procedures of amplification and sequencing of the internal transcribed spacer regions of 18S-26S nuclear ribosomal DNA (ITS) followed those in TSUTSUMI et al. (2007). Genbank accession Nos. are LC088232 for *L. loeselii* subsp. *nemoralis* from Udine, LC088231 for *L. loeselii* subsp. *nemoralis* from Pordenone and Belluno, LC088233 for *L. loeselii* subsp. *loeselii* from Italy, and LC088234 for *L. loeselii* subsp. *loeselii* from Russia.

Phylogenetic analyses were performed by Bayesian analysis using MrBayes. 3.1.2 (HUELSENBECK & RONQUIST 2001; RONQUIST & HUELSENBECK 2003). The sequence data of *Liparis* analyzed in TSUTSUMI et al. (2007) and TIAN et al. (2012) were analyzed together. MrModeltest 2.0 (NYLANDER 2004) was used to determine the nucleotide substitution model. Bayesian searches were conducted by MCMC with two independent sets of four chains, each run for ten million generations, sampling every 100 generations. Tracer (RAMBAUT & DRUMMOND 2009) was used to check the runs had reached stationarity and effective sample size of all the parameters was high (>100). The first 2.5 million generations before sufficient stationary generations were discarded as burn-in periods and the rest of trees were used to calculate posterior probabilities.

Biometric analysis was carried out in summer 2014 at the sites Erto and Val Canzoi for Italian samples. Vegetative and floral parts of 12 plants at each site were measured in situ on randomly selected plants avoiding plants growing at least five m distant from each other. For *L. kumokiri*, floral parts of five individuals widely collected in Japan were matured using fixed specimens by alcohol.

### 3. Results and Discussions

#### 3.1 Comparative morphology among *Liparis loeselii* s.str., subsp. *nemoralis* and *L. kumokiri*.

There are differences between *L. loeselii* s.str. and *L. loeselii* subsp. *nemoralis* in vegetative and reproductive morphologies. *L. loeselii* s.str. has two narrow elliptic leaves, at the base cuneate and gradually ending into a short winged petiole; leaves are greasy-shining and rather thick, with smooth margin, with conspicuous central vein and, at flowering time, remaining erect and partially convolute, apparently enfolding the inflorescence. In contrast, in *L. loeselii*

subsp. *nemoralis*, like *L. kumokiri*, the thinner leaves have an elliptic or broadly elliptic lamina, often with undulated margin, at the base more brusquely narrow up to subtruncate and confluent in a long petiole; at flowering time the leaves are not convolute, but spreading and bow-shaped. They are also distinguished by floral characters. Showy is the different position of the lateral sepals: they lie close together subparallel under the lip in *L. loeselii* s.str.; in contrast the lateral sepals in *L. loeselii* subsp. *nemoralis* are strongly divergent, somewhat enfolding the lip, like *L. kumokiri*. A comparison of the biometric data of flowers traits is shown in Table 1.

Table 1: Biometric data of *L. loeselii* subsp. *loeselii* in comparison to *L. loeselii* subsp. *nemoralis* and *L. kumokiri*.

Taxon	<i>L. loeselii</i> subsp. <i>loeselii</i>	<i>L. loeselii</i> subsp. <i>nemoralis</i>	<i>L. loeselii</i> subsp. <i>nemoralis</i>	<i>L. kumokiri</i>
Place	Durone and Levico (Trento)	Val Canzoi (Belluno)	Erto (Pordenone)	Japan
Flower stalk length (cm)	7-17	11-20(25)	(7)12-19(25)	19-35
Flowers number	2-11(19)	2-8(10)	1-9(16)	4-28
Labellum length (mm)	5-6	6.8-8	6.8-8.2	7-8
Labellum width (mm)	3-4.5	3.3-5.2	4.4-5.8(6.6)	6-7
Lateral sepal length (mm)	5-6	6.8-8	6.8-8.2	7-8
Lateral sepal width (mm)	1.4-2	2.1-2.5	2.2-2.5	2-3
Column length (mm)	3-3.5	3.5-4.2	3.8-4.0	3-5

*Liparis loeselii* s.str. has smaller flowers than *L. loeselii* subsp. *nemoralis*; the length of labellum, lateral sepals, and column is shorter in *L. loeselii* s.str. The data show that *L. loeselii* subsp. *nemoralis* fits well to the inferior limit of the range of variability of *L. kumokiri*. Furthermore, *L. loeselii* subsp. *nemoralis* has a subtrapezoidal lip, revolute both at the middle and at the distal part



(vs. subrectangular, only revolute at the middle in *L. loeselii* s.str.). This also coincides with the lip character of *L. kumokiri*. Those results indicate morphological affinity of *L. loeselii* subsp. *nemoralis* with *L. kumokiri*, not *L. loeselii*. The habitat of *L. loeselii* subsp. *nemoralis* are woods, and that of *L. kumokiri* road-sides, forest margins, and open forests.

*Liparis kumokiri* is auto-pollinated like *L. loeselii* subsp. *nemoralis*: the solid pollinia fall down from the anther versus the stigma, guided by their viscidia and by the winged, forward directed, apical sidewalls of the columns, and stick finally to the stigma, possibly favoured by small droplets of water like in *L. loeselii* s.str. (SEITÉ 2003, CLAESSENS & KLEYNEN 2011).

Several *Liparis* species in East Asia are confusing. *Liparis kumokiri* F. Maek. is described based on the specimen from Mt. Tsukuba, Ibaraki, Japan (MAEKAWA 1936), and is distributed in Japan, East Russia and Korea (SATOMI 1982). The morphology is quite close to *L. campylostalex* Rchb. f. 1877, described from East India, and now it is known to be distributed from India to Central China till Vietnam in the South and Siberia in the North, reaching also Taiwan, Korea and Japan (XINQUI et al. 2009). The species is, however, supposed to be confused with *L. kumokiri*, which is distinct from *L. campylostalex* and other relatives. Their phylogeny and morphologies will be published in another paper.

**Protologue of *Liparis kumokiri* F. Maek., Stud. Monocot. Jap. (VI): 96. 1936.**

“Planta acaulis cum scapo 15-20 cm. alta. Bulbus mediocris 1-1,2 cm. longus lateraliter subcompressus. Folia bina basi pauci-vaginantia petiolata; petiolus erecto-patens lamina brevior saepe late alatus; lamina patens elliptica vel late oblonga vel late lanceolato-oblonga 4.5-12 cm. longa 2.5-5.5 cm. lata utrinque obtusa raro apice acutiuscula margine integra vel paulum crispula utrinque 5-7 nervia, textu carnosula supra plana opaca viridula subtus concolor. Scapus folia superans angulato-alatus.

Flores 5-15, laxiusculi dilute viriduli vel purpurascens erectiusculi, bracteis minimis hyalinis subulatis, pedicello gracile cum ovario 7-10 mm longo. Sepala divaricato-patentia oblonga 5-5.5 mm. longa 2 mm. lata labello paulum superantia obtusiuscula vel obtusa basi breviter angustata 3-nervia. Petala filiformia divaricata sepalo subaequilonga. Labellum erectum supra medium subito recurvatum 4.5-5 mm. longum 3.5 mm. latum obovato-ellipticum obtusissimum versus apicem eroso-crenulatum medio vix carnosum basi late angustatum paulum unguiculatum 5 nervatum. Gynostemium breve crassum 2.5-3 mm. longum ascendentim incurvatum basi turbinatum apice prope stigmatibus utrinque anguste alatum.

Nom. Jap. *Kumokirisô*, *Kumochirisô*.

Hab. Hondo: prov. Hitachi, mt. Tsukuba (C. ÔVATARI, Jul. 13, 1895-Typus in Herb. Univ. Imp. Tokyo).”



Fig. 2 - *Liparis kumokiri* subsp. *nemoralis* (phot. G. Perazza).

a: Plant. Erto, Pordenone, Italy, 26.06.2012, UTM<sub>WGS84</sub> 33T TM 92.57.

b: Inflorescence. Prato Carnico, Udine (It), 22.06.2011, UTM 33T UM 35.24.

c: Flowers, d: Flower. Val Canzoi, BL (It), 29.06.2013, UTM 32T QS 21.70.





Fig. 3a: *Liparis kumokiri* subsp. *kumokiri*, Plants, Mt. Tsukuba, Ibaraki, Japan, 03.07.2006, 36.13N, 140.06E (phot. C. Tsutsumi).



Fig. 3b, c: Nishitamagun, Tokyo, Japan, 26. 06. 2005, 35.48N, 139.08E.  
b: Inflorescence, c: Flower (phot. C. Tsutsumi).

### 3.2. Molecular data and phylogeny

Molecular phylogeny showed that *L. loeselii* s.str. from Italy is close to *L. loeselii* from Russia, and they formed a monophyletic group with American *L. liliifolia* (Fig. 4). In contrast, *L. loeselii* subsp. *nemoralis* is quite close to *L. kumokiri* and relatives, and they are far from *L. loeselii* and *L. liliifolia*. Those results also suggested that *L. loeselii* subsp. *nemoralis* is closer to *L. kumokiri*, not *L. loeselii*. Preliminary molecular data showed that *L. loeselii* subsp. *nemoralis* is not close to samples reported as *L. campylostalyx* and *L. japonica* from China (data not shown).

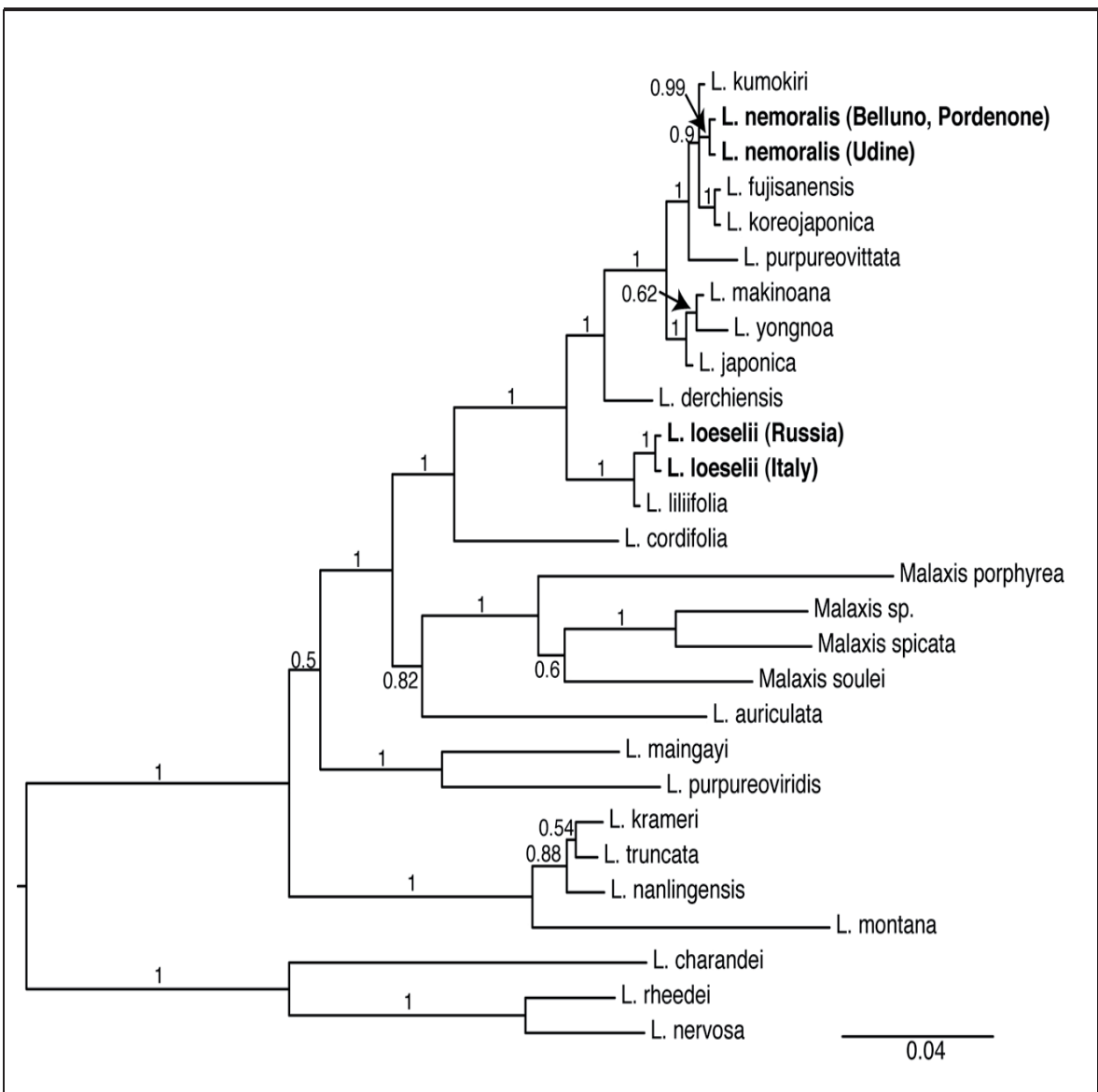


Fig. 4. Bayesian consensus tree based on ITS region (843 bp). Values above branches indicate posterior probabilities (>0.9) calculated by Bayesian analysis.





Fig. 5: *Liparis kumokiri* subsp. *kumokiri*, habitat. Mt. Tsukuba, Ibaraki, Japan, 03.07.2006 (phot. C. Tsutsumi).





Fig. 6: *Liparis kumokiri* subsp. *nemoralis*, analisi di foglia e spiga fiorale, Erto, Pordenone, Italy, 28.06.2014 (phot. G. Perazza).

### 3.3. History and distribution of *Liparis loeselii* subsp. *nemoralis*

Somewhat mysterious appears its geographic isolation. As far as currently known the small area of *L. loeselii* subsp. *nemoralis* extends linearly for only about 140 km from the historical site Tezze di Grigno (Trento) to the northeasternmost site in Val Raccolana (Udine), last site detected by the couple Boemo (2014 com. pers. ined.) resulting in a expressed remote position. How may be interpreted such a unique disjunction so far away from the main area of similar entities? The distance from *L. kumokiri* in Korea is about 10,500 km.

Could *L. loeselii* subsp. *nemoralis* be an adventitious species imported with seeds on the occasion of extraordinary freak weather or introduced by men? Last possibility may not be excluded because *L. kumokiri* is one of the popular orchids in Japan and sometimes sold in flower pots. If this really would be the case, then, considering the herbaria specimens of *L. loeselii* subsp. *nemoralis* collected two centuries ago and its presence in several valleys along a line of about 140 km, it must happened long time ago. The close relationships with *L. loeselii* subsp. *nemoralis* and Japanese *L. kumokiri* supports the hypothesis by P. Efimov (in litt.) that it could be a relict entity (paleoendemite), not a case of evolutionary convergence from *L. loeselii*.

#### 4. Taxonomical consequence

Both morphological and molecular data showed that *L. loeselii* subsp. *nemoralis* has more similarities with *L. kumokiri* than *L. loeselii*. But *Liparis loeselii* subsp. *nemoralis* has smaller flowers than those of *L. kumokiri* and has also smaller leaves and stem. Consequently, considering also the geographic isolations of their distributions, we revise *L. loeselii* subsp. *nemoralis* as follows:

***Liparis kumokiri* F. Maek. subsp. *nemoralis*** (Perazza, Decarli, Filippin, Bruna & Regattin) Perazza & Tsutsumi **comb. nov.**

Basionym: *Liparis loeselii* (L.) Rich. subsp. *nemoralis* Perazza, Decarli, Filippin, Bruna & Regattin, J. Eur. Orch. 44 (3): 489. 2012.

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