Research paper

Three new species of *Liparis* s.l. (Orchidaceae: Malaxideae) from Southwest China based on morphological characters and phylogenetic evidence

Ji-Dong Ya, Dong-Liang Lin, Zhou-Dong Han, Lei Cai, Zhi-Rong Zhang, De-Ming He, Xiao-Hua Jin, Wen-Bin Yu

* Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Lanhei Road 132, Heilongtan, Kunming, Yunnan, 650201, China
* State Key Laboratory of Systematic and Evolutionary Botany and Herbarium, Institute of Botany, Chinese Academy of Sciences, Nanxin-cun20, Xiangshan, Beijing, 100093, China
* CAS Key Laboratory of Systematic and Evolutionary Botany and Herbarium, Institute of Botany, Chinese Academy of Sciences, Nanxin-cun20, Xiangshan, Beijing, 100093, China
* University of Chinese Academy of Sciences, Beijing, 100049, China
* Yunnan Key Laboratory for Integrative Conservation of Plant Species with Extremely Small Populations, Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, 650201, China
* Wenshan National Nature Reserve Administration, Wenshan, Yunnan, 663000, China
* Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Mengla, Yunnan, 666303, China
* Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Mengla, Yunnan, 666303, China
* Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezi, Nay Pyi Taw, 05282, Myanmar

** Corresponding author. Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Mengla, Yunnan 666303, China. E-mail addresses: xiaohuajin@ibcas.ac.cn (X.-H. Jin), yuwenbin@xtbg.ac.cn (W.-B. Yu).

Peer review under responsibility of Editorial Office of Plant Diversity.

1 These authors contributed equally to this work.

1. Introduction

*Liparis* (Orchidaceae) consists of more than 350 species, with about 63 species in China (Chen et al., 2009, Kew science, 2019; Pridgeon et al., 2005). *Liparis* are terrestrial, lithophytic, or epiphytic herbs, and widespread around the world, mainly distributed in tropical and subtropical Asia, Africa and South America (Chen et al., 2009, Pridgeon et al., 2005).

The generic delimitation of *Liparis* has been hotly debated recently. Phylogenetic studies of Malaxideae showed that *Liparis* s.l. based on the traditional morphological delimitations was polyphyletic (Cameron, 2005; Margolies et al., 2012; Tang et al., 2015). To date, *Liparis* has been divided into two major clades, i.e., one terrestrial clade with conduplicate or plicate leaves close to *Malaxis* Sol. ex Sw.; and the other epiphytic clade with duplicate or compressed leaves nested with *Oberonia* Lindl. The generic
delimitation of Malaxideae needs to clarify using a broad sampling across the tribe (Cameron, 2005; Tang et al., 2015). In this study, therefore, we tentatively followed the traditional delimitation of Liparis proposed in Genera Orchidacearum (Pridgeon et al., 2005).

Recently, seven new species of Liparis from China have been described (Tian et al., 2012, 2016; Li and Yan, 2013; Su et al., 2015; Fan et al., 2017; Huang et al., 2018; Li et al., 2019). During our field survey in southwestern China, three little known species of Liparis were found. After careful morphological investigation, we concluded that these three species are new to science. We inferred the phylogenetic position of these three species using nrITS and plastid matK sequences.

2. Materials and methods

2.1. Morphological observations

Specimens were collected from Yunnan, China during field expeditions from 2018 to 2020. Morphological characters of each species were observed, measured and photographed based on five living individuals. Morphological photographs of the lip, column and pollinia were taken under an Olympus SZX16 stereozoom microscope. All voucher specimens were deposited at the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN) and the Herbarium (PE), Institute of Botany, Chinese Academy of Sciences.

2.2. Taxonomic sampling

To clarify phylogenetic relationships of the three new species with other Liparis species, nrDNA ITS and plastid matK were sequenced or downloaded from GenBank for phylogenetic analyses. A total of 71 accessions representing eight genera were analyzed, six of which belong to tribe Malaxideae and Eulophia graminea Lindl. from tribe Cymbidieae was used as outgroup. GenBank accession numbers are listed in Table 1.

2.3. Phylogenetic analyses

Bayesian inference (BI) and Maximum Likelihood (ML) methods were used to reconstruct the phylogenies. Parameters for the two analyses followed previous studies (Yu et al., 2013, 2015). The BI analysis was performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003). The total data set was partitioned, and DNA substitution model of Bayesian information criterion (BIC) for two of DNA regions was estimated using jModeltest 2 (Darriba et al., 2012). Markov chain Monte Carlo (MCMC) analysis was performed using MrBayes for 10,000,000 generations for the total data set, with two simultaneous runs, and each run comprising four incrementally heated chains. BI analysis was started with a random tree and sampled every 1000 generations. ML analysis was conducted with RAxML 8.2.10 (Stamatakis et al., 2008) using the GTR substitution model with gamma-distributed rate heterogeneity among sites and the proportion of invariable sites estimated from the data. Support values for nodes/clades were estimated from 1000 bootstrap replicates.

3. Results and discussion

3.1. Phylogenetic analyses

The ITS matrix was 780 bp in length including 457 variable sites and 357 parsimony-informative sites. The matK matrix was 593 bp in length including 183 variable sites and 104 parsimony-informative sites. The best-fit BIC model of ITS and matK data sets was GTR + 1 + G and TPM1uf + G, respectively. The major-rule consensus tree of both BI and ML analyses with support values is shown in Fig. 1.

Phylogenetic analyses indicate that six genera from tribe Malaxideae are monophyletic; in contrast, species of Liparis are not
monophyletic, but instead are nested with species from five other genera (Fig. 1). Furthermore, phylogram showed that *Liparis aureolabella* J.D. Ya & Z.D. Han and *L. mengziensis* J.D. Ya & Lei Cai are nested within the terrestrial *Liparis* clade of *Liparis s.s.* including the type of *Liparis*, *Liparis loeselii* (L.) Rich., which is sister to *Malaxis s.s.* with the type *Malaxis spicata* Sw. Two of the new species are nested with *Liparis auriculata* Blume ex Miq. and *Liparis petiolata* (D. Don) P.F. Hunt & Summerh (Fig. 1). *L. mengziensis* is sister to *L. petiolata*, followed by *L. auriculata*, and *L. aureolabella* is sister to the above three species. *Liparis bingzhongluoensis* is nested within the second terrestrial *Liparis* clade of *Liparis s.l.*, which is monophyletic and includes four other species: *Liparis nanlingensis* H.Z. Tian & F.W. Xing.

![Fig. 1. Phylogeny inferred from nrITS and plastid matK DNA sequence data. BI posterior probabilities and ML bootstrap values are presented above branches. The bottom scale bar represents the number of substitutions per site.](image-url)
Liparis tsii H.Z. Tian & A.Q. Hu, Liparis sasakii Hayata and Liparis krameri Franch. & Sav. This clade is sister to a particular clade that has only one species Liparis meihuashanensis S.M. Fan.

3.2. Morphological comparisons

Morphological investigations revealed that the three new terrestrial species belong to Liparis s.l., and are characterized by racemose inflorescences, resupinate lips without spur, column without a conspicuous foot, and 4 pollinia without caudicle or viscidium (Chen et al., 2009). *L. aureolabella* J.D. Ya & Z.D. Han is a distinctive species in Liparis and is characterized by having conspicuously raised reticulate veins on the adaxial side of leaf blade and lip with an auriculate base (Table 2). *L. mengziensis* J.D. Ya & Lei Cai is similar to *L. petiolata* in having slender rhizomes and broadly ovate leaves. However, *L. mengziensis* just has only small leaf in the flowering plant, and the purple and reflexed corolla lip united at the tip. Additionally, *L. mengziensis* is also similar to *L. auriculata*, but it can be distinguished from it by its slender rhizomes, as well as having a single small leaf, lip purple, and an apex conuate along the margins (Table 2). Geographically, *L. auriculata* is found only in Taiwan of China, Central and South Japan, and South Korea (Chen et al., 2009; Chang et al., 2014), and *L. aureolabella*, *L. mengziensis* and *L. petiolata* have overlapped distribution in South Yunnan. *L. bingzhongluoensis* X.H. Jin is closely related to *L. nanlingensis* by sharing purplish-red flowers, lip longitudinal concave in the middle cuspidate with a tail (see Tian et al., 2012), but it differs from the latter by having lip with two transparent ridges on disc, basal callus longitudinally concave and a pair of triangular column wings.

3.3. Conservation status

During our field surveys, two populations of Liparis aureolabella were found in the Mengzi County and Wenshan County, Yunnan, and only one population of *L. mengziensis* was found in the Mengzi County, Yunnan. It seems that these two new species could also grow in other limestone mountains in South Yunnan and the adjacent regions, whereas more field surveys are needed in the future. One population of *L. bingzhongluoensis* was discovered in China, and two populations in Myanmar, respectively. Therefore, we regarded the conservation status of the three new species as Data Deficient at this time (IUCN 2012).

4. Taxonomic treatments

4.1. Liparis aureolabella J.D. Ya & Z.D. Han, sp. nov. (紫脉羊耳蒜) (Fig. 2)

4.1.1. Diagnosis

*Liparis aureolabella* is characterized by the adaxial side of leaf blade having conspicuously raised reticulate veins, the base of lip having auriculate appendage on each side, and falcate-lanceolate pollinia.

4.1.2. Type

CHINA. Yunnan: Mengzi, subtropical, limestone mountainous area, 1990 m, 27 Jun 2018, J.-D. Ya & Z.-D. Han 18HT1878 (holotype: KUN!)

4.1.3. Distribution and habitat

*Liparis aureolabella* is currently known only from Mengzi County and the adjacent Wenshan County, southern Yunnan, China. It is a predominantly terrestrial species that grows in limestone mountainous area at an elevation of 1990 m a.s.l.

Leaves 2, petiole narrow in amplexical sheath at base, 2.0—3.0 cm long, not articulate; blade ovate-elliptic, glabrous, 2.5—4.7 × 1.3—2.5 cm, adaxial dark green, abaxial purple, margin entire or sometimes undulate, apex acuminate; veins purple, main vein obviously concave adaxial and conspicuously raised abaxial, reticulate veins conspicuously raised adaxial. Inflorescence 7.0—10.0 cm long; rachis with 8—12-flowered; floral bracts lanceolate, 6.0—8.0 mm long, peduncle and ovary ca. 10 mm long. Flowers full open, Sepals yellowish green and purple at base, petals and lip purple. Dorsal sepal narrowly lanceolate, margin revolute to cylindric, 4.5—6.0 × 0.8—1 mm, apex obtuse; lateral sepals lanceolate-oblong, strongly curved backward.

### Table 2

Morphological comparison of Liparis aureolabella, *L. mengziensis* and their closely related species.

| Characters          | Liparis aureolabella | *L. mengziensis* | *L. petiolata* | *L. auriculata* |
|---------------------|----------------------|-----------------|---------------|-----------------|
| Rhizomes            | without              | slender         | slender       | without         |
| Pseudobulbs         | ovoid                | ovoid           | ovoid         | ovoid           |
| Leaf blade          | 0.8—1.2 × 0.5—0.6 cm| 0.9—1.1 × 0.6—0.8 cm | 0.9—1.0 × 1.0—1.5 cm | 0.9—1.0 × 1.0—1.5 cm |
| Leaves              | 2 leaves             | 1 leaf          | 2 leaves      | 2 leaves        |
| Petiole             | 2.0—3.0 cm           | 2.0—3.5 × 3.0—4.5 cm | 2.5—3.0 × 3.0—4.5 cm | 2.0—3.5 × 3.0—4.5 cm |
| Scape               | 7.0—10.0 cm          | 10.0—14.0 cm    | 10.0—24.0 cm  | 10.0—24.0 cm    |
| Lateral sepals      | 8—12-flowered        | 2 to 10-flowered | 2 to 10-flowered | 6 to more than 10-flowered |
| Pedicel and ovary   | lanceolate           | lanceolate      | lanceolate    | lanceolate      |
| Pedicel length      | ca. 1.0 cm           | 0.6—0.8 cm      | 0.6—0.8 cm    | 0.5—0.6 cm      |
| Dorsal sepal        | 4.5—6.0 × 0.8—1.0 mm | 4.5—5.0 × 0.8—1.0 mm | 8.0 × 1.8 mm  | 6—7 × 1.5—2 mm, |
| Petals              | 4.0—5.0 × 0.3—0.4 mm | 5.0—6.0 × 0.3—0.4 mm | 7.0—8.0 × ca. 0.4 mm | ca. 6 × 0.5 mm   |
| Lip                 | purple               | purple          | purplish green| greenish, purplish, or deep purplish red |
|                    | oblong               | elliptic        | elliptic to orbicular | orbicular or orbicular |
|                    | ca. 8.0—9.0 × 5.0—6.0 mm | ca. 7.0—8.0 × 3.0—3.5 mm | ca. 10 × 8.0—9.0 mm | ca. 10 × 8.0—9.0 mm |
|                    | a deeply colored central longitudinal band | 2 calli near base | 2 calli near base | 2 subtriangular small calli near base |
| Base                | auriculate, margin entire, apex subtruncate and mucronate | reflexed and conuate along upper, margin often slightly irregularly incised, apex mucronate | margin often slightly irregularly incised, apex mucronate | apex rounded or sometimes apiculate |
|                    |                     |                 |               |                 |
1.8–2.2 mm, apex obtuse; petals narrowly linear, margin revolute to cylindric, 4.0–5.0 × 0.3–0.4 mm; lip oblong, ca. 8.0–9.0 × 5.0–6.0 mm, with a deeply colored central longitudinal band, without a callus, base auriculate and clasping column, apex subtruncate and mucronate. Column slightly curved, without wings; anther cap oblong; pollinia 4 in 2 pairs, 0.40–0.25 mm, yellow, falcate-lanceolate, waxy. Fl. June–July.
4.1.4. Additional specimen examined (paratypes)
CHINA. Yunnan: Wenshan, subtropical, limestone mountainous area, 1500 m, 23 July 2020, J.-D. Ya, D.-M. He, M.-J. Feng & M.-Q. Han 20CS19138 (paratype: KUN!).

Herbs, terrestrial. Plant 8.5–12.5 cm tall. Pseudobulbs clustered, ovoid, 0.8–1.2 × 0.5–0.6 cm.

4.1.5. Etymology
The specific epithet "aureolabella" refers to the base of corolla lip having a pair of auriculate appendages in this new species.

4.2. Liparis mengziensis J.D. Ya & Lei Cai, sp. nov. (蒙自羊耳蒜) (Fig. 3)

4.2.1. Diagnosis
Liparis mengziensis is similar to L. petiolata (D.Don) P.F. Hunt & Summerh. and L. auriculata Blume ex Miq., but differs from them by having one smaller and ovate to broadly ovate leaf, and purple and reflexed corolla lip united at the tip of the lip.

4.2.2. Type
CHINA. Yunnan: Mengzi, subtropical, limestone mountainous area, 1990 m, 21 Jul 2018, J.-D. Ya & Lei Cai 18HT1992 (holotype: KUN!).

Herbs, terrestrial. Plant 10.0–15.0 cm tall, with slender rhizomes. Pseudobulbs 0.5–1.5 cm apart on rhizome, ovoid, sometimes compressed, 0.9–1.1 × 0.6–0.8 cm. Leaves 1, petiole sheathlike, 1.5–4 cm long, amplexicaul, not articulate, base enclosed by 2–3 sheaths, 1.1–2.5 cm long; blade ovate to broadly ovate, 2.0–3.5 × 3.0–4.5 cm, herbaceous, base subtruncate or shallowly cordate, margin irregularly obtuse-crenate or nearly entire, apex subacuminate or shortly cuspidate. Inflorescence 10.0–14.0 cm long, lower part ridged, upper part narrowly winged; rachis 7–10-flowered; floral bracts lanceolate, 3.0–4.0 × 0.8–1.0 mm, pedicel and ovary 5.0–6.0 mm long. Flowers full open, Sepals yellowish-green, petals purple and yellowish-green at tip, lip purple. Dorsal sepal narrowly lanceolate, margin revolute to cylindrical, 4.5–5.0 × 0.8–1 mm, apex obtuse; lateral sepals lanceolate-oblong, strongly curved backward,
5.5—6.0 × 1.5—2.0 mm, apex obtuse; petals narrowly linear, margin revolute to cylindric, 5.0—6.0 × 0.3—0.4 mm; lip elliptic, 7.0—8.0 × 3.0—3.5 mm, with 2 calli near base, reflexed and connate along upper, margin often slightly irregularly incised, apex mucronate. Column arcuate, ca. 3.5 mm long, base thickened, apex enlarged and winged. Capsule subobovoid-oblong, ca. 8.0 × 3.5 mm; fruiting pedicel ca. 2.0 mm long. Fl. Jun—Jul.

4.2.3. Distribution and habitat
Only one population of Liparis mengziensis was found at the type locality of Mengzi County, southern Yunnan, China. It is a predominantly terrestrial species that grows on limestone mountains at an elevation of 1990 m a.s.l.

4.2.4. Etymology
The specific epithet “mengziensis” refers to the type locality Mengzi County.

4.3. Liparis bingzhongluoensis X.H. Jin, sp. nov. (Fig. 4 and 5)

4.3.1. Diagnosis
Liparis bingzhongluoensis is similar to L. nanlingensis H.Z. Tian & F.W. Xing but differs from the latter by having two transparent ridges in disc, lip with long tail and a basal longitudinally concave callus, column wings triangular.

4.3.2. Type
CHINA: Yunnan: Gongshan County, Bingzhongluo town, Gaoligong mountain, 2400 m, 22 Jun 2020, X.-H. Jin 31205 (holotype: PE!, isotypes: PE!).

Epiphytic, lithophytic, or terrestrial. Pseudobulb ellipsoid, green, ca. 8.3—12.5 × 5.2—8.0 mm, enclosed by white membranous sheaths. Leaves 2; petiole ca. 1.2—2.2 × 0.2—0.4 cm, enclosed by a few sheaths; blade ovate, ca. 1.8—2.5 × 0.5—1.2 cm, acute, margin wavy, lateral veins obvious on surface. Inflorescence 3.0—4.3 cm long, winged, quadrate in section; rachis 0.4—1.7 cm long, 2—4-flowered; bracts lanceolate, ca. 1.5 mm long. Flowers purplish red to purplish green; pedicel and ovary purplish, pedicel and ovary ca. 5.2 mm long. Dorsal sepal linear-lanceolate, 8.5 × 1.4 mm, apex acute, margin revolute; lateral sepals falcate, ca. 10.8 × 1.4 mm, basal margin revolute, apex acute. Petals filiform, ca. 7.4 × 0.3 mm, obtuse, deflexed to pedicel and ovary. Lip oblong, ca. 6.2 × 3.0 mm, reflexed in the middle, longitudinally grooved, two transparent arising along the groove, apex papillose with a tip for ca. 1.1 mm, minutely white ciliate; basal callus oblong, longitudinally concave, ca. 0.7 mm long. Column terete, arcuate, ca. 4 mm long, base slightly dilated, slightly winged on each side; stelidia two, triangular; anther cap purplish, ovate, obtuse, ca. 0.5 mm long; pollinia 4 in two pairs, yellow, waxy, ovoid, ca. 0.3 mm long. Capsule ellipsoid, ca. 4 mm long.

4.3.3. Distribution and habitat
Liparis bingzhongluoensis grows under montane broadleaf evergreen forest at elevations of 2250—3100 m. During our field investigations Bingzhongluo Town, Gongshan, Nuijiang, Yunnan in 2020, a population was discovered. At least two additional populations were discovered in Mountain Victoria, Chin State and Hphokongrazi Nature Reserve, Kachin State, Myanmar.

4.3.4. Additional specimens examined (paratypes)
MYANMAR. Kachin State: Putao District, Hphokongrazi, 2260 m, 2016-06-16. X.-H. Jin PT-2221 (paratypes: PE!).

4.3.5. Etymology
The specific epithet “bingzhongluoensis” refers to the type locality Bingzhongluo Town, Gongshan, Nuijiang, Yunnan.

Declaration of competing interest
There is no conflict of interest.
Acknowledgments

This study was financially supported by National Forestry and Grassland Administration (2019073019,2019073003,2019073002), National Science & Technology Infrastructure, the Large-scale Scientific Facilities of the Chinese Academy of Sciences (2017-LSFGBOWS-02), Science and Technology Basic Resources Investigation Program of China “Survey and Germplasm Conservation of Plant Species with Extremely Small Populations in South-west China” (2017FY100100), grants from Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (Grant No. Y4ZK111B01), the National Natural Science Foundation of China (Grant Nos. 31670194, 31870195), and the CAS 135 Program (2017XTBG-T03). Yun-Xi Zhu is thanked for his outstanding illustrations.

References

Cameron, K.M., 2005. Leave it to the leaves: a molecular phylogenetic study of Malaxideae (Epidendroideae, Orchidaceae). Am. J. Bot. 92, 1025–1032.

Chang, C.S., Kim, H., Chang, K.S., 2014. Provisional Checklist of Vascular Plants for the Korea Peninsula Flora (KPF) (Version 1.0), p. 561.

Chen, S.C., Ormerod, P., Wood, J.J., 2009. In: Wu, Z.G., Raven, P.H., Hong, D.Y. (Eds.), Liparis in, Flora of China, vol. 25. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp. 211–228.

Darriba, D., Taboada, G.L., Doallo, R., et al., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9, 772.

Fan, S.M., Liu, J.F., Zhai, J.W., et al., 2017. Liparis meihuashanensis, a new orchid species from Fujian, China: evidence from morphological and molecular analyses. Phytotaxa 323, 182–188.

Huang, H.X., Chen, L.J., Liu, Z.J., et al., 2018. Liparis vivipara (Orchidaceae: Malaxideae), a new species from China: evidence from morphological and molecular analyses. Phytotaxa 351, 289–295.

IUCN, 2012. IUCN Red List Categories and Criteria Version 3.1. Ed. 2. IUCN, Cland, Switzerland and Cambridge, UK.

Kew science, 2019. Plants of the world online. Available online: http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:331229-2. (Accessed 17 June 2019).

Margońska, H.B., Kowalkowska, A.K., Gorniak, M., et al., 2012. Taxonomic Redefinition of the Subtribe Malaxidinae (Orchidales, Malaxideae). Koeltz Scientific Books, Germany, pp. 9–24.

Su, Y.Y., Huang, Y.L., Chen, L.J., et al., 2015. Liparis wenshanensis, a new orchid species from China: evidence from morphological and molecular analyses. Phytotaxa 204, 253–264.