Liparis macrosepala (Orchidaceae), a new species from southwest China with its phylogenetic position

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Abstract

A new orchid species, Liparis macrosepala, is illustrated and described from Yunnan Province, China, based on morphological and molecular analyses. This plant is characterised by the ovoid-fusiform, slightly compressed pseudobulbs with 4 or 5 leaves with slightly crisped margins on their apical half, dorsal sepal heart-shaped, lip with a bituberculate basal callus and a thickened folded lateral lobe on each side, centrally with one cavity with slightly raised margins, the column with a single pair of broadly triangular, obtuse wings. Maximum Likelihood and Bayesian Inference analyses of combined nrITS and plastid matK DNA sequences place this species in section Cestichis.

Keywords

Liparis section Cestichis, molecular phylogeny, morphology, matK, nrITS

Introduction

The genus Liparis Rich. (Epidendroideae, Malaxideae, Malaxidinae) comprises about 320 species distributed worldwide with more than 70 species in China (Pridgeon et al. 1999; Chen et al. 2009; Tian et al. 2015; Huang et al. 2018; Ya et al. 2021). Species
from this genus are terrestrial, lithophytic, epiphytic and rarely mycoheterotrophic, with inflorescences laxly or densely many-flowered, lip often reflexed and usually with a basal callus, lacking a spur, column winged at apex and sometimes at base and four pollinia in two pairs (Chen et al. 2009).

During our field surveys in Xishuangbanna, Yunnan, China, an unknown species was found. In this paper, we analysed the morphological differences of the newly-found species and its allied species and the phylogenetic position of the new entity is also discussed, based on molecular evidence from nrITS and plastid matK. After careful morphological comparison and phylogenetic analyses, we concluded that this species is new to science.

**Material and method**

**Morphological observations**

Materials of the new species were collected from Xishuangbanna, Yunnan, China during a field expedition. Morphological characters were observed, measured and photographed based on five living individuals under a stereomicroscope (SZX16-6151, Olympus, Japan) and photographed with a digital camera (D750, Nikon, Japan). A voucher specimen, designated as the holotype, was deposited at Shanghai Chenshan Herbarium (CSH). Conservation assessment has been conducted following IUCN guidelines (IUCN 2019).

**Taxonomic sampling**

DNA sequences of nrDNA ITS and plastid matK of the new species were sequenced and sequences of the same markers for 82 related species were downloaded from GenBank, including five outgroup species from other subtribes (Table 1).

**Phylogenetic analyses**

DNA sequences were aligned using the MAFFT programme in Geneious v. 2020.2.4 (https://www.geneious.com, accessed on 10 March 2021). Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) in RAxML v.7.0.4 (Stamatakis 2006) and MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012), respectively. The appropriate DNA substitution model under AIC criteria was estimated using jModelTest 2.1.10 (Posada 2008). ML analyses were conducted with bootstrap values calculated by running 1,000 replicates. For BI analysis, four chains were run with random initial trees, each for 1,000,000 generations, until the average standard deviation of the split frequency values was less than 0.01 to ensure convergence, sampling trees every 1,000 generations. After the first 20% of samples were discarded as burn-in, the remaining replicates were used to estimate the posterior probabilities.
A new orchid species of *Liparis*

**Table 1. Taxon sampling in this study.**

| Species Name                          | nrITS    | matK       |
|---------------------------------------|----------|------------|
| 1. Acanthophippium mantinianum L.     | AF521081 | AF263618   |
| 2. Collabium simplex Rchb.f.          | EF670387 | AY557200   |
| 3. *Crepidium acuminatum* (D.Don) Szlach. | KJ459274 | KJ459304   |
| 4. *Crepidium bahanense* (Hand.-Mazz.) S.C.Chen & J.J.Wood | MH116611 | MH117500   |
| 5. *Crepidium bancanoides* (Ames) Szlach. | AB290885 | AB290893   |
| 6. *Crepidium brevidentatum* (Schwein.) M.A.Clem. & D.L.Jones | AB290886 | AB290894   |
| 7. *Crepidium resupinatum* (G.Forst.) Szlach. | JN114483 | JN004403   |
| 8. *Dendrobium dixanthum* Rchb.f.     | KY966535 | KY966825   |
| 9. *Dienia cylindrostachya* Lindl.    | MH768269 | MH767976   |
| 10. *Eria ferruginea* Lindl.         | AF521071 | AF263660   |
| 11. *Eulophia graminea* Lindl.       | MH768269 | MH767976   |
| 12. *Liparis macrosepala* Z.W. Wang, Y. Zhang & W.C. Huang | ON642332 | ON642331   |
| 13. *Liparis anopheles* J.J.Wood      | AY907075 | AY907139   |
| 14. *Liparis asamica* King & Pantl.   | KJ459276 | KJ459306   |
| 15. *Liparis aurorabella* J.D. Ya & Z.D. Han | MN065679 | MN065733   |
| 16. *Liparis auriculata* Blume ex Miq. | AB289458 | KF262076   |
| 17. *Liparis balansae* Gagnep.-1      | KF589874 | KF589880   |
| 18. *Liparis balansae* Gagnep.-2      | KJ459277 | KJ459307   |
| 19. *Liparis bingzhongluoensis* X.H. Jin | MW169041 | MW169042   |
| 20. *Liparis bistriata* E.C.Parish & Rchb.f. | KJ459279 | KJ459309   |
| 21. *Liparis bootanensis* Griff       | KJ459280 | KJ459310   |
| 22. *Liparis bracteata* T.E.Hunt      | AY907076 | AY907140   |
| 23. *Liparis brunnenecum* Schltr.     | AY907098 | AY907165   |
| 24. *Liparis cordifolia* Hook.f.      | AY907098 | AY907144   |
| 25. *Liparis delicatula* Hook.f.      | KJ459282 | KJ459312   |
| 26. *Liparis distans* C.B.Clarke      | KJ459283 | KJ459313   |
| 27. *Liparis disticha* (Thuons) Lindl. | AY907081 | AY907145   |
| 28. *Liparis elliptica* Wight         | KJ459285 | KJ459315   |
| 29. *Liparis fiscalabris* Tang & F.T.Wang | KJ459286 | KJ459316   |
| 30. *Liparis fissifera* Finet         | KJ459287 | KJ459317   |
| 31. *Liparis formosana* Rchb.f.       | AY907082 | AY907147   |
| 32. *Liparis fujianensis* F.Maek. ex Konta & S.Matsumoto | EU024936 | EU024937   |
| 33. *Liparis gibba* Finet-1           | AY907083 | AY907148   |
| 34. *Liparis gibba* Finet-2           | AY907084 | AY907149   |
| 35. *Liparis glaucescens* Rchb.f.     | KJ459289 | KJ459319   |
| 36. *Liparis guangxiensis* C.L.Feng & X.H.Jin | KF589875 | KF589881   |
| 37. *Liparis japonica* (Miq.) Maxim.  | AY907086 | AY907151   |
| 38. *Liparis koreana* (Nakai) Nakai   | EU017422 | EU017444   |
| 39. *Liparis kunokiri* F.Maek.        | AY907087 | AY907152   |
| 40. *Liparis latifolia* Lindl.        | AY907088 | AY907153   |
| 41. *Liparis latilabris* Rolfe        | KJ459291 | KJ459321   |
| 42. *Liparis liliifolia* (L.) Rich. ex Lindl. | AY907090 | AY907156   |
| 43. *Liparis longii* (L.) Rich.       | AY907091 | AY907157   |
| 44. *Liparis makinoana* Schltr.       | EU017405 | EU017428   |
| 45. *Liparis manu* Rchb.f.            | KJ459293 | KJ459323   |
| 46. *Liparis meihuashanensis* S.M.Fan | MFS95772 | MFS95773   |
| 47. *Liparis mengzienii* J.D. Ya & Lei Cai | MN065734 | MN065678   |
| 48. *Liparis nanlingensis* H.Z.Tian & F.W.Xing | AB701346 | /           |
| 49. *Liparis napoensis* L.Li, H.F.Yan & S.J. Li-1 | MT012899 | MT019986   |
| 50. *Liparis napoensis.Li, H.F.Yan & S.J. Li -2 | MT012900 | MT019987   |
| 51. *Liparis nervosa* (Thunb.) Lindl. | AY907092 | AY907158   |
| 52. *Liparis negentiae* E.M.Bailey     | AY907093 | AY907159   |
| 53. *Liparis odonata* (Wild.) Lindl.   | KJ021033 | KJ021029   |
| 54. *Liparis pandurata* Ames           | AY907094 | AY907160   |
| 55. *Liparis pauliana* Hand.-Mazz.     | AY907096 | AY907163   |
Results

Phylogenetic analyses

The length of nrITS matrix was 792 bp including 262 parsimony-informative sites and for matK, the length and parsimony-informative sites were 1443 bp and 120, respectively. Both analyses (MP and BI) recovered similar relationships. The ML tree with bootstrap percentages, on which the posterior probabilities from the BI analysis were also indicated, is shown in Fig. 1.

The phylogenetic analyses indicate that Liparis is not monophyletic, being mingled with species of other genera of Malaxideae. This result agrees with what was found in previous studies (Cameron 2005; Margońska et al. 2012; Tang et al. 2015; Li et al. 2020; Kumar et al. 2022). The new species, henceforth referred to as Liparis macrosepala Z.W. Wang, Y. Zhang & W.C. Huang, is grouped with species in Liparis sect. Cestichis Thouars ex Pfitzer as the sister of a clade consisting of L. delicatula Hook.f., L. fissipetala Finet, L. assamica King & Pantl. and L. resupinata Ridl.

Morphological comparisons

Liparis is defined as species with racemose inflorescences, resupinate lip lacking a spur, column without a conspicuous foot and four pollinia in two pairs with small viscidium, but no caudicle. The morphology of Liparis macrosepala is in
A new orchid species of Liparis

Figure 1. Maximum Likelihood tree of Liparis and its allied genera in subtribe Malaxidinae inferred from the combined analysis of nrITS and matK. ML bootstrap values (ML BP)/Bayesian posterior probabilities (PP) are indicated above the branches, respectively. The sectional taxonomy of Liparis follows Garay and Romero-Gonzalez (1999) and Li et al. (2020).
accordance with the characteristics of sect. *Cestichis* like the slightly flattened, narrowly winged rachis with alternating bracts. The morphological characters can distinguish *Liparis macrosepala* from its close relatives *L. delicatula*, *L. fissipetala*, *L. assamica* and *L. resupinata*.

**Taxonomic treatment**

*Liparis macrosepala* Z.W. Wang, Y. Zhang & W.C. Huang, sp. nov.

urn:lsid:ipni.org:names:77306143-1

Figs 2, 3

**Chinese name:** 大萼羊耳蒜

**Type.** CHINA. Yunnan Province (云南), Xishuangbanna (西双版纳), Mengla County (勐腊县) epiphyte on the tree trunk, 1620 m elev., 23 Nov 2021, Zhengwei Wang, Xiaochen Li, Yu Zhang & Zhijin Wu, WZW04247 (holotype: CSH!)

**Diagnosis.** *Liparis macrosepala* is characterised by the ovoid-fusiform, slightly compressed pseudobulbs with 4 or 5 alternate leaves on their apical half, these with slightly crispate margins, dorsal sepal ovate with cordate base, broadly elliptic, ca. 4 mm long, 2 callus-shaped and thickened folds, base with 2 oblong lobes on both sides, centrally with 1 thickened, concave callus, column with a single pair of arcuate wings.

Epiphytic herbs. Roots slender, flexuose. Pseudobulbs clustered, ovoid-fusiform, slightly compressed laterally, 1–2 × 0.5–1 cm, upper half with 4–5 widely spaced leaves. Leaf blade ovate-oblong, 1.8–2.3 × 0.8–1.2 cm, apex acuminate, base contracted into a short petiole, articulate, margins of their apical half slightly crispate. Peduncle 7–10 cm long, with several sterile bracts 2–5 mm long; raceme with 7–10 flowers arranged in zigzag manner. Floral bracts broadly ovate with cordate base, 2–3 × 1–1.5 mm, acute. Flowers greenish-orange; pedicel and ovary ca. 7 mm long. Dorsal sepal broadly ovate with cordate base, 3.2–5 × 3–3.6 mm, 1-veined, abaxially carinate, apex acute; lateral sepal oblong-ovate or ovate-lanceolate, 5–6 × ca. 0.6 mm long, abaxially slightly carinate. Petals narrowly linear, 3–4 × ca. 0.2 mm; lip elliptic, 2–3 × ca. 1 mm, apex apiculate, base bearing a bituberculate callus, then expanded on each side into a thickened, folded, rounded lobe, with 1 excavation with raised margins between the lobes. Column straight, ca. 2 mm long, with a pair of subtriangular, obtuse wings on each side near the middle and a ridge on the back of the column. Anther cap hemispherical, pale yellow; pollinia 4 in 2 pairs with one pollinium of each pair smaller than the other, waxy, brownish, with minute apical viscidium.

Phenology: Flowering in November–December.

**Distribution and habitat.** It is found on tree trunks on a limestone ridge-top evergreen broad-leaved forest at an elevation of 1500–1700 m in Mengna County, Xishuangbanna Autonomous Prefecture, Yunnan Province, People’s Republic of China. The habitat presents a tropical monsoon climate.
A new orchid species of *Liparis*

**Figure 2.** Morphology of *Liparis macrosepala*. A plants in situ B flowering plant C pseudobulbs and leaves D inflorescence E flowers, front view F flowers, side view G perianth dissection H column from side I lip in oblique view J anther cap and pollinia. Photographs by Weichang Huang.
Figure 3. *Liparis macrosepala*  

A flower, front view  
B flower, side view  
C lip, side view  
D inflorescence  
E column, side view  
F lip and column, side view  
G lip, back view  
H lip, front view  
I flowering plant  
J pollinia and anther cap  
K perianth dissection  
L column and ovary, oblique view  
M ovary, transection. Drawn by Lan Yan.
**Etymology.** The species epithet refers to the large and conspicuous dorsal sepal of the flower.

**Taxonomic notes.** *Liparis macrosepala* differs from *L. delicatula* in its 4 to 5 leaves with slightly crispate margins on their apical half and single pair of wings on the column. Its entire, not Y-shaped petals and sessile lip (i.e. without a claw) easily distinguish *L. macrosepala* from *L. fissipetala*. The dorsal sepal of *L. assamica* is narrowly ovate-oblong, in contrast with the heart-shaped dorsal sepal of *Liparis macrosepala*. *Liparis resupinata* is distinguished from *L. macrosepala* by its 10–50-flowered raceme and the column with a single pair of broad wings, each with a retrorse thread. The main differences between these closely-related species, according to our phylogenetic analyses, are summarised in Table 2.

**Conservation assessment.** The new species was found in a ridge-top evergreen broad-leaved forest on a limestone mountain. Despite numerous surveys in the areas, only six mature individuals were found without fruits or evidence of cross-pollination. This extremely small effective population occurs in a touristic zone which is a serious threat to the survival of the species. Consequently, the species can be assessed as Critically Endangered (CR, D), based on current information and following IUCN guidelines (IUCN 2019).

| Table 2. Comparison of *L. macrosepala* and related species. |
|---------------------------------------------------------------|
| **Characters** | **L. delicatula** | **L. fissipetala** | **L. assamica** | **L. resupinata** | **L. macrosepala** |
| Pseudobulbs | oblong or cylindrical-fusiform 5–9 3–5 mm | ovoid, 8–10 mm long | ovoid-fusiform, slightly compressed 1.5–2.5 cm × 6–10 mm | subcylindrical or ± spindle-shaped, 1.8–5 cm × 3–6 mm | ovoid-fusiform, slightly compressed, 1–2 cm × 0.5–1 cm |
| Leaf | 2 or 3, margin flat | 3 or 4, strongly crisped-margined | 3 or 4, apical half slightly crisped-margined | 3 or 4, margin slightly serrate | 4 or 5, apical half slightly crisped-margined |
| Scape | 2–5 cm, several to 10-flowered, flowers white | 5–10 cm long, with 10–15 flowers, flowers yellow, | 10–13 cm, more than 10-flowered, flowers orange | 7–18 cm, 10–50-flowered, flowers pale green or greenish-yellow | 7–10 cm, more than 10-flowered, flowers greenish-orange |
| Bracts | ovate-lanceolate, 2–3 mm | ovate-lanceolate, 1.5–3.5 mm | lanceolate, 2–3 mm | lanceolate, 3–5 mm | broadly ovate, 2–3 mm |
| Dorsal sepal | ovate-oblong, 2.5–3 mm × 1.5–1.8 mm | oblong-lanceolate, 3–4 mm × 0.8–1 mm | narrowly ovate-oblong, 4.8–5.8 mm × ca. 1.6 mm | oblong or elliptic-oblong, ca. 4 × 1.8 mm | broadly ovate, ca. 3.2–5 mm × 3–3.6 mm |
| Petals | narrowly linear-lanceolate, 2.5–3 mm × ca. 0.5 mm, entire | narrow linear, 4–5 mm long, Y-shaped broader ovate-oblong, ca. 4.5–5.5 mm × ca. 0.7 mm, entire | narrowly linear, 5–5.5 mm × ca. 0.7 mm, entire | narrowly linear, ca. 3.5 × 0.3 mm, entire | narrowly linear, 3–4 mm × ca. 0.2 mm, entire |
| Lip | broadly elliptic or orbicular, ca. 2.5 mm, base with an orbicular, auriculate, callus-shaped fold on either side, with a concave callus near base | epichile broadly oblong-oblong or subsquare, 1.5–2 mm × 1.5–1 mm, base with two auricles on both sides; claw short, with a fleshy callus centrally near base | broadly obovate-oblong, ca. 4 × 2.7 mm, with two callus-shaped thickened folds, two suborbicular lobes on both sides, centrally with one concave callus near base | broadly elliptic-oblong or broadly obovate-oblong, 2.5–3 mm, with two callus-shaped lobes, base with two lateral splits below middle; two suborbicular lobes, centrally with one bilobed callus near base | broadly elliptic, ca. 2–3 mm × 2–3 mm, two callus-shaped and thickened folds, base with two oblong lobes on both sides, centrally with one bilobed callus near base |
| Column | ca. 2.2 mm, two pairs of wings | ca. 1.5 mm, broadly winged with two horn-like appendages | ca. 2 mm, two pairs of wings | ca. 2.8 mm, a pair of wings, each with a retrorse thread | ca. 2 mm, a single pair of subtriangular wings |
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