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Article

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Liparis meihuashanensis, a new orchid species from Fujian, China: Evidence from morphological and molecular analyses

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Abstrct

A new orchid species, *Liparis meihuashanensis*, from Fujian, China is described and illustrated based on morphological and molecular analyses. Detailed morphological comparisons indicate that *L. meihuashanensis* is similar to *L. auriculata* and *L. pauliana*, but it can be distinguished from them by the shorter inflorescence, triangular floral bract, and a lip with a truncate-emarginate and mucronate apex and 2 small subconical calli on contracted base. Molecular analyses based on nuclear ITS and plastid *mat*K DNA sequence data support the *L. meihuashanensis* as a distinct species.

Key words: Chinese orchid, Orchidaceae, Malaxideae, Malaxidinae, widelip orchid

Introduction

Liparis Richard (1817: 39) is an orchid genus, comprising ca. 320 species. It is mainly distributed in tropical and subtropical regions of Asia, Oceania and Americas, with only a few extending to the temperate zone (Chen *et al*. 2009). There are 74 species of this genus found in China according to the most recent revision and recently published new species (Chen *et al*. 2009, Huang *et al*. 2016, Hsu 2013, Jin 2011, Li *et al*. 2013, Su *et al*. 2015, Tang *et al*. 2012, 2015, Wu *et al*. 2012, Yang *et al*. 2010).

A new species was found in the National Nature Reserve of Meihuashan in Fujian, China, during a botanical trip there in 2016. It is similar to *L. auriculata* Blume ex Miquel (1866: 203) and *L. pauliana* Handel-Mazzetti (1921: 65), but it can be distinguished from them by shorter inflorescence, triangular floral bract, and a lip with a truncate-emarginate and mucronate apex and 2 small subconical calli on contracted base. The results of our phylogenetic analyses lent further support to its distinctiveness.

Materials and Methods

Morphological observations:—Gross morphological data were obtained during the fieldwork. Measurements, shapes, colors and other details given in the description were based on living materials. The images of flowering plant were photographed with the Canon S100v digital camera. The floral anatomy was conducted under a XTL-340Z stereomicroscope.

Taxonomic sampling:—We used phylogenetic analysis to explore the systematic position of the new species, a total of 39 taxa of *Liparis*, *Oberonia*, *Oberonioides* and *Malaxis* as ingroups, and two species of *Acanthephippium* and *Phaius* as outgroups. GenBank accession numbers are provided in Table 1.

Molecular markers:—nrDNA ITS and cpDNA *mat*K were used in the phylogenetic analyses, with 41 DNA sequences were utilized in the study. Most of them were download from NCBI, except the sequences of *Liparis meihuashanensis*. Total DNA extraction, PCR, DNA markers sequencing, editing, assembly, and the primers used for PCR were conducted according to Tang *et al*. (2015).

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TABLE 1. GenBank accession numbers for sequence data.

Names	Voucher	ITS	*mat*K
Liparis anopheles	Leiden cult. 980165	AY907075	AY907139
L. auriculata	TBG144267(TNS)	AB289458	-
L. balansae	L. Li 152 (IBSC)	KF589874	KF589880
L. bracteata	P. Weston s.n.	AY907076	AY907140
L. brunnescens	Leiden cult. 20030224	AY907098	AY907165
L. caespitosa	Leiden cult. 20030195	AY907077	AY907141
L. chalandei	T. Motley & K. Cameron 2160 (NY)	AY907078	AY907142
L. clypeolum	J.-Y. Meyer 1029 (NY)	AY907079	AY907143
L. condylobulbon	Leiden cult. 20030654	AY907080	AY907144
L. disticha	Leiden cult. 20010180	AY907081	AY907145
L. formosana	K. Cameron 2151 (NY)	AY907082	AY907147
L. fujisanensis	EWH: Lee 239	EU024936	EU024937
L. guangxiensis	L. Li 153 (IBSC)	KF589875	KF589881
L. japonica	K. Cameron 2176	AY907086	AY907151
L. kumokiri	EWH: Lee 228	AY907087	AY907152
L. koreana	EWH:Lee 197	EU017422	EU017444
L. latifolia	Singapore B. G. cult. 837	AY907088	AY907153
L. liliifolia	Chase O-214 (K)	AF521067	AF263667
L. loeselii	B. Ewachas.n.	AY907091	AY907157
L. meihuashanensis	S. M. Fan 2016015	KY959772	KY959773
L. nugentae	P. Weston s.n.	AY907093	AY907159
L. pandurata	Leiden cult. 20020341	AY907094	AY907160
L. pauliana	K. Cameron 2169 (NY)	AY907096	AY907163
L. rheedei	Leiden cult. 970454	AY907097	AY907164
L. stricklandiana	L. Li 135 (IBSC)	KF589873	KF589879
L. sula	K. Cameron s.n. DNA#1174	AY907104	AY907171
L. terrestris	Singapore B. G. cult. 3482	AY907105	AY907172
L. truncicola	Leiden cult. 20030222	AY907106	AY907173
L. viridiflora	NYBG cult. 2025	AY907107	AY907174
Malaxis bancanoides	Yukawa 95-101 (TNS)	AB290885	AB290893
M. brachypoda	K. Cameron 2136 (NY)	AY907108	AY907175
M. corymbosa	R. Coleman 1068 (AZ)	AY907110	AY907176
M. spicata	MWC377	AF521068	AY368415
M. soulei	R. Coleman 1069 (AZ)	AY907119	AY907186
M. tenuis	R. Coleman 1019 (AZ)	AY907129	AY907196
Oberonia setifera	NYBG cult. s.n. ex Andy's Orchids	AY907136	AY907204
O. equitans	T. Motley & K. Cameron 2255 (NY)	AY907130	AY907198
Oberonioides microtatantha	Z.J. Liu 4868	KJ459302	KJ459333
O. sp.	Z.J. Liu 6036	KJ459303	KJ459334
Acanthephippium mantinianum	MWC397	AF521081	AF263618
Phaius tancarvilleae	M. Watanabe s. n. (TNS)	AB290884	AB290892

Phylogenetic analyses:—Phylogenetic analyses were performed using the Bayesian inference (BI) and maximum-parsimony (MP) methods. The BI analysis was performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). The following settings were used: sampling frequency = 1000; tem = 0.1; burn-in = 2000; and number of Markov chain Monte Carlo generations = 10000000. MP analysis was performed in PAUP v.4.0b10 (Swofford 2002).

Result of molecular analyses

The phylogenetic analysis shows in Figure 1. The new species *L. meihuashanensis* naturally become an independent clade (PP=0.99, BP=100).

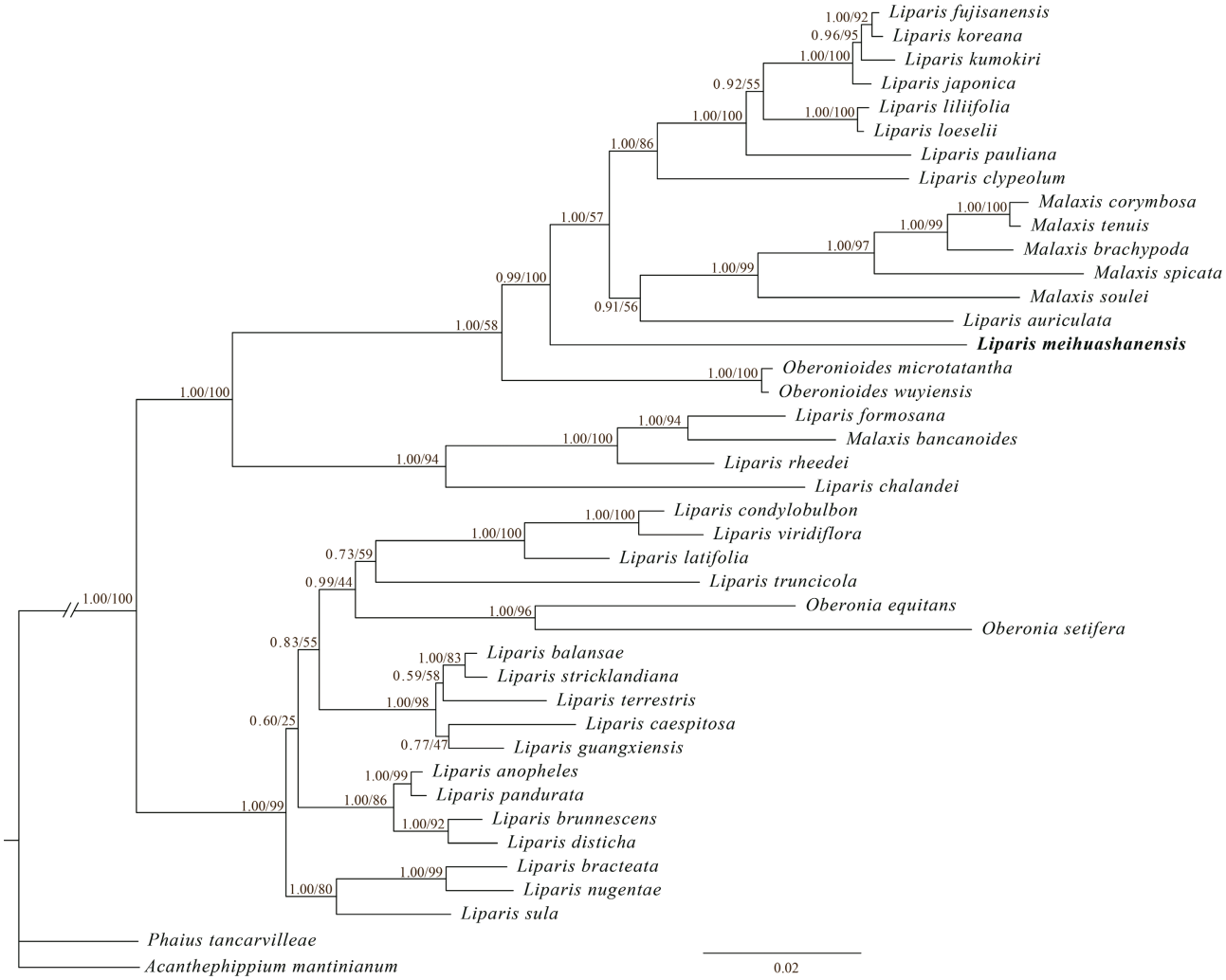

FIGURE 1. Phylogenetic placement of *L. meihuashanensis* in the Bayesian analysis of the combined nrITS and plastid *matK*. Support percentages and posterior probabilities displayed on the branches are PP_{BI}/BP_{MP}. The scale bar denotes the estimated number of substitutions in Bayesian analysis.

Taxonmy

Liparis meihuashanensis S. M. Fan, *sp. nov*. (梅花山羊耳蒜, Figs. 2, 3)

Type:—China. Fujian (福建): National Nature Reserve of the Meihuashan of Fujian, on mossy rock, alt. 1770 m, 2 May 2016, S. M. Fan 2016015 (holotype: Fujian University of Traditional Chinese Medicine; isotype: NOCC).

The new species is similar to *L. auriculata* Blume ex Miquel (1886: 203) and *L. pauliana* Handel-Mazzetti (1921: 65), but it can be distinguished from them by the shorter inflorescence (4.0–5.5 cm), triangular floral bracts, a lip with a truncate-emarginate and mucronate apex and 2 small subconical calli on contracted base.

Terrestrial or lithophytic herb. Plant 5.5–8.5 cm tall. Pseudobulbs clustered, ovoid, ca. 1.2 cm long, ca. 0.8 cm in diameter, ±enclosed by a few white membranous sheaths. Leaves 2; petiole sheathlike, 5–12 mm long, not articulate; blade ovate-elliptic, 2.0–3.5 × 1.0–2.0 cm, base contracted and decurrent into petiole, margin entire, apex acute. Inflorescence 4.0–5.5 cm; rachis with 3–5-flowered; bracts triangular, ca. 2 mm long. Flowers green or purple; pedicel and ovary 5.5–7.0 mm long. Dorsal sepal broadly linear, ca. 9.0 × ca. 2.0 mm, apex obtuse; lateral sepals suboblong, ca. 8.0 × ca. 2.5 mm, slightly oblique; petals nearly filiform, ca. 8.0 × ca. 0.5 mm; lip broadly obovate, 7–8 × 7–8 mm, with 2 small subconical calli on contracted base, margin entire, apex subtruncate and mucronate. Column weakly curved, ca. 5.5 mm long, base dilated, apex with small subquadrate wings. Pollinia four in two pairs, yellow. Capsule ellipsoid-globose.

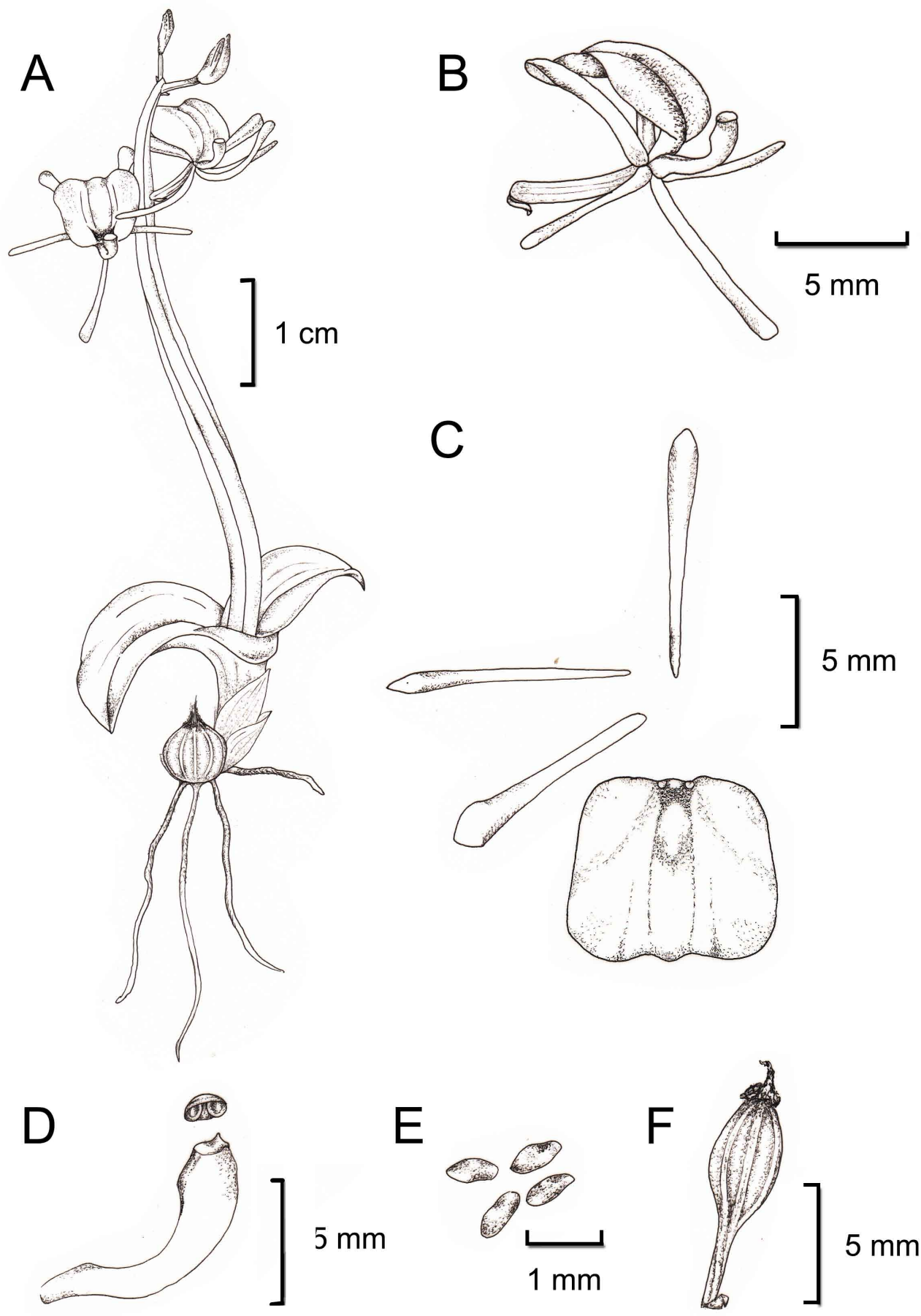

FIGURE 2. *Liparis meihuashanensis* S. M. Fan. A. Flowering plant. B. Flower, side view. C. Dorsal sepal, petal, lateral sepal, and lip. D. Column and anther cap. E. Pollinarium. F. Fruit.

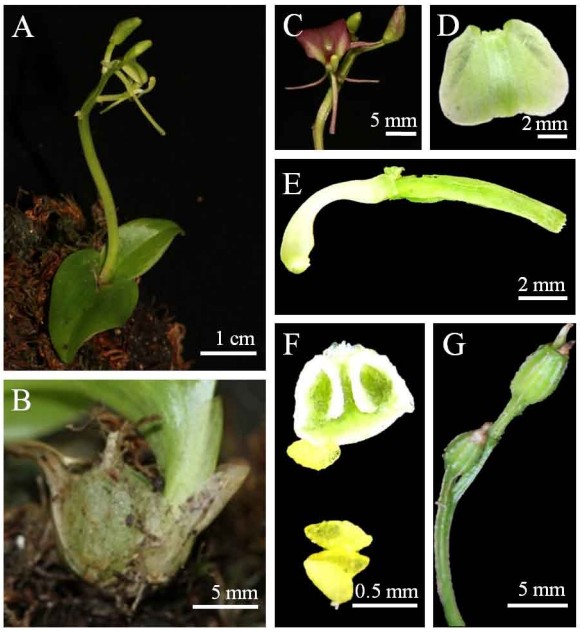

FIGURE 3. *Liparis meihuashanensis* S. M. Fan. A. Flowering plant (green flower). B. Pseudobulbs. C. Flower (purple flower), front view. D. Lip (green flower). E. Column.F. Anther cap and pollinarium. G. Fruit.

Phenology:—Flowering in May.

Distribution and habitat:—*Liparis meihuashanensis* is only known from the National Nature Reserve of Meihuashan in Fujian, China (Fig. 3). The plants grow on the mossy rock on the edge of evergreen forest. *Pleione formosana* was found growing together with this new species.

Etymology:—The specific name refers to the Natural Reserve of Meihuashan where the new species was found.

Conservation status:—*Liparis meihuashanensis*is known only from one site, with one population of ca. 20 individuals was discovered during author's botanical trip there. Probably more populations may be found by further botanical trips there. Therefore, it seems premature to conduct a full conservation assessment right now. We try to regard this species as Data Deficient (DD: IUCN 2012).

Notes:—*Liparis meihuashanensis* is closely related to *L. auriculata*, but it can be distinguished by having smaller (2–3.5 × 1–2 vs. 4–10 × 3–8 cm) ovate-elliptic leaves, a shorter inflorescence (4.0–5.5 vs. 20–30 cm), triangular (vs. lanceolate) flroal bracts, longer (ca. 9 vs. 6–7 mm) dorsal sepal with obtuse (vs. acute) apex, a bigger (7–8 × 7–8 vs. 5.5–6.0 × ca. 5 mm) lip with a truncate-emarginate and mucronate (vs. rounded or sometimes apiculate) apex and 2 small subconical (vs. subtriangular) calli on contracted base. The new species is also closely related to *L. pauliana*, but it can be distinguished by having a shorter inflorescence (4.0–5.5 vs. 7–28 cm), triangular (vs. ovate or ovate-lanceolate) floral bracts, a shorter pedicel and ovary (5.5–7.0 vs. 10–18 mm), a shorter (ca. 9.0 vs. 11.5–18.0 mm) dorsal sepal with an obtuse (vs. subacute) apex, shorter (ca. 8.0 vs. 11.5–18 mm) lateral sepals with an obtuse (vs. subacute) apex, a smaller (7–8 × 7–8 vs. 13–20 × 8–12 mm) lip with a truncate-emarginate and mucronate apex (vs. obuse or sometimes mucronate) and 2 subconical calli (vs. 2 short longitudinal lamellae, sometimes lamellae inconspicuous) on contracted base, longer column (ca. 5.5 vs. 3.5–4.5 mm) (Table 2).

TABLE 2. Comparison of diagnostic characteristics for *L. meihuashanensis*, *L. auriculata* and *L. pauliana*.

Characteristics	*L. meihuashanensis*	*L. auriculata*	*L. pauliana*
Leaves	2.0–3.5 × 1.0–2.0 cm, base not rounded to cordate and decurrent into petiole	4–10 × 3–8 cm, base rounded to cordate and decurrent into petiole	2.7–9.0 × 1.5–5.0 cm, base not rounded to cordate and decurrent into petiole
Inflorescence	4.0–5.5 cm long, 3–5 flowered	20–30 cm long, several to more than 10-flowered	7–28 cm long, several flowered, very rarely many flowered or 1- or 2-flowered
Floral bracts	Triangular, ca. 2 mm long	Lanceolate, 1.5–2.5 mm long	Ovate or ovate-lanceolate, 1.5–3.0 mm long
Pedicel & ovary	5.5–7.0 mm long	5–6 mm long	10–18 mm long

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TABLE 1. (Continued)

Characteristics	*L. meihuashanensis*	*L. auriculata*	*L. pauliana*
Dorsal sepal	Broadly linear, ca. 9.0 × ca. 2.0 mm, apex obtuse	Linear, 6–7 × 1.5–2.0 mm, apex acute	Linear-lanceolate, 11.5–18.0 × 2.0–2.5 mm, apex acuminate
Lateral sepals	Obliquely suboblong, ca. 8.0 × ca. 2.5 mm, apex obtuse	Similar to dorsal sepal, slightly shorter and broader	Linear-lanceolate, 11.5–18.0 × 2.0–2.5 mm, apex acuminate
Petals	Ca. 8.0 × ca. 0.5 mm	Ca. 6.0 × 0.5 mm	11.5–18.0 × ca. 0.3 mm
Lip	Obovate, 7–8 × 7–8 mm, with 2 small subconical calli, apex truncate-emarginate and mucronate	Orbicular or ovate-orbicular, 5.5–6.0 × ca. 5 mm, with 2 subtriangular small calli near base, apex rounded or sometimes apiculate	Obovate-elliptic, 13–20 × 8–12 mm, often with 2 short longitudinal lamellae near base, sometimes lamellae in conspicuous, apex obtuse or sometimes mucronate
Column	Ca. 5.5 mm long	3–5 mm long	3.5–4.5 mm long
Flowering	May	May–July	May

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