



Hemipilia galeata (Orchideae, Orchidaceae), a new species from Fujian Province, southeastern China

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Abstract

A new orchid species, *Hemipilia galeata*, is described and illustrated from Wuyi Mountains, Fujian Province, southeastern China, and included in a phylogenetic analysis using the nuclear ribosomal ITS and three plastid markers (*matK*, *trnL* intron and *trnL-F* spacer) DNA. This species is a member of *H.* section *Hemipilia sensu Tang et al.* and is distinguished from other species in *Hemipilia* by its hemispheric dorsal sepal. It is similar to *H. hemipilioides*, *H. occidensichuanensis* and *H. thailandica*, but *H. galeata* differs by its elliptic, purple-spotted leaf, suborbicular petals, oblong lateral lip lobes and an ovate midlobe.

Keywords: China, *Hemipilia galeata*, new species, Orchidaceae

Introduction

The genus *Hemipilia* Lindley (1835: 296) *sensu stricto* (Orchideae, Orchidaceae) comprises ca. 10 species that are distributed from eastern Himalayas through Myanmar and Thailand to southeastern China (Luo 1999, Pridgeon *et al.* 2001). Morphologically, *Hemipilia s.s.* is easily distinguished by a protruding, tongue-like rostellum (cf. Luo & Chen 2000). However, molecular phylogenetic studies revealed *Hemipilia s.s.* as monophyletic but nested deeply within a strongly supported clade that also included *Ponerorchis brevicealcarata* (Finet 1901: 420) von Soó (1966: 353) and *Hemipiliopsis purpureopunctata* (Lang in Lang & Ji 1978: 127) Luo & Chen (2003: 450) (Luo 1999, Bateman *et al.* 2003, Jin *et al.* 2014). Given the fact that *Ponerorchis* Reichenbach (1852: 227) was paraphyletic, Bateman *et al.* (2003) suggested expansion of *Hemipilia* to include the above-mentioned two species; Jin *et al.* (2014) formally made the transfers, which resulted in a broad *Hemipilia*, although Luo (2014) recognized *Hemipiliopsis* Luo & Chen (2003: 450) as a distinct monotypic genus. Most recently, Tang *et al.* (2015) further demonstrated that *Amitostigma hemipilioides* (Finet 1901: 515) Tang & Wang (1936: 5), *A. thailandicum* Seidenfaden & Thaithong in Seidenfaden (1997: 8) and *P. limprichtii* (Schltr. in Limpricht 1922: 330) von Soó (1966: 353) were sister to *Hemipilia s.s.*, rendering not only *Ponerorchis* but also *Amitostigma* Schlechter (1919: 91) as paraphyletic. Therefore, based on molecular as well as morphological evidence, Tang *et al.* (2015) proposed to combine the East Asian *Amitostigma* and its alliance into *Hemipilia sensu latissimo*, in which seven sections were also recognised. As a result, *Hemipilia* currently includes ca. 65 species that are generally characterized by relatively small globose tubers, unsheathed inflorescences, non-membranous bracts and two viscidia enclosed within two separate bursicles (Tang *et al.* 2015, Chase *et al.* 2015).

During a recent field trip to Wuyi Mountains, Fujian Province, southeastern China, an orchid that morphologically fits into the category of *Hemipilia* was discovered. However, this early-flowering, tiny orchid has a unique set of characteristics, differing from all known species in the genus. The results of our phylogenetic analyses lent further support to its distinctiveness. Here, we describe it as a new species in *H.* sect. *Hemipilia sensu Tang et al.* (2015).

Materials & methods

Taxon sampling:—To explore the phylogenetic position of the new species described here 50 accessions, representing 46 taxa and all seven sections of *Hemipilia*, plus 21 accessions of related genera in tribe Orchideae were selected based on the datasets of Tang *et al.* (2015). Voucher information (deposited at KUN) and GenBank accession numbers are provided in Table 1.

TABLE 1. Voucher information and GenBank accession numbers for sequence data of *Hemipilia galeata* generated during this study. Missing data are indicated with “–”.

Accession	Voucher/Source	nrITS	<i>matK</i>	<i>trnL-F</i>
<i>Hemipilia galeata</i>	Tang & Zhu 203	KT183499	KT183498	KT183500
<i>Brachycorythis henryi</i>	GenBank	KM651260	KM651424	KM651586
<i>B. neglecta</i>	GenBank	KM651261	KM651425	KM651587
<i>Dactylorhiza viridis</i>	GenBank	KM651262	KM651426	KM651588
<i>Disa buchenaviana</i>	GenBank	DQ414921	DQ415064	DQ415205
<i>Galearis roborowskyi</i>	GenBank	KM651265	KM651429	KM651591
<i>G. spathulata</i>	GenBank	KM651266	KM651430	KM651592
<i>Gymnadenia orchidis</i>	GenBank	KM651267	KM651431	KM651593
<i>Habenaria delavayi</i>	GenBank	KM651268	KM651432	KM651594
<i>Hemipilia alpestris</i>	GenBank	KM651221	KM651385	KM651545
<i>H. amplexifolia</i>	GenBank	KM651222	KM651386	KM651546
<i>H. basifoliata</i>	GenBank	KM651223	KM651387	KM651547
<i>H. brevicealcarata</i>	GenBank	KM651285	KM651449	KM651611
<i>H. calcicola</i>	GenBank	KM651279	KM651440	KM651605
<i>H. calophylla</i>	GenBank	KM651269	KM651433	KM651595
<i>H. camptoceras</i>	GenBank	KM651275	KM651439	KM651601
<i>H. capitata</i>	GenBank	KM651224	KM651388	KM651548
<i>H. cf. amplexifolia</i>	GenBank	KM651225	KM651415	KM651549
<i>H. cf. faberi</i>	GenBank	KM651226	KM651395	KM651550
<i>H. cf. occidentichuanensis</i>	GenBank	KM651296	KM651462	KM651621
<i>H. chidori</i>	GenBank	KM651287	KM651451	KM651612
<i>H. chusua</i>	GenBank	KM651288	KM651452	KM651616
<i>H. compacta</i>	GenBank	JN696455	KJ452796	–
<i>H. cordifolia</i>	GenBank	AF348036	–	–
<i>H. crassicalcarata</i>	GenBank	KM651270	KM651434	KM651596
<i>H. cucullata</i>	GenBank	KM651278	KM651443	KM651602
<i>H. faberi</i> -110	GenBank	KM651229	KM651391	KM651553
<i>H. faberi</i> -116	GenBank	KM651230	KM651389	KM651554
<i>H. farreri</i>	GenBank	KM651231	KM651392	KM651555
<i>H. flabellata</i>	GenBank	KM651271	KM651435	KM651597
<i>H. forrestii</i>	GenBank	KJ460049	KJ452805	–
<i>H. fujisanensis</i>	GenBank	KM651280	KM651444	KM651606
<i>H. gonggashanica</i>	GenBank	KM651233	KM651394	KM651557
<i>H. gracilis</i>	GenBank	KM651235	KM651397	KM651559
<i>H. graminifolia</i>	GenBank	KM651294	KM651458	KM651619
<i>H. hemipilioides</i>	GenBank	KM651238	KM651400	KM651562
<i>H. joo-iokiana</i>	GenBank	–	KF695169	–
<i>H. keiskei</i>	GenBank	KM651239	KM651401	KM651563

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TABLE 1. (Continued)

Accession	Voucher/Source	nrITS	<i>matK</i>	<i>trnL-F</i>
<i>H. keiskeoides</i>	GenBank	KM651240	KM651402	KM651564
<i>H. kinoshitai</i>	GenBank	KM651241	KM651403	KM651565
<i>H. kiraishiensis</i>	GenBank	KM651295	KM651460	–
<i>H. kwangsiensis</i>	GenBank	KM651272	KM651436	KM651598
<i>H. lepida</i>	GenBank	KM651242	KM651404	KM651566
<i>H. monantha</i> -139	GenBank	KM651243	KM651405	KM651569
<i>H. monantha</i> -173	GenBank	KM651244	KM651407	KM651567
<i>H. oblonga</i>	GenBank	KM651281	KM651445	KM651607
<i>H. occidentichuanensis</i>	GenBank	KM651298	KM651461	KM651623
<i>H. omeishanica</i>	GenBank	KM651299	KM651464	KM651624
<i>H. parceflora</i>	GenBank	KJ460052	KJ452808	KM651571
<i>H. physoceras</i>	GenBank	KM651248	KM651410	KM651573
<i>H. pinguicula</i>	GenBank	KM651252	KM651411	KM651575
<i>H. purpureopunctata</i>	GenBank	KJ460051	KJ452807	–
<i>H. sichuanica</i>	GenBank	KJ460059	KJ452815	–
<i>H. simplex</i>	GenBank	KM651253	KM651416	KM651578
<i>H. tetraloba</i>	GenBank	KM651255	KM651418	KM651580
<i>H. thailandica</i>	GenBank	KM651256	KM651419	KM651581
<i>H. tibetica</i>	GenBank	KM651257	KM651421	KM651582
<i>H. trifurcata</i>	GenBank	KJ460055	KJ452811	KM651583
<i>H. wenshanensis</i>	GenBank	KM651258	KM651422	KM651584
<i>H. yuana</i>	GenBank	KM651259	KM651423	KM651585
<i>Herminium monorchis</i>	GenBank	KM651273	KM651437	KM651599
<i>H. souliei</i>	GenBank	KM651274	KM651438	KM651600
<i>Himantoglossum robertianum</i>	GenBank	AY351384	AY368382	AY014584
<i>Hsenshua chrysea</i>	GenBank	KJ460056	KJ452812	–
<i>Ophrys apifera</i>	GenBank	AJ539529	AJ310049	AJ409432
<i>Peristylus densus</i>	GenBank	KM651282	KM651446	KM651608
<i>P. mannii</i>	GenBank	KM651283	KM651447	KM651609
<i>Platanthera contigua</i>	GenBank	KM651263	KM651427	KM651589
<i>P. roseotincta</i>	GenBank	KM651284	KM651448	KM651610
<i>P. urceolata</i>	GenBank	KM651264	KM651428	KM651590
<i>Satyrium nepalense</i>	GenBank	KM651301	KM651465	KM651626
<i>Sirindhornia pulchella</i>	GenBank	KJ460045	KJ452801	–

DNA extraction, PCR amplification & sequencing:—Genomic DNA was extracted from silica-dried leaf fragments using the modified 2× CTAB procedure of Doyle & Doyle (1987). DNA markers from one nuclear (nrITS) and three plastid markers (*matK*, *trnL* intron and *trnL-F* intergenic spacer) were chosen for the phylogenetic analyses. The nrITS and combined two *trnL-F* regions were amplified using primer pairs 17SE/26SE (Sun *et al.* 1994) and c/f (Taberlet *et al.* 1991), respectively. An internal fragment of ca. 800 bp of the *matK* gene was amplified using the primers 390F/1326R (Cuénoud *et al.* 2002). The PCR mixtures and cycling parameters followed those of Tang *et al.* (2015). PCR products were isolated and purified using QIAquick PCR purification kits (BioTeke, China), following the manufacturer's instructions. Sequencing reactions were performed using the dideoxy chain termination method running on an ABI PRISM 3730 automated sequencer, using the primers described above for PCR. All regions were sequenced for both DNA strands.

Phylogenetic analyses:—Maximum parsimony (MP) analysis, Bayesian inference (BI) and maximum likelihood (ML) analysis were employed to generate phylogenetic trees. Data for the plastid regions were combined, whereas

the nrITS and combined plastid DNA datasets were analyzed separately. One portion of the non-coding *trnL-F* proved difficult to align, as well as the length-variable poly A/T regions in this marker, and these were excluded from the final alignments prior to phylogenetic analysis. Gaps were not coded for these markers. Other parameters of each category of analysis generally followed the description of Tang *et al.* (2015).

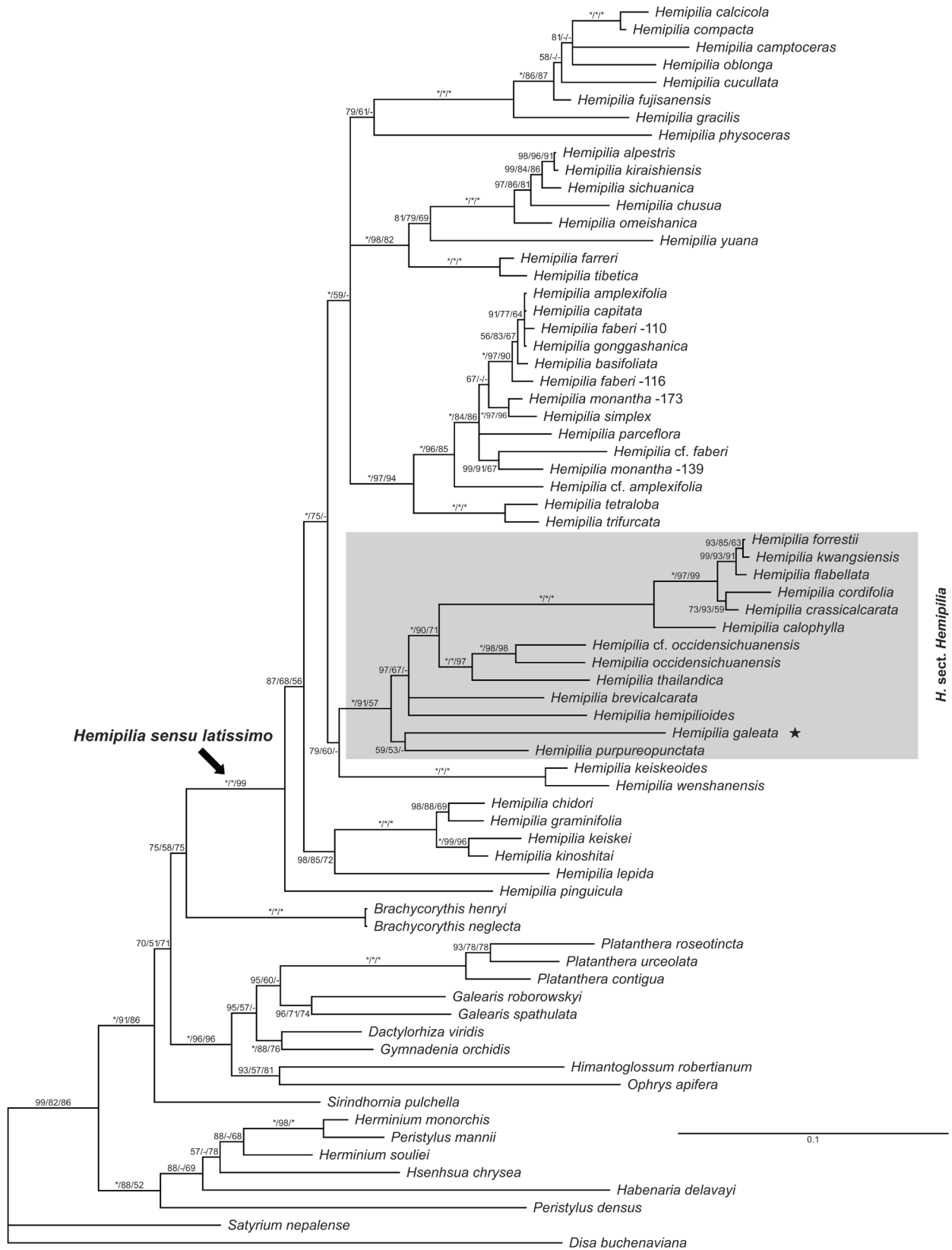


FIGURE 1. Phylogenetic placement of *Hemipilia galeata* (indicated with the symbol “★”) from Bayesian analysis of the nrITS dataset. Support percentages and posterior probabilities displayed on the branches are PP_{BI}/BP_{ML}/BP_{MP} (“-” indicates support values of less than 90 and “*” indicates a support value of 100). The scale bar denotes the estimated number of substitutions in Bayesian analysis.

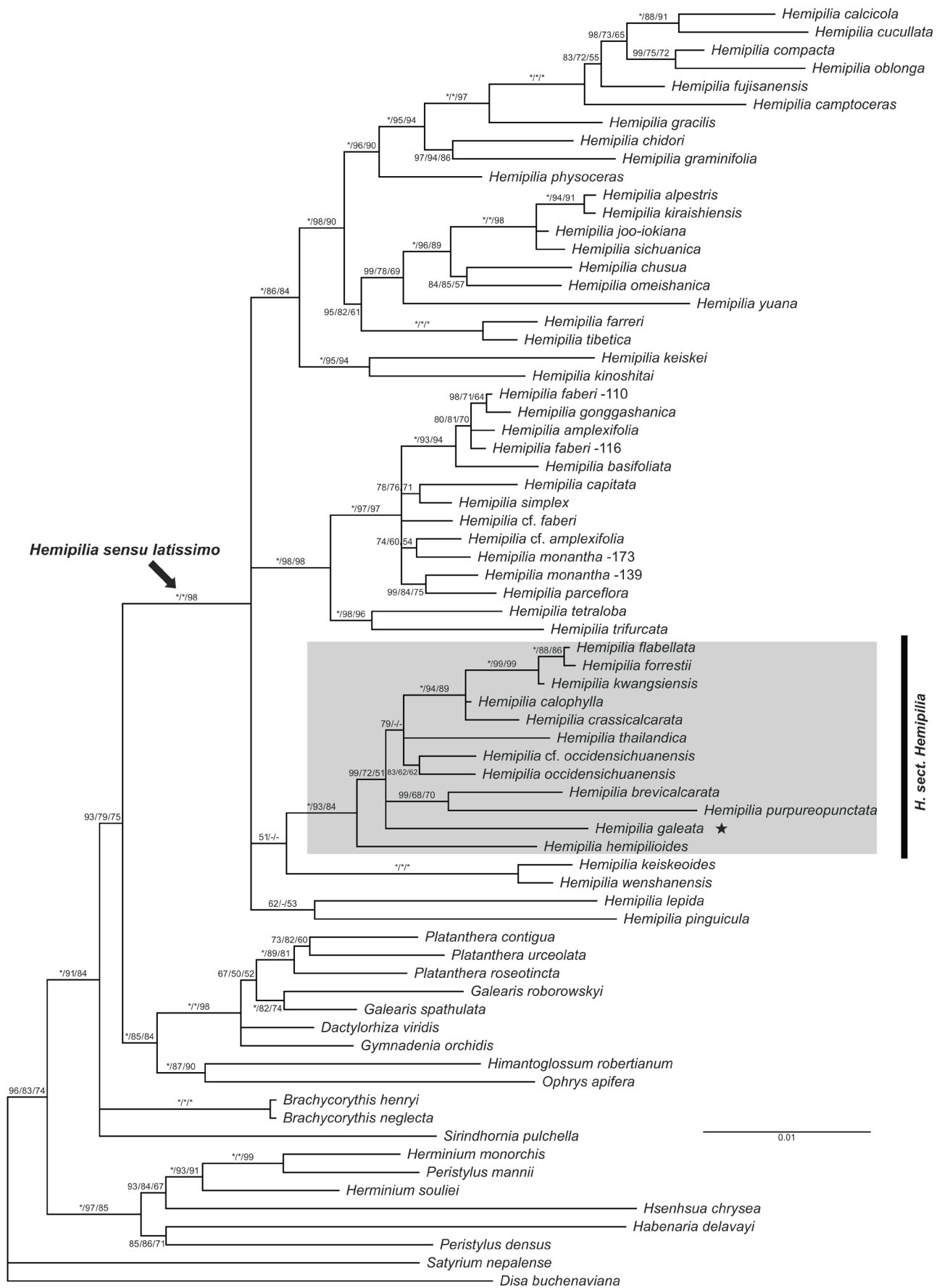


FIGURE 2. Phylogenetic placement of *Hemipilia galeata* (indicated with the symbol “★”) in the Bayesian analysis of the combined plastid DNA dataset. Support percentages and posterior probabilities displayed on the branches are PP_{BI}/BP_{ML}/BP_{MP} (“-” indicates support values of less than 50 and “*” indicates a support value of 100). The scale bar denotes the estimated number of substitutions in Bayesian analysis.

Results of molecular analyses

Sequences and alignment:—The length of the nrITS sequence of the new species described here is 643 bp. The lengths of trimmed sequences of the *matK* and *trnL-F* regions are 802 bp and 858 bp, respectively. Table 2 summarizes the properties of each dataset in this study.

Phylogenetic reconstruction:—Trees generated from the nrITS and combined plastid DNA datasets in the present study are similar to those of Tang *et al.* (2015). Excluding weakly supported or collapsed nodes, the MP strict consensus trees, ML best-score trees and BI majority-rule consensus trees generated similar topologies for *Hemipilia sensu* Tang *et al.* (2015) (results not shown). The Bayesian tree was chosen as the basis for our discussion.

The new species is revealed as one of the members of the *Hemipilia* clade *sensu* Tang *et al.* (2015) with strong support (Fig. 1, */91/57; Fig. 2, */93/84). The nrITS tree recovers the new species and *H. purpureopunctata* (Lang) Jin, Schuiteman & Jin in Jin *et al.* (2014: 50) as sister taxa but with low support (Fig. 1, 59/53/-), whereas in the combined plastid DNA tree, the new species is included in a trichotomy formed by species of the *Hemipilia* clade with the sole exception of *H. hemipilioides* (Finet) Tang & Peng in Tang *et al.* (2015: 96).

TABLE 2. Properties of datasets used in this study and resulting tree statistics.

Dataset	nrITS	Combined cpDNA	<i>matK</i>	<i>trnL-F</i>
No. of taxa	71	71	71	63
Alignment length ^a	746	1961	832	1129
No. of variable characters	442 (59%)	523 (27%)	258 (31%)	265 (23%)
No. of parsimony-informative characters	352 (47%)	270 (14%)	140 (17%)	130 (12%)
No. of most-parsimonious trees	251	7093	8397	5950
Tree Length	2000	1077	556	518
Consistency index ^b	0.40	0.61	0.58	0.65
Retention index	0.67	0.74	0.72	0.76
No. of excluded ambiguously aligned positions ^c	–	201 (9%)	–	201 (15%)

^aDetermined after the ambiguously aligned characters were excluded

^bEstimated including autapomorphies

^cFigures are approximate due to ambiguous alignment

Taxonomy

Hemipilia galeata Y.Tang, X.X.Zhu & H.Peng, *sp. nov.* (Figs. 3–5)

Type:—CHINA. Fujian: Wuyishan City, 250 m, 27°41' N, 117°57' E, 18 April 2015, Tang & Zhu 203 (holotype: KUN!; isotype: CSH!).
Diagnosis:—Differing from all known species of *Hemipilia* in having a hemispheric dorsal sepal. Similar to *H. hemipilioides*, *H. occidentischuanensis* Tang & Peng in Tang *et al.* (2015: 96) and *H. thailandica* (Seidenf. & Thaitong) Tang & Peng in Tang *et al.* (2015: 96), from which it can be distinguished by an elliptic, purple-spotted leaf, suborbicular petals, oblong lateral lip lobes and an ovate midlobe (Table 3).

Terrestrial, erect herbs, 5–13 cm tall. Tubers ovoid, 4–6 mm long, 3 mm in diameter, neck with few roots. Stem slender, 1 mm in diameter, green with purple markings, with 1 tubular basal sheath. Leaf subbasal, solitary, elliptic, 1.5–3.0 × 0.8–2.2 cm, apex acute, base contracted into amplexicaul sheath, adaxially green with purple markings, rarely uniformly green, abaxially pale green. Inflorescence terminal, 1–13-flowered. Flowers pink and white, not scented. Ovary straight to slightly incurved, cylindrical, 5.0–9.5 mm long; floral bract connivent to ovary, elliptic to ovate, 2.8–3.0 × 1.8–2.3 mm, apex acuminate; dorsal sepal free, 1-veined, hemispheric, 2.3 × 2.1 mm, apex acute and forming a brim, adaxially white with pink markings; lateral sepals free, spreading, 1-veined, ovate, oblique, concave, 3.1–3.4 × 1.8 mm, apex obtuse, white to pale pink; petals connivent with dorsal sepal and forming a hood, suborbicular, oblique, 2.6 × 2.5 mm, apex obtuse, white above median vein and pink below; labellum spreading, rhomboidal, spurred at base, trilobed above middle; lateral lobes oblong, 2.4–4.2 × 0.7–1.5 mm, apex obtuse, pink; midlobe not divided, obovate, 4.2–5.3 × 2.1–3.3 mm, apex sometimes undulate, pink; disk white with pink markings; spur white, straight to slightly incurved, cylindrical, 5.0–9.5 mm long, slightly shorter than or equal to ovary, apex slightly bilobed, entrance

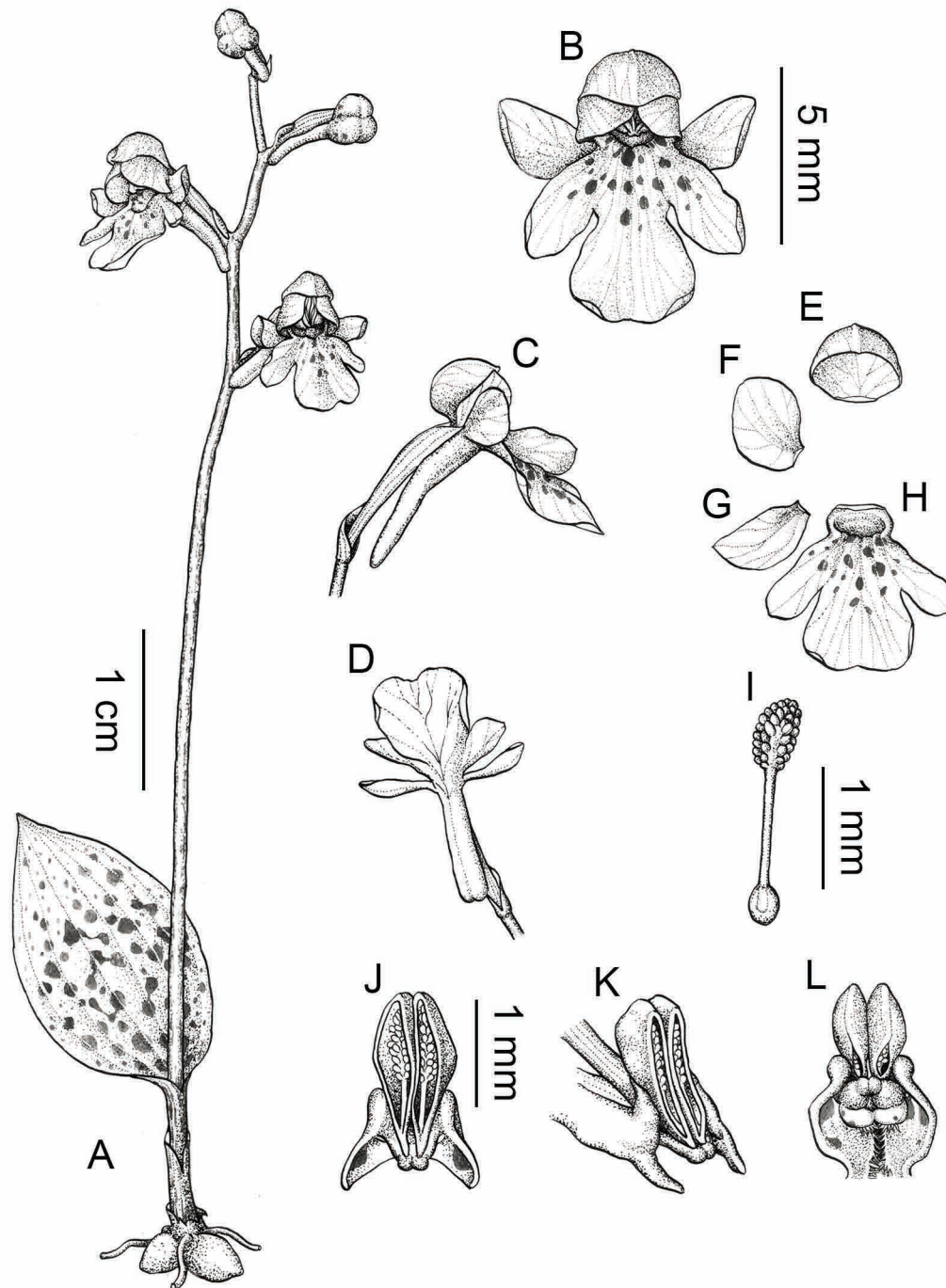


FIGURE 3. *Hemipilia galeata*. A. Habit. B–D. Flower, front, side and ventral views. E. Dorsal sepal. F. Petal. G. Lateral sepal. H. Labellum. I. Pollinium. J–L. Gynostemium, front, side and ventral views. Drawn by Y. Zhang.

1.5–2.0 mm wide, with both a few hairs on abaxial interior surface and purple markings on lateral interior surface near entrance; anther erect, 1.8 mm long, 2-locular, locules parallel and closely spaced, purple; pollinia 2, sectile; caudicles strap-like, 1.1 mm long, transparent; viscidia 2, closely spaced, elliptic, 0.3×0.2 mm, transparent, each enclosed within a separate bursicle; bursicles formed by folding of rostellar arms; rostellum median lobe inconspicuous, lateral lobes grooved; stigma ventral, lobes 2, divergent, lamelliform, 0.8×0.3 mm, with purple markings at apex and a few hairs at base; auricles 2, stout, 0.6 mm long, each placed laterally at base of anther.

Flowering:—Peaking in late April.

Distribution & habitat:—Currently known from a single population, which occurs within a small area on moss-covered Danxia rocks.

TABLE 3. Comparisons among *Hemipilia galeata* and morphologically similar species.

	<i>H. galeata</i>	<i>H. hemipilioides</i>	<i>H. occidentichuanensis</i>	<i>H. thailandica</i>
Numbers of leaves	one	one	one	one
Leaf shape	elliptic	ovate-orbicular to oblong	cordate, ovate or elliptic	lanceolate-oblong
Leaf color (adaxial)	green with purple markings	green with purple markings	green with purple markings	uniformly green
Sterile bract (amount)	absent	present (1–2)	absent	absent
Dorsal sepal shape	hemispheric	oblong-ovate	suboblong	oblong-ovate
Petal shape	suborbicular	oblong	ovate-cymbiform	elliptic
Lateral lip lobe shape	oblong	oblong-quadrate	suboblong	oblong
Midlobe shape	ovate	quadrate	subsquare	broadly ovate-oblong
Spur shape	cylindrical and slightly bilobed at apex	cylindrical	cylindrical and slightly swollen toward apex	cylindrical
Spur length (relative)	slightly shorter than or equal to ovary	shorter than ovary	equal to ovary	shorter than ovary

Etymology:—The epithet refers to the hemispheric, helmet-like dorsal sepal, by which the new species can be readily distinguished.

Taxonomic notes:—*Hemipilia galeata* belongs to *H. sect. Hemipilia sensu Tang et al.* (2015). Table 3 provides the detailed comparisons among *H. galeata* and morphologically similar species. Besides the easily distinguished, hemispheric dorsal sepal (Fig. 3E), *H. galeata* also has a unique set of characteristics: leaf solitary, elliptic, adaxially green with purple-markings; sterile bract absent; petal suborbicular; lateral lip lobes oblong; spur cylindrical, slightly shorter than or equal to ovary.

Particularly, *H. galeata* can be distinguished by suborbicular petals plus an ovate midlobe of the lip from *H. hemipilioides*, by an elliptic leaf plus oblong lateral lip lobes from *H. occidentichuanensis* (\equiv *Ponerorchis limprichtii*), and by a purple-spotted leaf plus suborbicular petals from *H. thailandica*.

The remaining species in *H. sect. Hemipilia* are easily distinguished from *H. galeata*. *Hemipilia brevicealcarata* Finet has a saccate spur, whereas *H. galeata* has a cylindrical spur. *Hemipilia purpureopunctata* is characterized by a spur that is contracted near its apex, whereas in *H. galeata* the spur is slightly bilobed at the apex. Moreover, *H. galeata* (Figs. 3J–L, 5A–C) lacks a protruding, tongue-like rostellum that characterizes the well-circumscribed *Hemipilia s.s.* species.

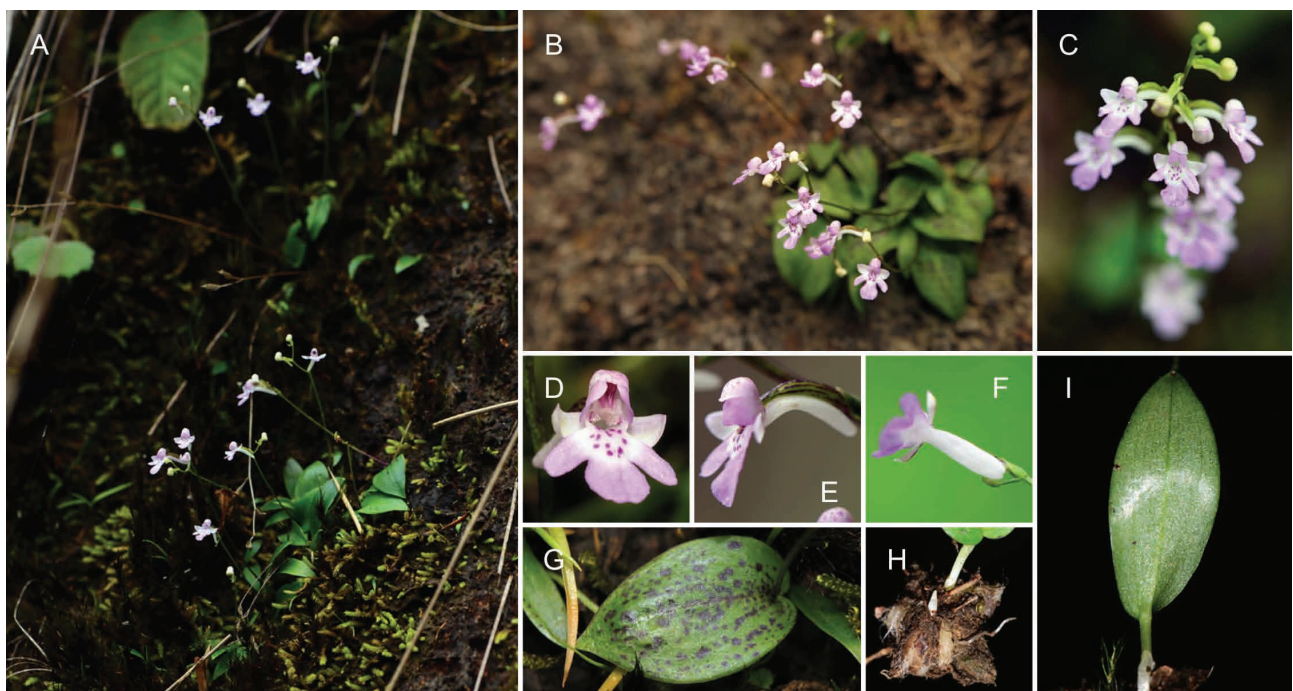


FIGURE 4. Habitat and morphology of *Hemipilia galeata*. A, C, E, F, H, I: photographed by X.-X. Zhu. B, D, G: photographed by Y. Tang.

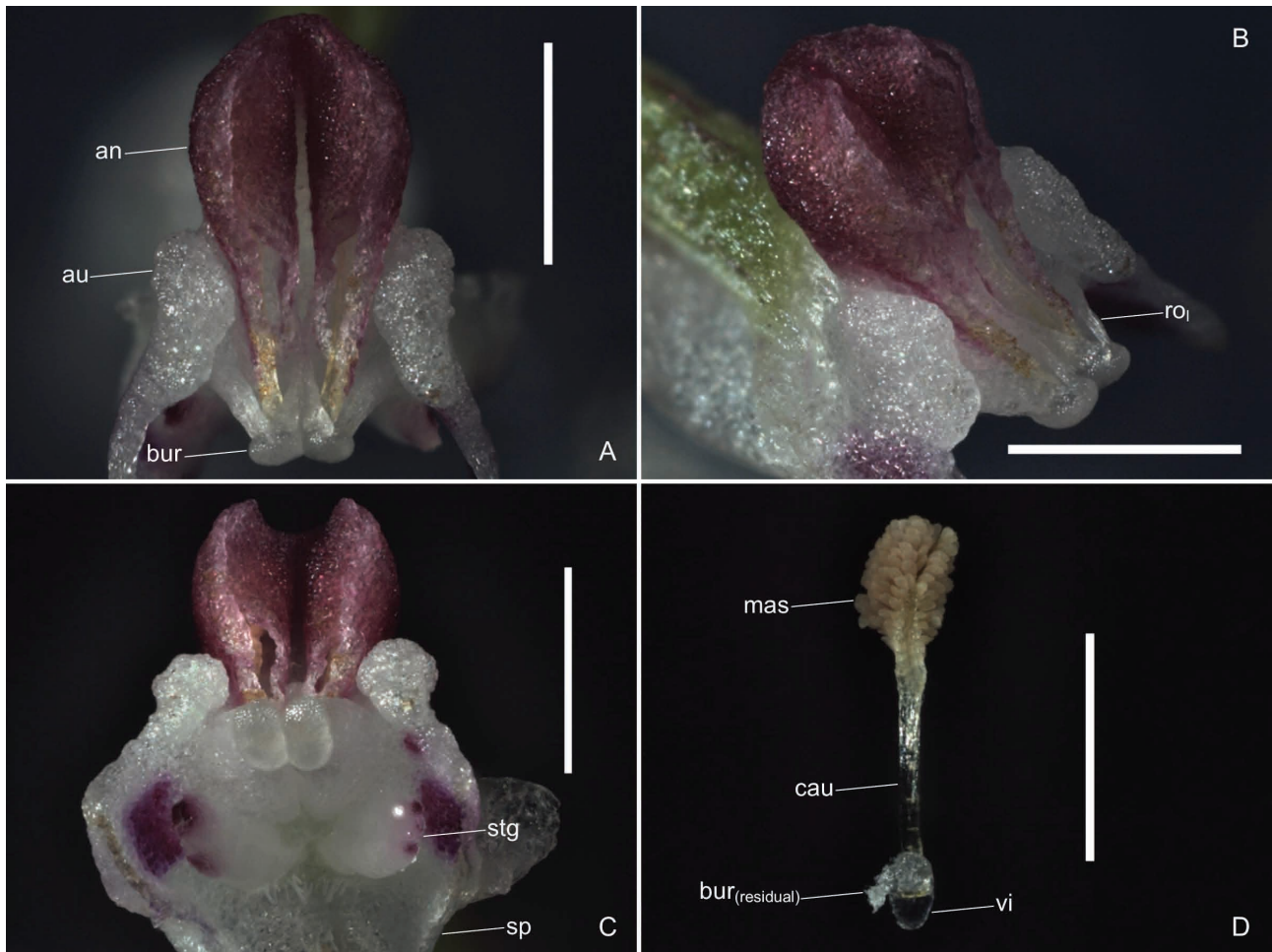


FIGURE 5. Gynostemium morphology of *Hemipilia galeata*. A. Front view of gynostemium. B. Side view of gynostemium. C. Ventral view of gynostemium. D. Front view of pollinium. Abbreviations: an, anther; au, auricle; bur, bursicle; ro_l, lateral rostellum lobe; stg, stigma; sp, spur; mas, massulae; cau, caudicle; vi, viscidium. Scale bar, 1 mm.

Discussion

There is strong support for the inclusion of *H. galeata* in the *Hemipilia* clade *sensu* Tang *et al.* (2015). However, within the clade, relationships among the species are not well resolved. *Hemipilia galeata* occurs on a long branch in both the nrITS and plastid DNA trees. Spurious attraction, therefore, may have influenced the placement of *H. galeata* as sister to *H. purpleopunctata* in the nrITS tree. Furthermore, the nrITS and combined plastid DNA trees place *H. galeata* in different places, which could be due to incongruence.

Morphologically, *H. galeata* differs from all known species of *Hemipilia* by a set of characteristics (see Taxonomy). In addition, *H. galeata* is the eastern-most species in the *Hemipilia* clade and has one of the earliest antheses in *Hemipilia* (late April), both features reminiscent of *H. pinguicula* (Rchb.f. in Moore 1878: 135) Y.Tang & H.Peng in Tang *et al.* (2015: 96) and *H. gracilis* (Blume 1856: 190) Y.Tang, H.Peng & T.Yukawa in Tang *et al.* (2015: 96); however, *H. galeata* is distinct genetically.

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