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Hemipilia zhuxiensis (Orchideae, Orchidaceae), a new species from Hubei Province, China

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Abstract

A new orchid species, is described and illustrated. *Hemipilia zhuxiensis* from the Shibali Long Canyon National Nature Reserve, Hubei Province, China, which is morphologically similar to *Hemipilia henryi* and *Hemipilia calophylla*. This species is assigned to *H*. section *Hemipilia* but differs from other species in having gibbous lip, shorter spur and unobvious lobes. Phylogenetic analyses were also conducted based on the three plastid DNA regions (*matK*, *trnL* intron and *trnL-F* spacer). The analyses revealed the phylogenetic relations between this species and its close allies.

Keywords: China, *Hemipilia*, new species, Hubei

Introduction

Hemipilia Lindley (1835: 296) are terrestrial herbs, characterized by relatively small globose tubers, unsheathed inflorescence, no membranous bracts, somewhat elongate and protruding rostellum, and two viscidia enclosed within two separate bursicles (Luo and Chen 2000; Chase et al. 2015; Tang et al. 2015). It comprises approximately 80 species, well represented from the

eastern Himalayas through Myanmar and Thailand to southeastern China (Luo 1999; Pridgeon et al. 2001).

During our fieldtrips to Qinling-bashan mountains, Hubei Province, southeastern China in 2020, we discovered a *Hemipilia* species that is rarely known. It differs from all known species in *Hemipilia* by having a ligulate-obovate and gibbous lip. The results of our phylogenetic analyses and morphological comparison indicated that it is new species. Here, we describe the new species and named it as *Hemipilia zhuxiensis* Hong Liu.

Materials & methods

Macromorphological observations: — The morphological analysis and description of the new species are based on the examination of live plants. In order to understand the diversity in the species, and to ascertain the discreteness of the new species, congenerics were examined at different herbaria (PE!, WHK!, IBSC!, KUN!). Specimens of *Hemipilia zhuxiensis* were deposited at the Herbarium of South-Central Minzu University (HSN).

Taxon sampling: — Botanical surveys were undertaken in the Hubei Province of China. In total，66 accessions, representing 6 species of *Hemipilia*, and related genera in tribe Orchideae were selected based on the study of Tang et al. (2016). GenBank accession numbers are provided in Table 1.

Table 1. Voucher information and GenBank accession numbers for sequence data of *Hemipilia zhuxiensis*.

Phylogenetic analyses: —Total DNA of *Hemipilia zhuxiensis* and the relative *Hemipilia henryi* were extracted from silica-dried leaf fragments using the modified 2× CTAB procedure of Doyle and Doyle (1987). The total DNA was sequenced using the Illumina NovaSeq platform at Majorbio Company (Shanghai, China). The nuclear ITS and three plastid loci (*matK* and *trnL-F* intergenic spacer) were chosen for the phylogenetic analyses.

Bayesian inference (BI) and maximum likelihood (ML) analyses were employed to generate phylogenetic trees. We reconstructed phylogenetic trees using the dataset with combined the plastid regions. Bayesian inference was performed using one million generations, four runs, four

chains, a temperature of 0.001, 25% trees discarded as burn-in, and trees sampled every 1,000 generations (1,000 trees sampled in total) with GTR+F+G4 model. the ML analysis using IQ-TREE v 2.0.6 (Nguyen et al. 2015) with 1,000 bootstrap replicates, and default ModelFinder (Kalyaanamoorthy et al. 2017) to find TVM+F+R3 as the best-fit substitution model. Tree visualization was achieved in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). *Satyrium nepalense* and *Disa buchenaviana* were chosen as outgroups according to Tang et al. (2016).

Results

Morphological analysis: — The key characteristics, such as tuber and leaf, inflorescence, flowers the newly discovered orchid and *Hemipilia henryi* were compared (Table 2). *Hemipilia zhuxiensis* is generally similar to *H. henryi* and *H. calophylla*, but differs from them by its shorter spur and gibbous lip.

Phylogenetic reconstruction: —The aligned matrix of combined sequence was 2,181 characters. Of the 1030 variable characters, 709 were parsimony-informative, including indels. Phylograms of BI and ML analyses agreed with each other with few excepted on the support on some nodes (Fig. 1). Most of the clades received middle to strong supports (PP >95, BPML > 75). *Hemipilia zhuxiensis* is most closely related to *H. henryi* with strong support (BPML = 100, PP = 100). Given the phylogenetic position of *H. zhuxiensis* and morphological differences from *H. calophylla* (Table 2), we described it as a new species.

Table 2. Comparisons among *Hemipilia zhuxiensis*, *H. henryi* and *H. calophylla*

Taxonomy

Type: —CHINA. Hubei: Zhuxi, Shibali Long Canyon National Nature Reserve, 733 m, 18 June 2020, HSN13099 (holotype: HSN). In order to protect this species, we do not publish the specific latitude and longitude.

Diagnosis: —Differing from all known species of *Hemipilia* in having an entire and spreading lip. Similar to *H. henryi* and *H. calophylla*, from which it can be distinguished by an elliptic, purple-spotted leaf, suborbicular petals, oblong lateral lip lobes and an ovate mid lobe (Table 2).

***Hemipilia zhuxiensis* Hong Liu, sp. nov.**

Terrestrial herbs, 17-25 cm tall. Tubers ellipsoid, 4-11 × 3-5 mm, neck with few roots. Stem slender, 1 mm in diameter, green with purple spots, with 1 tubular sheath at base. Leaf solitary, elliptic, 6-12 ×5-8 cm, apex subacute, base cordate or contracted into amplexicaul sheath, adaxially green with purple markings, rarely uniformly green, abaxially pale green. Inflorescence terminal, 14-23 cm long; rachis 6-10 cm long, laxly 4-9-flowered; floral bracts lanceolate, 3-5 × 1-3 mm, apex acuminate or long acuminate. Flowers white to pink; pedicel and ovary straight to slightly arcuate, 13-21 mm long. Dorsal sepal ovate-elliptic, 6-9 × 3-7 mm, 3-veined, apex obtuse; lateral sepals broadly ovate, oblique, spreading, 7-10 × 5-8 mm, 3-veined, apex obtuse, white to pale pink; petals obliquely ovate, 6-7 × 4-5 mm, 1-veined, apex obtuse, white to pink; lip ligulate-obovate, gibbous, 10 × 7 mm, adaxially purplish pink, abaxially pale pink, slightly 3-lobed; lateral lobes widely triangular; middle lobe elliptic, margins sometimes erect, apex obtuse to emarginate; a ridge ranging from base of lateral lobes to the middle of lateral lobe ; spur short and infundibuliform, slightly curved downward, narrowly conic, 4-6 mm long, entrance 2-2.5 mm wide. Column ca. 3 mm long; rostellum tongue-like, purple, ca. 2 mm, apex acute.

Etymology: —This species is named after the type locality.

Phenology: —Flowering in June.

Distribution & habitat: —Currently known from a single population, which occurs within a small area on palisades of Shibali Long Canyon National Nature Reserve.

Discussion

The phylogenetic relationships based on the combined datasets (nuclear ribosomal ITS and plastid DNA) show that *Hemipilia zhuxiensis* is a member of *Hemipilia* (Tang et al. 2015), sister to *H. henryi* (Fig. 1). *H. zhuxiensis* occurs on a long branch in the phylogenetic trees. Given the morphological differences from *H. henryi*, we conclude that it should be treated as a distinct new species. However, the genetic and morphological variation with *H. zhuxiensis* has not been exhaustively studied. We only measured and included one accession of *H. zhuxiensis* from China. Given the morphologically of *H. zhuxiensis* differs from all known species of *Hemipilia* by a set of characteristics, we describe it as new species.

Acknowledgements

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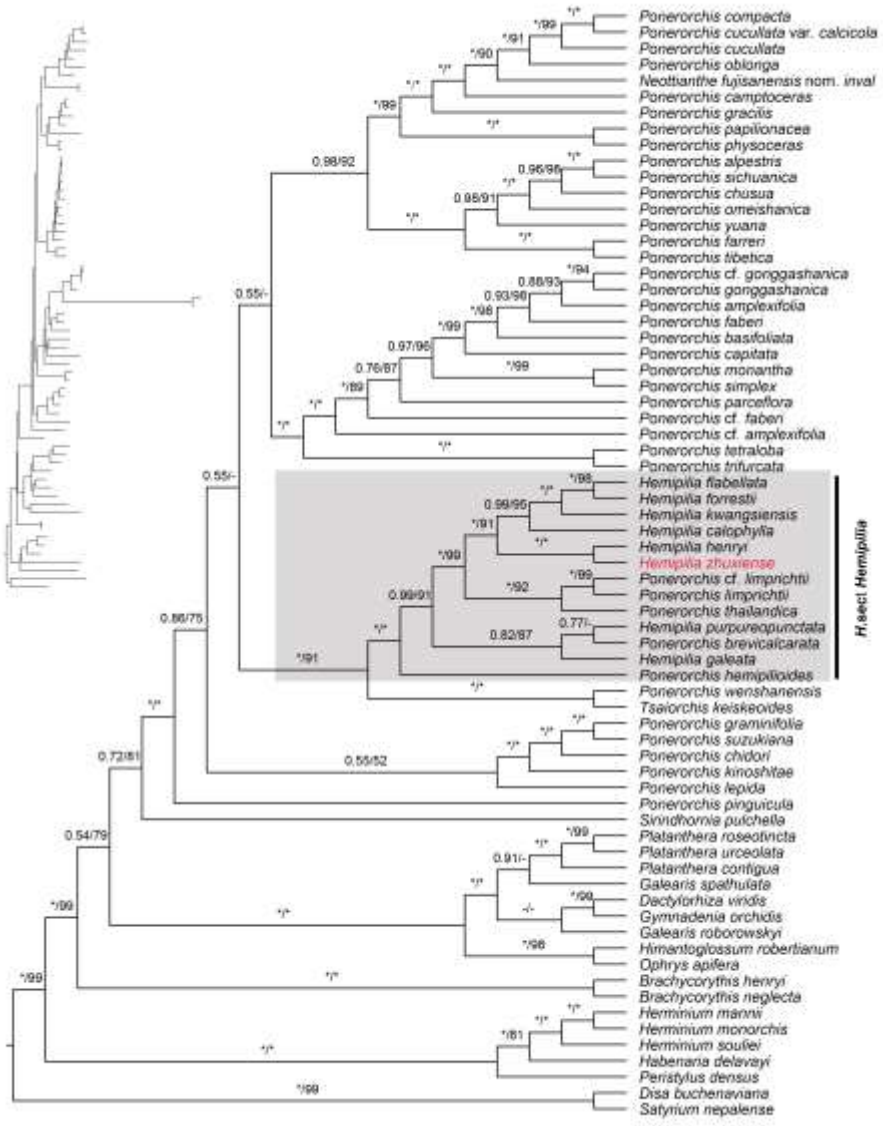

166

167 **Figure 1.** Phylogenetic relationships of *Hemipilia zhuxiensis* based on the combined nrITS and
168 plastid DNA. The numbers near the nodes are Maximum likelihood bootstrap percentages
169 (BPML), Bayesian posterior probabilities (PP). "*" indicates that the node has BP 100 or PP 100
170 "-" indicates that the node is incongruent between the topology of the ML tree and the Bayesian.
171 The new species are highlighted in red. The clade of *H.*sect *Hemipilia* is highlighted with the
172 grey rectangle.

173

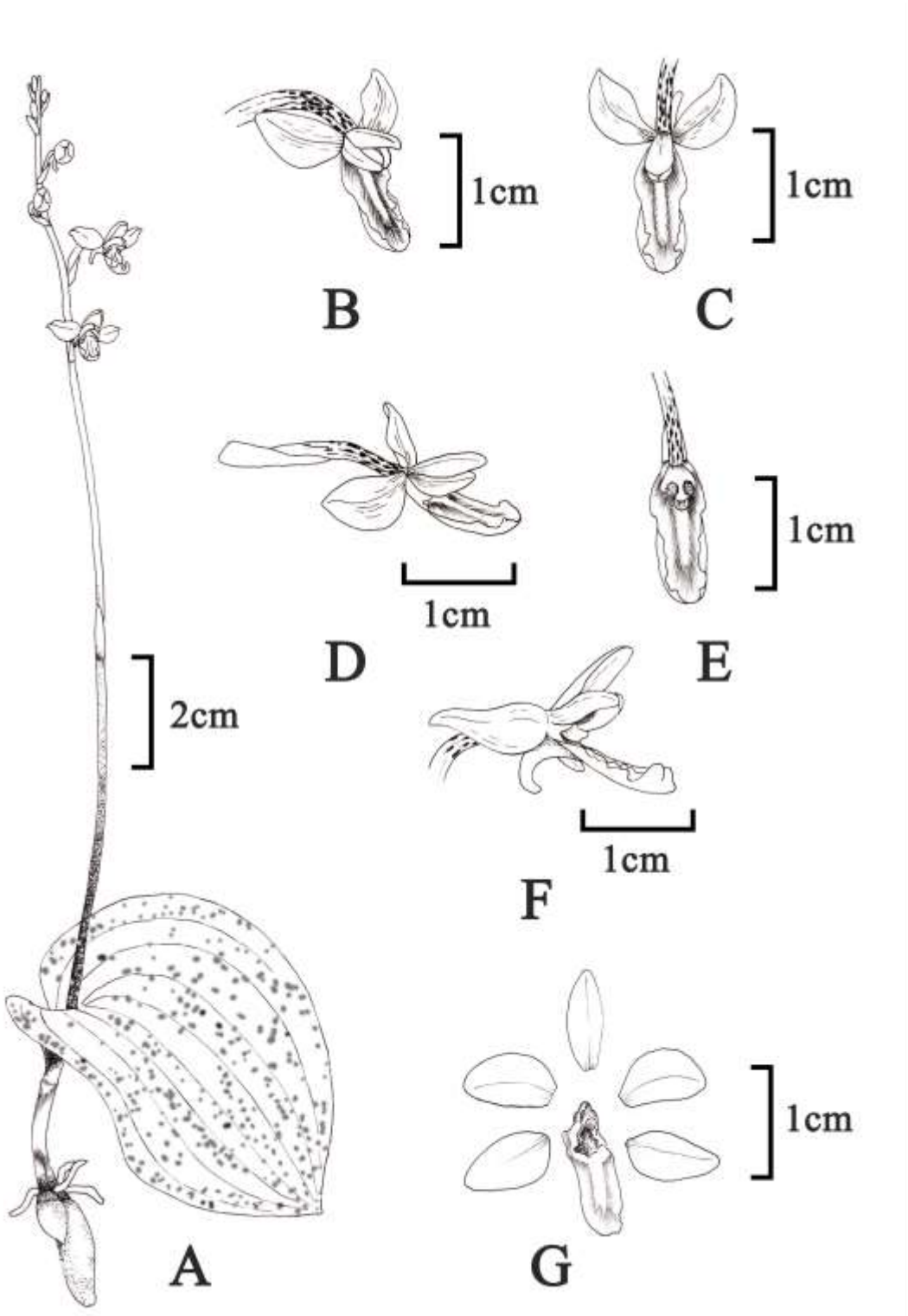

Figure 2. *Hemipilia zhuxiensis*. A. Flowering plant. B. Flower, front view. C. Flower, side view. D. Lip, front view. E. Lip, side view. F. Dorsal sepal. G. Petal. H. Lateral sepals. Drawn by Ta-Li Cai.

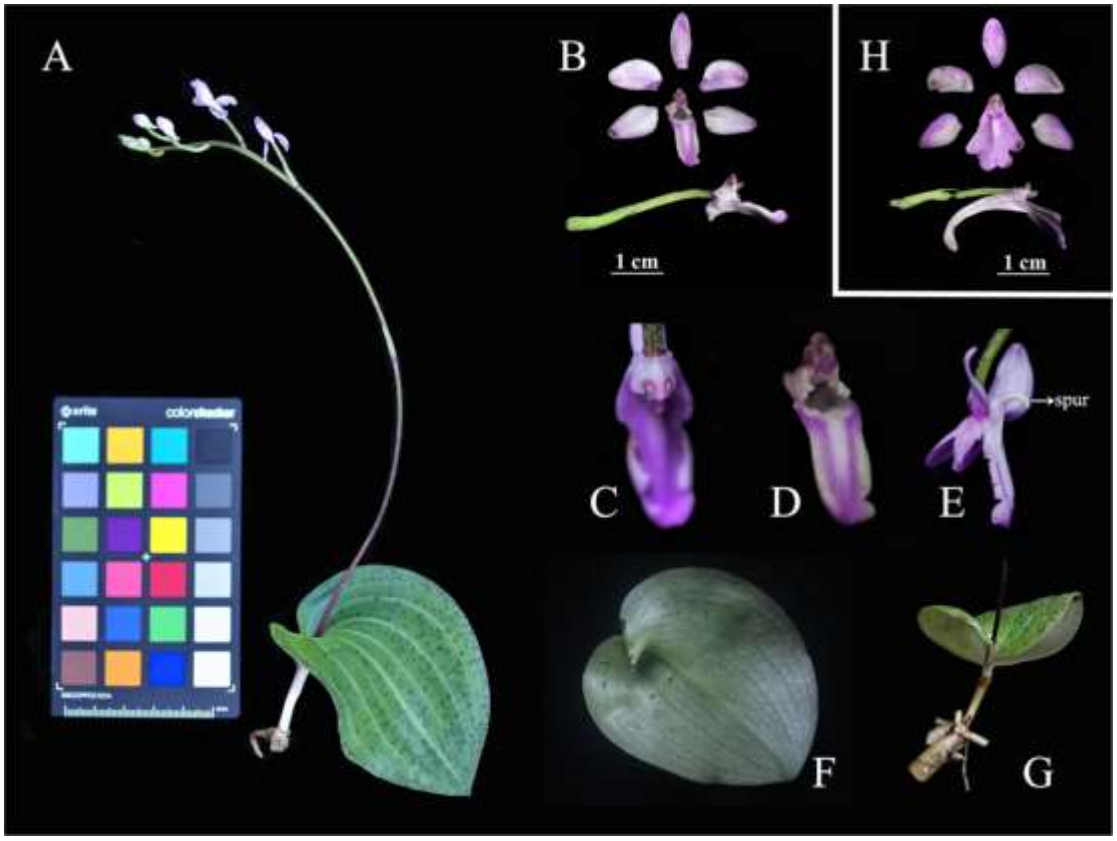

Figure 3. *Hemipilia zhuxiensis*. A. Flowering plant. B. Anatomy of a flower. C. Lip, front view. D. Lip and column. E. Flower, side view. F. Leaf, back view. G. Tubers and roots. H. Anatomy of *H. henryi*.

182 **Table 1.** Voucher information and GenBank accession numbers for sequence data of *Hemipilia*

183 *zhuxiensis*.

Species	nrITS	*matK*	*trnL-F*
Brachycorythis henryi (Schltr.) Summerh.	KM651260	KM651424	KM651586
Brachycorythis neglecta H.A.Pedersen	KM651261	KM651425	KM651587
Dactylorhiza viridis (Linnaeus) R. M. Bateman, Pridgeon & M. W. Chase	KM651262	KM651426	KM651588
Disa buchenaviana Kraenzl.	DQ414921	DQ415064	DQ415205
Galearis roborowskyi (Maxim.) S.C.Chen, P.J.Cribb & S.W.Gale	KM651265	KM651429	KM651591
Galearis spathulata (Lindley) P. F. Hunt	KM651266	KM651430	KM651592
Gymnadenia orchidis Lindl.	KM651267	KM651431	KM651593
Habenaria delavayi Finet	KM651268	KM651432	KM651594
Hemipilia calophylla E. C. Parish & H. G. Reichenbach	KM651269	KM651433	KM651595
Hemipilia flabellata Bur. et Franch.	KM651271	KM651435	KM651597
Hemipilia forrestii Rolfe	KJ460049	KJ452805	MF945326
Hemipilia galeata	KT183499	KT183498	KT183500
Hemipilia kwangsiensis T. Tang et F. T. Wang ex K. Y. Lang	KM651272	KM651436	KM651598
Hemipilia purpureopunctata	KJ460051	KJ452807	MF945328
Herminium mannii (Rchb.f.) Tang & F.T.Wang	KM651283	KM651447	KM651609
Herminium monorchis (L.) R.Br.	KM651273	KM651437	KM651599
Herminium souliei (Finet) Rolfe	KM651274	KM651438	KM651600
Himantoglossum robertianum (Loisel.) P.Delforge	AY351384	AY368382	AY014584
Neottianthe fujisanensis (Sugim.) Maek.	KM651280	KM651444	KM651606
Ophrys apifera Huds.	AJ539529	AJ310049	AJ409432
Peristylus densus (Lindl.) Santap. et Kapad.	KM651282	KM651446	KM651608
Platanthera contigua Tang & F.T.Wang	KM651263	KM651427	KM651589
Platanthera roseotincta (W. W. Smith) T. Tang et F. T. Wang	KM651284	KM651448	KM651610
Platanthera urceolata (Hook.f.) R.M.Bateman	KM651264	KM651428	KM651590
Ponerorchis alpestris (Fukuy.) X.H.Jin, Schuit. & W.T.Jin	KM651221	KM651385	KM651545
Ponerorchis amplexifolia (Tang & F.T.Wang) X.H.Jin, Schuit. & W.T.Jin	KM651222	KM651386	KM651546
Ponerorchis basifoliata (Finet) X.H.Jin, Schuit. & W.T.Jin	KM651223	KM651387	KM651547
Ponerorchis brevicalcarata (Finet) Soó	KM651285	KM651449	KM651611
Ponerorchis camptoceras (Rolfe ex Hemsl.) X.H.Jin, Schuit. & W.T.Jin	KM651275	KM651439	KM651601
Ponerorchis capitata (Tang & F.T.Wang) X.H.Jin, Schuit. & W.T.Jin	KM651224	KM651388	KM651548
Ponerorchis cf. *amplexifolia* Tang 132	KM651225	KM651415	KM651549

Ponerorchis cf. *faberi* Tang & Su 128	KM651226	KM651395	KM651550
Ponerorchis cf. *gonggashanica* Tang 160	KM651296	KM651420	KM651551
Ponerorchis cf. *limprichtii* Lee 201228	KM651287	KM651462	KM651621
Ponerorchis chidori (Makino) Ohwi	KM651288	KM651451	KM651612
Ponerorchis chusua (D.Don) Soo	JN696455	KM651452	KM651616
Ponerorchis cucullata (L.) X.H.Jin, Schuit. & W.T.Jin	KM651278	KM651443	KM651602
Ponerorchis cucullata var. *calcicola* (W.W.Sm.) X.H.Jin, Schuit. & W.T.Jin	KM651279	KM651440	KM651605
Ponerorchis faberi (Rolfe) X.H.Jin, Schuit. & W.T.Jin	KM651230	KM651391	KM651554
Ponerorchis farreri (Schltr.) X.H.Jin, Schuit. & W.T.Jin	KM651231	KM651392	KM651555
Ponerorchis gonggashanica (K.Y.Lang) X.H.Jin, Schuit. & W.T.Jin	KM651233	KM651393	KM651557
Ponerorchis gracilis (Blume) X.H.Jin, Schuit. & W.T.Jin	KM651235	KM651397	KM651559
Ponerorchis graminifolia Rchb.f.	KM651294	KM651458	KM651619
Ponerorchis hemipilioides (Finet) Soo	KM651238	KM651400	KM651562
Ponerorchis kinoshitae (Makino) X.H.Jin, Schuit. & W.T.Ji	KM651241	KM651403	KM651565
Ponerorchis lepida (Rchb.f.) X.H.Jin, Schuit. & W.T.Jin	KM651242	KM651404	KM651566
Ponerorchis limprichtii (Schltr.) Soo	KM651298	KM651461	KM651623
Ponerorchis monantha (Finet) X.H.Jin, Schuit. & W.T.Jin	KM651244	KM651405	KM651567
Ponerorchis oblonga (K.Y.Lang) X.H.Jin, Schuit. & W.T.Jin	KM651281	KM651445	KM651607
Ponerorchis omeishanica (T.Tang, F.T.Wang & K.Y.Lang) S.C.Chen, P.J.Cribb & S.W.Gale	KM651299	KM651464	KM651624
Ponerorchis papilionacea (Tang, F.T.Wang & K.Y.Lang) X.H.Jin, Schuit. & W.T.Jin	-	KM651408	KM651570
Ponerorchis parceflora (Finet) X.H.Jin, Schuit. & W.T.Jin	KJ460052	KJ452808	KM651571
Ponerorchis physoceras (Schltr.) X.H.Jin, Schuit. & W.T.Jin	KM651248	KM651410	KM651573
Ponerorchis pinguicula (Rchb.f. & S.Moore) X.H.Jin, Schuit. & W.T.Jin	KM651252	KM651411	KM651575
Ponerorchis sichuanica (K.Y.Lang) S.C.Chen, P.J.Cribb & S.W.Gale	KJ460059	-	-
Ponerorchis simplex (Tang & F.T.Wang) X.H.Jin, Schuit. & W.T.Jin	KM651253	KM651416	KM651578
Ponerorchis suzukiana (Ohwi) J.M.H.Shaw	-	KM651459	KM651625
Ponerorchis tetraloba (Finet) X.H.Jin, Schuit. & W.T.Jin	KM651255	KM651418	KM651580
Ponerorchis thailandica (Seidenf. & Thaithong) X.H.Jin, Schuit. & W.T.Jin	KM651256	KM651419	KM651581
Ponerorchis tibetica (Schltr.) X.H.Jin, Schuit. & W.T.Jin	KM651257	KM651421	KM651582

Ponerorchis trifurcata (Tang, F.T.Wang & K.Y.Lang) X.H.Jin, Schuit. & W.T.Jin	KJ460055	KJ452811	KM651583
Ponerorchis wenshanensis (W.H.Chen, Y.M.Shui & K.Y.Lang) X.H.Jin, Schuit. & W.T.Jin	KM651258	KM651422	KM651584
Ponerorchis yuana (Tang & F.T.Wang) X.H.Jin, Schuit. & W.T.Jin	KM651259	KM651423	KM651585
Satyrium nepalense D. Don	KM651301	KM651465	KM651626
Sirindhornia pulchella H. A. Pedersen & Indham.	KJ460045	-	-
Tsaiorchis keiskeoides (Gagnep.) X.H.Jin, Schuit. & W.T.Jin	KM651240	KM651402	KM651564

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186 **Table 2.** Comparisons among *Hemipilia zhuxiensis*, *H. henryi* and *H. calophylla*

	Hemipilia zhuxiensis	***Hemipilia henryi***	***Hemipilia calophylla***
Numbers of leaves	One	One	One
Leaf shape	Elliptic	Ovate	suborbicular to ovate
Leaf color (adaxial)	green with purple markings	green with purple spots	dark green netted with purplish brown
Sterile bract (amount)	Absent	Present（2-4)	Absent
Dorsal sepal color	white to pale pink	Pink	white to green
Petal shape	obliquely ovate	obliquely rhombic-ovate	Ovate
lip shape	ligulate-obovate, gibbous	broadly obovate-cuneate	oblong to obovate
Spur shape	short and infundibuliform, apex hooked	straight and horizontal or slightly curved downward	conic to urceolate
Spur length (relative)	shorter than ovary	equal to ovary	shorter than ovary

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