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# *Hemipilia avisoides* (Orchidaceae), a new species from Sichuan Province, China

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## 1 *Hemipilia avisoides* (Orchidaceae), a new species

# 2 from Sichuan Province, China

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#### 12 Abstract

- 13 A new orchid species, *Hemipilia avisoides*, is described from Songpan County
- 14 and Maoxian County, Sichuan Province, China. Morphologically, it is most
- similar to *H. occidensichuanensis* ( $\equiv$  *Ponerorchis limprichtii*), but *H. avisoides*
- 16 can be distinguished by the combination of its involute middle lip lobe that is
- 17 smaller than the lateral lobes, pendulous lateral lip lobes, floral bracts that are
- always shorter than the ovary, a leaf that is appressed to substrate and is
- adaxially green with white veins and a solitary sheath at the stem base. The
- 20 floral morphology of *H. avisoides* is also presented by utilising *in vivo* micro-
- 21 CT scanning and 3D visualisation.

### 22 Keywords

23 Arid alley, Minjiang River Valley, Orchidinae, taxonomy

### 24 Introduction

- 25 The genus Hemipilia Lindl. sensu stricto (Orchideae, Orchidaceae) comprises
- 26 ca. 10 species that are characterised by a protruding, tongue-like rostellum
- 27 (Luo & Chen 2000; Chen et al. 2009). Nevertheless, molecular phylogenies
- 28 revealed *Hemipilia s.s.* as monophyletic, but nested deeply within a strongly-
- 29 supported clade that also included several species from closely-allied genera,

for example, *Ponerorchis* Rchb. f., *Amitostigma* Schltr. and the monotypic *Hemipiliopsis* (K. Y. Lang) Y. B. Luo & S. C. Chen (Luo 1999; Bateman et al.
2003; Jin et al. 2014, 2017; Tang et al. 2015). Given the fact that *Ponerorchis*and *Amitostigma* are paraphyletic, both Jin et al. (2014) and Tang et al. (2015)
formally proposed to expand the circumscription of *Hemipilia*, although to a
different extent. Here, we follow the treatment of *Hemipilia sensu latissimo*, in
which seven sections were recognised (Tang et al. 2015).

37 During the field trip in 2013 to collect A. physoceras Schltr. in Minjiang 38 River Valley, Songpan County, Sichuan Province, China, one of the authors 39 (Y. Tang) collected another orchid that morphologically fits into the category of 40 Hemipilia s.l. It had been temporarily identified as cf. Ponerorchis limprichtii 41 (Schltr.) Soó in the previous study by Tang et al. (2015). However, this taxon in Songpan not only differs in the morphology of the labellum and leaf, but 42 43 also diverges in DNA sequences, both of which suggest it as a potential new 44 species (Tang et al. 2015). Here, we describe it in H. sect. Hemipilia sensu 45 Tang et al. (2015) and present its floral morphology by utilising an in vivo 46 micro-CT method.

#### 47 Methods

#### 48 Material collection

During our field investigation to Minjiang River Valley, Songpan, Sichuan,
China in 7–9 June 2022, two populations of the new taxon with 12 flowering
individuals were found. One population occurs at the same locality that was
visited in 2013 by one of the authors (Y. Tang) and the other is ca. 11.2 km
southwards in the Valley.

A total of four living individuals with intact flowers from the two populations were collected, each was packaged with soils and EPE pearl cotton in a plastic bottle and transported by air to the Key Laboratory of Stratigraphy and Paleontology, Ministry of Natural Resources for *in vivo* micro-CT scanning. After scanning, these individuals were pressed as specimens. The voucher specimens were deposited at the Herbarium of Sichuan University (SZ).

#### 61 Morphological observations

The morphological description of the new taxon was mainly based on living
materials. The length and width of leaves and the height of the inflorescence
were measured on seven living, flowering plants in the field. The morphology

of subterranean parts was described on the four plants collected (see Materialcollection). The morphology of a single flower was described mainly on the

67 basis of the 3D mesh model reconstructed by micro-CT data.

#### 68 Micro-CT scanning and 3D Visualisation

X-ray Computed Tomography (CT) was completed at the Key Laboratory of
Stratigraphy and Paleontology, Ministry of Natural Resources. One of the four
living individuals collected (see Material collection) was finally selected for
scanning and was then chosen as the holotype of the new taxon. Its
inflorescence with the uppermost three flowers was scanned *in vivo* in a Nikon
XTH 225ST CT scanner at a resolution of 18.6 µm and X-ray of 90 kv and 70
µA.

3D reconstructions were performed in the software VGSTUDIO MAX 3.0 with STL files being exported. For the 3D model of inflorescence, however, only the uppermost two flowers were reconstructed due to the trade-off between resolution and computing time. Acquired 3D mesh models were visualised and processed by the software GOM INSPECT PRO in GOM SUITE 3.1.1109.0.

#### 82 **Results**

#### 83 **Taxonomic treatment**

84 *Hemipilia avisoides* Y. Tang, X. M. Wang & H. Peng, sp. nov.

85 Figs. 1A–D, 2 and 3; see also Data availability

86 Type. CHINA, Sichuan Province, Aba Tibetan and Qiang Autonomous

87 **Prefecture**, Songpan County, 9 June 2022, Y. *Tang, X. M. Wang* & Y. *T. Zhu* 

88 235 (holotype: SZ!); Songpan County, 9 June 2022, Y. Tang, X. M. Wang & Y.

89 T. Zhu 236 (paratype: SZ!); Maoxian County, 1 June 1958, S. Y. Chen, Z. He,

90 *M. F. Zhong et al. 5078* (paratype: SZ!).

Diagnosis. Similar to *Hemipilia occidensichuanensis* Y. Tang & H. Peng
(≡ *Ponerorchis limprichtii* (Schltr.) Soó), from which *H. avisoides* can be
distinguished by a set of characteristics: mid-lobe involute, suboblong in apical
view, trapeziform when flattened, smaller than lateral lobes; lateral lobes
pendulous; floral bracts 5 mm long, always shorter than ovary; leaf appressed
to substrate, adaxially green with white veins; stem with one sheath at base
(Fig. 1; Table 1).

98 **Description.** Terrestrial, erect herbs, 8.5–31 cm tall. Tubers oblong, 2.5 99 cm long, 0.8 cm in diameter, neck with few roots. Stem subterranean, 2.7-5 100 cm long, 0.2 cm in diameter, with one sheath at base. Sheath tubular, 101 membranous, 1–2 cm long, pale yellow. Leaf appressed to substrate, solitary, 102 cordate, ovate or elliptic, 3-6.5 × 2-5.5 cm, apex acute, slightly fleshy, 103 glabrous, abaxially purple, adaxially green with white veins, occasionally also 104 with purple spots. Inflorescence terminal, erect, 3–14 cm long, 1–21-flowered, 105 glabrous, dark purple. Flowers not secund, plum or purple plum, fragrant; 106 floral bracts connivent to ovary, elliptic, 5 × 2.6 mm, shorter than ovary, apex 107 acuminate, glabrous, dark purple; ovary curved, cylindrical, 10.5 mm long 108 including pedicel, 1 mm in diameter, glabrous, dark purple. Dorsal sepal erect, 109 oblong, cymbiform, 4.5 × 2.6 mm, apex rounded, sometimes concave at each 110 side of central vein below middle, glabrous; lateral sepals spreading, obliquely 111 ovate, cymbiform, 5.6 × 3.6 mm, apex obtuse, glabrous. Petals erect, 112 connivent with dorsal sepal and forming a hood, apex bending similar to holding a fist in the other hand, obliquely ovate, 4 × 2.8 mm, apex obtuse, 113 114 glabrous. Labellum spreading, broadly ovate when flattened, 7.1 × 5.4 mm, 3-115 lobed below middle, spurred, base collar-like raised on each side of spur 116 entrance, glabrous, tinged white at base, disc dotted with purple; lateral lobes 117 pendulous, rhombic, 3.4 × 2.5 mm, apex truncate, margin slightly undulate; 118 mid-lobe horizontal, involute, suboblong in apical view, 2.2 × 1.2 mm, 119 trapeziform when flattened, apex rounded or sometimes apiculate; spur 120 horizontal, straight or curved upwards, cuneate, 9 mm long, ventrally carinate 121 along central axis, entrance 2.5 mm wide, apex swollen, obtuse, 2.7 mm wide; 122 anther reclined, 2.8 mm long, 2-locular, locules parallel and closely spaced, 123 aubergine; pollinia 2, sectile, ovate, 1.2 × 0.7 mm; caudicles cuneate, 1.2 mm 124 long; viscidia 2, closely spaced, oblong, transparent, each enclosed within a 125 separate bursicle; bursicles formed by folding of rostellar arms, oblong, 0.6 × 126 0.3 mm; rostellum median lobe triangle, 0.7 mm long, lateral lobes grooved; 127 stigma ventral, lobes 2, divergent, lamelliform, 1.2 × 0.5 mm, with hairs at 128 base; auricles 2, each placed laterally at base of anther, behind collar of 129 labellum base, 0.5 mm long.

130 **Flowering.** Peaking in early June.

Distribution and habitat. *Hemipilia avisoides* is currently known from
two localities in Songpan County, which are ca. 11.2 km apart along the
Minjiang River Valley and one locality in Maoxian County according to the
collection by S. Y. Chen et al. in 1958. Individuals of the new taxon occur
under arid-valley shrubs and on moss-covered rocks (see Discussion).

136 **Etymology.** The epithet refers to the resemblance of flowers and

inflorescence of *Hemipilia avisoides* to wild geese that are flapping wings andflying in formation.

139 Chinese name. 雁字舌喙兰 (Chinese pinyin: yàn zì shé huì lán).

# Additional specimens examined. *Hemipilia avisoides*: CHINA, Sichuan Province, Aba Tibetan and Qiang Autonomous Prefecture, Songpan

142 County, 30 June 2013, Y. Tang 151 (KUN!).

143 Hemipilia occidensichuanensis: CHINA, Sichuan Province, Ganzi 144 Tibetan Autonomous Prefecture, Kangding City, 18 June 2022, Y. Tang, X. 145 M. Wang, W. Q. Yuan & Y. T. Zhu 237 (SZ!); Kangding City, 17 June 2017, Y. 146 L. Peng, Q. Yu & L. L. Li THP-KD-1390 (CDBI!); Kangding City, 13 June 2014, 147 Y. Tang 199 (KUN!); Luhuo County, 12 August 2005, D. E. Boufford, J. H. 148 Chen, K. Fujikawa, S. L. Kelley, R. H. Ree, H. Sun, J. P. Yue, D. C. Zhang & 149 Y. H. Zhang 34770 (A!); Xiangcheng County, 15 July 2004, D. E. Boufford, J. 150 H. Chen, S. L. Kelley, J. Li, R. H. Ree, H. Sun, J. P. Yue & Y. H. Zhang 30764 151 (A!); Daofu County, 10 June 1996, J. S. Yang 91-270 (IBSC!; PE!); Kangding 152 City, 28 May 1981, Z. J. Zhao, J. B. Shi & D. G. Fan 114262 (SZ!); Xinlong 153 County, 28 June 1974, Z. S. Qin 06383 (CDBI!); Xinlong County, 27 June 154 1974, Z. S. Yu 06409 (CDBI!); Yajiang County, 15 June 1961, S. Jiang 05196 155 (KUN!). CHINA, Sichuan Province, Aba Tibetan and Qiang Autonomous 156 Prefecture, Xiaojin County, 21 May 1959, Xiao Jin Zu 0130 (SZ!); Xiaojin 157 County, 21 May 1957, J. Zhou 34 (IBSC!); Maerkang City, 16 May 1957, X. Li 158 71047 (PE!; SZ!). CHINA, Gansu Province, Longnan City, Wenxian County, 159 12 May 2007, Bai Shui Jiang Cai Ji Dui 4839 (PE!); Wenxian County, 9 May 160 2007, Bai Shui Jiang Cai Ji Dui 4514 (PE!); Wudu District, 15 June 1959, Z. Y. 161 Zhang 4390 (WUK!); Wudu District, 5 June 1959, Z. Y. Zhang 3379 (WUK!); 162 Wudu District, 30 May 1959, Z. Y. Zhang 3180 (PE!; WUK!). CHINA, Gansu 163 Province, Gannan Tibetan Autonomous Prefecture, Zhougu County, 27 164 May 1999, Bailongjiang Exped. 1408 (PE!). CHINA, 1959, Chuan Jing A 0130 165 (KUN!); July 1907, E. H. Wilson 1762 (specimen 2nd from left: AMES!).

166 Conservation status. Hemipilia avisoides seems narrowly distributed 167 within the Arid valley in the upper reaches of Minjiang River (see Discussion), 168 with few populations and individuals being found. The habitat of *H. avisoides* 169 could be easily disturbed by people as it is close to roads and villages. 170 However, further field investigations in Minjiang River Valley and areas nearby 171 are necessary to assess the exact distribution and population status of H. 172 avisoides. Therefore, we here temporarily assign *H. avisoides* to the category 173 DD (Data Deficient) according to the International Union for Conservation of 174 Nature (IUCN Standards and Petitions Committee 2022).

#### 175 Discussion

The new species Hemipilia avisoides has oblong tubers, two erect anthers, 176 177 two stigmas that are beneath the rostellum and two sectile pollinia with 178 viscidium each enclosed within a bursicle. These characteristics fit well into 179 the category of the Amitostigma alliance or Hemipilia s.l. (Tang et al. 2015). 180 Hemipilia avisoides has also a solitary, slightly fleshy leaf that is appressed to substrate, which is quite reminiscent of Hemipilia s.s. (Chen et al. 2009). 181 182 However, the median rostellum lobe of *H. avisoides* never protrudes between 183 anther cells like that of Hemipilia s.s.

184 Molecular phylogenies did, however, reveal a close relationship between 185 Hemipilia s.s. and H. avisoides in a clade (figs. 1–3 in Tang et al. 2015, H. 186 avisoides identified as Ponerorchis cf. limprichtii). The sister relationship 187 between *P. limprichtii* (= *H. occidensichuanensis*) and *H. avisoides* is also 188 strongly supported in both nuclear and chloroplast trees (Tang et al. 2015), 189 while they are most similar in morphology (see Taxonomic treatment and 190 below). Moreover, H. avisoides and P. limprichtii each occupies a long branch 191 in molecular trees, exhibiting considerable DNA sequence divergences (Tang 192 et al. 2015).

193 Amongst the specimens of *P. limprichtii*, one collection by S. Y. Chen et 194 al. in 1958 caught our attention for it was gathered from Maoxian County at an 195 elevation of 1780 m, this being close to the localities where we discovered H. 196 avisoides. The environment of that region differs from the alpine habitat that 197 P. limprichtii usually favours. After careful examination, we believe that this 198 specimen represents *H. avisoides* here described, although it was initially 199 identified as *P. limprichtii*. We highlighted in Fig. 3 the key features, which facilitated our identification of the specimen. Nevertheless, rather than on 200 201 living plants, some subtle features could faintly be observed on pressed 202 specimens of *H. avisoides* and its similar species. For example, the three-203 dimensional structure of flowers would collapse once pressed and the colours 204 of leaves would fade away when drying. This might obscure the discrepancies 205 between H. avisoides and P. limprichtii or even other more distantly related 206 species like P. chusua (D. Don) Soó. We hope that the 3D mesh model 207 reconstructed in this study (see Data availability) would become helpful for 208 recognising *H. avisoides* in future research.

*Ponerorchis limprichtii* is also distributed in Gansu Province, which is
 north of Sichuan Province. According to the vegetation regionalisation of
 China (Zhang 2007) and the information of specimens (see Additional
 specimens examined), the habitats of *P. limprichtii* in Gansu probably range

213 from arid-valley shrubs to deciduous broadleaved forests at an elevation 214 between 1250 and 1850 m. Besides herbarium specimens, there are some 215 photo records of *P. limprichtii* in Gansu, despite initially being assigned only to 216 genus level or to other species by the photographers, on the web site of Plant 217 Photo Bank of China, PPBC (in Chinese; see http://ppbc.iplant.cn/tu/5920959 218 (by R. B. Zhu in Zhouqu County), http://ppbc.iplant.cn/tu/5919232 (by R. B. 219 Zhu in Wenxian County), http://ppbc.iplant.cn/tu/5919279 (by R. B. Zhu in 220 Wenxian County), http://ppbc.iplant.cn/tu/7885080 (by X. J. Liu in Chengxian 221 County) and the remaining photos in the albums of each plant). The plants 222 shown in those photos have a subsquare mid-lobe that is obviously larger 223 than the lateral lobes; therefore, we recognised them as *P. limprichtii*. 224 Surprisingly, their leaves are green with white, reticulate venation, which 225 mainly resemble those of *H. avisoides*. Another online photo of *P. limprichtii*, 226 taken by S. L. Kelley in Luhuo County, Sichuan, showed a nearly uniformly 227 green leaf 228 (http://hengduan.huh.harvard.edu/fieldnotes/specimens/search/specimen det 229 ail.zpt?specimen id=21330&full image=skelley04179). On the other hand, a 230 few individuals of *H. avisoides* were observed in the field to possess 231 conspicuously purple spots, along with white veins, on their leaves. We have 232 also noticed that the lateral lobes of P. limprichtii in Gansu as shown in the 233 PPBC photos are often pendulous. Taking these facts into account, the 234 abovementioned cases render the diagnostic characteristics of H. avisoides 235 seemingly cryptic.

236 According to the spatial delimitation of the Arid valley in the upper 237 reaches of Minjiang River (Zheng et al. 2017), of the two H. avisoides 238 populations we discovered, one is distributed within the range of the Arid 239 valley and the other is closely situated next to the Arid valley. The locality of 240 the collection by S. Y. Chen et al. in 1958 was not precisely recorded, but the 241 elevation of 1780 m implied that the specimen was collected from the range of 242 the Arid valley in that region. However, it is notable that, as climate changes, 243 the spatial range of the Arid valley varies (Zheng et al. 2017). Focusing on vegetation type, based on our field observations, the vegetation where our 244 245 collections of *H. avisoides* occurs could be classified into Form. Sophora 246 davidii (Franch.) Skeels, Form. Prunus tangutica (Batal.) Korsh. and/or Form. 247 Ostryopsis davidiana Decaisne, which are typical of arid-valley shrubs and span an elevation between ca. 1700 and 2500 m (Yang 2007). To sum up, the 248 249 habitats of *H. avisoides* are mostly within the Arid valley in the upper reaches 250 of Minjiang River. To our knowledge, other orchids in the same and sympatric 251 habitats include H. physoceras (Schltr.) Y. Tang & H. Peng, Habenaria 252 acianthoides Schltr. and Cephalanthera erecta (Thunb. ex A. Murray) Bl., 253 although each of these species is more widely distributed overall. We believe

the ecological characteristics of these orchids, including *Hemipilia avisoides*,

are worthy of future study.

#### 256 Data availability

- 257 The 3D mesh model of the uppermost two flowers on an inflorescence and
- 258 photos of the corresponding micro-CT-scanned individual of *Hemipilia*
- 259 *avisoides*, are available on Zenodo via DOI:
- 260 https://doi.org/10.5281/zenodo.6832154.

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### 277 Author contributions

- 278 Xue-Man Wang: Investigation; Writing original draft; Writing review &
- editing. **Ying Tang**: Conceptualisation; Investigation; Resources;
- 280 Visualisation; Writing original draft; Writing review & editing. **Pei-Hao**
- 281 **Peng**: Funding acquisition; Supervision. **Hua Peng**: Conceptualisation;
- 282 Funding acquisition; Supervision.

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**TABLE 1.** Comparisons in morphology between *Hemipilia avisoides* sp. nov.
and similar *H. occidensichuanensis*.

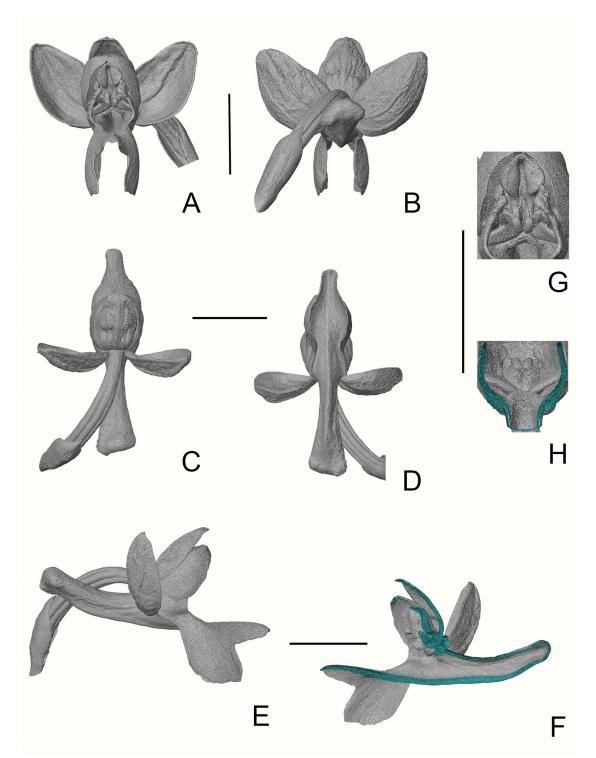
Species	Hemipilia avisoides	H. occidensichuanensis
Stem shape	Subterranean, with 1 sheath at base	Partly subterranean, with 1 or 2 (or 3) sheaths at base
Leaf position	Appressed to substrate	Sub-basal
Leaf colour adaxially	Green with white veins, occasionally also with purple spots	Usually green with purple markings, sometimes nearly uniformly green or with white, reticulate venation
Flora bract shape	Elliptic, 5 mm long, always shorter than ovary	Lanceolate or ovate-lanceolate, lower ones nearly as long as ovary, gradually smaller upwards to shorter than ovary
Dorsal sepal shape	Oblong, apex rounded, sometimes concave at each side of central vein below middle	Suboblong, apex subacute
Lateral lip lobe shape	Pendulous, rhombic	Usually horizontal, auricular or transversely suboblong
Middle lip lobe shape	Involute, suboblong in apical view, 2.2 × 1.2 mm, trapeziform when flattened, smaller than lateral lobes, apex rounded or sometimes apiculate	Usually open and flat, subsquare, 4–5 × 3–4 mm, larger than lateral lobes, apex obtuse-rounded, sometimes slightly emarginate or shortly apiculate





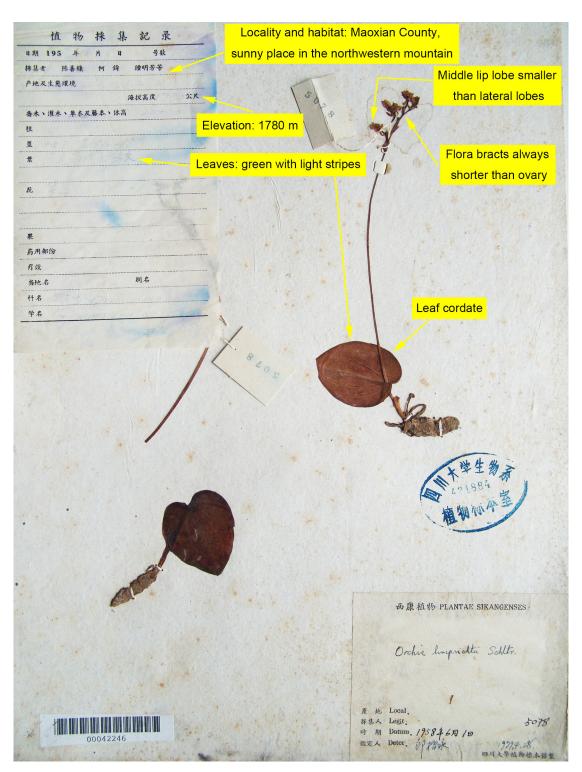
328 **Figure 1.** Comparisons between living plants of *Hemipilia avisoides* sp. nov.

- 329 and similar *H. occidensichuanensis* in the wild. **A–D**, habit, flowers, leaf and
- habitat of *H. avisoides*; **E–H**, habit, flowers, leaf and habitat of *H.*
- 331 *occidensichuanensis*. Photographs A–H by Y. Tang.



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Figure 2. Different views of *Hemipilia avisoides*, based on 3D mesh model
reconstructed by micro-CT data. A–E, front, back, apical, ventral and rightside views of flower; F, left-side view of labellum that is split; G, H, front and
ventral views of gynostemium. Scale bars, 5 mm.



- 338 **Figure 3.** Specimen of S. Y. Chen, Z. He, M. F. Zhong et al. 5078 (SZ!)
- identified as *Hemipilia avisoides* in this study. Key features, which would
   facilitate the identification of this specimen, are highlighted and arrowed in
- 341 yellow.