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***Holcoglossum yui* (Orchidaceae: Aeridinae), a new species from Yunnan, China**

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1 ***Holcoglossum yui* (Orchidaceae: Aeridinae), a new species from**
2 **Yunnan, China**

3
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10
11 **Abstract**

12 Based on field observations and molecular identification, *Holcoglossum yui*
13 (Orchidaceae: Aeridinae) from Southern Yunnan, China, is described and illustrated as
14 a new species. It is most similar to *H. rupestre*, but clearly differs from the latter by its
15 inflorescence slightly long, callosum strong thickened with two grooves and three
16 ridges, and erect spur. The phylogenetic placement of this new species was assessed
17 based on morphological and molecular data.

18
19 **Keywords**

20 *Holcoglossum*, *Holcoglossum rupestre*, morphology, phylogenetic analysis

21 Introduction

22 Orchidaceae is the largest family of angiosperms including over 700 genera and about
23 30,000-35,000 species (Chase et al. 2015; Yeh et al. 2019). *Holcoglossum* Schltr.
24 (Orchidaceae) was established by Schlechter based on *Saccolabium quasipinifolium*
25 Hayata (Schlechter 1919). This genus is a small Asian genus with less than 20 species,
26 mainly distributed in southwestern China with a few species extending to Malaysia,
27 Thailand, Myanmar, and Vietnam (Christenson 1987; Seidenfaden 1993; Tsi 1999; Jin
28 et al. 2004, 2005, 2007a, 2007b; Jin 2005; Fan et al. 2009; Xiang et al. 2012).
29 Morphologically, *Holcoglossum* is characterized by leaves subterete, flowers opening
30 widely, petals similar to sepals, column short, thick, winged, with a very short but
31 distinct foot.

32 Based upon our fieldwork in southern Yunnan, an unknown species of
33 *Holcoglossum* was discovered. Further studies of morphological comparison and
34 molecular phylogenetic indicated that the plant represents an undescribed species. The
35 new species is described below.

36

37 Material and methods

38 Morphological description

39 The morphological description is based on live material. Labellum, corpus callosum,
40 sepals and petals were observed by Olympus SZX2-TR30 stereoscopic microscope
41 (Olympus Corp., Tokyo, Japan) and measured with a digital caliper. Furthermore, two
42 similar species were sampled for morphological comparison.

43 DNA extraction, amplification, and sequencing

44 Genomic DNA was extracted from fresh leaves using a TIANGEN Plant Genomic
45 DNA Kit (TIANGEN BIOTECH (BEIJING) CO., LTD) according to the
46 manufacturer's instructions. The PCR protocol used the following conditions: 1 min at
47 96°C, followed by 35 cycles of 30 second at 94°C, 30 second at 55°C, 2 min at 72°C,
48 and with a final extension at 72°C for 10 min. The ITS primers used were ITS1 and
49 ITS4, as described by Urbatsch et al. (2000).

50

51 Phylogenetic analyses

52 We adopted a molecular approach to shed light on the position of the new species
53 within *Holcoglossum* s.l. Bayesian Inference (BI) and Maximum Likelihood (ML)
54 analyses were used to construct a phylogenetic tree based on ITS sequences. GenBank
55 sequences covering most species in *Holcoglossum* were selected, with *Neobenthamia*
56 *gracilis* and *Polystachya neobenthamia* as the outgroup (Table 1).

57 ITS sequences were aligned with MAFFT software (Katoh and Standley 2013).
58 ModelFinder was used to select the best-fit model using Bayesian information
59 criterion (BIC) (Kalyaanamoorthy et al. 2017). BI analysis was inferred using
60 MrBayes 3.2.6 (Ronquist et al. 2012) under HKY+F model (2 parallel runs, 5000000

61 generations). ML analysis was inferred using IQ-TREE (Nguyen et al. 2015) under
 62 the model automatically selected by IQ-TREE for 20000 ultrafast (Minh et al. 2013)
 63 bootstraps, as well as the Shimodaira–Hasegawa–like approximate likelihood-ratio
 64 test (Hasegawa et al. 1985; Guindon et al. 2010).

65

66 **Table 1.** Names and GenBank accession numbers of the taxa used in the ITS phylogenetic
 67 analyses.

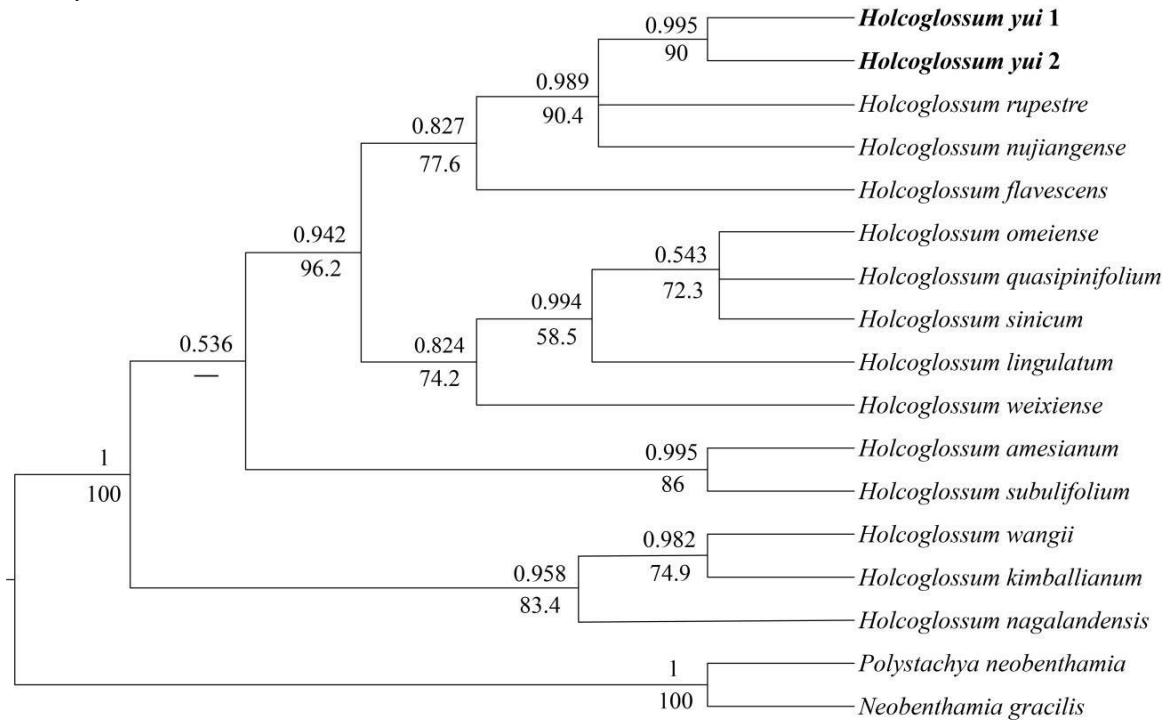
Species	GenBank accession number
<i>Holcoglossum yui</i> 1	MZ521034
<i>Holcoglossum yui</i> 2	OM445255.1
<i>Holcoglossum amesianum</i>	HQ452912.1
<i>Holcoglossum flavescens</i>	EU558921.1
<i>Holcoglossum kimballianum</i>	EF079436.1
<i>Holcoglossum lingulatum</i>	EU558907.1
<i>Holcoglossum nagalandensis</i>	KC110629.1
<i>Holcoglossum nujiangense</i>	EU558910.1
<i>Holcoglossum omeiense</i>	HQ452902.1
<i>Holcoglossum quasipinifolium</i>	HQ452909.1
<i>Holcoglossum rupestre</i>	EU558909.1
<i>Holcoglossum sinicum</i>	HQ452906.1
<i>Holcoglossum subulifolium</i>	EF670360.1
<i>Holcoglossum wangii</i>	KY966610.1
<i>Holcoglossum weixiense</i>	HQ450900.1
<i>Neobenthamia gracilis</i>	GU556663.1
<i>Polystachya neobenthamia</i>	MT358292.1

68

69 **Results**

70 **Phylogenetic Reconstruction**

71 Our results based on ITS produced trees with identical topology ML, which included
 72 over 15 species, they did confirm the position of the new species within
 73 *Holcoglossum* (Fig. 1). Clades associated with *H. nujiangense* are also included
 74 because morphological criteria indicate that this species displays several similarities
 75 to *H. yui*.

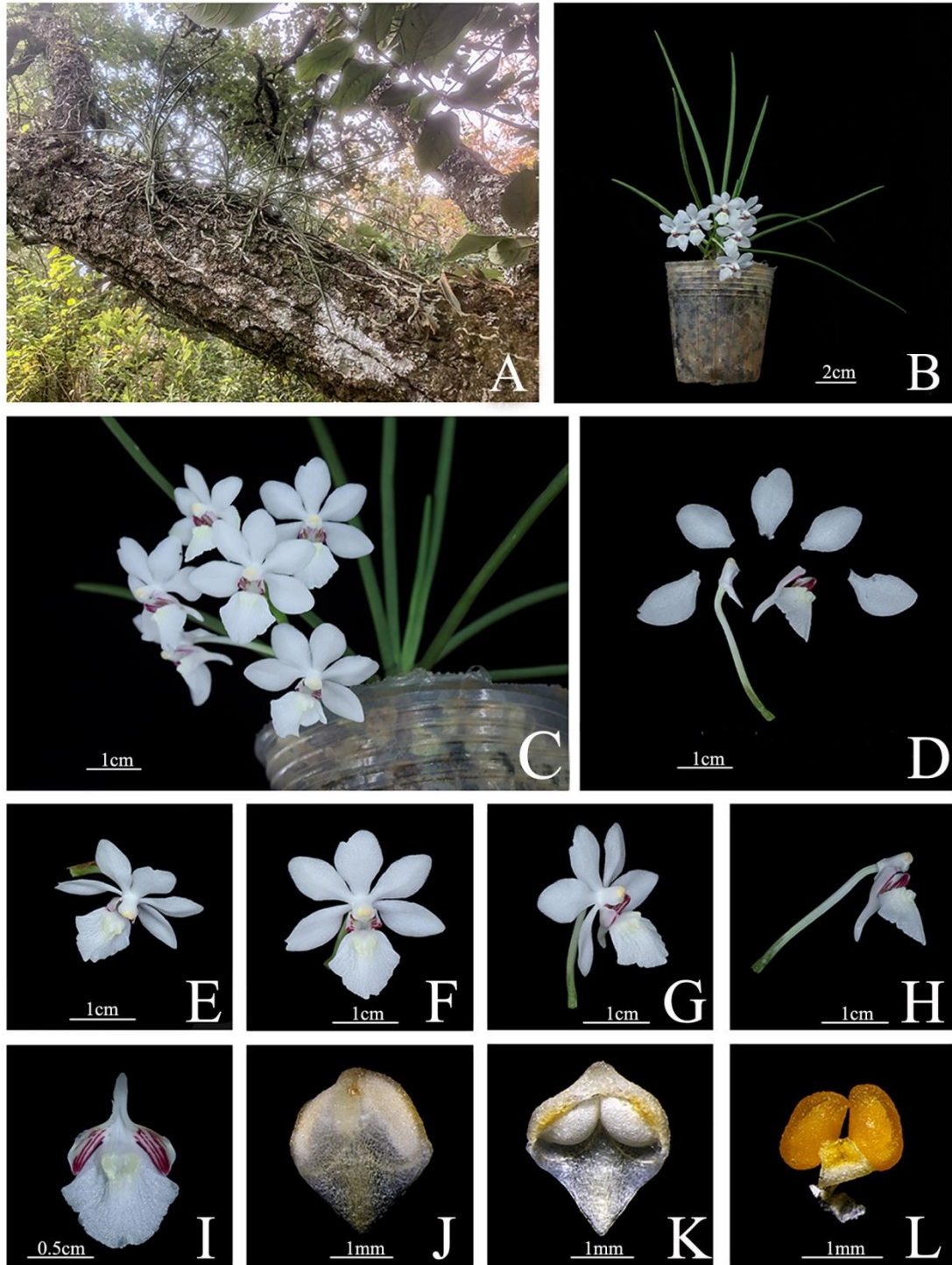


76
 77 **Figure 1.** Bayesian tree obtained from analysis of the combined data set showing the detailed
 78 relationships of *H. yui*. The Bayesian posterior probabilities are shown below the branches
 79 and Maximum Likelihood (ML) bootstrap values above. The new species is shown in bold.

80 Taxonomic treatments

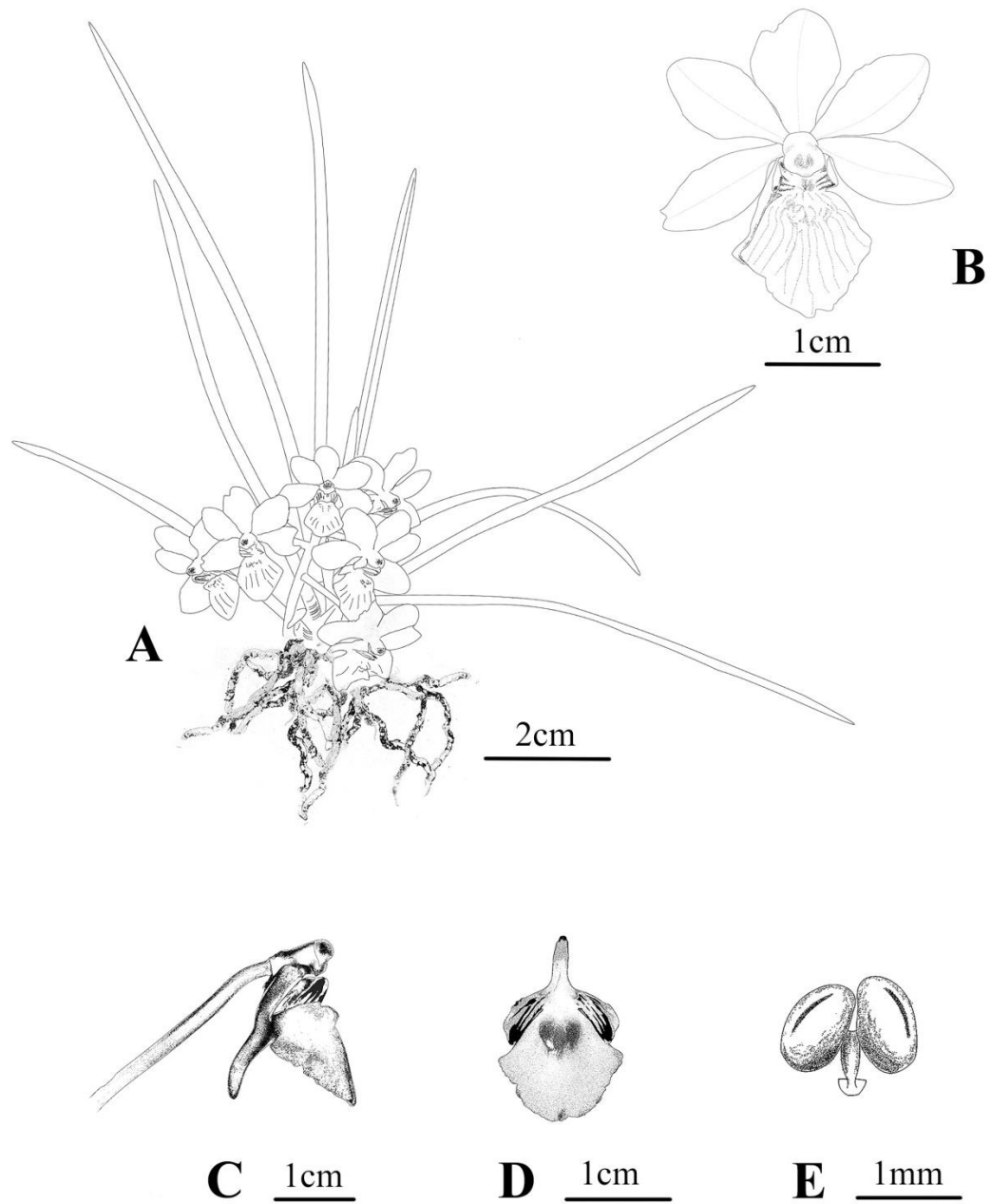
81 *Holcoglossum yui* D.K. Zhao & Y. Zhang, *sp. nov.*

82 Figs 2, 3



83

84 **Figure 2.** *Holcoglossum yui* A plant habit B plant C flowers D dissection of flower E top
85 view of flower F front view of flower G side view of flower H labellum, spur I lip J front
86 view of anther cap K back view of anther cap L pollinarium.



87
88 **Figure 3.** *Holcoglossum yui* A plant B front view of flower C labellum, spur D lip E
89 pollinarium.

90 **Type.** China. Yunan Province, Yuxi city 2450 m, epiphytic on tree, 18 March 2021, Y.
 91 Zhang, D. K. Zhao, Z. H. Yuan & R. B. Wang (holotype YUKU!(YUKU02074689),
 92 isotype YUKU!).

93 **Diagnosis.** *Holcoglossum yui* is morphologically similar to *H. rupestre* (Jin and
 94 Wood 2009) and *H. nujiangense* (Jin et al. 2007a) (Table 2), but typically differs from
 95 the two species by its inflorescence slightly long, callosum strong thickened with two
 96 grooves and three ridges, and spur almost erect.

97
 98 **Table 2.** Diagnostic morphological characters comparing *Holcoglossum yui* with related
 99 species.

Characters	Species		
	<i>H. yui</i>	<i>H. rupestre</i>	<i>H. nujiangense</i>
Stem length	2.5 cm	2 cm	1–2 cm
Leaves	6–14 cm × 2–3 mm	12–28 cm × 2–2.5 mm	20–30 cm × 1.5 mm
Inflorescence	lateral, 5–10-flowered, ca. 10.5 cm	ascending, 2–10-flowered, 5–10 cm	lateral, 1 to 3-flowered
Callosum	forming two grooves and three ridges, central ridge white, bilateral ridges pale yellow.	yellowish	yellow
Spur	nearly erect, ca. 9 mm	bent forward, ca. 8 mm	bent forward, ca. 6 mm

100

101 **Description.** Epiphytic herbs. Root white, terete, 11 cm long, 3–4 mm in diam..
 102 Stems short, ca. 2.5 cm long. Leaves 4–12, fleshy, semi-terete, 6–14 cm long, 2–3 mm
 103 in diam., apex acuminate, a joint above the base, under which there is an imbricate
 104 sheath. Inflorescence lateral, ca. 10.5 cm long; inflorescence covered by several ovate
 105 scaly sheaths 5–7 mm long; inflorescence peduncle weak, 8-flowered. Floral bracts
 106 ovate, reflexed, ca. 1.1 cm long. Pedicel and ovary 2.5–3.5 cm long. Flowers opening,
 107 ca. 2.9 cm in diam, slightly curved backward; sepals and petals white; mid-sepals
 108 elliptic, 1–1.4 cm long, 4–7 mm wide, apex obtuse; lateral sepals oblique longly
 109 elliptic, ca. 13 mm long, ca. 7 mm wide, apex obtuse; petals subovate-elliptic, ca. 12
 110 mm long, ca. 6 mm wide, apex obtuse; lip white, 3-lobed; lateral lobes erect, ovate
 111 triangular, inside surface red stripes, apex obtuse; middle lobe broadly ovate rhombic,
 112 ca. 12 mm long, ca. 10 mm wide, apex obtuse, micro concave, marginal microwaves,
 113 broad ovate triangular callosum in base, pale yellow; callosum strong thickened,
 114 forming two grooves and three ridges, central ridge white, bilateral ridges yellowish;
 115 spur, nearly erect, ca. 9 mm long, narrowed toward apex, apex acute; column ca. 5
 116 mm long, column foot ca. 2 mm long, slightly enlarged at the top; anther cap white,
 117 tip contracted.

118 **Phenology.** Flowering from April to May, fruiting from May to the January or
 119 February of next year.

120 **Etymology.** The new species is named after Mr. Zhang Yu, who found and
121 collected this orchid.

122 **Distribution and habitat.** The species was found at the southern Yunnan, China. It
123 is an epiphyte under tropical seasonal rain forests, at elevation of approximately 2450
124 m. *H. yui* are epiphytic mostly on barks of *Castanopsis delavayi* Franch. (Fagaceae)
125 and *Quercus variabilis* Bl. (Fagaceae).

126 **Conservation status.** During our field investigation, only about 100 matured
127 individuals were found in the type locality. Based on the limited information available
128 so far, we thus tentatively regard it as “Data Deficient (DD)” following the IUCN Red
129 List (IUCN 2020). We have not given the exact locality of *H.yui* as we are concerned
130 that the beautiful species may be the target of illegal collection.
131

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137

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