



Danxiaorchis yangii sp. nov. (Orchidaceae: Epidendroideae), the second species of *Danxiaorchis*

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Abstract

Danxiaorchis yangii (Calypsoinae, Epidendreae, Epidendroideae), a holomycotrophic new species from Jinggangshan National Nature Reserve, western Jiangxi, eastern of China, is here illustrated and described based on both morphological and phylogenetic evidences. The new species can be easily distinguished from *D. singchiana* by its much smaller flowers and larger Y-shaped callus adaxially bearing a additional obovoid appendage, and by its four pollinia narrowly elliptic in shape and equal in size.

Keywords: *Danxiaorchis*, Jinggangshan Mountains, Jiangxi orchids, phylogenetic analysis

Introduction

Danxiaorchis (J.W. Zhai, F.W. Xing et Z.J. Liu in Zhai *et al.* 2013) is a recently described new mycotrophic orchid genus which is distinct from all known orchid genera by having a labellum with a large Y-shaped callus and two sacs at the base, and cylindrical, fleshy seeds (Zhai *et al.* 2013). Phylogenetic analyses based on combined DNA sequences (ITS, *matK* and *rbcL*) and morphological characters matrix indicated that *Danxiaorchis* is a member of the tribe Calypsoeae in the subfamily Epidendroideae (Zhai *et al.* 2013). Under Calypsoeae, *Danxiaorchis* was inferred to be most closely related to *Yuania* Maximowicz (1873: 68), and this relationship was subsequently confirmed by Zhang *et al.* (2015). However, the only sequence for *Yuania* included in both Zhai *et al.* (2013) and Zhang *et al.* (2015) data sets was a partial *rbcL* sequence of 222 bp out of the full length of 1428 bp, and Freudenstein *et al.* (2017) noted that such a high proportion of missing data of *Yuania* would output a spurious relationship for *Danxiaorchis* and *Yuania*. After including more representatives and sequences of *Yuania* and reanalyzing generic relationships among subtribe Calypsoinae, Freudenstein *et al.* (2017) found that *Danxiaorchis* emerges in *Corallorhiza* (Gagnebin 1755: 61) clade and is sister to *Oreorchis* (Lindley 1859: 26) + *Corallorhiza*, while *Yuania* emerges in *Calypso* (Salisbury 1807: 89) clade. Now, *Danxiaorchis* is placed in subtribe Calypsoinae, tribe Epidendreae, subfamily Epidendroideae in Orchidaceae (Chase *et al.* 2015; Li *et al.* 2016), and it represents a distinct leafless lineage in Calypsoinae (Freudenstein *et al.* 2017).

In *Danxiaorchis*, only one species, *D. singchiana* J.W. Zhai, F.W. Xing et Z.J. Liu from Danxia Mountain in Guangdong, China, has been recorded so far (Zhou *et al.* 2016). In April 2015, a yellow mycotrophic orchid was collected by the first author during a field work in the Jinggangshan National Nature Reserve of Ji'an City, Jiangxi Province, China. Flower of the plant bears a remarkable large Y-shaped appendage on its labellum, which is a diagnostic character of *Danxiaorchis*. After a critical morphological comparison with *D. singchiana*, we confirmed that the plant represents an undescribed new species in *Danxiaorchis* and here described it below.

Materials and methods

Morphological observations:—Field observations were carried out in Jinggangshan National Nature Reserve during April to May in 2015 and 2016. Morphological variation was measured on 20 individuals using a ruler and a micrometer. The specimens were deposited in IBSC, JXAU and JXU (acronyms according to Thiers 2016+).

TABLE 1. Taxa and GenBank accession numbers of the subtribe Calypsoinae that were used in this study. Sequences used in Freudenstein *et al.* (2017) were marked by “*” and in Zhai *et al.* (2013) by “#”. The symbol “-” indicates missing data, and “●” denotes new sequences generated in this study.

Taxa	ITS	matK
<i>Aplectrum hyemale</i>	EU266404#	EU266416#
<i>Calypso bulbosa</i>	LC176591*	LC176612*
<i>Changnienia amoena</i>	LC176592*	LC176613*
<i>Changnienia malipoensis</i>	JX293179#	JX293183#
<i>Chysis bractescens</i>	EF079363*	AY121747*
<i>Corallorhiza bentleyi</i>	JF319668#	EF525706#
<i>Corallorhiza bulbosa</i>	EU391332#	EF525699#
<i>Corallorhiza involuta</i>	EU391347#	EF525698#
<i>Corallorhiza maculata</i> var. <i>maculata</i>	EU391329#	EF525700#
<i>Corallorhiza maculata</i> var. <i>occidentalis</i>	EU391330#	EF525697#
<i>Corallorhiza mertensiana</i>	EU391333#	EF525704#
<i>Corallorhiza odontorhiza</i>	EU391326#	EF525701#
<i>Corallorhiza striata</i> var. <i>striata</i>	EU391349#	EF525702#
<i>Corallorhiza striata</i> var. <i>vreelandii</i>	EU391352#	EF525705#
<i>Corallorhiza trifida</i>	EU391324#	EF525695#
<i>Corallorhiza wisteriana</i>	EU391327#	EF525703#
<i>Cremastra aphylla</i>	LC176594*	LC176614*
<i>Cremastra appendiculata</i>	KM526764#	JX293182#
<i>Cremastra unguiculata</i>	LC176593*	LC176616*
<i>Cremastra variabilis</i>	LC176595*	LC176615*
<i>Dactylostalix ringens</i>	LC176596*	LC176617*
<i>Danxiaorchis singchiana</i>	JX293178*#	JX293186*#
<i>Danxiaorchis yangii</i> 1	KY968693●	KY968689●
<i>Danxiaorchis yangii</i> 2	KY968694●	KY968690●
<i>Danxiaorchis yangii</i> 3	KY968695●	KY968691●
<i>Danxiaorchis yangii</i> 4	KY968696●	KY968692●
<i>Ephippianthus sawadanus</i>	LC176598*	LC176619*
<i>Ephippianthus schmidtii</i>	LC176599*	LC176620*
<i>Govenia liliacea</i>	AF521056#	AY121723#
<i>Govenia</i> sp.	EF525672#	EF525690#
<i>Govenia utriculata</i>	LC176600*	LC176621*
<i>Oreorchis coreana</i>	LC009378*	LC176625*
<i>Oreorchis erythrochrysea</i>	LC176601*	LC176624*
<i>Oreorchis fargesii</i>	LC176603*	LC176622*
<i>Oreorchis indica</i>	LC176602*	LC176623*
<i>Oreorchis nana</i>	KM526766#	JX293185#
<i>Oreorchis patens</i>	LC176604*	LC176626*
<i>Tipularia cunninghamii</i>	LC176597*	LC176618*
<i>Tipularia discolor</i>	LC176606*	LC176628*
<i>Tipularia japonica</i>	LC176607*	LC176629*
<i>Tipularia szechuanica</i>	LC176605*	LC176627*
<i>Yuania amagiensis</i>	LC176610*	-
<i>Yuania flava</i>	LC176608*	-
<i>Yuania japonica</i>	LC176609*	-
<i>Yunorchis pingbianensis</i>	KM526768*#	KM526763*#

Molecular datasets and phylogenetic analyses:—Two DNA makers, ITS and *matK*, were employed in this study because they were successfully used by both Zhai *et al.* (2013) and Freudenstein *et al.* (2017). 44 taxa from subtribe Calypsoinae were selected as ingroups and *Chysis bractescens* as outgroup. Most of them has been either used by Zhai *et al.* (2013) or Freudenstein *et al.* (2017). Four individuals of the putative new species were collected for DNA extraction, PCR amplification and sequencing. The procedures followed Zhai *et al.* (2013). Taxa and GenBank accession numbers were listed in Table 1.

Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) methods. The BI analysis was performed using MrBayes v.3.2 (Ronquist *et al.* 2012). The following settings were used: sampling frequency = 1000; temp = 0.1; burn-in = 2000; and number of Markov chain Monte Carlo generations = 2000000. ML analyses were performed on the web server RAxML Black Box (Stamatakis *et al.* 2008). Before each submission, the “Maximum likelihood search” and “Estimate proportion of invariable sites” options were selected, with a total of 1000 bootstrap replicates performed.

Results

Trees generated from separate and combined datasets are topologically similar to those presented in Freudenstein *et al.* (2017), respectively. Visual examination of the trees indicates that ML trees do not conflict with the BI trees for either of the separate or combined analyses, hence the 50% majority-rule consensus phylogram from the BI analysis of the combined datasets is showed here (Fig.1). The four representatives of the putative new species grouped together and was well supported as sister to *D. singchiana* (ML bootstrap percentage = 96, posterior probability = 1.00).

Taxonomy

Danxiaorchis yangii B. Y. Yang et Bo Li, *sp. nov.* (Figs. 2 A–I, 3)

Diagnosis:—*Danxiaorchis yangii* obviously differs from *D. singchiana* in its Y-shaped callus adaxially bearing a remarkable obovoid appendage, and in its four pollinia narrowly elliptic in shape and equal in size.

Type:—CHINA. Jiangxi Province, Ji'an City, Jinggangshan Mountain, Jinggangshan National Nature Reserve, forest margins, under mixed shrubs, 26°27'06"N, 114°30'43"E, ca. 360 m a.s.l., 7 April 2016, B.Y. Yang 075 (Holotype: IBSC!; Isotypes: JXU!, JXAU!).

Description:—*Plant* erect, 10–25 cm tall, holomycotrophic. *Rhizome* tuberous, fleshy, cylindrical, 5–15 mm long, 3–11 mm thick, with many minute and short branches, without roots. *Scape* terete, pale red-brown, slightly tinged with green-yellow, 2- to 3-sheathed; sheaths cylindrical, clasping stem, membranous, 25–45 × 4–7 mm. *Inflorescence* racemose, 4–8 cm long, 5- to 30-flowered; *floral bracts* oblong-lanceolate, 12–19 × 2.5–3.0 mm, apex acuminate, with sparse to dense purple-red spots; *pedicel and ovary* bright yellow, 15–25 mm long, glabrous; *sepals* yellow, obovate-elliptic, dorsal sepals 11–16 × 3.5–5.5 mm, acute to obtuse; lateral sepals 12–18 × 3.6–6.3 mm, acute; *petals* yellow, narrowly elliptic, 8.0–15 × 3.5–6.0 mm, acute; *labellum* 3-lobed, with 3–4 pairs of purple-red stripes on side lobes and purple-red spots on middle lobe; side lobes erect, slightly clasping the column, subsquare, 3.5–4.0 × 5.0–5.5 mm; mid-lobe oblong, 5.0–5.5 × 4.5–5 mm, apex acute to obtuse; labellum with two sacs at the base and a Y-shaped fleshy callus centrally; *callus* 4.0–4.5 mm tall, extending from the base of disc to the base of mid-lobe, adaxially covering an additional obovoid appendage, ca. 3.0–3.5 mm in diameter; *column* cream colored, straight, semi-cylindrical, 5.0–5.5 mm long, footless; *stigma* concave, triangular, terminal; *anther cap* ellipsoid, ca. 1.3 mm in diameter; *pollinia* four, in two pairs, narrowly elliptic, granular-farinaceous, composed of friable massulae, each pair containing two pollinia equal in size with a thick caudicle attached to a common subsquare viscidium. *Capsule* purple red, fusiform, 35–45 mm long, 13–15 mm thick. *Seeds* dark brown, cylindrical, 1.7 × 0.8 mm, fleshy.

Etymology:—The specific epithet refers to the family name of the first author, Pro. Boyun Yang, who devotes himself to the conservation of orchids biodiversity in Jiangxi Province, China.

Distribution and habitat:—To date, the species was discovered only from Jinggangshan National Nature Reserve in western Jiangxi, eastern of China. It occurs in nearly the same longitude as *D. singchiana* but in higher latitude (Fig. 4). *D. yangii* frequently grows at the margin of subtropical evergreen broad-leaved forest, under mixed shrubs and bamboo forest, in wet places at elevations of 360 m a.s.l.

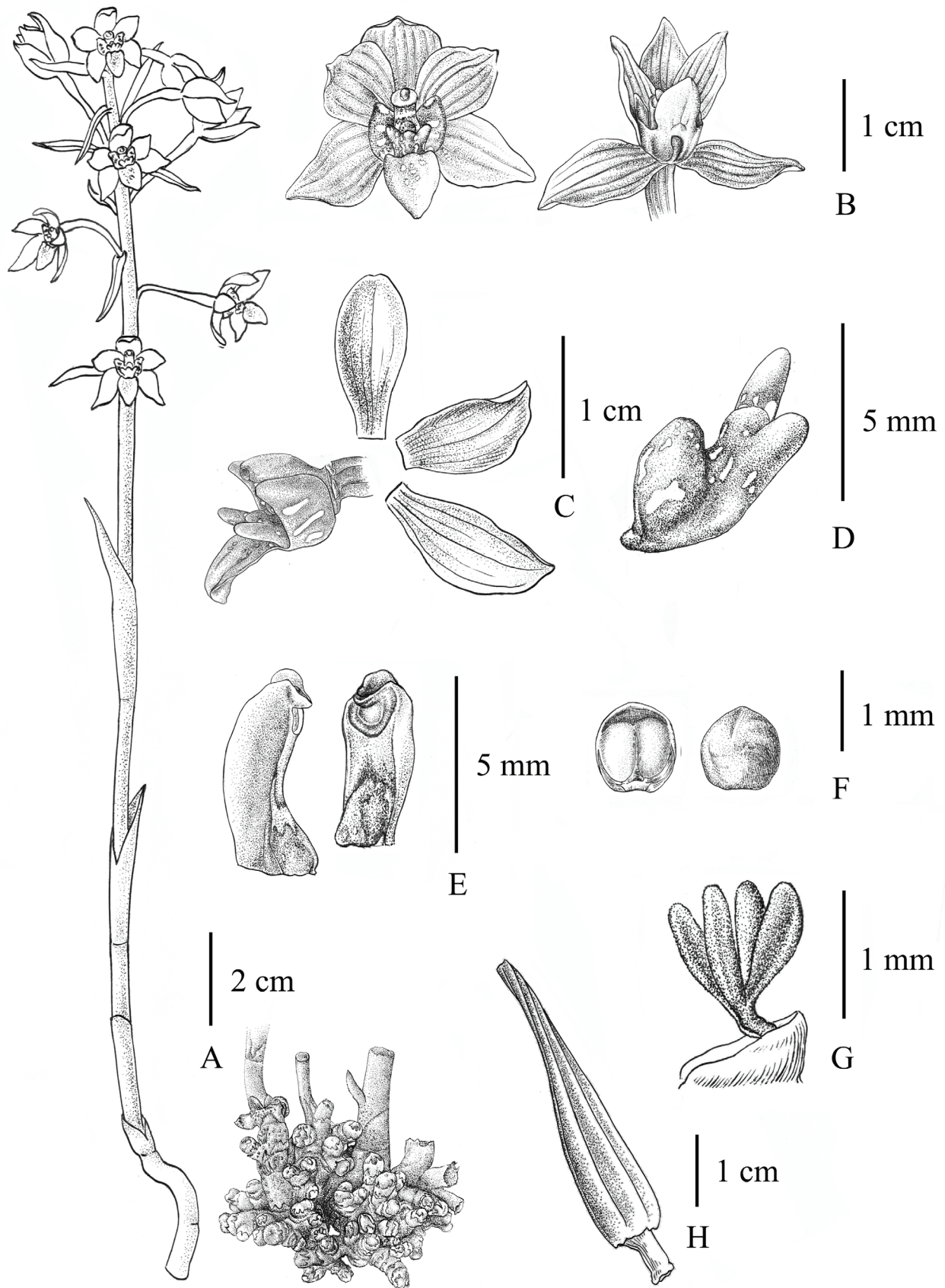


FIGURE 2. *Danxiaorchis yangii* B. Y. Yang et Bo Li, *sp. nov.* A. Flowering plant and rhizome; B. Flower, front view (left) and bottom view (right); C. Dissection of a flower, showing dorsal sepal, petal, lateral sepal and labellum with column; D. Appendage of the labellum, side view; E. Column, side view (left) and front view (right); F. Anther cap, ventral view (left) and dorsal view (right); G. Pollinarium; H. Capsule.

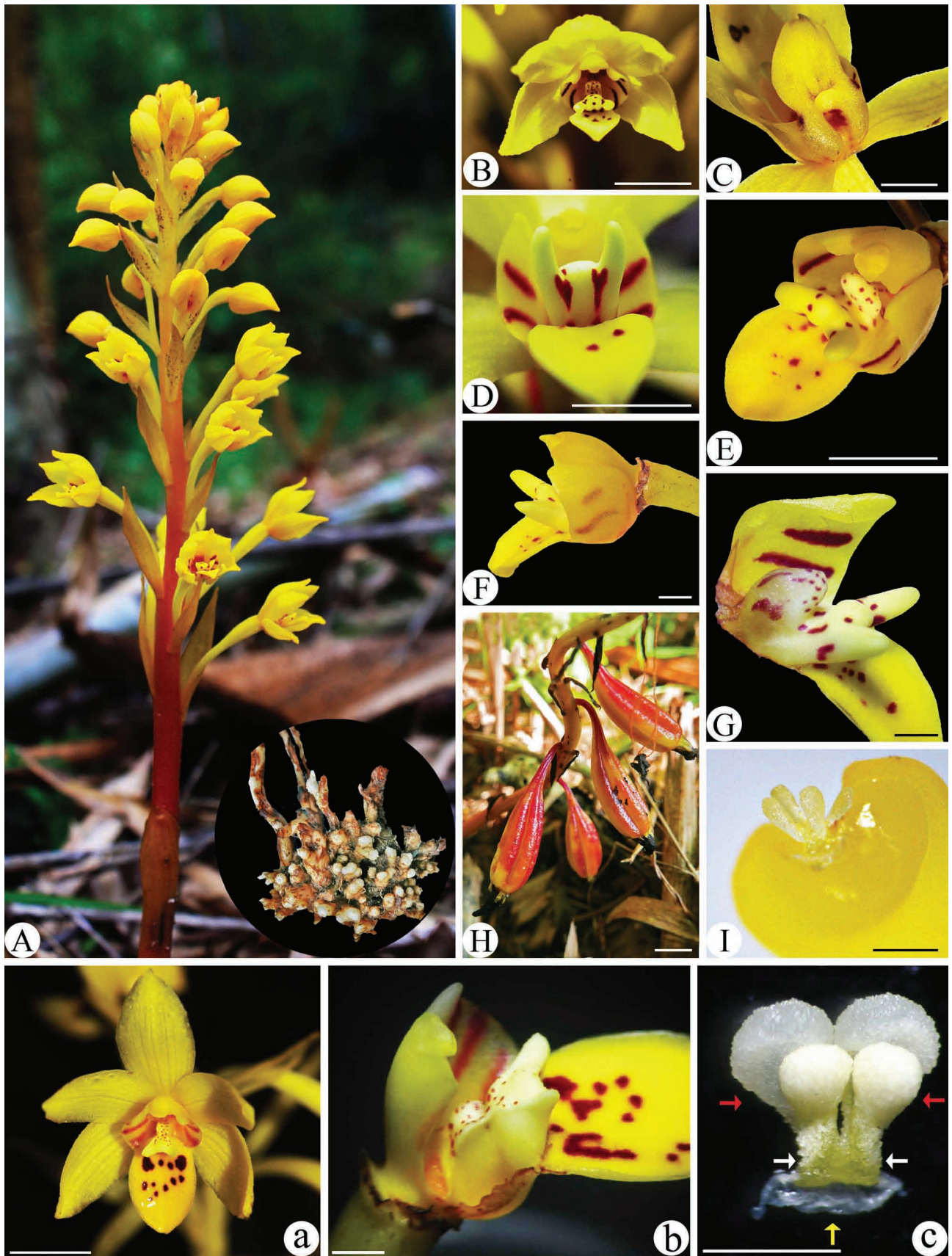


FIGURE 3. Morphology of *Danxiaorchis yangii* B. Y. Yang et Bo Li, *sp. nov.* (A–I) and *D. singhiana* (a–c). A. Flowering in nature habitat and rhizome (showed in the black circle); B, a. Front view of a flower; C. Bottom view of labellum, showing two sacs; D. Front view of the Y-shaped appendage of labellum; E. Upper view of a labellum with column; F. Side view of a labellum with column; G, b. Side view of the Y-shaped appendage of the labellum; H. Capsule; I, c. Pollinarium. Scale bars: B, a = 1 cm; C, D, E = 5 mm; F, G, I, c = 1 mm; H = 1 cm; b = 2 mm. Images b and c were cited from the Figure 1 in Zhai *et al.* (2013).

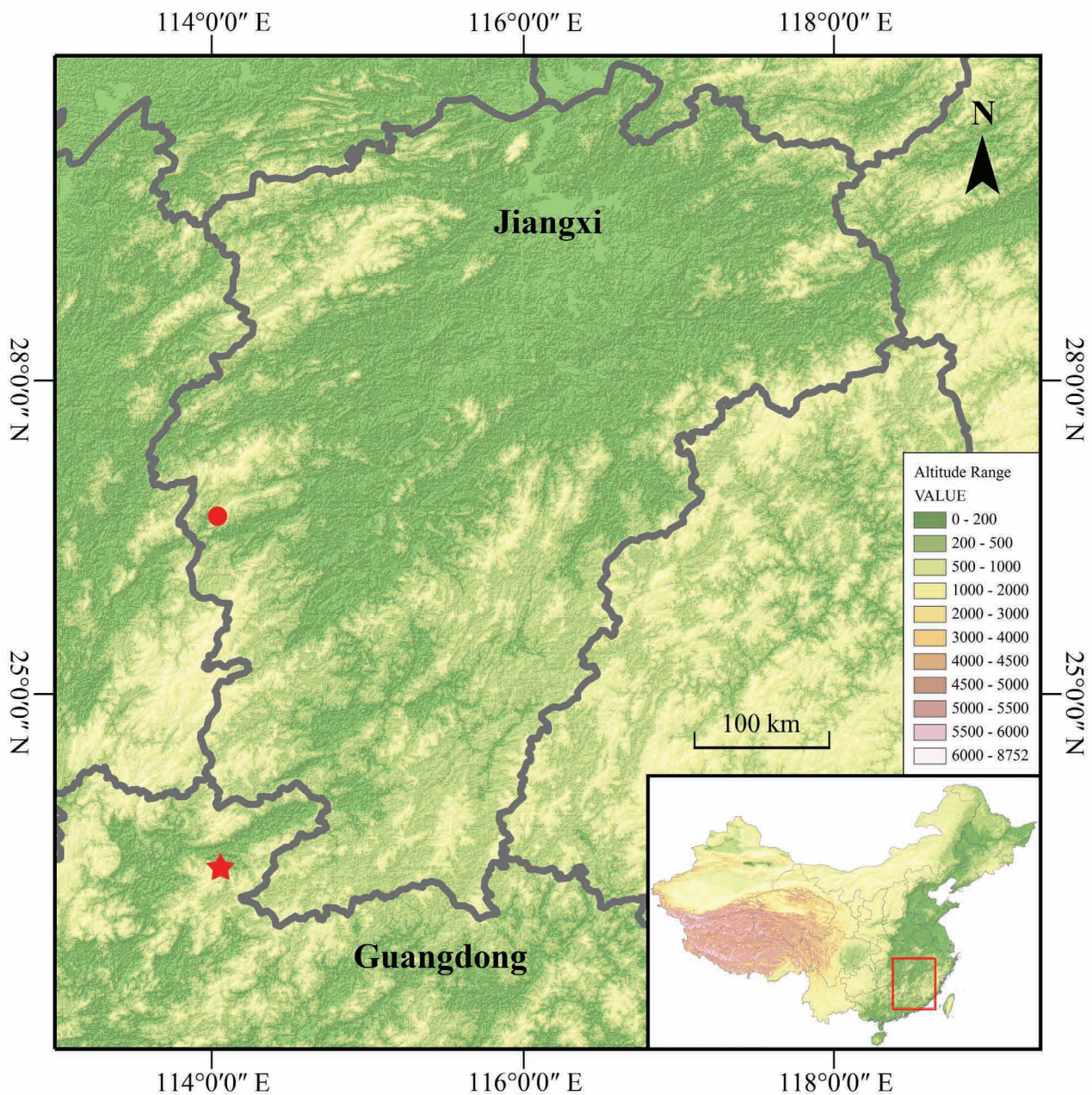


FIGURE 4. Distribution map of *Danxiaorchis yangii* (circle) and *D. singchiana* (star).

Phenology:—Flowering was observed from mid April to mid May, and fruiting from mid May to late May.

Conservation status:—The species is currently known only from one site, and the observed total population is calculated as less than 250 individuals, so it should be considered an very small or restricted population and given an IUCN (2014) provisional conservation status of Endangered (EN, criterion D).

Additional specimens examined (paratypes):—CHINA. Jiangxi Province, Ji'an City, Jinggangshan Mountain, Jinggangshan National Nature Reserve, forest margins, under mixed shrubs, 26°27'06"N, 114°30'43"E, ca. 360 m a.s.l., 10 May 2016, *B.Y. Yang* 076 (JXU!).

Discussion

With the characteristic large Y-shaped appendage on its labellum, *D. yangii* is a certainty member of the genus *Danxiaorchis*, and it was well supported by molecular phylogenetic analyses based on combined datasets of ITS and *matK* (Fig. 1). After thorough morphological comparison between *D. yangii* and *D. singchiana*, we found that *D.*

yangii can be easily distinguished from *D. singchiana* in many aspects (Fig. 3). Superficially the plants of *D. yangii* are shorter than those of *D. singchiana*, and its flowers are much smaller, whereas its Y-shaped appendages are much larger than those of *D. singchiana*. Besides, the adaxial side of the Y-shaped appendages in *D. singchiana* is flat or slightly raised, while in *D. yangii*, the adaxial side additionally bears a remarkable obovoid appendage with its size ca. 3.0–3.5 mm in diameter (Fig. 3 G vs. b). Furthermore, there is an essential difference between *D. yangii* and *D. singchiana*: the four pollinia of *D. yangii* are narrowly elliptic in shape and equal in size, while those of *D. singchiana* are subobovoid-globose with two pollinia unequal in size in each pair (Fig. 3 I vs. c). Consequently, we found out that *D. yangii* can be treated as a distinct species, which is the second species in *Danxiaorchis*.

Acknowledgements

The authors are thankful to staffs of Jingtangshan National Nature Reserve for their help in field surveys, to Pro. Tomohisa Yukawa in National Museum of Nature and Science of Japan for providing DNA samples of *Yonia*, to Pro. Zhongjian Liu in the National Orchid Conservation Center of China and the Orchid Conservation and Research Center of Shenzhen and Pro. Yibo Luo in the Institute of Botany, Chinese Academy of Sciences for their advices and comments on identifying the new species. This work was financially supported by National Natural Science Foundation of China (grant no. 31260485, 31460044, 31360491).

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