

NORDIC JOURNAL OF BOTANY

Research

Hemiboea yongfuensis (Gesneriaceae): a cryptic and critically endangered new species from North Guangxi, China

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Nordic Journal of Botany

2020: e02435

doi: 10.1111/njb.02435

Subject Editor: Xiang-Yun Zhu

Editor-in-Chief: Torbjörn Tyler

Accepted 10 January 2020

Published 21 March 2020

The limestone region of southern China is a biodiversity hotspot from where numerous rare new species have been or are being described. Here, *Hemiboea yongfuensis*, a new species of Gesneriaceae from a limestone hill in Guilin, North Guangxi, southern China is described and illustrated. It is similar to *H. subcapitata* and *H. pterocaulis* in morphology and coexists with both of them in the same limestone hill, but can be distinguished from the latter two by its quadrilateral stem without winged edges, corolla outside light pink and sparsely glandular-pubescent and pale pink calyx which is 5-lobed from base, as well as by molecular data from *trnL-F* and ITS. We provide detailed information, including morphological descriptions, illustrations, diagnostic comparisons and molecular phylogenetic affinities of the new species.

Keywords: cryptic species, molecular phylogeny, morphology, taxonomy

Introduction

Hemiboea C. B. Clarke (1888) in Gesneriaceae is a genus of perennial herbs which are mainly distributed in southern China and northern Vietnam. They usually grow in shaded rock crevices or in humus on rock surfaces of limestone, conglomerate, granite or sandstone, in evergreen broad-leaved forests or mixed evergreen and deciduous broad-leaved forests at low altitude in valleys, hills and mountains (Wei et al. 2010).

Hemiboea, just as many other Gesneriaceae taxa and lots of other families (e.g. Begoniaceae) are highly represented in the biodiversity hotspot of the limestone region of southern China, and is rapidly growing with numerous rare new species being described. Since the previous comprehensive taxonomic revision by Li and Wang (2005) in which 23 species and five varieties were recognized, *Hemiboea* has increased to at least 37 species and five varieties. Apart from the two *Metabriggsia* Wang (1983) species which were transferred to *Hemiboea* by Weber et al. (2011), ten species have been newly found in Guangxi, Guizhou and Yunnan in recent years (Xu et al. 2010, 2012, Wen et al. 2011, 2013, Pan et al. 2012, Zhou et al. 2013, Zhang et al. 2014, Chen et al. 2018, Li et al. 2018, Wu et al. 2019). Most if not all the newly described species have narrow or site-endemic distribution and low abundance. With respect



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to these special characteristics of the species and the large area of the limestone region, many more rare new species of *Hemiboea* may be expected to be discovered.

During an investigation of plant resources in March 2018, we found a special and rare population of *Hemiboea* in a limestone hill in Yongfu County, Guilin, Guangxi. These plants co-occurred with *H. subcapitata* Clarke (1888) and *H. pterocaulis* (Z. Y. Li) Huang, Xiang & Zhang (Li 2004, Huang et al. 2017) in the same limestone hill and were even intermixed with some individuals of the latter two species at the same site. The vegetative plants were morphologically very similar to *H. subcapitata* and only slightly discernible differences of yellowish green leaf adaxial surface (versus darker green of *H. subcapitata*) and quadrilateral stem (versus cylindrical stem of typical *H. subcapitata*) were observed. We collected plant leaves for DNA experiment from a few of these odd plants and the intermixed typical *H. subcapitata* plants, obtained its partial chloroplast *trnL* and *trnL-F* intergenic spacer (*trnL-F*) and nuclear ribosomal internal transcribed spacer (ITS) sequences and compared them with each other and with all available corresponding sequences of other *Hemiboea* taxa. We found that the DNA sequences of the odd plants, particularly the ITS sequences, differed from all known taxa, suggesting that the odd plant individuals may represent an undescribed new species. Subsequently, we tracked its phenology and found that they began to flower tightly following typical *H. subcapitata* and that the flowers were different from the latter species as well as any other known *Hemiboea* taxa, which verified its identity as a new species. It is described and illustrated here.

Material and methods

Fresh leaf materials were collected and preserved in silica gel for quick drying. Total genomic DNA was extracted using the CTAB method (Doyle and Doyle 1987). The ITS and *trnL-F* were selected for phylogenetic analyses as these two loci are the most frequently used in phylogenetic studies of *Hemiboea* and other Gesneriaceae (Zhang et al. 2014, Huang et al. 2017, Lu et al. 2017). The PCR amplifications and sequencing procedures were according to those described in Guo et al. (2016). Ten samples of the assumed new species were selected for sequencing and all the ITS sequences were successfully directly sequenced, while only six samples could be sequenced directly for *trnL-F*. We also amplified the same two loci of three individuals of *H. subcapitata* from the same site, and all the ITS sequences were successfully obtained, but we failed to obtain any *trnL-F* sequences. Thus, 13 ITS sequences and six *trnL-F* sequences were newly acquired in the present study. The newly acquired sequences have been submitted to GenBank.

We queried highly similar sequences from GenBank using a BLAST search and found that the most similar sequences were those of *H. subcapitata*. We then downloaded a total of 64 sequences (34 ITS sequences and 30 *trnL-F* sequences) of

species of *Hemiboea*, *Lysionotus* Don and David (1822) and *Petrocosmea* Oliver and Daniel (1887) to test the distinctness of the odd individuals as an independent species and their phylogenetic position in the genus. Four species, namely, *Lysionotus chingii* Chun ex Wang (1983), *L. petelotii* Pellegr (1930), *Petrocosmea kerrii* Craib (1918) and *P. nervosa* Craib (1919) were selected as outgroups and the *Petrocosmea* species (the most distant outgroup taxa) was set to root the tree following previous studies (Möller et al. 2011, Weber et al. 2011, Huang et al. 2017). The GenBank accession numbers of the downloaded sequences are listed in Table 1.

DNA sequences were aligned using the program MUSCLE 3.8.31 (Edgar 2004) and adjusted manually with Bioedit 5.0.9 (Hall 1999). We reconstructed the phylogeny using maximum likelihood (ML) and Bayesian inference (BI). We reconstructed separate maximum likelihood trees for ITS and *trnL-F*, and compared the trees to check whether there was any phylogenetic conflict between the chloroplast and nuclear data. ML analyses were performed using RAxML-VI-HPC (Stamatakis 2006) with the substitution model GTR+G and 1000 rapid bootstrap searches (BS). For the Bayesian analyses, the optimal substitution model K81uf+I+G was selected by ModelTest (Posada and Crandall 1998) according to the Akaike information criterion (AIC; Akaike 1974). BI analyses were conducted in MrBayes ver. 3.2.6 (Ronquist et al. 2012). All BI analyses were run for 100 000 000 generations with four chains in two parallel runs and sampled every 5000 generations after a burn-in of the first 5000 trees. The convergence of the two parallel runs was assessed and reached as the splitting frequency was less than 0.005 after the burn-in. All other parameters were set as default.

Results

The aligned ITS and *trnL-F* matrices contained 679 and 809 characters, including 227 and 79 variable sites with 150 and 30 informative sites, respectively. The consistency index (CI), retention index (RI) and homoplasy index (HI) were 0.704, 0.824 and 0.296, and 0.945, 0.928 and 0.055 for the ITS and *trnL-F* data, respectively.

All *Hemiboea* taxa including the assumed new species (*H. yongfuensis*) together formed a clade (BS=100% and 97%; PP=1.00 and 1.00) in both the ITS and *trnL-F* trees (Fig. 1, 2). In the ITS tree, all the accessions of the three species *H. yongfuensis*, *H. subcapitata* and *H. pterocaulis* were resolved as monophyletic lineages (BS=97, 68 and 87%; PP=1.00, 0.96 and 0.99), respectively, and *H. yongfuensis* and *H. subcapitata* were resolved as sister taxa (BS=62%; PP=0.75), followed by *H. pterocaulis*, with high support values (BS=97%; PP=1.00). In the *trnL-F* tree, *H. yongfuensis* also formed as a monophyletic lineage (BS=55%), which was the closest relative to individuals of *H. subcapitata* and *H. gracilis* var. *gracilis* (BS=64%; PP=0.88), but *H. subcapitata* failed to form a monophyletic lineage in this low resolved phylogeny, forming a complete polytomy with *H. subcapitata* and *H. yongfuensis*.

Table 1. GenBank accession numbers of the sequences used in the present study.

Species	GenBank accession numbers (ITS/ <i>trnL-F</i>)
<i>Hemiboea bicornuta</i> (Hayata) Ohwi	FJ501356/FJ501534
<i>Hemiboea cavaleriei</i> Lévél	FJ501355/FJ501533
<i>Hemiboea fangii</i> Chun ex Li	HQ632979/–
<i>Hemiboea flaccida</i> Chun ex Li	JF697567/JF697579
<i>Hemiboea follicularis</i> Clarke	HQ632982/HQ632885
<i>Hemiboea gracilis</i> Franchet	–/FJ501536
<i>Hemiboea gracilis</i> var. <i>gracilis</i> Franchet	–/HQ327445
<i>Hemiboea yongfuensis</i> Huang and Lu	MK441665–MK441674/MK441675–MK441680
<i>Hemiboea longgangensis</i> Li	HQ632986/HQ632889
<i>Hemiboea longzhouensis</i> Wang ex Li	HQ632985/HQ632888
<i>Hemiboea magnibracteata</i> Wei and Wen	HQ632984/HQ632887
<i>Hemiboea malipoensis</i> Tan	–/KJ948111
<i>Hemiboea omeiensis</i> Wang	HQ632983/HQ632886
<i>Hemiboea ovalifolia</i> Wang	HQ632980/HQ632883
<i>Hemiboea purpureotincta</i> Wang	HQ632981/HQ632884
<i>Hemiboea rubibracteata</i> Li and Liu	HQ632987/HQ632890
<i>Hemiboea pterocaulis</i> (Li) Huang, Xiang and Zhang	–/KR476562
<i>Hemiboea pterocaulis</i> (Li) Huang, Xiang and Zhang	KY607397/–
<i>Hemiboea pterocaulis</i> (Li) Huang, Xiang and Zhang	KY607398/KY607416
<i>Hemiboea pterocaulis</i> (Li) Huang, Xiang and Zhang	KY607399/KY607413
<i>Hemiboea pterocaulis</i> (Li) Huang, Xiang and Zhang	KY607400–KY607403/–
<i>Hemiboea pterocaulis</i> (Li) Huang, Xiang and Zhang	KY607404/KY607415
<i>Hemiboea pterocaulis</i> (Li) Huang, Xiang and Zhang	KY607405/KY607414
<i>Hemiboea subcapitata</i> Clarke	KY607406/KY607418
<i>Hemiboea subcapitata</i> Clarke	KY607407/KY607420
<i>Hemiboea subcapitata</i> Clarke	KY607408–KY607410/–
<i>Hemiboea subcapitata</i> Clarke	KY607411/KY607419
<i>Hemiboea subcapitata</i> Clarke	KY607412/KY607417
<i>Hemiboea subcapitata</i> Clarke	FJ501357/FJ501535
<i>Hemiboea subcapitata</i> Clarke	MK441681–MK441683/–
<i>Hemiboea subcapitata</i> Clarke var. <i>guangdongensis</i> (Li) Li	MF625025/–
<i>Hemiboea wangiana</i> Li	–/KJ475428
<i>Hemiboea</i> sp	–/JN644339
<i>Lysionotus chingii</i> Chun ex Wang	FJ501332/FJ501498
<i>Lysionotus petelotii</i> Pellegr	HQ632974/FJ501496
<i>Petrocosmea kerrii</i> Craib	FJ501334/FJ501502
<i>Petrocosmea nervosa</i> Craib	FJ501335/AJ492299

At both loci, the range of the pairwise genetic distances within *H. yongfuensis* was non-overlapping or only slightly-overlapping with that of interspecific pairwise genetic distances. The range of pairwise ITS genetic distances within each of the three species *H. yongfuensis*, *H. subcapitata* and *H. pterocaulis* were 0.0000–0.0084, 0.0000–0.0327 and 0.0000–0.0141, which was smaller than the pairwise interspecific genetic distances between *H. yongfuensis* and *H. subcapitata* (0.0193–0.0469) and between *H. yongfuensis* and *H. pterocaulis* (0.0194–0.0311). According to the *trnL-F* sequences, however, the range of pairwise genetic distance within each of the same three species are 0.0000–0.0037, 0.0000–0.0178 and 0.0000–0.0012, which is generally smaller than but slightly overlapping with the interspecific genetic distances. The non-overlap or slight overlap of the ranges of the intraspecific versus interspecific genetic distances indicate a genetic gap, supporting that *H. yongfuensis* to an independent species and distinguishable from the most closely related species known.

Thus, the genetic isolation and diagnostic morphological traits strongly support the separation of *H. yongfuensis* from its two closely-related species, verifying the validity as the independent new species.

Taxonomic treatment

Hemiboea yongfuensis Z. P. Huang & Y. B. Lu sp. nov. (Fig. 3, 4)

A species similar to *H. subcapitata* Clarke and *H. pterocaulis* (Li) Huang, Xiang and Zhang, but distinguished by the shape of the stem, the color of the corolla and the characteristics of the calyx. It has a quadrilateral stem without winged edges, while *H. subcapitata* has a cylindrical stem without winged edges and *H. pterocaulis* has an angular stem with winged edges. The outer surface of the corolla is light pink and sparsely glandular-pubescent, while it is white and glandular pubescent

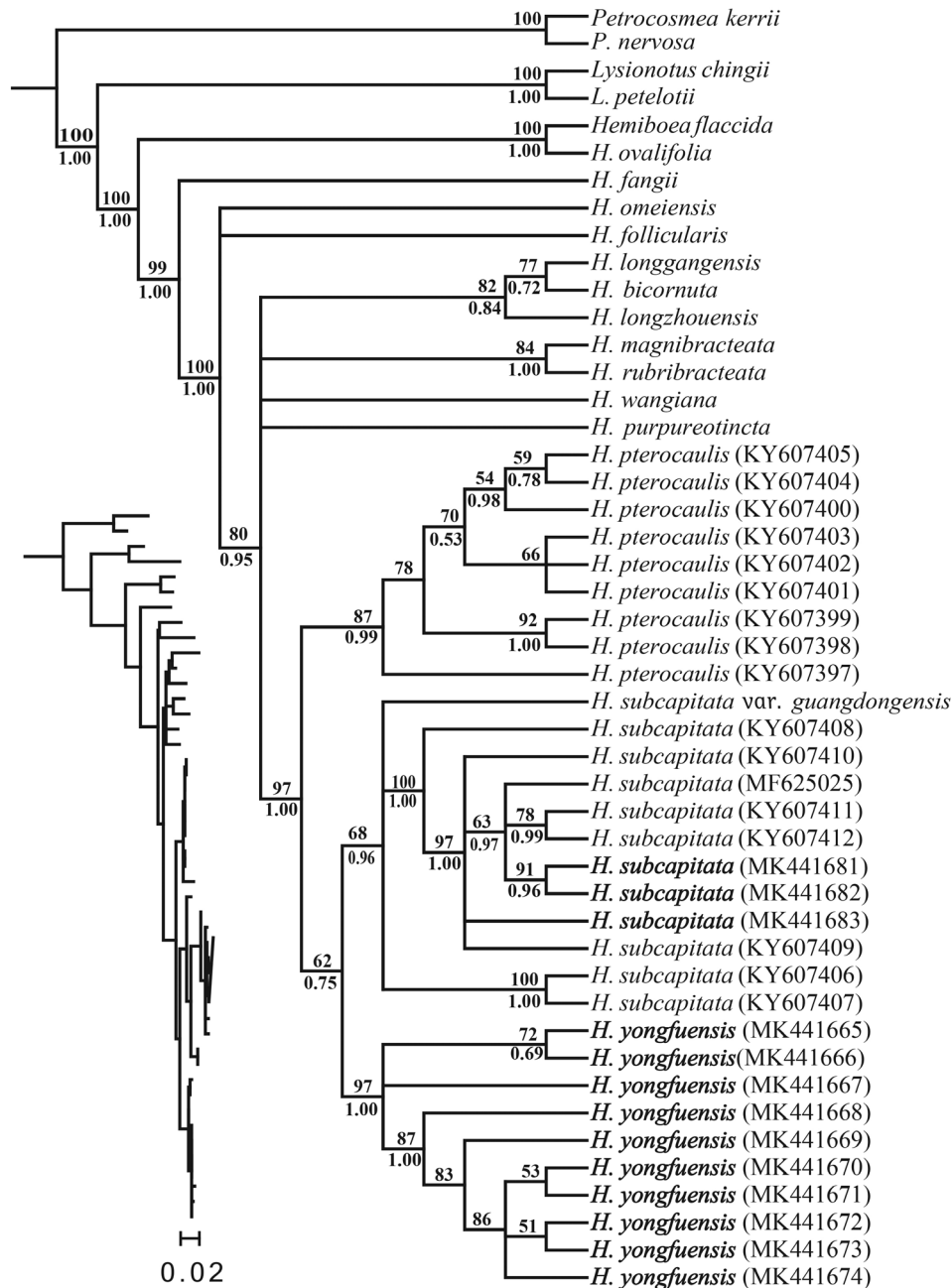


Figure 1. The majority-rule consensus tree generated from maximum likelihood inference analysis based on the ITS sequences with PP values (>0.50) and BS (>50%) shown below and above the branches. Newly acquired samples of *H. yongfuensis* are highlighted in bold.

and white and pubescent in *H. subcapitata* and *H. pterocaulis*, respectively. The calyx is pale pink and 5-lobed from the base, while it is greenish white and 5-lobed from the base in *H. subcapitata*, and greenish white with a connate base in *H. pterocaulis* (see more diagnostic characters in Table 2).

Type: China, Guangxi Zhuang Autonomous Region, Guilin: Yongfu County, Luojin Town, 25°4'13.70"N, 110°17'57.23"E, 132–160 m a.s.l., 10 Oct 2018, Q. Zhang 380846, flowering (holotype: IBK, isotype: PE).

Etymology

The name is derived from the type locality, Yongfu County, Guilin, Guangxi.

Description

Perennial herb. Stems ascending, 10–46 cm long, 4–9 mm in diameter, quadrilateral, covered with scattered purplish brown spots, unbranched, simple with 4–6 nodes, glabrous. Leaves opposite, 5–6 crowded at the apex of stem; petiole 0.3–1.5 cm long, 3–5 mm in diameter, triangular, glabrous;

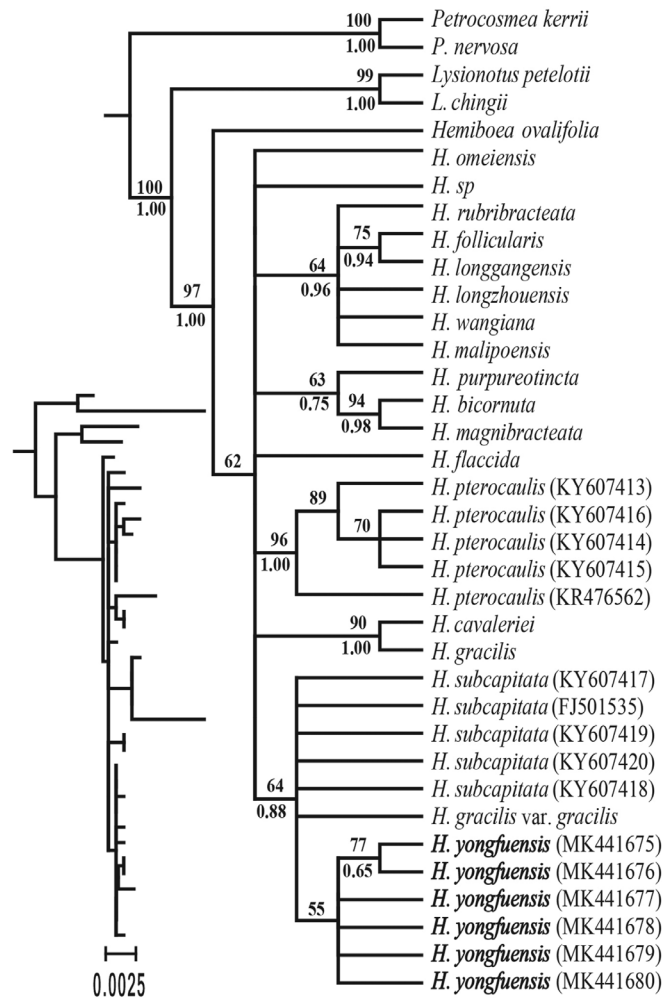


Figure 2. The majority-rule consensus tree generated from maximum likelihood inference analysis based on the *trnL-F* sequences with PP values (>0.50) and BS (>50%) shown below and above the branches, respectively. Newly acquired sequences of *H. yongfuensis* are highlighted in bold.

leaf blade slightly fleshy, elliptic, ovate-lanceolate or obovate-lanceolate, adaxially yellowish green, abaxially light green, 4–18×4–10 cm; margin entire; apex acute or acuminate; base cuneate or elongate; lateral veins slightly convex on the front, with transverse section triangular, 5–6 on each side of midrib. Cymes axillary or terminal, 2–9 flowered; peduncle 3.5–4.0 cm long, glabrous, with scattered purplish brown spots; involucre subcordate, 1.5–2.5 cm in diameter, light brown with pale purple spots, glabrous, acute at apex. Pedicel 2–5 mm long, ca 1.5 mm in diameter, glabrous. Calyx pale pink, equally 5-lobed from the base, oblong-elliptic, 9–12×3.5–5.0 mm, glabrous. Corolla outside pale pink, sparsely glandular-pubescent; corolla tube 2.8–3.7 cm long, 13–15 mm in diameter at the mouth, 5–6 mm in diameter at the base, orange-spotted at throat, with a hairy ring nearly adnate to the corolla tube base, the upper side of corolla inside light pink to pale yellow, lower side yellow with a small pilose spot below the middle lobe; limb distinctly 2-lipped, adaxial lip 3–5×5–10 mm, 2-lobed at apex, nearly semicircular;

abaxial lip 3-lobed, 3.5–5.5×5.5–8.0 mm, almost semicircular. Stamens 2, inserted ca 13 mm above the corolla base, glabrous; filaments narrowly linear, 8–13 mm long, glabrous; anthers oblong, 2.8–3.2×2.0–2.5 mm, coherent at apex, glabrous. Staminodes 3, linear, glabrous, capitate at apex, adnate to ca 8 mm above the corolla base, middle one 2 mm long, lateral ones 5–8 mm long. Disc ring-like, 1.0–1.2 mm high, glabrous. Pistil 3.2–3.5 cm long, ovary linear, 8–12 mm long, 2 mm in diameter, glabrous; style 1.9–2.4 cm long; stigma obtuse, capitate, slightly wider than the stylar canal. Capsule linear-lanceolate, 1.5–2.5 cm long, base width 3–4 mm, acuminate at apex, slightly curved, glabrous.

Distribution, habitat and phenology

Hemiboea yongfuensis has only been found in one of the Jinzhong hills in Luojin Town, Yongfu County, Guilin city, Guangxi, southern China. It grows on limestone rocks, coexisting with the congener of *H. subcapitata* and *H. pterocaulis* in the forest, and accompanied with *Primulina*

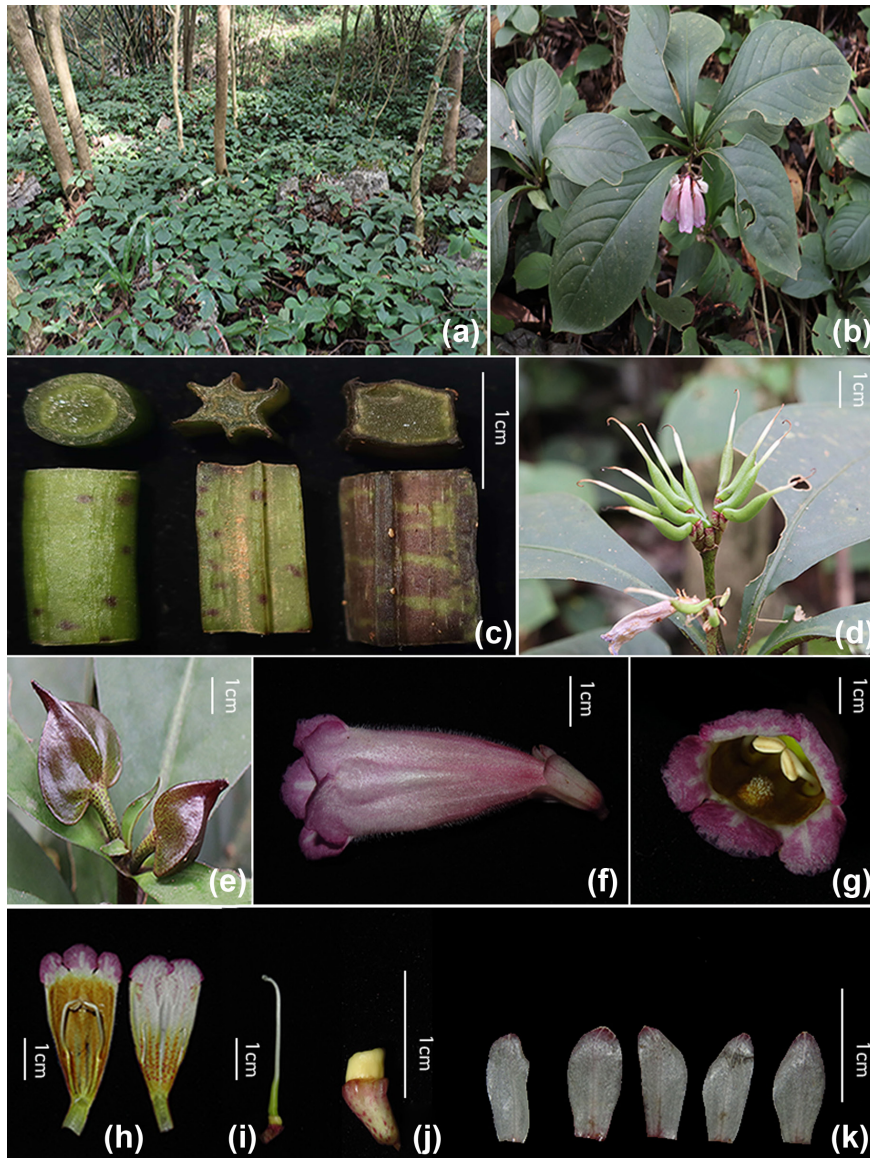


Figure 3. *Hemiboea yongfuensis* (a) habitat, (b) flowering individual, (c) comparison of the anatomically transected stems among *H. yongfuensis*, *H. subcapitata* and *H. pterocaulis* with *H. subcapitata* on the left, *H. pterocaulis* in the middle and *H. yongfuensis* on the right, (d) fruits, (e) involucre, (f) side view of flower, (g) front view of flower, (h) opened corolla with stamens and staminodes, (i) pistil with disc, (j) disc, (k) opened calyx.

subrhomboidea Wang (1981), *Pilea longicaulis* Handel (1929), *Spiradiclis fusca* Lo (1991), *Neottopteris antrophyoides* Ching (1940) and *Liparis distans* Clarke (1889). Its flowering period is in October–November, and fruiting period in November–December.

Proposed IUCN conservation status

According to the field observations, *H. yongfuensis* has only one known population comprising hundreds of mature individuals at the type locality (a limestone hill in Yongfu County, Guilin, Guangxi, China), and the total area of occupancy is less than 0.5 km². The population and habitat are susceptible to human activities, e.g. tourism or deforestation. Therefore,

it is suggested to be classified as Critically Endangered (CR) according to the IUCN standards (IUCN 2019).

Similar species

Morphologically, *H. yongfuensis* is similar to *H. subcapitata* and *H. pterocaulis*. All of them co-occur in the same hill and even intermixed at the same site, but *H. yongfuensis* differ by the shape of the stem, the color of the corolla and the characteristics of the calyx (Table 1). Furthermore, the flowering period is different with *H. yongfuensis* in October–November, *H. subcapitata* in August–October and *H. pterocaulis* in April–May. Analyses of DNA sequence data (the ITS and *trnL-F*) also suggest that *H. yongfuensis* is closely related to

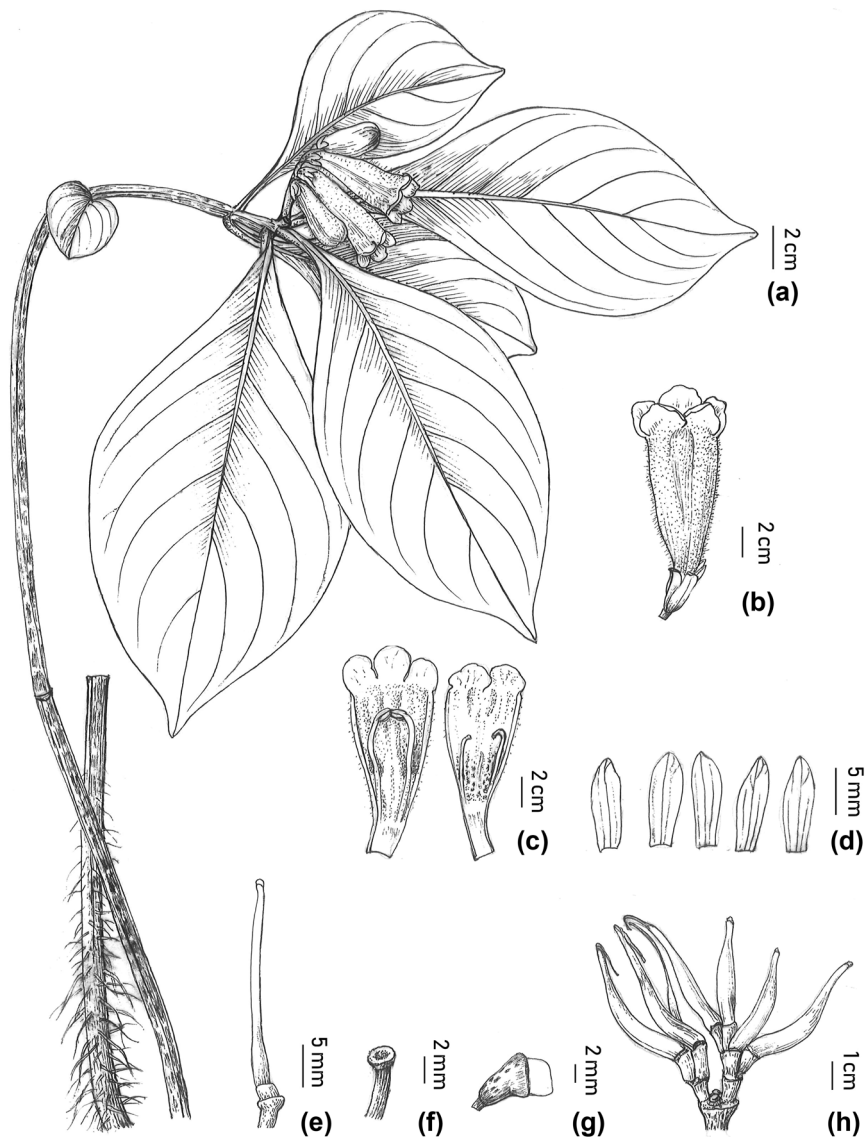


Figure 4. *Hemiboea yongfuensis*. (a) habit (plant individual), (b) side view of flower, (c) opened corolla with stamens and staminodes, (d) opened calyx, (e) pistil with disc, (f) stigma, (g) disc, (h) fruits.

Table 2. Morphological comparison of *Hemiboea yongfuensis*, *H. subcapitata* and *H. pterocaulis*.

	<i>H. yongfuensis</i>	<i>H. subcapitata</i>	<i>H. pterocaulis</i>
Stem	quadrilateral, without wings	cylindrical, without wings	angular, with wings
Involucre	light brown with pale purple spots.	completely green without any spots	almost all green, only a little purple at apex, and a little purple speck near the base
Corolla outside	light pink, sparsely glandular-pubescent	white, glandular pubescent	white to pinkish, puberulent
Corolla inside	upper side white and yellow with orange spots, lower side yellow with a hairy ring	yellow, dark purple	upper side white, lower side yellow with brown spots
Calyx	pale pink, 5-lobed from base, segments oblong to elliptic	greenish white, 5-lobed from base, segments lanceolate to narrowly ovate	greenish white, base connate, 5-lobed from near middle, segments triangular to ovate

H. subcapitata and *H. pterocaulis*, and all the individuals of the new species form a monophyletic lineage separated from the two closely-related species. In conclusion, morphological, phenological and molecular characters support that *H. yongfuensis* should be recognized as a new species in *Hemiboea*.

Acknowledgements – The authors thank Wen-Hong Lin (IBK) for preparing the illustration.

Funding – This study was supported by National Natural Science Foundation of China (NSFC, 31560114), Special Funds for Local Science and Technology Development Guided by the Central Committee (ZY1949013), Guilin Scientific Research and Technology Development Program (20180107-6), Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain (no. 17-259-23) and the Project of High level Innovation Team and the Scholars of Excellence Program of Universities in Guangxi in 2018.

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