

# *Hemiboea liana* (Gesneriaceae): A new species from northern Guangxi, China

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ABSTRACT: *Hemiboea liana*, a species of Gesneriaceae new to science found in northern Guangxi, China, is described and illustrated. While sharing morphological similarities with *H. chanii* and *H. sinovietnamica*, the new species can be distinguished by a distinct combination of traits, such as its brown rounded squared hard stem and trigonous purple-light brown involuce. Detailed information is provided, including a morphological description, illustrations, diagnostic comparisons, and a molecular phylogenetic analysis placing the new species among 30 out of 44 species currently recognized in *Hemiboea*.

KEY WORDS: Hemiboea chanii, Hemiboea rubribracteata, Hemiboea sinovietnamica, ITS, trnL-F, phylogeny, taxonomy.

# INTRODUCTION

Hemiboea, a genus of Gesneriaceae, is primarily distributed in South China, Vietnam, India and Japan. These plants live in evergreen broad-leaved forests or mixed evergreen and deciduous broad-leaved forests, particularly thrive in limestone habitats, in rock crevices, or in humus on rock surfaces, but are also found on conglomerates, granites, or sandstones (Wei et al., 2010). Over the past two decades, 16 new species and two new varieties of Hemiboea have been described predominantly from limestone habitats in Southwest China (Li, 2004; Li and Liu, 2004; Xu et al., 2010, 2012; Huang et al., 2011, 2017, 2020; 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, 2019; Wu et al., 2019; Tan et al., 2021; Miao et al., 2022; Peng et al., 2022). As of July 2023, based on the checklists of the Gesneriaceae Resource Centre (GRC, 2024), the genus Hemiboea encompasses currently a total of 44 species and five varieties.

In the present study, a new species, *H. liana*, discovered in Yizhou, northern Guangxi, China, is described and presented. It has a unique combination of characteristics uncommon in *Hemiboea*, including a branched brown rounded squared hard stem and trigonous purplish-light brown involucre. These characteristics differentiate the new species from its morphologically most similar allies (*H. chanii*, *H. rubribracteata* and *H. sinovietnamica*). A molecular phylogenetic analysis based on the plastid *trn*L-F and nuclear ITS sequences was conducted to investigate the genetic relationships between the newly discovered species and selected members of *Hemiboea*.

# MARERIALS AND METHODS

#### Morphological observation

Living plants of the new putative species were collected in the field and cultivated in the nursery of the Guangxi Institute of Botany. The morphological characteristics were observed and recorded in the field and nursery and the phenology was traced in the nursery. Morphologically similar species, i.e., H. chanii, H. rubribracteata, and H. sinovietnamica. The cultivated living plants of this presumed new species, H. rubribracteata, and H. sinovietnamica, along with the original protologues of the three known species selected for comparison, were chosen for morphological comparisons (Li and Liu, 2004; Xu et al., 2012; Nguyen et al., 2021). Additionally, we consulted the isotype of H. chanii (http://en.herbariumle.ru/?t=occ&id=91550), as well as the holotype and isotype of H. rubribracteata and H. sinovietnamica stored in the herbarium of Guangxi Institute of Botany (IBK). A voucher specimen of the putative new species was deposited in IBK.

#### **Phylogenetic analysis**

For molecular studies, samples of five plants of the putative new species were collected solely from a single population located in Yizhou, northern Guangxi, China. Total DNA was extracted from silica-gel-dried leaves using a modified CTAB method (Doyle and Doyle, 1987). The nuclear ribosomal internal transcribed spacers (ITS) and chloroplast *trn*L-F regions were selected for phylogenetic analyses. The PCR amplifications and sequencing procedures followed Guo *et al.* (2016). All five samples of the newly collected material were sequenced.



Table 1. Morphological and phenological comparisons among H. liana, H. chanii, H. rubribracteata, and H. sinovietnami	ica.
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Characters	H. liana	H. chanii	H. rubribracteata	H. sinovietnamica
Stem	basal lignifications, brown without spot, 10–46 cm tall, rounded square and branching	green, spotless, 50–90 cm tall, subterete, branching	hard and green, scattered dark purple spots, 40–150 cm tall, nearly cylindrical, sometimes branching	green without spot, 25–45 cm tall, subtetragonal, non- branching
Leaf blade size	4.7–8.3 × 2.8–3.8 cm	7.5–12 × 2.5–5.0 cm	4–18 × 3–10 cm	8.0–17.5 × 2.5–8.0 cm
Involucre	purple-light brown, trigonous	light green, diamond	completely red, spheroidal	green, trigonous
Corolla	pink-spotted	pink	white	yellowish
Filaments Anthers	narrowly linear coherent at apex, coherent at apex, semicircular	filiform, coiled coherent at apex, coherent at apex, basifixed	narrowly linear coherent at apex, ovate - elliptic	linear coherent at apex, subrotund
Flowering period	Nov. to Jan.	Jan. to Feb.	Jun. to Oct.	Oct. to Nov.

All the ITS sequences were directly sequenced successfully, while only four samples could be successfully sequenced for trnL-F. In total, 35 ITS sequences representing 30 species and one variety of Hemiboea, two species of Lysionotus, and two species of Petrocosmea, and 24 trnL-F sequences, comprising 20 Hemiboea species, two Lysionotus species, and two Petrocosmea species were downloaded from GenBank to which the newly generated sequences were added, to test the phylogenetic affinities of the newly collected material. In both the ITS and *trn*L-F phylogenetic analyses, the two species of Petrocosmea (P. kerrii and P. nervosa) and two species of Lysionotus (L. chingii and L. petelotii) were set as the outgroup, with the two species of *Petrocosmea* set as the root (Möller et al., 2011). The GenBank accession numbers for the sequences used in the phylogenetic study can be found in Appendix Table 1.

DNA sequences were aligned with MUSCLE 3.8.31 (Edgar, 2004) and adjusted manually in Bioedit 5.0.9 (Hall, 1999). The divergent segments in the alignment were masked by alignmentFilter 1.1.0 (Zhang et al., 2023), with a stringency of 0.0001 and excluded from analysis. Subsequently, nucleotide substitution models were selected using ModelFinder in IQTree v2.2.2.7 (Kalyaanamoorthy et al., 2017) based on the Akaike Information Criterion (AIC; Akaike, 1974). The Partition Homogeneity Test (PHT) in PAUP\* 4.0b10 (Swofford, 2002) was utilized to determine whether the partitions were homogeneous. Homoplasy levels were also assessed using PAUP\* 4.0b10. The maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out to construct phylogenetic trees based on the ITS and trnL-F datasets separately. ML analyses were performed using RAxML-NG (Kozlov et al., 2019) with the best substitution models (GTR+F+I+G4 for ITS and GTR+F+G4 for *trn*L-F) and 1,000 rapid bootstrap searches (BS). The optimal substitution models (GTR+F+I+G4 for ITS and GTR+F+G4 for trnL-F) were chosen for the Bayesian analyses. BI analyses were conducted using MrBayes 3.2.7 (Ronquist et al., 2012), performing two independent parallel runs with four chains each for a total of 100,000,000 generations. Sampling was conducted every 5,000 generations, with the first 5,000 trees (25%) discarded as burn-in. To ensure convergence, the average standard deviation of split frequencies between the two parallel runs was monitored to have reached a value below 0.005. All other parameters were kept at their default settings.

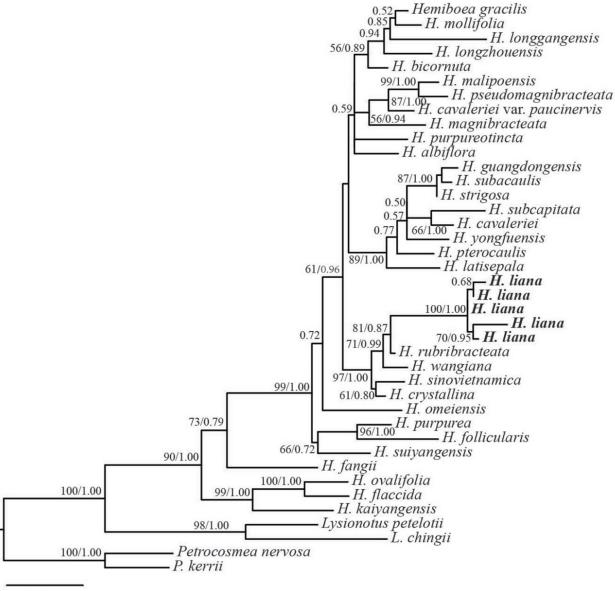
# RESULTS

#### **Phylogenetic analyses**

The ITS and *trn*L-F matrices had 895 and 987 characters, respectively, which contained 637 and 888 constant sites, 229 and 76 variable but parsimony uninformative sites, and 137 and 29 parsimony informative sites, respectively. Further, the signals of the information sites for the sequence were evaluated. The consistency index (CI), homoplasy index (HI), and retention index (RI) were 0.632, 0.368, 0.718, and 0.953, 0.047, and 0.942 for ITS and *trn*L-F, respectively. Finally, the partition homogeneity test conducted on the ITS and *trn*L-F sequences revealed significant differences (p-value = 0.01), indicating that these two segments are not suitable for concatenating to construct a phylogenetic tree.

The phylogenetic analysis reveals that the topology of phylogenetic trees constructed using the ML and BI methods is consistent for both the ITS and trnL-F sequences. However, there are certain conflicts between the ITS tree and the trnL-F tree. For example, certain shared species, such as *H. kaiyangensis* and *H. flaccida*, exhibit incongruent placement between the ITS and trnL-F trees. Additionally, some nodes in the both two trees have low support values, resulting in unclear phylogenetic placements of species. Nonetheless, all trees consistently demonstrate that the included samples of Hemiboea form a distinct clade (BS = 75 and 90%, PP =0.78 and 1.00). In the ITS tree, all individuals of *H. liana* formed one clade (BS = 100%; PP = 1.00), which was closely related to *H. rubribracteata* (BS = 81%; PP = 0.87) (Fig. 1). In the trnL-F tree, the accessions of H. liana also formed a single clade (BS = 92%; PP = 1.00), which was also the closest relative to H. rubribracteata, though with low support (BS = 65%; PP = 0.95) (Fig. 2). Unfortunately, molecular sequences of H. chanii were not available, and based on the samples included here, both





0.02

Fig. 1. Maximum Likelihood tree of 36 *Hemiboea* ingroup samples, including the newly described *Hemiboea liana* (highlighted in bold) and four outgroup samples based on ITS sequences with PP values (>0.50) and BS (>50%) shown near the branches. Newly acquired sequences of *Hemiboea liana* are highlighted in bold.

ITS and *trn*L-F phylogenetic trees indicated that *H. liana* is most closely related to *H. rubribracteata*. Although this might change once molecular data for other species (such as *H. chanii* and *H. shimentaiensis*) mentioned above become available.

### TAXONOMIC TREATMENT

#### Hemiboea liana Z.P. Huang, Y.B. Lu & B. Pan, sp. nov. 李氏半蒴苣苔 Figs. 3-4

*Type*: CHINA. Guangxi province: Hechi city, Yizhou county, Longtou town, Dushan village, in limestone

forests, 108°15′28.00″E, 24°31′20.39″N, elevation *ca*. 132m, 26 October 2019, B. Pan *PB20191026* (holotype, IBK00417133).

**Diagnosis:** Morphologically, *H. liana* and *H. chanii* are most similar, but they can be distinguished by the shape and length of the stem (shape: rounded square vs. subterete; length: 10-46 cm vs. 50-90 cm), leaf blade size ( $4.7-8.3 \times 2.8-3.8$  cm), shape and color of the involucre (shape: trigonous vs. diamond; color: purple-light brown vs. light green), corolla color (pink-spotted vs. pink), and the shape of the filament and anther (filament: narrowly linear vs. filiform; anther: semicircular vs. basifixed). In



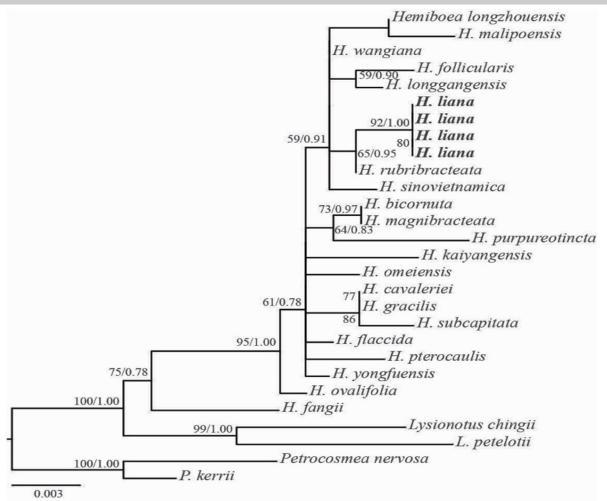


Fig. 2. Maximum Likelihood tree of 24 *Hemiboea* ingroup samples based on the *trn*L-F sequences with PP values (>0.50) and BS (>50%) shown near the branches. Newly acquired sequences of *Hemiboea liana* are highlighted in bold.

addition, this new species has the same involucre shape as H. sinovietnamica, but their involucres are different in color (purple-light brown vs. green). In addition, they can be distinguished by their stem cross section (rounded square vs. subtetragonal), corolla color (pink-spotted vs. yellowish), and anther shape (semicircular vs. subrotund). Although the phylogenetic relationship between H. liana and H. rubribracteata is closest among the sampled they exhibit significant morphological species, differences. For instance, the shape and length of the stem (shape: rounded square vs. nearly cylindrical; length: 10-46 cm vs. 40-150 cm), the shape and color of the involucre (shape: trigonous vs. spheroidal; color: purplelight brown vs. completely red), the corolla color (pinkspotted vs. white), and the anther shape (semicircular vs. ovate-elliptic). The morphological and phenological comparison between the new species and its morphological allies is shown in Table 1.

**Description:** Subshrub. Stems ascendent, surface sepia and eguttulate, 10–46 cm long, 4–9 mm in diameter, basally lignified, rounded square in cross-section,

branching axillary, with 4-6 nodes, glabrous. Leaves opposite, leaf blade papery when dried, elliptic or obovate-lanceolate, adaxially green, abaxially lilac, 4.7- $8.3 \times 2.8-3.8$  cm, margin obtuse dentate or subentire, apex acuminate, base asymmetry; petiole, 2-2.5 cm long, glabrous; veins slightly depressed on adaxial surface, abaxially conspicuous, lateral veins 5 to 8 on each side of midrib. Cymes terminal, usually 1-flowered, occasionally 2-flowered, 6–10 inflorescences; peduncle, fuscous, 0.5– 1.3 cm long, glabrous; involucre trigonous, purplish-light brown,  $1.3-1.7 \times 1.8-2.3$  cm each side, acuminate apex, 1-3 mm long, glabrous. Calyx white or pale pink, 10-13 mm long, glabrous. Calyx dried membranous, 5-lobed from near base, long elliptic, 10-12 mm long, 1-2.5 mm wide, glabrous. Corolla, 4-5 cm long, sparsely glandularpubescent, pink-spotted outside, inside white, with scattered pink and purple spots, and ring of hairs 4.5-5.8 mm above base; corolla tube 24-40 mm long, 15-22 mm in diameter at mouth, 3-5 mm in diameter at base, limb distinctly 2-lipped, adaxial lip 2-lobed, lobes obovate, 1- $2 \times 6-9$  mm; abaxial lip 3-lobed, lobes obovate,  $6-7.7 \times$ 

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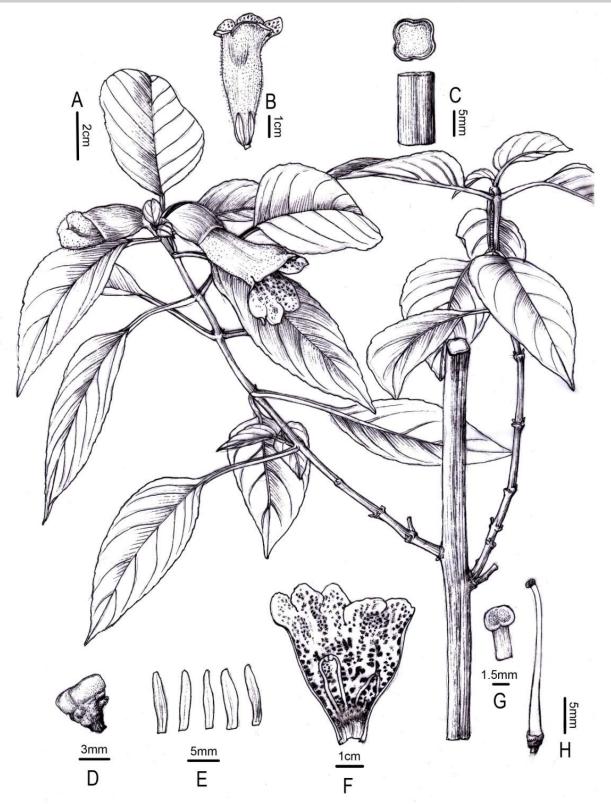


Fig. 3. *Hemiboea liana* A. habit plant individual, B. side view of flower, C. cross-section of the stem, D. annular disc, E. opened calyx, F. opened corolla with stamens and staminodes, G. stigma, H. pistil with the disc.



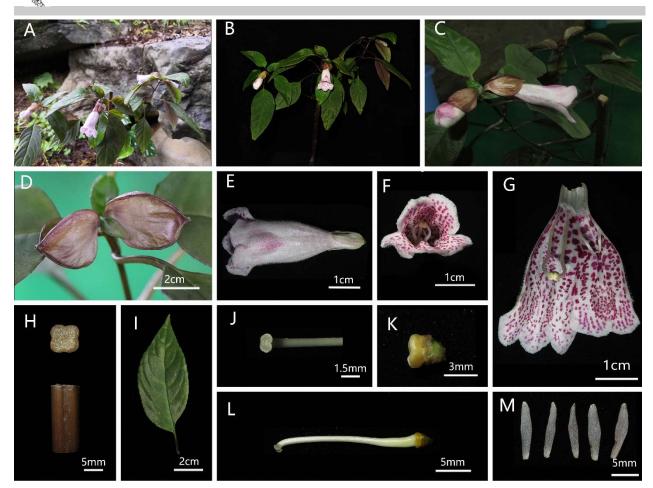


Fig. 4. Hemiboea liana A. habitat, B. flowering individual, C. inflorescences, D. involucre, E. side view of flower, F. front view of flower, G. opened corolla with stamens and staminodes, H. opened calyx, I. pistil with disc, J. annular disc, K. stigma, L. leave blade morphology, M. cross-section of the stem.

7.4–9.5 mm. Stamens 2, adnate to 7.5–9 mm from the base of the corolla tube; filaments geniculate above the middle, 18–20 mm long, narrowly linear, glabrous; anthers semicircular,  $2-2.5 \times 1.2-1.5$  mm, coherent at apex, glabrous; staminodes 3, adaxial one, 2 mm long; two lateral ones, 5 to 7 mm long. Disc ringlike, 1–1.2 mm high, glabrous. Pistil 2.7–3.6 cm long, ovary linear, 0.8–1.2 cm long, 2.2 mm in diameter, glabrous; style 1.9–2.4 cm long; stigma shallowly bilobed, slightly wider than style.

*Distribution and Habitat*: This new species has been found only in Dushan village, Longtou town, Yizhou county, Hechi city, Guangxi province, southern China. It grows on limestone rocks in forests at an elevation of *ca.* 132 m.

*Phenology:* Flowering from November to January the following year; fruiting not seen.

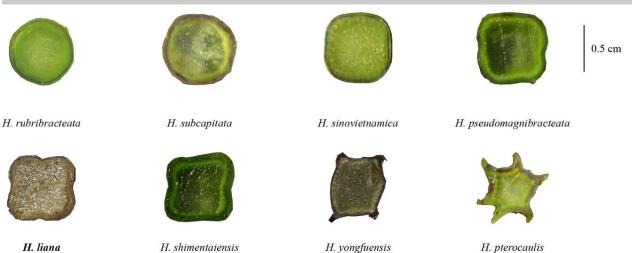
*Conservation status*: Currently, *H. liana* is only known from the type locality, and the population has less than one hundred mature individuals. However, more populations are likely to be found in similar habitats in the limestone areas of northern Guangxi. With the limited fieldwork, we currently consider this species to be Data Deficient (DD) (IUCN, 2022).

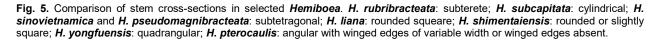
*Etymology*: The name of this newly discovered species, *Hemiboea liana*, is in honor of LI Zhen-Yu, for his significant contributions to the study of Gesneriaceae, including *Hemiboea*.

Notes: In most Hemiboea species, the shape of the stem is terete or subterete, with a few species having ribbed or winged stems, such as subtetragonal (H. purpurea, H. sinovietnamica, H. pseudomagnibracteata), quadrangular (H. follicularis var. retroflexa, H. yongfuensis), rounded to square (H. malipoensis), rounded or slightly square (H. shimentaiensis), and angular with winged edges of variable width or winged edges absent (H. pterocaulis) (Fig. 5). Despite the striking resemblance between the stems of H. liana and H. shimentaiensis, there are notable differences. Firstly, their habitats differ (limestone vs. non-limestone). Additionally, leaf margin (obtuse dentate or subentire vs. entire or somewhat undulate), inflorescence (1-flowered, occasionally 2-flowered vs. 6-14 flowers), and the shape and color of the involucre (shape: trigonous vs oblatespherical; color: purplish-light brown vs. green) are also different (Miao et al., 2022).

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In most Hemiboea species, the involucre are (near-)spheroidal or (sub-)cordate, and rarely trigonous (H. liana and H. sinovietnamica), 4-winged (H. crvstallina), and diamond-shaped (*H*. chanii). Additionally, each involucre of H. liana typically bears only one flower, but occasionally, there may be two flowers within a single involucre. Most known species of the Hemiboea typically have inflorescences with multiple flowers (each involucre containing at least three flowers), such as H. subcapitata (3-10 flowers), H. strigosa (3 flowers or more), H. gamosepala (7-12 flowers), H. follicularis (7–20 flowers), and H. kaiyangensis (6–12 flowers). Additionally, there are a few species where each inflorescence contains only 1-3 flowers, including H. mollifolia (3 flowers), H. gracilis (1-3 flowers), H. subacaulis (1-3 flowers), H. pingbianensis (1-2 flowers), H. longgangensis (2-3 flowers), and H. sinovietnamica (2–3 flowers).

Additionally, we conducted new sequencing for the morphologically unique species, H. liana, and performed a combined analysis with the ITS and trnL-F sequences of 30 known species in Hemiboea. The results revealed that, compared to the ITS sequences, the trnL-F sequences exhibited slightly higher CI and RI values (0.953 and 0.942 vs. 0.632 and 0.718), suggesting that the trnL-F sequences may possess higher consistency and information integrity when constructing the phylogenetic tree, thus representing higher reliability. Subsequently, reconstruction of the phylogenetic relationships revealed conflicts between the ITS and trnL-F trees, with low support values observed among some known species, indicating ambiguity in the phylogenetic relationships between species. This suggests that the efficacy of relying on ITS and trnL-F sequences to elucidate the phylogenetic relationships among species in Hemiboea is still insufficient, and more efficient molecular markers should be utilized to elucidate in the future. On the other hand, there is a need to increase the sampling of populations in *Hemiboea*, particularly by increasing the sampling density of *H. liana* (currently only one population identified) and its close relatives (e.g., *H. chanii*, *H. rubribracteata*, *H. sinovietnamica* and *H. shimentaiensis*). This will help determine the precise phylogenetic position of the unique new species, *H. liana*, and reveal the relationships among species within the entire *Hemiboea*.

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