Diversity, Geographic Distribution, and Species Boundaries of the *Hemiboea* Complex Postprint

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Abstract

Hemiboea subcapitata is a perennial herbaceous plant in the genus Hemiboea of the family Gesneriaceae. This species exhibits considerable morphological variation and previously included multiple varieties; it has a wide distribution range and occurs on various types of habitat substrates. This study encompasses typical Hemiboea subcapitata and its close relative H. pterocaulis, which was previously treated as its variety, as well as two other newly discovered variant types that are morphologically most similar to each of the former respectively. We described and compared the diversity of their key morphological and micromorphological characteristics; integrated herbarium specimen examination, literature review, and long-term field tracking surveys to conduct a statistical analysis of the geographic and habitat distributions among different types of Hemiboea subcapitata; additionally, based on DNA evidence, we preliminarily reconstructed the phylogenetic relationships and haplotype evolutionary relationships among the species and variant types. The results indicate: (1) The various types within the Hemiboea subcapitata complex can be distinguished through a combination of multiple traits including stems, leaves, flowers, and flowering phenology. (2) Among the four types of the Hemiboea subcapitata complex, there are no significant differences in leaf epidermal cell shape and stomatal distribution; however, differences exist in the presence or absence of epidermal hairs, the number of multiple epidermal layers, and the number and arrangement of vascular bundles, which may aid in classification. (3) Typical Hemiboea subcapitata is widely distributed from central to southern China, extending southward to northern Vietnam, and occurs on various habitat substrates including limestone and Danxia formations; H. pterocaulis is restricted to limestone substrates of karst mountains in a small area of Guilin City, Guangxi; while the other two variant types have even more restricted distributions, being found only on a single or several adjacent karst mountain limestone substrates in Yongfu County, Guilin City. (4) DNA haplotype network analysis reveals that each of the four types within the Hemiboea subcapitata complex possesses exclusive haplotypes; however, reconstructed molecular phylogenetic relationships indicate that the Jinzhongshan population clusters with H. pterocaulis as a single clade, with the two being most closely related but not reciprocally monophyletic. Whether the Jinzhongshan population constitutes an independent species requires further evidence for verification, whereas H. subcapitata and the Yongfu Hemiboea are reciprocally monophyletic, supporting their status as independent species.

Full Text

Preamble

Diversity, Geographical Distribution, and Species Boundaries of the *Hemiboea subcapitata* Complex

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Abstract

Hemiboea subcapitata is a group of perennial herbs in Gesneriaceae, which has great morphological variations and large distributional range, growing on various edaphic substrates in different habitats. In this study, focusing on *H. subcapitata*, *H. pterocaulis*, and two other related variations, we investigated and described the diversities of the morphology and micromorphology; outlined the geographic distributions and habitats by consulting specimens and literature, and long-term field surveys; in addition, the phylogenetic relationship and haplotype network among all types were inferred based on DNA data of the nuclear ITS sequences. The results are as follows: (1) Different variations of the *Hemiboea subcapitata* complex can be distinguished through the combination of multiple traits from stems, leaves, flowers and phenology. (2) The leaf cross-section anatomical micromorphologies are highly similar in terms of basic tissue compositions, epidermal cell shape and stomatal distribution, while there are differences in presence versus absence of the epidermal hair, number of the vascular bundles and arrangement, which are seemingly helpful for the classification. (3) Typical *H. subcapitata* is widely distributed in central and southern China and extends to northern Vietnam, growing on different types of rocks, e.g. limestone and sandstone in the limestone region and/or Danxia landform etc., and *H. pterocaulis* possesses a narrow range in Guilin, Guangxi and is endemic to limestone, while other two types are only distributed in one or a few adjacent limestone hills in Yongfu County, Guangxi and restricted to limestone substrates as well. (4) The haplotype network show that each type of *H. subcapitata* complex has its own unique and unshared haplotypes; the molecular phylogenetic relationships indicate that *Hemiboea* sp. and *H. pterocaulis* group together, but the monophyletic lineage consisting of all indivuduals of the former type (i.e. *Hemiboea* sp. from Jinzhongshan) is nested within the latter; *H. subcapitata* and *H. yongfuensis* are reciprocally monophyletic, supporting both of them as independent species. In conclusion, at least three indenpendent species can be indentified for the *H. subcapitata* complex. The sympatric distribution of all the four variations in South China, and even the coexistence of the three of them at the same sites in the same limestone hills do not necessarily mean sympatric speciation, though they likely diverged sympatrically via key trait change(s) such as phenological transition of differential flowering times that could have resulted in reproductive isolation.

Keywords: *Hemiboea subcapitata* complex, geographical distribution, morphology, phylogeny, species delimitation

Introduction

The genus *Hemiboea* C. B. Clarke (1888) belongs to the family Gesneriaceae. *Hemiboea subcapitata* C. B. Clarke primarily grows in rock crevices or in humus on the surfaces of limestone, conglomerate, granite, or sandstone rocks in valleys, hills, and mountains at elevations of 100–2,100 m in central and southern China and northern Vietnam (Wang et al., 1998; Wei et al., 2010). According to *Flora of China* (Wang et al., 1990) and *Plants of Gesneriaceae in China* (Li & Wang, 2005), *H. subcapitata* is a perennial herb with erect stems scattered with purple-brown spots and no branching. Its leaves are opposite, connate-perfoliate, slightly fleshy, entire or with shallow obtuse teeth above the middle, acute or acuminate at the apex, often unequal at the base, and petiolate; after connation, they become boat-shaped. Worm-shaped sclereids are scattered on the leaf surface, vascular bundles, and around the mesophyll. Cymes are axillary or pseudoterminal; the involucre is spherical with a mucronate apex, glabrous, and becomes boat-shaped after dehiscence. There are five free sepals; the corolla tube has sparse glandular pubescence externally and a hair ring above the base internally, with the upper lip shallowly two-lobed and the lower lip shallowly three-lobed, all lobes being semicircular. Anthers are connected at the apex, with three staminodes—one central and two lateral—with small capitate apices that are separate. The disc is annular; the ovary is linear, and the stigma is obtuse, slightly wider than the style. The capsule is linear-lanceolate. The flowering period is September–October, and the fruiting period is October–December.

This species exhibits extensive morphological variation and a wide distribution range, and its taxonomic classification at the species and infraspecific levels has undergone several revisions. In *Flora of China*, seven previously described species or varieties—including *H. henryi* Clarke (Clarke, 1888), *H. henryi* Clarke var. *major* Diels (Diels, 1900), *H. subcapitata* Clarke var. *intermedia* Pamp. (Pampanini & Silvestri, 1910), *H. marmorata* Lévl. (Léveillé, 1911), *Didymocarpus hwaianus* S. Y. Hu (Hu, 1980), *H. subcapitata* Clarke var. *denticulata* W. T. Wang ex Z. Y. Li (Li, 1983), and *H. subcapitata* Clarke var. *sordidopuberula* Z. Y. Li (Li, 1987)—were all synonymized under *H. subcapitata* (Wang et al., 1998).

Subsequently, Li (2004) discovered a population in Guilin, Guangxi that was morphologically similar to *H. subcapitata* but had winged stems and a completely different flowering period. This was described as a new variety, *H. subcapitata* var. *pterocaulis* Z. Y. Li. In later publications, *H. subcapitata* was considered to include only the typical variety (*H. subcapitata* var. *subcapitata*), the Guangdong variety (*H. subcapitata* var. *guangdongensis*), and the winged-stem variety (Li & Wang, 2005; Wei et al., 2010). Recently, based on morphological, phenological, and molecular phylogenetic evidence, the winged-stem variety was elevated to species status as *H. pterocaulis* (Z. Y. Li) J. Huang, X. G. Xiang & Q. Zhang (Huang et al., 2017). Additionally, phylogenetic studies have shown that the Guangdong variety is distantly related to *H. subcapitata* and *H. pterocaulis*, but is most closely related to *H. subacaulis* Hand.-Mazz., and is also recognized as an independent species (*H. guangdongensis* (Z. Y. Li) X. Q. Li & X. G. Xiang; Li et al., 2019).

During field investigations of the diversity of *H. subcapitata* and its relatives, we successively discovered two previously unreported new types that are morphologically similar to typical *H. subcapitata* and *H. pterocaulis*, respectively. Both new types were found in Jinzhongshan, Guilin, Guangxi, where they grow intermixed with typical *H. subcapitata* on the same karst hill limestone substrate. One type is distributed across several adjacent karst hills near Jinzhongshan and shows almost no difference in floral and fruit morphology or phenology from *H. pterocaulis*, but exhibits clear differences in stem shape, robustness, and plant size (tentatively referred to as the Jinzhongshan population, *Hemiboea* sp.; Figure 1 [Figure 1: see original paper]: D). The other type was found only on a single karst hill limestone substrate and is most similar to typical *H. subcapitata* in morphology but differs in stem morphology and corolla color (referred to as *Hemiboea yongfuensis*; Figure 1: B) (Huang, 2020).

In this study, we collectively refer to *H. subcapitata*, *H. pterocaulis*, the Jinzhongshan population, and *H. yongfuensis* as the *Hemiboea subcapitata* complex. Delimiting species boundaries among morphologically similar close relatives has long been a fundamental challenge in taxonomy. Karst habitats are complex and diverse, harboring rich plant diversity with numerous endemic species having narrow distributions (Hou et al., 2010; Wei et al., 2010; Yu et al., 2017). Recent phylogenetic studies of Gesneriaceae in southern China's karst regions have demonstrated that taxonomic units established based on morphology, particularly genera, are often non-monophyletic (Wang et al., 2011; Weber et al., 2011a, b). Complex morphological convergence, parallel evolution, and rapid morphological changes may be key factors contributing to unnatural traditional classifications (Lu et al., 2017). The repeated taxonomic revisions of *H. subcapitata* and its varieties also indicate that classification based solely on morphological characteristics is difficult (Huang et al., 2017; Li et al., 2019). Therefore, this study provides a detailed comparison of morphological and micromorphological diversity and geographic distribution within the *H. subcapitata* complex; reconstructs preliminary phylogenetic relationships and haplotype networks among its members using population sampling and nuclear ribosomal ITS sequences; and explores species boundaries based on these data.

Materials and Methods

1.1 Materials

We conducted field investigations in *H. subcapitata* distribution areas including Zhejiang, Guizhou, Hunan, Hubei, and Guangxi provinces, and transplanted 50, 30, 25, and 25 individuals of typical *H. subcapitata*, *H. yongfuensis*, *H. pterocaulis*, and the Jinzhongshan population, respectively, to the nursery of Guangxi Institute of Botany, Chinese Academy of Sciences, for phenological tracking. We compiled data from 611 herbarium specimens of *H. subcapitata* available in the Chinese Virtual Herbarium (CVH; http://www.cvh.ac.cn/). Geographic names were converted to coordinates using XGeocoding V2 (http://www.gpsspg.com/xgeocoding/), and distribution maps of the *H. subcapitata* complex were generated using ArcGis 10.2 (https://www.arcgis.com/features/index.html, ESRI, Inc., Redlands, CA, USA).

1.2 Morphological Measurements and Statistical Methods

We used ImageJ (http://imagej.nih.gov/ij/index.html) to measure stem width and leaf length and width from four plant types collected from different locations (*H. subcapitata*: 49 individuals; *H. yongfuensis*: 39 individuals; *H. pterocaulis*: 36 individuals; Jinzhongshan population: 38 individuals) to determine the range and mean values of these quantitative traits. Corresponding boxplots were generated using Graphpad Prism (https://www.graphpad.com/scientific-software/prism/).

1.3 Micromorphological Observations

1.3.1 Materials Mature leaves from six individuals each of *H. subcapitata* and *H. yongfuensis*, five individuals of *H. pterocaulis*, and six individuals of the Jinzhongshan population were selected for micromorphological measurements.

1.3.2 Leaf Cross-Section Processing Leaves were cross-sectioned along the midrib into 5 mm × 5 mm pieces, stained with 1% safranin solution, mounted on slides, and observed and photographed under a light microscope.

1.3.3 Leaf Epidermis Processing Intact mature leaves were cleaned and cut into approximately 1.5 cm × 1.5 cm pieces along with the midrib (to facilitate identification of adaxial and abaxial surfaces). After adding appropriate KOH, the material was boiled for several minutes (timing varied by material), then immersed in cold water for several minutes. The cold water was discarded, and the material was soaked in 30% NaClO solution until the leaves turned white. After rinsing with distilled water, the upper and lower epidermis were peeled off, mesophyll tissue was removed with a brush, stained with 1% safranin solution, and mounted for observation and photography under a light microscope.

1.4 Phylogenetic Reconstruction and Haplotype Network Construction Based on ITS Sequences

We collected and silica-dried leaf samples from different locations: nine samples of *H. subcapitata*, five of *H. yongfuensis*, five of *H. pterocaulis*, and five of the Jinzhongshan population (see Table 1 for detailed material sources). Total DNA was extracted using the CTAB method (Doyle & Doyle, 1987). Primer selection and PCR amplification for ITS followed the experimental protocol of Huang et al. (2017). Raw sequences obtained in this study were edited and assembled using SEQMAN (Swindell & Plasterer, 1997), yielding a total of 24 ITS sequences. These newly obtained sequences were added to the most recently published ITS matrix for *Hemiboea* (Li et al., 2019). The final matrix included sequences from 22 known species of *Hemiboea* plus the two new types, totaling 57 sequences. *Petrocosmea minor*, *Lysionotus microphyllus*, and *Anna submontana* were selected as outgroups (Li et al., 2019).

Alignment was performed using MUSCLE 3.8.31 (Edgar, 2004) and manually checked and adjusted in BioEdit (Hall, 1999). The optimal model GTR+G was calculated and selected using jModelTest2.1.7 (Posada, 2008). A maximum likelihood phylogenetic tree was constructed using RAxML7.2.6 (Stamatakis, 2006) with 1,000 bootstrap replicates to assess node reliability.

For haplotype analysis, we selected the ITS matrix of the *H. subcapitata* complex (18 sequences of *H. subcapitata*, 5 of *H. yongfuensis*, 6 of *H. pterocaulis*, and 5 of the Jinzhongshan population) totaling 34 sequences. Haplotypes were analyzed using DnaSP v5 (Librado & Rozas, 2009), and haplotype diversity and nucleotide diversity were calculated for the complex. The haplotype network was constructed using Popart (Leigh & David, 2015).

Results

2.1 Morphological Differences in the *Hemiboea subcapitata* Complex

The species and variation types within the *H. subcapitata* complex differ primarily in stem cross-section shape and presence/absence of wings, involucre color, calyx color and insertion pattern, and corolla outer surface color (Figure 1 [Figure 1: see original paper]). *Hemiboea subcapitata* has stems with few spots, no wings, and a circular cross-section; leaves are connate-perfoliate or not; involucre is green; white sepals are divided to the base; and the corolla outer surface is white. *Hemiboea yongfuensis* has stems densely spotted with purple, no wings, and a quadrangular cross-section; petiole base is non-amplexicaul; involucre is brown; pink sepals are divided to the base; and the corolla outer surface is pink. *Hemiboea pterocaulis* has winged stems with a polygonous cross-section; petiole base is non-amplexicaul; involucre is green; light green sepals are divided to the middle (connate at base); and the corolla outer surface is white. The Jinzhongshan population has slender stems with slight wings and a polygonous cross-section; petiole base is non-amplexicaul; involucre is green with scattered purple spots; light green sepals are divided to the middle (connate at base); and the corolla outer surface is white with purple spots. More detailed morphological comparisons are provided in Table 2 .

We also found that flowering and fruiting periods differ among species and variation types within the complex: *H. subcapitata* flowers in September–October and fruits in October–December; *H. yongfuensis* has slightly later flowering and fruiting periods, flowering in October–November and fruiting from December to January of the following year; *H. pterocaulis* and the Jinzhongshan population share the same flowering and fruiting periods, flowering in April–May and fruiting in June–July, which are completely different from the former two.

2.2 Quantitative Statistical Analysis of Stem and Leaf Morphology

Observations of stem and leaf morphology revealed differences among species and variation types in the *H. subcapitata* complex (Figure 2 [Figure 2: see original paper]). Stem width across the complex generally ranges from 2–12 mm. *Hemiboea pterocaulis* shows the greatest variation in stem width with the highest mean value (10 mm), followed by *H. subcapitata* (mean 5 mm). *Hemiboea yongfuensis* shows less variation than the former two but slightly more than the Jinzhongshan population (mean approximately 3 mm) (Figure 2:). Leaf length and width vary considerably within species and variation types, with significant overlap among them. Mean leaf length and width show a decreasing trend from *H. subcapitata* to *H. yongfuensis*, *H. pterocaulis*, and the Jinzhongshan population (Figure 2: ,), while the leaf length/width ratio shows the opposite trend (Figure 2:).

Most individuals of the Jinzhongshan population differ noticeably from the other three types in stem width, leaf length, and leaf width. However, the other types show large variation ranges that overlap among types, making these traits unsuitable for distinguishing them or for use as taxonomic criteria.

2.3 Micromorphological Observations of the *Hemiboea subcapitata* Complex

Cross-sectional anatomy of leaves in the *H. subcapitata* complex revealed that all leaves consist of three parts: epidermis, mesophyll cells, and veins (Figure

3 [Figure 3: see original paper]). Species and types differ in the number and arrangement of vascular bundles: *H. subcapitata* has multiple vascular bundles scattered in a triangular arrangement (Figure 3: A); *H. yongfuensis* has multiple vascular bundles tightly arranged in a triangle (Figure 3: B); *H. pterocaulis* has multiple vascular bundles tightly arranged in a near-circular pattern (Figure 3: C); and the Jinzhongshan population has multiple vascular bundles tightly arranged in an irregular shape (Figure 3: D).

The leaf epidermal micromorphology is generally consistent across the complex. Upper epidermal cells are polygonal with straight anticlinal walls and lack stomata, while lower epidermal cells are polygonal with straight anticlinal walls and have anomocytic stomata (Figure 4 [Figure 4: see original paper]). Some differences exist among types: *H. yongfuensis* has "joint"-shaped unicellular hairs on the upper epidermis composed of 3–5 stacked cells (Figure 4: B1); *H. subcapitata* has a few multicellular hairs on the upper epidermis (Figure 4: A1); and *H. pterocaulis* and the Jinzhongshan population have smooth surfaces without hair-like structures (C1, D1).

2.4 Geographic Distribution of the *Hemiboea subcapitata* Complex

Based on literature, herbarium records from CVH, and our long-term field surveys, we mapped the distribution of the *H. subcapitata* complex (Figure 5 [Figure 5: see original paper]). Typical *H. subcapitata* is mainly distributed across 16 provinces and municipalities in central and southern China, extending to northern Vietnam, growing in crevices or on humus surfaces of various rock types including granite, sandstone, and limestone in karst and Danxia landforms. *Hemiboea pterocaulis* is currently known only from karst limestone hills in the urban area of Guilin and Yangshuo County, Guangxi. Both *H. yongfuensis* and the Jinzhongshan population have even more restricted distributions, being found only on one or a few adjacent karst limestone hills in Yongfu County, Guilin.

2.5 Phylogenetic Relationships and Haplotype Evolution in the *Hemiboea subcapitata* Complex

The phylogenetic tree reconstructed from ITS sequences indicates that *Hemiboea* is monophyletic (BS = 100%). The *H. subcapitata* complex, *H. latisepala* H.W. Li, and a clade comprising *H. guangdongensis*, *H. subcaulis*, and *H. strigosa* Chun ex W.T. Wang (BS = 97%) are the most closely related groups (BS = 98%), forming a polytomy of six branches. The *H. subcapitata* complex includes four clades: all individuals of the Jinzhongshan population form a weakly supported monophyletic group (BS = 56%) nested within *H. pterocaulis*, with the two together forming a clade (BS = 99%); all *H. subcapitata* individuals except one form a clade (BS = 84%); and all *H. yongfuensis* individuals form an independent monophyletic clade (BS = 94%).

Haplotype analysis identified 18 haplotypes in the *H. subcapitata* complex (12 in

H. subcapitata, 3 in *H. yongfuensis*, 2 in *H. pterocaulis*, and 1 in the Jinzhongshan population), with each type possessing unique, unshared haplotypes. Haplotype diversity was 0.9376 and nucleotide diversity was 0.02206. Except for a few haplotypes connected by a small number of mutational steps, most haplotypes are connected through numerous "hidden haplotypes" (undetected or extinct ancient haplotypes). These findings indicate that extant haplotypes have a relatively long evolutionary history, with no or limited gene flow among types for a considerable period.

Discussion

Delimiting species boundaries in species complexes is a universal challenge in biodiversity research across major taxonomic groups. Some closely related species or groups may show little morphological variation due to short independent evolutionary histories, while others that have evolved independently for longer periods may be morphologically indistinguishable due to morphostasis or homoplasy, forming cryptic species. Some cryptic species can be identified through DNA evidence or combined analysis of DNA and ecological niche differentiation (Liu et al., 2013; Zhang & Li, 2014). In insect taxonomy, for example, cryptic species are frequently discovered in American butterflies (Hebert et al., 2004), and numerous cryptic species have also been reported in plants (Lu et al., 2010; Yu et al., 2018). In this study, although *H. yongfuensis* grows intermixed with its morphologically most similar relative *H. subcapitata* (which may also be its closest phylogenetic relative) and another closely related new type, the Jinzhongshan population, on the same hill, it does not share any haplotypes with them, indicating natural genetic isolation. *Hemiboea yongfuensis* has produced and fixed its own unique haplotypes, suggesting a relatively long period of independent evolution. Therefore, despite high morphological similarity to *H. subcapitata* with only slight differences in stem shape, flower, calyx, and leaf color, DNA evidence shows that *H. yongfuensis* forms a monophyletic group phylogenetically, meeting the criteria for phylogenetic species recognition (Taylor et al., 2000), and should be recognized as an independent species. The newly discovered Jinzhongshan population is morphologically and phenologically most similar to *H. pterocaulis*, and DNA evidence also indicates they are the most closely related, with the Jinzhongshan population forming a monophyletic group. However, this monophyletic group is nested within *H. pterocaulis*, so its status as an independent species is not supported phylogenetically. Nevertheless, previous studies have noted that incomplete lineage sorting and introgression readily occur among closely related species and can cause them to not be reciprocally monophyletic in gene trees (Seehausen, 2004). Therefore, whether the Jinzhongshan population represents an independent species requires more evidence for accurate determination, such as species tree reconstruction or species delimitation analyses based on multi-locus or genomic data from multiple populations and individuals, as well as hybridization compatibility tests among types.

The processes and mechanisms of species origin and formation are often complex and diverse, which may be key reasons for the difficulty in species classification. Allopatric speciation is considered the primary mode in plants. With deeper research, parapatric and sympatric speciation have been increasingly discovered and reported, with mechanisms such as ecological niche diversification, natural selection, polyploidization, and mutations in key speciation genes being progressively revealed (Rieseberg & Willis, 2007; Rundle & Nosil, 2005; Ting et al., 2000). In the *H. subcapitata* complex, both *H. yongfuensis* and *H. pterocaulis* (along with the Jinzhongshan population) have narrow distributions contained within the distribution range of typical *H. subcapitata*. We propose two possible scenarios for the speciation and current geographic distribution patterns of this complex: (1) *Hemiboea yongfuensis* and *H. pterocaulis* diverged locally from a widespread ancestral species, or even directly from ancestral populations of *H. subcapitata* within its broad distribution (i.e., sympatric speciation); or (2) the three taxa diverged through vicariance from populations in different regions, with the current overlapping distribution resulting from range expansion of typical *H. subcapitata* or range shifts of the other two species. Phylogeographic studies with population-level sampling will help test and distinguish between these scenarios. If *H. yongfuensis* and/or *H. pterocaulis* originated sympatrically from a subset of *H. subcapitata* populations, or if widespread *H. subcapitata* inherited most ancestral genetic diversity, then phylogenetic relationships based on broader sampling and more gene loci would show typical *H. subcapitata* as paraphyletic relative to *H. pterocaulis* or *H. yongfuensis*. This pattern has been reported in other groups (Luebert et al., 2014). Conversely, if further molecular phylogenetic analyses show the three taxa as monophyletic groups, with sympatric *H. subcapitata* derived from other regions (nested within groups from other areas), this would support allopatric speciation. Differences in flowering periods among typical *H. subcapitata*, *H. yongfuensis*, and *H. pterocaulis* (non-overlapping or only slightly overlapping) prevent or limit gene flow among them, providing the possibility for sympatric speciation, though whether phenological shifts are the cause or consequence of speciation requires further investigation.

Conclusion

This preliminary study of the *Hemiboea subcapitata* complex, integrating morphology, micromorphology, geographic distribution, and molecular phylogeny, demonstrates high morphological and genetic diversity within the complex. The complex can be divided into at least three independent species: *H. subcapitata*, *H. pterocaulis*, and *H. yongfuensis*, while the status of the Jinzhongshan population as an independent species or conspecific with *H. pterocaulis* requires more substantial evidence. Single-type evidence is insufficient for correctly delimiting species boundaries among morphologically similar species complexes. The sympatric distribution of complex members, particularly intermixed populations (such as *H. subcapitata* and *H. yongfuensis* in this study), provides an excellent opportunity to use DNA evidence to assess gene flow or genetic isolation among different morphological variants, thereby facilitating natural species delimitation.

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