

Hemsleya kunmingensis (Cucurbitaceae), a new species from China

Hong-Tao Li^{1,2} Jun-Bo Yang¹ & De-Zhu Li^{1,*}

¹) Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming, Yunnan, 650204, China (corresponding author's e-mail: dzl@mail.kib.ac.cn)

²) The Graduate School of Chinese Academy of Sciences, Beijing, 100049, China

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Hemsleya kunmingensis H.T. Li & D.Z. Li *sp. nova* (Cucurbitaceae) is described and illustrated from the Yunnan Province, China. It appears to have been rarely collected and the previous collections examined have remained undetermined to species level or were misidentified due to the absence of flowers and fruits. The most prominent features of the species are the very small, yellowish green, laxly patelliform corolla with evaginate petals that revolute into an anchor shape at the apex, the well-developed dichasium, the obconic fruit, and the lenticulated seed without seed-wing. Its relationship to closely similar species is discussed in the light of morphological and molecular data.

Key words: Cucurbitaceae, *Hemsleya*, new species, taxonomy

Introduction

Hemsleya belongs in the Cucurbitaceae and comprises ca. 24 species with their principal distribution from southern to southwest China. One species is found only in Burma, Thailand and Nepal. Yunnan and Sichuan provinces of China are the centres of diversity, with more than 90 percent of the species (Lu 1982, Chang & Shen 1983, Wu & Chen 1985, 1986, Li 1993). Most species inhabit moist and shady under-storey environments in subtropical forests. The genus is characterized by dioecy, with male flowers possessing five stamens and female flowers three stigmas bifurcating at the apex, five closely imbricate petals, a seriate dichasium, and a 9–10-ribbed capsule. The plants are herbaceous vines, with a rhizoma-

tous stem-tuber, alternate leaves, the palmately compound leaves comprising 3–11 leaflets.

Starting in 1988, the Kunming Institute of Botany conducted a series of studies on *Hemsleya*, which resulted in a monograph (Li 1993). The ongoing research revealed that the genus may harbour more species than hitherto assumed. A number of collections in southwest China produced *Hemsleya* specimens with novel characters in the stem-tuber, fruit shape and seed shape, seed-wing, and corolla shape and color. Other features distinctive from the established species included the leaflet number, shape, and serration. Such specimens were observed and collected in the middle of Yunnan province. After detailed literature and herbarium studies, it was decided to describe a new species in *Hemsleya*. In the

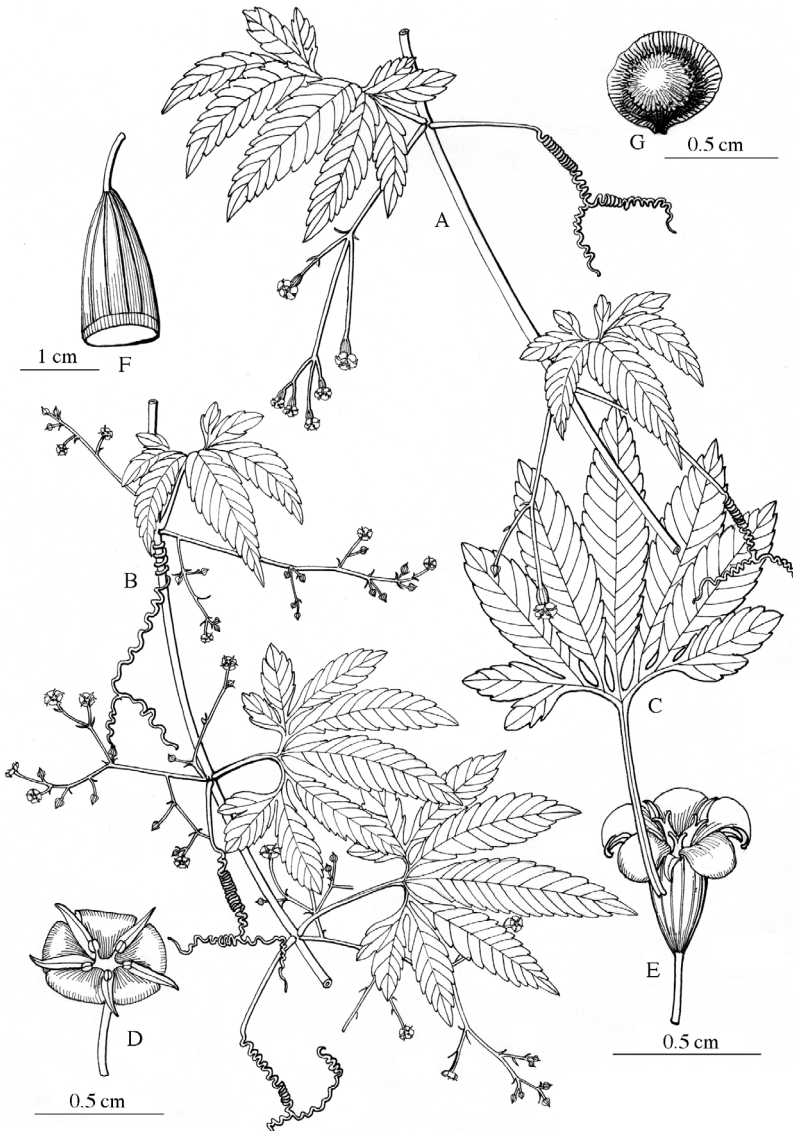


Fig. 1. *Hemsleya kunmingensis*. — **A:** Female habit. — **B:** Male habit. — **C:** Leaf. — **D:** Male flower. — **E:** Female flower. — **F:** Fruit. — **G:** Seed. Drawn by Ling Wang.

following two years, further research was carried out including morphological and molecular data analyses. Meanwhile, a new location (Songming, Guodong, *H. T. Li 045*) with numerous specimens was discovered on another mountain about 20 km from the first location.

Hemsleya kunmingensis H.T. Li & D.Z. Li, *sp. nova* (Fig. 1)

Species H. amabili affinis, sed differt petalis flavor-viridulis, reflexis, corollis patelliformibus;

seminibus lenticulariibus, alis destitutibus, marginibus angustissimis, infra 0.1 cm longis, fructibus obconibus, majoribus.

HOLOTYPE: China. Yunnan, Kunming, Xishan Mountain, under secondary forest, *H. T. Li 043* (KUN). — **PARATYPES:** China. Same locality as for holotype, *H. T. Li 044* (KUN); Yunnan, Songming, Guodong, *H. T. Li 045* (KUN).

Herbaceous perennial vine up to 5 m long, growing amplexant. Stems with two types, ovate rhizomatous stem-tuber and slim 4-angled stems with sulcate, perennial stem-tuber weighing several kilograms, slim 4-angled stems moderately

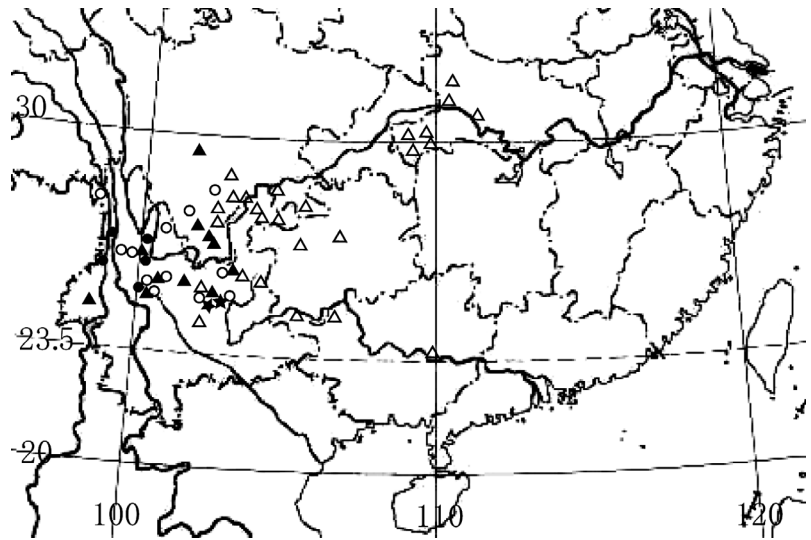


Fig. 2. Distribution map of *Hemsleya kunmingensis* (★), *H. chinensis* (△), *H. amabilis* (○), *H. delavayi* (▲), and *H. lijiangensis* (●).

to sparsely tomentose when young but smooth when older. Tendrils axillary, smooth, 5–25 cm long, bifurcating at the apex, winded. Palmately compound leaves alternate, comprising 7–9 leaflets, slightly fleshy, green above and dark-green below, slightly lustrous above, adpressed, sparsely pubescent on both sides, petiole 2–4 cm; leaflet lanceolate to oblong, denticulate at margin, middle leaflet 6–12 × 1–1.5 cm, petiolule 0.2–0.4 cm. Inflorescence dioecious, seriate dichasium, axillary, 5–30 × 2–5 cm, 10–30-flowered; pedicels 0.5–1 cm; sepals 5, 0.3–0.5 cm × 0.2–0.3 cm, widely triangular at base, shortly cuspidate, revolute at apex, greenish, entire; petals 5, 0.3–0.5 × 0.3–0.5 cm, evaginate and revolute into an anchor shape at apex, yellowish green, elliptic. Corolla laxly patelliform, herbaceous, membranous, glabrous, 0.3–0.5 cm in diameter. Stamens 5, filaments 0.1–0.2 cm, anthers whitish, 0.1 cm in diameter. Styles 3, 0.1–0.2 cm long, stigma bifurcating at apex. Fruits many, obconic, greenish, longitudinally 10-striate, almost smooth, 2–4 × 1.2–1.5 cm, fruit stalks 1–1.5 cm long. Seeds many, seed-wing absent, lenticulated, 0.5–0.8 × 0.2–0.3 cm, blackish brown, with a verrucose surface. Flowering June to September, fruits mature August to November.

DISTRIBUTION AND HABITAT. *Hemsleya kunmingensis* primarily occurs on communal land and is known only from two populations in an area of about 10 km × 20 km in the middle of Yunnan

Province (Fig. 2). It was first found in the Xishan Mountains (*H. T. Li 043, 044*), Kunming city district, in the middle of Yunnan Province, growing in a damp valley under secondary forest at an altitude of ca. 2280 m.

Molecular studies

Plant samples

For this study, *H. kunmingensis* and the morphologically similar *H. chinensis*, *H. amabilis*, *H. delavayi* and *H. lijiangensis* were sampled.

DNA extraction, amplification, and sequencing

For each sample, total genomic DNA was isolated from 0.2 g silica-gel-dried or 0.4 g fresh leaves using the modified CTAB method (Doyle & Doyle 1987), with 4% CTAB instead of 2%. Leaf tissue was grounded in liquid nitrogen before using CTAB.

The *trnH-psbA* region was amplified as described by Shaw *et al.* (2005) with primers *trnH* (5'-CGC GCA TGG TGG ATT CAC AAT CC-3') (Tate & Simpson 2003) and *psbA* (5'-GTT ATG CAT GAA CGT AAT GCT C-3') (Sang *et al.* 1997). The ITS region includes the

ITS1, 5.8S, and ITS2 nuclear rDNA regions. It was amplified with primers ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS-5 (5'-GGA AGG AGAAGT CGT AAC AAG G-3') (White et al. 1990). The *rpl16* intron region was amplified as described by Jordan et al. (1996) with primers *rpl16*-F71 (5'-GCT ATG CTT AGT GTG TGA CTC GTT G-3') and *rpl16*-R1516 (5'-CCC TTC ATT CTT CCT CTA TGT TG-3'). The *trnS-trnR* region was amplified with primers *trnS*-F (5'-CGC CGC TTT AGT CCA CTC A-3') (Doyle et al. 1992) and *trnR*-R (5'-ATT GCG TCC AAT AGG ATT TGA A-3') (Dumolin-Lapegue et al. 1997). The thermal cycler (PE9600 or PE9700) for *trnS-trnR* was programmed for an initial step of 4 min at 94 °C, followed by 36 cycles of 60 s at 94 °C, 90 s at 50 °C, 90 s at 72 °C, and a final extension of 7 min at 72 °C. The PCR products were visualized by agarose gel electrophoresis, cleaned with Watson's Purification Kit, and both strands were sequenced using the same primer combination as for PCR amplifications.

Cycle sequencing was carried on PE9600 or PE9700 thermal cyclers. Each 5 µl reaction contained 1 µl of purified PCR product, 1 µl of 0.2 µM of primer, 1 µl Big Dye and 2 µl ddH₂O and was run through a program of 33 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Reactions were cleaned using CleanSEQ (Perkin-Elmer, Norfolk, CT) and run on an ABI PRISM 3700 DNA capillary automated sequence analyzer.

Phylogenetic analyses

The entire ITS region, including both spacers and the 5.8S gene, was 631 bp for all taxa. The *trnH-psbA* spacer region was about 500 bp long, the *rpl16* intron region approximately 1050 bp and the *trnS-trnR* region about 1600 bp.

Clustal X (Thompson et al. 1997) was used to produce an alignment, which was corrected manually using the Bioedit program (Hall 1999). The indels were coded using GapCoder (Simmons & Ochoterena 2000) in the data matrix. Phylogenetic reconstruction was carried out using the whole alignment. MP analyses were conducted using PAUP 4.0b10 (Swofford 2001).

Discussion

Hemsleya kunmingensis is morphologically similar to *H. amabilis* and *H. lijiangensis*. It has often been misidentified as *H. amabilis* in the vegetative stage. Similarly, mature specimens without flowers and fruits have most commonly been determined as *H. amabilis*. However, *H. kunmingensis* is clearly distinguishable from *H. amabilis*, differing considerably in many morphological features, especially in generative organs (Table 1). The two species have a very different fruit shape and corolla pattern and size. To some extent, *H. kunmingensis* is also similar to *H. delavayi*. Comparing *H. kunmingensis* with *H. delavayi*, however, reveals differences in seed morphology, texture of the petals and petiole length. *Hemsleya lijiangensis* is, except for the fruit morphology, distinct in most morphological features from *H. kunmingensis*. *Hemsleya kunmingensis* may be confused with *H. chinensis* too; however, they can be easily differentiated by characters of the flower, fruit and seed. The main differences in morphological features of *H. kunmingensis* and the other species are summarized in Table 1 and Figs. 3 and 4.

When identifying herbarium specimens, *H. kunmingensis* may be confused *H. amabilis*, *H. chinensis*, *H. lijiangensis* or *H. delavayi*. In terms of the fruit shape, corolla pattern, color and texture, seed morphology and stem-tuber features, the new species fits best into *H. amabilis* and *H. lijiangensis*.

Consequently, the combination of morphological and molecular data analyses suggest that *H. kunmingensis* belongs to subsection *Amabilis*. It currently has three species: *H. amabilis*, *H. lijiangensis* and *H. heterosperma*. The first two are common in southwest China and hundreds of specimens of them have been collected in the country. All types and many representative specimens were examined in various herbaria (KUN, IBSC) by us. *Hemsleya heterosperma* is distributed in Burma, Thailand and Nepal, but not in China. Although there are some morphological similarities between *H. kunmingensis* and the three other taxa, their flowers, fruits, seeds and some other features are quite different.

There are three basic corolla patterns in

Hemsleya: radiate corollas with patulous petals, bowl-like corollas with patulous petals, and spherical, umbraculiform or patelliform corollas with revolute petals. Among all spherical, umbraculiform and patelliform corollas, the petals are densely imbricate, lustrous and carnifate. On the other hand, corollas comprising herbaceous, membranous petals are usually radiate or bowl-like. Consequently, the combination of herbaceous, membranous and revolute petals with a laxly patelliform corolla as observed in *H. kunmingensis* is new for the genus. Additionally, the corolla of *H. kunmingensis* is the smallest hitherto known in *Hemsleya*.

Sequence data (Table 2) from ITS, *rpl16*, *trnH-psbA* and *trnS-trnR* show that *H. kunmingensis* differs significantly from the related species. There are seven indels ranging from 1 to 16bp and five site substitutions on *trnS-trnR*, ten site substitutions on ITS, seven site substitutions and two indels on *trnH-psbA*, and six indels and six site substitutions on *rpl16* in *H. kunmingensis*. A phylogenetic analysis of molecular sequences, using *Cucumis sativus* from GenBank (AY833602, DQ119058) as outgroup (Table 2), also reveals that *H. kunmingensis* is closely related to *H. amabilis* (Fig. 5).

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Table 1. Comparison of main morphological characters of *Hemsleya kunmingensis* with *H. chinensis*, *H. amabilis*, *H. delavayi*, *H. lijiangensis* and *H. heterosperma*.

	<i>H. kunmingensis</i>	<i>H. amabilis</i>	<i>H. chinensis</i>	<i>H. delavayi</i>	<i>H. lijiangensis</i>	<i>H. heterosperma</i>
Leaves	7–9 leaflets	5–9 leaflets	5–9 leaflets	5–7 leaflets	5–7 leaflets	3–5 leaflets
Middle leaflet	6–12 × 1–1.5 cm	4–5 × 1–1.5 cm	5–12 × 2–2.5 cm	3–10 × 0.8–1.5 cm	6–12 × 2–2.2 cm	5–7 × 1.5 cm
Petiole length	2–4 cm	2–4 cm	4–8 cm	0.2–0.5 cm	2–4 cm	3–4 cm
Petal	herbaceous, membranous, revolute	herbaceous, membranous, patulous	carnifate, revolute	carnifate, revolute	carnifate, patulous	herbaceous, membranous, patulous
Corolla pattern	laxly patelliform, 0.3–0.5 cm in diameter	radiate, imbricate, 1–1.2 cm in diameter	spherical, densely imbricate, 1.2–1.5 cm in diameter	patelliform, densely imbricate, 0.5–0.7 cm in diameter	radiate, imbricate, 0.8–1 cm in diameter	radiate, imbricate, 0.3–0.4 cm in diameter
Fruit shape	obconic	spherical	elliptical	obconic	obconic	obconic
Seed shape	lenticulate	ovate	lenticulate	long elliptic	irregular rotund	irregular ovate
Seed-wing	absent	absent	absent	ligneous	absent	absent

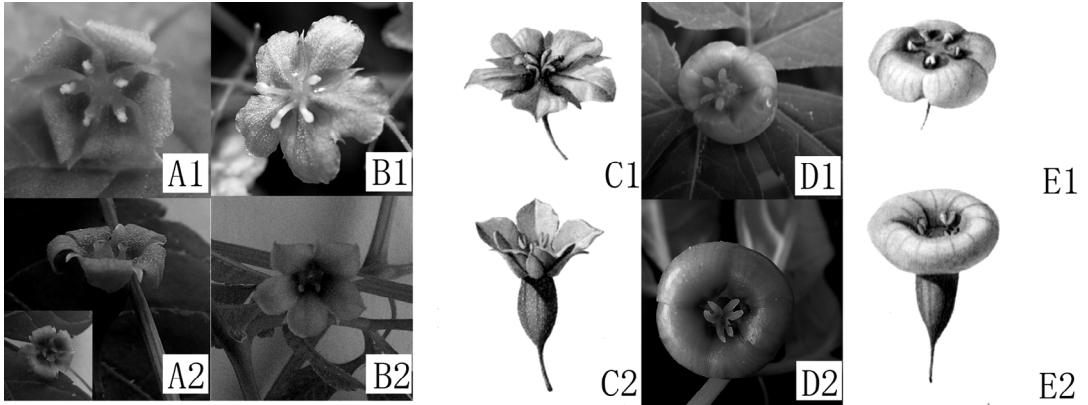


Fig. 3. Flower morphology of *Hemsleya kunmingensis* and its related species (1 represents male; 2 represents female). — **A:** *H. kunmingensis*. — **B:** *H. amabilis*. — **C:** *H. lijiangensis* (Li 1993: fig. 30). — **D:** *H. chinensis*. — **E:** *H. delavayi* (Li 1993: fig. 29).

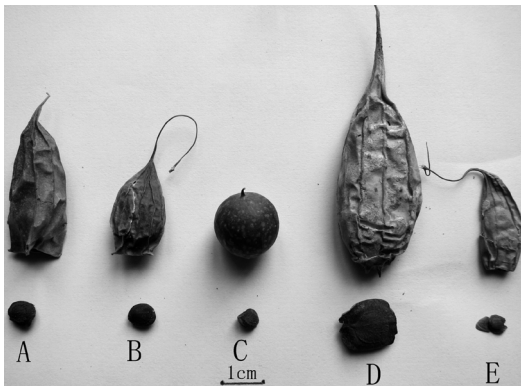


Fig. 4. Fruits and seed morphology of *Hemsleya kunmingensis* and its related species. — **A:** *H. kunmingensis*. — **B:** *H. lijiangensis*. — **C:** *H. amabilis*. — **D:** *H. chinensis*. — **E:** *H. delavayi*.

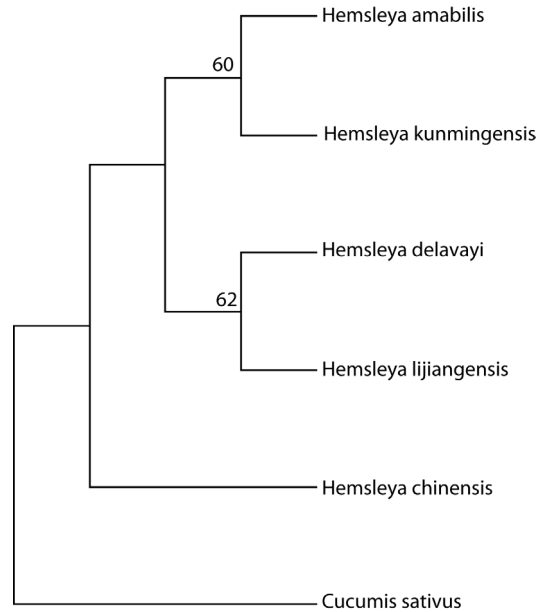


Fig. 5. Strict consensus bootstrapped tree (CI = 0.978, RI = 0.444, RC = 0.435) of 2 most parsimonious trees founded by ITS, *rp16*, *trnH-psbA* and *trnS-trnR* combination analysis.

Table 2. Sources of plant material and GeneBank accession numbers.

Taxon	ITS	<i>rp16</i>	<i>trnH-psbA</i>	<i>trnS-trnR</i>	Locality information	Voucher	Herbarium
<i>H. kunmingensis</i>	EF424062	EF424076	EF424067	EF424079	Kunming, Yunnan	<i>H. T. Li 043</i>	KUN
<i>H. amabilis</i>	EF424066	EF424074	EF424071	EF424077	Guodong, Yunnan	<i>H. T. Li 061</i>	KUN
<i>H. lijiangensis</i>	EF424065	EF424075	EF424070	EF424078	Lijiang, Yunnan	<i>H. T. Li 047</i>	KUN
<i>H. delavayi</i>	EF424063	EF424072	EF424068	EF424080	Songming, Yunnan	<i>H. T. Li 048</i>	KUN
<i>H. chinensis</i>	EF424064	EF424073	EF424069	EF424081	Omei, Sichuan	<i>H. T. Li 021</i>	KUN

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