



# *Hoya longicalyx*, a new species of *Hoya* (Apocynaceae: Asclepiadoideae) from Yunnan, China

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(Manuscript received 8 January 2020; Accepted 24 June 2020; Online published 7 July 2020)

**ABSTRACT:** *Hoya longicalyx* Wang Hui & E. F. Huang, a new species of *Hoya* R. Br. (Apocynaceae: *Asclepiadoideae*) from Yingjiang county (Yunnan, China), is described and fully illustrated. The new species is morphologically similar to *H. chinghungensis* (Tsiang & P.T. Li) M.G. Gilbert, P.T. Li & W.D. Stevens, but can be easily distinguished by its rounded lamina base, longer and linear calyx lobes, ovate and translucent corona lobes. Result from molecular phylogenetic analysis revealed that the new species was sister to *Hoya engleriana* Hosseus.

**KEY WORDS:** *Hoya*, new taxon, Phylogenic Analysis, ITS, 5'-ETS, *psbA-trnH*, *trnT-trnL*, *matK*.

## INTRODUCTION

*Hoya* Brown is one of the most species-rich genera in the tribe *Marsdenieae* (subfamily *Asclepiadoideae*, Apocynaceae), consisting of more than 300 species (Kleijn and van Donkelaar, 2001; Rodda *et al.*, 2011, 2013; Wanntorp, 2014; Rodda and Ercole, 2014; Rodda and Omlor, 2014) widely distributed in the tropical and subtropical regions of Asia, Oceania and Pacific island (Li *et al.*, 1995; Forster and Liddle, 1991, 1996; Rodda and Simonsson, 2011). In China, *Hoya* species are mainly distributed in southwest and southeast coastal area, and about 32 species have been recorded according to the Flora of China (Li *et al.*, 1995), but this number is still increasing in recent publications (He *et al.*, 2009a, 2009b, 2011, 2012; Rodda *et al.*, 2019; Zhang *et al.*, 2015, 2019).

During the fieldwork in Yunnan province, southwest China, in May 2019, one of the authors (E. F. Huang) collected a specimen of *Hoya*. The plant grows on arboreal branch covered by moss in mid-montane evergreen forest. Its leaves are small and fleshy, and its calyx lobes are long and linear. Morphologically, the species superficially differs from any congeneric taxa recorded in China and its adjacent countries. After a series of detailed morphological and molecular phylogenetic studies, we confirmed that the species is new to science, and thus it is formally described here.

## MATERIALS AND METHODS

**Phylogenetic analysis:** To study the phylogenetic position of the new species within *Hoya*, we performed

a phylogenetic analysis of the genus based on combined DNA fragments of the nuclear ribosomal intergeneric transcribed spacer (ITS), external transcribed spacer (5'-ETS), and three chloroplast fragments (*matK*, *psbA-trnH* and *trnT-trnL*). PCR amplification and sequencing was carried out using the primer pairs ITS1 and ITS4 for the ITS spacer (White, 1990), AsETS-F and AsETS-R for 5'-ETS (Yamashiro, 2004), *matK390F* and *matK1326R* for *matK* (Cuénoud *et al.*, 2002), *psbA3't/ trnHf* (Sang *et al.*, 1997; Tate and Simpson, 2003) for *psbA-trnH*, and *trnT<sup>UGU</sup>(a)* and *trnL<sup>UAA</sup>(b)* for *trnT-trnL* (Taberlet, 1991). We extracted total genomic DNA from silica gel-dried leaves (Chase and Hills, 1991) using a Plant Genomic DNA Kit (Biomed Shenzhen China). The standard polymerase chain reaction was used to amplify target regions and carried out polymerase chain reaction in a 50µL volume containing 2 µL DNA, 25µL 2× EasyTaq PCR SuperMix, 1µL each Primer, and 21µL ddH<sub>2</sub>O. The cycling program for all primers consisted of initial denaturation 2 min at 95 °C followed by 35 cycles of amplification at 95 °C for 30 s, 48–55 °C for 30–120 s, and 72 °C for 1 min, and ended by a final extension at 72 °C for 5 min. We carried out the sequencing reactions using an ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Shenzhen China). Following the manufacturer's protocols, sequences were analyzed using ABI 3730xl DNA Analysis Systems.

To investigate the phylogenetic position of the new species, all of the DNA sequences obtained were aligned with 61 taxa, including 59 species of *Hoya* (ingroup), and two species of *Dischidia* Brown (outgroup), the sampling represented most of the clades within *Hoya* (Rodda and Ercole, 2014). We generated DNA sequences for the new



species and related species *Hoya chinghungensis*, *Hoya lanceolata*, *Hoya engleriana* for this study and collected others from GenBank, all Genbank accession numbers are presented in Supplementary.

Sequences were aligned and manually adjusted in BioEdit version 5.0.9 (Hall, 1999). Phylogenetic analysis was performed by maximum likelihood (Felsenstein, 1973) using RAxML-HPC v.7.2.6 (Stamatakis, 2006) implemented on the CIPRES web cluster (Miller *et al.*, 2010). The GTRGAMMA substitution model was applied to each gene independently follow Rodda and Ercole (2014) in this study. Following Wannthrop *et al.* (2014), bootstrap support values (BS) of 60–79 are considered as moderate support and of 80–100 as high support.

The topologies based on individual DNA data were largely congruent except some of the terminal branches and the phylogenetic analysis based on the combined data gave higher bootstrap support than those based on individual makers. Hence, we present only the results from combined DNA data analyses below. The best tree from RaxML analyses of 61 taxa (Fig. 1) was in accordance with recent phylogenetic investigations of *Hoya* (Wannthrop *et al.*, 2014). The new species is sister to *H. engleriana* (BS=97%).

## TAXONOMIC TREATMENT

Both morphology and phylogenetic analysis (Fig. 1) demonstrate that the new species is a member of *Hoya*, and sister to *Hoya engleriana* with strong support.

*Hoya longicalyx* Wang Hui & E. F. Huang, *sp. nov.*

長萼球蘭 Figs. 2 & 3

**Type:** CHINA. Yunnan Province: Yingjiang county, A-jiang-po, elev. ca. 1900 m, on arboreal branch covered by moss in mid-montane evergreen forest, 16 May 2019, Huang Er-feng 1905003 (holotype SZG!; isotype PE!, KUN!, TAI!).

**Diagnosis:** Morphologically similar to *Hoya chinghungensis* (Tsiang & P.T. Li) M.G. Gilbert, P.T. Li & W.D. Stevens from which it differs in having longer lamina (1.5–2 cm vs. 1–1.5 cm), acuminate lamina apex (vs. acute to obtuse lamina apex), longer calyx lobes (5–7 mm vs. 1.5–2 mm), ovate and translucent corona lobes (vs. pink and triangular corona lobes) and oblong and upside apart ovaries (vs. ovate and attached ovaries).

Subshrubs epiphytic, hanging, densely pubescent except old stems and lamina surface. *Stems* rounded in cross section, branched, stout, internodes 1.2–2 cm; adventitious root absent; *old stems* ca. 4 mm in diam., pale white, glabrous; *leafy stems* 2 mm in diam., green. *Leaves* opposite, fleshy; *petiole* short, recurved, ca. 3 mm long, pubescent; *lamina* ovate-lanceolate 1.5–2 × ca. 1 cm, base rounded, margin entire, sometime purple, slightly pubescent, reflexed in older leaves, apex

acuminate, adaxial surface green, abaxial surface greyish-green; *venation* pinnate, anastomosing, inconspicuous when fresh; midrib depressed on adaxial surface, raised on abaxial surface; secondary veins in 3–4 pairs, borne at 60°–85° to midrib, basal 1 pair from the base of midrib; tertiary venation reticulate. *Pseudumbels* terminal, pendent, flat-topped; *peduncle* short, ca. 5 mm long, light green. *Flower* 3–4 per pseudumbels; *bracteoles* 2 at each pedicel base, linear, 1.6–2 × 0.2–0.3 mm; *pedicel* 1.6–1.8 cm long, 1.4–1.6 mm in diam., yellowish white; *calyx* lobes linear, 5–7 × 1–1.2 mm, margin entire, apex acuminate to obtuse, revolute, yellowish white, pubescent outside and upper 1/4 part of inside, glabrous lower 3/4 of inside; *corolla* rotate, flat to slightly incurved, 1.8–2 cm in diam., white, glabrous outside, pubescent inside, lobes triangular, ca. 5.5 × 6 mm, apex acute, flat; *Corona* staminal, 6–7 mm in diam., ca. 3 mm high, lobes ovate 2.7–3 mm × ca. 1.7 mm, translucent, inner process cuspidate, outer process apex retuse, concave above; *guide rail* forming a ridge, with a acuminate ending; *pollinarium* 0.8–0.9 mm long, *pollinia* clavate, 0.55–0.6 × 0.27–0.3 mm, narrowing towards the base, base and apex truncate, *caudicula* attached at the center of the retinaculum, ca. 0.15 mm, inner edge winged, *retinaculum* ca. 0.2 mm long. *Pistils* 2; *ovaries* attach to each other below center, free upside, oblong, 1.6–1.7 mm long, ca. 0.6 mm in diam., yellowish white, apex excurved, pubescent; *Stigma* head discoid, rounded. *Fruit* and *seed* not seen.

**Geographical distribution:** *Hoya longicalyx* is known only from the type locality in Yingjiang county, Yunnan province, southwest China, base on the fieldwork and herbaria investigations in HITBC (Herbarium, Xishuangbanna Tropical Botanical Garden, CAS), IBSC (South China Botanical Garden, CAS), PE (Institute of Botany, CAS) and KUN (Herbarium, Kunming Institute of Botany, CAS).

**Ecology:** Epilithic on arboreal branch covered by moss (*Trachypodopsis serrulata*) under mid-montane evergreen forest.

**Etymology:** *Hoya longicalyx* is named from its long and linear calyx lobes, which is a significant feature to distinguish the new species from relevant species.

**Phenology:** Flowering April to June, fruiting unknown.

**Conservation status:** Deficient (DD) (IUCN, 2017).

**Note:** Our phylogenetic analysis shows that the new species belongs to a clade including *H. bella* Hook., *H. chinghungensis*, *H. edeni* King ex Hook. f., *H. engleriana*, *H. lanceolata* Wall. ex D. Don and *H. linearis* Wall. ex D. Don. According to Wannthrop *et al.* (2014), this clade comprised only a few species restricted to the subtropical foothills of the Himalayas and the Tibet Plateau. The new species is also found in this area. Morphologically, members of this clade can be easily distinguished from other taxa of *Hoya* by having a once-,



**Fig. 1.** Maximum likelihood trees obtained from the combined analysis of 61 taxa and the concatenated dataset of nuclear ribosomal intergeneric transcribed spacer (ITS), external transcribed spacer (5'-ETS), and chloroplast *matK*, *psbA-trnH* and *trnT-trnL* intergeneric spacers. Numbers are bootstrap percentages (>50%).

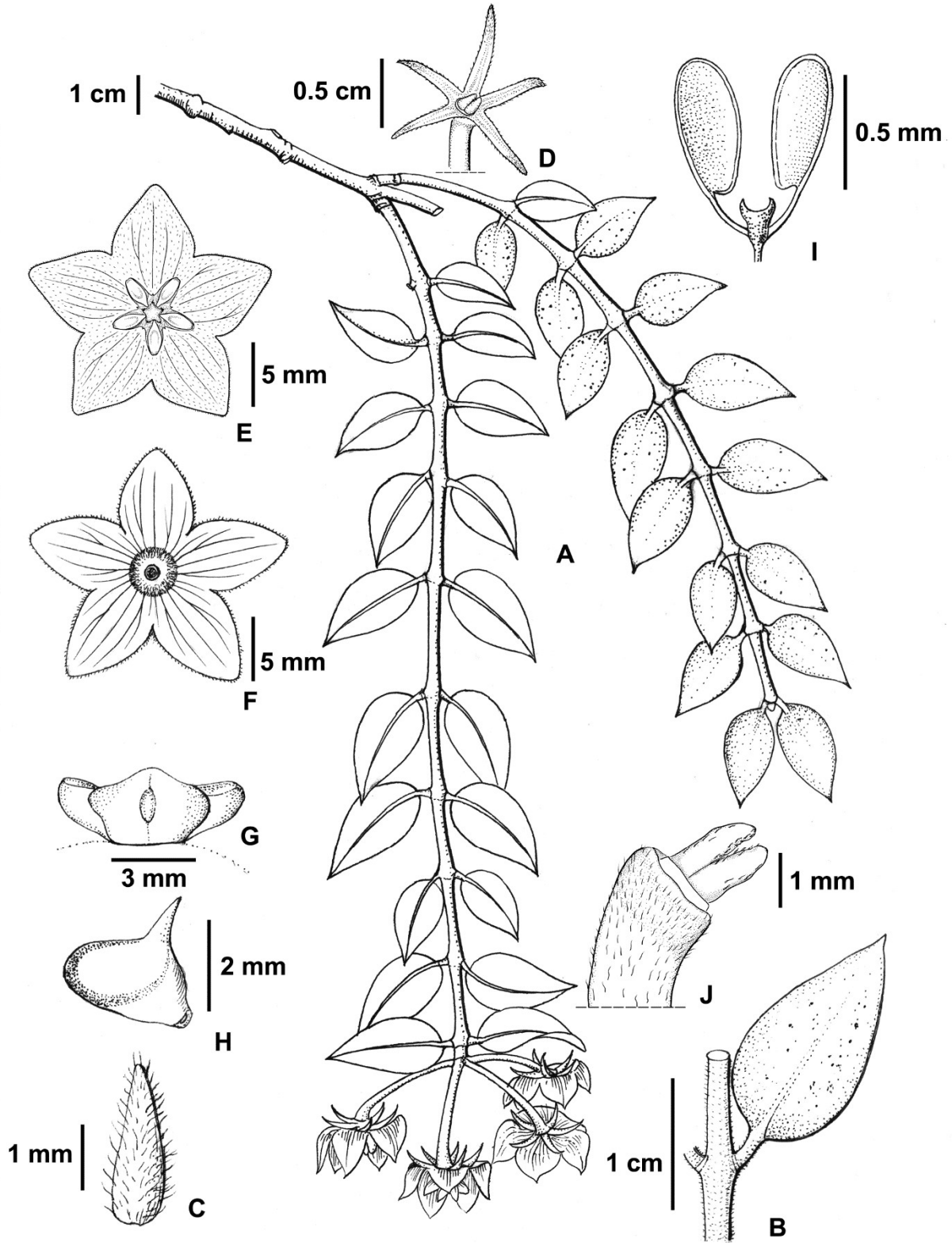
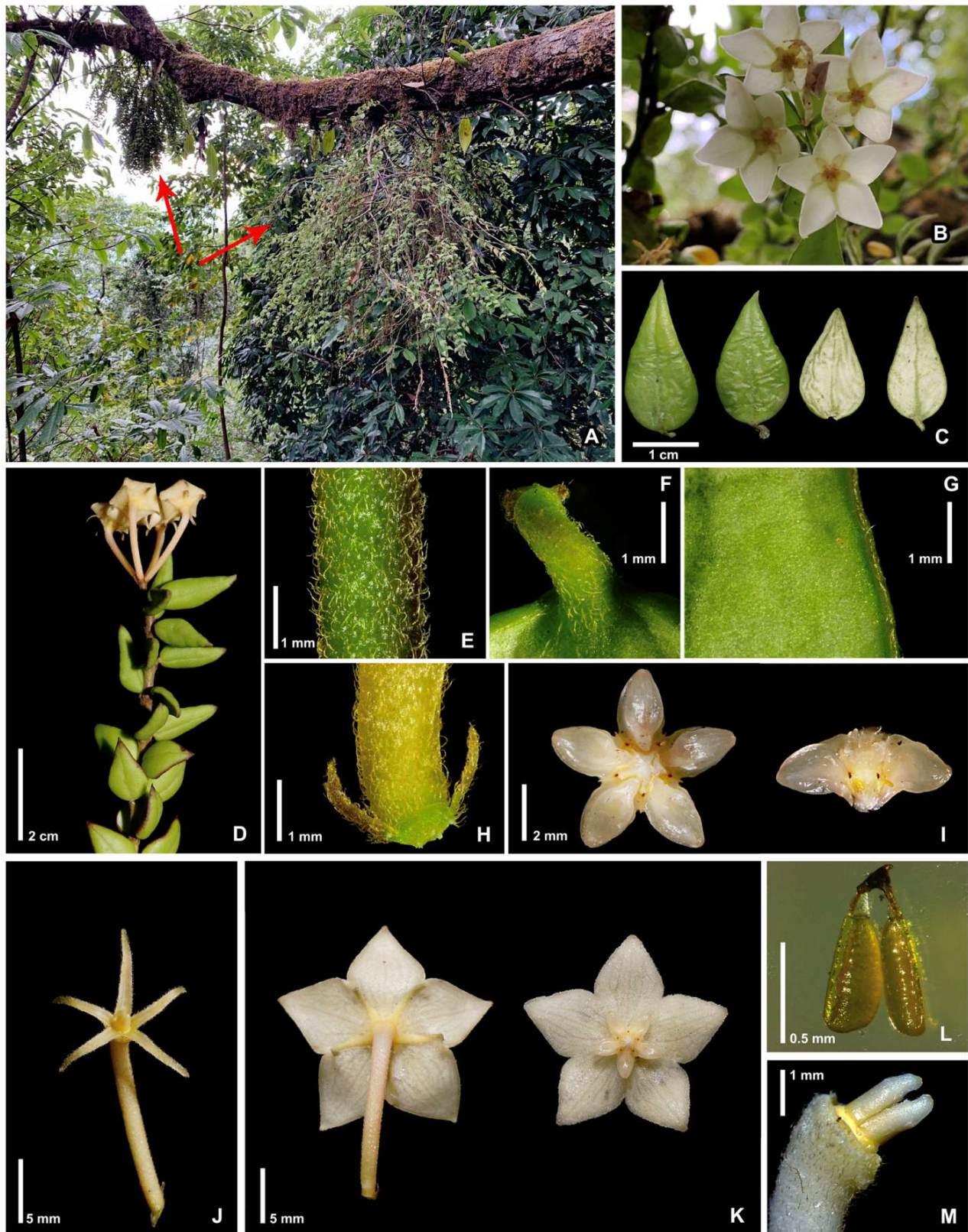


Fig. 2. *Hoya longicalyx*. A. Branch with inflorescences. B. Leafy stem and leaf. C. Bracteole. D. Calyx and ovaries. E. Corolla (adaxial side). F. Corolla (abaxial side). G. Corona (side view). H. Corona lobe (side view). I. Pollinarium. J. Pistils. Drawn by Z. M. Li and H. Dong.





**Fig. 3.** *Hoya longicalyx*. **A.** Habits of the new species *in situ*, arrows indicate the plants. **B.** Inflorescence. **C.** Leaves. **D.** Branch and inflorescence. **E.** Leafy stem. **F.** Petiole. **G.** Lamina margin. **H.** Bracteoles. **I.** Corona (top view and side view). **J.** Calyx. **K.** Corolla (adaxial and abaxial sides). **L.** Pollinarium. **M.** Pistils. Photographed by H. Wang and E. F. Huang.

**Table 1.** Detailed comparison of *H. longicalyx*, *H. chinghungensis* and *H. engleriana*.

Characters	<i>H. longicalyx</i>	<i>H. chinghungensis</i>	<i>H. engleriana</i>
Lamina	Ovate-lanceolate, base rounded, apex acuminate, glabrous on both surface when young.	Broadly ovate, base rounded to truncate, pubescent on both surface when young.	Oblong, base cuneate, apex usually obtuse with mucro, pubescent on both surface when young.
Pseudumbels	Terminal, 3–4 flowered.	Terminal, 4–5 flowered.	Subterminal, 5–7 flowered.
Calyx lobes	Linear, 5–7 mm long.	Ovate, 1.5–2 mm long.	Ovate, 1.5–2 mm long.
Corolla lobes	Triangular	Triangular-ovate	Triangular-ovate
Corona lobes	Ovate, outer process apex retuse.	Triangular, outer apex subacute.	Triangular, outer process apex retuse.
Ovaries	Oblong, upside free to each other.	Ovate, upside attach to each other.	Ovate, upside attach to each other.

flowers-beard pedicel. Among the members of this clade the new species is most similar to *H. chinghungensis*, which is distributed in southern Yunnan province, China and northern Myanmar, and *H. engleriana*, which is distributed in northern Thailand. Detailed comparison between them see table 1. Other taxa of this clade (*H. bella*, *H. edeni*, *H. lanceolata* and *H. linearis*) also share a few characters with the new species. In order to facilitate identification, by specimen investigation, we here provide a diagnostic key to all the 7 species of *Hoya* in this clade known from subtropical foothills of the Himalayas and the Tibet plateau.

#### Key to *H. bella*, *H. chinghungensis*, *H. edeni*, *H. engleriana*, *H. lanceolata*, *H. linearis* and *H. longicalyx*.

- 1a. lamina linear, 2.5–6 × 0.3–0.5 cm ..... *Hoya linearis*
- 1b. lamina lanceolate, ovate or oblanceolate, not linear ..... 2
- 2a. lamina usually oblanceolate, widest above middle, 7–8 × 1.5–2.5 cm, lateral vein obvious when dry, corona inner process long caudate. .... *Hoya edeni*
- 2b. lamina oblong, lanceolate, ovate-lanceolate, deltoid or ovate, widest below middle, lateral vein obscure when dry, corona inner process not extended. .... 3
- 3a. Calyx lobe linear, long > 5 mm, corona lobes translucent. .... *Hoya longicalyx*
- 3b. Calyx lobe ovate or oblong, long < 3 mm, corona lobes pink to purple red. .... 4
- 4a. lamina oblong, ca. 2.3 × 0.6 cm, apex with mucro ..... *Hoya engleriana*
- 4b. lamina broadly ovate, ovate-lanceolate or lanceolate, not oblong, apex without mucro ..... 5
- 5a. lamina lanceolate, base cuneate ..... *Hoya lanceolata*
- 5b. lamina broadly ovate or ovate-lanceolate base rounded to truncate, not cuneate ..... 6
- 6a. lamina broadly ovate, long usually < 2.5 cm, apex acute, corona lobes triangular ..... *Hoya chinghungensis*
- 6b. lamina ovate-lanceolate, long usually > 3 cm, apex acuminate, corona lobes ovate ..... *Hoya bella*

**Additional specimens examined:** *H. bella*: MYANMAR: 1959, *K.U. Kramer s.n.* (NY 04192686!). NEPAL: Kathmandu, 17 Mar. 1966, *H.J. Lange 59* (B100271832!), 29 May 1966, *H. J. Lange 153* (B100271833!). USA (Cultivated): 24 Aug. 1945, *A. Seaman 713/42* (NY03536495!), 1 Apr. 1970, *R.A.M. Keefe 54178* (NY03536494!), 8 Jan. 1910, *R.S. Williams 29946* (NY03536496!); Nonlocation, 7 Jun. 1959, *Brewer s. n.* (L1658265!), 1974, *H.W. Groeneveld 21-1* (L1102530!), 25 Aug. 1965, *J.J. Bos 1741* (L1658266!), 1 Sep 1967, *L. Dehosalle s.n.* (BR0000022429803!). *H. chinghungensis*: CHINA: Yunnan, 4 Aug. 1993, *Biodiversity Exp. 2397* (KUN1267298!), 1 Sep. 1936, *C.W. Wang 78311* (Isotype, A00016231!), 1 Aug. 1936 *C.W. Wang 111587*, (IBSC0519523!), 1934, *H.T Tsai 57025*

(IBSC0519522!); 5 Jul. 1998, *H. Wang 2197* (HITBC89093!), *H. Wang 2203* (HITBC98077!), 5 Dec. 1993, *H. Wang & B. G. Li 2219* (HITBC61458!). *H. edeni*: INDIA: 1859, *C.H. Anders 3437* (P04550995!); 1859, *J.D. Hook s.n.*, (P04550994!). *H. engleriana*: THAILAND: Chiang Mai, 1 Jan. 1905, *C.C. Hosseus s.n.*, (Isotype, P00700522!); 11 Sep. 1974, *K. Larsen & S. S. Larsen 34404* (P00700531!). LAOS: Khammouan, 24 May 2006, *M. F. Newman et. al.*, *LAO 1455*, (P04551003!); INDIA: Assam, *s.n.* (P04551004!). *H. linearis*: CHINA: Yunnan, 15 Apr. 1941, *T.N. Liou 018897* (IBSC0520489!), 8 Apr. 1941, *T.N. Liou 018655* (PE01024742!), 29 Sep. 1940, *K.M. Feng 8089* (KUN268013!), 5 Jul. 1998, *H. Wang 2199* (HITBC87965!). INDIA: 1963, *J.D. Hook s.n.* (P06602854!, P05207669!). VIETNAM: Lao Cai, 14 Aug. 1926, *E. Poilane 12932* (P05207667!). *H. lanceolata*: INDIA: Assam & Sikkim, *C.H. Anders 3524* (P05207654! & P05207654!), Sikkim, 1839, *J.D. Hook 2056a* (P05207658!), 1843, *W. Griffith s.n.* (P05207657!), 28 Apr. 1913, *C.C. Hosseus 16040* (P05207660!). NEPAL: Gadaki, 24 May, 1954, *S. Sykes 610* (P05207653!), *N. Wallich 36a* (Isotype, PE01456953! & PE01456940!), 1821, *N. Wallich s.n.* (P05207656!), Uttarakhand, 1843, *R. Strachey & J.E. Winterbottom 1* (P05207659!). *H. lasiogyne*: CHINA: Hainan, 13 Apr. 1982, *Q. Huang 820037* (IBSC0520477!), 15 Apr. 1982, *Q. Huang 820137* (IBSC0520478!). *H. pandurata*: CHINA: Yunnan. 15 Oct. 1956, *B.Y. Qiu 52862* (KUN0268049!), 1 Aug. 1936, *C.W. Wang 73461* (Isotype, PE00029510!, IBSC0520534!), 1 Jul. 1936, *C. W. Wang 75297* (IBSC0520533!, PE01024793), 7 Jun. 1982, *H. Wang 2203* (HITBC84373!), 20 Feb. 1988, *S.Q. Tong & A.M. Li, 32871* (HITBC!).

#### ACKNOWLEDGMENTS

The authors thank HITBC, KUN, K, P, PE for allowing access high quality images of herbarium specimens; Ying-Ming Hu and Hong-Mei Yang for photographing; Dr. Hui Dong for improving the illustration; Dr. Hong-Lei Li for advice and discussion on phylogenetic analysis. Profound thanks go to professor Ping-Tao Li from South China Agricultural University for his valuable comments. We thank the Shenzhen Urban Management Bureau (Project Number: 201415).

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Supplementary materials are available from Journal Website.