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

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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 Hoya engleriana Hosseus. Result from molecular phylogenetic analysis revealed that the new species was sister to Hoya chinghunensis Tsiang & P.T. Li

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 intergenic 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'–trnHf (Sang et al., 1997; Tate and Simpson, 2003) for psbA-trnH, and trnT–trnL(a) and trnL–trnA(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 ddH2O. 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 Wanntrup 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 (Wanntrup 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!, TAII!).

**Diagnosis:** Morphologically similar to Hoya chinghungensis (Tsieg & 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 calyx lobes, which is a significant feature to distinguish the new species from relevant species.

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

**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 Wanntrup 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%).
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. engleri*, 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. engleri*, *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 chinghungensis**

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: Hoya bella:**

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**Literature Cited:**


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Supplementary materials are available from Journal Website.