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HOYA LAMTHANHIAE (ASCLEPIDOIDEAE, APOCYNACEAE), A NEW SPECIES FROM SOUTHERN VIETNAM

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Abstract

Hoya lamthanhiae V.T. Pham & Kloppenb. is described and illustrated. The molecular data is provided; this species was found in Dak Lak province, southern Vietnam. Diagnostic features of the new species are a milky latex lithophytic vine and pure white with red-purple centre flowers. The described species clearly differs from closely related *H. hainanensis* in number of characters: a leaf blade shortly attenuated and wavy margins at the base, a hairy abaxial leaf surface, a bigger calyx with few sparse hairs or glabrous, a bigger corolla with acute lobe, shorter pollinia, as well as molecular characters.

Key words: Asclepiadoideae, *Hoya*, New species, Plant diversity, Vietnam.

Introduction

Hoya R.Br. (Brown 1810) is a genus belonging to Apocynaceae family, subfamily Asclepioideae. Although more than 500 published *Hoya* names have been recorded (<http://www.theplantlist.org/>), less than 300 species have been found throughout tropical Asia, tropical Pacific islands and north-eastern Australia (Li *et al.*, 1995; Forster & Liddle, 1996; Forster, 2006; Liddle, 2009; Kamel, 2014). Mainland southeast Asia, particularly the Indochinese Peninsula, is recognized as a one of the richest areas Hoya diversity, with 46 species previously recorded from Thailand (Thaithong, 2001; Kloppenburg, 2005; Kidyoo & Thaithong, 2007; Kidyoo, 2016), 40 species in southern China (Li *et al.*, 1995; He *et al.*, 2012; Zhang *et al.*, 2015), 21 species in Lao PDR (Costantin, 1912; Newman *et al.*, 2007; Averyanov *et al.*, 2017) and 8 species in Cambodia (Costantin, 1912; Averyanov *et al.*, 2017). At least 40 species have been reported in Vietnam (Costantin, 1912; Pham, 2003; Pham & Averyanov, 2012[a,b]; Pham *et al.*, 2015; Averyanov *et al.*, 2017), including *Hoya ignorata* T.B. Tran *et al.*, (Tran *et al.*, 2011), which is currently regarded as a synonym of *Hiepia corymbosa* V.T. Pham & Aver. (Pham & Averyanov, 2011). Meanwhile, Vietnam is certainly richer in *Hoya* species, but still remains insufficiently studied.

Materials and Methods

Morphological analysis: Six specimens of species described here were collected by Lam Thi Ngoc Thanh and Nguyen Van Canh in southern Vietnam, Dak Lak province on 20 January 2016. The voucher specimens are deposited in the Vietnam National Museum of Nature (VNMN) and Hanoi University of Science (HNU). Photos were taken of both living plants and the herbarium dry specimens by Nguyen Van Canh and Nguyen Hoang Tuan. Fresh inflorescences and flowers were preserved in 60–70% ethanol. All co-authors have cooperated in laboratory studies, including measurements, data analysis and morphologic descriptions. A ZEISS Axio Scope A1 and a binocular OLYMPUS SZ61 stereomicroscope were used for micromorphology observation, and a Canon PowerShot G10 and Canon Rebel XT 8.0 MP were used for macro- and micro-photography. Type specimens of the related species were accessed and examined using high-resolution digital images from A, E, MO, NY, UC, and US herbaria. All morphological characters were described using the general terminology by Harris & Harris (2006).

Molecular analysis: The fresh leaves of the new species were collected from the wild in Dak Lak province and its closely related *H. hainanensis* Merr. (1923: 263) were collected from cultivated plants in Kon Tum province, Vietnam. The plant specimens examined in present study are summarized in Table 1. The samples were dried by Silica gel and were grounded to fine powder in liquid nitrogen. The total DNA was extracted from 100 mg powder by CTAB method followed by ethanol precipitation. DNA sequence fragments from six loci include 18S-rDNA, ITS-rDNA, matK, rbcL, trnL-F, and psbA-trnH were amplified by PCR used single primer pair for each gene, respectively (Table 2). PCR products were purified used Exo-AP, and sequenced used BigDye kit.

Hoya lamthanhiae V.T. Pham & Kloppenb., *Sp. Nov.* (Figs. 1, 2); *Hya hainanensis* auct. non Merr.: Aver. *et al*., 2017, Turczaninovia 20(3): 122.

Table 1. Plant materials of *Hoya* collected in Vietnam.

Taxon	Locality of voucher	Voucher No.	GenBank accession number					
			18S-rDNA	ITS-rDNA	matK	rbcL	trnL-F	psbA-trnH
Hoya Dak Lak	M´Drak, Dak Lak Prov.	H01	MH036480	MH036482	MH036488	MH036484	MH036486	MH036490
H. hainanensis	Ban Me Thuot, Kon Tum Prov.	H02	MH036481	MH036483	MH036489	MH036485	MH036487	MH036491

Table 2. Target genes and primer sequences.

No.	Target gene	Primer sequence (5' – 3')	Ta (°C)	Length of PCR product (bp)
1.	18S subunit ribosomal RNA gene (18S-rDNA)	>18S_forward CCT TCT GCG AAA TCA GAG TGT TTG >18S_reverse CTT CTC CTT CCT CTA AAT GAT AAG	52	600
2.	ribosomal Internal transcript spacer (ITS-rDNA)	>ITS_forward AGA GGA AGG AGA AGT CGT AAC A >ITS_reverse TTC CTC CGC TTA TTG ATA TGC	53	750
3.	Maturase K (matK)	>matK_forward CGA TCT ATT CAT TCA ATA TTT C >matK_reverse TCT AGC ACA CGA AAG TCG AAG T	49	950
4.	Ribulose biphosphate carboxylase large chain (rbcL)	>rbcL_forward ATG TCA CCA CAA ACA GAG ACT AA >rbcL_reverse CTT CGG CAC AAA ATA CGA AAC GAT CTC TCC A	55	650
5.	Leucine - Phenylalanine transfer RNA intergenic spacer (trnL-F)	>trnL-F_forward CGA AAT TGG TAG ACG CTA CG >trnL-F_reverse ATT TGA AAC TGG TGA CAC GAG	54	950
6.	Photosystem II protein D1 - Transfer RNA Histidine intergenic spacer (psbA-trnH)	>psbA-trnH_forward GTT ATG CAT GAA CGT AAT GCT C >psbA-trnH_reverse CGC GCA TGG TGG ATT CAC AAT CC	53	~400

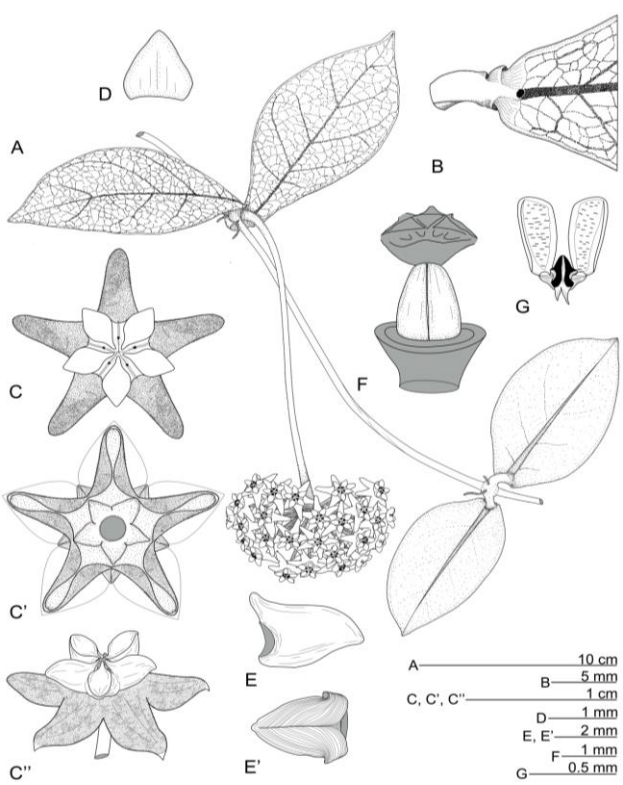

Fig. 1. *Hoya lamthanhiae* V.T. Pham & Kloppenb. A. Habit. B. Leave base. C, C', C''. Flower, frontal, side, and dorsal views. D. Calyx. E, E'. Corona lobe, side and dorsal views. F. Ovary. G. Pollinaria. Draw by V.T. Pham from *FOP/02/2016/Hoya* (VNMN).

Type: Vietnam, Dak Lak province, M´Drak district, Chu Mu Mountain, 12°41'N, 108°54'E, primary evergreen forest, at elevation approximately 800 m a.s.l., 20 January 2016, *FOP/02/2016/Hoya*, (holotype: VNMN; Isotype: HNU).

Lithophytic vine to 5 m long with milky latex. Stems slender, rooting at nodes and internodes, green and hairy when young, light grey and glabrous when old, terete, 1.6–1.8 mm in diam.; internodes to 25 cm long. Leaves opposite; petiole stout, often twisted, grooved, sparsely hairy, 4–6 mm long, 2–2.5 mm in diam.; leaf blade oblanceolate or elliptic, more or less fleshy, 9–11 cm long, 4–5 cm in wide, slightly curved, shortly attenuate and wavy margin at base, acute at apex, dark green and glabrous above, light green and scattered hairy below; distinct reticulate nerves on adaxial surface, hardly visible on abaxial surface except keeled midrib, secondary veins in 4–6 pairs, extended to the margin. Inflorescence extra-axillary round umbel, commonly of 8–30 flowers; peduncle terete, stout, swollen at base and apex, hairy, 13–15 cm long, 1.5–4 mm in diam., green to dark green with some dirty purple marks; pedicels white speckled with dull purple, slender, glabrous, 1.8–2 cm long, approximately 1 mm in diam. Flowers pure white with red–purple centre. Calyx of 5 segments; sepals triangular, overlap at bases, sparsely hairy or glabrous on abaxial surface, acute, 1.0-1.2 mm long and wide. Corolla 5-lobed, 1.45–1.55 cm in diam.; lobes fleshy, triangular ovate, acute, adaxially pubescent, few hairs at tips, pure white, abaxially glabrous, dull light purplish, 5.5–5.8 mm long, 3.8 mm wide, slightly reflexed, margin revolute. Corona white with red-purple centre, of 5 segments, approximately 2.2–2.4 mm tall and

7.6–7.8 mm in diam., concave in surface; segments glossy, 2.2–2.4 mm tall, 2.1–2.2 mm wide, almost white with red-purple inner angle, outer angle acute and curved, inner angle apiculate. Gynostegium bears on top 5 pollinaria covered by membranous yellowish anther caps. Pollinaria of two pollinia connected by two short translators; retinaculum dark-chestnut, spear-shaped, hollowed along, angled, obtuse at apex, approximately 0.23 mm long, shoulders ca. 0.19 mm wide, waist ca. 0.11 mm wide, hip ca. 0.14 mm wide, extensions ca. 0.04 mm; translators transparent, ca. 0.10 mm long, 0.02 mm wide; caudicle bulb 0.04 mm in diam.; pollinia nearly oblong, bright yellow, approximately 0.45 mm long, widest 0.21 mm; with germinating crested extending from translator to pollinia apex, pollinia inner ends almost square, rounded at corners. Carpels 2, free, half bottle shaped, approximately 1.5 mm tall, 0.6 mm in diam., densely connivent each other, light green tinged with purple, apex shortly cuspidate and joined to style head bottom.

Phenology: Flowering was observed in January, and fruiting started in March.

Table 3. Comparison of diagnostic features of *Hoya lamthanhiae* and *H. hainanensis*.

Characters	*Hoya lamthanhiae*	*H.hainanensis*
Leaf		
Length (cm)	9–11	6–8
Width (cm)	4–5	2.5–4
Petiole length (cm)	0.4–0.6	0.8–1.0
Base and apex	more or less attenuate	acute
Margins	flat, wavy at base	revolute
Abaxial surface	hairy	glabrous
Secondary veins	4–6 pairs	4 pairs
Venation reticulation	distinct	obscure
Calyx		
Length (mm)	1.0–1.2	1.0
Width (mm)	1.0–1.2	1.0
Surface	sparsely hairy or glabrous	ciliate
Corolla		
Diameter (mm)	14	8–10 (12)
Lobe apex	acute	rounded
Corona		
Diameter (mm)	7.6–7.8	7.0
Colour	white with red-purple centre	pure white
Pollinia		
Length (mm)	0.45	0.53
Retinaculum		
Length (mm)	0.23	0.26
Shoulder width (mm)	0.19	0.14
Waist width (mm)	0.11	0.09
Hip width (mm)	0.14	0.12
Extensions width (mm)	0.04	0.02
Translator		
Length (mm)	0.10	0.11
Width (mm)	0.02	0.03
Bulb diam. (mm)	0.04	0.08

Diagnostic morphology characters: Described species is most closely related to *Hoya hainanensis* Merr. (1923: 263) from Hainan, sometimes regarded as a synonym of *H. ovalifolia* Wight & Arn. (Li *et al*., 1995). However, type specimens of *H. ovalifolia* accessed from E and NY virtual herbariums and a description from Wight (1834: 37) are apparently quite different. In contrast, leaf form and habit of *H. hainanensis* is similar to the new species. The examination by the second author of type specimens of *H. hainanensis* deposited at UC and observations of other types at A, MO and US virtual herbariums, reveal the following differences from our new species; leaf blade shortly attenuate and wavy margins at base, hairy abaxial leaf surface, bigger calyx with few sparse hairs or glabrous, bigger corolla with acute lobe, as well as shorter pollinia as it is presented in Table 3.

DNA sequence comparison: There are no nucleotide sequence difference between 18S-rDNA partial sequence of *Hoya lamthanhiae* and *H. hainanensis* whereas some difference were found between two species for ITS-rDNA, matK, rbcL, trnL-F, and psbA-trnH partial gene sequences (Table 4).

Distribution: The species is currently known only from the lone type locality in Dak Lak province, southern Vietnam.

Habitat and ecology: *Hoya lamthanhiae* occurs in primarily evergreen broad-leaved forests, often on granite outcrops along streams at an elevation of approximately 800 m a.s.l.

Etymology: Species is named after its discoverer - Mrs. Lam Thi Ngoc Thanh.

Vernacular name: Vietnamese: Cẩm cù lâm thành.

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Table 4. Sequence comparison of *Hoya lamthanhiae* and *H. hainanensis*.

DNA sequence locus	18S-rDNA	matK	rbcL	trnL-F	psbA-trnH	ITS-rDNA
Length (base pair, *H. lamthanhiae / H. hainanensis*)	546/546	914/914	617/617	908/908	361/389	710/710
Nucleotide sequence identity	1.00	0.987	0.998	0.993	0.867	0.99
Nucleotide sequence difference count	**0**	**11**	**1**	**6**	**52**	**7**
Amino acid sequence difference count	*NA*	**7**	**1**	*NA*	*NA*	*NA*

(NA: Not applicable)

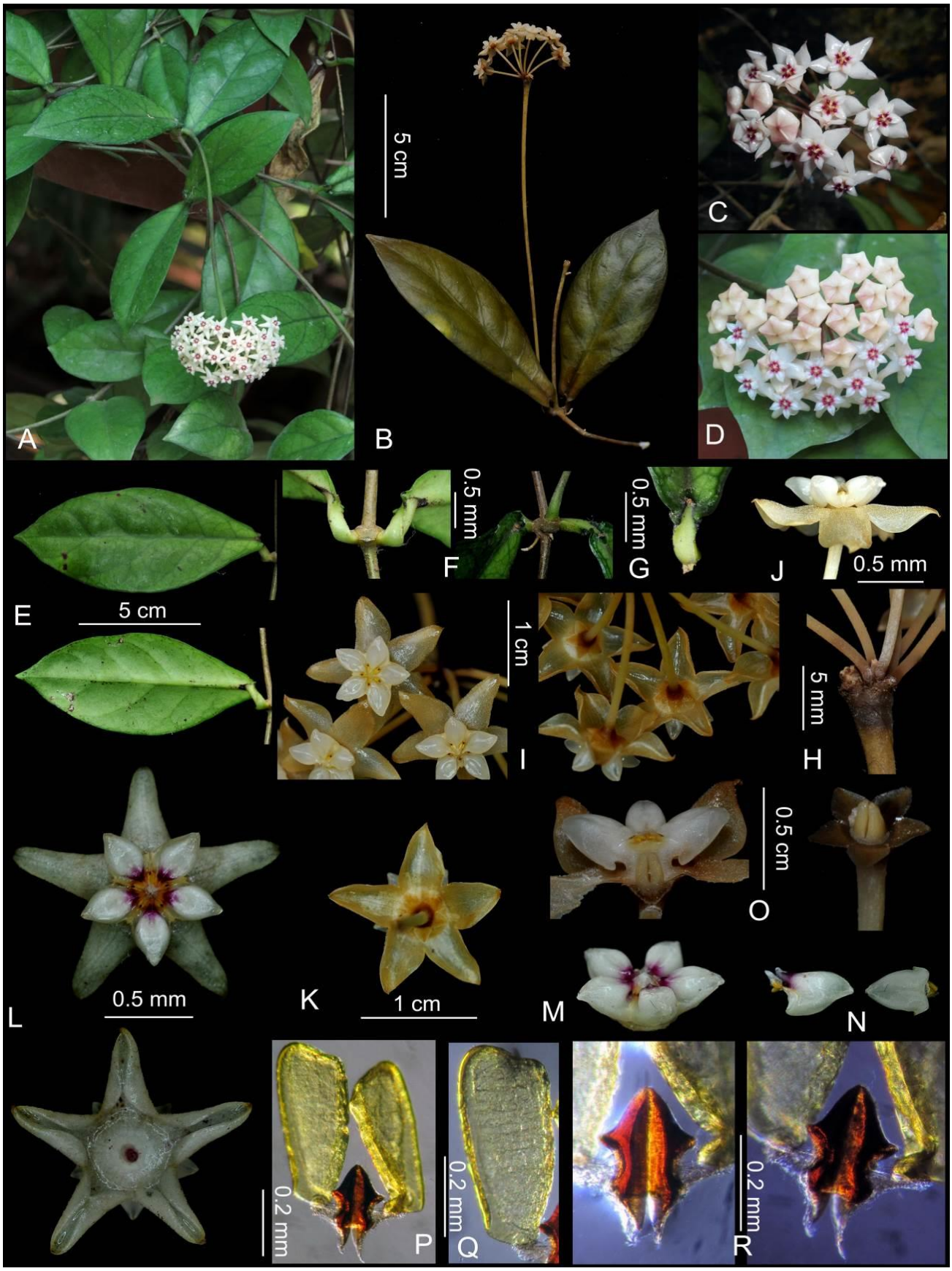

Fig. 2. *Hoya lamthanhiae* V.T. Pham & Kloppenb. **A**. Habit. **B**. Inflorescence with leaf (preserved in ethanol). **C, D.** Inflorescence. **E.** Leave (above: adaxial view; dorsal: abaxial view). **F.** Petioles. **G.** Leaf bases. **H.** A part of peduncle and pedicels. **I.** Flowers (left: frontal view; right: dorsal view). **J.** Corolla and corona (side view). **K.** Calyx and petal (view from behind). **L.** Corolla (frontal view and dorsal view). **M.** Corona (haft-side view). **N.** Corona lobe (left: half-side view; right: dorsal view). **O.** Ovary. **P.** Pollinaria. **Q.** Pollinia. **R.** Retinaculum. All illustrations were made from the type specimens.

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