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Hedyotis konhanungensis (Rubiaceae): A new species from the central highlands of Vietnam

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Abstract
Hedyotis konhanungensis B.H.Quang, T.A.Le, K.S.Nguyen & Neupane (Rubiaceae, Spermacoceae), a new species from Vietnam, is described and illustrated based on morphological and phylogenetic evidence. The new species is morphologically distinct from all southeastern Asian *Hedyotis* L. in its set of morphological traits such as the leaf type (shape and thickness), growth habit, and floral parts (color of inflorescence axis and the shape of calyx lobes). This species shows similarities with *Hedyotis shenzhenensis, H. shiuyingiae* and *H. yangchunensis* from China in habit, leaf blades, floral bracts, dichasial cymes, and fruits, but it is phylogenetically distinct and can be distinguished from them by the following combination of characteristics; cylindrical or subterete stem up to 15 cm tall, obovate to oval leaves spreading along the stem, broadly ovate or deltoid stipules with an entire margin and cuspidate apex, suborbicular or broadly oval lowest floral bracts, ovate or nearly oval calyx lobes, corolla tube 6–7 × 1–1.5 mm, stamens of the long-styled flowers inserted near base of corolla tube, and the short-styled flowers with stamens inserted near the throat of the corolla tube and filaments not exserted the throat.

Keywords
Gia Lai Province, Indochina, phylogenetics, Spermacoceae, *Hedyotis-Oldenlandia* complex, taxonomy

Introduction

Asian-Pacific genus *Hedyotis* L. (ca.180 species) lies within a polymorphic herbaceous tribe Spermacoceae (ca. 1000 species) in the Rubiaceae family. In Vietnam, this tribe is represented by 70–80 species belonging to the genera *Dimetia, Debia, Exallage, Leptopetalum, Hedyotis, Involucrella, Neanotis, Oldenlandia,* and *Spermacoce* (Govaerts et al. 2022). The genus *Hedyotis* and its related genera that form the *Hedyotis-Oldenlandia* complex have a long history of taxonomic confusion and disagreement due to the use of inconsistent and overlapping characters in the generic delimitations. The genus *Hedyotis* is now treated in a narrower sense based on the molecular phylogenies (Guo et al., 2013; Wikström et al., 2013; Neupane et al., 2015) where members of it are united by their predominantly shrubby to tree-like habit, septicidal capsules, flattened seeds, and tropical upland distributions in Asia and the Pacific. The species of this genus are mostly found in the mid or high-elevation slopes (to 4000 m a.s.l.) of Asia (Sri Lanka,

southern India, SE China, Indochina, Malesia), Papuasia, and NW Pacific (Micronesian islands). In Vietnam there are about 20 species reported for the flora of this country (Pitard 1922; Pham 2003; Tran 2005; Do et al. 2013; Govaerts et al. 2022) but the actual number could be as high as 40 species as many species listed under *Oldenlandia* have dipolophragmus capsule (pers. obs.).

During a botanical field survey in Kon Ha Nung Biosphere Reserve of the highlands of Central Vietnam, we came across a population of Spermacoceae with a peculiar morphology (collection number *LTA 531)* which are characterized by having perennial herbs, opposite obovate to nearly oval leaf blades, interpetiolar entire stipules with cuspidate apex, 4-merous flowers, bilobed stigmas, inferior ovary, and many seeded diplophragmous capsules with persistent ovate or nearly oval calyx lobes. It is clear that the morphological features of our specimens represent a species of the genus *Hedyotis* (Wikström et al. 2013; Neupane et al. 2015). After carefully investigating the relevant taxonomic literature of the genus in Vietnam and surrounding countries (Pitard 1922; Fukuoka 1970; Pham 2003; Tran 2005; Chen et al. 2011; Wikström et al. 2013; Neupane et al. 2015), and morphologically comparing our species with its close allies viz. *Hedyotis shenzhenensis* T.Chen, *H. shiuyingiae* T.Chen, and *H. yangchunensis* Ko & Zhang, and conducting phylogenetic analysis for its position in the Spermacoceae, we conclude that our specimens represent an undescribed species of *Hedyotis* and named here as *Hedyotis konhanungensis*.

Materials and methods

Morphological study

Our collected specimens were compared with all described species from southeast Asia and southern China by studying relevant literature and examining digital herbarium images. Morphological characters were recorded using Nikon SMZ745/SMZ745T stereoscopic microscope and photographs of its vegetative and floral parts were taken both in the field and from the samples preserved in 70% ethanol using Canon EOS 7D. The type specimens have been stored in the herbaria; Vietnam National Museum of Nature (VNMN), Institute of Ecology and Biological Resources, Vietnam Academy of Sciences and Technology (HN), and Kon Ka Kinh National Park.

Phylogenetic analysis

To establish its phylogenetic position within Spermacoceae, total genomic DNA was from silica-dried material with the DNeasy Plant Kit (Qiagen, Valencia, California, U.S.A.). Four DNA regions (nuclear genome: ITS, ETS; plastid genome: *petD*, *rps* 16) that were used in our earlier studies (Neupane et al. 2015, Neupane et al. 2017), were selected for amplification from this sample. Amplifications were performed in a 25 µl reaction mixture composed of 1 µl of each primer (10 µM), 1 µl of DNA template, 12.5 µl of GoTaq Green Master Mix (Promega, Madison, Wisconsin, U.S.A.), 9,5 µl of water. The amplification protocol for nuclear and chloroplast regions follows Kårehed et al. (2008) and Groeninckx et al. (2009) respectively. Amplified PCR products were purified using ExoSAP-IT™ PCR Product Cleanup (Thermo Fisher Scientific) following the manufacturer's protocols and sequenced at Apical Scientific Company for sequencing (Selangor, Malaysia).

The DNA sequences of our sample along with the additional sequences of *Hedyotis yangchunensis*, *Hedyotis shenzhenensis*, and *Hedyotis shiuyingiae* representing ITS, petD, and rps16 regions (from Guo et al. 2013 available in GenBank) were added to our existing DNA data matrix (see supplementary document). Each of these DNA regions was aligned using MAFFT v.7 (Katoh and Standley 2013) and concatenated into a single matrix to infer the phylogeny. Phylogenetic analysis was conducted on the combined matrix under the maximum likelihood (ML) framework with four partition schemes (ITS, ETS, petD, rps16) for their own substitution model (GTR+GAMMA) parameters using RAxML v.8.2.12 (Stamatakis, 2014). The RAxML

tree search and bootstrap analysis (1000 rapid bootstrap replicates for clade support) were conducted on CIPRES (Miller et al. 2010).

Results

Molecular phylogeny

The Maximum likelihood tree obtained from RAxML analysis on the concatenated data confirms the position of our sample from Vietnam within *Hedyotis* distinct from *Hedyotis yangchunensis*, *Hedyotis shenzhenensis*, and *Hedyotis shiuyingiae* (Fig. 1) The new species is nested within the clade (BS = 85 %) representing *Hedyotis* from Sri-Lanka, southeast Asia (primarily Borneo), and New Guinea. The new species appears sister to the Sri Lankan clade, however, this phylogenetic position is considered unresolved due to weak bootstrap support (BS = 50%).

Taxonomic treatment

***Hedyotis konhanungensis* B.H.Quang, T.A.Le, K.S.Nguyen & Neupane, sp. nov.**

Figs. 2, 3, Table 1

Diagnosis. *Hedyotis konhanungensis* is morphologically similar to *H. shenzhenensis H. shiuyingiae* and *H. yangchunensis* from southeastern China (Guangdong and Hongkong) on leaf blades, floral bracts, dichasial cymes, and fruits, but differs from them by its broadly ovate or deltoid (*vs.* triangular or broadly triangular) stipules with entire (*vs.* hairy or lacerated) margins and cuspidate (*vs.* acute) apex, suborbicular or broadly oval (*vs.* subovate or ovate to lanceolate) lowest floral bracts, ovate or nearly oval (*vs.* triangular or subulate to lanceolate) persistent calyx lobes on fruits, and stamens in long-styled flowers inserted in lower ¼ or near the base (*vs.* in the middle or near the throat) of the corolla tube (Table 1).

Type. VIETNAM. Central Highlands of Vietnam, Kon Ha Nung Biosphere Reserve, Gia Lai province: K'Bang District, Kon Pne Commune, 14°20’53”N, 108°20’48”E, primary evergreen forest slopes at elevation 1150 m a.s.l., 28 November 2021, *Nguyen Quoc Luan, Ngo Duy Hoang Vu, Le Tuan Anh, LTA 531* (holotype: VNMN!; isotypes: HN!).

Description. Perennial herbs, erect, 15–25 cm tall, completely glabrous, exception to the flower inside. **Stem** simple, rarely branched, cylindrical, 8–18 cm long, 5–7 mm in diam., internodes 1–1.5 cm long. ***Leaves*** 5-7 pairs, decussate, spreading along stem, abaxially dark green, adaxially glossy, dark purple to purplish black; petiole 4–6 mm long; blade flattened, thick, fleshy, (subleathery when dried), obovate-lanceolate to nearly oval, 6–10 × 2–4 cm, base decurrent or cuneate, apex broadly acute or obtuse; midrib depressed adaxially and prominent abaxially; secondary veins 4–5 pairs, inconspicuous on adaxial side. ***Stipule*** interpetiolar, fused to leaf bases or very shortly around stem, broadly ovate or deltoid, 2.5–3 mm tall, 5–6 mm wide at base (being flatten), dark purple outside, apex cuspidate or aristate, with aristae of 3–4 mm long and 0.7–0.9 mm wide, margins entire. ***Inflorescence*** compound dichasial cyme, terminal, 5–7 cm long, 3–4 orders of branching, purplish, 30–50-flowered. Peduncle cylindrical or terete, 2–4 cm long, basally subtending by two largest floral bracts, leaf-like, suborbicular or broadly oval, slightly concave, 2–2.5 × 1.5–2 cm, apex rounded to broadly acute or obtuse, abaxially dark purple to dark bluish purple, pale green adaxially; upper floral bracts smaller, ovate or lanceolate, 0.8–1.5 × 0.5–1 cm, apex acute. Pedicels terete, 3–6 mm long, usually bluish purple or purplish, bracteolate or ebracteolate; bracteoles narrowly ovate or lanceolate, somewhat concave, ca. 2.5 × 1.5 mm, broadly acute or obtuse. ***Flowers*** 4-merous, heterodistylous. ***Calyx*** hypanthium, bluish purple, cupular, 0.8–1.2 mm long and wide, glossy and glabrous; free lobes 4, sub-equal, ovate or nearly oval, somewhat longitudinally concave, 1.7–2.2 mm long, 0.8–1.4 mm wide, broadly acute to obtuse at apex, adaxially white or purplish white, abaxially bluish purple. ***Corolla*** narrowly infundibuliform or tubular, bluish purple outside; tube 6–7 mm long, 1–1.5 mm wide, slightly enlarged in both ends, inside white pubescent; lobes 4, strongly recurved, triangular-ovate, 1.5–

1.8 × 1.2–1.5 mm, acute, adaxially white or purplish white, sparsely puberulent near base on adaxial surface; ***Longistylous flower*** stamens 4, inserted in the lower ¼ of corolla tube, 1.5–2 mm above the base of tube; filaments very short, ca. 0.2 mm long; anthers linear, 1–1.2 × 0.2 mm, dorsifixed, introrse, longitudinally dehiscent; style filiform, 5.5–6.5 mm long, white and glabrous; stigma 2-parted, lobes oblong, 0.6–0.7 mm long, minutely papillate, pure white, slightly exserted the throat; ovary inferior, 2-loculed, numerous ovules, axile placentation; ***Short-styled flowers*** stamens 4, inserted in the upper ¼ of corolla tube, 1–1.5 mm below the throat; filaments and anthers similar to those in the long-styled flowers; style filiform, 1.5–1.8 mm long; stigma 2-parted, lobes oblong, 0.6–0.7 mm long, densely papillate. ***Disks*** fleshy, rounded, concave in the center, glabrous, white. ***Fruits*** capsule, nearly cupuliform with slightly concave top, 2.5–3 mm in diam., glabrous, purplish, with persistent, unveined, ovate or nearly oval calyx lobes. Seeds many, black, irregularly angular, reticulate, minute, 0.3–0.5 mm long.

Additional specimens examined. VIETNAM. Central Highlands of Vietnam. Kon Ha Nung Biosphere Reserve, Central Highlands of Vietnam, Gia Lai province: K'Bang District, Kon Pne Commune, 14°20’43.06”N; 108°20’38.33”E, 906 m a.s.l., 26 March 2022, *Bui Hong Quang et al. BHQ 453* (HN, and herbarium of Kon Ka Kinh National Park).

Etymology. This species is named after the “Kon Ha Nung Biosphere Reserve” where it was discovered.

Vernacular name. Vietnamese: An điền Kon Hà Nừng

Phenology. Flowering in October to November, fruiting from November to December.

Distribution and Ecology. *Hedyotis konhanungensis* is only recorded from the type locality of the Kon Pne Commune, at the Central Highlands of Vietnam. The species grows understorey of the evergreen forests in the valleys or on flat areas to slopes of sandstone mountains. Within its occupancy areas, the new species was associated with some shrubs or herbs as *Pavetta bauchei* Bremek., *Lasianthus biflorus* (Blume) M.Gangop. & Chakrab., *Staurogyne* sp., *Popowia* sp., *Huperzia* sp.

Table 1. Morphological comparison of *Hedyotis konhanungensis* with its putative closest allies. The characters of *H. shenzhenensis* T. Chen, *H. shiuyingiae* T. Chen and *H. yangchunensis* W.C.Ko & Zhang are derived from Tao Chen (2007, 2008), Gao (1995) and their type specimens respectively.

Morphological characters	***H. konhanungensis***	***H. shenzhenensis***	***H. shiuyingiae***	***H. yangchunensis***
Plant height, including the inflorescences (cm)	15–25 cm	20–40 cm	15–35 cm	30–39 cm
Stem	cylindrical or subterete, 8–18 cm long	cylindrical or subterete,1–2 cm long	slightly tetragonal,10–22 cm long	tetragonal or subterete, 15–30 cm long
Leaf arrangement	spread along the stem	fascicled on short stem forming a rosette-like	spread along the stem	spread along the stem
petiole length (mm)	4–6	0.5	5–10	15–20
blade shape and size (cm)	obovate to oval, 6–10 × 2–4	elliptic, oblong-elliptic or	broadly	narrowly elliptic, oblanceolate or

		obovate, 8.5–15 × 5–9	lanceolate or obovate-lanceolate, 2–19 × 1.4–8	elliptic-oblong, 3–12 × 1–4.5
Stipule shape and size (mm)	broadly ovate or deltoid, apex cuspidate, margin entire, 2.5–3 × 5–6	triangular, apex acute, margin shortly hairy, 3–5 × 5–10	triangular, apex acute, margin dense trichomes, 4–8 × 5–10	triangular, apex and margin lacerate or deeply divided into several linear lobes, ca. 16 × 8
Inflorescence	flowers lax or not congested into head-like inflorescence	flowers lax or not congested into head-like inflorescence	flowers congested into head-like inflorescence	flowers congested into cymose or capitate inflorescence
Peduncle length (cm)	2–4	10–18	10	3–4
Lowest floral bracts	suborbicular or broadly oval, 2–2.5 × 1.5–2 cm	subovate, 2.5–3 × 1.8–2 cm or sometimes larger	ovate to lanceolate, 0.6–2.5 × 0.2–1 cm	ovate, ca. 1.5 × 0.5–0.6 cm,
Calyx lobes	ovate or nearly oval, 1.7–2.2 × 0.8–1.4 mm	subulate, ca. 1.5 mm long	subulate, ca. 3 mm long	unknown
Corolla abaxial colour	bluish purple	white	purplish white	unknown
tube	6–7 × 1–1.5 mm, slightly enlarged both ends	ca.3 × 1.5–1.8 mm, slightly dwindled near throat	tube ca. 4.5 × 2–2.5 mm, enlarged at throat	unknown
Stamens of long-styled flowers	inserted in lower ¼ (or near the base) of corolla tube	inserted in upper ¼ (or near the throat) of corolla tube	inserted in ½ (or middle) of corolla tube	unknown
Stamens of short-styled flowers	inserted in the upper ¼ (or near the throat) of corolla tube; filaments ca. 0.2 mm long, not exserted corolla throat	inserted near the throat of corolla tube; filaments ca. 0.8 mm long, exserted corolla throat	inserted near base of corolla tube; filaments ca. 0.3 mm long, not exserted corolla throat	unknown
Persistent calyx lobes on fruits	ovate or nearly oval, 1.8–2.3 × 0.8–1.5 mm, unveined	narrowly triangular or subulate, 1.2–2 × 0.5–0.7 mm, unveined	narrowly triangular or subulate, 3–4 × ca.1 mm, unveined	lanceolate, 3–6 × 1.2–3 mm, veined

Discussion

This species is resolved within the clade containing other southeast Asian species and is unique among the Indochinese *Hedyotis* in its thick and fleshy oval leaves and completely dark purple floral parts (calyx and corolla) including the inflorescence stalk. Despite the geographic distance, its morphological similarity with Chinese members and non-monophyly of *H. shenzhenensis* and *H. shiuyingiae* with *H. yangchunensis* (Fig. 1) suggest the independent evolution of fleshy leaves and somewhat stunted growth habit among these species. Here we provide both morphological and molecular evidence to confirm the novelty of this taxon and propose it as species new to science.

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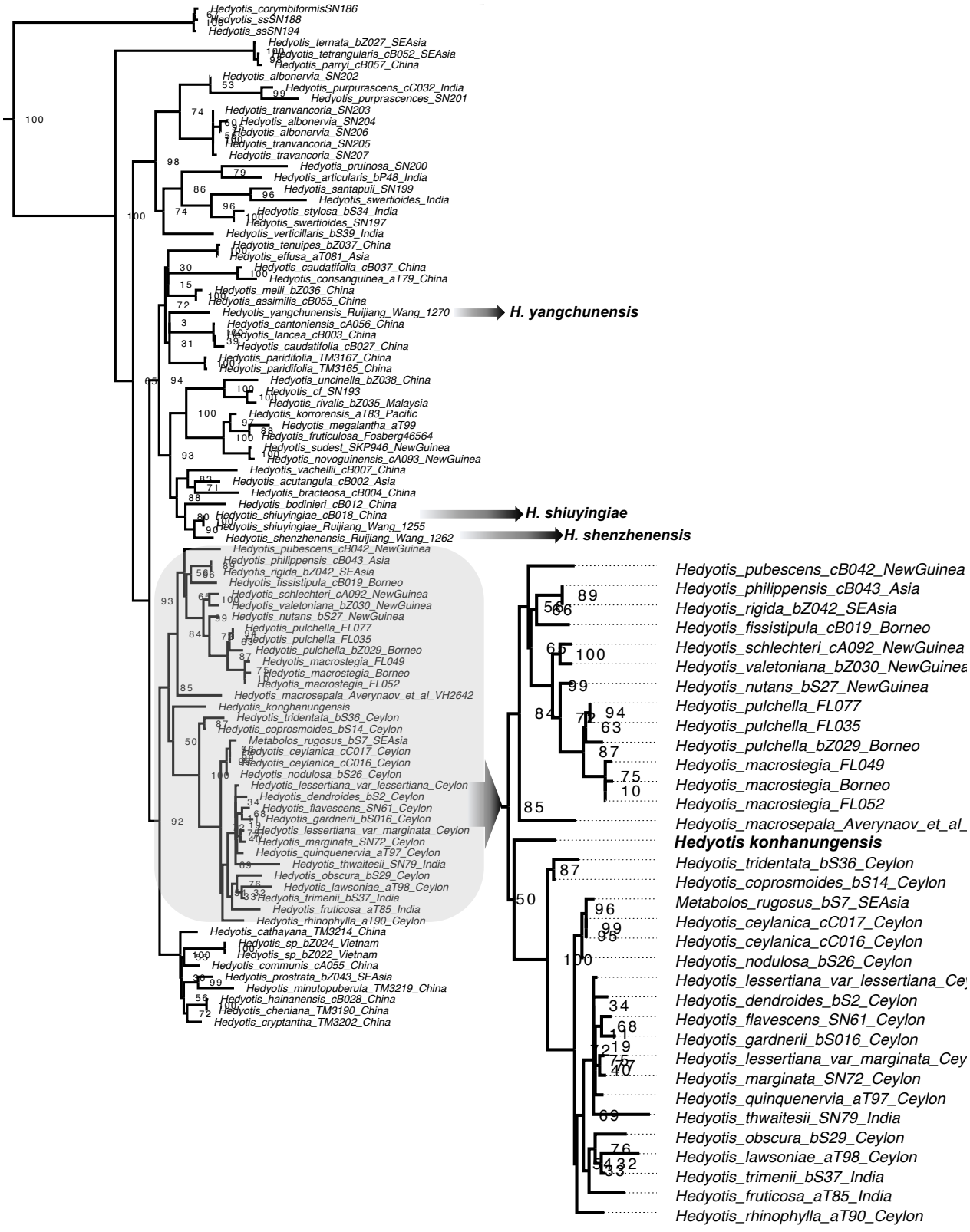

Figure 1. Maximum likelihood phylogenetic tree of *Hedyotis* based on the combined nuclear (ITS, ETS) and plastid (*petD*, *rps16*) data. The taxa discussed in the paper and the new species are in bold. Values at the nodes represent bootstrap support (BS).

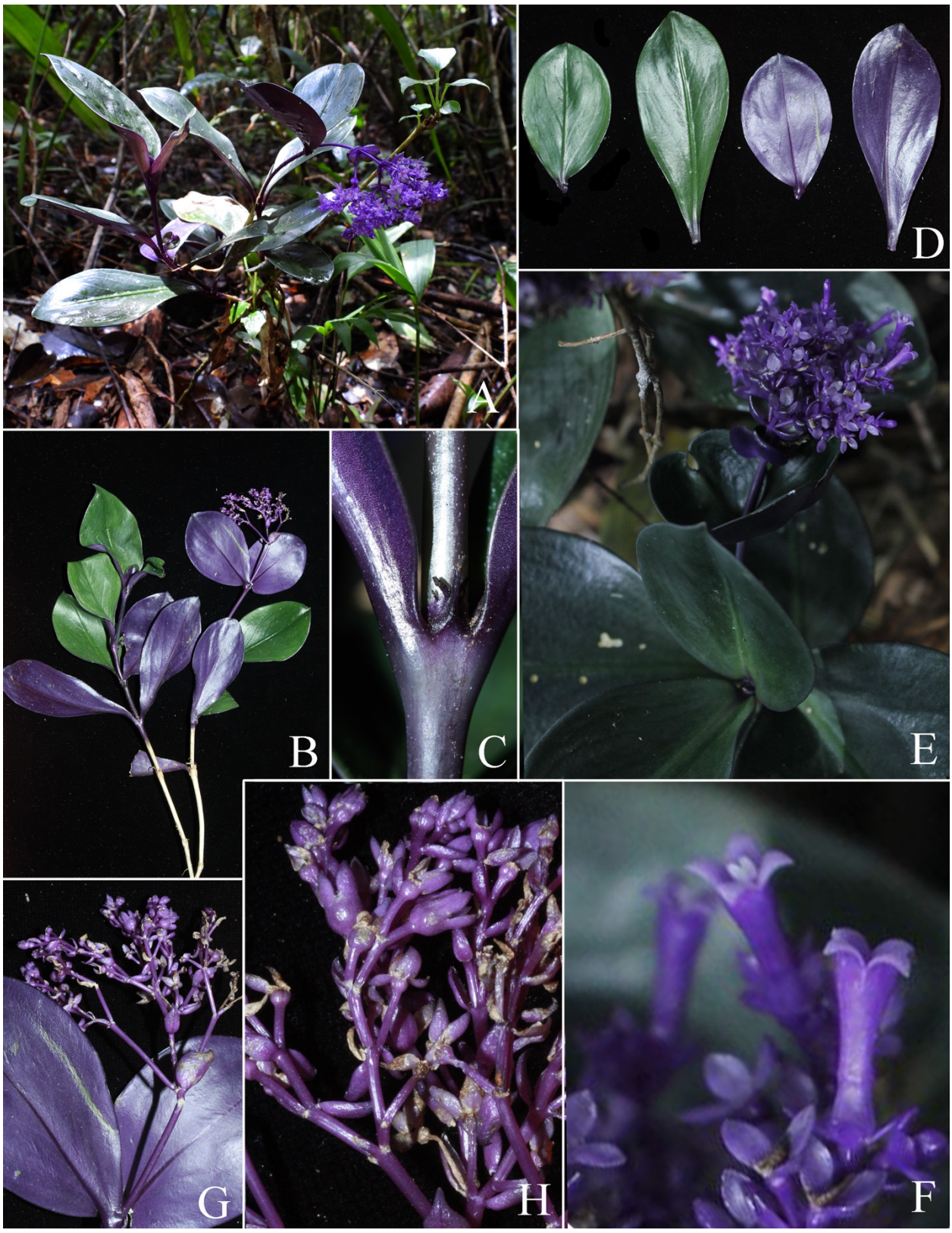

Figure 2. *Hedyotis konhanungensis* **A** habitat **B** habit **C** stipule **D** adaxial and abaxial leaf surfaces **E** inflorescences top view **F** long-styled and short-styled flower **G** infructescence **H** fruiting branch. Photos by Q.L. Nguyen and B.H. Quang from *Luan et al. LTA 531.*

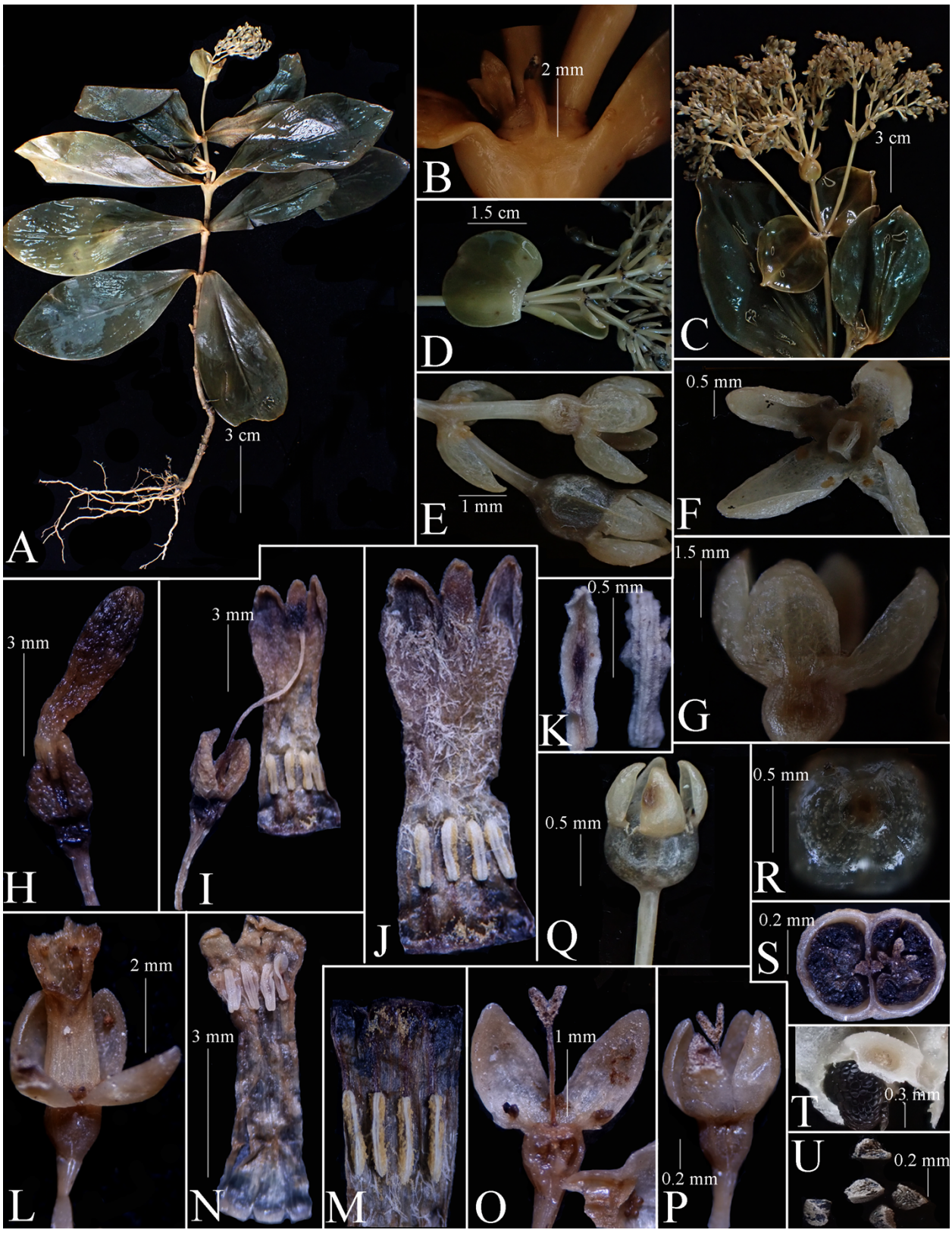

Figure 3. *Hedyotis konhanungensis* **A** habit **B** stipule **C** inflorescence **D** floral bracts **E** bracteoles and fruits **F, G** calyx top and side views **H**–**K** long-styled flower, entire and open showing pistil, corolla and anthers **L** – **P** short-styled flower, dissection showing calyx, corolla, anthers and pistil **Q** – **T** fruit, entire and dissection **U** seeds. Photos and design by B.H. Quang from *Luan et al. LTA 531*.