

FLORA OF NAM KADING NATIONAL PROTECTED AREA VI: *DIDYMOCARPUS MIDDLETONII* (GESNERIACEAE), A NEW SPECIES FROM LIMESTONE

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Didymocarpus middletonii Souvann., Soulad. & Tagane, a new species of Gesneriaceae from Nam Kading National Protected Area, is described and illustrated. The new species is morphologically similar to *Didymocarpus brevicalyx*, *D. formosus* and *D. puhoatensis* but distinguished from the three by its fewer-flowered inflorescence, longer pedicel, and urceolate and multicellular eglandular hairy calyx. Based on the latest IUCN criteria, *Didymocarpus middletonii* is proposed to be Critically Endangered (CR). Our record of *Didymocarpus* represents a new genus record for the flora of Laos.

Keywords. Biodiversity, *Didymocarpus brevicalyx*, *Didymocarpus formosus*, *Didymocarpus puhoatensis*, Indochina, Laos, new taxa, taxonomy.

INTRODUCTION

Didymocarpus Wall. (Gesneriaceae) is a medium-sized genus with more than 70 species distributed from India to Southeast Asia (Nangngam & Maxwell, 2013; Nangngam & Middleton, 2014; Möller *et al.*, 2016; Hong *et al.*, 2018). The genus is characterised by the combination of lithophyte perennial habit, the ovate to ovate-cordate lamina which is mostly glandular hairy, the tubular corolla with an oblique limb (rarely trumpet-shaped or bell-shaped), two fertile stamens, three staminodes, a capitate stigma, an orthocarpic ovary and a bivalve capsule which dehisces loculicidally (Weber *et al.*, 2000). *Didymocarpus* is similar to *Henckelia* Spreng. in having dehiscent fruits on the dorsal side and chiritoid stigma (Weber *et al.*, 2011a, Möller *et al.*, 2017). The greatest diversity of species of *Didymocarpus* in this region is in China and Thailand, in which 30 known and 23 known taxa have been reported, respectively (Nangngam & Maxwell, 2013; Nangngam & Middleton, 2014; Möller *et al.*, 2016). In Indochina, only five species have been recorded from Vietnam (Hong *et al.*, 2018) and none in Laos

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and Cambodia (Cho *et al.*, 2016; Zhu, 2017). *Didymocarpus bonii* Pellegr. is the only species listed in the *Checklist of the Vascular Plants of Laos* (Newman *et al.*, 2007), but this species has since been removed to *Petrocodon* Hance based on the results of a phylogenetic analysis of the *trnL-F* and internal transcribed spacer (ITS) regions (Weber *et al.*, 2011b) and is now recognised as *Petrocodon bonii* (Pellegr.) Mich.Möller & A.Weber. The lower diversity in the region is probably a consequence of insufficient botanical exploration. Therefore, diversity and distribution patterns of Gesneriaceae in Indochina should be treated as underestimated.

Nam Kading National Protected Area in central Laos is one of the biodiversity hotspots in the country (Hallam & Hedemark, 2013). Recently, a series of botanical studies has elucidated its plant diversity and discovered numerous new species and records from this area, including *Strobilanthes namkadingensis* Soulad. & Tagane (Acanthaceae; Souladeth *et al.*, 2017), *Begonia namkadingensis* C.-J.Yang, Soulad. & Tagane (Begoniaceae; Yang *et al.*, 2018), *Monoon namkadingense* Soulad. & Tagane and *Neo-uvaria laosensis* Tagane & Soulad. (Annonaceae; Tagane *et al.*, 2018b), as well as 30 new records for the flora of Laos (Tagane *et al.*, 2018a)

In this paper, we describe a new species of Gesneriaceae, *Didymocarpus middletonii* Souvann., Soulad. & Tagane, found on limestone rocks along a stream in Nam Kading National Protected Area. Photographs, a hand-drawn illustration, and DNA barcodes of *rbcL*, *matK* and ITS (Kress & Erickson, 2012) are also provided for the new species.

MATERIALS AND METHODS

Morphological observations

The new species was recognised by detailed comparisons with morphologically similar species: descriptions found in a review of the literature (Nangngam & Maxwell, 2013; Nangngam & Middleton, 2014; Möller *et al.*, 2016; Hong *et al.*, 2018); dry specimens from the herbaria BKF, FOF, FU, HNL and KAG; and digitised plant specimens available online (e.g. JSTOR Global Plants, no date). The measurements of the description below are based on the herbarium materials we collected.

DNA barcoding

Total DNA was extracted from silica gel-dried leaves collected in the field. DNA extraction was carried out by using a modified CTAB protocol (Doyle & Doyle, 1987), as described in detail by Toyama *et al.* (2015). Amplification and sequencing of the three DNA barcode regions, *rbcL*, *matK* and ITS, were performed according to published protocols (Kress *et al.*, 2009; Dunning & Savolainen, 2010, for *rbcL* and *matK*; Rohwer *et al.*, 2009, for ITS).

TAXONOMIC TREATMENT

***Didymocarpus middletonii* Souvann., Soulad. & Tagane, sp. nov.**

Didymocarpus middletonii is similar to *Didymocarpus brevicealyx* Nangngam & D.J.Middleton, *Didymocarpus formosus* Nangngam & D.J.Middleton from Thailand

and *Didymocarpus puhoatensis* X.Hong & F.Wen from Vietnam but differs from these three in having an urceolate calyx (versus campanulate) and a smaller corolla (c.3.4 cm long versus longer than 4.5 cm). – Type: Laos, Nam Kading National Protected Area, Bolikhamxai Province, Pak Kading District, 18°20'38.2'', 104°09'29.5'', 665 m elevation, 29 vi 2017, *Tagane, S., Souladeth, P., Okabe, N., Yang, C.-J.* L1198 [fl.] (holo FOF; iso E, HNL, P). **Figs 1, 2.**

Deciduous, perennial, lithophytic herb, 8–16 cm tall. *Stem* erect, 0.3–0.7 cm long, single, densely covered with multicellular glandular hairs. *Leaves* opposite, anisophyllous; petioles terete, 1.5–3.8 cm long, covered with multicellular glandular hairs, light green; blade asymmetrical or subsymmetrical, broadly ovate, 8–11.5 × 6.5–8.5 cm, membranous, base slightly oblique, slightly cordate to broadly obtuse-cuneate or sometimes truncate, apex acute, margins serrate, dark green adaxially, light green abaxially, densely covered with white multicellular glandular hairs on both surfaces, venation pinnate, midrib prominent beneath, secondary veins 5–6 on each side of midrib, subopposite, sometime alternate, obscure above, prominent beneath. *Inflorescences* terminal, cymose, up to 14.2 cm long, 1- to 3-flowered; peduncles slender, 8–11.4 cm long, dark brown to reddish towards green, covered with multicellular glandular and glandular hairs; pedicels 7–12 mm long, reddish towards green, with indumentum as on the peduncle. *Bracts* paired, orbicular, c.3 × 2.7 mm, apex rounded, margin entire to crenate, green, covered with multicellular glandular hairs. *Calyx* shallowly 5-lobed, radially symmetrical, c.7.8–9.2 mm long, c.7 mm in diameter, dark red, sparsely covered in multicellular glandular hairs, tube urceolate, c.7 mm long, lobes triangular, c.2 × 2 mm, apex acute. *Corolla* funnellform, c.3.4 cm long, light red at base, reddish to blackish purple elsewhere, 9 dark stripes inside, glabrous; tube c.3 cm long, base narrow, c.2.4 mm in diameter, dilated and slightly ventricose towards the throat, widest at throat, c.9 mm in diameter; corolla lobes suborbicular or triangular, lower lip 3-lobed, lobes triangular, more or less equal, c.6 × 5.5 mm, upper lip 2-lobed, lobes suborbicular, c.4.5 × 4.5 mm long, apex rounded. *Fertile stamens* 2, inserted c.1.4 cm from the base of the corolla; filaments c.5.8 mm long, glabrous; anthers connected, ellipsoidal, c.2 mm long, 1 mm in diameter, tips and bases rounded, silky hairy on the back. *Staminodes* 3, adnate to corolla c.1.2 cm from base, reduced to thin filaments, lateral ones 2 mm long, the central one 1 mm long, glabrous. *Disc* cylindrical, c.1.8 mm long, margin irregular. *Pistil* c.2.2 cm long, sparsely glandular puberulent; ovary narrowly linear, c.1.8 cm long, sparsely glandular puberulent; stigma capitate, concave, papillose.

Distribution. Laos, Bolikhamxai Province (so far known only from the type locality).

Habitat and ecology. *Didymocarpus middletonii* grows on shaded rocks, along a stream in semi-evergreen forest. Given that most of the individuals seen during our field survey had flower buds, the species should be in full bloom in July.

Etymology. The specific epithet honours Dr David Middleton (Singapore Botanic Gardens), from whom we received the most generous advice regarding this new species.

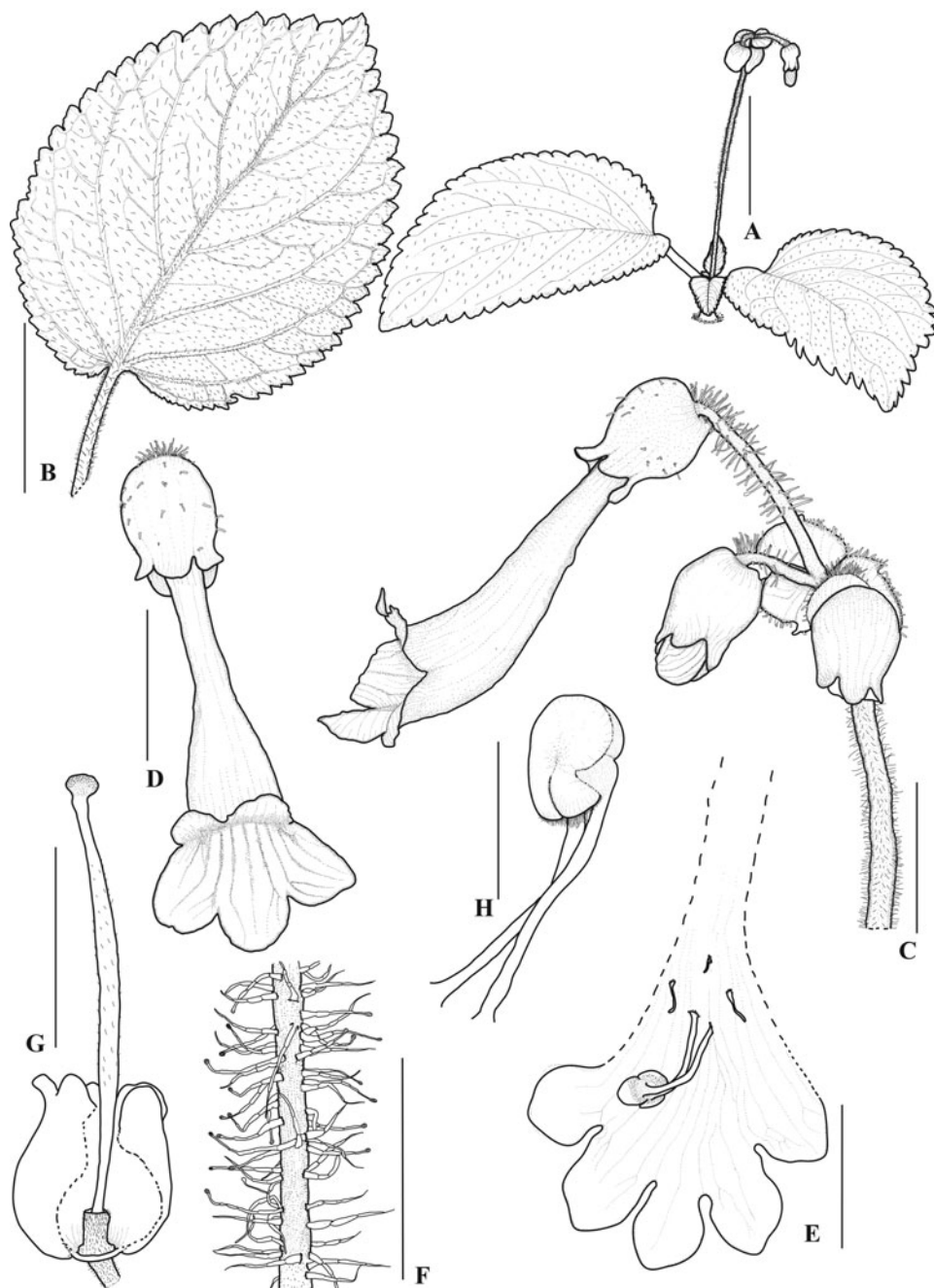


FIG. 1. *Didymocarpus middletonii* Souvann., Soulad. & Tagane. A, Habit; B, abaxial surface of lamina; C, inflorescence with flower (lateral view); D, flower (top view); E, dissected corolla, showing stamens and staminodes; F, multicellular glandular and eglandular hairs on pedicel; G, calyx and pistil; H, fertile stamens. Scale bars: A, 4 cm; B, 3 cm; C–E and G, 1 cm; F and H, 4 mm. Drawn by K. Souvannakhoummane from Tagane *et al.* L1198 (FOF).

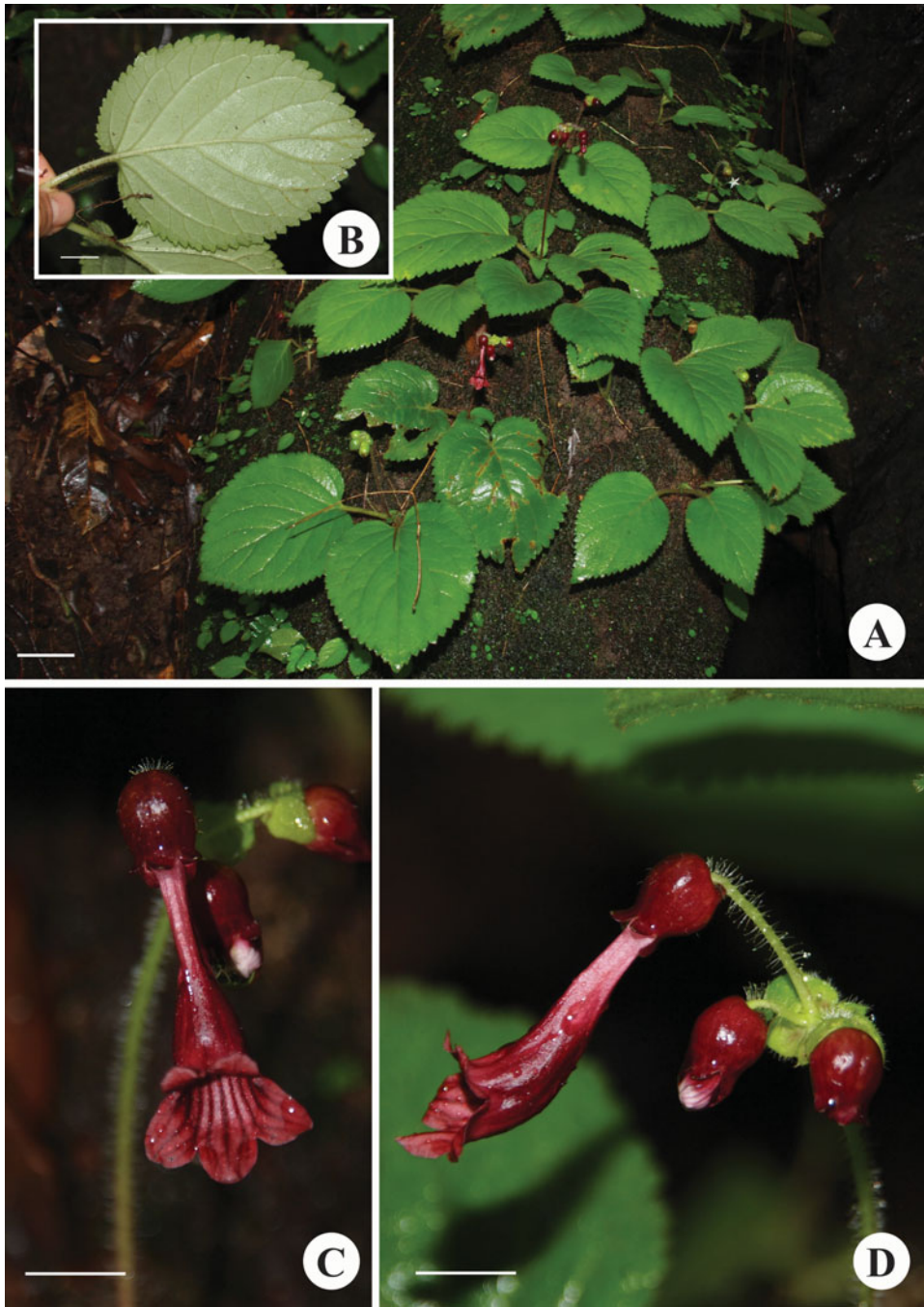


FIG. 2. *Didymocarpus middletonii* Souvann., Soulad. & Tagane in the type locality. A, Habit; B, abaxial surface of lamina; C, flower (front view); D, flower (lateral view). Scale bars: A, 4 cm; B–D, 1 cm. Photographed by S. Tagane on 29 June 2017.

TABLE 1. Diagnostic characters for *Didymocarpus middletonii* Souvann., Soulad. & Tagane and its relatives^a

Character	<i>Didymocarpus middletonii</i>	<i>Didymocarpus brevicalyx</i>	<i>Didymocarpus formosus</i>	<i>Didymocarpus puhoatensis</i>
Indumentum of stem	Densely glandular pubescent	Densely glandular hairs and scattered pigment glands	Sparsely eglandular hairs	Sparsely puberulent to glabrescent
Leaf blade (cm)	8–11.5 × 6.5–8.5	7–9 × 3.5–4.5	7–19 × 4–12	6–10 × 5–8
Leaf base	Cordate to broadly obtuse-cuneate or sometime truncate	Obtuse-cuneate	Base oblique, truncate on one side, cordate on the other side	Cordate
Secondary veins of lamina	7–9 pairs	4–6 pairs	6–8 pairs	4–8 pairs
Inflorescence	Up to 14.2 cm long, 1- to 3-flowered	Up to c.13 cm long, few-flowered	14 cm long, several-flowered	c.12 cm long, 4- to 10(–30)-flowered
Pedicels	7–12 mm long, reddish, covered with multicellular glandular hairs and multicellular eglandular hairs	3–6 mm long, reddish, glabrous	2–3 mm long, densely covered with multicellular glandular hairs	10–15 mm long, pale green, covered with multicellular glandular hairs

TABLE 1. *Continued*

Character	<i>Didymocarpus middletonii</i>	<i>Didymocarpus brevicealyx</i>	<i>Didymocarpus formosus</i>	<i>Didymocarpus puhoatensis</i>
Bracts	Orbicular, c.6 mm long and wide, green, with multicellular eglandular hairs	Triangular, c.2 mm long and 1.5 mm wide, reddish, glabrous	Orbicular, c.7 × 8 mm, purple	Orbicular, c.5 mm long and wide, greenish reddish, glabrous
Calyx	Urceolate, c.7.8–9.2 mm long, dark purplish, with multicellular eglandular hairs; lobes acute at apex	Campanulate, c.4 mm, reddish, glabrous; lobes acute at apex	Campanulate, 13 mm, pinkish, glabrous, lobes acute at apex	Campanulate, 6 mm long, purple, glabrous, lobes obtuse at apex
Corolla	Funnelform, c.3.4 cm long, dark purple-blackish, glabrous	Funnelform, c.4.5 cm long, dark purple-blackish, glabrous	Funnelform, c.6 cm long, dark red–maroon without streaks at throat, glabrous	Funnelform, 4–5 cm long, dark purple-blackish, glabrous
Filaments	Glabrous	Gland-tipped hairs on the upper part	Glabrous	Glabrous
Indumentum of ovary	Sparsely glandular puberulent	Densely glandular pubescent	Sparsely covered with a vesicular indumentum	Sparsely glandular puberulent

^a The measurements of *Didymocarpus brevicealyx* and *D. puhoatensis* are taken from Nangngam & Middleton (2014) and Hong *et al.* (2018), respectively.

Vernacular name. ດອກລຳໂພງຫີນສີມ່ວງ [Dok Lam Phong Hin Si Mouang (meaning: limestone purple funnel flower)].

GenBank accession nos. LC373520 (*rbcL*), LC373521 (*matK*) and LC373522 (ITS); we sequenced Tagane et al. L1198.

Preliminary conservation assessment. Critically Endangered (CR). *Didymocarpus middletonii* is known only from a single population, with c.30 individuals, in a very restricted area of limestone karst in Nam Kading National Protected Area. Therefore, we propose the status CR for this species according to the IUCN criterion CR B2a D (IUCN, 2012). The habitat is very close to the construction of Nam Kading Dam, which is likely to impinge on the survival of this species.

Didymocarpus middletonii is similar to *D. brevicalyx* but easily distinguished by its wider lamina (6.5–8.5 cm wide versus 3.5–4.5 cm wide), pedicel and bracts covered with glandular hairs (versus glabrous), smaller corollas with longer staminodes (corolla 3.4 cm long, staminodes 1–2 mm long versus corolla 4.5 cm long, staminodes 3–5 mm long). *Didymocarpus middletonii* is similar to *D. formosus* but easily distinguished by its small habit (versus 40 cm tall), bracts green (versus purple) and smaller corolla (versus 6 cm long). *Didymocarpus middletonii* is similar to *D. puhoatensis* but easily distinguished by its bracts covered with eglandular hairs (versus glabrous) and shorter pedicels (versus 1–1.5 cm long). A detailed morphological comparison of these allied species is given in Table 1.

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