A new species of Paraboea and a new species record of Middletonia (Gesneriaceae) from a limestone karst in Central Laos

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INTRODUCTION
The taxonomy of Gesneriaceae for the flora of Laos is being revised by the first and second authors. Several intensive surveys around Laos have been undertaken so far, these already resulting in the discovery of two new species of *Didymocarpus* Wall.: *D. middletonii* Souvann., Soulad. & Tagane and *D. albiflorus* Souvann. & Phonep. (Souvannakhoummane et al., 2019; Souvan-nakhoummane & Phonepaseuth, 2020). In this paper, we describe the new species *Paraboea khotamiae* Phonep. & Souvann., the third species of this genus known for the flora of Laos (Xu et al., 2008; Newman et al., 2017–, continuously updated), and also report the occurrence of *Middletonia reticulata* (Gesneriaceae) for the flora of Laos for the first time.

The genus *Paraboea* (C.B. Clarke) Ridl. is one of the largest genera of Gesneriaceae, comprising ca 130 species (Middleton, 2018; Puglisi & Phutthai, 2018). The genus was revised by Xu et al. (2008) and included 89 species and five varieties. Later, the limits of the genus were substantially revised by Puglisi et al. (2011). Thirty-one species have been recorded for China (Xu et al., 2017; He et al., 2018; Lu et al., 2019; Guo et al., 2020) and fewer than 20 species for Vietnam (Middleton, 2018). It is notable that ca 75 species are recorded in Thailand, among which 26 have been newly described since 2012 (Triboun & Middleton, 2012, 2015; Triboun, 2013; Puglisi et al., 2015; Puglisi & Phutthai, 2018). In Laos, only three species have been recorded, namely *Paraboea mahaxayana* Xu & Burtt, *P. multiflora* (R.Br.) B.L. Burtt and *P. thorelii* (Pellegr.) B.L. Burtt (Xu et al., 2008; Newman et al., 2017–, continuously updated), although *P. multiflora* has now been transferred to *Middletonia* as *M. multiflora* (R.Br.) C. Puglisi.

The genus *Middletonia* is a small genus which has been distinguished from the genus *Paraboea* (C.B. Clarke) Ridl. based on molecular and morphological data. Currently five species have been recognised from India, Bangladesh, Bhutan, China, Myanmar, Thailand, Laos, Vietnam, Cambodia and...
Malaysia (Puglisi et al., 2016). The diagnostic traits of *Middletonia* are the erect anthers in *Middletonia* (vs borne at a right angle in *Paraboea*); minutely glandular indumentum on the anthers and ovary; and reticulate tertiary veins, which are visible at least along the leaf margin; generally smaller corolla, and shorter fruits (Puglisi & Middleton, 2017). In Laos, two species of *Middletonia* have been recorded, *M. evrardii* (Pellegr.) C.Puglisi and *M. multiflora* (R.Br.) C.Puglisi (Puglisi et al., 2016; Puglisi & Middleton, 2017), both of which were previously recognised in *Paraboea*.

Central Laos, including Bolikhamxay and Khammouan Provinces, is known to be an area of high biological diversity in Laos, where limestone karst provides a particular habitat known for endemic plant species. In August 2019, the first author took photographs of an unknown *Paraboea* with old fruit in limestone karst in Gnommalat district, Khammouan Province. Subsequently, in August and November of the following year, the same species was collected by the first author. Using the relevant taxonomic literature, we compared our specimens to morphologically similar taxa from China, Malaysia, Thailand and Vietnam (Burtt, 1984; Xu et al., 2008; Tribouin & Middleton, 2012; Xu et al., 2017; Middleton, 2018) and compared the diagnostic characters with herbarium specimens kept in FOF, HNL and KAG, as well as online specimen data and images from AAU, AUH, BKF, E, K and P. As a result, this unknown species is considered to be most similar to *Paraboea bintangensis* B.L.Burtt., *P. prazeri* (B.L.Burtt) C.Puglisi and *P. robusta* (B.L.Burtt) C.Puglisi but we have concluded that the unknown taxon is morphologically different from these three. Thus, we here describe and illustrate it as a new species, *Paraboea khotamiae* Phonep. & Souvann., along with its ecological information and conservation status. In addition, we record *Middletonia reticulata* for the flora of Laos, which was previously known only from Thailand but was discovered during our field survey in Nam Kading National Protected Area (NPA), Bolikhamxay Province, central Laos, in July 2017.

**TAXONOMIC TREATMENT**

*Paraboea khotamiae* Phonep. & Souvann., sp. nov.

This species is most similar to *Paraboea bintangensis* B.L.Burtt from Peninsular Malaysia in its almost stemless habit, ovate or ovate-oblong leaves, axillary inflorescences and white flowers but differs in having 6–8 pairs of lateral veins anastomosing (vs ca 5 veins in *P. bintangensis*), 3 upper calyx lobes fused ½–¾ lobes length and 2 lower lobes free (vs 5 calyx lobes free), and larger corolla 1.6–1.8 cm long, 1.6–2.4 cm across (vs ca 1 cm long, ca 1.5 cm across). *Paraboea khotamiae* is also similar to *P. prazeri* (B.L.Burtt) C.Puglisi from Myanmar in having long petiolar leaves, cymose inflorescence, 3 upper calyx lobes fused, and lingulate stigma, but differs in having a broader stem 1–1.5 cm in diameter (vs 3–3.5 mm in diam. in *P. prazeri*), leaf blade ovate to ovate-oblong, glabrescent above (vs elliptic, pubescent with short hairs above), non-twisted capsules (vs twisted capsule). It also shares some similar characters with *Paraboea robusta* (B.L.Burtt) C.Puglisi from Thailand (Northern region) such as a white corolla, lingulate stigma, 3 upper calyx lobes fused, and cymose inflorescence, but differs in its shorter stem 0.8–1.2 cm long (vs at least 6 cm long in *P. robusta*), smaller ovate or ovate-oblong leaf blade 7–10 × 5–6 cm, glabrescent adaxially (vs narrowly elliptic-oblancoate, 12–25 × 2–5.5 cm, densely pilose-pubescent adaxially). Table 1. Type: Laos. Khammouan Province, Gnommalat District, in limestone karst, 30 Aug. 2020, *Phonepaseuth, P. P010* (holotype FOF [FOF00051941]; isotype HNL!). Figs. 1 & 2.

Lithophytic, perennial herb. Stem 0.8–1.2 cm tall, 1–1.5 cm in diameter. Leaves decussate, appearing in a rosette when congested, white when young, green when mature, petiolate; petiole 6–8 cm long, green, sparingly pubescent; blade ovate to ovate-oblong, 7–10 × 5–8 cm, apex obtuse, base cordate, margin crenate, adaxial side dark green, glabrescent, abaxial side light green, pubescent; midrib sunken above, prominent below, lateral veins 6–8 pairs, sunken above, prominent below, tertiary veins reticulate, sunken above, prominent below, visible. Inflorescence axillary, cymose, 2–4 per plant, each with 3–4 flowers; peduncle 3–6 cm long, greenish red to reddish brown, white puberulent; inflorescence bracts at the top of the peduncle, leaf-like, broadly ovate, ca 8 × 4 mm, covered with appressed velvety hairs; pedicels 3–4 cm long, covered with appressed velvety hairs. Calyx cotyliform, 8–12 mm long, 5-lobed, imbricate at base, greenish-brown, light green towards the apex, covered with stiff whitish multicellular hairs; upper 3 lobes fused ½–¾ of length, each lobe linear-oblong, 8–9 × ca 2 mm, apex rounded; lower 2 lobes free to the base, linear-elliptic,
8–9 × 1.8–2.4 mm, apex rounded. *Corolla* white, 1.6–1.8 cm long, 1.6–2.4 cm across, glabrous; tube 6–8 mm long, lobes orbicular or widely ovate, 6–7 × 8–10 mm, apex rounded. *Stamens* 2, inserted near base of corolla tube; filaments ca 3 mm long, white, glabrous and geniculate; anthers yellow, broadly ovoid, ca 2 × 3 mm, dehiscing along centre of anther locules, coherent. *Staminodes* 3, 2 laterals ca 0.8 mm long, middle one ca 0.4 mm long, white to pale green, glabrous. *Pistil* 1–1.6 cm long, white at base, green towards apex, glabrous; ovary 3–4 mm long; style ca 8 mm long, ca 0.5 mm in diam.; stigma linguiform, thicker than style, 1 mm in diam. *Capsule* cylindric, linear ovoid, 10–11 × 2–2.5 mm, non-twisted, glabrous, drying brown.

**Etymology.**—The specific epithet “*khotamiae*” is coined in honour of Khotamy Sayyasone, who was the teacher of the first author in the field of biology and taxonomy.

**Distribution.**—Laos (only known from the type locality).

**Ecology.**—*Paraboea khotamiae* grows on shaded limestone cliffs, associated with *Begonia* spp. (Gesneriaceae), *Amorphophallus* spp. (Araceae) and some lithophytic ferns; ca 200 m elev. Flowering from August to September, fruiting September to November.

**Table 1.** Detailed comparison of *Paraboea khotamiae* Phonep. & Souvann., *P. bintangensis* B.L.Burtt, *P. praerzi* (B.L.Burtt) C.Puglisi and *P. robusta* (B.L.Burtt) C.Puglisi.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Paraboea khotamiae</th>
<th>Paraboea bintangensis</th>
<th>Paraboea praerzi</th>
<th>Paraboea robusta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>0.8–1.2 cm long, 1–1.5 cm in diameter</td>
<td>stemless</td>
<td>1–5 cm long, 3–3.5 mm in diameter</td>
<td>at least 6 cm long and 1 cm in diameter</td>
</tr>
<tr>
<td>Petiole length</td>
<td>6–8 cm long</td>
<td>3–7 cm long</td>
<td>1–4 cm long elliptic, pubescent with short hairs above</td>
<td>less than 2.5 cm long narrowly elliptic, oblanceolate, densely scabrid-pubescent above</td>
</tr>
<tr>
<td>Leaf blade</td>
<td>ovate, ovate-oblong, glabrescent above</td>
<td>ovate, elliptic, or slightly obovate or oblong, glabrescent above</td>
<td>3–8-flowered</td>
<td>4–6-flowered</td>
</tr>
<tr>
<td>Leaf base</td>
<td>cordate</td>
<td>oblique</td>
<td>attenuate</td>
<td>attenuate</td>
</tr>
<tr>
<td>Leaf blade size</td>
<td>7–10 × 5–6 cm</td>
<td>4–10 × 2–4.5 cm</td>
<td>5–15 × 3–6 cm</td>
<td>10–16 × 2–5.5 cm</td>
</tr>
<tr>
<td>Number of lateral veins</td>
<td>6–8 pairs</td>
<td>5 pairs</td>
<td>6–8 pairs</td>
<td>11–15 pairs</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>cymose</td>
<td>umbelliform</td>
<td>cymose</td>
<td>cymose</td>
</tr>
<tr>
<td>Number of flowers per inflorescence</td>
<td>3–4-flowered</td>
<td>3–8-flowered</td>
<td>3–5-flowered</td>
<td>4–6-flowered</td>
</tr>
<tr>
<td>Peduncles length</td>
<td>3–6 cm long</td>
<td>5–8 cm long</td>
<td>4–6 cm long</td>
<td>1.7–8 cm long</td>
</tr>
<tr>
<td>Inflorescence bracts</td>
<td>leaf-like, widely ovate, ca 8 × 4 mm</td>
<td>leaf-like, widely ovate, ca 13 × 9 mm</td>
<td>leaf-like, suborbicular, ca 5 × 5 mm</td>
<td>ovate-elliptic, ca 20 × 8 mm</td>
</tr>
<tr>
<td>Calyx lobe size</td>
<td>8–9 × 1.8–2.4 mm</td>
<td>5–7 × ca 1.5 mm</td>
<td>ca 7 × 5 mm for upper lobes, ca 4.5 × 2 mm for lower lobes</td>
<td>ca 16 × 12 mm</td>
</tr>
<tr>
<td>Corolla</td>
<td>white, 1.6–1.8 cm long, 1.6–2.4 cm across</td>
<td>white, ca 1 cm long, ca 1.5 cm across</td>
<td>ca 1.4 cm long, ca 8 mm across</td>
<td>white, ca 2 cm long, ca 1.6 mm across</td>
</tr>
<tr>
<td>Corolla tube length</td>
<td>6–8 mm long</td>
<td>ca 2 mm long</td>
<td>ca 8 mm long</td>
<td>10–12 mm long</td>
</tr>
<tr>
<td>Filament length</td>
<td>ca 3 mm long</td>
<td>ca 2.5 mm long</td>
<td>ca 2.25 mm long</td>
<td>ca 5 mm long</td>
</tr>
<tr>
<td>Staminode length</td>
<td>&gt; 1 mm long</td>
<td>&lt; 1 mm long</td>
<td>&lt; 1 mm long</td>
<td>not seen</td>
</tr>
<tr>
<td>Capsules</td>
<td>cylindrical, linear ovoid, 10–11 × 2–2.5 mm, non-twisted, glabrous</td>
<td>linear, 1.4–1.5 cm long, non-twisted, glabrous</td>
<td>1.1–2 cm long, twisted</td>
<td>not seen</td>
</tr>
</tbody>
</table>

**Note:** 1Xu et al. (2008); 2Burtt (1984), 3Isotypes Prazer 93 (K000858069, US00064713)
Figure 1. *Paraboea khotamiae* Phonep. & Souvann.: A. Flower (front view); B. Flower (lateral view); C. Calyx lobes; D. Corolla opened out (adaxial side); E. Pistil; F. Stamens (front view); G. Stamens (lateral view); H. Capsule with persistent stigma. Materials from *Phonepaseuth P010* (FOF). Line drawing by K. Souvannakhounmane.
Vernacular name.—

Additional specimen examined (paratype).—Khammouan Province, Gnommalat District, in limestone karst, 21 Nov. 2020, Phonepaseuth et al. P015 (FOF!)

Provisional conservation assessment.—Critically Endangered (CR B1ab(iii), B2ab(iii), D) (IUCN, 2019). *Paraboea khotamiae* is so far known only from one locality and occupies an area of less than 0.1 km$^2$. Fewer than 50 mature individuals are known. The locality is not in a protected area and is being promoted as a nature trail for tourists (to view the limestone karst in Gnommalat District), which may disturb the habitat and threaten this new species. In addition, some of the limestone karsts in the district are being destroyed for an active cement plant which may completely destroy their habitat in the future. An effective conservation action plan is required.

Figure 2. *Paraboea khotamiae* Phonep. & Souvann.: A. Habitat; B. Flowering plant; C. Leaves adaxial side (below) and abaxial side (above); D. Inflorescences; E. Flower (front view); F. Flower (back view); G. Lateral view of flower bud (left) and flower (right); H. Dissected flower showing corolla, stamens, pistil and calyx; J. Fruits. Photos (A–H) from Phonepaseuth P010 (FOF) and (J) Phonepaseuth et al. P015 (FOF) by P. Phonepaseuth.
NEW RECORD


Specimen examined.—Bolikhamxay Province: Nam Kading NPA, 18°12′17.9″, 104°33′34.5″, 146 m elev., 26 June 2017, Tagane et al. L973 (FOF!, FU, TAI). Fig. 3.

Distribution.—Laos (Bolikhamxay Province) and Thailand (Northern).

Vernacular name.—ʻຊາລືສີ (Sa leusee, suggested here).

Ecology in Laos.—On the semi-shaded rocks of limestone karst, at the edge of evergreen forest. Flowering in June.

Notes.—_Middletonia reticulata_ is here newly recorded for the flora of Laos. It was reported to be endemic to Thailand by Puglisi & Middleton (2017) but they also predicted its likely occurrence in Laos and Myanmar. Here we confirm its distribution in Laos based on our voucher specimens collected from Nam Kading NPA. Measurements of the characters of the above-mentioned Lao specimen are within the previously known variation (Barnett, 1961; Puglisi & Middleton, 2017).

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Figure 3. _Middletonia reticulata_ (Barnett) C. Puglisi: A. Habit; B. Lower side of plant showing abaxial leave surface. C. Inflorescence (front view); D. Lateral view of inflorescence; Photos from Tagane et al. L973 (FOF!) by S. Tagane.
Carmen Puglisi (Royal Botanic Gardens Kew) and David J. Middleton (Singapore Botanic Gardens) for their helpful comments. We sincerely thank two anonymous reviewers for their thoughtful comments and helpful suggestions. We would also like to thank the curators and staff of the following herbaria: FOF, HNL and KAG for their kind permission to study the herbarium specimens in their care. Field collection in Nam Kading NPA was supported by the Environment Research and Technology Development Fund (4-1601) of the Ministry of the Environment, Japan, and MEXT/JSPS KAKENHI (Grant Number JP15H02640).

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