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

PHONGPHAYBOUN PHONEPASEUTH^{1,*}, KEOOUDONE SOUVANNAKHOUMMANE², SHUICHIRO TAGANE³, PHETLASY SOULADETH⁴ & TETSUKAZU YAHARA⁵

ABSTRACT

A new species of *Paraboea* (Gesneriaceae), *P. khotamiae*, from a limestone karst in central Laos, is described and illustrated. The species resembles *Paraboea bintangensis*, *P. prazeri* and *P. robusta*, but differs in leaf shape and floral features, especially corolla shape and size, the interior of the corolla tube, and capsule characters. In addition, we report the occurrence of *Middletonia reticulata* (Gesneriaceae) for the flora of Laos for the first time.

KEYWORDS: Angiosperms, flora of Laos, limestone flora, taxonomy. Accepted for publication: 2 June 2021. Published online: 11 June 2021

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; Souvannakhoummane & 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 (Barnett) C.Puglisi in central Laos, the third species of Middletonia C.Puglisi recorded for the country (Puglisi & Middleton, 2017).

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

¹ Faculty of Environmental Sciences, National University of Laos, Dongdok Campus, Xaythany District, Vientiane Capital, Laos.

² Centre for Development and Environment, Laos country office, Unit 11, #136, Simuang Road, Hom 1, P.O. Box 1438, Ban Phapo, Vientiane, Laos.

³ The Kagoshima University Museum, Kagoshima University, Kagoshima, Japan.

⁴ Faculty of Forestry Science, National University of Laos, Dongdok Campus, Xaythany District, Vientiane Capital, Laos.

⁵ Center for Asian Conservation Ecology, Kyushu Open University, 6-10-1 Hakozaki, Fukuoka, 812-8581 Japan.

^{*} Corresponding author: p.phonepaseuth@nuol.edu.la

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; Triboun & 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 ½-3/4 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 petiolate 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 ovateoblong, 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-oblanceolate, 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 [FOF0005194!]; 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, sparsely pubescent; blade ovate to ovateoblong, $7-10 \times 5-8$ 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, $8-9 \times \text{ca 2 mm}$, 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. (Begoniaceae), 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. prazeri* (B.L.Burtt) C.Puglisi and *P. robusta* (B.L.Burtt) C.Puglisi.

Characters	Paraboea khotamiae	Paraboea bintangensis¹	Paraboea prazeri ^{2,3}	Paraboea robusta ²
Stem	0.8–1.2 cm long, 1–1.5 cm in diameter	stemless	1–5 cm long, 3–3.5 mm in diameter	at least 6 cm long and 1 cm in diameter
Petiole length	6-8 cm long	3–7 cm long	1-4 cm long	less than 2.5 cm long
Leaf blade	ovate, ovate-oblong, glabrescent above	ovate, elliptic, or slightly obovate or oblong, glabrescent above	elliptic, pubescent with short hairs above	narrowly elliptic, oblanceolate, densely scabrid- pubescent above
Leaf base	cordate	oblique	attenuate	attenuate
Leaf blade size	$7-10 \times 5-6 \text{ cm}$	$4-10 \times 2-4.5 \text{ cm}$	$5-15 \times 3-6 \text{ cm}$	$10-16 \times 2-5.5 \text{ cm}$
Number of lateral veins	6–8 pairs	5 pairs	6–8 pairs	11–15 pairs
Inflorescence	cymose	umbelliform	cymose	cymose
Number of flowers per inflorescence	3–4-flowered	3–8-flowered	3–5-flowered	4–6-flowered
Peduncles length	3-6 cm long	5–8 cm long	4–6 cm long	1.7-8 cm long
Inflorescence bracts	leaf-like, widely ovate, ca 8 × 4 mm	leaf-like, widely ovate, ca 13 × 9 mm	leaf-like, suborbicular, ca 5 × 5 mm	ovate-elliptic, ca 20 × 8 mm
Calyx lobe size	8–9 × 1.8–2.4 mm	5–7 × ca 1.5 mm	ca 7×5 mm for upper lobes, ca 4.5×2 mm for lower lobes	ca 16 ×12 mm
Corolla	white, 1.6–1.8 cm long, 1.6–2.4 cm across	white, ca 1 cm long, ca 1.5 cm across	ca 1.4 cm long, ca 8 mm across	white, ca 2 cm long, ca 1.6 mm across
Corolla tube length	6–8 mm long	ca 2 mm long	ca 8 mm long	10–12 mm long
Filament length	ca 3 mm long	ca 2.5 mm long	ca 2.25 mm long	ca 5 mm long
Staminode length	> 1 mm long	< 1 mm long	< 1 mm long	not seen
Capsules	cylindric, linear ovoid, 10–11 × 2–2.5 mm, non-twisted, glabrous	linear, 1.4–1.5 cm long, non-twisted, glabrous	1.1–2 cm long, twisted	not seen

Note: ¹Xu et al. (2008); ²Burtt (1984), ³Isotypes *Prazer 93* (K000858069, US00064713)

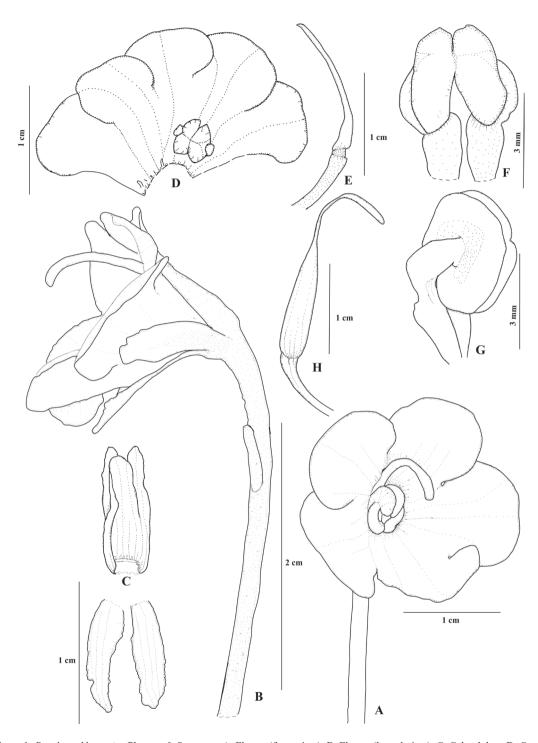


Figure 1. Paraboea khotamiae Phonep. & Souvann.: A. Flower (front view); B. Flower (lateral view); C. Calyx lobes; D. Corolla opened out (adaxial side); E. Pistil; F. Stamens (front view); G. Stamens (lateral view); H. Capsule with persistent stigma. Materials from *Phonepaseuth P010* (FOF). Line drawing by K. Souvannakhoummane.

Vernacular name.— ดอกละตั้ງโถตะมี (Dok lakang khotamy, suggested here).

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 km². 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.

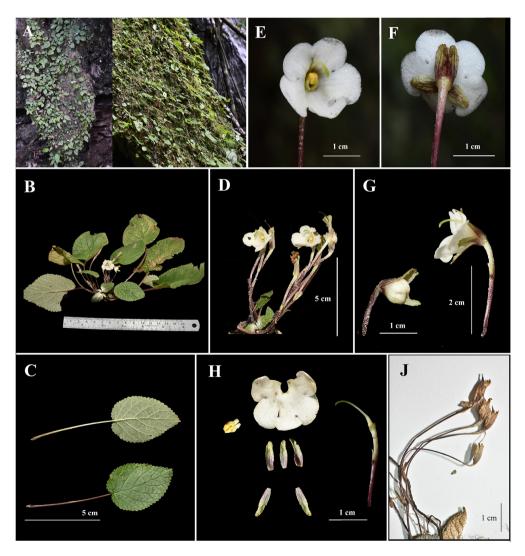


Figure 2. *Paraboea khotamiae* Phonep. & Souvann.: A. Habitat; B. Flowering plant; C. Leaves adaxial side (below) and abaxial side (above); D. Inflorescences; E. Flower (front view); F. Flower (back view); G. Lateral view of flower bud (left) and flower (right); H. Dissected flower showing corolla, stamens,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

NEW RECORD

Middletonia reticulata (Barnett) C.Puglisi, Thai Forest Bull., Bot. 45(1): 39 (2017).—*Boea reticulata* Barnett, Nat. Hist. Bull. Siam Soc. 20: 20 (1961). Type: Thailand, Chiang Mai Province, Mae Wang, *Kerr, A.F.G. 6356* (lectotype **K** [K000196614, digital image!], designated by Barnett (1961: 256); isolectotypes **ABD**, **BM**).

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.— ຊາລືສີ (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).

ACKNOWLEDGEMENTS

We are grateful to Sackda Phasavaeng, Visala Chounlamany and Sisavath Chansavang for guiding us during the field surveys in the Nakai and Gnommalat Districts, Khammouan Province, and to the director and staff of Nam Kading NPA for allowing us to conduct field surveys in the protected area. The authors wish to thank Che-Wei Lin (Herbarium of Taiwan Forestry Research Institute),

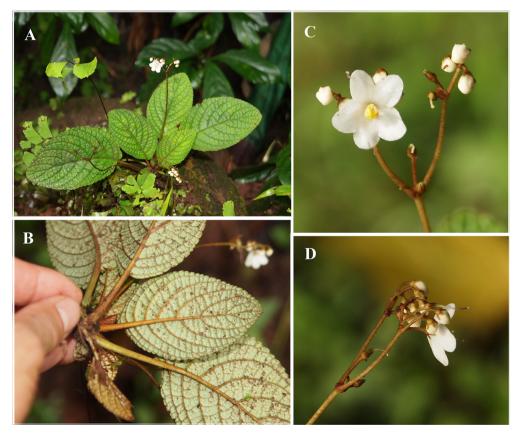


Figure 3. *Middletonia reticulata* (Barnett) C.Puglisi: A. Habit; B. Lower side of plant showing abaxial leave surface. C. Inflorescence (front view); D. Lateral view of inflorescence; Photos from *Tagane et al. L973* (FOF!) by S. Tagane.

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).

REFERENCES

- Barnett, E.C. (1961). Contributions to the Flora of Thailand: LV. Kew Bulletin 15: 249–259.
- Burtt, B.L. (1984). Studies in the Gesneriaceae of the Old World: XLVII. Revised generic concepts for *Boea* and its allies. Notes from the Royal Botanic Garden, Edinburgh 41: 401–452.
- Guo, Z., Wu, Z., Xu, W., Li, Z. & Xiang, X. (2020). *Paraboea dolomitica* (Gesneriaceae), a new species from Guizhou, China. PhytoKeys 153: 37–48.
- He, D.M., Feng, Y.F., Pan, F.Z., Hong, X. & Wen, F. (2018). *Paraboea wenshanensis*, a new species of Gesneriaceae from Yunnan, China. PhytoKeys 95: 83–91.
- IUCN (2019). Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. Downloadable from http://www. iucnredlist.org/documents/RedListGuidelines. pdf. [Accessed 4 Sep 2020]
- Lu, Z.C., Liu, E.D., Han, M.Q., Zhu, X.X., Nguyen, K.S. & Xu, W.B. (2019). Discovery of *Paraboea minutiflora* (Gesneriaceae) from southeastern Yunnan, China with supplementary description. Guihaia (online) DOI:10.11931/guihaia. gxzw201909027
- Middleton, D.J. (2018). Two new species of Paraboea (Gesneriaceae) from Vietnam. Edinburgh Journal of Botany 75(3): 421–425.
- Newman, M.F., Pullan, M., Ketphanh, S., Svengsuksa, B., Thomas, P., Sengdala, K., Lamxay, V. & Armstrong, K. (2017–, continuously updated). A checklist of the vascular plants of Lao PDR. Online database available at https://padme.rbge. org.uk/laos/.

- Puglisi, C. & Middleton, D.J. (2017). A revision of Middletonia (Gesneriaceae) in Thailand. Thai Forest Bulletin (Botany) 45(1): 35–41.
- Puglisi, C. & Phutthai, T. (2018). A new species of *Paraboea* (Gesneriaceae) from Thailand. Edinburgh Journal of Botany 75(1): 51–54.
- Puglisi, C., Middleton, D.J., Triboun, P. & Möller, M. (2011). New insights into the relationships between *Paraboea*, *Trisepalum*, and *Phylloboea* (Gesneriaceae) and their taxonomic consequences. Taxon 60(6): 1693–1702.
- Puglisi, C., Suddee, S., Triboun, P. & Middleton, D.J. (2015). A new species of *Paraboea* (Gesneriaceae) from Thailand. Gardens' Bulletin Singapore 67: 101–106.
- Puglisi, C., Yao, T.L., Milne, R., Moller, M. & Middleton, D.J. (2016). Generic recircumscription in the Loxocarpinae (Gesneriaceae), as inferred by phylogenetic and morphological data. Taxon 65 (2): 277–292.
- Souvannakhoummane, K. & Phonepaseuth, P. (2020). *Didymocarpus albiflorus* (Gesneriaceae), a new species from Vientiane capital, Lao PDR. Taiwania 65(2): 109–113.
- Souvannakhoummane, K., Souladeth, P., Tagane, S., Yang, C.-J. & Yahara, T. (2019). Flora of Nam Kading National Protected Area VI: *Didymocarpus middletonii* (Gesneriaceae), a new species from limestone. Edinburgh Journal of Botany 76(1): 45–54.
- Triboun, P. (2013). *Paraboea middletonii* (Gesneriaceae), a new species from Thailand. Thai Forest Bulletin (Botany) 41: 45–47.
- Triboun, P. & Middleton, D.J. (2012). Twenty new species of *Paraboea* (Gesneriaceae) from Thailand. Gardens' Bulletin Singapore 64: 333–370.
- _____. (2015). Three new species of *Paraboea* (Gesneriaceae) from Thailand. Thai Forest Bulletin (Botany) 43: 18–23.
- Xu, Z., Burtt, B.L., Skog, L.E. & Middleton, D.J. (2008). A revision of *Paraboea* (Gesneriaceae). Edinburgh Journal of Botany 65: 161–347.
- Xu, W.B., Guo, J., Pan, B., Zhang, Q. & Liu, Y. (2017). Diversity and distribution of Gesneriaceae in China. Guihaia 37(10): 1219–1226.