**Lysimachia fanii**, a new species of Primulaceae from limestone area of Guangxi, China

Yun-Feng Huang\(^1\), Li-Na Dong\(^2\), Wei-Bin Xu\(^2\)

\(^1\) Guangxi Key Laboratory of Traditional Chinese Medicine Quality Standards, Guangxi Institute of Chinese Medicine & Pharmaceutical Sciences, Nanning 530022, Guangxi, China
\(^2\) Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, Guangxi, China

Corresponding author: Li-Na Dong (donglina2014@163.com); Wei-Bin Xu (gxibwbxu@163.com)

**Citation:** Huang Y-F, Dong L-N, Xu W-B (2019) *Lysimachia fanii*, a new species of Primulaceae from limestone area of Guangxi, China. In: Cai J, Yu W-B, Zhang T, Li D-Z (Eds) Revealing of the plant diversity in China’s biodiversity hotspots. PhytoKeys 130: 75–84. [https://doi.org/10.3897/phytokeys.130.34655](https://doi.org/10.3897/phytokeys.130.34655)

**Abstract**

*Lysimachia fanii*, a new species of *Lysimachia* (Subgen. *Idiophyton*, Primulaceae), is described and illustrated from Guangxi, China based on morphological and molecular data. *Lysimachia fanii* differs from *L. verbascifolia*, *L. rupestris* and *L. alpestris* mainly by the habit being nearly rosulate, leaves congested at the apex of the rhizome, leaf blades spatulate to narrowly oblanceolate and flowers solitary. Phylogenetic analyses supported *L. verbascifolia* as sister to *L. fanii*. This new species is endemic to limestone areas in Liucheng county of Guangxi, China.

**Keywords**

*Lysimachia*, subgen. *Idiophyton*, Primulaceae, taxonomy, limestone flora

**Introduction**

The genus *Lysimachia* L. (1753: 146) includes about 190 species and was originally placed in Primulaceae (Cronquist 1981, Takhtajan 1997), but later transferred into Myrsinaceae, based on morphological and molecular evidence (Anderberg and Ståhl 1995, Anderberg et al. 1998, 2002, Källersjö et al. 2000, Hao et al. 2004). Myrsinaceae was later merged into Primulaceae s.l., hence *Lysimachia* was replaced into...
The majority of species within the genus are distributed in temperate and subtropical regions of the Northern Hemisphere, with some species in Africa, Australia and South America. In China, the genus has 138 species (Hu and Kelso 1996) and is highly diversified in south-western China, especially in limestone areas. According to the flower and gland morphology, the genus is separated into five subgenera, viz. subgen. *Idiophyton* Hand.-Mazz., subgen. *Lysimachia*, subgen. *Palladia* (Moench) Hand.-Mazz., subgen. *Heterostylanandra* (Hand.-Mazz.) F.H.Chen & C.M.Hu and subgen. *Naumburgia* (Moench) Klatt. (Chen and Hu 1979, Chen et al. 1989).

The south-western limestone karst area is one of China’s biodiversity hotspots. These areas are fragile and sensitive to environmental change and, in the wake of the rapid economic development of China, they are facing serious threat. Documentation of the plant diversity in these regions is urgently needed. Thus, we are surveying traditional medicinal plants in the limestone areas of Guangxi and trying to increase our knowledge of these poorly studied areas. During fieldwork in May 2018, we discovered an unknown species in *Lysimachia*. This species is allied to subgen. *Heterostylanandra* by having rosette leaves, but it differs in having heteromorphic flowers. It shows alliance to subgen. *Idiophyton*, subgen. *Lysimachia* and subgen. *Palladia* by having 5-merous flowers, but has unique filaments, anthers and glands. After morphological observation and consulting relevant literature (Chen and Hu 1979, Chen et al. 1989, Hu and Kelso 1996, Tong et al. 2017), we confirm that the rare plant is a new species and has been placed into subgen. *Idiophyton*, based on morphology and molecular analyses.

**Material and methods**

**Taxon sampling**

We followed the classification of *Lysimachia* of Chen et al. (1989) and Hu and Kelso (1996). Leaves were collected from the holotype (L.Y. Fan et al., FLY2018001 in GXMI) and paratypes (L.Y. Fan et al., FLY2018002 in IBK & GXMI) to represent the new species. Twenty related taxa within subgen. *Idiophyton*, one taxon within subgen. *Heterostylanandra* and four taxa within subgen. *Lysimachia* were selected to ascertain the phylogenetic relationships within *Lysimachia* (Anderberg et al. 2002). Based on Yan et al. (2018), *Pelletiera verna* A. St.-Hil. and *Anagallis monelli* L. were selected as outgroups.

**DNA sequencing**

Total genomic DNA was extracted from silica-dried plant leaves by a modified CTAB protocol (Doyle and Doyle 1987). Four chloroplast DNA regions (*atpF-atpH*, *rpl32-trnL*, *trnL-F* and *rrnS-trnG*) and one nuclear loci (ITS) were selected and amplified following Yan et al. (2018). Genebank Accession Numbers are listed in Table 1.
Table 1. Species of Lysimachia and related taxa sampled and GenBank accession numbers of sequences used in this study.

| Taxa                | atpF-atpH | rpl32-trnL | trnL-F | trnS-trnG | ITS     |
|---------------------|-----------|------------|--------|-----------|---------|
| Anagallis monelli   | MG950735  | MG950945   | MG951268 | MG951373  | MG877752|
| L. alpestris        | MG950743  | MG950953   | MG951276 | MG951381  | MG877760|
| L. bavensis         | MG950773  | MG950983   | MG951306 | MG951410  | MG877790|
| L. capillipes       | MG950748  | MG950958   | MG951281 | MG951386  | MG877765|
| L. chapaensis       | MG950749  | MG950959   | MG951282 | MG951387  | MG877766|
| L. confertifolia    | MG950757  | MG950967   | MG951290 | –          | MG877774|
| L. crispidens       | MG950759  | MG950969   | MG951292 | MG951396  | MG877776|
| L. engleri          | MG950765  | MG950975   | MG951298 | MG951402  | MG87782 |
| L. foemum-graecum   | MG950770  | MG950980   | MG951303 | MG951407  | MG87787 |
| L. heterobotrys     | MG950779  | MG950989   | MG951311 | MG951415  | MG87796 |
| L. insignis         | MG950784  | MG950994   | MG951316 | MG951420  | MG87801 |
| L. lancifolia       | MG950788  | MG960998   | MG951320 | MG951424  | MG877805|
| L. laxa             | MG950789  | MG950999   | MG951321 | MG951425  | MG877806|
| L. longipes         | MG950792  | MG951002   | MG951324 | MG951428  | MG877809|
| L. microcarpa       | MG950796  | MG951006   | MG951328 | MG951432  | MG877813|
| L. millettii        | MG950797  | MG951007   | MG951329 | MG951433  | MG877814|
| L. nemorum          | MG950799  | MG951009   | MG951331 | MG951435  | MG877816|
| L. mutantiflora     | MG950801  | MG951011   | MG951333 | MG951437  | MG877818|
| L. peduncularis     | MG950805  | MG951015   | MG951337 | –          | MG877822|
| L. petelotii        | MG950808  | MG951018   | MG951340 | –          | MG877825|
| L. pittosporoides   | MG950810  | MG951020   | MG951342 | MG951445  | MG877827|
| L. punctata         | MG950813  | MG951023   | MG951345 | MG951448  | MG877830|
| L. trichopoda       | MG950826  | MG951038   | MG951359 | MG951461  | MG877845|
| L. verbascifolia    | MG950827  | MG951039   | MG951360 | MG951462  | MG877846|
| L. vittiformis      | MG950828  | MG951040   | MG951361 | MG951463  | MG877847|
| Pelletiera verna    | MG950832  | MG951044   | MG951365 | MG951467  | MG877851|
| L. fanii 01         | MK516268  | MK516270   | MK516272 | –          | MK516275|
| L. fanii 02         | MK516269  | MK516271   | MK516273 | MK516274  | MK516276|

Phylogenetic analysis

Sequences of each DNA region were aligned using MUSCLE 3.8.31 (Edgar 2004a, 2004b) and adjusted manually where necessary. Indels were treated as gaps and all regions were combined as a single region for further study.

Maximum Parsimony (MP) analyses were conducted using PAUP v.4.0b10 (Swofford 2002). Heuristic searches were carried out with 1000 replicates and tree-bisection-reconnection (TBR) branch swapping. A strict consensus tree was summarised from all the most parsimonious trees. Node support was assessed by 500 bootstrap replicates using TBR branch swapping.

Bayesian Inference (BI) analyses were conducted using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). The Markov chain Monte Carlo (MCMC) chains were run for 100 000 generations while trees were sampled every 100 generations. The MCMC chains were stopped when the average standard deviation of the split frequencies was 0.008 after 100 000 generations, which meant that the chains were converged to a stationary distribution. A majority-rule consensus tree was constructed after removing a burn-in of 25% of the trees. Posterior Probability (PP) values were used to estimate branch support.
Figure 1. Phylogenetic tree inferred by MP and BI analyses based on the combined dataset of four plastid loci (atpF-atpH, rpl32-trnL, trnL-F and trnS-trnG) and nuclear ITS. Numbers above branches indicate maximum parsimony bootstrap/Bayesian inference posterior probability.

Results

Molecular systematic relationship

In total, 29 atpF-atpH, rpl32-trnL, trnL-F and ITS sequences and 25 trnS-G sequences were included. The combined matrix has a length of 3649 aligned characters (ITS: 653bp, atpF-atpH: 512bp, rpl32-trnL: 728bp, trnL-trnF: 946bp, trnS-G: 810bp), of which 363 are parsimony informative. The inferred phylogenies using MP and BI analyses are congruent (Fig. 1). The two samples of the new species (L. fani)
are clustered into subgenus *Idiophyton* with strong support values in both MP and BI analyses (BS= 100%, PP = 0.99). *L. verbascifolia* is placed as the sister group to *L. fanii* with high support in the BI analysis (PP = 0.92).

**Taxonomic treatment**

*Lysimachia fanii* Y.Feng Huang, W.B.Xu & L.N.Dong, sp. nov.
urn:lsid:ipni.org:names:60479343-2
Figs 2, 3

**Type.** CHINA. Guangxi Zhuangzu Autonomous Region: Liucheng County, Taiping Town, 23°42'50"N, 109°29'20"E, 320 m a.s.l., 21 May 2018, flowering, L.Y. Fan et al. FLY2018001 (holotype, GXMI!; isotypes, IBK!, GXMI!).

**Diagnosis.** *Lysimachia fanii* differs from congeneric species in subgen. *Idiophyton* mainly by the habit being nearly rosulate, leaves congested at the apex of the rhizome, leaf blades spatulate to narrowly oblanceolate and flowers being solitary.

**Description.** Herbaceous perennial, glabrous. Rhizome subterete, 6–8 cm long, 4–6 mm in diameter, branched at the apex of the rhizome. Leaves papery, thickly papery to thinly leathery when dry, spirally arranged, congested at the apex of the rhizome, ± forming a rosette, subsessile, spatulate to narrowly oblanceolate, 6–21 × 0.6–2.0 cm, tapering towards the base, apex acute to obtuse, glabrous adaxially, glandular abaxially, veins invisible on both sides. Flowers solitary, axillary. Pedicel 3.0–6.0 cm long, ca. 1 mm in diameter, densely glandular. Calyx lobes lanceolate, 5–6 × ca. 3 mm, 5 (rarely 6), separate to near the base, apex acuminate, glabrous inside, glandular outside. Corolla yellow, deeply parted, tube 0.5–1.0 mm; lobes broadly ovate, 7.0 × 6.0 mm, apex obtuse, glabrous on both sides. Filaments ca. 1.5 mm long, lower 0.5 mm connate into a tube; anthers 3–3.5 mm long, ca. 1 mm in diameter, basifixed, opening by apical pores. Ovary globose, ca. 1 mm in diameter; style 2.8 mm long, slightly shorter than stamens. Capsule globose, 3.5–4 mm in diameter.

**Phenology.** Flowering from May to June.

**Etymology.** The new species is named after Mr. Li-Yong Fan, who first discovered and collected this rare species.

**Distribution and habitat.** *Lysimachia fanii* is known only from the type locality in Taiping Town, Liucheng County, Guangxi Zhuangzu Autonomous Region, China (Fig. 4). It grows on moist limestone rock surfaces at the entrance to caves.

**Additional specimens examined.** CHINA. Guangxi: Liucheng County, Taiping Town. 320 m a.s.l., 21 May 2018, L.Y. Fan et al. FLY2018002 (IBK, GXMI).

**Taxonomic notes.** Based on the molecular phylogeny, *L. fanii* belongs to subgenus *Idiophyton*, that is also supported by the morphological characters of basifixed anthers, short filaments and anthers open by apical pores. *L. fanii* is morphologically similar to *L. verbascifolia* C.M.Hu & L.K.Phan that is endemic to limestone areas in Vietnam (Phan and Hu 2011), but can be easily distinguished by its spatulate to nar-
rowly oblanceolate leaf blade and glabrous adaxially and glandular abaxially. *L. fanii* and *L. alpestris* Champ. ex Benth. resemble each other in having congested leaves and spatulate to narrowly oblanceolate leaf blades and invisible veins and solitary inflorescences but *L. fanii* differs from *L. alpestris* by its rhizome which is branched at the

*Figure 2. Lysimachia fanii. A Habit B flower, frontal view C flower, back view (showing six calyx lobes) D corolla opened showing stamens E calyx and pistil F capsule. (Drawn by X.C. Qu from the holotype).*
Lysimachia fanii, a new species from China

Figure 3. Lysimachia fanii. A Habitat B habit C flower, frontal view D flower, back view E flower, lateral view F stamens.
apex without stolons from the base, leaf blade glabrous adaxially and glandular abaxially, basifixed anthers which open by apical pores. *L. fanii* is also similar to *L. rupestris* F.H. Chen & C.M. Hu from limestone areas distributed in south-western China and northern Vietnam (Tong et al. 2017), but it can be distinguished from the latter by its rhizome which is branched at the apex and without stolons from the base, leaf blade spatulate to narrowly oblanceolate and glabrous adaxially, lateral veins invisible on both sides. A comparison of the main characters of the four species is shown in Table 2.

**Table 2.** Comparison of characters amongst *Lysimachia fanii*, *L. verbascifolia*, *L. rupestris* and *L. alpestris*.

| Morphological traits | *L. fanii* | *L. verbascifolia* | *L. rupestris* | *L. alpestris* |
|----------------------|------------|--------------------|----------------|---------------|
| **Rhizome**          | 6–8 cm long, branched at the apex | 4–10 cm long, geniculate at the base | 2–5 cm long, with stolons from the base | 1–4 cm long, with stolons from the base |
| **Leaf blade**       | spatulate to narrowly oblanceolate, 6–21 × 0.6–2.0 cm | elliptic to broadly elliptic, 7–17 × 3.5–8.0 cm | elliptic-oblanco-olate, 3–6.5 × 1.2–2.2 cm | spatulate to narrowly oblanceolate, 3–6 × 0.6–1.5 cm |
| **Leaf indumentum**  | glabrous adaxially, glandular abaxially | greyish villous on both sides | minutely glandular on both sides | dense long coarse greyish hairs on both sides |
| **Lateral veins**    | invisible on both sides | obvious, densely greyish villous | prominent abaxially | invisible on both sides |
| **Inflorescence**    | flowers solitary | subumbellate | flowers solitary | flowers solitary |
| **Corolla**          | yellow, deeply parted, tube 0.5–1.0 mm | pale yellow, divided nearly to the base | yellow, divided nearly to the base | yellow, deeply parted, tube 1–1.5 mm |
| **Filaments**        | ca. 1.5 mm long, lower 0.5 mm connate into a tube | ca. 3 mm long, connate basally into a ring | ca. 1 mm long, connate basally into a ring | ca. 3 mm long, lower 1.5 mm connate into a tube |
| **Anthers**          | 3-3.5 mm long, basifixed, opening by apical pores | ca. 5 mm long, basifixed, opening by apical pores | 4–5 mm long, basifixed, opening by apical pores | ca. 2 mm long, dorsifixed, opening by lateral slits |
| **Flower**           | May to June | June to October | April to May | April |

**Figure 4.** The distribution of *Lysimachia fanii* in Guangxi, China.
Lysimachia fanii, a new species from China

Acknowledgements

The authors are grateful to Mr. Xincheng Qu (Guangxi Institute of Chinese Medicine & Pharmaceutical Sciences) for the fine line drawings, to Mr. Xiaomao Huang and Mr. Wanlong Wei for their help in the field, to Dr. Mark Hughes (Royal Botanic Garden, Edinburgh) for his helpful comments. This study was supported by Science & Technology Basic Resources Investigation Program of China (Grant no. 2017FY100100) and also in part by The Chinese Medicine Public Health Service Subsidy Special “National Chinese Medicine Resources Census Project” (CS 2017, No. 6) and the Construction of a Joint Laboratory for Quality Research of Traditional Chinese Medicine in Guangxi and Macao (GuiKe AD17195002).

References

Anderberg AA, Ståhl B (1995) Phylogenetic interrelationships in the order Primulales, with special emphasis on the family circumscriptions. Canadian Journal of Botany 73: 1699–1730. https://doi.org/10.1139/b95-184

Anderberg AA, Ståhl B, Källersjö M (1998) Phylogenetic relationships in the Primulales inferred from rbcL sequence data. Plant Systematics and Evolution 211: 93–102. https://doi.org/10.1007/BF00984914

Anderberg AA, Rydin C, Källersjö M (2002) Phylogenetic relationships in the order Ericales s.l.: Analyses of molecular data from five genes from the plastid and mitochondrial genomes. American Journal of Botany 89(4): 677–687. https://doi.org/10.3732/ajb.89.4.677

Chen FH, Hu CM (1979) Taxonomic and phytogeographic studies on Chinese species of Lysimachia. Zhiwu Fenlei Xuebao 17: 21–53.

Chen FH, Hu CM, Fang YY, Cheng CZ (1989) Lysimachia (Primulaceae). In: Flora Reipublicae Popularis Sinicae. Vol. 59, Science Press, Beijing, 1–133.

China Phylogeny Consortium (2016) Tree of life for the genera of Chinese vascular plants. Journal of Systematics and Evolution 54(4): 277–306. https://doi.org/10.1111/jse.12219

Cronquist A (1981) An Integrated System of Classification of Flowering Plant. Columbia University Press, New York.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Edgar RC (2004a) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Edgar RC (2004b) MUSCLE: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5(1): 113. https://doi.org/10.1186/1471-2105-5-113

Hao G, Yuan YM, Hu CM, Ge XJ, Zhao NX (2004) Molecular phylogeny of Lysimachia (Myrsinaceae) based on chloroplast rnl-L-F and nuclear ribosomal ITS sequences. Molecular Phylogenetics and Evolution 31(1): 323–339. https://doi.org/10.1016/S1055-7903(03)00286-0

Hu CM, Kelso S (1996) Primulaceae. In: Wu ZY, Raven PH (Eds) Flora of China. Vol. 15. Science Press, Beijing & Missouri Botanical Garden Press, St Louis, 39–189.
Källersjö M, Bergqvist G, Anderberg AA (2000) Generic realignment in primuloid families of the Ericales s.l.: A phylogenetic analysis based on DNA sequences from three chloroplast genes and morphology. American Journal of Botany 87(9): 1325–1341. https://doi.org/10.2307/2656725

Phan KL, Hu CM (2011) Lysimachia vietnamensis and L. verbascifolia spp. nov. (Primulaceae) from Vietnam. Nordic Journal of Botany 29(5): 601–604. https://doi.org/10.1111/j.1756-1051.2011.01174.x

Ronquist E, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Swofford DL (2002) PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4.0b10. Sinauer Associates, Sunderland.

Takhtajan AL (1997) Diversity and Classification of Flowering Plants. New York: Columbia University Press.

Tong YH, Xia NH, Lam LV (2017) Lysimachia rupestris F.H.Chen & C.M.Hu (Primulaceae): A new record for the flora of Vietnam and reconsideration of its taxonomical position. Adansonia 39(2): 125–128. https://doi.org/10.5252/a2017n2a3

Yan HF, Zhang CY, Anderberg AA, Hao G, Ge XJ, Wiens JJ (2018) What explains high plant richness in East Asia? Time and diversification in the tribe Lysimachieae (Primulaceae). New Phytologist 219: 436–448. https://doi.org/10.1111/nph.15144