



## *Lysimachia liujiangensis* (Primulaceae), a new species from limestone area of central Guangxi, China

Zhao-Cen LU<sup>1</sup>, Quan YUAN<sup>1,2</sup>, Su-Juan WEI<sup>1</sup>, Li-Na DONG<sup>1,\*</sup>, Wei-Bin XU<sup>1,\*</sup>

1. 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.

2. College of Life Sciences, Guangxi Normal University, Guilin 541004, Guangxi, China.

\*Corresponding authors' email: LND: donglina2014@163.com, WBX: gxibwbxu@163.com

(Manuscript received 3 November 2020; Accepted 31 January 2021; Online published 24 February 2021)

**ABSTRACT:** *Lysimachia liujiangensis*, a new species of *Lysimachia* subg. *Idiophyton* Hand.-Mazz. from central Guangxi, China, is described and illustrated based on morphological and molecular data. The phylogenetic analyses showed that *Lysimachia liujiangensis* belongs to subg. *Idiophyton* and closely related to *L. millietii* (H.Lév.) Hand.-Mazz.. However, *Lysimachia liujiangensis* can be distinguished from *L. millietii* by its stems densely dark purple-glandular at the top and terete in upper part, leaf blade oblanceolate, pedicel 1.3–2.2 cm long, dark purple-glandular, calyx lobes 5–7 mm long, dark purple-glandular on both sides and margin.

**KEY WORDS:** *Lysimachia* subg. *Idiophyton*, *Lysimachia millietii*, limestone flora, Primulaceae.

### INTRODUCTION

The genus *Lysimachia* L. (1753) includes about 160–210 species (Chen *et al.*, 1989; Hu and Kelso, 1996; Ståhl and Anderberg, 2004; Sun *et al.*, 2020), is one of the largest genera in the family Primulaceae (APG III, 2009; China Phylogeny Consortium, 2016; Stevens, 2017). The genus is mainly distributed in temperate and subtropical regions of Northern Hemisphere, and rare in Africa, Australia and South America (Chen *et al.*, 1989; Hu and Kelso, 1996). The species of *Lysimachia* are highly diversified in East Asia (Yan *et al.*, 2018). Especially in China, there is nearly 150 species (Zhou *et al.*, 2015; Wang *et al.*, 2016; Yan *et al.*, 2017; Wang *et al.*, 2018; Huang *et al.*, 2019; Huang *et al.*, 2020) and one of the diversity centers of the genus.

During the field work in 2019, an unfamiliar species of *Lysimachia* was collected from limestone hills in Liujiang, Guangxi, China. After carefully checking morphological characters of this species and consulting relevant literature (Chen and Hu, 1979; Hu, 1985, 1992, Chen *et al.*, 1989; Hu and Kelso, 1996), we confirmed that it is a new species belonging to *Lysimachia* subg. *Idiophyton* Hand.-Mazz. (1928). More lines of evidences on morphological characters and molecular phylogenetic relationships of this new species are given below.

### MATERIALS AND METHODS

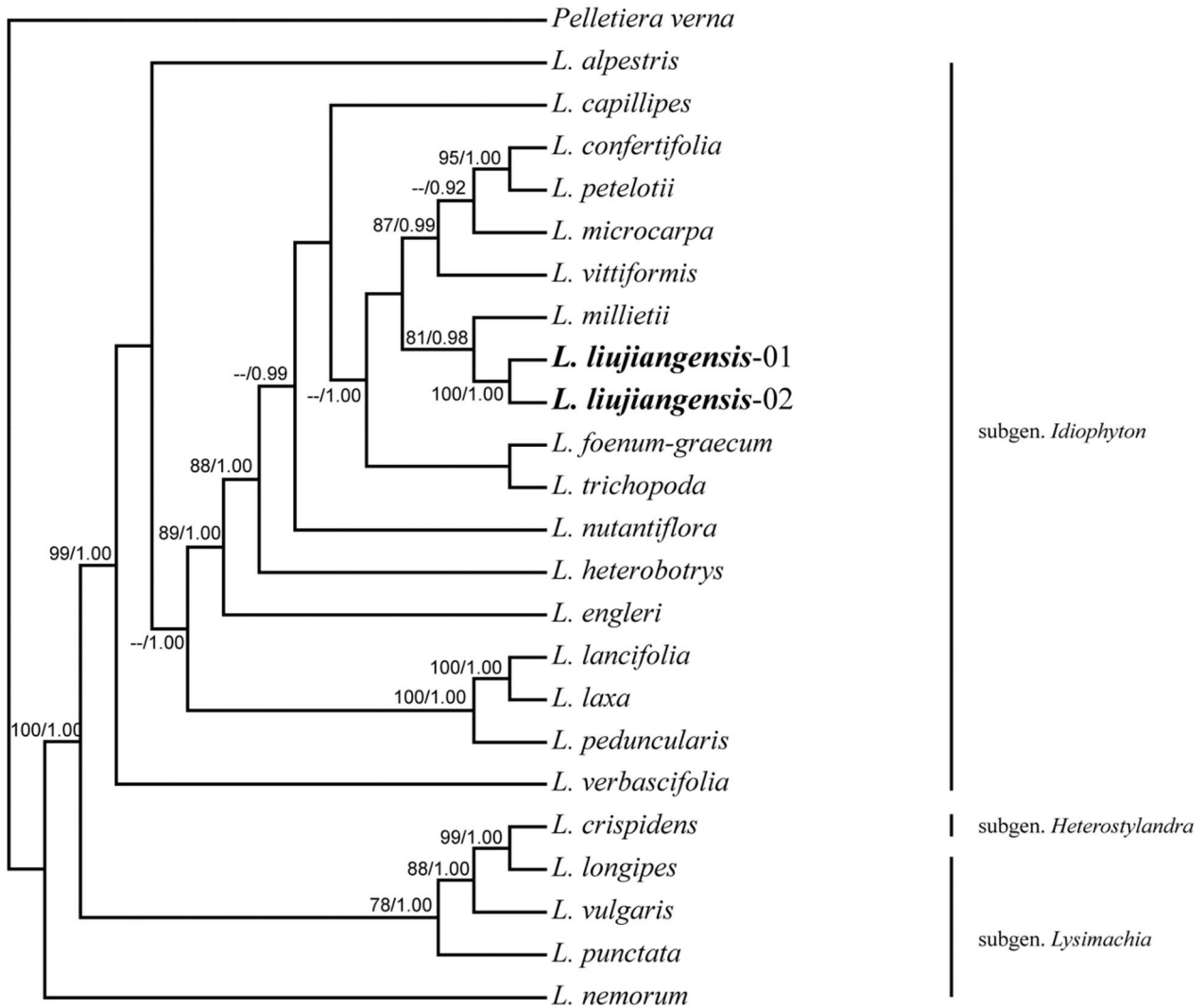
#### Taxon sampling

The vouchers of the new species were collected by Liujiang Expedition from type locality and are kept in herbarium, Guangxi Institute of Botany (IBK). We followed the classification of *Lysimachia* by Chen *et al.* (1989) and Hu and Kelso (1996). Two samples (*L.*

*liujiang*-01 from isotype and *L. liujiang*-02 from holotype) were selected to represent the new species for the phylogenetic analysis. In order to infer the phylogenetic position of the new species, we selected 21 related taxa within subgenera *Idiophyton*, *Heterostylandra* (Hand.-Mazz.) Chen & C.M.Hu (1979) and *Lysimachia* within the genus *Lysimachia* to reconstruct the phylogenetic relationships (Table 1). *Pelletiera verna* A. St.-Hil (1822) was selected as outgroup (Yan *et al.*, 2018).

**Table 1.** Species of *Lysimachia* and related taxa sampled and GenBank accession numbers of sequences used in this study.

Taxa	ITS	atpF-atpH	trnL-trnF	rpl32-trnL
<i>L. alpestris</i>	MG877760	MG950743	MG951276	MG950953
<i>L. capillipes</i>	MG877765	MG950748	MG951281	MG950958
<i>L. confertifolia</i>	MG877774	MG950757	MG951290	MG950967
<i>L. crispidens</i>	MG877776	MG950759	MG951292	MG950969
<i>L. engleri</i>	MG877782	MG950765	MG951298	MG950975
<i>L. foenumgraecum</i>	MG877787	MG950770	MG951303	MG950980
<i>L. heterobotrys</i>	MG877796	MG950779	MG951311	MG950989
<i>L. lancifolia</i>	MG877805	MG950788	MG951320	MG950998
<i>L. laxa</i>	MG877806	MG950789	MG951321	MG950999
<i>L. longipes</i>	MG877809	MG950792	MG951324	MG951002
<i>L. microcarpa</i>	MG877813	MG950796	MG951328	MG951006
<i>L. millietii</i>	MG877814	MG950797	MG951329	MG951007
<i>L. nemorum</i>	MG877816	MG950799	MG951331	MG951009
<i>L. nutantiflora</i>	MG877818	MG950801	MG951333	MG951011
<i>L. peduncularis</i>	MG877822	MG950805	MG951337	MG951015
<i>L. punctata</i>	MG877830	MG950813	MG951345	MG951023
<i>L. trichopoda</i>	MG877845	MG950826	MG951359	MG951038
<i>L. verbascifolia</i>	MG877846	MG950827	MG951360	MG951039
<i>L. vittiformis</i>	MG877847	MG950828	MG951361	MG951040
<i>L. vulgaris</i>	MG877848	MG950829	MG951362	MG951041
<i>L. liujiangensis</i> 01	MT801181	MT798850	MT798854	MT798852
<i>L. liujiangensis</i> 02	MT801182	MT798851	MT798855	MT798853
<i>Pelletiera verna</i>	MG877851	MG950832	MG951365	MG951044



**Fig. 1.** Phylogenetic tree inferred by MP and BI analyses based on the combined dataset of three plastid loci (*atpF-atpH*, *rp32-trnL*, and *trnL-F*) and nuclear ITS. Numbers above branches indicate maximum parsimony bootstrap/bayesian inference posterior probability.

### Pollen grains and seeds observing

Pollen grain samples were taken from the holotype. Seed samples were taken from the specimen (*Liujiang Exped. 45021190703002LY*). The seeds were fixed by Glutaraldehyde Fixed solution and dehydrated using graded ethanol. Pollen grains and seeds were mounted on stubs, coated with gold in a sputter coater, and observed with a Zeiss EV018 scanning electron microscope (SEM) at 25kV for pollen grains and 20kV for seeds. Terminology for pollen and seed morphology follows Wei (2003) and Oh *et al.* (2008), respectively.

### DNA sequencing

The leaves of the new species were collected and dried with silica-gel. Total genomic DNA was extracted by a modified CTAB protocol (Doyle and Doyle, 1987). Three chloroplast DNA regions (*atpF-atpH*, *trnL-F*, and *rp32-trnL*) and one nuclear loci (ITS) were amplified and sequenced. The primers of chloroplast DNA regions

were according to Yan *et al.* (2018). The primers of ITS were designed as follow: ITS-LJU (CCG CGC CAA AAT TCA CA) and ITS-LJD (AGA CRA GAC CGC ACG CGA CAA AAT).

### Phylogenetic analysis

The sequences of related taxa were downloaded from GenBank. Sequences were aligned using MUSCLE online (<https://www.ebi.ac.uk/Tools/msa/muscle/>, Madeira *et al.*, 2019) and adjusted manually. The indels were treated as gaps. The congruence between plastid sequences and nuclear loci ITS was tested with the incongruence length difference (ILD) test with 100 replicates of default heuristic search using PAUP v4.0a (Swofford, 2002). The p value was 0.36 more than 0.05 (Farris *et al.*, 1994). Thus we combined all datasets for phylogenetic analysis. Maximum Parsimony (MP) analysis was conducted using PAUP v4.0b10. Heuristic searches were carried out with 1000 replicates and tree-



bisection-reconnection (TBR) with no reconnection limit. The strict consensus tree was referred from all the most parsimonious trees. Node support was assessed by 1000 bootstrap replicates using TBR branch swapping. Bayesian Inference (BI) analysis was conducted using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). The Markov chain Monte Carlo (MCMC) chains were run for 1 000 000 generations while trees were sampled every 100 generations. Average standard deviation of split frequencies was 0.002396 after 1 000 000 generations. The consensus tree was constructed after burn-in 25% of the trees. The branch supports were estimated according to Posterior Probability (PP) values.

## RESULTS

The combined matrix includes 24 taxa of *atpF-atpH* (507 bp), *rpl32-trnL* (693 bp), *trnL-trnF* (923 bp) and ITS (637 bp), totally 2760 bp aligned characters. These data have provided 262 parsimony informative characters within 292 variable characters. The phylogenetic trees of MP and BI analyses are congruent (Fig. 1). The new species (*L. liujiangensis*) belongs to subg. *Idiophyton* with strong support values in both MP and BI analysis (BS= 100%, PP= 1.00). *L. liujiangensis* is closely related to *L. millietii* with high support in the BI analysis (PP= 0.98).

## TAXONOMIC TREATMENT

*Lysimachia liujiangensis* W.B. Xu, Z.C. Lu & L.N. Dong, *sp. nov.*

柳江香草 Figs. 2–4 & 5A

**Type:** CHINA. Guangxi Zhuang Autonomous Region: Liujiang County, Baipeng Town, Fenlong Village, 24°10'02.15"N, 109°12'31.56"E, 170 m a.s.l., 1 April 2019, flowering, *Liujiang Exped. 450221190401052LY* (holotype, IBK00427520; isotypes, IBK00427522, GXMG).

**Diagnosis:** *Lysimachia liujiangensis* is related to *Lysimachia millietii* (H.Lév.) Hand.-Mazz. (in Brotherus et al. 1936) (Fig. 5B), but differs by the stems densely dark purple-glandular at the top and terete in upper part, leaf blade oblanceolate, pedicel 1.3–2.2 cm long, dark purple-glandular, calyx lobes 5–7 mm long, dark purple-glandular on both sides and margin.

**Description:** Herbaceous perennial. **Stems** ascendent, terete, 25–45 cm tall, densely dark purple-glandular at the top, branched from the base. **Leaves** thickly papery to thinly leathery when dry, alternate, oblanceolate, 2.8–6.1 × 0.8–2.1 cm, base attenuate to cuneate, apex acute, margin revolute, glabrous to sparsely dark purple-glandular adaxially, dark purple-glandular abaxially, **lateral veins** 2–3 on each side of midrib, flat adaxially and slightly prominent abaxially, veinlets invisible, **petiole** 4–8 mm long, densely dark purple-glandular. **Flowers** solitary, axillary. **Pedicel**

1.3–2.2 cm long, ca. 0.5 mm in diameter, dark purple-glandular. **Calyx lobes** lanceolate, 5–7 × 1.2–1.9 mm, separate to near the base, apex acuminate, dark purple-glandular on both sides and margin. **Corolla** yellow, deeply parted, tube 0.5–1 mm; lobes lanceolate, 7–9 × 3.5–4 mm, apex acuminate, glabrous on both sides. Filaments ca. 1.5 mm long, lower 0.5 mm connate into a tube; **anthers** ca. 5 mm long, ca. 1 mm in diameter, basifixed, opening by apical pores; pollen grains small, 10.1 (8.4–10.8) × 11.4 (9.5–12.7) μm, spheroidicity or subspherical, P/E = 0.89, tricolporate, pori long, exine coarsely rugulate (Fig. 4 A, B). **Ovary** globose, ca. 1.2 mm in diameter; **style** ca. 5 mm long. **Capsule** globose, 4–4.5 mm in diameter. **Seeds** brown or dark brown, sectoroid, 0.95–1.25 × 0.6–1 × 0.35–0.45 mm (length × width × thickness), tuberculate on ventral surface, weakly colliculate on dorsal surface, hilum nearly linear (Fig. 4 C–F).

**Phenology:** Flowering from March to April, and fruiting from July to August.

**Etymology:** The specific epithet '*liujiangensis*' refers to the type locality of this new species.

**Distribution and habitat:** *Lysimachia liujiangensis* is known only from the type locality in Baipeng Town, Liujiang County, Guangxi Zhuang Autonomous Region, China (Fig. 6). It grows in shrub of slope of limestone hills.

**Additional specimens examined:** CHINA. Guangxi: Liujiang County, Baipeng Town, Fenlong Village, 24°10'02.15"N, 109°12'31.56"E, 170 m a.s.l., 1 April 2019, flowering, *Liujiang Exped. 450221190401054LY* (IBK00427523, GXMG); *ibid.*, 3 July 2019, fruiting, *Liujiang Exped. 450221190703002LY* (IBK00427524, GXMG); *ibid.*, 14 April 2020, flowering, *W.B. Xu & Z.C. Lu 14176* (IBK00427521).

**Taxonomic notes:** *Lysimachia liujiangensis* belongs to subg. *Idiophyton* Hand.-Mazz. in the morphological characters of basifixed anthers, shorter filaments and anthers open by apical pores. Phylogenetic analyses are consistent with the morphological characters (Fig. 1). The placement of *L. liujiangensis* into subg. *Idiophyton* is strong supported by both in maximum parsimony analysis and Bayesian analysis (BS= 99%, PP= 1.00) and closely related with *Lysimachia millietii* (H.Lév.) Hand.-Mazz. (Fig. 1). *Lysimachia liujiangensis* is similar to *Lysimachia millietii* (H.Lév.) Hand.-Mazz. (Fig. 5B) in the characters of stem branched from the base, leaves base cuneate, margin revolute and thick papery, lateral veins 2–3 pairs and veinlets invisible, but can be easily distinguished from the latter by the stems densely dark purple-glandular at the top and terete in upper part, leaf blade oblanceolate, pedicel 1.3–2.2 cm long, dark purple-glandular, calyx lobes 5–7 mm long, dark purple-glandular on both sides and margin, flowering March–April. A detailed comparison of the two species is shown in Table 2. Thus, we confirmed that *L. liujiangensis* is a new species within *Lysimachia* subg. *Idiophyton* based on morphological and molecular analyses.

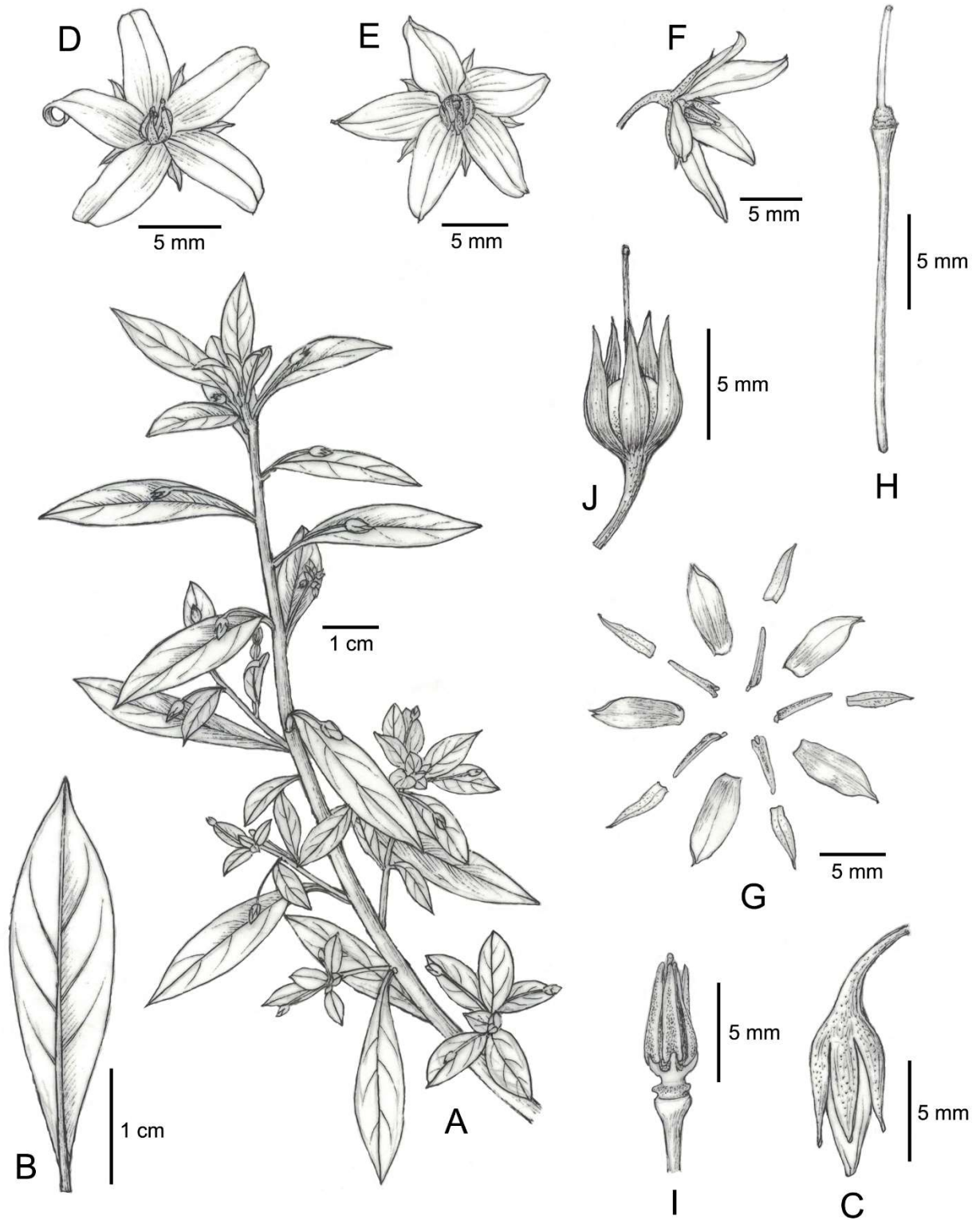
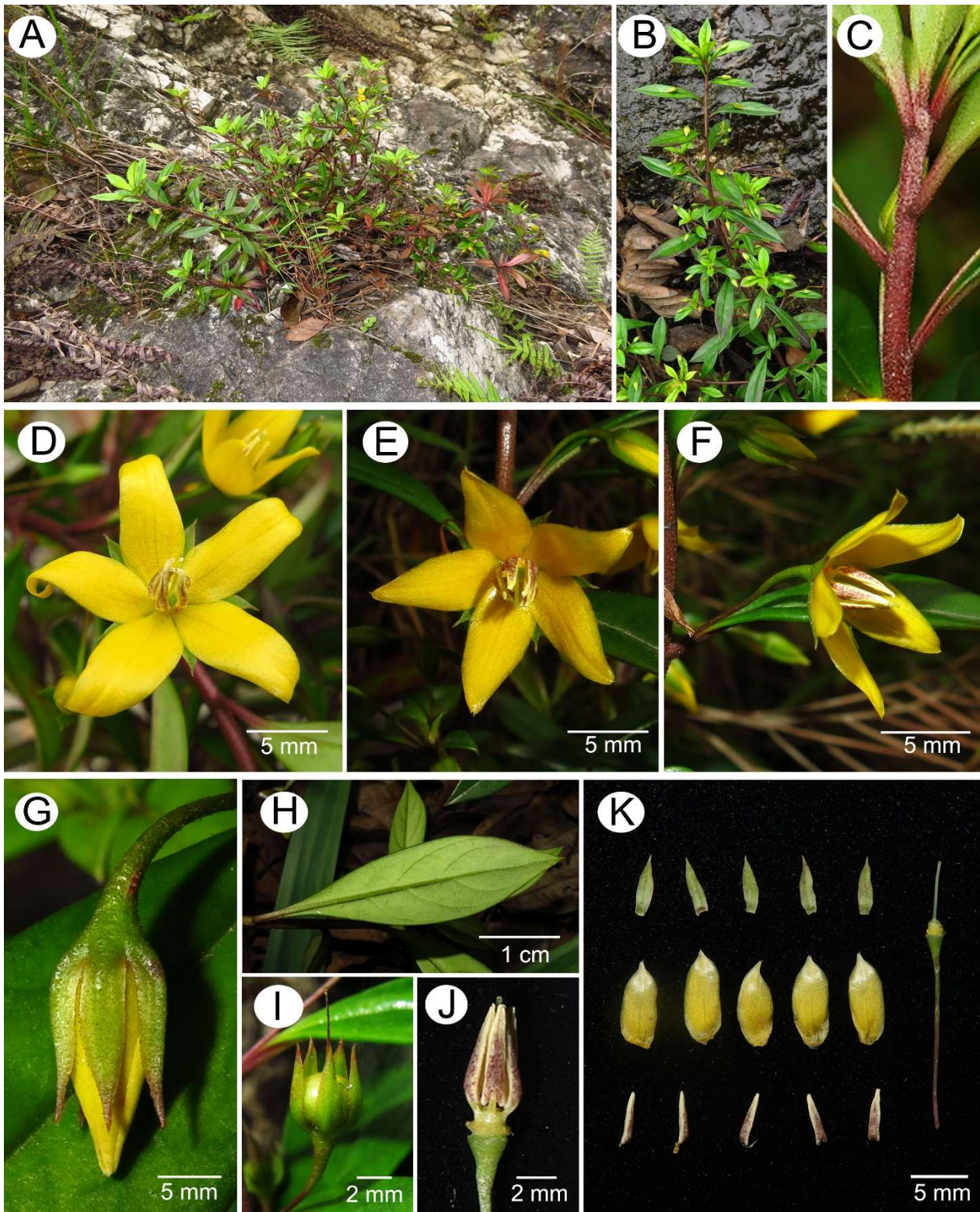


Fig. 2. *Lysimachia liujiangensis*: A. A part of plant, B. Lower surface of leaf, C. Calyx, D and E. Flower, frontal view, F. Flower, lateral view, G. Flower anatomy, H. Pistil, I. Stamens, J. Capsule. (Drawn by W.H. Lin from the holotype and paratype)



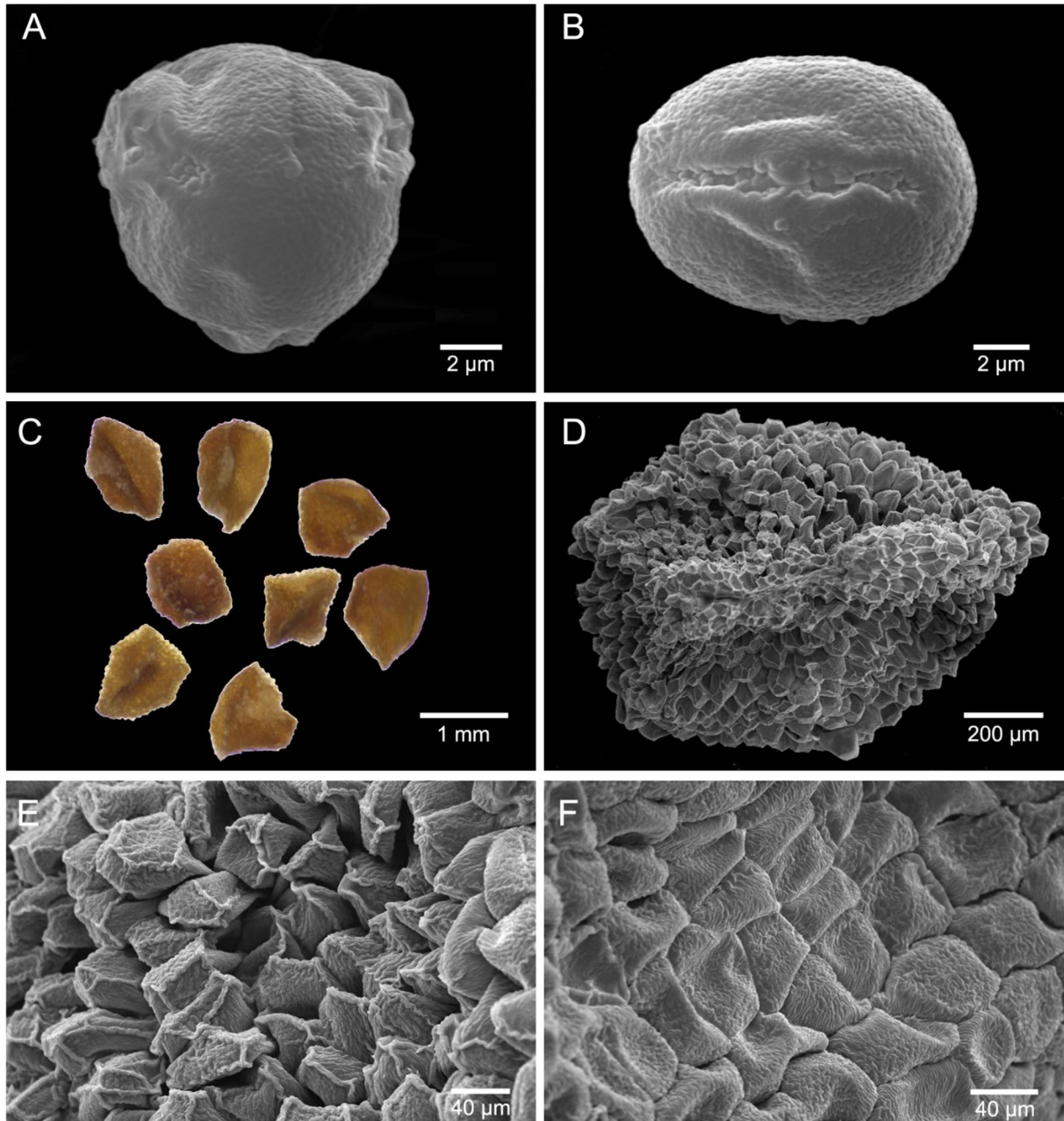


**Fig. 3.** *Lysimachia liujiangensis*: **A.** Habitat, **B.** Habit, **C.** Stem, showing densely dark purple-glandular at the top, **D** and **E.** Flower, frontal view, **F.** Flower, lateral view, **G.** Calyx, **H.** Lower surface of leaf, **I.** Capsule, **J.** Stamens, **K.** Flower anatomy.



**Table 2.** Comparison of characters between *Lysimachia liujiangensis* and *L. millietii*.

Taxonomic traits	<i>L. liujiangensis</i>	<i>L. millietii</i>
Stem	25–45 cm tall, densely dark purple-glandular at the top, terete in upper part	40–60 cm tall, no glandular, obviously angular in upper part
Leaf blade	oblanceolate, 2.8–6.1 × 0.8–2.1 cm, glabrous to sparsely with dark purple-glandular on both sides	lanceolate, 3–4.5 × 0.5–1.5 cm, abaxially glaucous, adaxially dark green
Petiole	4–8 mm long, densely dark purple-glandular	3–5 mm long, no glandular
Pedicele	1.3–2.2 cm long, dark purple-glandular	2.5–3 cm long, no glandular
Calyx lobes	5–7 mm long, dark purple-glandular on both sides and margin	ca. 3 mm long, no glandular
Corolla lobes	lanceolate, 7–9 × 3.5–4 mm, apex acuminate	narrowly oblong, ca. 5 × 2 mm, apex subobtusate
Anthers	ca. 5 × 1 mm	ca. 3.8 × 0.8 mm
Flowering	March–April	June

**Fig. 4.** A–B: Pollen grain of *Lysimachia liujiangensis* under SEM. A. Polar view, B. Equatorial view. C. Seeds of *Lysimachia liujiangensis* under Microscope. D–F: Seeds of *Lysimachia liujiangensis* under SEM. D. Ventral view with elongate hilum. E. Tuberculate pattern on ventral surface. F. Colliculate pattern on dorsal surface.

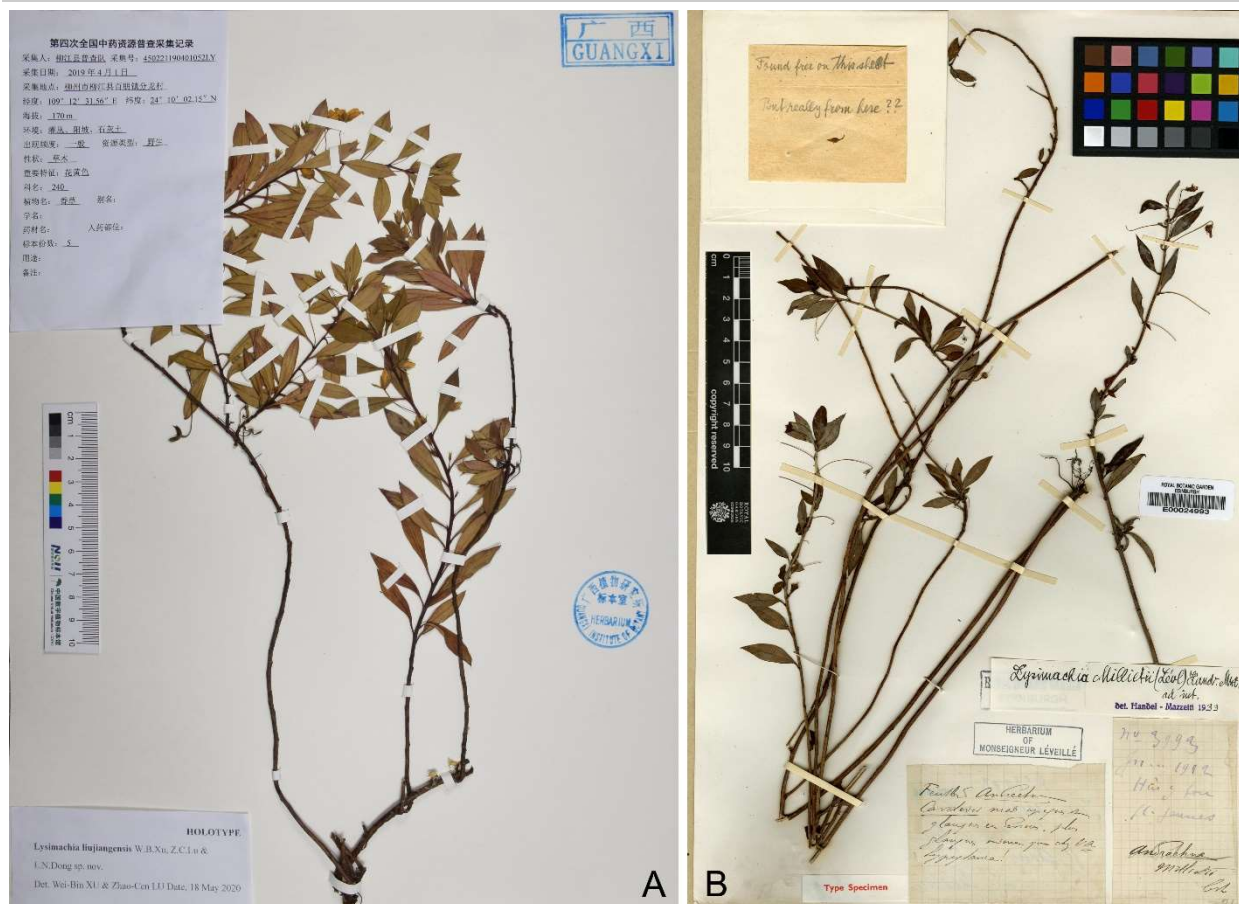


Fig. 5. Type sheets of *Lysimachia liujiangensis* (A) and *Lysimachia millietii* (B)



Fig. 6. The distribution of *Lysimachia liujiangensis* in Guangxi, China.

**ACKNOWLEDGMENTS**

The authors are grateful to Mr. Wen-Hong Lin (IBK) for the fine line drawings, to Miss. Yu-Jing Wei for SEM technical help, and also to Mr. Ping Yang and Mr. Jin-Quan Huang for

their help in the field. This study was supported by The Chinese Medicine Public Health Service Subsidy Special “National Chinese Medicine Resources Census Project” ([2018]43), Science & Technology Basic Resources Investigation Program of China (Grant no. 2017FY100100), and the Large-scale Scientific Facilities of the Chinese Academy of Sciences (2017-LSFGBOWS-02).

**LITERATURE CITED**

APG III. 2009. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161(2): 105–121.

Brotherus, V.-F., H. Handel-Mazzetti, T. Herzog, K. Keissler, H. Lohwag, W.E. Nicholson, H. Skuja, F. Verdoorn, A. Zahlbruckner and A. Fachmännern. 1936. Symbolae Sinicae: Botanische Ergebnisse der Expedition der Akademie der Wissenschaften in Weinnach Südwest-China, 1914–1918. v.7: 731. J. Springer, Wien.

China Phylogeny Consortium: Z.-D. Chen, T. Yang, L. Lin, L.-M. Lu, H.-L. Li, M. Sun, B. Liu, M. Chen, Y.-T. Niu, J.-F. Ye, Z.-Y. Cao, H.-M. Liu, X.-M. Wang, W. Wang, J.-B. Zhang, Z. Meng, W. Cao, J.-H. Li, S.-D. Wu, H.-L. Zhao, Z.-J. Liu, Z.-Y. Du, Q.-F. Wang, J. Guo, X.-X. Tan, J.-X. Su, L.-J. Zhang, L.-L. Yang, Y.-Y. Liao, M.-H. Li, G.-Q. Zhang, S.-W. Chung, J. Zhang, K.-L. Xiang, R.-Q. Li, D.E. Soltis, P.S. Soltis, S.-L. Zhou, J.-H. Ran, X.-Q. Wang, X.-H. Jin, Y.-S. Chen, T.-G. Gao, J.-H. Li, S.-Zhou,



- Zhang, A.-M. Lu. 2016. Tree of life for the genera of Chinese vascular Plants. *J. Syst. Evol.* **54(4)**: 277–306.
- Chen, F.-H. and C.-M. Hu. 1979. Taxonomic and phytogeographic studies on Chinese species of *Lysimachia*. *Acta Phytotax. Sin.* **17**: 21–53.
- Chen, F.-H., C.-M. Hu, Y.-Y. Fang and C.-Z. Cheng. 1989. *Lysimachia* (Primulaceae). In: Chen, F.-H. & C.-M. Hu (eds.) *Flora Reipublicae Popularis Sinicae*, Science Press, Beijing, **59**: 1–133.
- Doyle, J.-J. and J.-L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**: 11–15.
- Farris, J.-M., M. Källersjö, A.-G. Kluge, C. Bult. 1994. Testing significance of congruence. *Cladistics* **10(3)**: 315–319.
- Handel-Mazzetti, H. 1928. A revision of the Chinese species of *Lysimachia*. *Notes Roy. Bot. Gard. Edinb.* **16**: 51–122.
- Hu, C.-M. 1985. Further notes on the genus *Lysimachia* L. in mainland S. E. Asia. *Acta Phytotax. Sin.* **23**: 355–368.
- Hu, C.-M. 1992. Primulaceae. In: Morat, A. (ed.) *Flore du Cambodge, du Laos et du Vietnam*. Muséum National D'Histoire Naturelle, Paris, **26**: 115–144.
- Hu, C.-M. and S. Kelso. 1996. Primulaceae. In: Wu Z.-Y. & P.-H. Raven (eds.) *Flora of China*. Science Press, Beijing & Missouri Botanical Garden Press, St Louis, **15**: 39–189.
- Huang, R.-Z., M. Liao, W. Han, Y.-Z. Yang and G.-D. Tang. 2020. *Lysimachia daqiaoensis* (Primulaceae), a new cave species from Guangdong, China. *Phytotaxa* **430(1)**: 41–45.
- Huang, Y.-F., L.-N. Dong, and W.-B. Xu. 2019. *Lysimachia fanii*, a new species of Primulaceae from limestone area of Guangxi, China. *PhytoKeys* **130**: 75–84.
- Linnaeus, C. 1753. *Species plantarum*. Imprensus Laurentii Salvii, Holmiae. **1**: 1–784.
- Madeira, F., Y.-M. Park, J. Lee, N. Buso, T. Gur, N. Madhusoodanan, P. Basutkar, A.-R.-N. Tivey, S.-C. Potter, R.-D. Finn and R. Lopez. 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* **47(W1)**: W636–W641.
- Oh, I.-C., A.-L. Anderberg, J. Schönenberger and A.-A. Anderberg. 2008. Comparative seed morphology and character evolution in the genus *Lysimachia* (Myrsinaceae) and related taxa. *Pl. Syst. Evol.* **271(3-4)**: 177–197.
- Ronquist, E. and J.-P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19(12)**: 1572–1574.
- Saint-Hilaire, A.-D. 1822. Voyage Dans L'interieur Du Bresil. *Mémoires du Muséum d'histoire naturelle* **9**: 337–380.
- Ståhl, B. and A.-A. Anderberg. 2004. Myrsinaceae. In: Kubitki K (ed.) *The families and genera of vascular plants*. Heidelberg, Germany: Springer-Verlag, **6**: 266–281.
- Stevens, P.-F. 2017-onwards. Angiosperm Phylogeny Website. Version 14. Available: <http://www.mobot.org/MOBOT/research/APweb/>.
- Sun, W.-B., Z.-K. Wu and Y.-P. Ma. 2020. Primulaceae. In: Li D.-Z. (ed.) *The families and genera of Chinese vascular plants*. Beijing, China. Science Press, **3**: 1661–1671.
- Swofford, D.-L. 2002. PAUP\*. Phylogenetic analysis using parsimony (\*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, MA.
- Wang, Z.-H., X.-W. Li, E.-D. Liu, F.-Z. Shangguan and X.-K. Chang. 2016. *Lysimachia septemfida* (Primulaceae), a new species from Yunnan, China. *Plant Divers.* **38(4)**: 201–206.
- Wang, Y., W. Ma, S.-B. Zhou and K. Liu. 2018. *Lysimachia tianmaensis* (Primulaceae), a new species from Anhui, China. *PhytoKeys* **98**: 117–124.
- Wei, Z.-X. 2003. Pollen flora of seed plants. Yunnan Science and Technology Press, Kunming.
- Yan, H.-F., Y. Xu, Z.-M. Zhu, C.-M. Hu and G. Hao. 2017. *Lysimachia sinopilosa* (Primulaceae), a New Species from Yunnan, China. *Ann. Bot. Fenn.* **54(1-3)**: 45–48.
- Yan, H.-F., C.-Y. Zhang, A.-A. Anderberg, G. Hao, X.-J. Ge and J.-J. Wiens. 2018. What explains high plant richness in East Asia? Time and diversification in the tribe Lysimachieae (Primulaceae). *New Phytol.* **219(1)**: 436–448.
- Zhou, J.-J., X.-L. Yu, Y.-F. Deng, H.-F. Yan and Z.-L. Lin. 2015. *Lysimachia huangsangensis* (Primulaceae), a New Species from Hunan, China. *PLOS ONE* **10(7)**: e0132713.