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Parasenecio lanpingensis (Asteraceae), a new species from Yunnan, China

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ABSTRACT: A new species of *Parasenecio*, *P. lanpingensis*, was described in Lanping County, Yunnan Province, China. Morphologically, *P. lanpingensis* shares several key features with species in sect. *Monanthae* of *Parasenecio*, such as the white pappus, villous stem and ovate–triangular leaf. However, it can be easily distinguished from other *Parasenecio* species by its hairy, purplish–red stem, petiole, abaxially leaf blade and adaxially leaf vein. Phylogenetic analysis including 163 related species of trib. Senecioneae based on two nuclear DNA fragments (*ITS* and *ETS*) demonstrates that *P. lanpingensis* is clearly clustered within *Parasenecio*, and *P. lanpingensis* is closely related to *P. cyclotus* and *P. koualapensis.* Overall, both morphological and phylogenetic evidences strongly support the classification of *P. lanpingensis* as a newly described species. A comprehensive description of this species is provided, including the detailed illustrations and photographs, geographical distribution, habitat and ecology, as well as its IUCN conservation status.

KEY WORDS: *ETS, ITS*, *Parasenecio*, *Parasenecio cyclotus*, *Parasenecio koualapensis*, sect. *Monanthae*, phylogeny.

INTRODUCTION

Parasenecio W. W. Sm. & J. Small is a group of perennial herbs in the subtribe. Tussilaginae, tribe Senecioneae, Asteraceae. This genus is characterized by its stout rhizomes, numerous capitula, and small, tubular florets that are yellow, white, or reddish-orange (Koyama, 1995). *Parasenecio* is mainly distributed in East-Asia and Himalayan area. Three major diversity centers are recognized, i.e., China-Himalaya, central China, and northeast China-Japan (Koyama, 1995; Chen *et al*., 2011). Some species are also distributed in parts of eastern Europe and northern Asia (Grant, 1993; Czerepanov, 1995). With over 70 described species, the genus is taxonomically divided into six groups primarily based on their leaf morphology and chromosome numbers: *Parasenecio*, *Delphiniifolii* (H.Koyama) H.Koyama, *Hirsuti* (H.Koyama) H.Koyama, *Koyamacalia* (H.Rob. & Brettell) Y.L.Chen, *Monanthi* (Kitam.) Y.L.Chen and *Taimingasa* (Kitam.) H.Koyama (Koyama, 1995).

Several studies based on molecular systematics showed that *Parasenecio* is not a monophyletic group. In a systematic study of the East Asian subtribe. Tussilaginae, Liu *et al*. (2006) found that *Sinacalia tangutica* was clustered in the same branch with the other four species of *Parasenecio*. And in their study, *Parasenecio* and *Sinacalia* formed a clade with nine other eastern Asian genera of Tussilagininae, including *Cremanthodium* Benth., *Farfugium* Lindl., *Ligularia* Cass., *Ligulariopsis* Y.L.Chen, *Miricacalia* Kitam., *Nemosenecio* (Kitam.) B.Nord., *Sinosenecio* B.Nord., *Syneilesis* Maxim. and *Tephroseris* (Rchb.) Rchb. As the relationships in the clade were poorly resolved, Liu *et al.* (2006) named the clade *Ligularia-Cremanthodium-Parasenecio* as LCP complex. Ren *et al*. (2017) conducted a molecular phylogenetic study of *Parasenecio* based on four chloroplast fragments (*trnC*^{GCA}*-petN*, *trnG*^{UCC}*-trnT*^{GGU}*-psbD*, *trnT*^{UGU}*-trnL*^{UAA} and *rpl32-ndhF*) and two nuclear gene fragments (internal transcribed spacer (*ITS*) and external transcribed spacer *(ETS*)). They included 37 *Cremanthodium* spp., 1 *Farfugium* sp., 70 *Ligularia* spp., 1 *Miricacalia* sp., 2 *Nemosenecio* spp., 51 *Parasenecio* spp., 3 *Sinacalia* spp., 24 *Sinosenecio* spp., 4 *Syneilesis* spp. and 4 *Tephroseris* spp. Based on their results, they proposed a transferring of *Sinacalia caroli* (C. Winkl.) C. Jeffrey & Y. L. Chen to *Parasenecio* as *P. caroli* (C. Winkl.) C. Ren & Q. E. Yang and segregating *P.* section *Delphiniifoliae* (Koyama) H. Koyama and *P.* section *Taimingasa* (Kitam.) H. Koyama into genera *Japonicalia* C. Ren & Q. E. Yang and *Taimingasa* (Kitam.) C. Ren & Q. E. Yang, respectively. In this study, we adopt the taxonomic treatment on *Parasenecio* raised by Ren *et al.* (2017).

During a survey in July 2024, we found an unusual plant under a valley forest in Lanping County, Yunnan Province, whose morphology is very close to the species in *Parasenecio* with several aspects such as the capitula, floret and pappus. However, its stem, petiole, abaxial leaf blade, and adaxial leaf vein are densely hairy and purplish–red, those traits are distinctly different from all the known plants of genus *Parasenecio*. To determine whether it is a new species, detailed morphological and molecular comparisons between this species and other *Parasenecio* members were conducted. Overall, the classification of this species as newly discovered is strongly supported by both morphological and phylogeny evidences.

MATERIALS AND METHODS

Morphological observations

Photos of various parts of the species (plant, rhizome, leaf) were taken at the discovery site in July and September 2024. Additionally, photographs of the capitula, phyllary, floret, and achene were taken after dissection. The key characteristics of the species, such as the length and width of stem, floret, style branch, petiole, and peduncle, were measured with digital calipers and steel tape. Five intact plant specimens were collected and preserved in the Biological Science Museum of Dali University.

Phylogenetic analyses

Fresh leaves were collected from the wild and dried using silica gel. Total DNA was extracted from 50 mg of stored leaves using the improved CTAB method (Doyle and Doyle, 1987). Sequences of *ITS* and *ETS* were generated. The *ITS* sequence was amplified in the reactions as described by Blattner (1999), and the *ETS* sequence was amplified in the reactions as described by Markos and Baldwin (2001). The *ITS* sequence was amplified using the primer pairs ITSA-F (5′-GGAAGGAGAAGTCGTAACAAGG-3′) and ITSA-R (5′-CTTTTCCTCCGCTTATTGATATG-3′). The *ETS* sequences was amplified using the primer pairs Ast-1 (5′-CGTAAAGGTGCATGAGT GGTGT-3′) and 18S-ETS (5′-ACTTACACATGCATGGCTTAATCT-3′).

The amplification of the two sequences was performed using 1x reaction buffer, 1 mM of each primer, 200 mM of each dNTP, 20–40 ng of template DNA, 1.25 U of Ex–Taq polymerase (TaKaRa Co., Ltd., China), with distilled deionised water added to a total volume of 25ul. The reaction condition was as followed: 98°C for 3 min, 30 cycles of 98°C for 10 s 56°C for 15 s, and 72°C for 1 min, followed by a final extension at 72°C for 6 min. Amplified DNA fragments were checked on 2% agarose gels and visualised using the Tanon Super Vision Camera System (Shanghai Tanon Biotech Co., Ltd.). The successfully amplified PCR products were sent to Beijing Qingke Biological Company (Beijing Tsingke Biotech Co., Ltd.) for sequencing.

Sequences were edited and assembled using BIOEDIT 7.0.9 (Hall *et al.*, 2011). In addition, homologous sequences of 162 other closely related species were obtained from the NCBI database (Table S1) (1 *Crassocephalum* sp., 13 *Cremanthodium* spp.,1 *Crocidium* sp., 1 *Emilia* sp., 1 *Erechtites* sp., 1 *Farfugium* sp., 1 *Homogyne* sp., 1 *Jacobaea* sp., 43 *Ligularia* spp., 1 *Ligulariopsis* sp., 1 *Luina* sp., 1 *Mikaniopsis* sp.,1 *Miricacalia* sp., 1 *Nemosenecio* sp., 1 *Nordenstamia* sp., 1 *Othonna* sp., 31 *Parasenecio* spp., 2 *Japonicalia* spp., 3 *Taimingasa* spp., 1 *Petasites* sp., 1 *Pippenalia* sp., 1 *Psacaliopsis* sp., 1 *Roldana* sp., 2 *Senecio* spp., 3 *Sinacalia* spp., 35 *Sinosenecio* spp., 7 *Syneilesis* spp., 4 *Tephroseris* spp., 1 *Tussilago* sp., *Othonna capensis* was set as outgroup) followed the studies of Ren *et al.* (2017). A multiple sequence alignment was compiled using the MAFFT version 7 (Katoh *et al.*, 2002). Phylogenetic analysis was performed using IQ–TREE software (Nguyen *et al.,* 2015) to construct a maximum likelihood (ML) tree (Felsenstein 1981) based on the Tamura–Nei model (Tamura and Nei 1993). The best fitting model based on BIC prediction was GTR+F+R3 using ModelFinder (Kalyaanamoorthy *et al*., 2017), and the topological robustness of the ML analysis was assessed by performing bootstrap analysis with 1000 replicates (Felsenstein, 1985). We performed BI analysis using MrBayes v.3.2.5, and selected the best fitting model (GTR+I+G) by MrModeltest v.2 (Nylander, 1981) (Ronquist *et al.*, 2012). The Markov chain Monte Carlo (MCMC) analysis was performed with 10 million generations and four chains, sampling every 1,000 generations and discarding the 25% as burn–in. Bootstrap percentage (MLBS) values ≥ 70 and PP values ≥ 0.95 were regarded as strong support. Sequence statistics were calculated using MEGA software, version 11.0 (Tamura *et al*., 2013).

RESULTS

Phylogeny

The aligned dataset comprises 973 bp, with the *ITS* region accounting for 607 bp the ETS region accounting for 366 bp. A total of 372 bp informative–sites (38.23%) were used for analysis. The length of the homologous sequences of the remaining species varies from 701 bp to 1054 bp. The current phylogeny results in the ML and Bayesian trees show similar topological structure. *P. lanpingensis* is clearly genetically differentiated from other species in the analysis. Meanwhile, it is phylogenetically similar to the species of *Parasenecio*, and is significantly clustered to a clade with *P. cyclotus* and *P. koualapensis* (Figs. 1 & S1, BS=100% of the ML trees, and PP=1 of the Bayesian tree).

Morphological Comparison and Taxonomic Position

A morphological comparison between *P. lanpingensis*, *P. cyclotus*, and *P. koualapensis* was performed. In contrast, *P. lanpingensis* can be distinguished from *P. cyclotus* and *P. koualapensis* by its densely hairy stem, petiole and abaxially leaf vein, as well as the purplish-red petioles, phyllaries, abaxial leaves and adaxial veins. A detailed morphological comparison of the three species is shown in table 1. Morphologically, *P. lanpingensis* is assumed to be placed into the sect. *Monanthae* due to its white pappus, villous stem and ovate-triangular leaf. In phylogenetic analysis, *P. lanpingensis* is considered to be clustered into the core *Monanthae* clade (Figs. 1 & S1). Based on the comprehensive phylogenetic and morphological analysis, *P. lanpingensis* is presumed to belong to the sect. *Monanthae.*

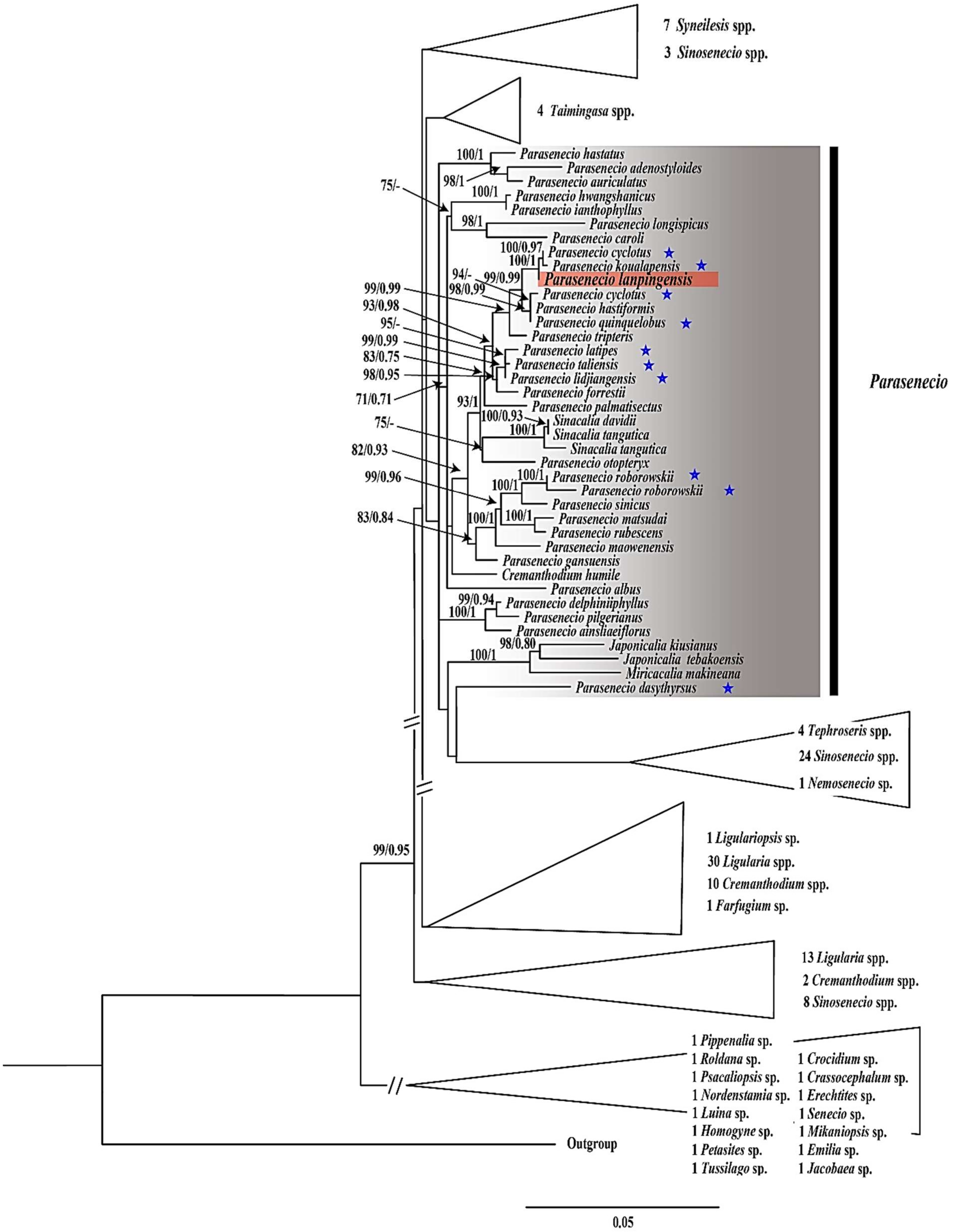

Fig. 1. Maximum Likelihood (ML) tree of ***Parasenecio lanpingensis*** and related 162 species, based on *ITS* and *ETS* sequences. Bayesian posterior probabilities (PPs) ≥ 0.95 and bootstrap values (maximum likelihood; MLBS) ≥ 50 are indicated above the branches. The newly found species is marked with red shading, and the species of sect. ***Monanthae*** are marked with blue five-pointed star. Double slashes ('//') on the branches indicate that the branch length has been artificially shortened. See Supporting Information Figure S1 for the complete topology.

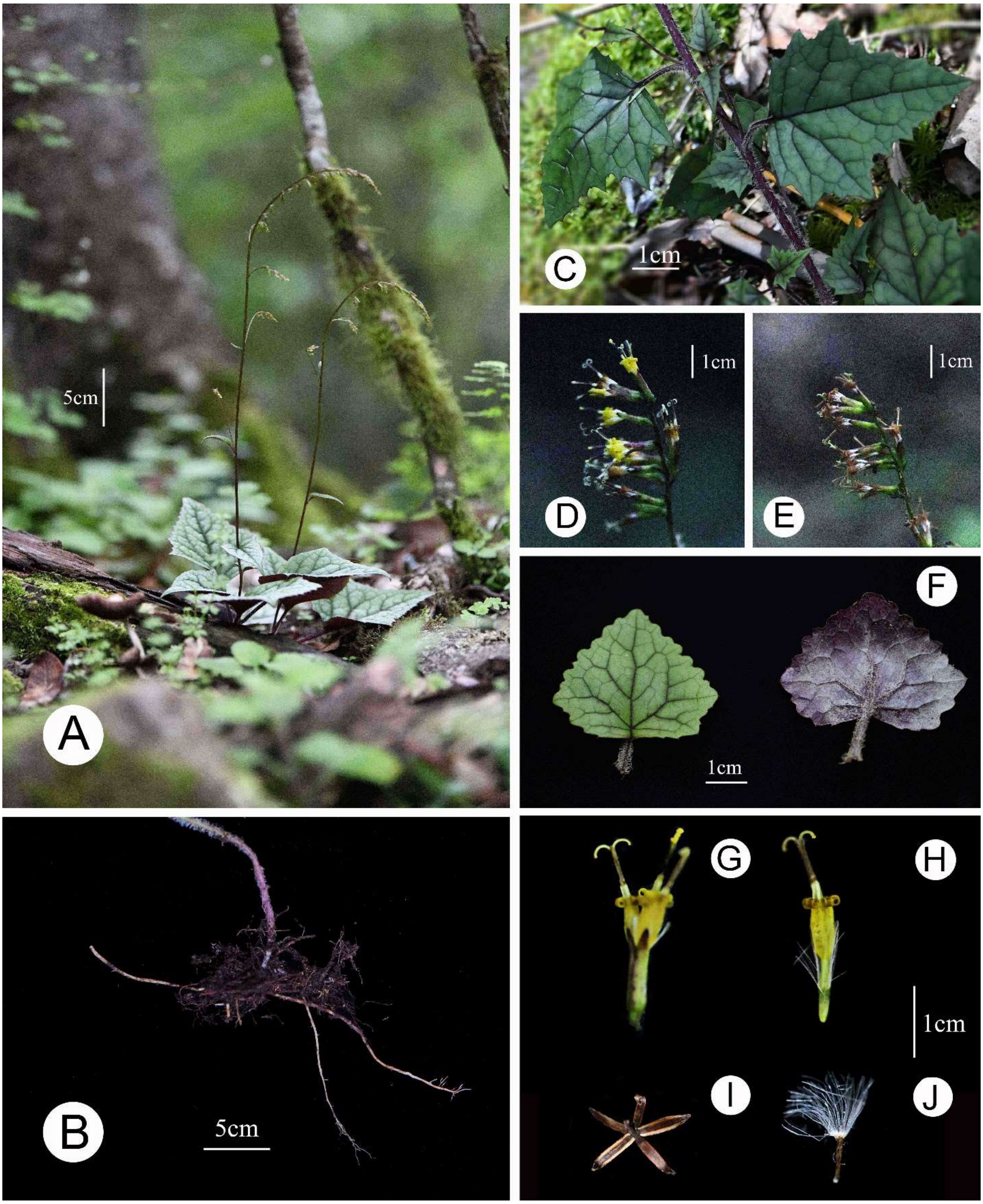

Fig. 2. Photographs of ***Parasenecio lanpingensis*** sp. nov. **A.** a complete plant of ***Parasenecio lanpingensis*** and its habitat; **B.** rhizomes with fibrous root; **C.** cauline leaves; **D.** inflorescence; **E.** infructescence; **F.** adaxial and abaxial basal leaf **G.** capitula, **H.** florets, **I.** phyllary and **J.** achene (photographed by Xianfeng Jiang).

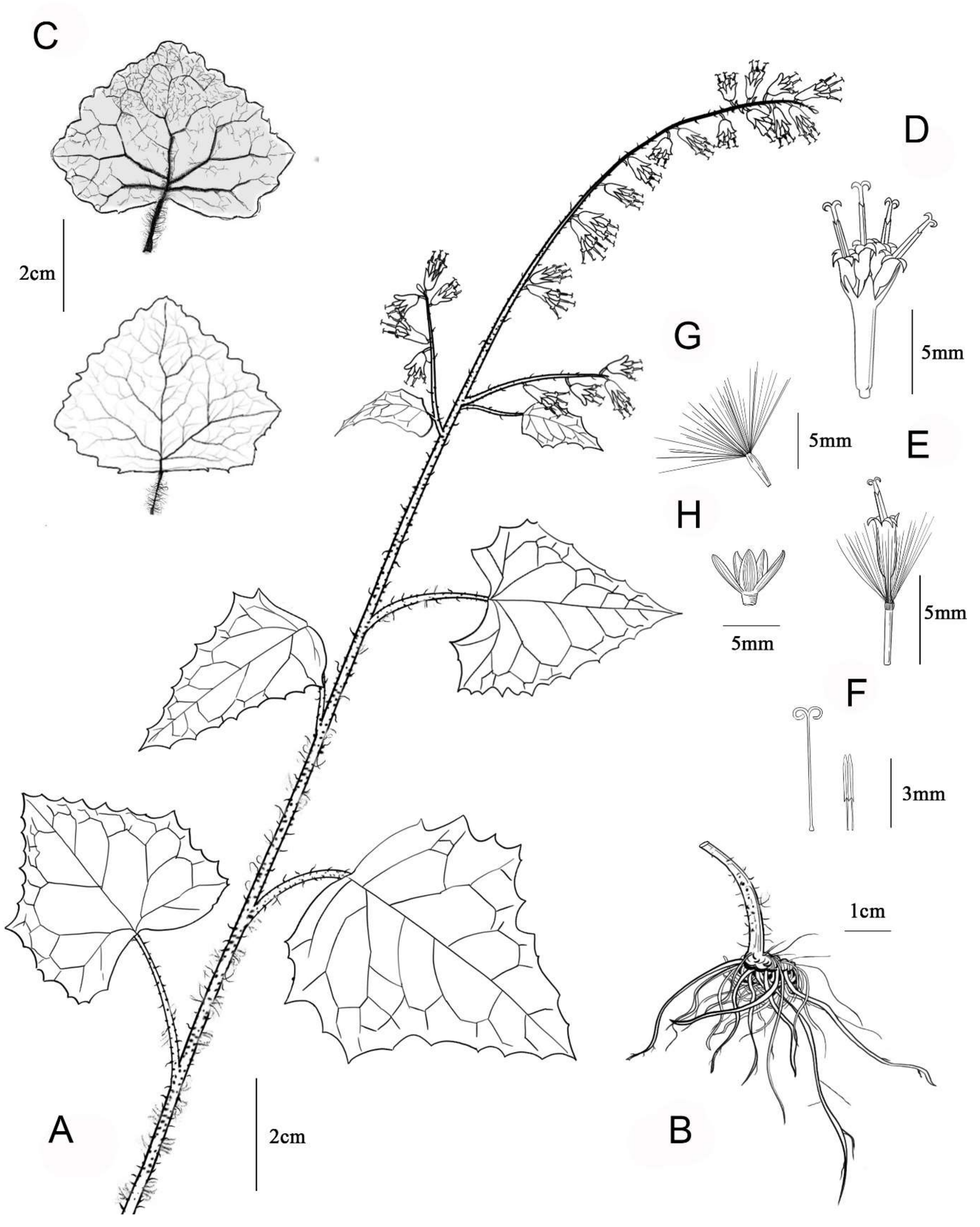

Fig. 3. *Parasenecio lanpingensis* sp. nov. **A** stem with inflorescence and cauline leaves; **B** rhizomes with fibrous root; **C** adaxial and abaxial basal leaf; **D** capitula; **E** floret; **F** stigma and anther; **G** achene; **H** phyllary. (Illustrated by Xianfeng Jiang)

Table 1. Main morphological differences between ***Parasenecio lanpingensis*** and related species.

Characteristic	***P. lanpingensis***	***P. cyclotus***	***P. koualapensis***
Distribution	Lanping, Yunnan province	West Sichuan province and northwest Yunnan province	Northwest and central Yunnan province
Elevation	3205 m a.s.l	2200–3600 m a.s.l	2850–3200 m a.s.l
*Stem	solitary, **purple, densely hairy**	solitary, nearly colorless in lower part, sparsely arachnoid above, subglabrous below	solitary, brown in lower part, sparsely somewhat glabrous above, multicellular long soft hairs below
Leaf blade shape	ovate or broadly triangular	obovate-spatulate	broadly ovate-triangular or broadly ovate
*leaf blade	**adaxially pale green, abaxially purplish-red.**	adaxially green, abaxially sparsely or densely arachnoid-tomentose	adaxially green, abaxially pale green, sometimes turning purple
*Leaf vein	**Purple, densely hairy on veins**	Green, glabrous	Green, glabrous
petiole	densely pubescent	entire or laxly toothed auricle	densely brown villous
*phyllaries	5, **purple**	5–6, green	5, green
No. of Florets	3–5	5–7	5

*the morphological characteristics that can significantly distinguish ***P. lanpingensis*** from the related species. The morphological characters of ***P. cyclotus*** and ***P. koualapensis*** are based on the comprehensive information of Flora of China (Chen ***et al.***, 2011). The unique traits of a species relative to the other three species are shown in bold.

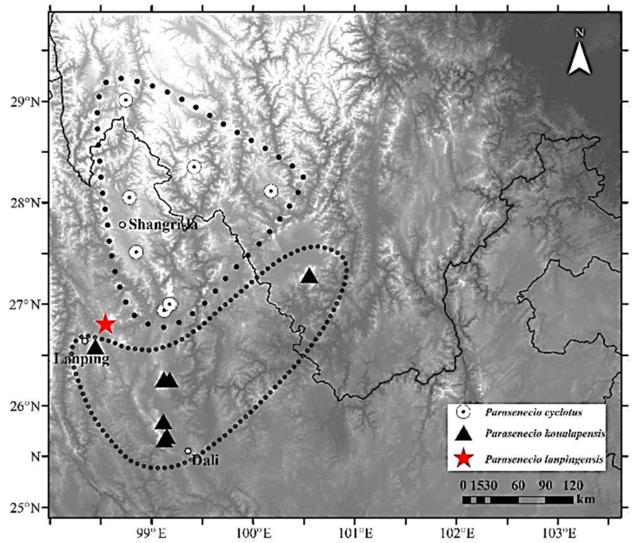

Fig. 4. Distribution of ***Parasenecio lanpingensis*** and its relatives (***Parasenecio cyclotus*** and ***Parasenecio koualapensis***). The species distribution data are obtained from specimen records (https://www.cvh.ac.cn/), and location of different species is indicated by different label in the map.

TAXONOMIC TREATMENT

Parasenecio lanpingensis X.F. Jiang, ***sp. nov.***

Figs. 2–3

Type**:** CHINA. Yunnan Province: Lanping County, Desheng Village, 3205 m elev., 25.539047N, 99.811019E, July 2024 (fl), *Jiang KKZW1160* (holotype: Museum of Dali University). (Fig. S2)

Diagnosis**:** The key distinguishing features of *P. lanpingensis* from other species in the genus *Parasenecio* are its densely hairy stem, petiole and abaxially leaf vein, as well as the purplish-red petioles, phyllaries, abaxial leaves, and adaxial veins.

Description**:** **Rhizomes** robust, ca. 2 mm in diam., with numerous fibrous roots. **Stem** solitary, erect, purplish-red, striate, 50–100 cm tall, densely hairy or hispidulous on the basal, simple or synflorescence branched. **Leaves** many, papery; 8–10×10–14 cm, petiole densely hairy; blade abaxially purplish-red, densely hairy on the vein, adaxially pale green, adpressed hirsutulous, with clearly visible purplish-red veins. **Basal leaves** broadly ovate-triangle, petiole 2–5 cm, slightly winged at the basal; **Cauline leaves** triangular or triangular-lanceolate, petiole 3–8 cm, not winged, gradually smaller upward. Uppermost leaves lanceolate or linear-lanceolate. **Capitula** numerous, racemes or compound racemes, with lateral heads drooping at anthesis. **Peduncles** 2–3 mm, with 1–3 linear bracteoles. **Phyllaries** 5, pale purple, oblong–lanceolate, outside glabrous, margin ciliate, narrowly scarious, slightly acute. **Florets** 3−5; corolla yellow, 6–7 mm, with ca. 3 mm tube, and campanulate limb. **Anthers** ca.1 mm, exserted from corolla, basally long caudate. **Style branches** 1–1.5mm, excurved, truncate, papillose. **Achenes** cylindric, ca. 4 mm, glabrous, ribbed. **Pappus** white, ca. 6 mm. (Fig. 2 & 3)

Distribution and ecology**:** *Parasenecio lanpingensis* mainly grows in the understory of the valley forest at 2800−3200 m a.s.l of Lanping County, Densheng Village, Yunnan Province (Fig. 4).

Phenology**:** The new species flowers from June to August and fruit from August to September.

Etymology**:** The new species is named after the Lanping County, Yunnan Province where it was discovered.

Local names and uses**:** The Chinese name of *P. lanpingensis* is 兰坪蟹甲草.

Conservation status**:** At present, the natural distribution area of *P. lanpingensis* is limited to a valley within Lanping County, covering an area of about 30 km^2. The population consists of approximately 2000 plants. According to the the IUCN Red List Categories Criteria version 2024.16 (IUCN, 2024), *P. lanpingensis* meets the criteria B (a. severely fragmented, b. continuing decline), C (less than 2500 individuals), and D2 (exists at typically five or fewer locations). Based on these criteria, *P. lanpingensis* is formally assigned the status as Endanger (EN B1ab+2ab; C2a(i)+b; D2).

Key to the *Parasenecio lanpingensis* and its close related species

1 leaf not divided 2
1 leaf divided, leaf blade obovate–spatulate, lyrately pinnate ...... ***P. cyclotus***
2 The petiole has wings 3
2 The petiole has no wings 4
3 Leaf blade ovate–triangular to broadly triangular, papery; petioles broadly winged, basally expanded into broad auricle; phyllaries 3; florets 5 or 6 .... ***P. latipes***
3 Leaf blade triangular or triangular–hastate, thick, abaxial blade arachnoid–tomentose; phyllaries 6–8; florets 9–12 ... ***P. lidjiangensis***
4 cauline leaves numerous, crowded at middle part of stem; leaves ovate or reniform–triangular; capitula laxly racemose .... ***P. tenianus***
4 cauline leaves 3–4, crowded at the middle and lower part of stem, capitula is laterally inclined 5
5 cauline leaves crowded at lower and middle parts of stem, leaf blade membranous or submembranous, broadly ovate, densely white hairs in abaxial blade, florets 6– 9 .... ***P. taliensis***
5 basal leaves survive during the flowering period, leaf blade papery, ovate–triangular, no dense white downy hairs, florets 5 6
6 Stems slender, glabrous; leaves ovate–orbicular or cordate, abaxial blade becoming purple when dry, glabrous .... ***P. jiulongensis***
6 Stems yellow–brown multicellular pubescent, leaves ovate–triangular or broadly ovate 7
7 leaf blade and leaf vein green, glabrous .... ***P. koualapensis***
7 adaxial leaf blade pale green; leaf vein, stem and abaxial leaf blade are purplish–red and densely hairy .... ***P. lanpingensis***

DISCUSSION

The newly–described species, *P. lanpingensis*, is found to be naturally distributed in a valley forest of Lanping County, Yunnan Province, China (Fig. 4). *Parasenecio* is primarily distributed in East Asia, with the Hengduan Mountains–Himalayan region being one of the three diversity centers. This region harbors approximately 30 *Parasenecio* species, comprising roughly half of the genus's total species richness (Chen *et al*., 2011). The dramatic topography of the Hengduan Mountains, featured by towering mountains and deep valleys, results in significant geographic isolation for the species in this area (Sun *et al*., 2017). The isolation might promote the speciation of numerous narrowly distributed endemic *Parasenecio* species in the Hengduan Moutain area, such as *P. jiulongensis*, *P. taliensis*, and *P. rockianus*.

The morphological features of *P. lanpingensis* are consistent with characteristics of sect. *Monanthae*, such as the white pappus, ovate–triangular leaf blades, and hairy stems (Koyama, 1995; Chen *et al*., 2011). Current phylogenetic results indicate that *Parasenecio* is not a monophyletic group. It is located at the base of the *Parasenecio–Sinosenecio* clade and incorporates some species from *Miricacalia*, *Cremanthodium*, and *Sinacalia* (Figs. 1 & S1). *P. lanpingensis* is clustered into the core area of *Parasenecio* with species from sect. *Montanthae* (Figs. 1 & S1). This indicates that *P. lanpingensis* can be clearly classified as belonging to *Parasenecio*. Taking into account the synapomorphy *P. lanpingensis* sharing with sect. *Montanthae*, such as the white pappus, villous stem and ovate–triangular leaf, we assume that *P. lanpingensis* should be classified into sect. *Montanthae* of *Parasenecio*.

Among the species of sect. *Monanthae*, *P. lanpingensis* is most morphologically similar to *P. koualapensis*, particularly in terms of the morphology of leaf blades, florets, and achenes. However, *P. koualapensis* is barely glabrous, with leaf green and stem sparsely somewhat glabrous above, multicellular long soft hairs below, which differs significantly from the corresponding features of *P. lanpingensis* (Table 1). We can figure out the morphological difference between *P. lanpingensis* and *P. koualapensis* from the stem, leaf, leaf vein and phyllary traits. Generally, *P. lanpingensis* exhibits a unique combination of traits that differentiates it from other *Parasenecio* species, including the purple–red and densely hairy stems, petioles and leaf veins. These characteristics set *P. lanpingensis* apart from other *Parasenecio* species.

The phylogenetic analysis based on two nuclear ribosomal DNA regions (*ITS* and *ETS*) shows that *Parasenecio* is placed at the basal of a clade with *Sinosenecio* and *Tephroseris*, which is consistent with the results reported by Ren *et al*. (2017). Various scenarios may lead to present phylogram, for examples, a large number of studies indicate that incomplete lineage sorting (Blanco-Pastor *et al.,* 2012), historical hybridization (Stull *et al*., 2023) or ongoing gene flow (Zhang *et al*., 2024) would cause the conflict in phylogenetic topological structure. However, based on our current results, it is insufficient to draw a definite conclusion as to whether *Parasenecio* has undergone significant incomplete lineage sorting or hybridization events. Insufficient resolution of the molecular markers used might also be a potential cause for this result. Given that each species was represented by only one OTU, future studies should incorporate broader intraspecific sampling and multi−locus genomic data to rigorously assess species boundaries and monophyly.

In conclusion, morphological and molecular phylogenetic evidence suggests that *P. lanpingensis* is a newly described species of *Parasenecio*, and on the basis of both morphological and molecular phylogenetic studies, we propose that *P. lanpingensis* should be placed in the sect. *Monanthae* of *Parasenecio*.

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