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Comprehensive molecular and morphological analysis of *Brachystemma calycinum* and *Stellaria ovatifolia* in the tribe Alsineae (Caryophyllaceae)

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Abstract

Over the course of the recent decade, the composition of Alsineae has been drastically changed by means of molecular phylogeny. However, the genus *Brachystemma* has not been sampled in any of the previous studies, and its phylogenetic position is still pending. In addition, the related species *Stellaria ovatifolia*, which has at times been placed in *Brachystemma*, *Schizotechium*, or *Stellaria*, has also not been sampled. Here, nuclear ribosomal internal transcribed spacer (ITS) and four plastid regions (*trnL-F*, *matK*, *rbcL*, *rps16*) were used to conduct phylogenetic analyses within Caryophyllaceae and the tribe Alsineae. Ancestral characters (petal margin and number of seeds) were reconstructed in the tribe Alsineae based on the phylogenetic results. Our results indicate that *Brachystemma* is nested in the tribe Alsineae and forms a monophylum with *S. ovatifolia*, and apically lobed petals and numerous seeds may be the ancestral characters in the tribe Alsineae. Based on our study, *Stellaria ovatifolia* should be considered within *Brachystemma*, and *Brachystemma* is clearly a separate genus and now includes two species.

Keywords

Alsineae, *Brachystemma*, molecular phylogeny, *Stellaria*

Introduction

The family Caryophyllaceae has traditionally been divided into three subfamilies (Lu et al. 2001). Recently, a new classification system has been proposed based on molecular and morphological evidence in Caryophyllaceae, and eleven tribes were recognized (Harbaugh et al. 2010; Greenberg and Donoghue 2011).

The tribe Alsineae in a traditional sense contained 12 genera including *Arenaria* L., *Brachystemma* D.Don, *Cerastium* L., *Holosteum* L., *Lepyrodiclis* Fenzl, *Minuartia* Loefl., *Moehringia* L., *Myosoton* Moench, *Pseudostellaria* Pax, *Sagina* L., *Stellaria* L., and *Thylacospermum* Fenzl (Lu et al. 2001). However, molecular studies have revealed that the traditional tribe Alsineae is polyphyletic (Harbaugh et al. 2010; Greenberg and Donoghue 2011). To date, the tribe Alsineae now consists of 16 genera including five new genera (*Engellaria* Iamonico, *Hartmaniella* M.L.Zhang & Rabeler, *Nubelaria* M.T.Sharples & E.A.Tripp, *Rabelera* M.T.Sharples & E.A.Tripp, and *Shivparvatia* Pusalkar & D.K.Singh), three reinstated genera (*Adenonema* Bunge, *Odontostemma* Benth. ex G.Don, and *Schizotechium* (Fenzl) Rchb.), and eight originally accepted genera: *Cerastium*, *Dichodon* (Bartl. ex Rchb.) Rchb., *Holosteum*, *Lepyrodiclis*, *Mesostemma* Vved., *Moenchia* Ehrh., *Pseudostellaria*, and *Stellaria* (Keshav and Kumar 2015; Sadeghian et al. 2015; Pusalkar and Srivastava 2016; Zhang et al. 2017; Sharples and Tripp 2019; Iamonico 2021; Yao et al. 2021; Arabi et al. 2022). *Brachystemma* morphologically related to other Alsineae still lacks comprehensive molecular and morphological study.

Brachystemma ovatifolium Mizushima was first published in 1955 and is related to *Brachystemma calycinum* D.Don (Mizushima 1955; Fig. 1 in present paper). Subsequently, Mizushima transferred it to *Stellaria* as *Stellaria ovatifolia* (Mizushima) Mizushima due to its two-lobed petals and similar seed morphology (Mizushima 1966), which was also accepted by Flora Reipublicae Popularis Sinicae (Wu and Ke 1996) and Flora of China (Shilong and Rabeler 2001). In the first book, it was incorporated into sect. *Schizothecium* Fenzl of *Stellaria*, together with *S. delavayi* Franch. and *S. monosperma* Buch.-Ham. ex D.Don (Wu and Ke 1996). Recently, *Stellaria* sect. *Schizotechium* has been raised into a separate genus, *Schizotechium* (Pusalkar and Srivastava 2016), and the new combination *Schizotechium monospermum* (Buch.-Ham. ex D.Don) Pusalkar & S.K.Srivast. was proposed based on morphological studies (Pusalkar and Srivastava 2016). The molecular studies also indicated that *Stellaria monosperma* was far from the core *Stellaria* and nested within *Schizotechium* (Greenberg and Donoghue 2011; Sharples and Tripp 2019; Arabi et al. 2022). Although *Stellaria ovatifolia* was hypothesized to be part of *Schizotechium* (Pusalkar and Srivastava 2016), it has never been sampled and has at times been placed in *Brachystemma*, *Schizotechium*, or *Stellaria*, and its phylogenetic position is still pending.

In this study, we conducted a combined molecular and morphological analysis in order to (1) confirm the phylogenetic position of *Brachystemma*; (2) clarify the relationship of *Stellaria ovatifolia* among *Stellaria*, *Schizotechium*, and *Brachystemma*; (3) estimate the character evolution of seed number and petal margin in the tribe Alsineae.

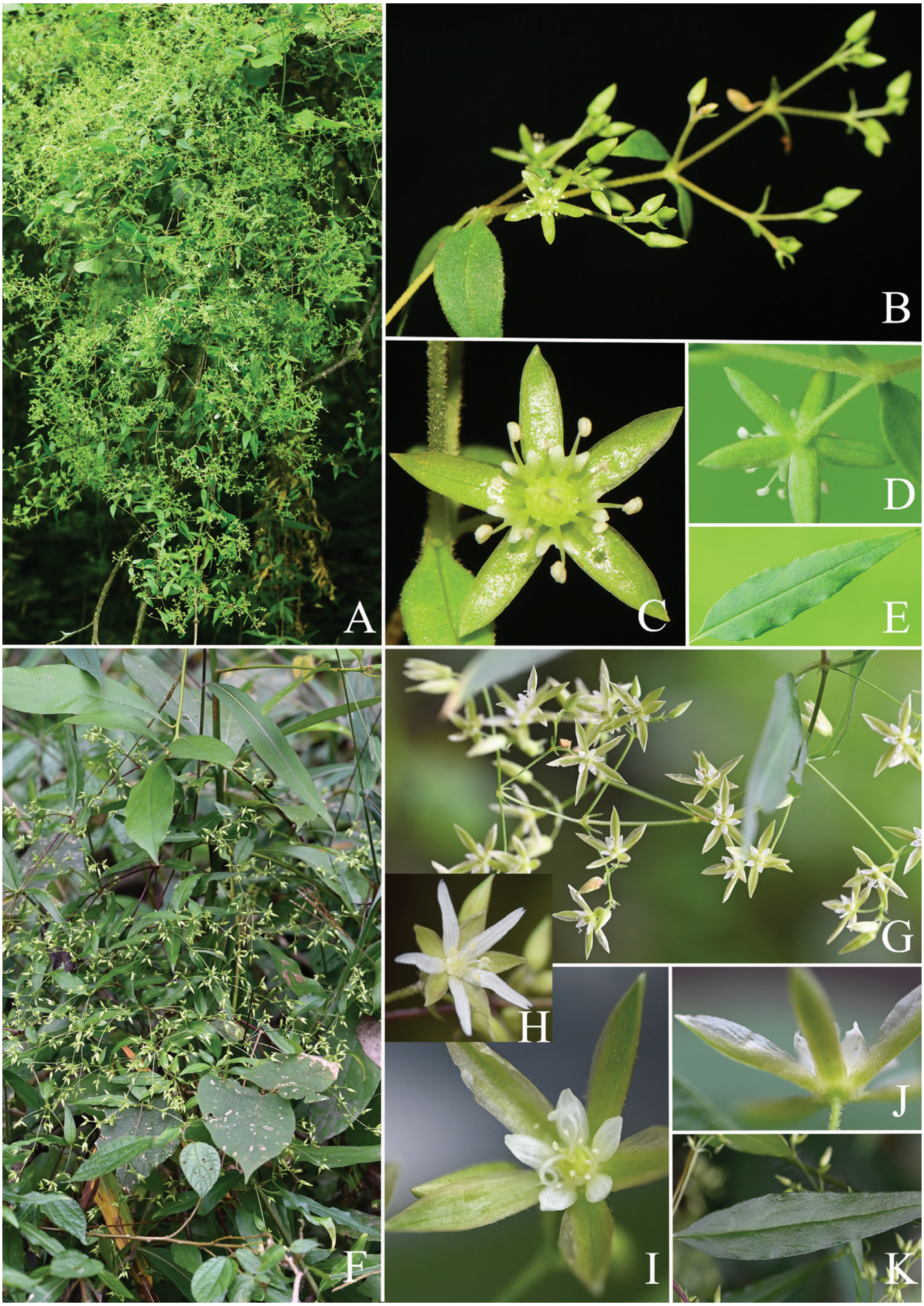

Figure 1. Morphological comparisons between *Stellaria ovatifolia* (**A–E**) and *Brachystemma calycinum* (**F–K**) **A, F** habit **B, G** inflorescence **C, H, I** flower (**H** the flower of *Brachystemma calycinum* 3) **D, J** sepal **E, K** leaf.

Methods

Taxon sampling and DNA sequencing

The samples of *Brachystemma calycinum* and *Stellaria ovatifolia* were collected from silica-dried leaves tissue, and the vouchers were deposited in the herbarium of the College of Agriculture, Guangxi University (**GAUA**) and the detailed information is shown in Suppl. material 1. The total DNA of the samples were extracted by the CTAB protocol (Maddison and Maddison 2014). The PCR amplification of ITS [5F (White et al. 1990), 4R (White et al. 1990)], *matK* [390F (Smissen et al. 2002), 1440R (Smissen et al. 2002)], *rbcL* [1F (Kress and Erickson 2007), 724R (Kress and Erickson 2007)], *rps16* [F (Popp and Oxelman 2001), R (Popp and Oxelman 2001)], *trnL-F* [C (Taberlet et al. 1991), F (Taberlet et al. 1991)] were performed as above cited. The sequencing of PCR products was performed by the Beijing Genomics Institute (BGI). Newly generated sequences are available in GenBank (https://www.ncbi.nlm.nih.gov/), and their accession numbers (in bold) and the sequences of Caryophyllaceae members downloaded from GenBank are listed in Table 1. The absent sequences were coded as missing data.

Phylogenetic analyses

Sequences alignment were performed with MAFFT v.7.313 (Katoh and Standley 2013). Phylogenetic analyses were conducted separately on the nuclear ribosomal internal transcribed spacer (ITS) and plastid regions (*matK*, *rbcL*, *trnL-F*, and *rps16*) and then combined; no notable incongruence was found (Fig. 2). The Bayesian Inference (BI) trees were constructed using MRBAYES 3.2.6 (Ronquist and Huelsenbeck 2003), and the maximum likelihood (ML) trees were constructed by RAXML-HPC2 (Stamatakis 2006). ML trees were constructed on CIPRES Science Gateway (Miller et al. 2010) under the GTRGAMMA model with 1,000 bootstrap replicates and default values for the remaining parameters. In Bayesian inference analysis, PARTITIONFINDER v.2.1.1 (Lanfear et al. 2016) was applied to selected models of nucleotide substitution under the Akaike Information Criterion (AIC). Selected models consisted of SYM+I+G for ITS, GTR+G for *matK*, *trnL-F*, and *rps16*, HKY+I+G for *rbcL*. Each Markov chain Monte Carlo (MCMC) analysis was run for 2,000,000 generations with the tree sampled every 100 generations. The first 25% trees of each run as burn-in were discarded.

Ancestral characters

Two morphological characters (petal margin and number of seeds) which were diagnostic characters in *Brachystemma* were selected to reconstruct the ancestral characters in the tribe Alsineae. MESQUITE v.3.6 (Maddison and Maddison 2014) was used to reconstruct the ancestral characters with default parameters, using the ML tree from the combined tree. Morphological characters were coded as the following: (a) the petals are entire or emarginate (coded as 0), apex lobed (less than 1/2 the

Table 1. List of sampled taxa and their GenBank accession numbers of sequences. The arrangement of sequences in the table shows sequences used to generate the trees shown in Fig. 3A, B. Sequences in bold were generated in this study.

Taxon	**GenBank accession numbers**				
	***nr*ITS**	***trnL-F***	***matK***	***rps16***	***rcbL***
A. Sequences used to generate Caryophyllaceae tree (Fig. 3A)					
Agrostemma githago L.	JN589107	EU221639	FJ589503	Z83154	KM360618
Arenaria lanuginosa (Michx.) Rohrb.	MZ388084	FJ404968	MH037652	FJ404891	MH028838
***Brachystemma calycinum* D.Don**	OP594537	OP595543	OP595548	OP595553	OP595558
Cerastium pusillum Ser.	JN589112	JN589683	JN589226	-	-
Corrigiola andina Planch. & Triana	JN589136	JN589707	JN589253	-	-
Dianthus armeria L.	GU440780	FJ404980	KP210382	FJ404903	MG249427
Dianthus caryophyllus L.	JN589053	MT312520	KU722867	KU904222	M77699
Eremogone bryophylla (Fernald) Pusalkar & D.K.Singh	MK341317	MK341206	MK341382	MK341262	-
Eremogone gypsophiloides Fenzl	KP148920	-	-	KP149022	-
Gymnocarpos przewalskii Bunge ex Maxim.	AJ310971	-	-	MH917997	-
Gypsophila paniculata L.	KX183986	KX183948	KX183906	FJ404908	MG547371
Holosteum marginatum C.A.Mey.	JN589093	JN589732	JN589261	-	-
Lepyrodiclis holosteoides (C.A.Mey.) Fenzl ex Fisch. & C.A.Mey.	MH808296	FJ404989	FJ404840	KP149043	JQ933385
Lychnis wilfordii (Regel) Maxim.	KX757649	-	-	LC423834	-
Moehringia lateriflora (L.) Fenzl	JX274536	FJ405000	MK520325	FJ404924	MN623790
Moehringia macrophylla (Hook.) Fenzl	MF964022	FJ405001	KY952464	FJ404925	MF963280
Polycarpon tetraphyllum (L.) L.	HE586018	FJ405009	MF963465	FJ404932	HM850271
Sabulina douglasii (Fenzl ex Torr. & A.Gray) Dillenb. & Kadereit	KF737459	FJ404992	FJ404842	FJ460221	-
Sagina japonica (Sw.) Ohwi	LC634109	-	MK435791	-	MN204811
Schiedea globosa H.Mann	AY517663	FJ405014	DQ907804	FJ404938	DQ907750
Schizotechium jamesianum (Torr.) Arabi, Rabeler & Zarre	KX158306	FJ405010	KX158343	KX158417	KX158380
Silene aprica Turcz. ex Fisch. & C.A.Mey.	KX757336	FN821322	MH658952	LC423907	KX158399
Spergula arvensis L.	JX274532	KY616142	JN894908	KY513576	KM360994
***Stellaria ovatifolia* (Mizushima) Mizushima**	OP594536	OP595542	OP595547	OP595552	OP595557
Stellaria vestita Kurz	MH117776	EU785988	MH116882	-	MH116433
Outgroup					
Celosia argentea L.	KY968928	LT993045	MH767769	FJ404898	AF206747
B. Sequences used to generate Alsineae tree (Fig. 3B)					
***Brachystemma calycinum* D.Don 1**	OP594537	OP595543	OP595548	OP595553	OP595558
***Brachystemma calycinum* D.Don 2**	OP594538	OP595544	OP595549	OP595554	OP595559
***Brachystemma calycinum* D.Don 3**	OP594539	OP595545	OP595550	OP595555	OP595560
Cerastium arvense L.	MH219805	FJ404976	AY936295	MH243535	JX848446
Cerastium brachypetalum Pers.	-	-	-	-	KF997372
Cerastium davuricum Fisch. ex Spreng.	KX158321	-	KX158358	KX158432	KX158395
Cerastium dichotomum subsp. *inflatum* (Link) Cullen	KX158322	-	KX158359	KX158433	KX158396
Cerastium dinaricum Beck & Szyszył.	KJ716515	KJ716526	-	-	-
Cerastium fontanum Baumg.	GU444015	FJ404977	KX821263	FJ404899	KF602216
Cerastium furcatum Cham. & Schltdl.	MH117479	-	MH116578	-	MH116103
Cerastium latifolium L.	-	AY521301, AY521348	-	-	KF602212
Cerastium pusillum Ser.	JN589112	JN589683	JN589226	-	-

Taxon	**GenBank accession numbers**				
	***nr*ITS**	***trnL-F***	***matK***	***rps16***	***rcbL***
Cerastium subtriflorum Dalla Torre & Sarnth.	MH537035	KJ716527	-	-	-
Cerastium szechuense F.N.Williams	JN589116	JN589674	-	-	-
Cerastium tomentosum L.	JN589031	AY521310, AY521357	JN589244	MH243538	KF997321
Dichodon cerastoides (L.) Rchb.	MH219812	AY521340, AY521388	-	MH243542	MG249356
Dichodon dubium (Bastard) Ikonn.	MH219815	AY521341, AY521389	-	MH243544	-
Hartmaniella oxyphylla (B.L.Rob.) M.L.Zhang	KX158311	-	KX158348	KX158422	KX158385
Hartmaniella sierra (Rabeler & R.L.Hartm.) M.L.Zhang	KX158314	-	KX158351	KX158425	KX158388
Holosteum marginatum C.A.Mey.	JN589093	JN589732	JN589261	-	-
Holosteum umbellatum L.	JN589051	JN589655	MK520188	FJ404909	MK525977
Lepyrodiclis holosteoides (C.A.Mey.) Fenzl ex Fisch. & C.A.Mey.	MH808296	FJ404989	FJ404840	KP149043	JQ933385
Mesostemma dichotomum (L.) Arabi, Rabeler & Zarre	MT624581	-	-	MT624662	-
Mesostemma kotschyanum (Fenzl ex Boiss.) Vved.	MT624582	-	-	MT624664	-
Mesostemma perfoliatum (Rech.f.) Rech.f.	MT624583	-	-	MT624665	-
Mesostemma platyphyllum Rech.f.	MT624584	-	-	MT624666	-
Moenchia erecta (L.) G.Gaertn., B. Mey. & Scherb.	JN589103	FJ405002	JN895271	FJ404926	JN892479
Odontostemma barbatum (Franch.) Sadeghian & Zarre	KP148852	-	-	-	-
Odontostemma trichophorum (Franch.) Sadeghian & Zarre	AY936243	-	-	-	-
Pseudostellaria heterophylla (Miq.) Pax	KX158334	EU785992	KX158371	KX158445	KX158408
Pseudostellaria japonica (Korsh.) Pax	KX158307	-	KX158344	KX158418	KX158381
Pseudostellaria maximowicziana (Franch. & Sav.) Pax	KX158309	-	KX158346	KX158420	KX158383
Pseudostellaria tianmushanensis G.H.Xia & G.Y.Li	KX158318	-	KX158355	KX158429	KX158392
Pseudostellaria tibetica Ohwi	KX158317	-	KX158354	KX158428	KX158391
Rabelera holostea (L.) M.T.Sharples & E.A.Tripp	KX183997	JN589664	KX183916	MH243549	FJ395575
Schizotechium americanum (Standl.) Arabi, Rabeler & Zarre	KX158335	JN589675	KX158372	KX158446	KX158409
Schizotechium jamesianum (Torr.) Arabi, Rabeler & Zarre 1	KX158306	FJ405010	KX158343	KX158417	KX158380
Schizotechium jamesianum (Torr.) Arabi, Rabeler & Zarre 2	JN589048	-	-	KX158417	-
Schizotechium monospermum (Buch.-Ham. ex D.Don) Pusalkar & S.K.Srivast 1	MT624596	-	-	MT624676	-
Schizotechium monospermum (Buch.-Ham. ex D.Don) Pusalkar & S.K.Srivast 2	MT624595	-	-	MT624675	-
Schizotechium turkestanicum (Schischk.) Arabi, Rabeler & Zarre	MT624597	-	-	MT624677	-
Shivparvatia ciliolata (Edgew. & Hook.f.) Pusalkar & D.K.Singh	KP148859	-	-	-	-
Shivparvatia glanduligera (Edgew.) Pusalkar & D.K.Singh	KP148867	-	-	-	-

Taxon	**GenBank accession numbers**				
	***nr*ITS**	***trnL-F***	***matK***	***rps16***	***rcbL***
Shivparvatia stracheyi (Edgew.) Pusalkar & D.K.Singh	KP148898	-	-	-	-
Stellaria alsine Grimm	AY438312	EU785987	HM850778	-	HM850385
Stellaria aquatica (L.) Scop.	AY594303	FJ405004	JN894058	MH243547	KM360890
Stellaria borealis Bigelow	JN589064	JN589713	JN589285	-	MG247728
Stellaria chinensis Regel	JN589133	EU785990	JN589241	-	-
Stellaria corei Shinners	JN589046	JN589715	JN589300	-	-
Stellaria crassifolia Ehrh.	JN589071	JN589701	KC475924	-	KC484145
Stellaria cuspidata Willd. ex D.F.K.Schltdl.	JN589099	JN589641	JN589268	FJ404952	-
Stellaria graminea L.	AY594304	JN589687	MK520714	MH243548	KM360998
Stellaria longifolia Muhl. ex Willd.	JN589146	GQ245567	MK520715	-	JX848448
Stellaria longipes Goldie	JN589086	JN589672	KC475949	-	JX848449
Stellaria media (L.) Vill.	MK044722	EU785989	HM850779	Z83152	AF206823
Stellaria nemorum L.	AY936246	HM590349	AY936298	-	JN893484
***Stellaria ovatifolia* (Mizushima) Mizushima 1**	OP594536	OP595542	OP595547	OP595552	OP595557
***Stellaria ovatifolia* (Mizushima) Mizushima 2**	OP594540	OP595546	OP595551	OP595556	OP595561
Stellaria palustris Ehrh. ex Hoffm.	JN589080	-	MK520716	KX158438	KX158401
Stellaria pubera Michx.	JN589127	FJ405027	FJ404878	-	KP643834
Stellaria soongorica Roshev.	KX158328	-	MF158660	KX158439	KX158402
Stellaria umbellata Turcz.	JN589109	JN589737	JN589254	-	MG246195
Stellaria vestita Kurz	MH117776	EU785988	MH116882	-	MH116433
Outgroup					
Arenaria serpyllifolia L.	KX158320	FJ404972	KX158357	KX158431	KX158394
Arenaria lanuginosa (Michx.) Rohrb.	MZ388084	FJ404968	MH037652	FJ404891	MH028838
Moehringia macrophylla (Hook.) Fenzl	MF964022	FJ405001	KY952464	FJ404925	MF963280
Sabulina douglasii (Fenzl ex Torr. & A.Gray) Dillenb. & Kadereit	KF737459	FJ404992	FJ404842	FJ460221	-
Sagina japonica (Sw.) Ohwi	LC634109	-	MK435791	-	MN204811

length of the petals) (1), deeply lobed (longer than 1/2 the length of the petals) (2); (b) the number of seeds in a capsule is 1–3 (0), 4–6 (1), more than 6 (2) (Lu et al. 2001; Arabi et al. 2022).

Results

Phylogenetic analyses

In the Caryophyllaceae tree, *Brachystemma calycinum* and *Stellaria ovatifolia* were nested in the tribe Alsineae with strong support (PP = 1.00, BS = 100) (Fig. 3A). Moreover, in the tree encompassing Alsineae tribe, *B. calycinum* and *S. ovatifolia* formed a monophylum (PP = 1.00, BS = 99) with strong support (PP = 1.00, BS = 100) (Fig. 3B), which is sister to the clade composed of *Schizotechium*, *Mesostemma*, *Lepyrodiclis*, *Shivparvatia*, *Odontostemma*, and *Pseudostellaria* in this tree (Fig. 3B). Our results suggested *Stellaria ovatifolia* was closely related to *Brachystemma*, instead of either *Stellaria* s.str. or *Schizotechium*.

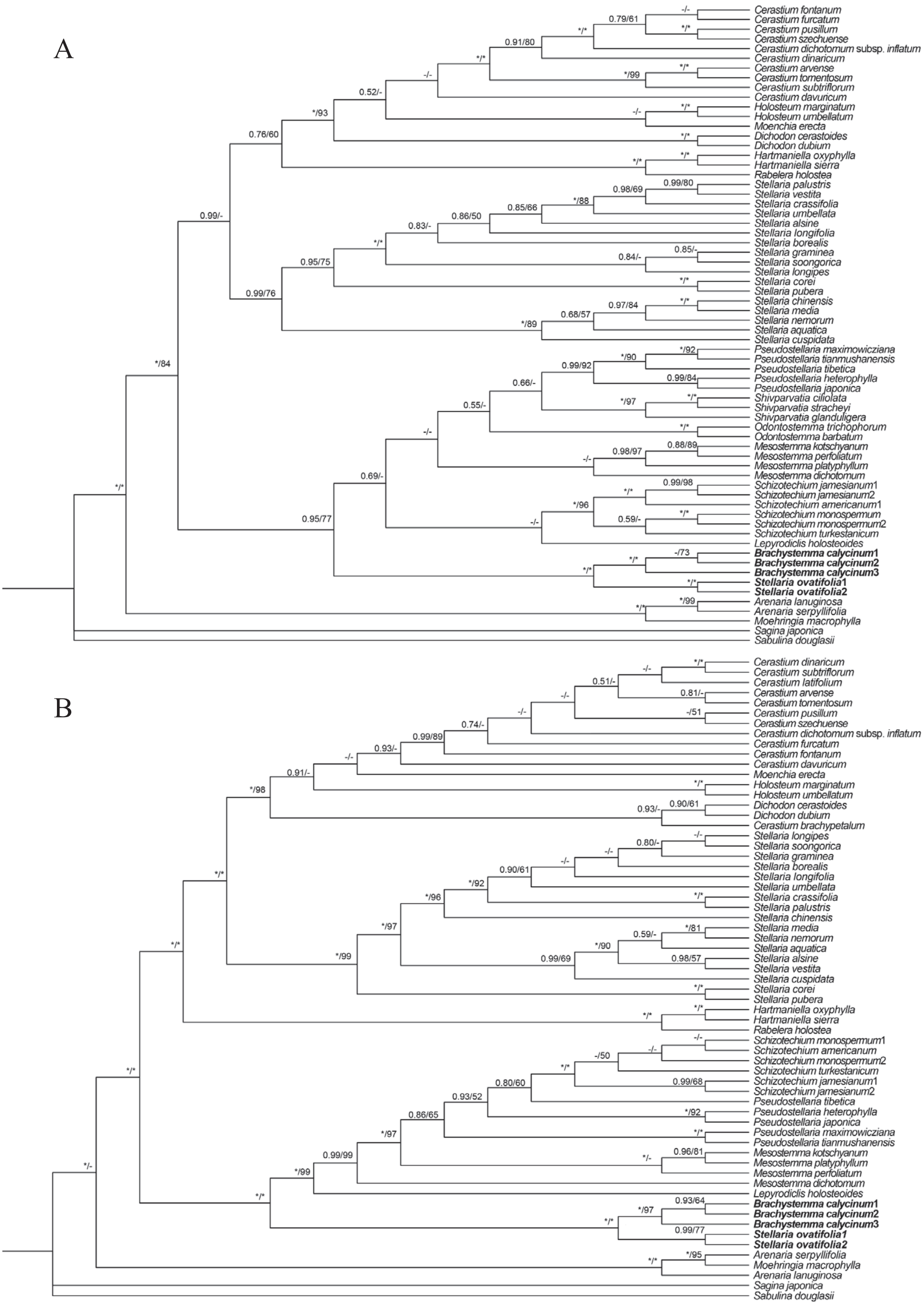

Figure 2. Phylogenetic relationships among the tribe Alsineae **A** ITS data **B** *trnL-F*, *matK*, *rbcL*, *rps16* combined data. The numbers on the nodes are Bayesian posterior probabilities (PP > 0.5), maximum likelihood bootstrap percentages (BS > 50%), respectively. "*" indicates that the node is PP = 1.00/ BS = 100%, "-" indicates that the node PP < 0.5/ BS < 50%.

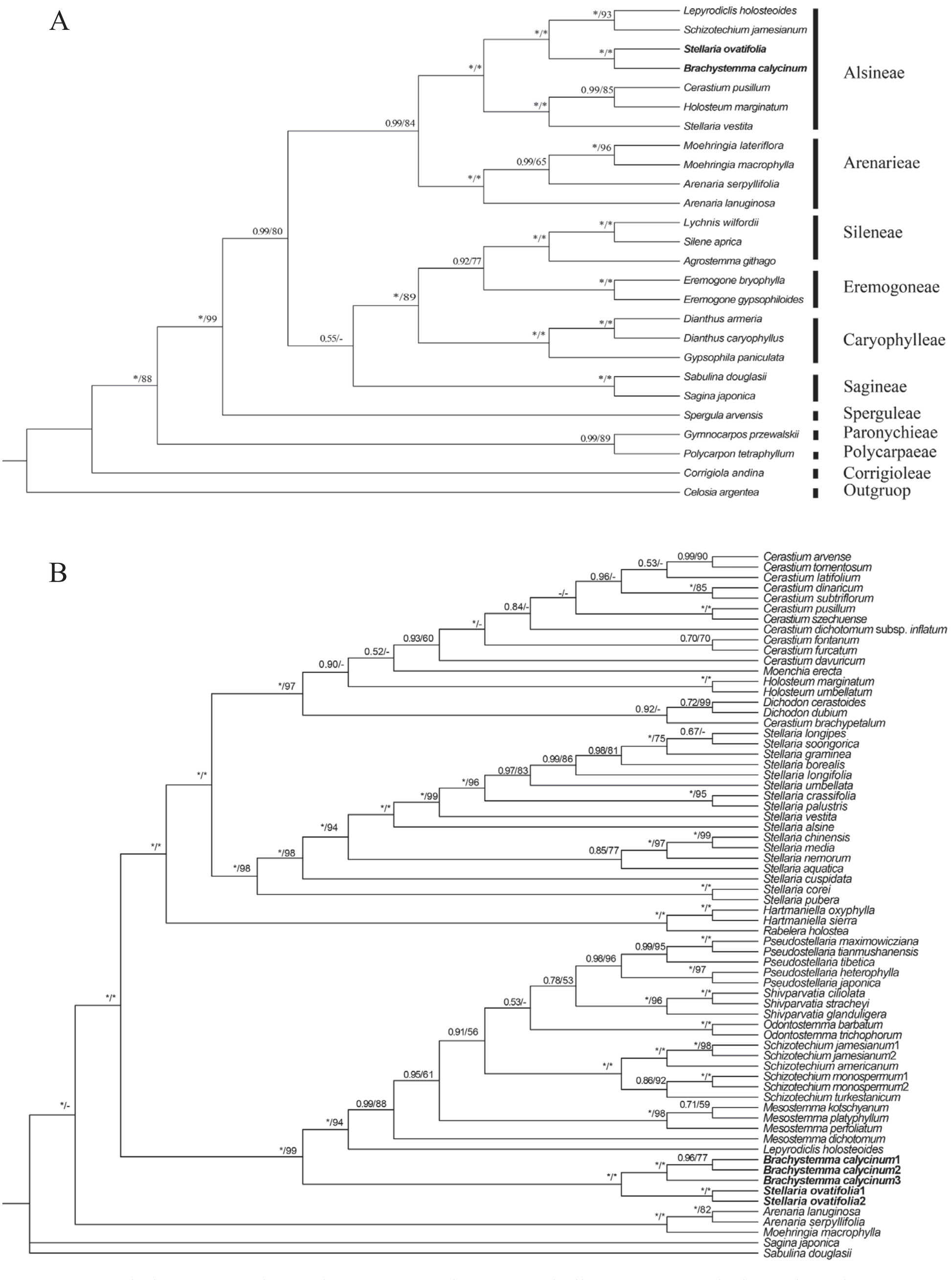

Figure 3. Phylogenetic relationships among the Caryophyllaceae (**A**) and the tribe Alsineae (**B**). Phylogenetic trees were conducted by ITS, *trnL-F*, *matK*, *rbcL*, *rps16* combined sequences. The numbers on the nodes are Bayesian posterior probabilities (PP > 0.5), maximum likelihood bootstrap percentages (BS > 50%), respectively. "*" indicates that the node is PP = 1.00/BS = 100%, "-" indicates that the node PP < 0.5/ BS<50%.

Ancestral character

The results of the ancestral character reconstruction indicated that petals with a lobed apex and numerous seeds may be the ancestral characters of the tribe Alsineae (Fig. 4). The presence of entire petals and 1–3 seeds became the diagnostic characters between *Brachystemma* and related genera. In addition, *B. calycinum* and *S. ovatifolia* shared the characters of 1–3 seeds and neither taxa has deeply bifid petals. It suggested a close relationship between *B. calycinum* and *S. ovatifolia*.

Discussion

Phylogenetic position and distinction of *Brachystemma*

As currently defined, *Brachystemma* is a monotypic genus in the tribe Alsineae, which is characterized by annual subscandent life form, lax thyrse with many flowers, petals shorter than 1/2 the length of the sepals with entire margins, two styles, four-valved capsules, and one mature seed (Fig. 1) (Lu and Gilbert 2001). Our phylogenetic results also revealed that *Brachystemma* formed a single branch with *S. ovatifolia* (Fig. 2 and Fig. 3) and demonstrated that *Brachystemma* is an independent genus (*S. ovatifolia* will be discussed in the following paragraphs), which is consistent with traditional morphological studies (Fenzl 1840; Bentham and Hooker 1862; Pax and Hoffmann 1934; Bittrich 1993; Lu and Gilbert 2001; Takhtajan 2009). Furthermore, the phylogenetic position of *Brachystemma* was nested in the tribe Alsineae and sister to the clade composed of *Schizotechium*, *Mesostemma*, *Lepyrodiclis*, *Shivparvatia*, *Odontostemma*, and *Pseudostellaria* (Fig. 3). Nevertheless, *Brachystemma* can be morphologically distinguished from the related genera of this clade. *Brachystemma* and *Lepyrodiclis* share characters such as annual life form, lax thyrse, and two styles, but *Brachystemma* differs from the latter by subscandent life form and four-valved capsules (Lu et al. 2001). It also can be distinguished from *Mesostemma*, *Pseudostellaria*, and *Schizotechium* by annual life form, petals with entire margins, lax thyrse, and two styles (Lu et al. 2001; Arabi et al. 2022). It can be clearly distinguished from *Shivparvatia* by annual habit, lax thyrse, and two styles (Lu et al. 2001; Keshav and Kumar 2015). Finally, it can be segregated from *Odontostemma* by lax thyrse, petals with entire margin and wingless seeds (Lu et al. 2001; Sadeghian et al. 2015).

Character evolution

Our results indicated that petals with a lobed apex and numerous seeds may be the ancestral characters of the tribe Alsineae, which was consistent with previous studies (Greenberg and Donoghue 2011; Zhang et al. 2017). *Brachystemma* has entire petal margins, but it is sister to the clade composed of genera having lobed petals *Schizotechium*, *Mesostemma*, *Odontostemma*, and *Pseudostellaria* (except *Pseudostellaria maximowicziana* and *Pseudostellaria tibetica*) (Fig. 4). Moreover, the tribe Alsineae is defined by a many-

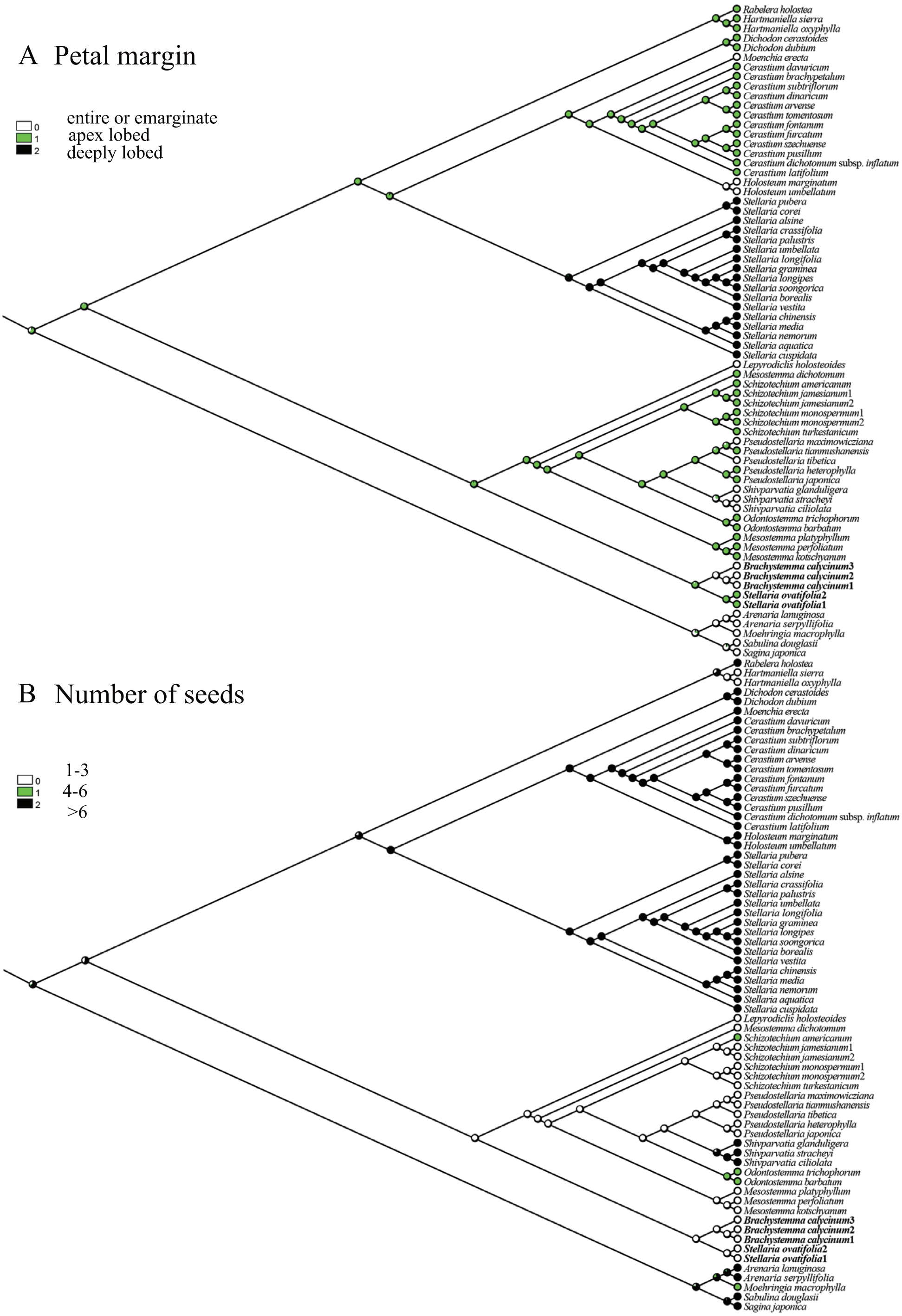

Figure 4. Evolutionary cladograms of the distribution of two character in Alsineae **A** petal margin **B** number of seeds.

seeded (rarely few- or one-seeded) capsule or a rarely indehiscent nutlet (Harbaugh et al. 2010; Greenberg and Donoghue 2011; Arabi et al. 2022), but above genera having lobed petals share the character of fewer seeds (a capsule) (Fig. 4). The tribe Alsineae may have developed in an evolutionary direction toward fewer seeds. In addition, *B. calycinum* may be a species with diverse petals based on our field observations. *B. calycinum* may also include long (longer than sepals) and apically lobed petals (Fig. 1H), instead of only short (shorter than 1/2 the sepal length) and entire petals in the protologue (Fig. 1I). While additional observations in the field and specimens are required to confirm the petal condition, the petal condition in *Brachystemma* is coded here in accordance with the protologue.

Classification of *Stellaria ovatifolia*

Although the placement of *Stellaria ovatifolia* among *Brachystemma*, *Schizotechium* and *Stellaria* has been uncertain for a long time, *S. ovatifolia* was considered more similar to *B. calycinum* in general appearance (Mizushima 1955; Wu and Ke 1996). It was clearly distinguished from the core *Stellaria* by subscandent life form (vs. non-scandent), lax thyrse (vs. cymes, rarely solitary), two styles (vs. three, rarely four or five), two-lobed (nearly to half of petal length) petals (vs. deeply-bifid petals), four-valved capsules (vs. six-valved capsules), and one mature seed (vs. many mature seeds) (Wu and Ke 1996; Lu and Gilbert 2001; Shilong and Rabeler 2001; Sharples and Tripp 2019). Despite being hypothesized to belong to *Schizotechium* (Pusalkar and Srivastava 2016), *S. ovatifolia* shows noticeable differences with *Schizotechium*, including a lax thyrse (vs. many-flowered compound cymes), two styles (vs. three styles), four-valved capsules (vs. six-valved capsules), and one mature seed (vs. one or two mature seeds) (Wu and Ke 1996; Shilong and Rabeler 2001; Pusalkar and Srivastava 2016). What is more, *S. ovatifolia* differs from *Brachystemma* by having two-lobed petals (nearly to half of petal length) and *Stellaria* type seeds, but they both share the following characters: subscandent life form, lax thyrse, two styles, four-valved capsules, and one mature seed (Fig. 1) (Wu and Ke 1996; Lu and Gilbert 2001; Shilong and Rabeler 2001). Hence, *S. ovatifolia* is highly similar to *Brachystemma*, instead of either *Stellaria* or *Schizotechium*. In terms of our molecular phylogeny, *Stellaria ovatifolia* is nested with *Brachystemma calycinum* in a clade with strong support (PP = 1.00, BS = 100) and not closely related to either *Stellaria* or *Schizotechium* in the nrDNA tree, cpDNA tree, and combined tree (Fig. 2 and Fig. 3). We believe that *S. ovatifolia* should be reclassified as a species of *Brachystemma* combining the evidence of similar general appearance and close phylogenetic relationship. As a result, the scientific name *Brachystemma ovatifolium* Mizushima is reinstated here. The main characters of *Brachystemma* now are: herbs annual or perennial; stems subscandent, branched; leaves opposite, petiolate; leaf ovate-lanceolate to lanceolate; stipules absent; inflorescence a thyrse or numerous in dichotomous, nearly subglobose cymes, terminal or axillary; flowers numerous, 5-merous, pedicellate; sepals free, subscarious, persisting in fruit; petals lanceolate or minute, much shorter than sepals, margin entire or bifid; stamens 5 or 10; styles 2; fruit a capsule, oblate, 4-valved, 1-seeded; seed reniform or globose.

Taxonomic treatment

***Brachystemma* D.Don, Prodr. Fl. Nepal. 216. 1825. Type: *B. calycinum* D.Don.**

Two species. *B. calycinum* D.Don, Prodr. Fl. Nepal. 216. 1825, and *B. ovatifolium* Mizushima, Acta Phytotax. Geobot. 16: 42. 1955.

Conclusion

Based on our study, *Brachystemma* is clearly a separate genus nested in the tribe Alsineae and now includes two Asiatic species *B. calycinum* and *B. ovatifolium*. The native range of *B. calycinum* is Assam (India), Cambodia, South-West (Tibet, Xizang province) and South-Central China, East Himalaya, Laos, Myanmar, Nepal, Thailand, Vietnam (Wu and Ke 1996; Lu and Gilbert 2001; Shilong and Rabeler 2001). The native range of *B. ovatifolium* is Nepal and China (Tibet) (Wu and Ke 1996; Lu and Gilbert 2001; Shilong and Rabeler 2001).

Acknowledgements

We thank Dr. Xinxin Zhu (Xinyang Normal University) for providing field images and samples of *S. ovatifolia*, and Zhi Xie for providing the samples of *B. calycinum*. This work is supported by National Natural Science Foundation of China (Grant No. 31760045, 31970220, and 32260047), Natural Science Foundation of Guangxi Province (Grant No. 2018GXNSFAA281132) and the Scientific Research Fund of Guangxi University of Chinese Medicine (Grant No. 2018MS011).

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Supplementary material 1

The vouchers detaled information

Authors: Wen-Qiao Wang, Zhong-Hui Ma

Data type: table (excel file)

Explanation note: The vouchers detaled information[DS/OL]. Science Data Bank, 2022[2023-02-02].

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RESEARCH ARTICLE

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Ranunculus luanchuanensis (Ranunculaceae), a new species from Henan, China

Wen-Qun Fei[1,2], Qiong Yuan[1,3], Qin-Er Yang[1,3]

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Abstract

Ranunculus luanchuanensis (Ranunculaceae), a new species from Laojun Shan in Luanchuan county, Henan province, central China, is here illustrated and described. It is morphologically similar to *R. limprichtii* in having 3-lobed and subreniform basal leaves, 3-lobed cauline leaves, and small flowers with reflexed and caducous sepals, but differs by having slender and basally slightly thickened roots (vs. fusiform), prostrate stems (vs. erect), obliquely ovoid and glabrous carpels and achenes (vs. widely ovoid and puberulous), longer styles in the carpels (ca. 1.2 mm vs. 0.6–0.8 mm) and achenes (ca. 1.8 mm vs. 0.6–0.8 mm), and glabrous receptacles (vs. sparsely puberulous). *Ranunculus luanchuanensis*, currently known only from its type locality, is geographically isolated from *R. limprichtii*, a species widely distributed in Gansu, Qinghai, Sichuan, Xizang (Tibet) and Yunnan, China. The distribution map of this new species and its putative closest ally, *R. limprichtii*, is also provided.

Keywords

Asia, buttercups, Ranunculales, *Ranunculus limprichtii*

Introduction

Ranunculus L., with ca. 600 species, is the largest genus in the Ranunculaceae and is widely distributed in all continents (Tamura 1995; Hörandl et al. 2005; Paun et al. 2005; Hörandl and Emadzade 2012). More than 150 species and 30 varieties of *Ranunculus* are currently recognized in China, one of the centers of species diversity for the genus (Wang 1995a, b,

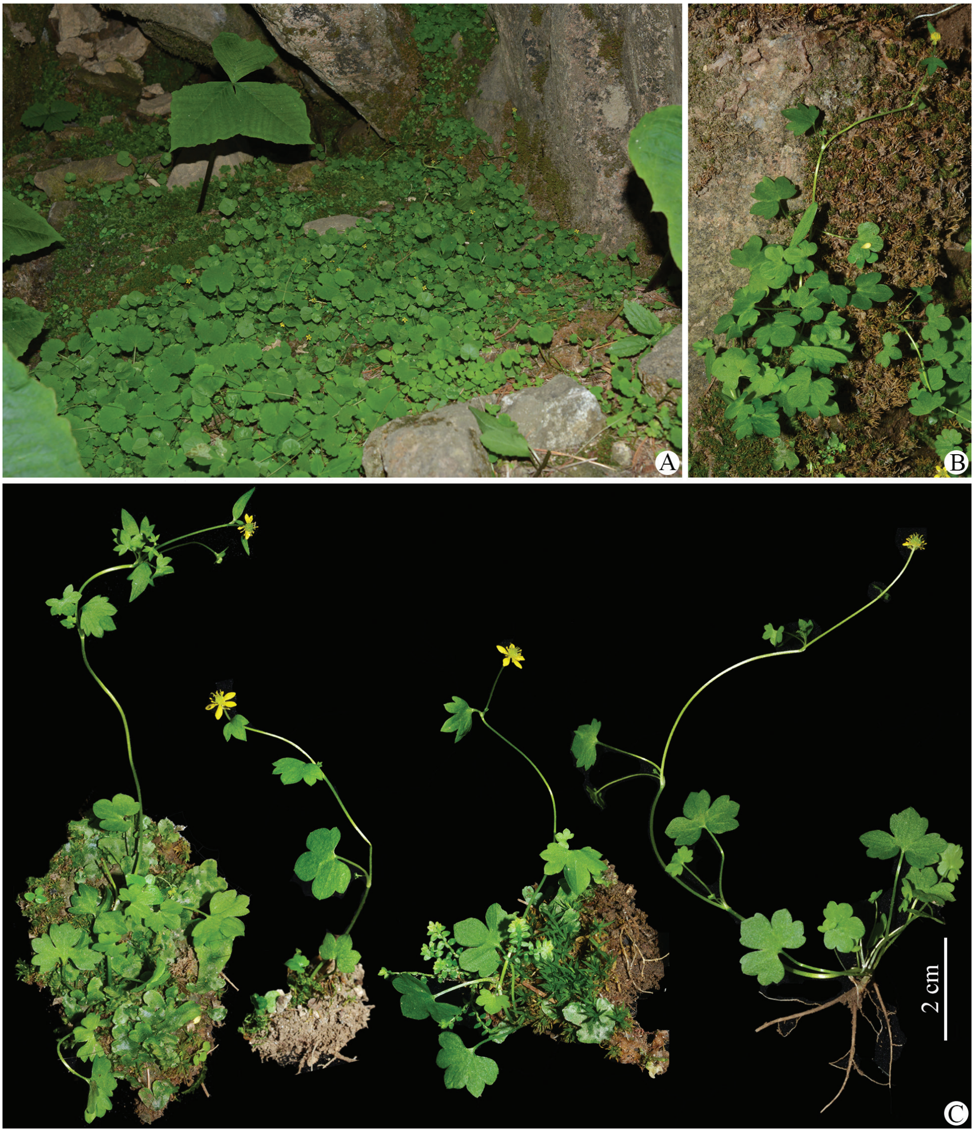


Figure 1. *Ranunculus luanchuanensis* sp. nov. in the wild (China, Henan, Luanchuan, Laojun Shan) **A, B** habitat **C** habit. Photographed by Wen-Qun Fei.

1996, 2007, 2008, 2013, 2015, 2016, 2018, 2019a, b, 2022; Yang 2000; Wang and Gilbert 2001; Wang and Liao 2009; Luo and Zhao 2013; Wang and Chen 2015; Wang et al. 2016; Yuan and Yang 2017a, b, c; Zhang et al. 2020; Fei et al. 2022, 2023a, b). New species of *Ranunculus* have been frequently found and described due to intensive field investigations of once not easily accessible areas (Wang 2022; Fei et al. 2023a, b).

During our botanical expedition in June 2022 to Laojun Shan in Luanchuan county, Henan province, central China, we encountered an unusual population of *Ranunculus* (Figs 1–4). The plants grow in a shady area among boulders and have prostrate stems,

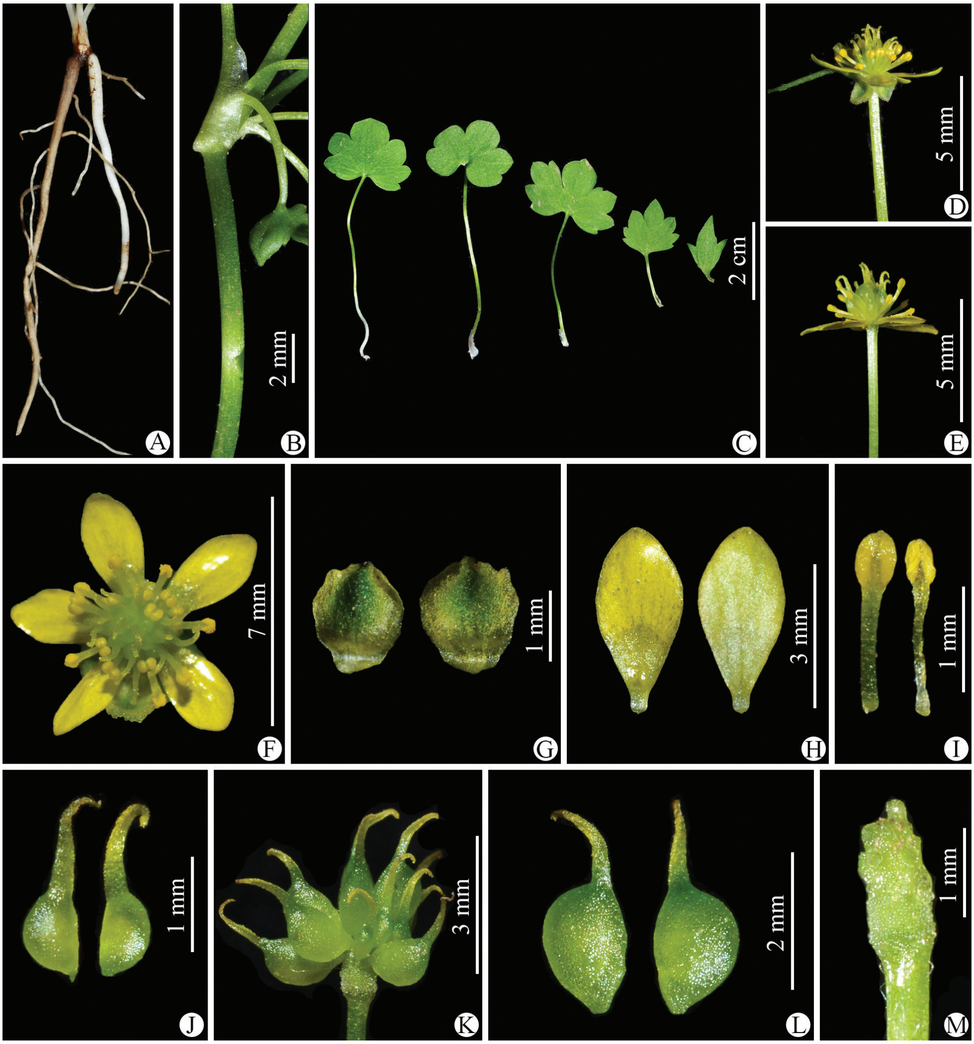


Figure 2. *Ranunculus luanchuanensis* sp. nov. in the wild (China, Henan, Luanchuan, Laojun Shan) **A** roots **B** portion of stem **C** leaves **D** flower with the sepals reflexed (lateral view) **E** flower with the sepals having fallen off (lateral view) **F** flower (top view) **G** sepal (left: abaxial side; right: adaxial side) **H** petal (left: adaxial side; right: abaxial side) **I** stamens **J** carpels **K** aggregate fruit **L** achenes **M** receptacle. Photographed by Wen-Qun Fei.

3-lobed and subreniform basal leaves, 3-lobed cauline leaves, small flowers with reflexed and caducous sepals, and glabrous carpels and achenes with long styles. They look like *R. limprichtii* Ulbr. (Figs 5–8) in having 3-lobed and subreniform basal leaves, 3-lobed cauline leaves (Figs 2C, 7C), small flowers (Figs 2D–F, 7D–F) with reflexed (Figs 2D, 7D) and caducous (Figs 2E, 7E) sepals, but differ by having slender and basally slightly thickened roots (vs. fusiform) (Figs 2A, 7A), prostrate stems (vs. erect) (Figs 1A, B, 6A, B), obliquely ovoid and glabrous carpels and achenes (vs. widely ovoid and puberulous)

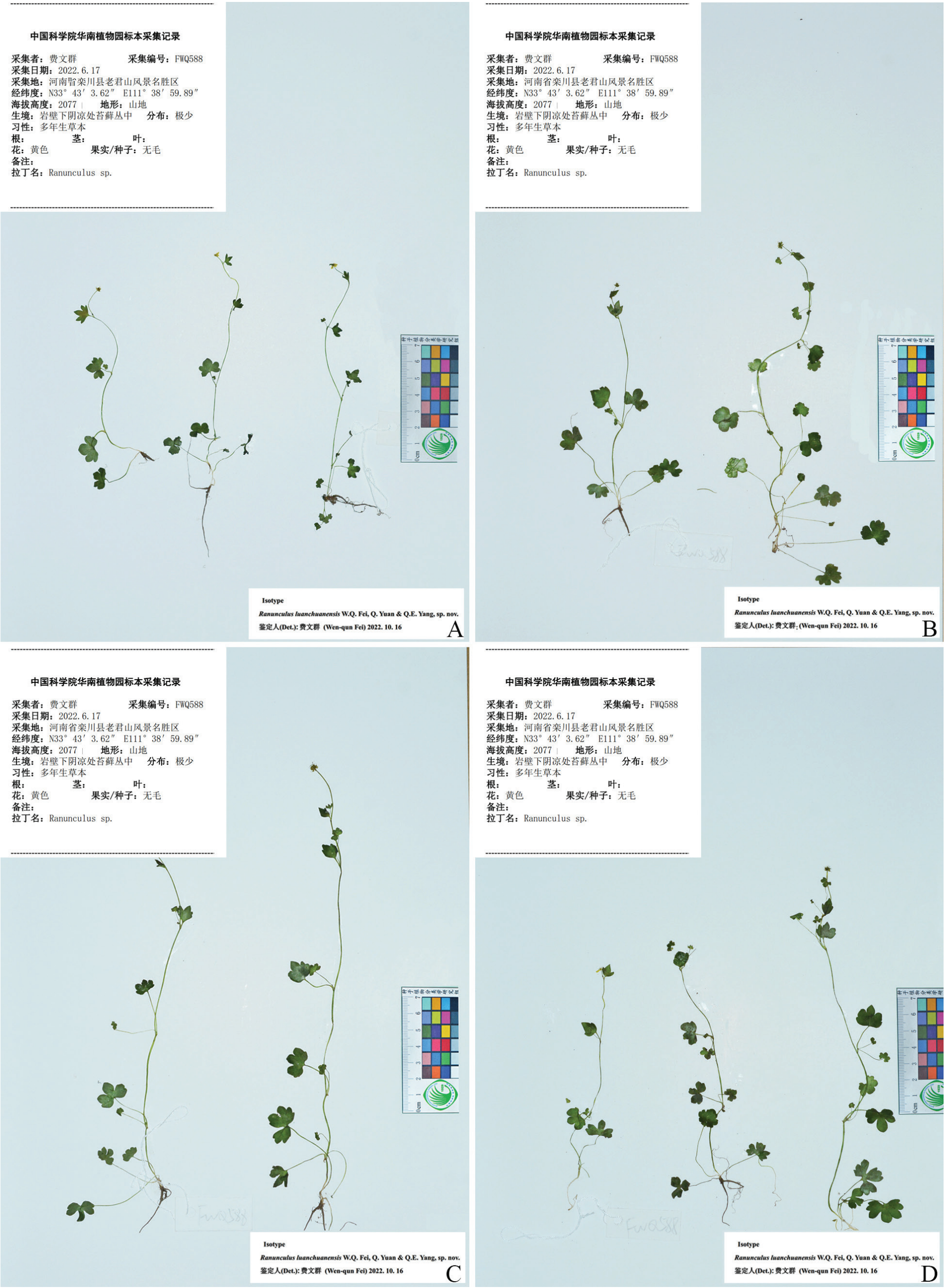
中国科学院华南植物园标本采集记录

采集者：费文群 采集编号：FWQ588
采集日期：2022.6.17
采集地：河南省栾川县老君山风景名胜区
经纬度：N33° 43′ 3.62″ E111° 38′ 59.89″
海拔高度：2077 m 地形：山地
生境：岩壁下阴凉处苔藓丛中 分布：极少
习性：多年生草本
根： 茎： 叶：
花：黄色 果实/种子：无毛
备注：
拉丁名：Ranunculus sp.

Holotype
Ranunculus luanchuanensis **W.Q. Fei, Q. Yuan & Q.E. Yang, sp. nov.**
鉴定人(Det.): 费文群 (Wen-qun Fei) 2022. 10. 16

Figure 3. Holotype sheet of *Ranunculus luanchuanensis* sp. nov.

(Figs 2J, L, 7J, L), longer styles in the carpels (ca. 1.2 mm vs. 0.6–0.8 mm) (Figs 2J, 7J) and achenes (ca. 1.8 mm vs. 0.6–0.8 mm) (Figs 2L, 7L), and glabrous receptacles (vs. sparsely puberulous) (Figs 2M, 7M). A detailed morphological comparison between the two species is given in Table 1. *Ranunculus limprichtii* is widely distributed in Gansu,



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生境：岩壁下阴凉处苔藓丛中　　分布：极少
习性：多年生草本
根：　　茎：　　叶：
花：黄色　　果实/种子：无毛
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习性：多年生草本
根：　　茎：　　叶：
花：黄色　　果实/种子：无毛
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鉴定人(Det.): 费文群; (Wen-qun Fei) 2022. 10. 16

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习性：多年生草本
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花：黄色　　果实/种子：无毛
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习性：多年生草本
根：　　茎：　　叶：
花：黄色　　果实/种子：无毛
备注：
拉丁名：Ranunculus sp.

Isotype
Ranunculus luanchuanensis **W.Q. Fei, Q. Yuan & Q.E. Yang, sp. nov.**
鉴定人(Det.): 费文群 (Wen-qun Fei) 2022. 10. 16

Figure 4. Isotype (**A–D**) sheets of *Ranunculus luanchuanensis* sp. nov.

Qinghai, Sichuan, Xizang (Tibet) and Yunnan, China (Fig. 9). Therefore, we determined that the population in question represents a hitherto undescribed species, which we name *R. luanchuanensis* and describe below.

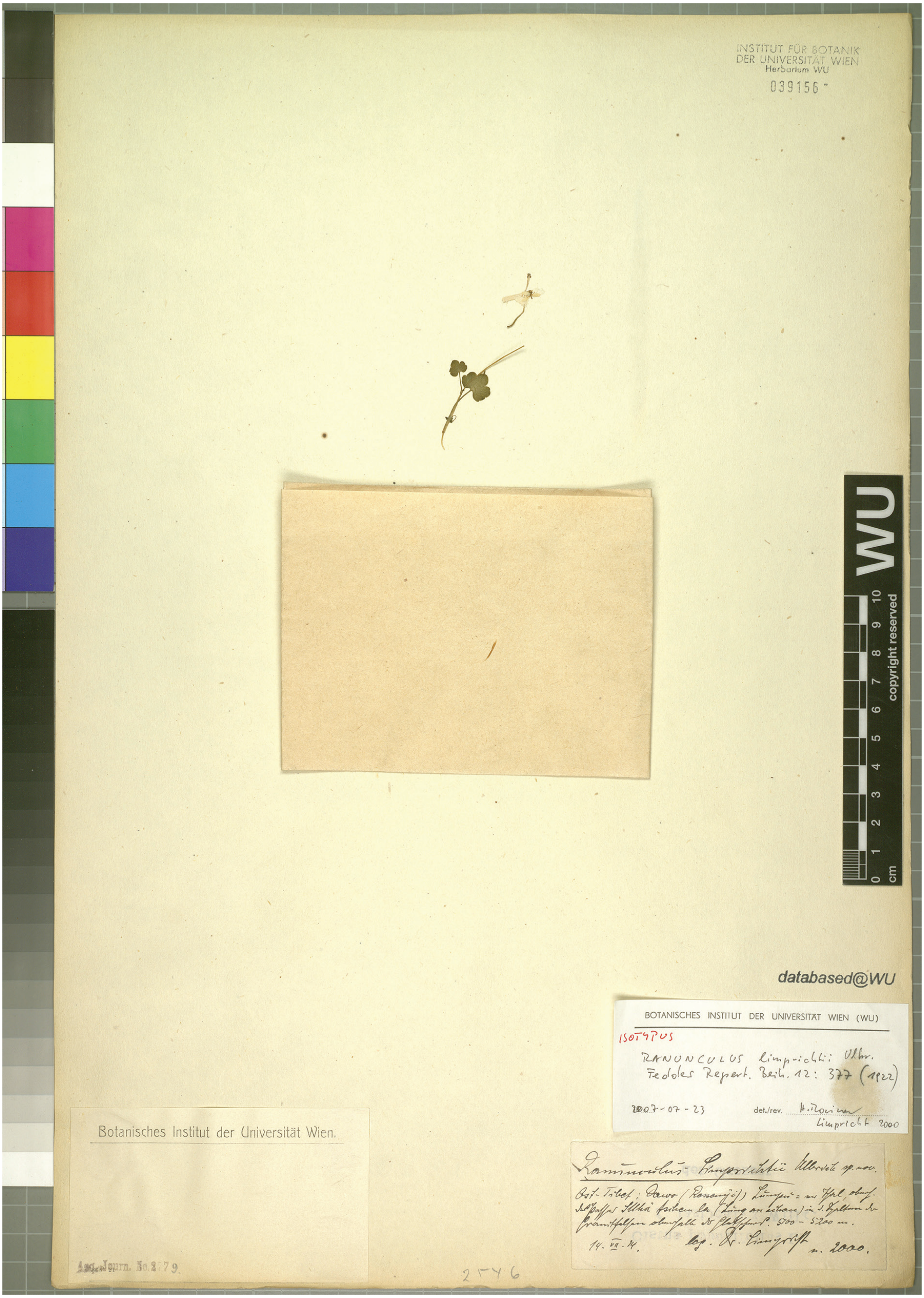

Figure 5. Isotype sheet of *Ranunculus limprichtii*. Note that the holotype was most probably destroyed during World War II.

Figure 6. *Ranunculus limprichtii* in the wild (China, Sichuan, Dawu, the type locality) **A, B** habitat **C** habit. Photographed by Wen-Qun Fei.

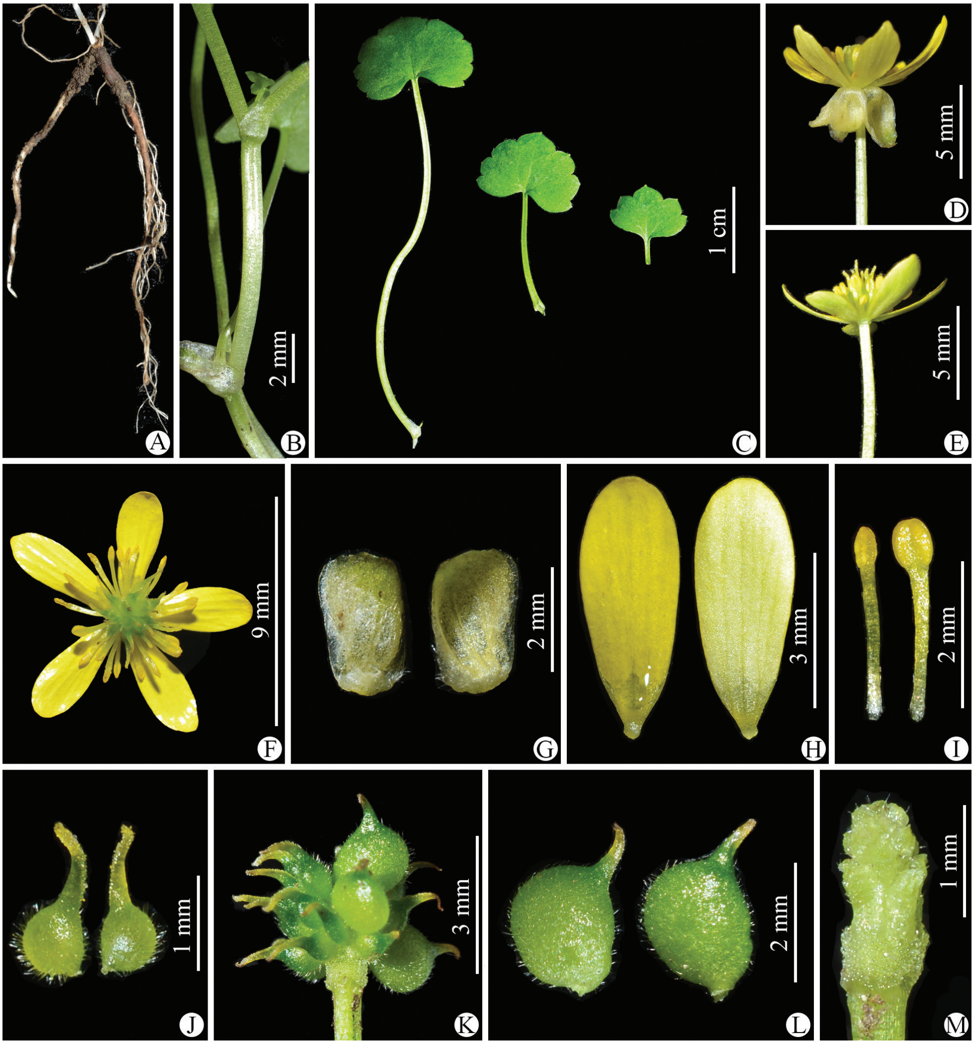

Figure 7. *Ranunculus limprichtii* in the wild (China, Sichuan, Dawu, the type locality) **A** roots **B** portion of stem **C** leaves **D** flower with the sepals reflexed (lateral view) **E** flower with the sepals having fallen off (lateral view) **F** flower (top view) **G** sepal (left: abaxial side; right: adaxial side) **H** petal (left: adaxial side; right: abaxial side) **I** stamens **J** carpels **K** aggregate fruit **L** achenes **M** receptacle. Photographed by Wen-Qun Fei.

Materials and methods

For morphological comparison, we examined physical specimens or high-resolution specimen images of *Ranunculus limprichtii* at CDBI, HNWP, KUN, PE and WU (acronyms according to Thiers 2022). We also observed living plants in one population of *R. limprichtii* (Dawu in Sichuan province, the type locality) and one population of *R. luanchuanensis* (Luanchuan in Henan province). The morphological description of *R. luanchuanensis* was based on the observation of herbarium specimens and living plants in the wild.

Figure 8. Selected specimens (**A, B**) of *Ranunculus limprichtii* from Dawu in Sichuan province, China (the type locality).

Taxonomy

Ranunculus luanchuanensis W.Q.Fei, Q.Yuan & Q.E.Yang, sp. nov.

urn:lsid:ipni.org:names:77314566-1

Figs 1–4

Diagnosis. The new species is morphologically similar to *R. limprichtii* in having 3-lobed and subreniform basal leaves, 3-lobed cauline leaves, and small flowers with reflexed and caducous sepals, but differs by having slender and basally slightly thickened roots (vs. fusiform), prostrate stems (vs. erect), obliquely ovoid and glabrous carpels and achenes (vs. widely ovoid and puberulous), longer styles in the carpels (ca. 1.2 mm vs. 0.6–0.8 mm) and achenes (ca. 1.8 mm vs. 0.6–0.8 mm), and glabrous receptacles (vs. sparsely puberulous).

Type. China. Henan province: Luanchuan county, Laojun Shan, 33°43'3.62"N, 111°38'59.89"E, in shady place among boulders on mountaintop, alt. 2077 m, 17 June 2022, *W.Q. Fei 588* (holotype: IBSC; isotypes: IBSC, PE).

Description. ***Herbs*** perennial, terrestrial or rupicolous. ***Roots*** 1–3, fibrous, slender, slightly thickened at base. ***Stems*** 12–20 cm long, prostrate, unbranched, glabrous. ***Basal leaves*** 2–6, 3-lobed, long petiolate; petioles 2–6 cm long, glabrous; blades 0.7–1.6 × 1–2.3 cm, subreniform in outline, thinly papery, adaxially green, glabrous or sparsely puberulous, abaxially light green, glabrous, base cordate, central segment 0.5–0.6 × 0.6–0.7 cm, widely obovate to rhombic-obovate, entire or 2–3-dentate, apex rounded or acuminate, lateral segments 0.5–0.7 × 0.7–1 cm, obliquely flabellate, inconspicuously 2-lobed, apex rounded or acuminate. ***Lower cauline leaves*** 2–3, similar to basal ones but smaller. ***Upper cauline leaves*** 1–2, 0.6–1.2 × 0.3–0.8 cm, 3-lobed, rarely entire, shortly petiolate or subsessile, oblate-ovate, flabellate or lanceolate, glabrous. ***Inflorescences*** terminal, 1(–2)-flowered. ***Flowers*** 6–7 mm in diameter; pedicels 0.5–2 cm long, glabrous or sparsely puberulous; receptacles ca. 1 mm long, clavate, glabrous; sepals 5, 2–2.5 × 1.5–1.8 mm, elliptic to obovate, reflexed, caducous, green tinged with yellowish, concave, adaxially glabrous, abaxially sparsely puberulous; petals 5(–6), 3–3.5 × 1.5–1.8 mm, narrowly obovate, yellow, glabrous, apex obtuse or acuminate, nectary pit without a scale, claws ca. 0.4 mm long; stamens 12–16, filaments ca. 1.5 mm long, narrowly linear, anthers ca. 0.5 mm long, oblong; gynoecium subglobose; carpels 14–18, ovaries ca. 0.8 mm long, obliquely ovoid, laterally flattened, biconvex, glabrous, styles ca. 1.2 mm long, glabrous, apex recurved. ***Aggregate fruit*** ca. 3.5 × 3.5 mm, subglobose; achenes ca. 1.8 × 1.2 mm, obliquely ovoid, laterally flattened, biconvex, glabrous, styles ca. 1.8 mm long, persistent, apex recurved.

Etymology. The specific epithet refers to the type locality of the new species, i.e., Luanchuan county in Henan province, central China.

Phenology. Flowering in early June; fruiting at the end of June.

Distribution and habitat. *Ranunculus luanchuanensis* is currently known only from its type locality, i.e., Laojun Shan in Luanchuan county, Henan province, central China (Fig. 9). It grows in a shady area among boulders on a mountaintop at an altitude of 2077 m above sea level.

Conservation status. *Ranunculus luanchuanensis* is currently known only from one small population at its type locality, i.e., Laojun Shan in Luanchuan county, Henan province, central China. This population consists of ca. 100 individuals within an area of less than 3 m^2. However, the threat risk seems low because this species is not economically valuable and grows in a secluded place. The conservation status of *R. luanchuanensis* is here categorized as “Data Deficient (DD)” before adequate information on this species is acquired (IUCN Standards and Petitions Committee 2022).

Notes. Since its description, *Ranunculus limprichtii* var. *flavus* Hand.-Mazz. has been known only from its type material from Songpan county in Sichuan province, China (Wang 1995b). Based on our observations of herbarium specimens and living plants in the wild, we agree with Liou (1980) that this variety should be reduced to the synonymy of *R. limprichtii*. We will deal with the identity of *R. limprichtii* var. *flavus* in detail elsewhere.

According to Tamura’s (1995) infrageneric classification of *Ranunculus*, *R. luanchuanensis* should be assigned to *R.* sect. *Ranunculus*, which is characterized by having swollen achenes with a distinct beak and receptacles hardly enlarged after anthesis. *Ranunculus limprichtii*, the putative closest ally of *R. luanchuanensis*, was placed by Wang (1995b) in *R.* sect. *Ranunculus*, with the section being incorrectly treated by him as *R.* sect. *Auricomus* (Spach) Schur. We accept the sectional placement of *R. limprichtii* since it is in accordance with the current placement of our new species in the same section.

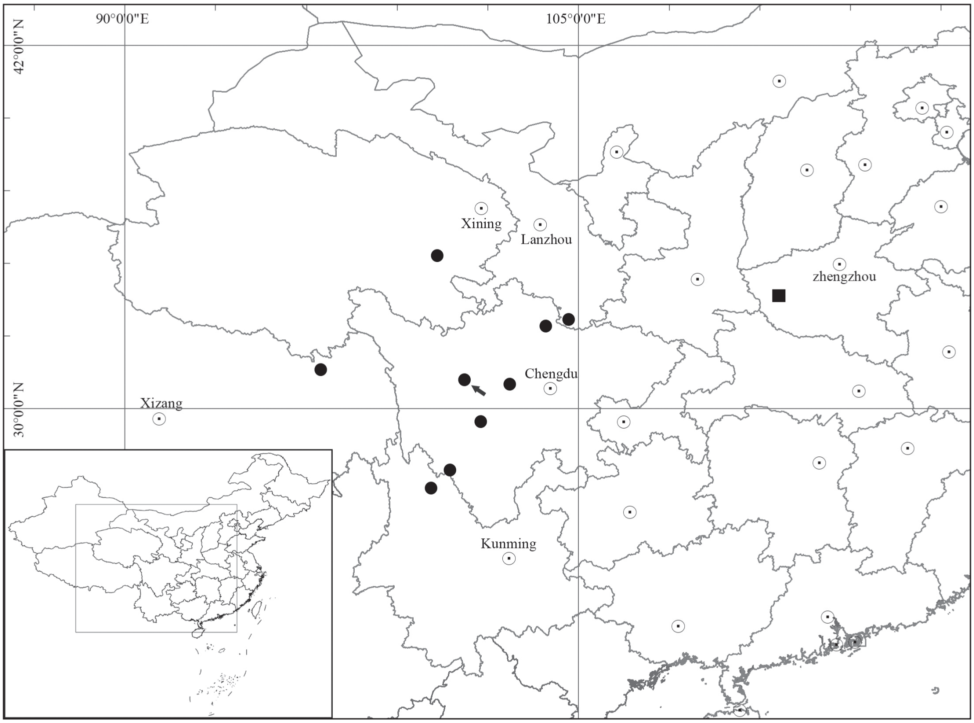

Figure 9. Distribution of *Ranunculus limprichtii* (black circle) and *R. luanchuanensis* sp. nov. (black square). Black arrow indicates the type locality of *R. limprichtii*, i.e., Dawu in Sichuan province, China.

Table 1. Morphological comparison between *Ranunculus limprichtii* and *R. luanchuanensis* sp. nov.

	R. limprichtii	***R. luanchuanensis***
Roots	1–3, fusiform	1–3, slender, slightly thickened at base
Stems	single, 7–10 cm tall, erect, glabrous or sparsely puberulous	single, 12–20 cm long, prostrate, glabrous
Basal leaves	1(–3)	2–6
Flowers	terminal, 1, 8–11 mm in diameter	terminal, 1(–2), 6–7 mm in diameter
Receptacles	ca. 1 mm long, clavate, sparsely puberulous	ca. 1 mm long, clavate, glabrous
Sepals	elliptic to obovate, abaxially sparsely puberulous	elliptic to obovate, abaxially sparsely puberulous
Petals	narrowly elliptic	narrowly obovate
Carpels	10–15; ovaries widely ovoid, puberulous; styles 0.6–0.8 mm long, apex recurved	14–18; ovaries obliquely ovoid, glabrous; styles ca. 1.2 mm long, apex recurved
Aggregate fruit	subglobose	subglobose
Achenes	widely ovoid, puberulous, styles 0.6–0.8 mm long	obliquely ovoid, glabrous, styles ca. 1.8 mm long

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The correct name for an *Aquilegia* (Ranunculaceae) hybrid of the parentage *Aquilegia flavescens* × *A. formosa*

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Abstract

Aquilegia × *miniana* (J.F.Macbr. & Payson) Cronk, **hybr. & stat. nov.** is the correct name for the hybrid *Aquilegia flavescens* S.Watson × *A. formosa* Fisch. & DC. var. *formosa*. In 1916 Payson and Macbride, while exploring the mountains of Idaho, found populations of *Aquilegia* that were pink in flower colour and appeared intermediate between the yellow-flowered *A. flavescens* and red-flowered *A. formosa*. They named these plants *A. flavescens* var. *miniana* J.F.Macbr. & Payson. There has been uncertainty over whether their type collections (in GH, RM, MO, US, E, CM, CAS, NY) do indeed represent hybrids or pink-flowered morphs of *A. flavescens*. Using a Wells diagram, the holotype (in the Gray Herbarium of Harvard University) is shown to be intermediate, allowing its identification as a clear hybrid. However, some of the isotype material is indistinguishable from *A. flavescens*. The holotype matches material from British Columbia that has been determined to be of hybrid origin using molecular and morphological data. *A. flavescens* var. *miniana* J.F.Macbr. & Payson is, therefore, an available name for the hybrid, which is here raised to the status of hybrid binomial.

Keywords

Aquilegia × *miniana*, columbines, hybridization, linear discriminant analysis, Ranunculales, Wells diagram

Introduction

Aquilegia flavescens and *A. formosa* are an ecologically separated species pair. *Aquilegia formosa* var. *formosa* is a widespread taxon of forest margins and light forest shade from sea level to montane forest, common across western North America from Utah to Alaska. By contrast, *A. flavescens* is a meadow plant of subalpine and alpine meadows, restricted to the western cordilleras from Utah to Alberta and British Columbia. The flowers of both species are strikingly different: *A. formosa* has bright red sepals, whereas *A. flavescens* has yellow (although pink-flowered morphs very rarely occur). Other distinguishing characters are more subtle. In *A. formosa*, the sepals are more tapered, the petal spurs straight and long (rather than incurved and slightly shorter), while the petal blades are small and yellow (rather than larger and paler) and the stamens are strongly exserted (less so in *A. flavescens*). In mountain regions where the two species co-occur, hybridization takes place. Hybrid populations between *A. flavescens* and *A. formosa* are very familiar to botanists in British Columbia (Canada), Idaho and Washington State (USA). No hybrid binomial up until now has been given for this despite its familiarity.

History

The hybrid was apparently encountered by Macbride and Payson (1917) during their important early botanical exploration of the mountains of Idaho in 1916 (Williams 2017). On *Aquilegia*, they write: "It has been recognized for some time that *A. flavescens* and *A. formosa* merge in the territory where their ranges join. As a result, there exist many intermediate forms, which cannot be definitely referred to by either of these species. One such state has evolved in central Idaho. There, in many localities, it entirely replaces the typical form of the species, so apparently, it has acquired a certain degree of stability. This form is similar to *A. flavescens* except that the sepals are salmon-color or flushed with pink. This color modification is striking and extremely beautiful, well worth it would seem, of varietal recognition."

Accordingly, they provided a new varietal name: *A. flavescens* S.Watson var. *miniana* J.F.Macbr. & Payson and provided an extensive series of type material from localities in central Idaho as exemplars. If these are regarded as hybrids, then the treatment as a variety of one of the parents is clearly unsatisfactory in modern usage. However, the varietal treatment clearly indicates Macbride and Payson's view that these populations, although intermediate, were closer to *A. flavescens* than to *A. formosa* (Macbride and Payson 1917). As hybrids, we would now see this in terms of introgression in the direction of *A. flavescens*.

A complication was outlined by Whittemore in his Flora of North America treatment of *Aquilegia* (Whittemore 1997). He noted that in natural populations, in addition to the pink-flowered hybrids, some pink-flowered plants are indistinguishable from the "pure" *A. flavescens* on morphological grounds (besides colour) and must therefore be considered a colour variant of *A. flavescens*. He considered that the type of *A. flavescens* var. *miniana* was one of these: "*Aquilegia flavescens* sometimes forms hybrid swarms with *A. formosa* var. *formosa*, which grows at lower elevations through much of its range. Intermediate specimens having pinkish-red flowers and petal blades 5–6 mm are occasionally found where these species grow together. The name *A. flavescens* var. *miniana* has sometimes been mistakenly applied to these intermediates, but the type of var. *miniana* is a typical, pink-sepaled plant of *A. flavescens*" (Whittemore 1997). However, the specimens annotated by Whittemore do not include the holotype (GH) but are instead isotype material (MO). Furthermore, MacBride and Payson are explicit in the protologue that they considered the populations from which they gathered the type material to be intermediate ("intermediate forms which cannot be definitely referred to either of these species"), with the implication of hybrid origin. In a recent study, Groh and Cronk (2020) undertook an extensive morphometric examination of herbarium material (including Macbride and Payson's type material). They presented evidence that the type population was (at least partly) of hybrid origin (Groh and Cronk 2020) but introgressed in the direction of *A. flavescens*, with (as Whittemore pointed out) some specimens close or indistinguishable from *A. flavescens*. Although not explicitly mentioned in that paper, the holotype (GH) was found to be intermediate and, therefore, an available name for *A. flavescens* × *A. formosa*. This paper aims to illustrate the morphological intermediacy of the holotype and, therefore, the hybridity of *A. flavescens* var. *miniana* and raise the name to hybrid binomial status to provide a convenient name for this widespread and conspicuous plant.

Methods

The complete list of type material (13 specimens) that has been examined in this study and a previous study (Groh and Cronk 2020) is as follows (summarised in Table 1):

IDAHO. Custer Co.: Stream bank in shade, Challis Creek, July 19, 1916, J.F.Macbride & E.B.Payson 3326 (GH [holo.], RM [iso.], MO 2 ex [iso.], US [iso.], E [iso.], CM [iso.], CAS [iso.], NY [iso.]); rocky, protected rocky hillside, Bonanza, July 28, 1916, J.F.Macbride & E.B.Payson 3487 (RM [para.]). Blaine Co.: along alpine brook, Sawtooth Peaks, Aug. 9, 1916, J.F.Macbride & E.B.Payson 3692 (RM [para.],

Table 1. Wells Index for type specimens of *A. flavescens* var. *miniana* J.F.Macbr. & Payson, compared with reference populations of pure species (see Fig. 2). Linear discriminant analysis (LDA) scores are given for comparison. Asterisks indicate that the specimen is abutting (*) or within (**) the range of *A. flavescens*.

Locality	Date	Coll. No.	Herbaria [type]	Wells index	LDA
Mt Kobau (BC) [*flavescens*]		MK1-MK20	UBC	0.9460-1.1535	-4.0443 - -7.3611
Roberts Lake (BC) [*formosa*]		RC1-RC20	UBC	0.1739-0.4285	3.8729 - 7.3717
Challis Creek	July 19, 1916	3326	GH [HOLO.]	0.7726	-1.5934
			RM [ISO.]	0.8217	-2.1263
			MO (#1) [ISO.]	0.8127	-2.4344
			MO (#2) [ISO.]	0.9070*	-3.6754*
			US [ISO.]	0.7306	-0.7466
			E [ISO.]	0.7958	-2.1444
			CM [ISO.]	0.7114	-0.7579
			CAS [ISO.]	0.8250	-2.1715
			NY [ISO.]	0.7438	-1.3846
Bonanza	July 28, 1916	3487	RM [PARA.]	0.7989	-1.6663
Sawtooth Peaks	Aug. 9, 1916	3692	RM [PARA.]	0.9287*	-4.2024**
		3692	CM [PARA.]	0.9704**	-4.5946**
Smoky Mts.	Aug. 13, 1916	3751	RM [PARA.]	0.7989	-1.9128

CM [para.]); crevices of granitic rock, Smoky Mts., Aug. 13, 1916, J.F.Macbride & E.B.Payson 3751 (RM [para.]).

Wells ordination

To complement the linear discriminant approach previously established (Groh et al. 2019; Groh and Cronk 2020), a Wells ordination approach has been used here (Wells 1980). The Wells method has been widely used in studies of hybridization, and for examples and discussion, see Christensen and Dar (1997); Diaz Lifante and Andres Camacho (2007); Little (2004). The morphometric data is taken from herbarium specimens and is previously generated and described by Groh et al. (2019) and Groh and Cronk (2020). Briefly, the following seven quantitative characters have been used: (a) anther exsertion; (b) corolla width; (c) petal lamina length; (d) petal spur length; (e) petal lamina width; (f) sepal length; and (g) sepal width. To avoid transport stress on the type specimens, all measurements were made using high-resolution images provided via JSTOR Global Plants (https://plants.jstor.org). The holotype material was later examined physically in GH.

In addition to the type material (13 Idaho specimens), two parental reference populations were chosen. For *A. flavescens*, a sample of 20 plants from the Mt Kobau population in southern British Columbia (BC) was used. It is morphologically typical of the species, and no *A. formosa* is known nearby. For *A. formosa* 20 individuals were sampled from a population at Roberts Lake, Vancouver Island, BC. These are typical of the species and allopatric to *A. flavescens*. Both parental populations have been shown to be pure species by molecular and morphological methods, with no evidence of hybridity (Groh et al. 2019; Groh and Cronk 2020).

The Wells method requires the construction of parental endpoints for analytical purposes by taking extreme values for each character associated with each species. Then the Euclidian distance is calculated between these endpoints and all the specimens in the analysis. Each specimen is then triangulated onto a plot by means of its distance from each of the species' endpoints and the distance between the endpoints themselves (Wells 1980). The raw measurements were log-transformed and ranged between 0 and 1. Weights were then applied for each character depending on their discriminatory power between the putative parents, calculated as the ratio of the mean of the within parent standard deviations (S_{mp}) to the standard deviation of both parents pooled (S_t), i.e., $Wi = 1–S_{mp}/S_t$ (Wicksell and Christensen 1999). For comparison, a linear discriminant analysis (LDA) on the untransformed data was performed using PAST 4 (Hammer et al. 2001).

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.79cnp5j0f.

Results

The holotype and other type specimens

Four collection numbers (Challis Creek 3326, Bonanza 3487, Sawtooth Peaks 3692, and Smoky Mts 3751) are cited in the protologue, with the Challis Creek gathering the main one on which the name is founded. Numerous duplicates were taken in the Challis Creek gathering. Still, only the single specimen at the Gray Herbarium (GH) is singled out, and it is the only specimen specifically designated as "Type". It must therefore be considered the holotype. The others are from the same gathering (3326) and, therefore, isotypes (although they likely represent different genetic individuals).

Identification of the holotype

The holotype (Fig. 1) cannot satisfactorily be identified as *A. flavescens*. The petal blades are c. 6 × 6 mm, whereas in *A. flavescens*, they are typically larger (8 × 8 mm), and in *A. formosa*, they are smaller (4 × 4 mm). The anthers of the holotype are strongly exserted, c. 10 mm, a feature of *A. formosa* (12–15 mm), rather than *A. flavescens* (typically 5–8mm). Finally, the flowers still show clear traces of pink coloration (not yellow as is general in *A. flavescens* or red as always in *A. formosa*). These features, taken together, are sufficient to allow the identification of the holotype as a hybrid. It strongly resembles material that has been identified as a hybrid by molecular methods in British Columbia in a previous study (Groh et al. 2019).

Figure 1. The two flowers preserved as part of the holotype specimen (GH). Scale bar: 1 cm.

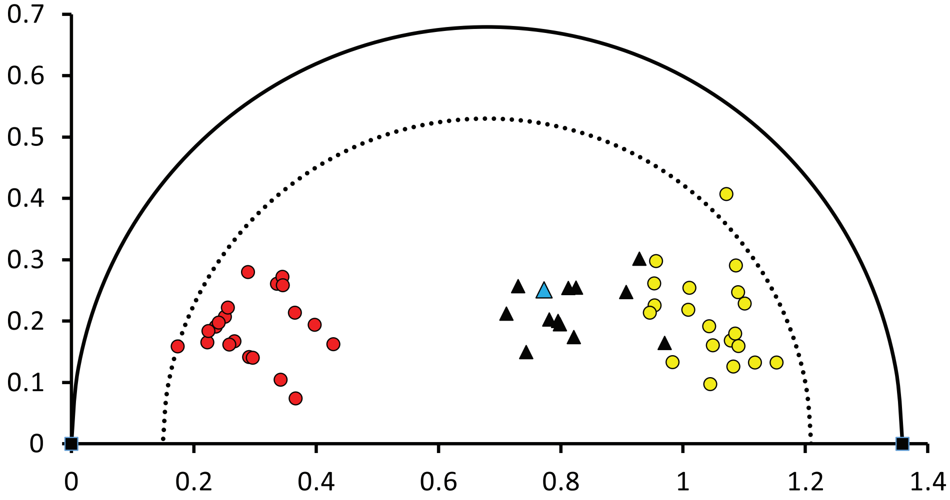

Figure 2. Wells diagram of *Aquilegia* samples. The red circles are the reference population of *A. formosa*, and the yellow circles are the reference population of *A. flavescens*. The black triangles denote type specimens, with the blue triangle indicating the holotype. The outer semicircle connects the two reference extremes on the x-axis, whereas the inner semicircle encloses all the parental specimens (except one outlier). The type specimens occupy an intermediate position, although skewed towards and intergrading with *A. flavescens*. The type specimens are also all within the inner semicircle, satisfying the theoretical expectations of intermediacy. The numerical position on the x-axis is the Wells Index (as given in Table 1).

Quantitative morphological intermediacy of the holotype and other type specimens

Quantitative data from the type specimens are summarised graphically in Fig. 2. These results complement the results previously published (Groh and Cronk 2020). The figure distinguishes the holotype from the isotypes and paratypes so that taxonomic conclusions can be drawn. Although the type specimens generally show intermediacy between the two parents, it also appears that certain specimens (from RM and MO) are indistinguishable from *A. flavescens*, as Whittemore correctly pointed out. However, as the other specimens show the intermediacy expected of hybrids, this is indicative of the type of material drawn from hybrid populations, as implied by Macbride and Payson (1917). Moreover, the holotype (GH) is distinctly intermediate between the two putative parents (Fig. 2). Macbride and Payson's name (*A. flavescens* var. *miniana*) is consequently confirmed as an available name for the hybrid.

Nomenclature

Naming a hybrid as a variety of one of its parents is not modern practice, and a hybrid binomial would be more beneficial for this widespread and characteristic hybrid. Accordingly, it is here raised to hybrid status:

Aquilegia × *miniana* (J.F.Macbr. & Payson) Cronk, hybr. & stat. nov.

A. flavescens var. *miniana* J.F.Macbr. & Payson, 1917, Contr. Gray Herb., 49: 61. Basionym.

Type material. *Holotype*. Macbride and Payson 3326 (GH). A hybrid of the parentage: *Aquilegia flavescens* S.Watson × *A. formosa* Fisch. & DC., with intermediacy between the two parents.

Type locality. In Payson (1918), further details are given of the type locality (Challis Creek) in relation to parental populations: "…Custer County, Idaho. There, near the town of Challis, at an altitude of 1620 meters, was found nearly typical *A. formosa*, while on the slopes of Parker Mountain, about 25 miles away and at an altitude of 2,400 to 2,700 metres, was found nearly typical *A. flavescens*. Intermediate forms were met along Challis Creek between these altitudes."

It is necessary to discuss an earlier name, *Aquilegia rubicunda* Tidestr. (Tidestrom 1910), collected by Tidestrom in the Wasatch Mountains of Utah. This is also an *Aquilegia* with pink sepals. It has been considered a pink-flowered form of *A. flavescens* (Welsh 1986) and, as such, might represent an earlier name for *A.* × *miniana*. However, the type specimen (US) bears no particular similarity to *A. flavescens* or its hybrid with *A. formosa*. Whittemore (1997) considered it to be *A. micrantha*. It might well repay further study, but we can rule out relevance to the hybrid considered here.

Acknowledgements

I would like to thank Dr K. Gandhi (Harvard University Herbaria) for advice on nomenclatural matters and Dr A. Brach and the curatorial staff at the Harvard University Herbaria for their kind assistance in viewing the holotype material in the Gray Herbarium. I also acknowledge a debt to J. Groh (Center for Population Biology and Department of Evolution and Ecology, University of California, Davis) for originally measuring the floral characters of the type specimens discussed here and first providing evidence for the hybridity of the holotype (Groh and Cronk 2020).

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RESEARCH ARTICLE

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Gastrodia bawanglingensis (Orchidaceae, Epidendroideae), a new species from Hainan Island, China

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Abstract

Gastrodia bawanglingensis, a new species of Orchidaceae from Hainan Island, China, is described and illustrated. It is morphologically similar to *G. theana*, *G. albidoides* and *G. albida* with dwarf habits, scarcely opening flowers, elongated fruit stems, curved and fleshy perianth tubes and similar columns and lips, but can be easily distinguished from them by having a pair of lateral wings bent outwards at the apex of the column and lateral wings with acuminate tips lower than the anther. According to the IUCN Red List Categories and Criteria, the new species is assessed as Endangered (EN). The plastome of *G. bawanglingensis* is greatly reduced and reconfigured with approximately 30876 bp in size and 25.36% in GC content. Morphological characteristics and molecular phylogenetic results based on chloroplast gene sequences support the recognition of *G. bawanglingensis* as a new species within *Gastrodia*.

Keywords

Gastrodieae, Hainan Tropical Rainforest National Park, holomycotrophic orchids, taxonomy, tropical rainforest

Introduction

Gastrodia Brown (1810: 330) (Epidendroideae, Gastrodieae) comprises approximately 100 species and is widespread from northeast India through the eastern Himalayas and southern China to Japan and eastern Siberia, southwards to Malaysia and Aus-

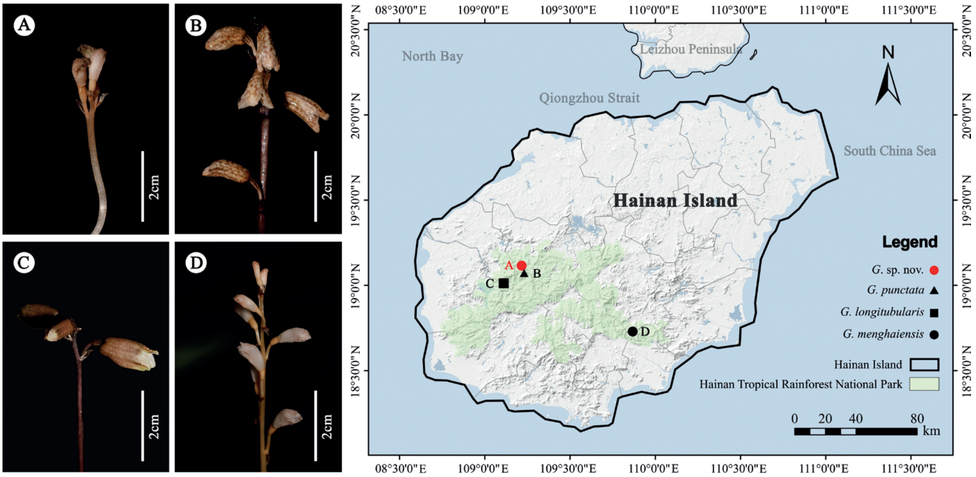

Figure 1. Pictures and distribution of *Gastrodia* species in Hainan Island based on our field investigation in the past three years. G., *Gastrodia* **A** *G.* sp. nov. **B** *G. punctata* **C** *G. longitubularis* **D** *G. menghaiensis*.

tralia, eastwards to the Pacific Islands as far as Samoa and westwards to Madagascar, Mascarene Islands and tropical Africa (Pridgeon et al. 2005; Chen et al. 2009; Cribb et al. 2010; Chase et al. 2015; Jin and Kyaw 2017; Suetsugu 2019, 2021; Bandara et al. 2020; Liu et al. 2021). There are 33 known species (16 endemic) of *Gastrodia* in China, mainly distributed in southern China, including Tibet, Fujian, Hainan, Yunnan, Sichuan and Taiwan (Liu et al. 2021; Zhou et al. 2021). In Hainan Island, three species, namely *Gastrodia longitubularis* Q.W.Meng, X.Q.Song & Y.B.Luo (Meng et al. 2007), *G. punctata* Aver. (Lu et al. 2017) and *G. menghaiensis* Z.H.Tsi & S.C.Chen (Huang et al. 2021), have been reported from the tropical rainforest (Fig. 1).

During our field investigation in April 2021, *Gastrodia* specimens with significantly different floral morphology from all the known species in China were collected in the forests of Bawangling, Hainan Tropical Rainforest National Park. Further studies, based on examination of specimens and literature of *Gastrodia* (Averyanov 2005; Hsu and Kuo 2011; Tan et al. 2012) and comparison with type specimens, showed that those specimens represent a new species that is morphologically distinct from previously-known taxa of the genus *Gastrodia* and is described below.

Materials and methods

DNA extraction and sequencing

The next generation sequencing technology (high-throughput sequencing) was applied to extract the total genomic DNA of plant materials and chloroplast splicing software GetOrganelle was used to assemble the plant genome (Jin et al. 2020). Moreover, online annotation software Geseq (https://chlorobox.mpimp-golm.mpg.de/geseq.html)

(Tillich et al. 2017) and CpGAVAS (http://www.herbalgeno-mics.org/cpgavas) (Liu et al. 2012) were used to determine the chloroplast genome start position and IR region and annotate the genes on the chloroplast genome. Finally, we used manual proofreading to verify the correctness of the annotations, according to the reference of NC_024662.1.

Phylogenetic analysis

To estimate the phylogenetic position of the *Gastrodia* sp. nov. within *Gastrodia*, phylogenies were reconstructed by Maximum likelihood (ML) and Bayesian Inference (BI) analyses using the coding sequences (CDSs). All plastomes were downloaded from the NCBI database except *Gastrodia* sp. nov. (Wen et al. 2022). In the phylogenetic tree, *Epipogium roseum* (D.Don) Lindl. and *Didymoplexis pallens* Griff. were selected as outgroup; *Epipogium* belongs to Nervilieae, a sister tribe to Gastrodieae while *Didymoplexis* is sister to *Gastrodia* (Wen et al. 2022). The sequences of the species and related ones were aligned in MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/) using MAFFT (Katoh and Standley 2013) by default setting. Phylogenetic construction was conducted by Maximum Likelihood with MEGA11 software (Tamura et al. 2021), selecting the best-fit model of GTR+G with 1000 bootstraps (Nei and Kumar 2000), and Bayesian Inference (BI) tree in MrBayes 3.2.7 using the GTR+G model (Ronquist et al. 2012), runs for 20 million generations. Phylogenetic trees were sampled every one thousand generations, the first 25% of trees generated were discarded as burn-in and the remaining trees were used to construct majority-rule consensus tree. Finally, the tree file was visualised and annotated on iTOL (https://itol.embl.de/) (Ivica and Peer 2021). All the sequences' accession numbers were listed in Fig. 2.

Morphological description

Morphological observations of *Gastrodia* sp. nov. were based on living plants (four individuals) and dried herbarium specimens all belonging to the type specimen, which is

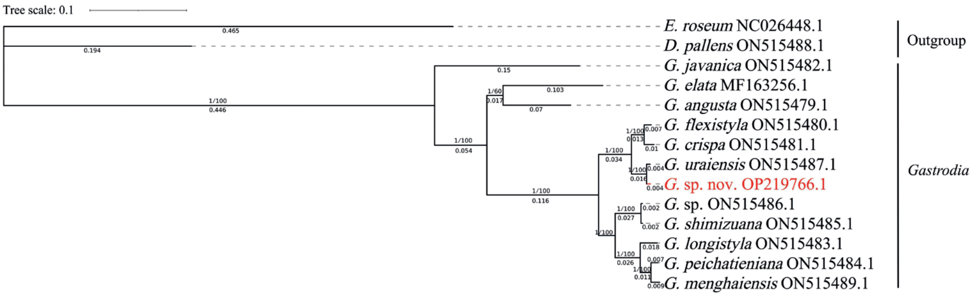

Figure 2. Phylogenetic tree reconstruction of *Gastrodia* using the maximum likelihood (ML) method based on chloroplast gene sequences of *Gastrodia* sp. nov. and 11 other species. Only the ML tree is shown, because its topology is nearly identical to that of the obtained BI tree. Numbers associated with the branches are BI posterior probabilities (PP) and ML bootstrap value (BS). The species name is followed by the accession number of the GenBank accession. D, *Didymoplexis*; E, *Epipogium*; G, *Gastrodia*.

kept in the HUFB (Teaching Herbarium of the College of Forestry, Hainan University). All length and width of structures were measured by vernier calipers. Morphological characters of the new species were based on dried herbarium specimens. Furthermore, we examined the type specimens of *Gastrodia albidoides* Y.H.Tan & T.C.Hsu, which is the most morphologically similar species to *Gastrodia* sp. nov. and housed in HFTC. High resolution photographs of living plants were provided by Zhong-Yang Zhang and Zhi-Heng Chen.

Results

Plastome of *Gastrodia* sp. nov.

The plastome of the novelty is 30876 bp in length with its GC content approximately 25.36% (GenBank accession number: OP219766) (Fig. 3), which is similar to the 11 other species of *Gastrodia* (29,696–36,812 bp, Table 1). The plastome contains 19 protein-coding genes, five transfer RNA and three ribosomal RNA genes. Several genes and typical plastome regions appear to have been either lost or pseudogenised in *G.* sp. nov. The *G.* sp. nov. plastome does not contain housekeeping genes and lacks an IR region. This indicates that plastomes of *Gastrodia* are in the last stages of plastome degradation (see Barrett and Davis 2012; Liu et al. 2021; Jiang et al. 2022; Wen et al. 2022).

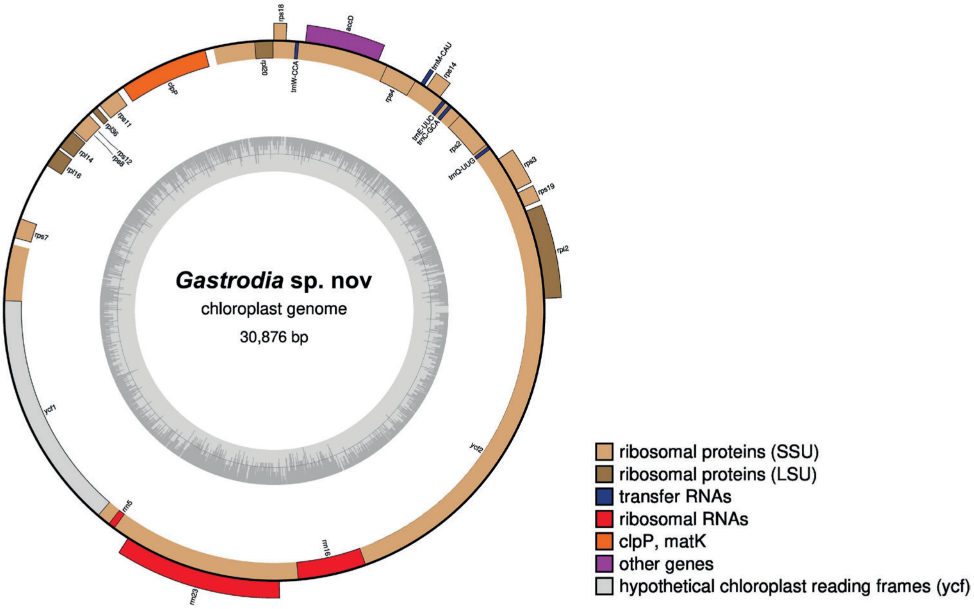

Figure 3. Plastome of *Gastrodia* sp. nov.

Table 1. Information on the chloroplast genomes of *Gastrodia* sp. nov. and other 11 species of *Gastrodia*.

Species	Length of chloroplast genome (bp)	GC content (%)	Number of genes		
			Protein coding genes	*t*RNA genes	*r*RNA genes
Gastrodia angusta	36.812	25.4	19	5	4
Gastrodia crispa	30.582	25.7	19	5	4
Gastrodia elata	35.304	26.8	20	5	3
Gastrodia flexistyla	30.797	25.4	19	5	4
Gastrodia javanica	31.896	24.8	18	4	4
Gastrodia longistyla	30.464	24.8	18	5	3
Gastrodia menghaiensis	30.118	24.9	19	4	3
Gastrodia peichatieniana	29.696	25.9	18	5	4
Gastrodia shimizuana	30.019	25.5	18	5	4
Gastrodia sp.	29.944	25.8	18	5	4
Gastrodia sp. nov.	30.876	25.4	19	5	3
Gastrodia uraiensis	30.746	24.9	19	5	4

Phylogenetic analysis

Our ML and BI phylogenetic trees constructed from the chloroplast gene sequences showed that the novelty belongs to the genus *Gastrodia*, and is related to *G. uraiensis*, *G. flexistyla* and *G. crispa*.

Taxonomic treatment

Gastrodia bawanglingensis Z.H.Chen, Z.Y.Zhang & X.Q.Song, sp. nov.

urn:lsid:ipni.org:names:77314677-1
Fig. 4

Type. China. Hainan Province: Bawangling, Hainan Tropical Rainforest National Park, in tropical rainforest, 850–950 m elevation, 25 April 2022, Z.Y. Zhang 006 (Holotype, HUFB!).

Diagnosis. *Gastrodia bawanglingensis* is similar to *G. albidoides* with dwarf habits, scarcely opening flowers, elongated fruit stems, curved and fleshy perianth tubes and similar columns and lips, but can be easily distinguished from the latter by having lateral sepals adnate to 4/5 of total length (vs. lateral sepals adnate to 1/2 of total length), lip with four ridges (vs. lip with two ridges), the absence of a column foot (vs. the presence of a column foot) and a pair of lateral wings bent outwards (vs. lateral wings upright) at the column apex (Table 2).

Description. Terrestrial, leafless, achlorophyllous herbs. Roots few, slender, 1–7 cm long, ca. 0.5–0.7 mm in diameter. Rhizome fleshy, tuberous, fusiform, 3–4 cm long, 5–7 mm in diameter, dark brown, covered with numerous scales. Scales verticillate, lanceolate, dark brown,1–2 mm long. Inflorescence erect, terminal, 2.0–6.5 cm long, ca. 2.2 mm in diameter, white to orange-brown, peduncle 3–4 noded, ovate to broadly ovate, sheath membranous, 3–5 × 2–3 mm; rachis often less than 5 mm long. Bracts membranous, ovate to ovate-oblong, apex pointed, pale yellowish-brown, 4–6 mm long, 1.5–3 mm wide. Ovary 3–6 mm long, 2–3 mm in diameter. Flowers (1–) 2–4 (–6), erect, bell-shaped, slightly curved, not opening widely, 8–10 mm long, 4–5 mm in diameter. Flowers whitish on both surfaces, apex brownish, lip red at the base, light green at the middle, reddish-brown apically and marginally; column white. Sepals and petals united, forming a 5–lobed perianth tube, 8–10 mm long, slightly verrucous in the middle and upper part, distinctly verrucose apically. Sepals fleshy, thickened, similar. Lateral sepals fused to 4/5 of their length, whitish on both surfaces, apex is brownish; free lobe of dorsal sepal triangle, ca. 2.5 × 2.0 mm; free lobes of lateral sepals ovate, ca. 2.0 × 2.0 mm. Petals connate with sepals, free portions brownish, whitish on both sides, triangular-ovate, ca. 1.5 mm long, 1 mm wide, connate portions distinctly thickened and the inside is obviously reddish-brown, forming a pair of ridge-like structures inside the perianth tube and the other side of the ridge-like structures is flesh-coloured. Lip rhombic-ovate, base adnate to perianth tube, 3.5–4.5 × 2.0–2.2 mm; hypochile with two whitish, globose, subsessile, nectarless calli, ca. 0.5 mm in diameter; epichile 5–7 nerved, truncate at base, entire, disc thickened with four ridges, a pair of low ridges outside the two main ridges; the two main ridges fused into one before reaching the tip, main ridges much raised and tinged orange near apex. Column 4.2–4.5 × 1.6–1.8 mm, apex with a pair of lateral wings bent outwards; lateral wings with acuminate tips lower than anther; column foot absent; rostellum 0.2 × 1 mm; stigma located near base. Anther hemispherical, 0.6–0.7 mm in diameter; pollinia 2. Capsule ellipsoid, 1.2–1.8 cm long, 0.5–0.8 cm in diameter; pedicel elongating to 10–25 cm in fruit. Seeds fusiform, 1.6–2.2 mm long.

Etymology. The new species is named after Bawangling, the mid-west State of Hainan Island where it was discovered in a vast area of primitive montane rainforest.

Vernacular name. 霸王岭天麻 (Chinese pinyin: bà wáng lǐng tiān má).

Distribution and habitat. *Gastrodia bawanglingensis* is a terrestrial mycoheterotrophic species that grows in montane rainforests which are dominated by *Dysoxylum gotadhora* (Buch.-Ham.) Mabb., *Livistona saribus* (Lour.) Merr. and A.Chev., *Hancea hookeriana* Seem. and *Engelhardia roxburghiana* Lindl. at elevations from 850 m to 950 m and associated with other orchids, such as *Anoectochilus hainanensis* H.Z.Tian, F.W.Xing & L.Li, *A. roxburghii* (Wall.) Lindl., *Oxystophyllum changjiangense* (S.J.Cheng & C.Z.Tang) M.A.Clem., *Dendrobium hainanense* Rolfe, *Cymbidium kanran* Makino and *Micropera poilanei* (Guillaumin) Garay. So far, only the type subpopulation has been found in the tropical rainforest of Bawangling, in Hainan.

Conservation status. Endangered [EN D1]. *Gastrodia bawanglingensis* was discovered in the mountain rainforest of Bawangling in Hainan Tropical Rainforest National Park. Until now, only the type subpopulation, consisting of ca. 100 individuals, has been discovered in Bawangling. Since its number of mature individuals is fewer than 250, we assess it as Endangered (EN) using criterion D1 (IUCN Standards and Petitions Subcommittee 2022).

Phenology. *Gastrodia bawanglingensis* was observed flowering and fruiting in April and May.

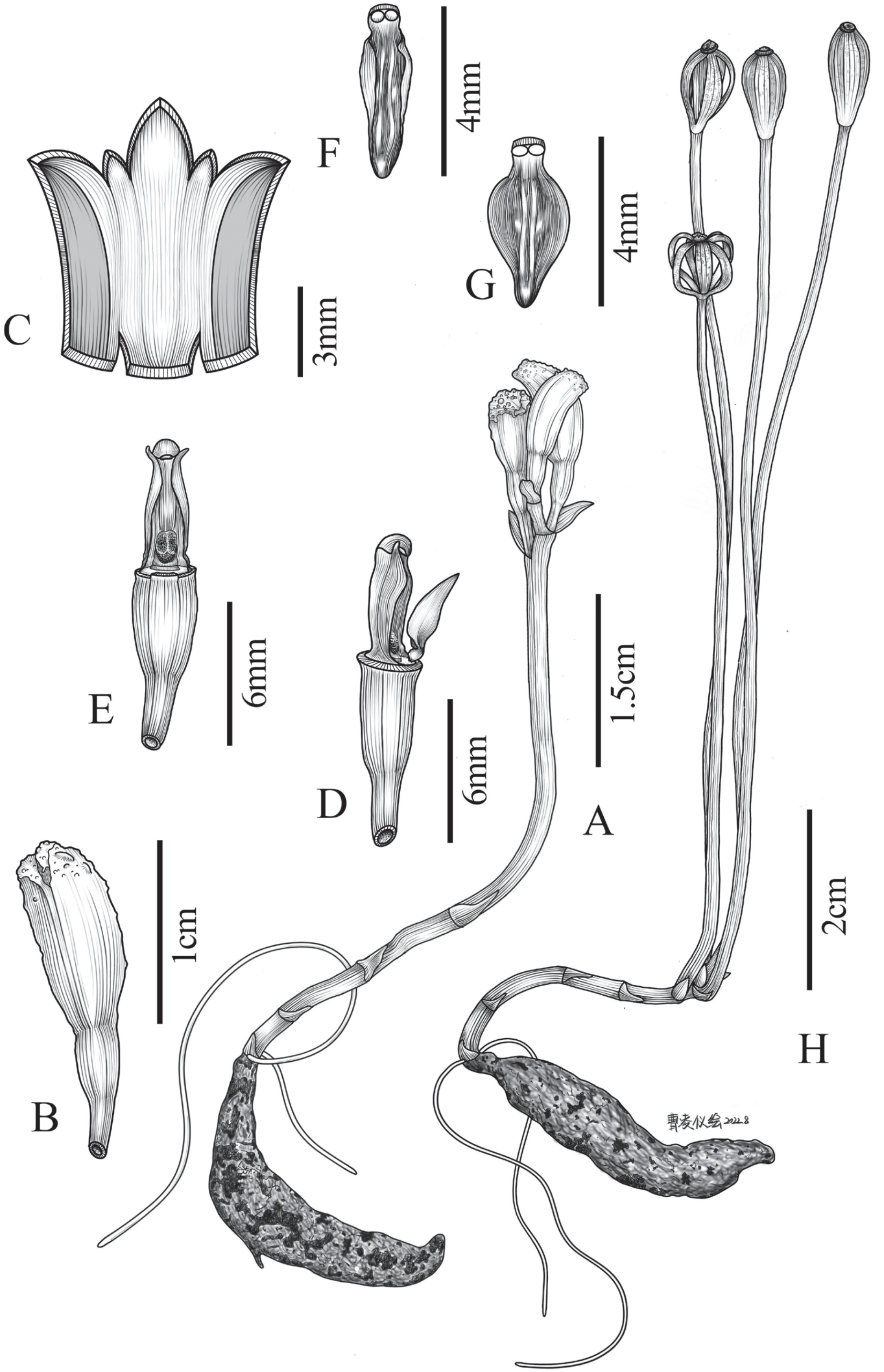

Figure 4. *Gastrodia bawanglingensis* Z.H.Chen, Z.Y.Zhang & X.Q.Song, sp. nov. **A** plant **B** flowers **C** flattened perianth tube **D** lip, column and ovary **E** column **F, G** lip **H** fruiting specimen. Illustration by Ling-Yi Cao, based on the holotype of Z.Y. Zhang 006 (HUFB).

Table 2. Differences between *Gastrodia bawanglingensis*, *G. albidoides*, *G. theana* and *G. albida*.

Character	***G. bawanglingensis***	***G. albidoides***	***G. theana***	***G. albida***
Perianth tube	slightly verrucous in the middle and upper part, distinct verrucose apically	slightly verrucose towards apex, otherwise smooth	distinctly striate and verrucose throughout	distinctly verrucose throughout
Lateral sepals	adnate, to 4/5 of their length	adnate, to 1/2 their length	adnate, 1/3–1/4 their length	adnate, 1/5–1/6 their length
Petals	brownish, fleshy, petals whitish on both sides, triangular-ovate, ca. 1.5 × 1.0 mm	whitish, thin in texture, triangular-ovate, 0.8–1.0 × 0.6–0.8 mm	salmon-pink, thin in texture, narrowly triangular, 0.4–0.8 × 0.2–0.3 mm	whitish outside, orange inside, fleshy, oblong-ovate, ca. 1.5 × 1.0 mm
Lip	red at the base, light green at the middle, reddish-brown apically and marginally, epichile rhombic-ovate, 5–7 nerved, disc thickened with four ridges, a pair of low ridges outside the two main ridges. truncate at base, hypochile with two whitish, globose, subsessile, nectarless calli, ca. 0.5 mm in diameter	pale green, epichile rhombic-ovate, 6–7-nerved, disc thickened with two ridges, rounded at base, hypochile with two whitish, globose, subsessile, nectarless calli, ca.1 mm in diameter	green, epichile ovate, 5-nerved, disc slightly elevate longitudinally at middle, with four ridges four ridges, arranged one behind the other. cordate at base, hypochile with two whitish, globose, subsessile, nectarless calli, ca.0.8 mm in diameter	white, epichile triangular, disc thickened with two ridges, truncate at base; hypochile with two whitish, globose, subsessile, nectarless calli, ca.1 mm in diameter
Column	apex with a pair of lateral wings bent outwards; lateral wings with acuminate tips lower than anther	apex with a pair of lateral wings; lateral wings with acuminate tips superior to anther	apex with a pair of lateral wings bent inwards; lateral wings with acuminate tips superior to anther	with a pair of lateral wings distally; edges of lateral wings parallel to column
Column foot	Absent	1.5–1.8 mm	1.5–1.8 mm	column foot very short
Rostellum	0.2 × 1.0 mm	0.2 × 1.5 mm	0.2 × 1.5 mm	Absent

Pollination implication. Flowers of *Gastrodia bawanglingensis* barely open and pollen massulae were observed on the stigma when flowers were dissected. Through field observation, it was found that the fruiting rate is very high. We bagged buds on 3 plants with 10 flowers in total prior to the anthesis, and found that each of them has evolved into fruit after 15 days. These observations indicate that the new species probably self-pollinates. *Gastrodia* is probably the only genus that contains species with completely cleistogamous flowers as confirmed by intensive monitoring. Self-pollination might be an adaptation to ensure reproduction, compensating for the defi- ciency of pollinators in the habitat (Suetsugu 2022; Suetsugu et al. 2022). Currently, complete cleistogamy has been reported in five *Gastrodia* species: *G. clausa*, *G. takeshimensis*, *G. flexistyloides*, *G. kuroshimensis* and *G. amamiana* (Hsu et al. 2012; Suetsugu 2013, 2014, 2016, 2019), *G. bawanglingensis* is likely to be the sixth species reported. Similar to other five species, *G. bawanglingensis* is also distributed on the island, further confirming island colonization may be one of the factors of evolution of complete cleistogamy. And compared with the mainland, there are more frequent geological and climate changes on the island, which may cause the rapid change of its living environment and lead to the loss of pollinators in its distribution area. Unreliable pollinator services and the cost of maintaining open flowers probably drove the completely cleistogamous *Gastrodia* species to abandon insect-mediated pollination (Suetsugu 2014, 2016). However, complete cleistogamy has arguably driven speciation (Kishikawa et al. 2019; Ogaki et al. 2019). We also found several other unpublished species that are different but very similar to *G. bawanglingensis* in our field survey in Hainan Island, which also confirms the above point of view. It is also notable that although lack of rostellum often facilitates selfing in the genus (Suetsugu 2022; Suetsugu et al. 2022), the new species has somewhat well-developed rostellum. Further observations are needed on how the species accomplishes autogamy.

Discussion

Gastrodia bawanglingensis is most similar to *G. albidoides* (Tan et al. 2012) from Yunnan, *G. theana* (Averyanov 2005) from Vietnam and *G. albida* (Hsu and Kuo 2011) from Taiwan. They share dwarf habits, scarcely opening flowers, fleshy curved perianth tubes with verruca and similar columns and lips. After comparison of available literature and specimens, we conclude that *G. bawanglingensis* could be clearly differentiated from *G. albidoides*, *G. theana* and *G. albida* by several floral characters (Table 2).

Key to the species of *Gastrodia* found in Hainan Island, China

1 Sepals adnate to 4/5 of their length; lip light green at the middle, reddish-brown apically and marginally; lip disc with two ridges ranging from base to apex .... ***G. bawanglingensis***
– Sepals adnate up to 1/2 of their length; lip green or white at the middle, uniform coloured or orange-red towards apex; lip disc without distinct ridges, but with lamellae or keel **2**
2 Flowers white, sub-erect; petals margin wrinkled; column foot very short, pedicel elongated in fruit .... ***G. menghaiensis***
– Flowers grey-brownish, horizontal or slightly bending; petals margin entire, column foot distinct; pedicel not elongated in fruit **3**
3 Tepal tube without white spots; column cylindrical and thick; lip disc with a pair of longitudinal lamellae near apex .... ***G. longitubularis***
– Tepal tube with white spots; column flat and thin; lip disc with four keels... .... ***G. punctata***

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RESEARCH ARTICLE

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Taxonomic notes on the genus *Deutzia* (Hydrangeaceae) from Central China

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Abstract

Based on examination of syntype specimens deposited at P, the lectotype for the name *Deutzia setchuenensis* Franch. is designated here. By consulting literature and specimen records, the type locality of *D. setchuenensis* var. *longidentata* Rehder, ‘Chin-Ting shan’ in the protologue is likely a misspelling of ‘Chiuting shan’ which is now called Jiuding shan located in southern Mao county, Sichuan province. In addition, a new variety, *Deutzia setchuenensis* var. *macrocarpa* Q.L.Gan, Z.Y.Li & S.Z.Xu from western Hubei, Central China, is described and illustrated. It differs from other varieties of *D. setchuenensis* Franch. by the orange anthers, broader outer filaments, obtuse inner filaments, and larger fruits.

Keywords

Deutzia setchuenensis, lectotype, new variety, var. *macrocarpa*

Introduction

Deutzia Thunb. as the second largest genus of the tribe Philadelpheae (Hydrangeaceae), consists of ca. 60 species and is mainly distributed across eastern Asia and Mexico, with ca. 50 species found in China (Hwang et al. 2001; Hufford 2004). *Deutzia setchuenensis* Franch. and its varieties, which were native to China, were widely introduced as ornamental plants (Hutchinson 1909; Rehder 1940; Brickell and Zuk 1996).

Recently, when we were identifying *Deutzia* specimens from Zhuxi county, Hubei province, we found a wild *Deutzia* morphologically similar to *Deutzia setchuenensis*, but with larger capsules. In order to identify this taxon, we have consulted protologue and specimens. However, we found that the type of *D. setchuenensis* is not designated, evidenced by the fact that all three gatherings (MNHN-P-P04573103, MNHN-P-P04573105 & MNHN-P-P04573106) stored at Muséum d'Histoire Naturelle, Paris (P) (Thiers 2016) were referred as the type. This necessitated designation of a single specimen as the type of this species from the aforesaid syntype. After examinations of syntypes at P, the lectotype of the species is designated in this study. In addition, we found the type locality of *D. setchuenensis* var. *longidentata* Rehder remains confused, and 'Chin-Ting shan' in the original record is likely a misspelling for Chiuting shan (now Jiuding shan). Finally, after checking Flora of China (Hwang et al. 2001), and relative literature and making comparisons with the specimens of *Deutzia* stored in PE and some virtual specimen databases (P, A, CVH, and JSTOR), we found that this unknown taxon resembles *Deutzia setchuenensis* in stem, leaf, flower, fruit and indumentum, white disc, and smaller seeds, but differs from three varieties of *D. setchuenensis* in the color of anthers, shape of outer and inner stamens, and size of fruits (Hutchinson 1909; Rehder 1911; Hwang 1992, 1995; Hwang et al. 2001). Therefore, we confirm that these peculiar plants represent a new variety of *Deutzia setchuenensis*, which is described and illustrated here.

Results

Lectotypification of *Deutzia setchuenensis* Franch.

***Deutzia setchuenensis* Franch. in Journ. De Bot. (Morot) 10: 282. 1896**

Type. China. 'Set-Chuen orientalis, circa Tchen-kéou-tin' (eastern Sichuan, near Chengkou tin), P. Farges s.n. (lectotype, P, P04573103 designated here; isolectotypes, P, P04573105 & P04573106; photos PE!).

Note. Adrien René Franchet (1896) published the species based on the type collected from Chengkou tin by P. Farges. There are three specimens collected from Chengkou tin by Farges deposited in Muséum d'Histoire Naturelle, Paris (P), and the label data of the specimens were exactly the same as original records, including the collector, collection locality, and all of them were flowering branches. Of them, two specimens (P04573103 and P04573105) were determined by Franchet, while another (P04573106) was determined by Alfred Rehder. It is clear that these three specimens are the syntypes of *Deutzia setchuenensis*. Based on examination of the syntypes, we selected the more perfect one (P04573103) as lectotype for the species. French missionary and plant collector, Paul Guillaume Farges collected more than 4000 specimens in Chengkou tin from 1892 to 1896 (Bretschneider 1898; Cox 1945). Chengkou tin (1822–1912) was previously located in the administrative division of Qing dynasty, an area renamed Chengkou county since 1913.

Correction of the type locality

Deutzia setchuenensis **var.** ***longidentata*** **Rehder in Sargent, Pl. Wils. 1: 8. 1911.**

Type. China. western Szechuan (Sichuan): Chiuting shan (original record misspelled it as Chinting), thickets, alt. 1200–1500 m, 25 May 1908, E.H. Wilson 2895 (holotype, A, A0042097; photo, PE!).

Note. According to 'Plantae Wilsonianae', from late spring to summer, 1908, E.H. Wilson collected plant specimens along the Min River valleys, and in late May when he was in Jiuding shan (Chiuting shan) around 31°51'N, 103°76'E, southern Mao county, not Jinding (Chinting), the main peak of Emei mountain (29°52'N, 103°33'E).

Taxonomic treatment of new variety

Deutzia setchuenensis **Franch. var.** ***macrocarpa*** **Q.L.Gan, Z.Y.Li & S.Z.Xu, var. nov.**
urn:lsid:ipni.org:names:77314712-1
Figs 1,2

Diagnosis. The new variety, *Deutzia setchuenensis* Franch. var. *macrocarpa* Q.L.Gan, Z.Y.Li & S.Z.Xu can be easily distinguished from other varieties (var. *setchuenensis*, var. *corymbiflora* (Lemoine) Rehder, var. *setchuenensis* and var. *longidentata* Rehder) by its orange anthers (vs. yellow anthers), broadly oblong outer filaments with 2 small repand denticles at apex, the width of teeth is more than twice its length (vs. oblong, with 2 deltoid, oblong or lanceolate teeth at apex, the length of teeth is equal to or more than its width), obtuse apex of inner filaments (vs. 2-dentata at apex), and larger fruits 5–7 mm in diam. (vs. 4–5 mm in diam.).

Type. China. Hubei Province: Hongyangou village, Quanxi town, Zhuxi county, alt. 850m, 25 June 2022, Q.L.Gan 3306 (fl., holotype, PE!; isotype, PE!).

Paratypes. China. Hubei province: Hengduanshan, Baguashan Natural Reserve, Zhuxi county, alt. 840m, 13 June 2022, Q.L.Gan 3305 (fl., PE!); the same locality, 2 August 2022, Q.L.Gan 3307 (fr., PE!).

Description. Deciduous shrubs, 90–150 cm tall. Old stems pale gray-brown, often with flaky bark; branches erect to spreading; branchlet opposite, sparsely gray stellate pubescent; flowering branchlets 8–14 cm, 6–10-leaved. Leaves opposite, stipules absent; petiole 3–5 mm long, sparsely stellate-pubescent; leaf blades papery, ovate to ovate-lanceolate, 2–10 cm long, 0.6–3 cm wide, base rounded to broadly cuneate, margin serrulate, apex acuminate or caudate-acuminate, adaxial surface green, not gloss, sparsely stellate pubescent, trichomes 2–4-rayed, abaxial light green, trichomes 4–6-rayed; lateral veins 2–4 paired, mid-vein and lateral veins impressed abaxially, and slightly prominent adaxially, veinlets inconspicuous. Cymes 2–3.5 cm long, 2–3 cm across, 6–12-flowered, sparsely stellate-pubescent; peduncle slender; pedicels 3–6 mm long, usually with 1 to 2 bracts at base or around the middle; bracts linear, 3–6 mm long, 0.5–1 mm wide; flower buds spheroical. Hypanthium hemispheric, 2.5–3.5 mm long and wide, densely 10–13-rayed stellate-tomentulose; calyx lobes 5, broadly deltoid,

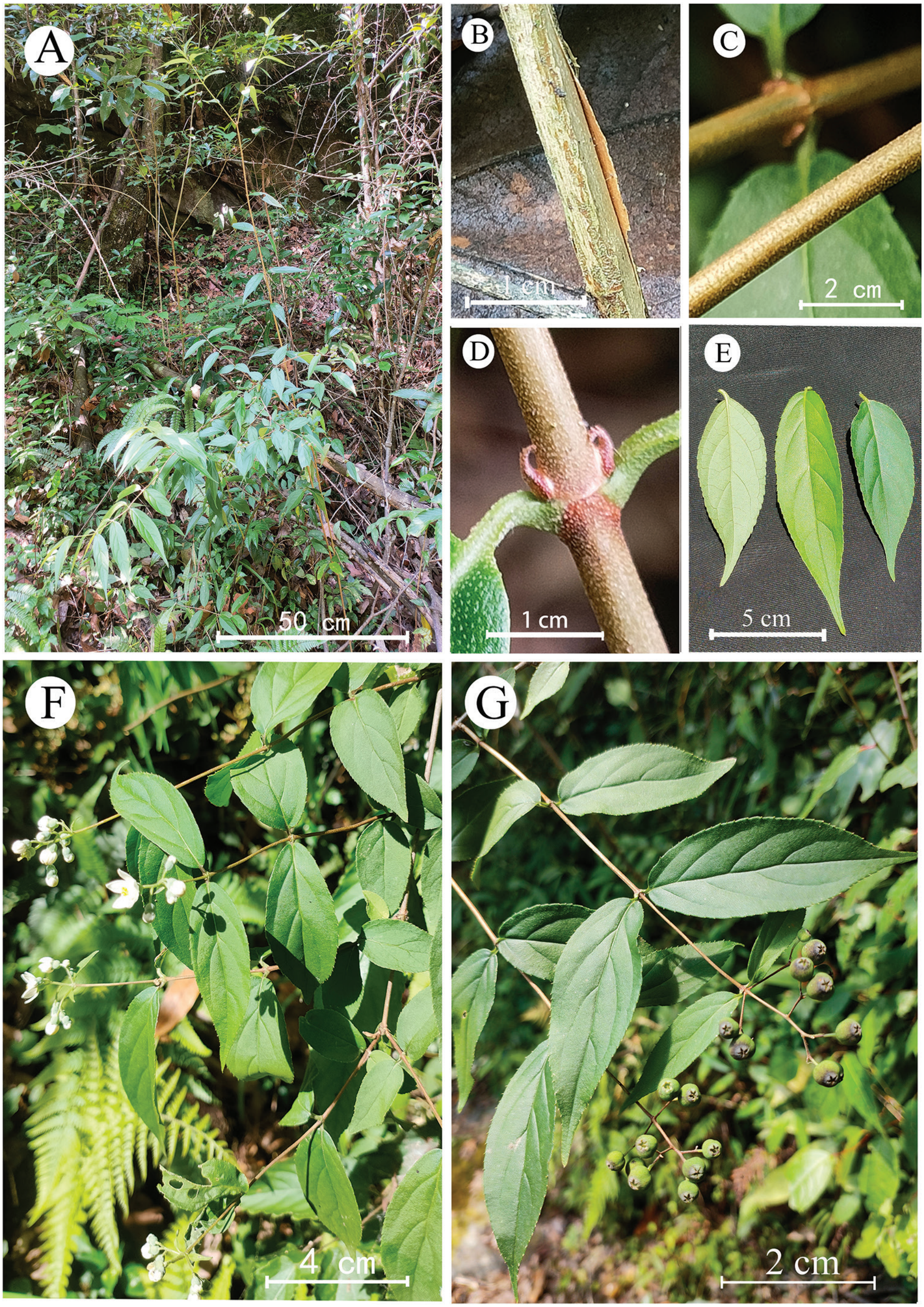

Figure 1. *Deutzia setchuenensis* var. *macrocarpa*, var. nov. **A** plant **B, C** branches **D** petioles **E** leaves **F** flowering branches **G** fruiting branches.

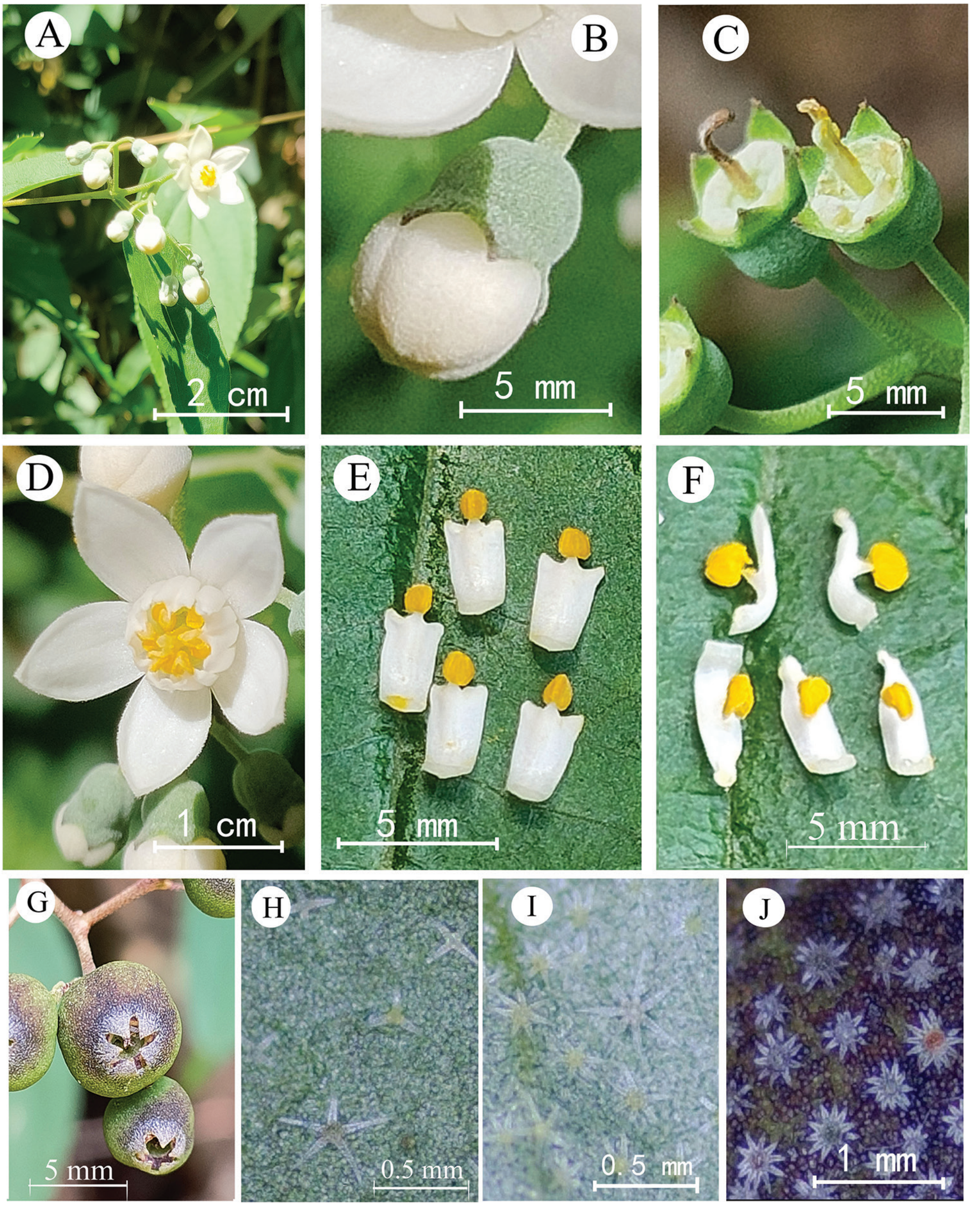

Figure 2. *Deutzia setchuenensis* var. *macrocarpa*, var. nov. **A** flowering branch **B** flower bud **C** young fruit **D** flower **E** outer stamens **F** inner stamens **G** capsules **H** stellate-pubescent on adaxial surface of leaf blade **I** stellate-pubescent on abaxial surface of leaf blade **J** stellate-tomentulose on surface of capsules.

1–1.5 × ca. 2 mm, apex acute, erect in bud, spreading in anthesis, inflexed and persistent in fruit. Corolla pure white; petals 5, white stellate-pubescent outside. Stamens 10 in 2-series, erect, filaments pure white, dorsiventraly flattened, anthers orange; outer stamens 4–5 mm long, filaments broadly oblong, with 2-repand denticles at apex, the width of teeth is more than twice its length, anthers broadly ovate; inner stamens shorter than outer ones, filaments obtuse at apex, anthers borne near middle of filaments abaxially, the width of anther exceeds the length. Disc annular, flattened, white. Ovary inferior, 2–3-loculed; styles 2–3, 3–3.5 mm long, usually coherent, glabrous. Capsule subglobose, 5–7 mm in diam., densely stellate-tomentulose, 2–3-valved. Seeds numerous, dark brown, ellipsoid or ovoid, 0.6–0.8 mm long, reticulate.

Phenology. Flowering from May to June; fruiting from September to October.

Distribution and habitat. *Deutzia setchuenensis* var. *macrocarpa* distributes in Quanxi town (Hongyangou village), Baguashan Provincial Nature Protection Area, and Taoyuan town (Hetaoyuan village) of Zhuxi county, Hubei province. It occurs at the edge of sparse thickets or forests on hillsides, or by streams. The main companion species include trees: *Phoebe zhennan* S. Lee, *Sycopsis sinensis* Oliv. and *Symplocos lucida* (Thunb.) Sieb. & Zucc.; shrubs: *Rubus swinhoei* Hance and *Camellia cuspidata* (Kochs) Wright ex Gard.; vines: *Smilax glaucochina* Warb. and *Actinidia polygama* (Sieb. et Zucc.) Maxim.; and a fern such as *Dryopteris fuscipes* C. Chr.

Etymology. The Latin name of the variety, 'macrocarpa', refers to the large fruit.

Vernacular name. Da Guo Sou Shu (Chinese).

Conservation assessment. *Deutzia setchuenensis* var. *macrocarpa* is currently known only from three localities consisting of less than 20 individuals in Zhuxi county, Hubei province. The provisional conservation status is Critically Endangered (CR), based on criterion D (number of mature individuals fewer than 50) (IUCN 2022).

Economic uses. *Deutzia setchuenensis* has rich intraspecific and morphological genetic diversity (Table 1). In the late 19th century, *Deutzia setchuenensis* var. *setchuenensis* and var. *corymbiflora* (Lemoine) Rehder were introduced into western Europe, and it was found that the ornamental value and winter hardness of the former were inferior to the latter. The new variety has larger flowers and fruits, and utilization of its germplasm is potential.

Table 1. Morphological comparisons of four varieties of *Deutzia setchuenensis* Franch.

Characters	**var. *setchuenensis***	**var. *longidentata* Rehder**	**var. *corymbiflora* (E. Lemoine ex Andre) Rehder**	**var. *macrocarpa* Q.L.Gan, Z.Y.Li & S.Z.Xu**
Inflorescence	6–12-flowered	6–12-flowered	12–50 (or more)-flowered	6–12-flowered
Teeth of outer filaments	oblong, slightly longer than the anthers	lanceolate, much longer than the anthers	deltoid, ca. as long as the anthers	repand, much shorter than the anthers
Apex of inner filaments	2-dentate	2-dentate	2-dentate	obtuse
Anthers	yellow	yellow	yellow	orange
Fruits	4–5 mm in diam.	ca. 4 mm in diam.	ca. 4 mm in diam.	5–7 mm in diam.
Distribution	Chongqing, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangxi, Sichuan, Yunnan	Sichuan (Mao Xian)	Hubei (Fang Xian, Badong)	Hubei (Zhuxi)

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RESEARCH ARTICLE

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Veronica hongii (Plantaginaceae), a new species from Central China

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Abstract

A new species *Veronica hongii*, from western Hubei Province, Central China is described and illustrated. The species is morphologically similar to *V. henryi* Yamazaki, but mainly differs in the glabrous plant, except pedicels, broadly ovate leaf blades, glandular-pubescent pedicels, obovate calyx lobes, smaller corolla, broadly ovate capsule and much smaller seeds.

Keywords

Central China, new species, taxonomy, *Veronica hongii*

Introduction

Veronica L. is a cosmopolitan genus consisting of ca. 250 species, mainly in Asia and Europe, of which 53 species are distributed in China (Hong and Fischer 1998). For a long time, *Veronica* has belonged to Scrophulariaceae (Fischer 2004). In recent years, *Veronica* L. has been transferred to Plantaginaceae (Albach et al. 2005; Angiosperm Phylogeny Group IV 2016). Some *Veronica* species have economic uses, including medicinal and ornamental value, while some other species are noxious weeds (Mabberley 1990). There are extremely rich *Veronica* species in Central China, including some endemic species, *Veronica szechuanica* Batal., *V. fargesii* Franch., *V. henryi* Yamazaki and *V. laxissima* D.Y. Hong (Tsoong and Hong 1979; Hong 1996). Recently, an unknown

Veronica species with some special characters was found during the fieldwork in Central China. The length of the seed of this species is only ca. 0.3 mm and should be the tiniest seed amongst species in *Veronica* (Thieret 1955; Martinez-Ortega and Rico 2001). The species is a terrestrial plant, with slender stems, axillary racemes, 4-parted calyx, rotated corolla, compressed and broadly ovate capsule and flattened seeds with convex both sides. After carefully checking related literature and specimens, we concluded this species should be placed in *Veronica* Sect. *Veronica* (Tsoong and Hong 1979) and it represents a species new to science. We describe and illustrate it here.

Materials and methods

Specimens of the putative new species were collected in Zhuxi County of Hubei Province in 2022. Comparisons with its relatives were made by consulting specimens stored in PE or some virtual specimen databases (HIB, KUN, IBK, IBSC, CVH, JSTOR, CDBI and WUK). Morphological observations and measurements were based on living plants of four individuals in the field. All morphological characters were measured with dissecting microscopes and were described using the terminology presented by Harris and Harris (1994).

Taxonomic treatment

Veronica hongii Q.L.Gan, Z.Y.Li & S.Z.Xu, sp. nov.

urn:lsid:ipni.org:names:77314713-1
Figs 1, 2

Diagnosis. *Veronica hongii* Q.L.Gan, Z.Y.Li & S.Z.Xu is similar to *V. henryi* Yamazaki in the perennial and diffuse plants, glabrous bracts and calyx and few-flowered racemes, but the new species can be easily distinguished from the latter by the glabrous plant, except pedicels, smaller leaf blades, corolla and seeds, longer and glandular-pubescent pedicels, obovate calyx lobes, broadly ovate capsule and flowering from September to October (see Table 1).

Type. China. Hubei Province: Zhuxi County, Huiwan Town, Chuanfeng Village, on river bank, alt. 361 m, 22 September 2022, Q.L.Gan3312 (holotype, PE!; isotype, PE!).

Description. Herbs perennial, plants diffuse. Stems terete, 5–18 cm long, 1.5–2 mm in diam., green or reddish-brown, glabrous, branched below the middle, branches slender, lower part prostrate and rooting at nodes, distally ascending, internodes 1.5–3 cm long. Leaves opposite, glabrous; petioles 2–6 mm long, lower ones longer, flattened, abaxial side shallowly grooved; leaf blades broadly ovate, 6–18 mm long, 4–13 mm wide, lower ones smaller, base broadly cuneate to rounded; margins shallowly serrate, crenate or subentire, apex acute to rounded; pinnately veined, midvein slightly impressed abaxially and prominent adaxially, lateral veins 2–3 on each side

Table 1. Morphological comparisons of *Veronica henryi* and *V. hongii*.

Characters	*V. henryi*	*V. hongii*
Stems	pubescent, becoming almost glabrous when old	glabrous
Leaf blades	ovate to narrowly ovate, 2–5 × 1.2–3 cm	broadly ovate, 0.6–1.8 × 0.4–1.3 cm
Pedicels	1–2 mm long at anthesis, 2–3 mm long in fruit, pubescent	3–5 mm long at anthesis, 5–7 mm long in fruit, glandular-pubescent
Calyx lobes	linear-lanceolate	obovate
Corolla	ca. 10 mm in diam., throat hairy	3.5–4 mm in diam., glabrous
Capsules	pliciform-rhomboid, 4–5 mm long, 9–11 mm wide, glandular-ciliate	broadly ovate, ca. 3 mm long and wide, glabrous
Seeds	ca. 1.5 mm long	ca. 0.3 mm long
Flowering	April to May	September to October

of mid-rib and alternate, veinlets inconspicuous. Racemes axillary from upper leaves, with 2–14 alternate flowers; peduncle 2–5 cm long, glabrous; axis 1–6 cm long, glabrous; bracts ovate-lanceolate to narrowly linear; pedicels filiform, straight or slightly incurved, 3–5 mm long at anthesis that elongate to 5–7 mm in fruit, sparsely with multicellular glandular hairs. Calyx glabrous 2–2.5 mm long, 4-parted, ca. 0.2 mm connated at base; lobes obovate, subequal, 1–1.3 mm wide. Corolla white and flushed purplish, with purple stripes, glabrous, rotated, 3.5–4 mm in diam., 4-parted; tube ca. 0.2 mm long; lower lobe smaller, obovate-rhombic, other 3 lobes rhombic, 2.5–3 mm long and wide, all lobes subacute at apex. Stamens 2, adnate to posterior side of corolla tube, slightly shorter than the lobes of corolla, glabrous; filaments white, 2–2.5 mm long; anthers purplish, ovate-oblong, ca. 0.9 mm long. Pistil glabrous; style ca. 2 times as long as the ovary; stigma capitellate; ovary rounded, slightly emarginate at apex. Capsule strongly compressed, broadly ovate, ca. 3 mm long and wide, glabrous, apex obtuse and small-notched, lateral angles rounded. Seeds 5–8, elliptic, ca. 0.3 mm long, flattened and convex on both sides, brown, glabrous.

Phenology. Flowering and fruiting from September to October.

Distribution and habitat. The populations of *Veronica hongii* were known from Chuanfeng Village, Huiwan Town, Zhuxi County, Hubei Province. It grows in grassland on river banks at elevations ca. 361 m.

Etymology. The species is named in honour of De-Yuan Hong (1937–), a famous botanist at the Institute of Botany, the Chinese Academy of Sciences (CAS), academician of CAS, who has devoted over 60 years to taxonomic and biosystematic studies of Paeoniaceae, Scrophulariaceae, Plantaginaceae, Campanulaceae, Commelinaceae and many other families, published *Plants of China* and *Flora of Pan-Himalaya*.

Conservation assessment. Based on the present field investigations, *Veronica hongii* is known from only one population composed of 11 individuals in Chuanfeng Village, Huiwan Town, Zhuxi County, Hubei Province. The provisional conservation status is Critically Endangered (CR), based on criteria D (number of mature individuals fewer than 50) (IUCN 2022).

Paratypes. China. Hubei Province: Zhuxi County, Huiwan Town, Chuanfeng Village, on river bank, alt. 361 m, 22 September 2022, Q.L.Gan3313 (PE!).

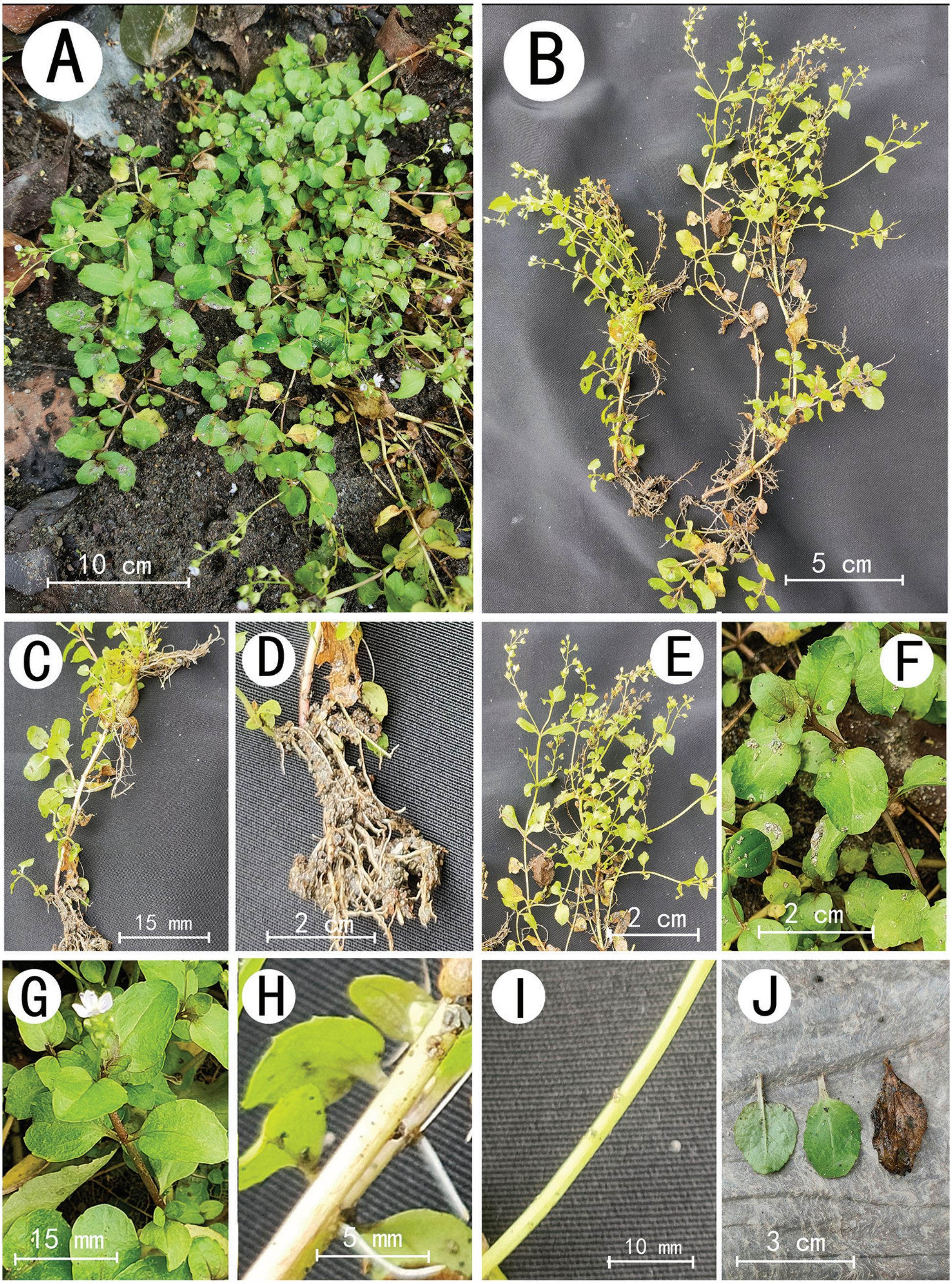

Figure 1. *Veronica hongii* sp. nov. **A, B** flowering plants **C, H** lower part of stem **D** roots **E, G** flowering branches **F** young branches **I** upper part of stem **J** leaf blades.

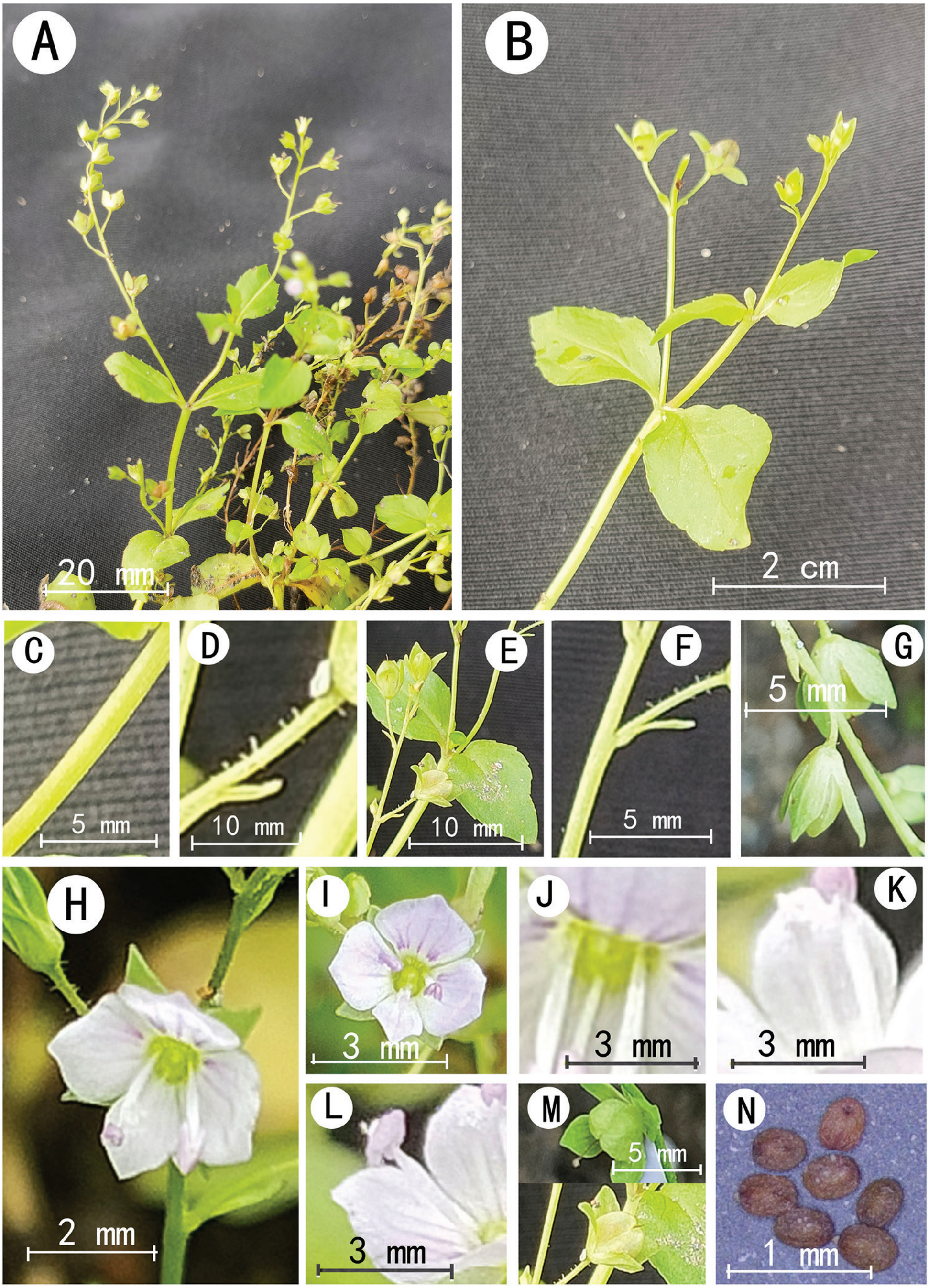

Figure 2. *Veronica hongii* sp. nov. **A–D** Fruiting branches **E** bract and pedicel **F** calyx **G** flower buds **H** flower **I** capsule **J** seeds.

Acknowledgements

We thank De-Yuan Hong, Xiao-Hua Jin and Lai Wei for their helpful suggestions concerning this manuscript. This study was supported by the Special Foundation of National Science and Technology Basic Research (2013FY112300) and Hubei Science And Technology Innovation Team of Excellent young and middle-aged in Colleges and Universities (T2021029).

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RESEARCH ARTICLE

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Aeschynanthus smaragdinus F.Wen & J.Q.Qin (Gesneriaceae), a new species from Yunnan Province, China

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Abstract

Aeschynanthus smaragdinus F.Wen & J.Q.Qin, a new species of Gesneriaceae from the monsoon rain forest in Mangbang township, Tengchong City, Yunnan Province, China, is described and illustrated here. It morphologically resembles *A. chiritoides* C.B.Clarke in size, shape and hairs on the leaf blades. But it can easily be distinguished from the latter by the green corolla limb with brownish-red to maroon lower lobes. At the same time, the hairs of the pedicel and calyx lobes, the length of the staminode and the size of the seed grain can also help distinguish both. It is provisionally assessed as Data Deficient (DD), according to the IUCN Red List Categories and Criteria, because field surveys for this new taxon have not been completed.

Keywords

Aeschynanthus chiritoides, Didymocarpoideae, Flora of Yunnan, taxonomy

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* These authors contributed equally to this work as co-first authors.

Introduction

Aeschynanthus Jack (1823) belongs to Subtribe Didymocarpinae G.Don, Tribe Trichosporeae Nees, subfamily Didymocarpoideae Arn., of the family Gesneriaceae Rich. & Juss. (Weber et al. 2013). In horticulture, this group is commonly called lipstick vine. All species of this genus are epiphytic and lithophytic plants. Approximately 160 known species are distributed in India, New Guinea, Solomon Islands, Southeast Asia, southern & southwestern China, and Sri Lanka (Weber 2004; Middleton 2007, 2009, 2016; Wei 2018; Olimpos and Mansibang 2021; Wei et al. 2022). Like many genera of Gesneriaceae, the genus is widespread, but local endemism at the species level is high (Mendum et al. 2001). There are currently 35 known species of *Aeschynanthus* in China (Li and Wang 2005; Hu et al. 2020). The Flora Pan-Himalaya project has developed a preliminary catalog of 32 species (Chen 2019). Many species are endemic to China (Wang et al. 1998). The corolla color of the *Aeschynanthus* species is variable, so it is challenging to describe. However, the characteristic of corolla color gradations is still important for the taxonomy of *Aeschynanthus*. The fruit type of this genus is a long narrow capsule (Wang et al. 1998; Middleton 2007, 2009).

During a field trip to Tengchong, Yunnan for Gesneriaceae investigation in 2017, an unknown pendulous *Aeschynanthus* species was found on tree trunks in a monsoon rain forest in Mangbang township, Tengchong city, Yunnan, China. This plant did not match any known species of *Aeschynanthus* in China or the neighboring countries. Some living plants were introduced and cultivated at the Gesneriad Conservation Center of China (GCCC), the National Gesneriaceae Germplasm Bank of GXIB, and the Shanghai Botanical Garden for further research. A comparison of living plants with type specimens and protologues of all known species of *Aeschynanthus* from China and neighboring countries led to the determination that these specimens neither fit the existing protologues nor conform to the known type specimens. The tiny shape and texture of the leaves make them very particular and similar to *A. chiritoides* C.B.Clarke (Middleton 2009; Cen et al. 2017). However, a combination of characteristics quickly distinguished it from the latter, especially in some important characters, viz. phyllotaxis variation, pedicel and calyx lobes indumentum, corolla and limb lobes color, staminode length, disk, seed grain size, and seed appendages length. We confirmed that it represents a new species of *Aeschynanthus* and describe it here.

Taxonomic treatment

Aeschynanthus smaragdinus F.Wen & J.Q.Qin, sp. nov.

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Figs 1–4

Diagnosis. The new species resembles *Aeschynanthus chiritoides* C.B.Clarke (Fig. 5) in leaf blades size, shape and indumentum, but can be easily distinguished from the latter by its pedicel densely erect glandular-pubescent (*vs.* densely villous), calyx lobes adaxially

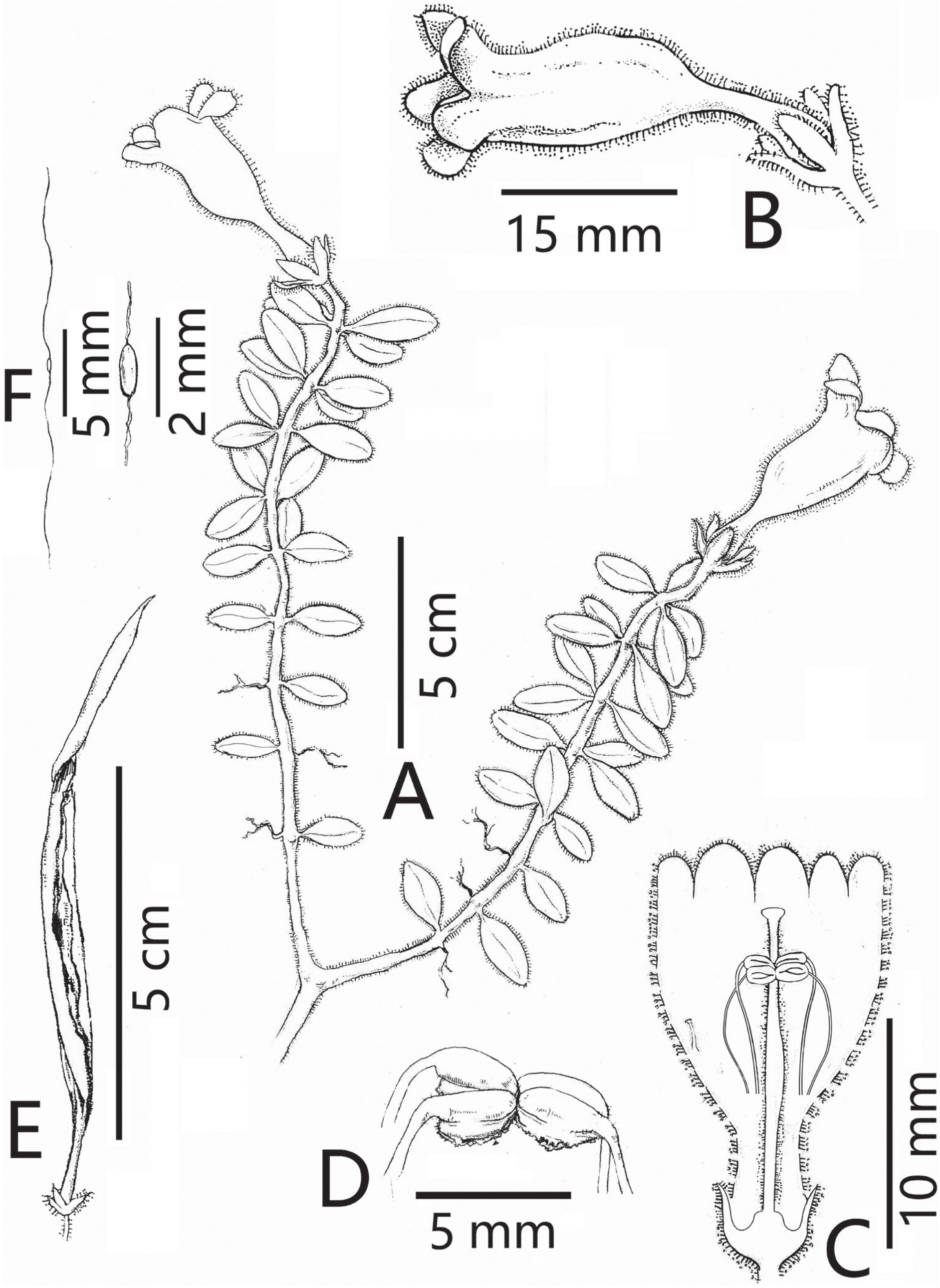

Figure 1. *Aeschynanthus smaragdinus* F.Wen & J.Q.Qin **A** habit **B** flower **C** flower dissection **D** fruit **E** seed **F** seed grain. **A–F** from Isotype. Scale bars: 1 cm (**A–E**); 1 mm (**F**). Illustrated by Rui-Feng Li.

nearly glabrous and abaxially erectly glandular puberulent (*vs*. adaxially and abaxially glandular- or eglandular-villous or a mixture of both), corolla pale yellowish green to greenish (*vs*. white or slightly yellowish or greenish with a few thin purple lines), corolla

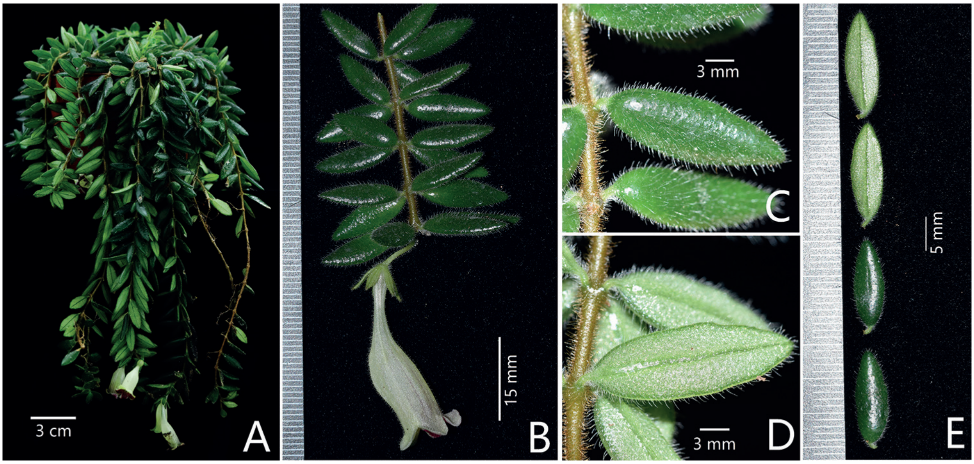

Figure 2. Plants *Aeschynanthus smaragdinus* F.Wen & J.Q.Qin **A** habit **B** a branch with a single flower **C** the adaxial surface of leaf blades and stem **D** the abaxial surface of leaf blades and stem **E** the adaxial and abaxial surfaces of leaf blades. Photographs by De-Chang Meng.

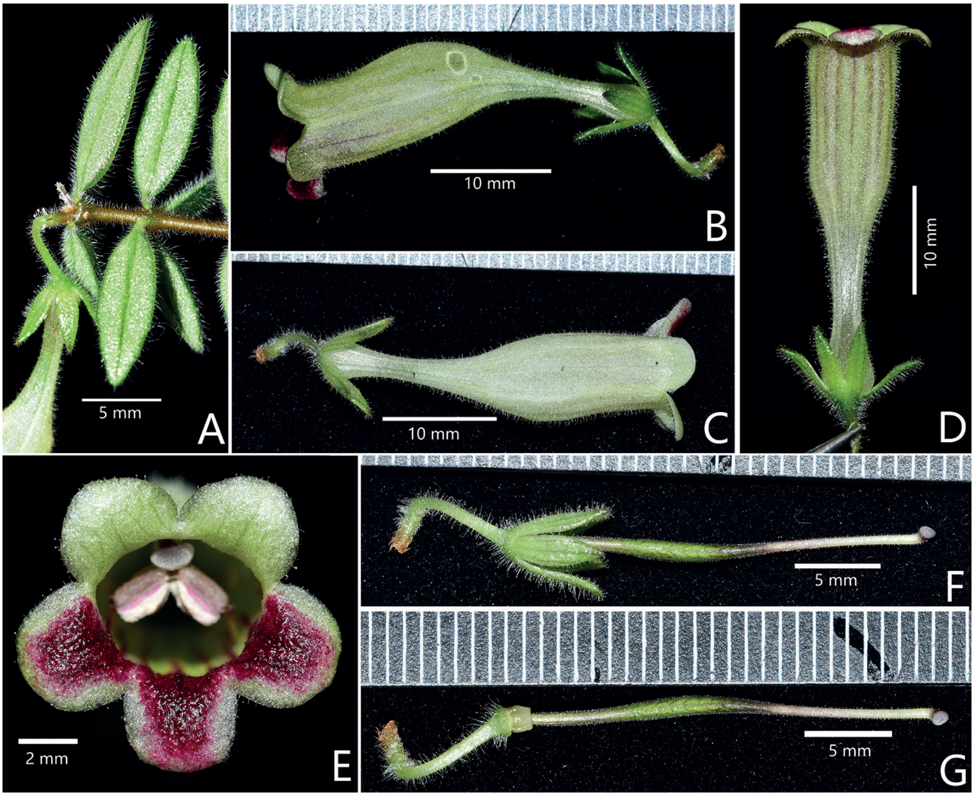

Figure 3. Propagative organs of *Aeschynanthus smaragdinus* F.Wen & J.Q.Qin (I) **A** a branch with terminal flower **B** lateral view of flower **C** top view of flower **D** upward view of flower **E** frontal view of corolla **F** pistil with calyx lobes **G** pistil without calyx lobes. Photographs by De-Chang Meng.

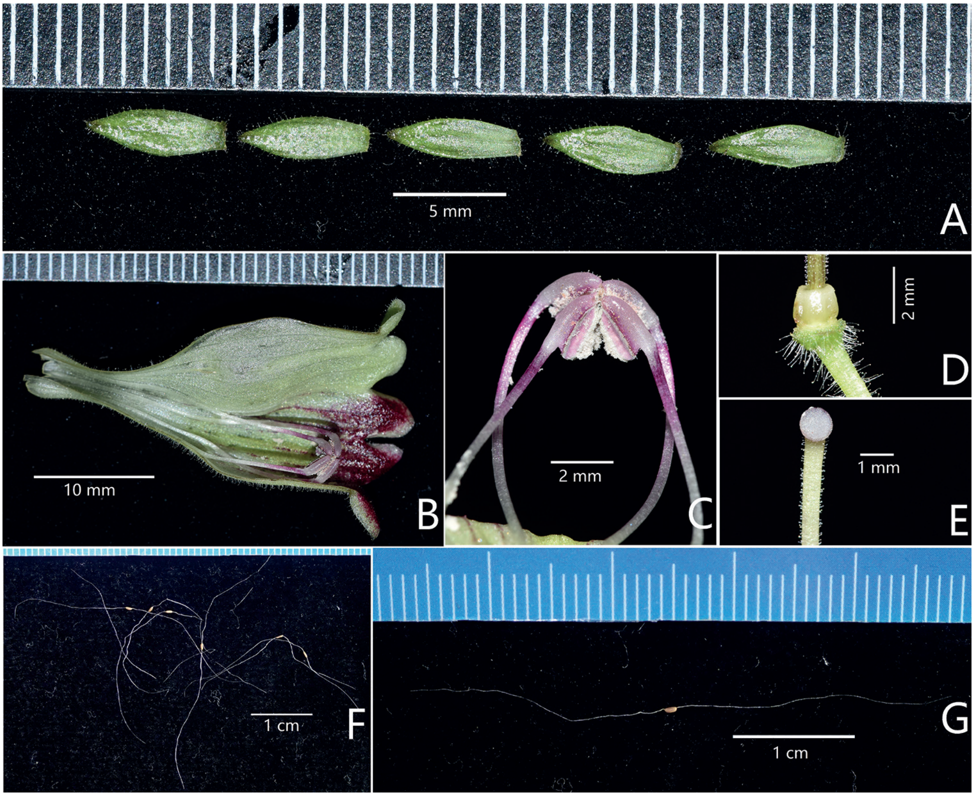

Figure 4. Propagative organs of *Aeschynanthus smaragdinus* F.Wen & J.Q.Qin (II) **A** calyx lobes (left two lobes showing abaxial surfaces; right three showing adaxial surfaces) **B** opened corolla **C** four fertile stamens **D** disk **E** stigma **F** seeds **G** seed. Photographs **A–E** by De-Chang Meng, **F, G** by Fang Wen.

limb upper lobes green and lower lobes brownish red to maroon (*vs.* all lobes white or slightly pale green with pale purplish lines), seed grain ca. 1.0× 0.5 mm (*vs.* 1.2–3 × ca. 0.3 mm). Detailed morphological comparisons with *A. chiritoides* are provided in Table 1.

Type. China. Yunnan Province: Tengchong city, Mangbang township, 24°93'N, 98°67'E, altitude ca. 1500 m, April 20, 2017, *Jia-Qi Qin QJQ170420-01* (holotype IBK!; Isotype: KUN!)

Description. Creeping or hanging subshrubs, epiphytic, with stem branched, greenish brown to pale brown, slender, with shorter internode (5–10 mm long), ca. 1 mm in diam., spreading rust-brown and white pubescent, occasionally roots at the node of the stem. Leaves in whorls of 3, sometimes opposite or in whorls of 4, occasionally three types on a single branch; petiole 0.5–1.0 mm long, green, sometimes subsessile, pubescent; leaf blade fleshy, thick, chartaceous when dried and size lessened, narrowly elliptic, elliptic to obovate and the cross-section olive-shaped, dark green on the adaxial surface and pale green on abaxial surface, not marbled, 1–1.9 × 0.6–1 cm, apex acute to subacute, base cuneate, adaxially and abaxially white pubescent; margin entire to slightly undulate; lateral veins invisible on both surfaces, main vein invisible on the adaxial surface but prominent and dark green on abaxial surface, secondary and tertiary venation obscure or invisible. Inflorescences usually terminal or subterminal, occasionally axillary, flowers solitary; peduncles almost obsolete, 0.5–1.0 mm long, ca. 1 mm in diam., slightly woody, arising from the axils, densely glandular-pubescent; bracts tiny and deciduous; pedicels 10–18 mm long, pubescent, pale green, densely erect glandular-pubescent. Calyx of 5 separate lobes free to base, campanulate, segments equal, both surfaces green, oblong-lanceolate to narrowly elliptic, ca. 6.0 × 1.5 mm, adaxially nearly glabrous, abaxially erect glandular puberulent, apex acute to subacute, margin entire. Corolla 30–35 mm long, externally green, pale yellowish green to greenish; upper lobes pale green to yellowish green, lower lobes pale green to yellowish green suffused pale brownish red, internally tube pale green, upper lobes pale green to yellowish green and lower lobes brownish red to maroon, with dark brownish red lines down into tube; corolla tube slightly obliquely swollen horn-shaped, often curved at the tube middle, ca. 2 cm long; ca. 7 mm in diam. at the mouth, the base of the corolla tube gibbose, and ca. 3 mm in diam.; limb distinctly 2-lipped, adaxial lip 2-lobed, lobes obliquely semicircular, spreading, ca. 4 mm in diam. at the base, apex rounded; abaxial lip 3-lobed from slightly below middle, 3 lobes bicolored, brownish red to maroon in the center and pale green on the edge of lobes, lateral lobes slightly obliquely oblong to oblong and the central one oblong, spreading, ca. 5 mm long, ca. 3.5 mm in diam. at the base, apex rounded; glandular puberulent outside, glabrous inside. Stamens 4, not exserted, all 4 fused together; filaments pale green from the middle to the base and gradually changing to pale purple from the middle to the top, glabrous, anthers pale purple; anterior filaments ca. 9 mm long, posterior filaments ca. 11 mm long, all adnate 14–15 mm above the corolla base; anthers ca. 2.5 × 1 mm, oblong, 2-locular, thecae parallel, dehiscing longitudinally, pollen pale yellow; Staminode 1, filiform, ca. 1 mm long, adnate to ca. 15 mm above the corolla base, glabrous. Disk annular, ca. 1.2 mm high, wax yellow, glabrous, margin entire. Pistil ca. 24 mm long; stipe 7–9 mm long; ovary narrowly spindly, ca. 1.5 cm long, glandular-puberulent, bicolor, brownish green at the base and top of the ovary but green with purplish stripes in the middle of the ovary; style ca. 9 mm long, extending out of corolla tube at the end of the single flowering phase, glandular-pubescent; stigma capitate, pale purple to whitish purple, ca. 1 mm in diam. Capsule linear, ca. 9 cm long, glabrous. Seed grain oblong-oval, ca. 1 × 0.5 mm, warty, apical appendage a filiform hair, ca. 25 mm long; hilar appendage a single filiform hair, ca. 24 mm long; appendages papillose.

Phenology. Flowering in December to February, fruiting from April to June.

Etymology. Compared with most other species of *Aeschynanthus*, the beautiful green leaves and flowers of this dwarf plant resemble an emerald. The specific epithet '*smaragdinus*' is derived from the Latin vocabulary and means a unique dazzling green.

Vernacular name. 翡翠芒毛苣苔 (Chinese name); Féi Cuì Máng Máo Jù Tái (Chinese pronunciation).

Distribution and habitat. Presently, *Aeschynanthus smaragdinus* is only found in the type locality, Mangbang Township, Tengchong City, Yunnan. The species grow on

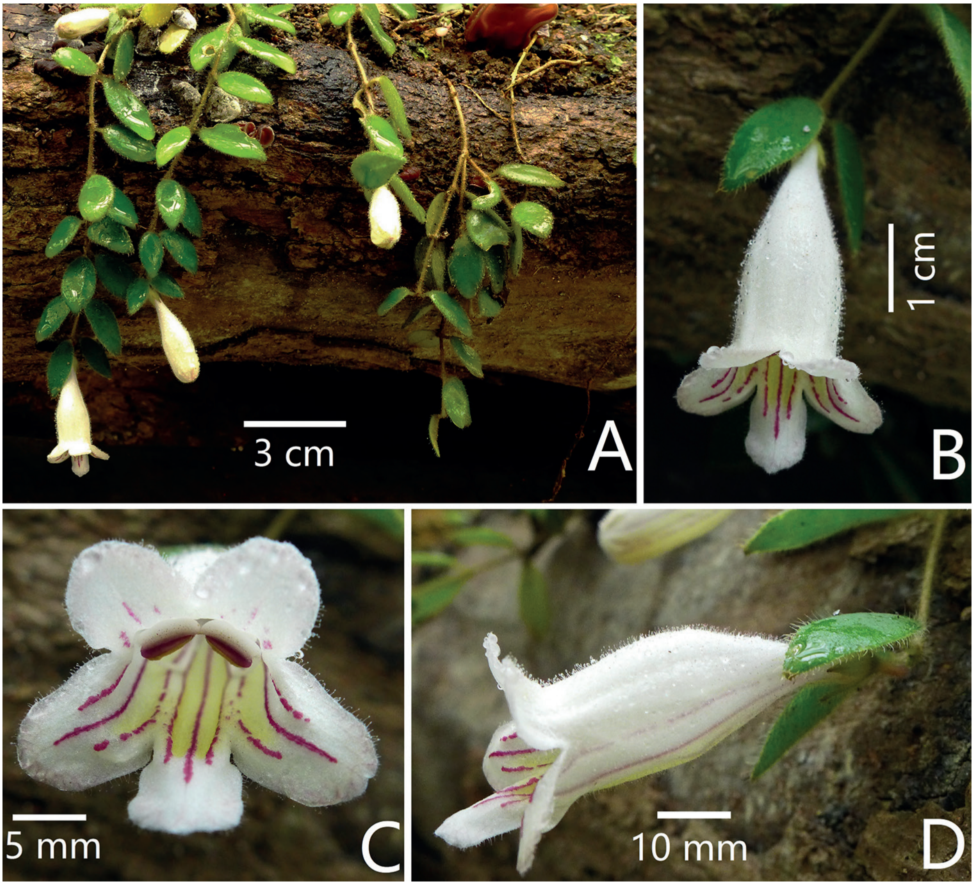

Figure 5. The morphologically similar species, *Aeschynanthus chiritoides* C.B.Clarke **A** habit **B** top view of flower **C** frontal view of corolla **D** lateral view of a flower. Photographs by Fang Wen.

Table 1. Detailed comparison of *Aeschynanthus smaragdinus* F.Wen & J.Q.Qin and its relative *A. chiritoides* C.B.Clarke.

Characters	*A. smaragdinus*	*A. chiritoides*
Phyllotaxis	in whorls of 3, sometimes opposite or in whorls of 4, occasionally three types on a single branch	opposite or in whorls of 3, often both types on a single branch
Pedicel indumentum	densely erectly glandular pubescent	densely villous
Calyx lobes indumentum	adaxially nearly glabrous, abaxially erectly glandular puberulent	adaxially and abaxially glandular- or eglandular-villous or a mixture of both
Corolla and limb lobes color	pale yellowish green to greenish; upper lobes pale green to yellowish green and lower lobes brownish red to maroon, with dark brownish red lines down into the tube	white or slightly yellowish or greenish with a few thin purple lines, all lobes white or slightly pale green with pale purplish lines
Disk	ca. 1.2 mm high and margin entire	1.8–3.6 mm high, 5-crenate or a simple annular ring
Seed grain size	ca. 1 × 0.5 mm	1.2–3 × ca. 0.3 mm
Seed appendages length	apical appendage, a filiform hair, ca. 25 mm long; hilar appendage, a single filiform hair, ca. 24 mm long	apical appendage a filiform hair, ca. 22 mm long; hilar appendage, a single filiform hair, ca. 17 mm long

moist, shady tree trunk surfaces in a monsoon rainforest at ca. 1500 m. Thus, it enjoys a cool environment with high air humidity in a moderately shaded monsoon rainforest.

Conservation status. *Aeschynanthus smaragdinus* is so far only known from the type locality. The total distribution area of this species is approximately five km^2 with a population size of about 500 mature individuals. However, we consider the data incomplete, and the new species is categorized as 'Data Deficient' (DD) according to the IUCN criteria (IUCN 2022).

Notes. The plant size of *Aeschynanthus smaragdinus* is dwarf, and the leaf blade length is less than 2 cm, but the flower length is from 3 cm to 3.5 cm, and the proportion of flowers and leaves is unusual in this genus. Besides this new taxon, other species have this property, for instance *A. chiritoides*, *A. gracilis* Parish & C.B.Clarke, *A. minutifolius* D.J.Middleton, *A. persimilis* Craib (Middleton 2007). However, the green flowers are especially distinctive. These characters differ from *A. chiritoides* in morphology (Table 1).

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RESEARCH ARTICLE

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Lysimachia fenghwaiana (Primulaceae), a new species from Hunan Province, China

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Abstract

A new species, *Lysimachia fenghwaiana* G.Hao & H.F.Yan (Primulaceae), from Hunan Province, China, is described and illustrated. This new species belongs to *Lysimachia* subgen. *Lysimachia* sect. *Nummularia* and is morphologically similar to *L. crista-galli* and *L. carinata*, but is distinctive in its leaf shape and arrangement of flowers. It can be further distinguished from *L. crista-galli* by the absence of calyx lobule spur, and from *L. carinata* by the black glandular striates in the corolla lobes, rather than punctate.

Keywords

central China, Ericales, flora, morphological features, taxonomy

Introduction

Lysimachia L. is one of the largest genera of Primulaceae, and it had been known to comprise about 180 species worldwide (Chen et al. 1989; Hu and Kelso 1996). As a whole, it is almost cosmopolitan, with the greatest diversity of species occurring in southwest China, especially in Sichuan, Guizhou and Yunnan Provinces. As a result of various molecular phylogenetic analyses over the past two decades, the alignment of the genus has been largely modified, with expansion to include some monotypic or small genera, for example, *Anagallis* L., *Glaux* L., *Pelletiera* A. St.-Hil. and *Trientalis* L. (Hao et al. 2004; Banfi et al. 2005; Anderberg et al. 2007; Manns and Anderberg 2009; Yan et al. 2018). The total number of species of *Lysimachia* has accordingly increased to approximately 250 (Yan et al. 2018).

Some new *Lysimachia* species have been continually described in recent years, mainly from the areas of central and south-western China (e.g. Zhou et al. 2015; Yan et al. 2017; Huang et al. 2019; Huang et al. 2020; Mou et al. 2020; Ju et al. 2021; Ke et al. 2021). During a field expedition conducted in Pingjiang County, Yueyang City, Hunan Province, in July 2021, a new taxon of *Lysimachia* was found, which is described here as a species new to science named *L. fenghwaiana* G.Hao & H.F.Yan, affiliated to *Lysimachia* subgen. *Lysimachia*. sect. *Nummularia* (Gilib.) Klatt.

Materials and methods

Historical taxonomic literature has been consulted (e.g. Handel-Mazzetti 1928; Chen and Hu 1979; Chen et al. 1989; Hu and Kelso 1996) to infer similar species and relatedness. The new species was examined in the field and at the herbarium, and measurements of morphological features were conducted with fresh specimens. Particularly, flowers were dissected and photographed. Morphological comparison with related species was performed based on living plants and specimens from IBSC, PE, IBK and CSFI and from the images of specimens from the JSTOR Global Plants (http://plants.jstor.org/). The conservation status of the new species was assessed following the guidelines for using the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022).

Taxonomic treatment

***Lysimachia fenghwaiana* G.Hao & H.F.Yan, sp. nov.**
urn:lsid:ipni.org:names:77314718-1
Figs 1–3

Type. China. Hunan Province, Yueyang City, Pingjiang County, Lutou Forest Farm, 28°32'N, 113°55'E, alt. 421 m, 22 May 2022, *Hai-Fei Yan and Chun-Lai Zhang Yan2022050* (holotype: IBSC! barcode IBSC0895001).

Diagnosis. *Lysimachia fenghwaiana* is most similar to *L. crista-galli* Pamp. & Hand. -Mazz. and *L. carinata* Y.I.Fang & C.Z.Cheng, but is different in its leaf shape and arrangement of flowers. It further differs from *L. crista-galli* in the absence of calyx lobule spur, and differs from *L. carinata* in the black glandular striates in the corolla lobes (vs. punctate).

Description. Herbs perennial, 20 to 70 cm tall. Stems erect, later arched to reclined, simple or shortly branched, initially covered with rust-coloured multicellu-

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846923
Primulaceae
芦头过路黄：Lysimachia fenghwaiana G.Hao & H.F.Yan. sp. nov.
鉴定人：颜海飞 2022.11.23
采集地：中国湖南省岳阳市平江县芦头林场桐木洞附近
林缘，路边、石壁上
海拔：421m 经纬度：28° 32′ 50.20″ N, 113° 55′ 48.35″ E
采集人：颜海飞、张春来
Yan2022050 2022.5.22

中国科学院华南植物园
标本号 846923
Lysimachia fenghwaiana G. Hao & H. F. Yan
采集号：Yan2022050 鉴定人：颜海飞
采集人：颜海飞、张春来 鉴定日期：2022/5/22

Figure 1. Holotype of *Lysimachia fenghwaiana* G.Hao & H.F.Yan, sp. nov. (*Hai-Fei Yan and Chun-Lai Zhang Yan2022050*, IBSC barcode IBSC0895001).

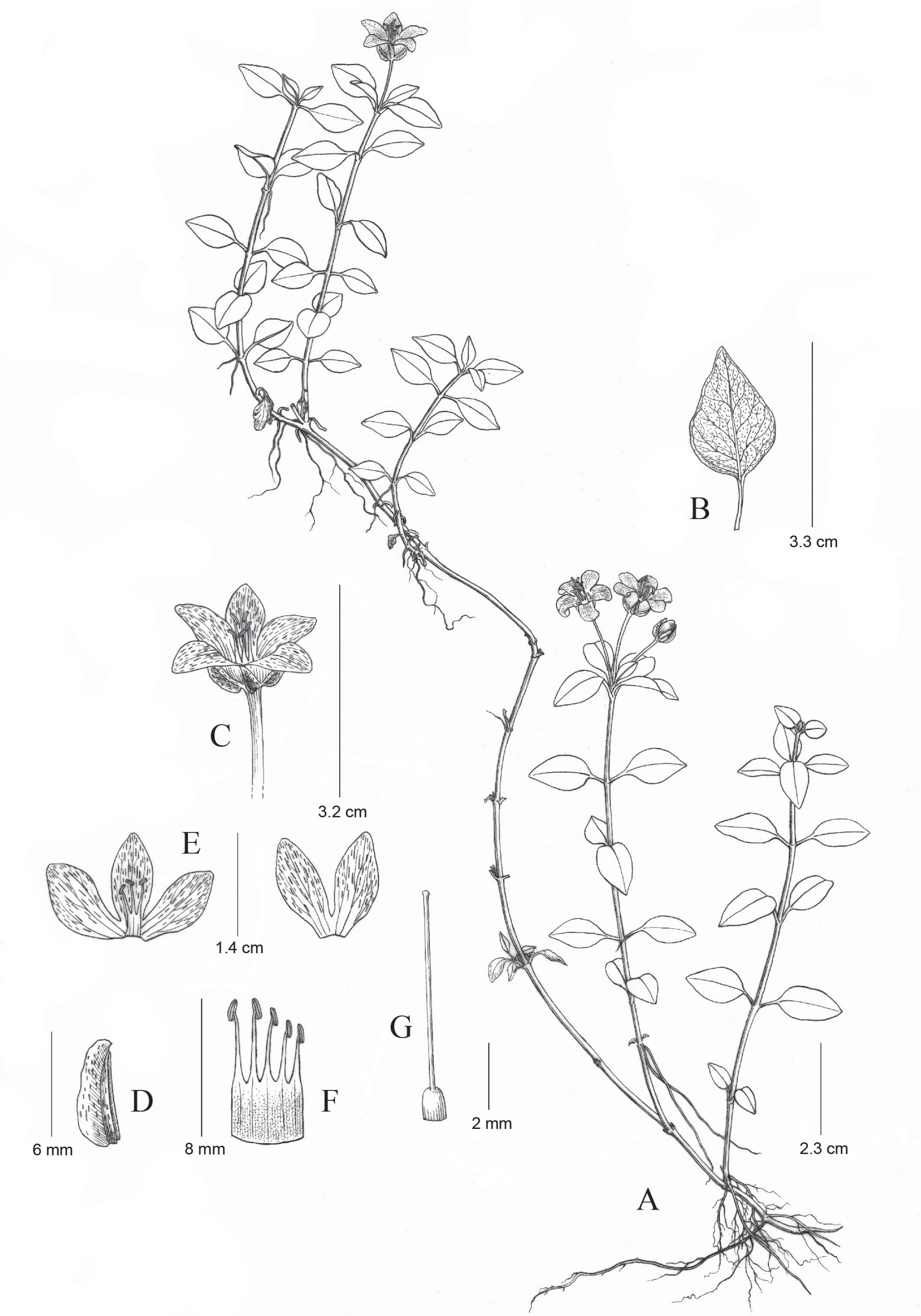

Figure 2. *Lysimachia fenghwaiana* G.Hao & H.F.Yan, sp. nov. **A** habit **B** abaxial surface of leaf **C** flower **D** calyx-lobe showing crest ridge **E** dissected corolla **F** stamens **G** pistil. Drawn by Yun-Xiao Liu from the holotype.

lar hairs, glabrescent. Leaves opposite; petioles 0.6–1.1 cm long, sparsely strigillose; blades broadly ovate, 1.2–2.8 × 0.8–1.8 cm, sparsely strigillose abaxially, densely short black glandular striate, base broadly cuneate, margin subentire, apex subacute to obtuse; midrib sunken abaxially, prominent abaxially when dry, secondary veins 3 or 4 pairs, veinlets inconspicuous. Flowers solitary or paired, in axis of apical leaves; pedicel 1–1.8 cm, glandular pubescent. Calyx 5-parted, green, lobes lanceolate, 5–6 mm long, abaxially cristate; crest widest at base, ca. 2 mm, black glandular striate, apex acute. Corolla yellow, tube ca. 2 mm long, lobes elliptic-lanceolate, ca. 11 × 4 mm, densely black glandular striate, apex obtuse. Stamens 5, filaments 3.2–3.5 mm long, connate basally into a tube, tube part 3.8–4.0 mm long, adnate to corolla tube, anthers oblong, ca. 1.8 mm long, dorsifixed, opening by lateral slits. Ovary ovoid, 1 mm long, glabrous, style ca. 7 mm long, stigma capitate. Capsules subspherical, ca. 5 mm in diameter, glabrous.

Distribution and habitat. The new species is currently known only from the type locality in Hunan Province, i.e. Lutou Forest Farm in Pingjiang County, Yueyang City. It grows at the edge of secondary mixed-evergreen forests, or under open forest on the hillside, at an altitude of ca. 400–450 m a.s.l.

Phenology. Flowering from May to June, fruiting from July to August.

Etymology. The new species is named in honour of Prof. Feng-Hwai Chen, a Chinese plant taxonomist and horticulturist, who devoted all his life to the development of botanical gardens in China and made considerable contributions to the study of Primulaceae and Asteraceae.

Local name. Simplified Chinese: 芦头过路黄; Chinese Pinyin: Lútou Guò Lù Huáng. “Lútou” means the flowers of *Phragmites communis* Trin. (Poaceae), which abundantly occurs locally. “Guò Lù Huáng” means plants of *Lysimachia*.

Conservation status. Based on our field investigations in Yueyang City and adjacent areas (e.g. Hubei and Guangxi Provinces) in the past ten years, only one population with ca. 1000 individuals of the new species has been found in an area of 10 km^2 in Lutou Forest Farm, Pingjiang County, Yueyang City. Moreover, the habitats are under threat from road construction and timber harvesting. Therefore, the conservation status of the new species is assessed as Critically Endangered (CR) (B2a & bi, iii), according to the guidelines for using the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022).

Additional specimens examined (paratypes). China. The same locality as holotype, 25 July 2021, *Hai-Fei Yan et al. Yan2021069* (IBSC!); The same locality as holotype, 4 June 2012, under forest, alt. ca. 500 m, *Jiaxiang Li et al. 1855* (CSFI! barcode CSFI069374).

Relationship with related species. Based on the classification of *Lysimachia* by Handel-Mazzetti (1928) and Chen and Hu (1979), the new species clearly belongs to *Lysimachia* subgenus *Lysimachia* sect. *Nummularia* ser. *Drymarifoliae* Hand.-Mazz., which is characterised by filaments connate into a tube, adnate to the base of corolla tube; anthers shorter than filaments, opening by lateral slits; and plants producing coloured punctate or striate glands. Amongst this series, approximately six species

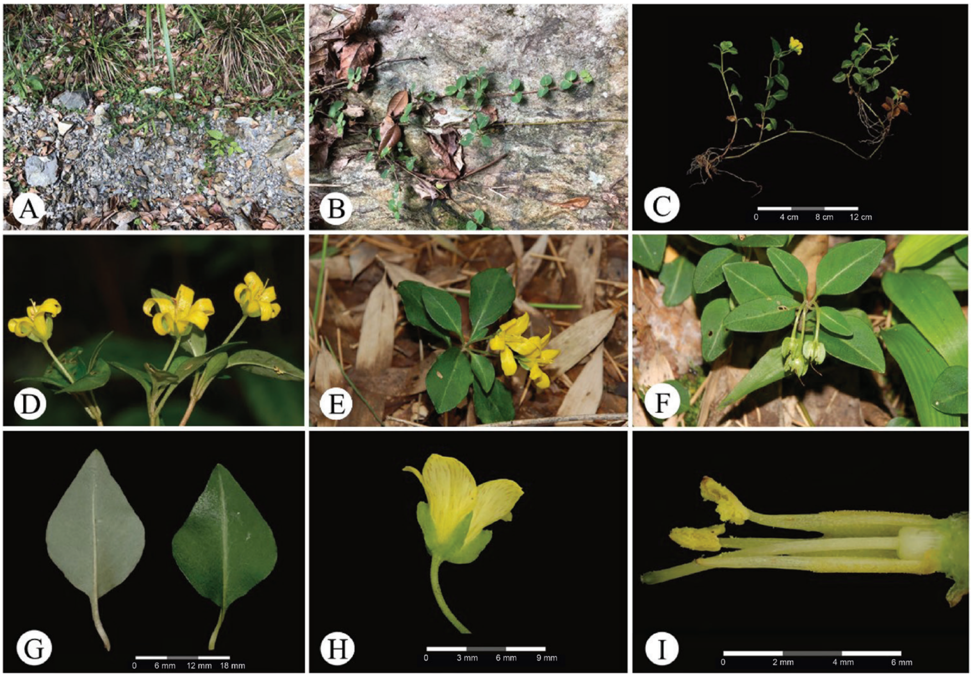

Figure 3. Living plant of *Lysimachia fenghwaiana* G.Hao & H.F.Yan, sp. nov. **A** habitat **B, C** habit **D, E** flowering plant **F** fruiting plant **G** leaves on abaxial (left) and adaxial (right) surfaces **H** flower (lateral view) **I** stamens and pistil (partial). Photographed by Jia-Xiang Li and Hai-Fei Yan.

Table 1. Main morphological differences between *Lysimachia fenghwaiana* and two similar species.

Features	*L. fenghwaiana*	*L. crista-galli*	*L. carinata*
Lamina shape	broadly ovate, base rounded to truncate, apex subacute to obtuse	broadly ovate to suborbicular, base cordate, apex subacute to obtuse	broadly ovate to ovate, base rounded to truncate, apex acute to acuminate
Lamina glands' type	short striate	mix of both striate and punctate	punctate
Arrangement of flowers	solitary or paired in axis of apical leaves	solitary, in axis of middle and upper leaves	solitary or paired, in axis of middle and upper leaves
Calyx lobule spur	Absent	Present	absent
Corolla lobule glands	densely striate	densely striate	punctate

constitute a group, highlighted by the calyx with crested ridges (Handel-Mazzetti 1928; Chen et al. 1989; Zhou et al. 2015). The new species belongs to the group by having a crested calyx (Figs 1D, 2G) and is morphologically similar to *L. crista-galli* and *L. carinata*, but is distinctive in its flowers occurring in the axis of the apical leaves, rather than in the axis of the middle and upper leaves in the latter two species. Further, from *L. crista-galli*, it differs in its cuneate leaf base and absence of corolla lobule spur (vs. leaf base cordate and calyx lobule spur present in *L. crista-galli*); and from

L. carinata, it differs by the shape of leaf lamina and corolla, i.e striate in *L. crista-galli* (vs. punctate in *L. carinata*) (see Table 1).

Whether the development of the crest to the calyx lobes, i.e. the winged keel of the calyx lobes, is a synapomorphy and those species constitute a monophyletic group in *Lysimachia* is uncertain, and further phylogenetic analysis should be undertaken to resolve this issue.

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RESEARCH ARTICLE

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New insights into the phylogenetic relationships of Japanese knotweed (*Reynoutria japonica*) and allied taxa in subtribe Reynoutriinae (Polygonaceae)

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Baowei Zhang[2], Kai Zhao[3], Trude Schwarzacher[1]

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Abstract

Japanese knotweed (*Reynoutria japonica*) is native to East Asia, but has been introduced to the West where it is a noxious invasive weed. Taxonomically, Japanese knotweed is placed within subtribe Reynoutriinae (Polygonaceae), which also contains the austral genus *Muehlenbeckia* (incl. *Homalocladium*) and north temperate *Fallopia*. In the current study, we conducted a phylogenetic analysis using sequence data from six markers, two nuclear (*LEAFYi2*, ITS) and four plastid (*matK*, *rbcL*, *rps16-trnK* and *trnL-trnF*) to further resolve the evolutionary relationships within this group, using the widest sampling of in-group taxa to date. The results of this analysis confirmed that subtribe Reynoutriinae is a monophyletic group, characterised by the presence of extra-floral, nectariferous glands at the base of leaf petioles. Within the subtribe, four main clades were identified: *Reynoutria*, *Fallopia* sect. *Parogonum*, *Fallopia* s.s. (including *Fallopia* sects. *Fallopia* and *Sarmentosae*) and *Muehlenbeckia*. The *Fallopia* s.s. and *Muehlenbeckia* clades are sister to one another, while the *Fallopia* sect. *Parogonum* clade is immediately basal to them and *Reynoutria* basal to all three. *Fallopia*, as currently circumscribed, is paraphyletic as *Muehlenbeckia* is nested within it. To resolve this, we propose that species of *Fallopia* sect. *Parogonum* should be treated as a new genus, *Parogonum* (Haraldson) Desjardins & J.P.Bailey, gen. et stat. nov. Within *Reynoutria*, the allied specific and infraspecific taxa that fall under the name Japanese knotweed s.l. form a monophyletic group and their taxonomic status is discussed.

Keywords

Fallopia, invasive aliens, *Muehlenbeckia*, phylogeny, polyploidy

Introduction

Japanese knotweed *sensu lato* is a group of large rhizomatous herbs in the genus *Reynoutria* Houtt. (Ohwi 1965; Anjen and Park 2003b). They are native to East Asia, but have been introduced to the West where they are invasive and persistent weeds (Bailey and Conolly 2000). There are two main species of knotweed: Japanese knotweed (*R. japonica* Houtt.) and giant knotweed (*R. sachalinensis* (F.Schmidt) Nakai) (Ohwi 1965; Anjen and Park 2003b). *Reynoutria japonica* can also be further recognised as a number of allied specific or infraspecific taxa, most of which are endemic to East Asia, but two are found outside of the native range, a tall lowland form, var. *japonica* (*R. japonica* s.s.) and a dwarf montane form, var. *compacta* (Hook.f.) Buchheim (= *Reynoutria compacta* (Hook.f.) Nakai) (Bailey 2003). Introduced knotweeds show greatly reduced genetic diversity compared to those in the native range, due to strong founder effects (Hollingsworth and Bailey 2000; Pashley 2003; Desjardins et al. 2022). This is most pronounced in *R. japonica* var. *japonica*, which occurs throughout Europe as a single female clone, that spreads by massive clonal reproduction and only produces seed through hybridisation with related taxa (Hollingsworth and Bailey 2000; Pashley 2003; Mandák et al. 2005).

Within the Polygonaceae, Japanese knotweed s.l. is placed in subtribe Reynoutriinae (Galasso et al. 2009), which is characterised by two putative synapomorphies: extrafloral nectaries at the base of leaf petioles (Salisbury 1909; Brandbyge 1992; Schuster et al. 2011b) and *Tiniaria*-type pollen (Hedberg 1946; Bailey 1989; Brandbyge 1992). In addition to the East Asian knotweeds (*Reynoutria*), the subtribe contains the austral genus *Muehlenbeckia* Meisn. (including *Homalocladium* (F.Muell.) L.H.Bailey) and the north-temperate genus *Fallopia* Adans.; all of which are segregates of *Polygonum* L. s.l. (Schuster et al. 2011b).

Fallopia and *Reynoutria* have been treated as a single entity ever since Meisner (1856) placed them together in *Polygonum* sect. *Tiniaria* Meisn. and, thereafter, by Hedberg (1946) under *Tiniaria* (Meisn.) Rchb., by Shinners (1967) under *Reynoutria*, by Ronse Decraene and Akeroyd (1988) under *Fallopia* and by Galasso et al. (2009) as separate genera under subtribe Reynoutriinae. *Muehlenbeckia*, however, has traditionally been considered distinct from *Fallopia* and *Reynoutria*, primarily on the basis of its succulent mature perianth and southern biogeographical distribution. Meisner (1840, 1856) instigated this by segregating *Muehlenbeckia* from *Polygonum* s.l. and the rest of the tribe Polygoneae and placing it in tribe Coccolobeae alongside *Coccoloba* P.Browne, which also has inflated tepals in fruit. This classification persisted until relatively recently, being adopted as late as Brandbyge (1993), and was not re-examined until the application of molecular techniques (Cuénoud et al. 2002; Lamb Frye and Kron 2003). However, earlier workers, such as Jaretzky (1925) and Edman (1929), had suggested that *Muehlenbeckia* may be derived from *Polygonum* sect. *Pleuropterus* (Turcz.) Benth. & Hook.f. (= *Reynoutria*), due to similarities in secondary chemistry and endosperm morphology. Furthermore, Haraldson (1978) suggested that the closest connection of *Fallopia* and *Reynoutria* was probably with *Muehlenbeckia*, amongst other genera, as a number of morphological traits, such as fimbriate stigmas and twining habit, are found within both groups. To indicate this relationship, she transferred *Fallopia* and *Reynoutria* into the Coccolobeae to be alongside *Muehlenbeckia*. A summary of the historical treatments of *Fallopia*, *Reynoutria* and *Muehlenbeckia* is presented in Table 1.

The latest molecular phylogenetic schemes, using plastid and nuclear sequence data, place *Reynoutria*, *Muehlenbeckia* and *Fallopia* in a strongly supported monophyletic group, known as the RMF clade (Schuster et al. 2011a, b, 2015). The stem age of this clade is reportedly 46.1–48.2 MYA (Schuster et al. 2013). Within this clade *Fallopia* and *Muehlenbeckia* are sister genera and appear to be more closely related to each other than either are to *Reynoutria*, which is immediately basal to them. *Coccoloba* and the rest of the Coccolobeae, previously regarded as members of subfamily Polygonoideae and sister to *Muehlenbeckia*, are now placed well away from it in subfamily Eriogonoideae (Cuénoud et al. 2002; Lamb Frye and Kron 2003; Galasso et al. 2009; Burke and Sanchez 2011).

Reynoutria

Reynoutria is an East Asian genus (Ohwi 1965; Anjen and Park 2003b) and, as currently circumscribed by Schuster et al. (2011b), corresponds to Bentham and Hooker's (1880) *Polygonum* sect *Pleuropterus*, containing both the erect, strongly rhizomatous knotweeds (*R. japonica* s.l. and *R. sachalinensis*), as well as weakly rhizomatous climbers (*R. multiflora* (Thunb.) Moldenke and *R. ciliinervis* (Nakai) Moldenke). Within *Reynoutria* s.l., the erect, strongly rhizomatous knotweeds form an in-group, with *R. multiflora* as a basal lineage (Schuster et al. 2011a, b). This distinction between the erect and climbing taxa is further supported by examinations of secondary chemistry, which reveal two distinct chemical entities within the genus, both of which are separable from *Fallopia* s.s. (Kim et al. 2000b; Park et al. 2011). Indeed, Galasso et al. (2018) preferred to separate the two groups and retained the climbing taxa in the genus *Pleuropterus* Turcz.

Reynoutria was formerly amalgamated under *Fallopia* by Ronse Decraene and Akeroyd (1988), who argued that the anatomical heterogeneity within the two genera breaks down any clear distinction between them, particularly when the full range of taxa is taken into account. They instead emphasised similarities in stamen type, tepal vasculature and outer tepal morphology in support of merging the genera. Intergeneric hybrids also occur between *Reynoutria* and *Fallopia* (= × *Reyllopia* Holub) and have been taken to support amalgamation (Bailey 1988, 2001). In Ronse Decraene and Akeroyd's (1988) treatment of *Fallopia*, the erect *Reynoutria* taxa are classified as *Fallopia* sect. *Reynoutria* (Houtt.) Ronse Decr., while *R. multiflora* is grouped with other perennial climbers (such as *F. baldschuanica* (Regel) Holub) in *Fallopia* sect. *Sarmentosae* (I.Grinț.) Holub. However, the latest phylogenetic schemes have shown that *Fallopia* sensu Ronse Decraene and Akeroyd (1988) is paraphyletic as species of *Muehlenbeckia* are nested within it (Galasso et al. 2009; Schuster et al. 2011a, b).

Table 1. Taxonomic treatment of *Fallopia*, *Muehlenbeckia*, *Parogonum* and *Reynoutria* in previous classifications.

	Fallopia	***Muehlenbeckia***	***Parogonum***	***Reynoutria***
Mesiner (1856)	*Polygonum* sect. *Tiniaria*	*Muehlenbeckia*	*Polygonum* sect. *Tiniaria*	*Polygonum* sect. *Tiniaria*
Bentham and Hooker (1880)	*Polygonum* sect. *Tiniaria*	*Muehlenbeckia*	n/a	*Polygonum* sect. *Pleuropterus*
Nakai (1926)	*Bilderdykia*	n/a	n/a	*Reynoutria*
Hedberg (1946)	*Tiniaria*	n/a	*Tiniaria*	*Tiniaria*
Webb and Chater (1963)	*Bilderdykia*	n/a	n/a	*Reynoutria*
Shinners (1967)	*Reynoutria*	n/a	*Reynoutria*	*Reynoutria*
Holub (1970)	*Fallopia*	n/a	*Fallopia*	*Reynoutria*
Haraldson (1978)	*Fallopia* sect. *Fallopia*; sect. *Pleuropterus*	*Muehlenbeckia*	*Fallopia* sect. *Parogonum*	*Reynoutria*; *Fallopia* sect. *Pleuropterus*
Ronse Decraene and Akeroyd (1988)	*Fallopia* sect. *Fallopia*; sect. *Sarmentosae*	n/a	n/a	*Fallopia* sect. *Reynoutria*; sect. *Sarmentosae*
Bailey and Stace (1992)	*Fallopia* sect. *Fallopia*; sect. *Sarmentosae*	n/a	*Fallopia* sect. *Parogonum*	*Fallopia* sect. *Reynoutria*; sect. *Sarmentosae*
Brandbyge (1993)	*Fallopia*	*Muehlenbeckia*	*Fallopia* sect. *Parogonum*	*Reynoutria*
Galasso et al. (2009)	*Fallopia*	*Muehlenbeckia*	n/a	*Reynoutria*
Schuster et al. (2011*b*)	*Fallopia*	*Muehlenbeckia*	n/a	*Reynoutria*
Proposed classification	*Fallopia* sect. *Fallopia*; sect. *Sarmentosae*	*Muehlenbeckia*	*Parogonum*	*Reynoutria*

Species of *Reynoutria* are herbaceous, rhizomatous perennials with dry, winged mature perianths, paniculate inflorescences, fimbriate stigmas and are functionally gynodioecious or hermaphrodite (Haraldson 1978; Ronse Decraene and Akeroyd 1988). Chromosome base number is $x = 11$ (Bailey and Stace 1992; Kim and Park 2000).

Reynoutria japonica s.l. is also comprised of a number of infraspecific and allied specific taxa. These include the tall, lowland form var. *japonica* (= *R. japonica* s.s.) and the dwarf, montane form var. *compacta* (= *R. compacta*), as well as East Asian endemics, such as var. *uzenensis* Honda (= *R. uzenensis* (Honda) Honda), var. *terminalis* (Honda) Kitag., *R. elliptica* (Koidz.) Migo ex Nakai and *R. forbesii* (Hance) T.Yamaz (Bailey 2003). Var. *uzenensis* is a tall lowland form, characterised by pubescent foliage with uniseriate, multicellular hairs and occurs only in the north-eastern part of Honshu, Japan (Pashley 2003). Var. *terminalis* is endemic to the Izu Islands, off the coast of Honshu and is characterised by large, lustrous leaves (Inamura et al. 2000). *Reynoutria forbesii* is the name applied to knotweed growing on the Chinese mainland, which is sometimes treated as synonymous with *R. elliptica* from Korea. Both taxa have a distinctive elliptic leaf shape with a rounded base, as opposed to truncate like var. *japonica* and thick rigid hairs on the lower surface of the leaves (absent in var. *japonica*) (Anjen and Park 2003a; Bailey 2003; Galasso et al. 2009).

Fallopia

Fallopia is a heterogeneous genus divided into three sections: sect. *Fallopia*, sect. *Sarmentosae* and sect. *Parogonum* Haraldson (Holub 1970; Haraldson 1978).

Fallopia* sect. *Fallopia

Fallopia sect. *Fallopia* was erected by Holub (1970) and contains approximately eight taxa: *F. convolvulus* (L.) Á.Löve, *F. cristata* (Engelm. & A.Gray) Holub, *F. dentatoalata* (F.Schmidt) Holub, *F. dumetorum* (L.) Holub, *F. filipes* (H.Hara) Holub, *F. pterocarpa* (Wall. ex Meisn.) Holub, *F. scandens* (L.) Holub (the type species) and *F. schischkinii* Tzvelev (Hara 1972; Tzvelev 1987; Kim et al. 2000c). Species of section *Fallopia* are annual vines with dry winged mature perianths (secondarily lost in *F. convolvulus* and *F. schischkinii*), spike-like to racemose inflorescences, capitate stigmas and perfect flowers (Haraldson 1978; Ronse Decraene and Akeroyd 1988). The section has a north temperate distribution (Hara 1982; Qaiser 2001; Anjen and Park 2003a; Freeman and Hinds 2005) and the chromosome base number is $x = 10$ (Bailey and Stace 1992).

An examination of secondary chemistry found that the flavonoid profiles of sect. *Fallopia* form a distinct group, which provides additional evidence for the segregation of sect. *Fallopia* within the genus (Kim et al. 2000a). Previous molecular phylogenetic studies also show that *Fallopia* sect. *Fallopia* forms a strongly supported monophyletic clade within the genus, which is sister to sect. *Sarmentosae* (Galasso et al. 2009; Schuster et al. 2011b).

Fallopia* sect. *Sarmentosae

Fallopia sect. *Sarmentosae* was erected by Holub (1970) and contains *F. aubertii* (L.Henry) Holub and *F. baldschuanica*, which may be conspecific (Bailey 1989; Bailey and Stace 1992). Species of sect. *Sarmentosae* are woody climbing perennials (without rhizomes) with dry winged mature perianths, paniculate inflorescences, capitate stigmas, and perfect flowers (Haraldson 1978; Ronse Decraene and Akeroyd 1988). The section has a central Asian distribution (Qaiser 2001; Anjen and Park 2003a) and the chromosome base number is $x = 10$ (Bailey and Stace 1992).

An examination of secondary chemistry found that the flavonoid profile of *F. baldschuanica* was distinct from other *Fallopia* species (Kim et al. 2000b). Previous molecular studies have also shown that *Fallopia* sect. *Sarmentosae* is a monophyletic group, sister to *Fallopia* sect. *Fallopia* (Galasso et al. 2009; Schuster et al. 2011b).

Fallopia koreana B.U.Oh & J.G.Kim is a climbing perennial herb endemic to Korea. It is rhizomatous, has enlarged winged perianths in fruit that become twisted at the apex and capitate stigmas with projected surfaces (Oh and Kim 1996). Somatic chromosome number is reported as $2n = 20$ (Kim et al. 2000b). *Fallopia koreana* was formerly classified in *Fallopia* sect. *Pleuropterus* (Kim et al. 2000b), but molecular work by Schuster et al. (2011b) indicates that it is sister to *F. baldschuanica* and may also belong in *Fallopia* sect. *Sarmentosae*. Due to a lack of available material, *F. koreana*, was not included in the present study.

Fallopia* sect. *Parogonum

Fallopia sect. *Parogonum* was erected by Haraldson (1978) and contains two taxa: *F. ciliinodis* (Michx.) Holub (the type species) and *F. cynanchoides* (Hemsl.) Haraldson. Species of sect. *Parogonum* are herbaceous perennial climbers, distinguished by their unique trichome type, a stiff unicellular hair with a papillate surface (Haraldson 1978; Bailey 1989). Members of the section also have dry mature perianths, which do not become enlarged and winged in fruit (cf. *F. convolvulus*), paniculate inflorescences, mildly-fimbriate stigmas and perfect flowers (Haraldson 1978). Section *Parogonum* has a disjunct East Asian-Eastern North American distribution with *F. ciliinodis* native to the East Coast of North America and *F. cynanchoides* restricted to Central China (Anjen and Park 2003a; Freeman and Hinds 2005). Chromosome base number is $x = 11$ (Bailey and Stace 1992; Kim et al. 2000a).

A molecular phylogenetic study including *F. ciliinodis* presented an unclear picture of its position within tribe Polygoneae (Schuster et al. 2015). Plastid data strongly supported its inclusion in the RMF clade (subtribe Reynoutriinae), but not within *Fallopia* s.s., while the inclusion of a nuclear dataset placed it outside of the RMF clade and weakly supported as sister to a DAP clade (subtribe Polygoninae), including: *Duma* T.M.Schust, *Atraphaxis* L. and *Polygonum* s.s. The separation of sect. *Parogonum* from *Fallopia* s.s. is supported by an examination of secondary chemistry, which found that the flavonoid profile of *F. ciliinodis* to be substantially different from the rest of *Fallopia*, most closely resembling the climbing *Reynoutria* taxa, *R. multiflora* and *R. ciliinervis* (Kim et al. 2000a, 2000b). However, species of sect. *Parogonum* were not included in Ronse Decraene and Akeroyd's (1988) morphological treatment of the genus and *F. cynanchoides* has been missing from all molecular studies to date, so the placement of *Fallopia* sect. *Parogonum* within subtribe Reynoutriinae remained unclear until the present study.

Muehlenbeckia

Muehlenbeckia was erected by Meisner (1840) to include five species of *Polygonum* with *M. australis* (G.Forst.) Meisn. as the type. The genus, as currently understood, contains approximately twenty-seven species, eighteen from Australia, New Zealand and the Pacific Islands and nine from Central and South America (Schuster et al. 2013).

The taxa of *Muehlenbeckia* are variable in habit, ranging from prostrate, mat-forming creepers to erect shrubs to woody lianas; all are perennial and none is herbaceous. *Muehlenbeckia* species have succulent mature perianths, as opposed to dry and winged as in *Fallopia* and *Reynoutria*, fasciculate to racemose to paniculate inflorescences, fimbriate stigmas and are often dioecious (Allan 1961; Brandbyge 1992; Green et al. 1994). Chromosome base number is $x = 10$ (Beuzenberg and Hair 1983; de Lange and Murray 2002).

Meisner (1856) divided *Muehlenbeckia* into three sections, namely sect. *Sarcogonum* Endl., sect. *Eumühlenbeckia* Endl. and sect. *Andinia* Wedd., based upon floral characters. The latest molecular phylogenetic schemes (e.g. Schuster et al. (2011a, b, 2013, 2015)) have revealed that *Muehlenbeckia* contains three well-supported subclades, denoted *x*, *y* and *z*, which generally correspond with biogeographic distribution and bear little resemblance to Meisner's (1856) sectional treatment. Clade *x* is a predominantly New Zealand clade, containing: *M. complexa* (A.Cunn.) Meisn., *M. ephedroides* Hook.f. and *M. axillaris* (Hook.f.) Endl., as well as *M. tuggeranong* Mallinson, an Australian endemic (Makinson and Mallinson 1997). Clade *y* is an Australian clade, containing: *M. arnhemica* K.L.Wilson & R.O.Makinson, *M. diclina* (F.Muell) F.Muell., *M. rhyticarya* F.Muell. ex Benth. and *M. zippelii* (Meisn.) Danser with strong support, as well as *M. adpressa* (Labill.) Meisn., *M. gracillima* Meisn., *M. costata* K.L.Wilson & R.O.Makinson and *M. gunnii* (Hook.f.) Endl. with weaker support. Clade *z* is a predominantly Central/South American clade, containing: *M. urubambensis* Brandbyge, *M. volcanica* (Benth.) Endl., *M. tiliifolia* Wedd., *M. tamnifolia* (Kunth) Meisn. and, somewhat surprisingly, *M. australis*, a native of New Zealand and Norfolk Island, whose inclusion in this clade was hypothesised to be the result of long-distance dispersal (Schuster et al. 2013). The phylogenetic placements of two further species are unresolved by previous analyses: *M. astonii* Petrie, a divaricating shrub native to New Zealand and *M. platyclada* (F.Muell.) Meisn. (= *Homalocladium platycladum* (F.Muell.) L.H.Bailey), an evergreen shrub with phylloclades, native to New Guinea and the Solomon Islands (e.g. Schuster et al. (2011a,b)).

In the current study, we further resolved the evolutionary relationships of *Reynoutria*, *Fallopia* and *Muehlenbeckia* within subtribe Reynoutriinae by including the widest sampling of ingroup taxa for the clade to date, in particular being the first to include infraspecific taxa and allies of *R. japonica*, as well as both taxa of *Fallopia* sect. *Parogonum*. A phylogenetic analysis was conducted on sequence data from six markers: two nuclear, the second intron of *LEAFY* (*LEAFYi2*) and the internal transcribed spacer (ITS) of the 17S-5.8S-26S rDNA region; and four plastid, *matK*, *rbcL*, *rps16-trnK* and *trnL-trnF*.

Materials and methods

Plant material

An accession list for the current study is presented in Suppl. material 2. Samples were collected either as fresh material or taken from herbarium specimens with the curator's permission. Where possible, voucher specimens were made and deposited in the University of Leicester Herbarium (**LTR**).

The accessions, collected for the current study, represent the widest sampling of ingroup taxa for any phylogenetic study in this subtribe to date (cf. Galasso et al. (2009); Schuster et al. (2011b, 2015)). In total, nine *Reynoutria*, nineteen *Muehlenbeckia* and nine *Fallopia* taxa were included. Published taxa that are missing from the current study include: *F. filipes*, *F. koreana*, *F. pterocarpa*, *F. schischkinii*, *M. andina* Brandbyge, *M. fruticulosa* (Walp.) Standl., *M. hastulata* (Sm.) I.M.Johnst., *M. monticola* Pulle, *M. nummularia* H.Gross, *M. polybotrya* Meisn., *M. sagittifolia* (Ortega) Meisn. and *M. triloba* Danser.

Molecular analysis

DNA extraction, amplification and sequencing

Total genomic DNA was isolated from dried leaf material using the DNeasy Plant Mini Kit (Qiagen). Six markers, four plastid: *matK*, *rbcL*, *rps16-trnK* and *trnL-trnF* and two nuclear: ITS and *LEAFYi2*, were amplified by PCR. The primer sequences and cycling conditions are presented in Suppl. material 3. For the ITS, the reaction mixture was supplemented with 4% DMSO to prevent amplification of paralogous pseudogenes (Buckler et al. 1997). PCR amplicons were visualised by gel electrophoresis, purified using the NucleoSpin Gel and PCR Clean-up kit (Machery-Nagel) and Sanger-sequenced by GATC Biotech (Konstanz, Germany). *LEAFYi2* was also sequenced from clones. Cloning was conducted using the pGEM-T Easy Vector System (Promega) and α-Select Competent Cells taken from E. coli (Bioline). Recombinant plasmids were selected by blue-white screening and the size of the insert determined by colony PCR with M13 primers. Plasmid DNA was isolated from cell cultures using the E.Z.N.A. Plasmid Mini Kit (Omega Bio-tek) and a minimum of five colonies were sequenced per accession.

Alignment and phylogenetic analysis

Generated sequence reads were viewed, trimmed and edited with Geneious R7 (created by Biomatters; available from http://www.geneious.com/). The sequences were then blasted against the NCBI GenBank database to ensure taxon and gene matches. In total, 259 sequences were used, 107 (41%) of these were newly generated for the current study and 152 (59%) were downloaded from the NCBI GenBank database (Suppl. material 1).

Multiple sequences were aligned for each gene region using the Clustal W algorithm (Larkin et al. 2007). Indels and areas of ambiguous homology were excised from the alignments prior to phylogenetic analysis. The collective chloroplast dataset (*matK*, *rbcL*, *rps16-trnK*, *trnL-trnF*), *LEAFYi2* and the ITS were analysed separately and then concatenated to produce a total evidence dataset. Not all gene regions were available for all taxa and some taxa had incomplete datasets (Table 2; Suppl. material 1). Missing data were treated as a continuous series of Ns in concatenated datasets (Wiens 2006).

Two methods were used to infer the evolutionary relationships of the taxa from the datasets, Maximum Likelihood (ML) and Maximum Parsimony (MP). ML analysis was conducted using PhyML 3.0 (Guindon and Gascuel 2003). The most appropriate model of DNA sequence evolution for each dataset was estimated using Model Selection in MEGA6 (Tamura et al. 2013) and the model with the lowest Bayesian information criterion chosen. Topology searches for the most likely tree were carried out using the nearest-neighbour interchange (NNI) search strategy. Maximum Parsimony (MP) analysis was conducted using PAUP* 4.0 (Swofford 2002). Topology searches for the most parsimonious trees were carried out using a branch and bound

Table 2. Statistical values for analysed datasets.

Dataset	Aligned length (bp)	No. (%) conserved characters	No. (%) variable characters	No. (%) parsimony informative characters	No. (%) of missing species
matK	1224	893 (73)	331 (27)	188 (15)	2 (4)
rbcL	1327	1140 (86)	182 (14)	105 (8)	15 (28)
rps16-trnK	1034	729 (71)	305 (29)	137 (13)	20 (37)
trnL-trnF	935	643 (69)	292 (31)	155 (16)	6 (11)
ITS	767	465 (61)	302 (39)	199 (26)	3 (6)
LEAFYi2	930	541 (58)	389 (42)	174 (19)	22 (40)
cp combined	4510	3403 (76)	1107 (24)	584 (13)	0 (0)
Total combined	6207	4420 (71)	1787 (29)	951 (15)	–

search strategy with the addition method FURTHEST. Node support for ML and MP analyses was estimated by resampling inferred trees by bootstrapping (BS) - 1000 replicates (Felsenstein 1985). Two species of *Coccoloba* were selected to form the outgroup as they belong to the sister subfamily Eriogonoideae and their separation from in-group taxa is well established (Cuénoud et al. 2002; Lamb Frye and Kron 2003; Sanchez et al. 2009, 2011; Burke and Sanchez 2011). They could also be reliably aligned with in-group taxa for the all markers, excluding *LEAFYi2*. Phylogenetic trees were generated for individual nuclear (*LEAFYi2*, ITS), combined chloroplast (*matK*, *rbcL*, *rps16-trnK* and *trnL-trnF*) and total evidence (*LEAFYi2*, the ITS, *matK*, *rbcL*, *rps16-trnK* and *trnL-trnF*) datasets. Congruence between trees was determined by comparison of BS values.

Data availability statement

All sequences generated for this study have been deposited on GenBank (NCBI). Sequence alignments are available in the Suppl. materials 4–7.

Results

Phylogenetic trees were generated by ML and MP. The two analyses were largely congruent, although bootstrap support (BS) values for ML were generally higher. The trees presented (Fig. 1 and Suppl. material 1: figs S1–S3) follow the topology generated by ML analysis. BS values (≥ 50%) are displayed above and below branches for ML and MP, respectively. Hyphens (-) indicate nodes where MP trees differ from ML in branching order. BS values from ML analysis are cited in the main text, unless otherwise stated.

The ITS, *LEAFYi2* and combined chloroplast (*matK*, *rbcL*, *rps16-trnK* and *trnL-trnF*) datasets were analysed separately (Suppl. material 1: figs S1–S3). The single-marker analyses of nuclear loci (the ITS and *LEAFYi2*) produced poorly-resolved trees and branches with strong support were largely confined to termini (Suppl. material 1:

figs S1, S2), while the combined chloroplast analysis was more informative with good resolution at internal nodes (Suppl. material 1: fig. S3). The three independent datasets (two nuclear and chloroplast) were largely congruent and so were concatenated to form a total evidence tree (Fig 1). The total evidence tree agreed with the combined chloroplast tree, excepting the placement of *R. japonica* var. *compacta* and *M. ephedroides*: 1) In the combined chloroplast tree, *R. japonica* var. *compacta* was sister to *R. sachalinensis* with strong support (84% BS), while in the total evidence tree, it was placed in a weakly-supported *R. japonica* s.l. clade (55%), to which *R. sachalinensis* was basal; 2) In the combined chloroplast analyses *M. ephedroides* fell within a clade with *M. axillaris* and *M. tuggeranong* (78% BS), while in the total evidence analysis, it was sister to *M. australis* (97% BS).

The results of the total evidence analysis gave greater resolution and a higher number of strongly-supported nodes than the individual nuclear and combined chloroplast trees alone. In our view, the total evidence tree is the best estimate of the phylogenetic relationships in this study; hereafter, this is the tree described (unless otherwise stated) and forms the basis of our discussions.

Phylogenetic analysis

Subtribe Reynoutriinae formed a well-supported (93% BS) clade within the Polygonaceae; sister to a subtribe Polygoninae clade (100% BS). Within subtribe Reynoutriinae, there were four well-supported subclades: A) a *Reynoutria* clade (77% BS with *R. multiflora* and 99% BS without); B) a *Fallopia* sect. *Parogonum* clade (100% BS); C) a *Fallopia* s.s. clade (100% BS) and D) a *Muehlenbeckia* clade (100% BS). The *Fallopia* s.s. and *Muehlenbeckia* clades were sister to one another with the *Fallopia* sect. *Parogonum* clade immediately basal to them (100% BS) and the *Reynoutria* clade basal to all three (55% BS).

The *Reynoutria* clade contained *R. multiflora*, *R. ciliinervis*, *R. sachalinensis*, *R. japonica* var. *compacta*, *R. elliptica*, *R. forbesii*, *R. japonica* var. *terminalis*, *R. japonica* var. *uzenensis* and *R. japonica* var. *japonica*. Within the clade, the erect *Reynoutria* taxa formed a strongly-supported subclade (100% BS), with *R. ciliinervis* (99% BS) and *R. multiflora* (77% BS) as independent basal lineages. However, relationships within the erect subclade were poorly resolved with only weakly-supported internal nodes. Nevertheless, there were two clear subclades within it, a strongly-supported one containing *R. elliptica* + *R. forbesii* (94% BS) and a moderately-supported one containing *R. japonica* var. *terminalis*, *R. japonica* var. *japonica* + *R. japonica* var. *uzenensis* (71% BS).

The *Fallopia* sect. *Parogonum* clade contained the sister taxa *F. cynanchoides* and *F. ciliinodis* with strong support (100% BS). The *Fallopia* s.s. clade contained two strongly-supported subclades, C1) a sect. *Fallopia* clade (100% BS) and C2) a sect. *Sarmentosae* clade (100% BS). The sect. *Fallopia* clade contained *F. convolvulus*, *F. dentatoalata*, *F. dumetorum*, *F. cristata* and *F. scandens*. All relationships within the clade there were strongly supported. Within this clade, *F. cristata* and *F. scandens* were almost identical (> 99.85% pairwise identity for all available sequence data) and were

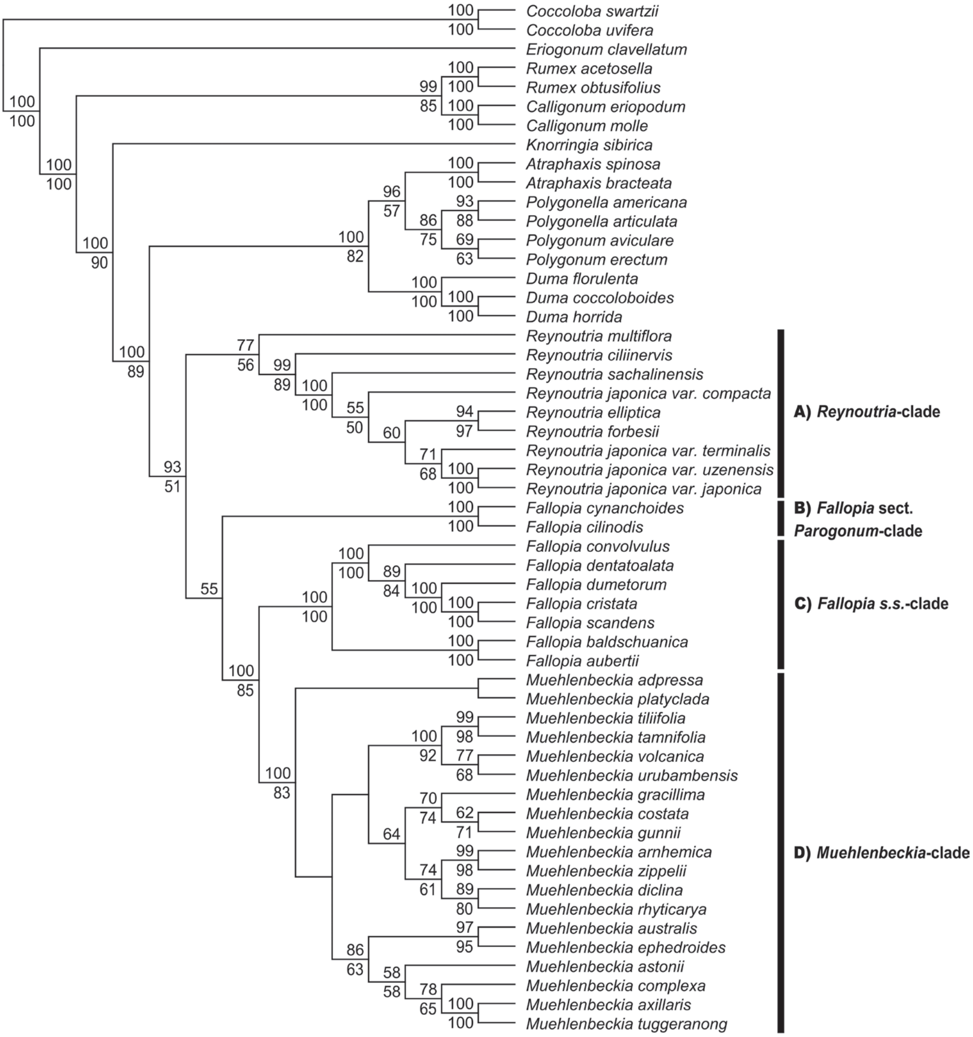

Figure 1. A total evidence phylogenetic tree generated by a Maximum Likelihood analysis of concatenated nuclear (ITS and *LEAFYi2*) and chloroplast (*matK*, *rbcL*, *rps16-trnK* and *trnL-trnF*) sequence data. Bootstrap support values (≥ 50%) are displayed above and below the nodes for Maximum Likelihood and Maximum Parsimony analyses, respectively. Maximum Parsimony analysis recovered eight equally parsimonious trees (3099 steps). The main clades within subtribe Reynoutriinae are marked with bars.

placed as sister taxa (100% BS). The sect. *Sarmentosae* clade contained the sister taxa *F. baldschuanica* and *F. aubertii* with strong support (100% BS).

The *Muehlenbeckia* clade contained three subclades with moderate to strong support, although the relationships between them were entirely unresolved: D1) a Central/South American clade (100% BS); D2) an Australian clade (64% BS) and D3) a predominantly New Zealand clade (86% BS). The American clade contained

M. tiliifolia, *M. tamnifolia*, *M. volcanica* and *M. urubambensis*. All relationships within the clade were strongly supported. Within this clade, there were two pairs of sister taxa, *M. tiliifolia* + *M. tamnifolia* (99% BS) and *M. volcanica* + *M. urubambensis* (77% BS). The Australian clade contained *M. gracillima*, *M. costata*, *M. gunnii*, *M. arnhemica*, *M. zippelii*, *M. diclina* and *M. rhyticarya*. All relationships within the clade were moderately/strongly supported. Within this clade, there were two subclades, one containing *M. gracillima*, *M. costata* and *M. gunnii* (70% BS) and another containing *M. zippelii*, *M. arnhemica*, *M. diclina* and *M. rhyticarya* (74% BS). The predominantly New Zealand clade contained *M. australis*, *M. ephedroides*, *M. astonii*, *M. complexa*, *M. axillaris*, as well as the Australian endemic *M. tuggeranong*. All relationships within the clade were moderately/strongly supported. In this clade, there were two subclades, one containing *M. australis* + *M. ephedroides* (97% BS) and another containing *M. astonii*, *M. complexa*, *M. axillaris* + *M tuggeranong* (58% BS). Within this second subclade, *M. axillaris* and *M. tuggeranong* were sister taxa with strong support (100% BS). The placement of *M. platyclada* and *M. adpressa* within the genus was unresolved.

LEAFYi2 copy number

LEAFYi2 was single-copy in all diploid taxa and was sequenced directly, but in two polyploid taxa, *R. japonica* var. *japonica* and *F. convolvulus*, two amplicons of different size were observed and these were sequenced from clones (Suppl. material 1: fig. S2). In *R. japonica* var. *japonica*, the two copies were sister to one another (73% BS), while in *F. convolvulus*, the two copies were separate on the tree. Copy 1 was sister to *F. dumetorum* (100% BS), while the position of copy 2 was unresolved in the ML analysis (< 50% BS), but placed in a clade with *F. scandens* and *F. cristata* in the MP analysis (70% BS).

Discussion

Phylogenetic relationships

The species of Reynoutriinae form a strongly-supported monophyletic clade within the Polygonaceae. This clade is characterised by the presence of extra-floral nectaries at the base of leaf petioles (Salisbury 1909; Brandbyge 1992; Schuster et al. 2011b) and *Tiniaria* pollen type (Hedberg 1946; Brandbyge 1992). The subtribe has a cosmopolitan distribution and is found in the Northern and Southern Hemispheres (Allan 1961; Brandbyge 1992; Anjen and Park 2003a, 2003b; Freeman and Hinds 2005). Within the Reynoutriinae clade, there are four strongly-supported subclades: a *Reynoutria* clade, a *Fallopia* sect. *Parogonum* clade, a *Fallopia* s.s. clade (containing *Fallopia* sect. *Fallopia* and sect. *Sarmentosae*) and a *Muehlenbeckia* clade. *Fallopia* s.s. and *Muehlenbeckia* are sister to one another, while *Fallopia* sect. *Parogonum* is basal to them and *Reynoutria* is basal to all three.

Reynoutria clade

Reynoutria taxa form a strongly-supported monophyletic clade within subtribe Reynoutriinae, which confirms the findings of previous molecular studies (e.g. Galasso et al. (2009); Schuster et al. (2011a, b, 2015)). The clade has an East Asian distribution and is characterised by the presence of rhizomes, which are unique within the subtribe (Ohwi 1965; Anjen and Park 2003a, b).

Within the *Reynoutria* clade, the erect taxa form a strongly-supported subclade (100% BS). Indeed, previous authors (e.g. Galasso et al. (2018)) have considered the erect taxa as distinct from the climbing taxa (*R. multiflora* & *R. ciliinervis*) and retain the climbers in their own genus, *Pleuropterus*. However, this is not supported by the current study as *R. multiflora* and *R. ciliinervis* do not form a reciprocally monophyletic subclade, but rather they form separate basal lineages within the *Reynoutria* clade. We, therefore, continue to treat both the climbing and erect taxa as *Reynoutria* s.l. (in line with Schuster et al. (2011b)), until further evidence is accumulated.

Within the erect *Reynoutria* clade, *R. japonica* and its allies form a weakly-supported monophyletic subclade, with *R. sachalinensis* as sister. Within this subclade, the notorious invasive alien var. *japonica* is most closely related to the other tall lowland forms from Japan, var. *uzenensis* and var. *terminalis*, which most likely represent subspecies of *R. japonica*.

Reynoutria forbesii from China and *R. elliptica* from Korea are sister taxa and form a monophyletic group, which comes out as sister to *R. japonica* with weak support. Furthermore, *R. forbesii* and *R. elliptica* are very similar morphologically and they most likely represent a single taxon - the epithet *forbesii* is the older name has priority (Anjen and Park 2003a; Bailey 2003; Galasso et al. 2009). Whether *R. forbesii* is specifically distinct from *R. japonica* remains unclear and further analysis using a wider range of material from across the native range is required. In the interim, we continue to treat this taxon as *R. forbesii*, with *R. elliptica* as a synonym.

The placement of the high-altitude dwarf form *R. japonica* var. *compacta* differed between the individual nuclear and combined chloroplast analyses, being sister to *R. sachalinensis* on the chloroplast tree (as also demonstrated by Galasso et al. (2009)) and closer to *R. japonica* on the nuclear trees. This is most likely due to reticulate evolution with the chloroplast haplotype of *R. sachalinensis* being captured during the formation of *R. japonica* var. *compacta*. Var. *compacta* is also distinct in being of small stature and flowering earlier, as well as having undulate leaf margins, somewhat leathery leaves and a red-tinged inflorescence (Ohwi 1965; Desjardins et al. 2022), characteristics which are maintained even when transplanted at lower altitudes (Shiosaka and Shibata 1993). This morphological distinction, its montane habitat and reticulate history can all be taken to support species status as *R. compacta* (Galasso et al. 2009). However, the distinction between the tall lowland forms of *R. japonica* and dwarf montane *compacta*, while apparent in the small subset of adventive clones, is less clear in the native range where leaf morphology and height grade into one another along an altitudinal cline (Bailey 2003). As is the case with *R. forbesii*, further analysis using a wider sampling of material from the native range is required to determine the true taxonomic status of var. *compacta* and whether it should be treated as a species in its own right or a subspecies of *R. japonica*.

Fallopia sect. *Parogonum* clade

Fallopia ciliinodis and *F. cynanchoides* form a strongly-supported monophyletic clade within subtribe Reynoutriinae, characterised by papillate trichomes (Haraldson 1978; Bailey 1989). *Fallopia cynanchoides* is restricted to central China (Anjen and Park 2003a) and *F. ciliinodis* to the East Coast of North America (Freeman and Hinds 2005). *Fallopia* sect. *Parogonum*, therefore, represents a good example of a well-known floristic affinity, in which counterparts (conspecifics or intercontinental species pairs) are discontinuously distributed between East Asia and Eastern North America (Graham 1972). This disjunct distribution is the product of complex processes, including migration/dispersal, extinction, speciation and vicariance, but the general pattern is thought to be due to the exchange of taxa between Eurasia and North America over the Bering and North Atlantic land bridges in the mid-Tertiary, followed by extirpation in western North America and North East Asia in the cooling climates of the late Tertiary to early Quaternary (Wen 1999, 2001).

The position of *Fallopia* sect. *Parogonum* within subtribe Reynoutriinae has been the subject of some speculation. Schuster et al. (2011b) predicted that the species of *Fallopia* sect. *Parogonum* may belong to the *Reynoutria* clade due to perceived similarities in morphology, for example, paniculate inflorescences, multicellular trichomes, chromosome base number (x = 11; Bailey and Stace (1992)) and secondary chemistry (Kim et al. 2000a, b). However, in the current study, *Fallopia* sect. *Parogonum* appeared to be more closely related to *Muehlenbeckia* and the rest of *Fallopia* than to *Reynoutria*. This placement was strongly supported by the combined chloroplast analysis, but only weakly supported by the total evidence analysis.

Fallopia s.s. clade

The species of *Fallopia* sampled, minus those of sect. *Parogonum*, formed a strongly-supported monophyletic clade within subtribe Reynoutriinae and are characterised within the subtribe by capitate stigmas. Within the *Fallopia* s.s. clade, there are two strongly-supported subclades, corresponding to *Fallopia* sect. *Fallopia* and *Fallopia* sect. *Sarmentosae*, which are sister to one another.

The species of sect. *Fallopia* form a strongly-supported subclade within the *Fallopia s.s.* clade, which confirms the results of previous molecular studies (Galasso et al. 2009; Schuster et al. 2011b) and supports Holub's (1970) treatment of them as a separate section. Members of this subclade are characterised by their annual twining habits, few-flowered inflorescences and distinctive flavonoid profiles (Kim et al. 2000a). All members of this subclade are found in the north temperate region (Anjen and Park 2003a; Freeman and Hinds 2005; Stace 2019).

The analysis also indicated that *F. cristata* is not specifically distinct from *F. scandens.* The phylogenetic analysis placed them as sister to one another and they were almost identical for the markers analysed. The two taxa are thought to be separable on the basis of their mature perianths, which are said to be smaller and more narrowly winged in *F. cristata* (Freeman and Hinds 2005). However, these differences are only apparent in extreme specimens and intermediate forms are often encountered that gradually grade into *F. scandens* (Freeman and Hinds 2005). Furthermore, morphometric (Kim et al. 2000c) and chemotaxonomic (Kim et al. 2000a) studies suggest that *F. cristata* falls within the normal variability of *F. scandens*. In our view, it is not worthy of taxonomic recognition.

The species of sect. *Sarmentosae* form a strongly-supported subclade within the *Fallopia* s.s. clade, which supports Holub's (1970) treatment of them as a section within the genus. Members of this subclade can be identified by a combination of characters: capitate stigmas, dry mature perianths and a woody perennial habit. They also have distinctive flavonoid profiles (Kim et al. 2000b) and are native to Asia (Qaiser 2001; Anjen and Park 2003a).

Muehlenbeckia clade

The species of *Muehlenbeckia* sampled formed a strongly-supported monophyletic clade within subtribe Reynoutriinae, which confirms the results of Schuster et al. (2011a, b) and supports Meisner's (1840, 1856) treatment of them as a distinct group. Members of this clade are characterised by their succulent mature perianths and are found exclusively in the Southern Hemisphere (Allan 1961; Brandbyge 1992; Green et al. 1994). Within *Muehlenbeckia*, evolutionary relationships generally correspond to geographic distribution and there are three subgroups, a Central/South American clade, an Australian clade and a predominantly New Zealand clade.

The placement of *Muehlenbeckia* taxa in the current study is largely congruent with that of Schuster et al. (2011b) and bootstrap values are roughly equivalent. However, there is disagreement in the positions of two taxa. In Schuster et al. (2011b), *M. australis*, a native to New Zealand and Norfolk Island, is placed within the Central/South American clade with strong support, while in the current study, it falls, as one would more naturally expect, in the predominantly New Zealand clade. We have not seen the specimen used by Schuster et al. (2011b) (W.R. Barker 8995 & R.M. Barker; AD), but we are confident in the identity of the *M. australis* sample included in the current study. It was collected from Ōtari-Wilton's Bush, Wellington, New Zealand by Dr Peter de Lange (Unitec Institute of Technology, New Zealand) and is supported by seven further collections of *M. australis* from around New Zealand, which form a monophyletic group within the New Zealand clade (Schmid et al., unpublished). Schuster et al.'s (2011b) analyses also failed to resolve the position of *M. astonii* within *Muehlenbeckia*, while, in the current analysis *M. astonii* was placed in the predominantly New Zealand clade with strong support. An examination of the sequence data used by Schuster et al. (2011b) revealed that the ITS sequence (EF635479) is likely a pseudogene, which inflated sequence divergence and resulted in the artificial separation of *M. astonii* from the rest of New Zealand *Muehlenbeckia*. This pseudogene was identified by its relatively low GC content (60.1% versus 65.5%) and the high number of SNPs in the conserved

5.8S region (Buckler et al. 1997; Álvaraez and Wendel 2003; Feliner and Rosselló 2007). We found that pseudogenised ITS copies would readily amplify in this group if 4% DMSO, or some other denaturant, was omitted from the PCR mixture.

The placement of *M. ephedroides* was incongruent between the chloroplast and individual nuclear analyses. In the chloroplast analyses, *M. ephedroides* fell within a clade with *M. axillaris* and *M. tuggeranong*, while in the nuclear analyses, it was sister to *M. australis*. As is the case in *R. japonica* var. *compacta*, *M. ephedroides* likely has a reticulate history and, during its formation, appears to have captured the haplotype of an ancestor of *M. axillaris*/*M. tuggeranong*. This scenario is supported by observations of modern hybridisation in New Zealand *Muehlenbeckia* (Yong 1967).

Taxonomy of Reynoutriinae

Reynoutriinae, or the RMF clade, is monophyletic and contains three genera *Reynoutria*, *Muehlenbeckia* and *Fallopia*. However, as currently circumscribed, *Fallopia* is paraphyletic as *Muehlenbeckia* is nested between *Fallopia* sect. *Parogonum* and the rest of the genus. The subtribe, therefore, requires an immediate taxonomic revision. There are two possible systematic interpretations to restore monophyly in this group, either treat *Fallopia*, *Muehlenbeckia* and *Reynoutria* as a single genus, *Fallopia*, which has priority, or treat *Fallopia* sect. *Parogonum* as a genus in its own right.

Both an amalgamated and a divided *Fallopia* can be supported by the available molecular data and there are putative synapomorphies for both treatments. An amalgamated *Fallopia* would include all members of the RMF clade and would be characterised by the presence of extra-floral pit nectaries at the base of leaf petioles and the *Tiniaria* pollen type (Salisbury 1909; Hedberg 1946; Brandbyge 1992), while a divided subtribe Reynoutriinae would be split into the different subclades of the RMF clade and these would be characterised by a number of putative synapomorphies: *Fallopia* by its capitate stigmas, *Parogonum* by its papillate trichomes, *Reynoutria* by its rhizomes and *Muehlenbeckia* by its succulent mature perianth (Brandbyge 1992; Anjen and Park 2003a, b; Freeman and Hinds 2005).

The two alternative treatments of the subtribe are both perfectly tenable and there are arguments for and against amalgamation. The arguments for amalgamating the genera are threefold: 1) The morphological characters used to separate *Fallopia*, *Muehlenbeckia* and *Reynoutria* are rather inconsistent. Meisner (1840, 1856) considered species of *Muehlenbeckia* distinct on the basis of their succulent mature perianths, fimbriate stigmas and dioecious breeding systems. However, fimbriate stigmas and functional dioecy are also found in *Reynoutria*. The only character that seems to consistently separate *Muehlenbeckia* is its succulent mature perianth (Brandbyge 1993), but, as Haraldson (1978) argues, succulent mature perianths have evolved several times within the Polygonaceae, for example, *Coccoloba*, *Duma*, *Muehlenbeckia* and *Persicaria* Mill. and is not a reliable character when delimiting genera. Schuster et al. (2011b) also cited basic chromosome basic number as a means of distinguishing *Reynoutria* ($x = 11$) from *Muehlenbeckia* and *Fallopia* ($x = 10$). However, the inclusion of *Fallopia* sect. *Parogonum* (*x* = 11) in a clade with *Muehlenbeckia* and *Fallopia* s.s. breaks down this distinction. Furthermore, intrageneric variation in basic chromosome number is not uncommon in the Polygonaceae, for example, *Persicaria*, *x* = 10, 11, 12 (Kim et al. 2008); 2) There are good synapomorphies for an amalgamated *Fallopia*, such as the presence of extra-floral pit nectaries and the *Tiniaria* pollen type (Salisbury 1909; Hedberg 1946; Brandbyge 1992); 3) Hybridisation occurs between the subclades, *Reynoutria* × *Fallopia* and *Reynoutria* × *Muehlenbeckia* (Bailey 2001, 2013).

Meanwhile, the arguments against amalgamating the genera are fivefold: 1) *Muehlenbeckia* has been treated as a distinct entity since its formation, while *Fallopia* and *Reynoutria* have often been treated as separate genera (Galasso et al. 2009); 2) It would require more taxonomic upheaval to amalgamate *Muehlenbeckia* within *Fallopia s.l* and a greater number of name changes; 3) *Muehlenbeckia* is a well-established genus and in widespread usage amongst botanists in the Southern Hemisphere; 4) *Muehlenbeckia* has been conserved against previous priority challenges (Rickett and Stafleu 1959); 5) *Muehlenbeckia* has a distinct biogeographical distribution, being confined to the Southern Hemisphere and is clearly separate from northern *Fallopia* and *Reynoutria*.

On balance, we are of the opinion that, despite compelling arguments in favour of amalgamation, species of subtribe Reynoutriinae are better treated as multiple genera to limit nomenclatural upheaval, preserve names in widespread use and to better distinguish the clades. *Fallopia* sect. *Parogonum* has, therefore, been treated as a genus in its own right and the relevant binomial changes have been made below.

Putative allopolyploid origin of *F. convolvulus*

Fallopia convolvulus (*Fallopia* sect. *Fallopia*) is tetraploid (2*n* = 40), but it is not known if it arose by autopolyploidy or allopolyploidy (Bailey and Stace 1992). In the current study, two divergent copies of the single-copy nuclear gene *LEAFYi2* were detected in *F. convolvulus*, which were clearly separated on the phylogenetic tree. One copy was sister to Eurasian *F. dumetorum*, while the other appeared to be most closely related to American *F. cristata*/*F. scandens*. The presence of two divergent copies can be taken as evidence for an allopolyploid origin of *F. convolvulus*, which may have originated as a result of hybridisation between the ancestors of *F. dumetorum* (2*n* = 20) and *F. scandens*/*F. cristata* (2*n* = 20), followed by chromosomal doubling. Bailey (1989) conjectured that *F. convolvulus* is derived from *F. scandens* and diversified relatively recently to become a weed of cereal crops. An allopolyploid origin of *F. convolvulus* is in line with this, as it would provide a mechanism for reproductive isolation and near-instantaneous speciation. Indeed, modern hybrids between *F. convolvulus* and *F. dumetorum*, *F.* × *convolvuloides* (Brügger) Holub, are triploid and sterile (Holub 1970).

However, this conclusion is not wholly supported by the other available datasets. In the combined chloroplast analysis *F. convolvulus* was placed basal to the rest of sect. *Fallopia* and was not sister to *F. dumetorum* or *F. scandens*/*F. cristata*. Furthermore, in the ITS analysis, only one functional copy was detected in

F. convolvulus, but this is not unexpected given the homogenising processes of concerted evolution in tandemly-arranged repetitive DNA, such as the ITS (Álvarez and Wendel 2003). A genomic in situ hybridisation (GISH) experiment using labelled *F. dumetorum* and *F. scandens* genomes to probe *F. convolvulus* chromosomes would be highly informative.

Conclusion

Subtribe Reynoutriinae is a monophyletic group, which is characterised by the presence of extra-floral, nectariferous glands at the base of leaf petioles. Within the subtribe, four main clades were identified, which represent separate genera: East Asian *Reynoutria*, disjunct East Asian/Eastern North American *Parogonum* (Haraldson) Desjardins & J.P.Bailey, gen. et stat. nov., north temperate *Fallopia* and austral *Muehlenbeckia*. Within the subtribe, *Reynoutria* can be identified by the presence of rhizomes, *Parogonum* by stiff papillate hairs, *Fallopia* by capitate stigmas and *Muehlenbeckia* by succulent mature perianths.

Nomenclatural novelties

***Parogonum* (Haraldson) Desjardins & J.P.Bailey, gen. et stat. nov.**
urn:lsid:ipni.org:names:77315139-1

Fallopia sect. *Parogonum* Haraldson, *Symb. Bot. Upsal.* 22: 78 (1978). Basionym.

1) *Parogonum ciliinode* (Michx.) Desjardins & J.P.Bailey, comb. nov.
urn:lsid:ipni.org:names:77315140-1

Polygonum ciliinode (‘*cilinode*’) Michx., *Fl. Bor.-Amer.* (*Michaux*) 1: 241 (1803). Basionym.
Tiniaria ciliinodis (‘*cilinodis*’) (Michx.) Small, *Fl. S.E. U.S. [Small].:* 382 (1903). *Bilderdykia ciliinodis* (‘*cilinodis*’) (Michx.) Greene, *Leafl. Bot. Observ. Crit.* 1: 23 (1904). *Reynoutria ciliinodis* (‘*cilinodis*’) (Michx.) Shinners, *Sida* 3: 117 (1967). *Fallopia ciliinodis* (‘*cilinodis*’) (Michx.) Holub, *Folia Geobot. Phytotax.* 6: 176 (1970). Homotypic synonyms.

2) *Parogonum cynanchoides* (Hemsl.) Desjardins & J.P.Bailey, comb. nov.
urn:lsid:ipni.org:names:77315141-1

Polygonum cynanchoides Hemsl., *J. Linn. Soc.*, *Bot.* 26: 338 (1891). Basionym.
Fallopia cynanchoides (Hemsl.) Haraldson, *Symb. Bot. Upsal.* 22: 78 (1978). Homotypic synonym.

3) *Parogonum cynanchoides* subsp. *glabriusculum* (A.J.Li) Desjardins & J.P.Bailey, comb. et stat. nov.

urn:lsid:ipni.org:names:77315142-1

Polygonum cynanchoides var. *glabriusculum* A.J.Li, *F. Xizang* 1: 608 (1983). Basionym.

Fallopia cynanchoides var. *glabriuscula* (A.J.Li) A.J.Li, *Fl. Reipubl. Popularis Sin.* 25: 104 (1998). Homotypic synonym.

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Supplementary material 1

Additional phylogenetic trees from ITS, *LEAFYi2* and combined chloroplast analyses.

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Data type: pdf file

Explanation note: **fig S1.** A phylogenetic tree generated by a Maximum Likelihood analysis of ITS sequence data. Bootstrap support values (≥ 50%) are displayed above and below the nodes for Maximum Likelihood and Maximum Parsimony analyses, respectively. Maximum Parsimony analysis recovered two equally parsimonious trees (825 steps); **fig S2.** A phylogenetic tree generated by a Maximum Likelihood analysis of *LEAFYi2* sequence data. Bootstrap support values (≥5 0%) are displayed above and below the nodes for Maximum Likelihood and Maximum Parsimony analyses, respectively. Hyphens (-) indicate nodes where parsimony and likelihood trees differ in branching pattern. Maximum Parsimony analysis recovered three equally parsimonious trees (598 steps); **fig S3.** A phylogenetic tree generated by a Maximum Likelihood analysis of concatenated chloroplast sequence data (*matK*, *rbcL*, *trnL-trnF* & *rps16-trnK*). Bootstrap support values (≥ 50%) are displayed above and below the nodes for Maximum Likelihood and Maximum Parsimony analyses, respectively. Maximum Parsimony analysis recovered 191 equally parsimonious trees (1600 steps). The main clades within subtribe Reynoutriinae are marked with bars.

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Supplementary material 2

Accessions used in the current study

Authors: Stuart D. Desjardins, John P. Bailey, Baowei Zhang, Kai Zhao, Trude Schwarzacher

Data type: excel file

Link: https://doi.org/10.3897/phytokeys.220.96922.suppl2

Supplementary material 3

Primer sequences and PCR cycling conditions

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Data type: excel file

Link: https://doi.org/10.3897/phytokeys.220.96922.suppl3

Supplementary material 4

ITS multiple-sequence alignment

Authors: Stuart D. Desjardins, John P. Bailey, Baowei Zhang, Kai Zhao, Trude Schwarzacher

Data type: .txt file

Link: https://doi.org/10.3897/phytokeys.220.96922.suppl4

Supplementary material 5

LEAFYi2 multiple-sequence alignment

Authors: Stuart D. Desjardins, John P. Bailey, Baowei Zhang, Kai Zhao, Trude Schwarzacher

Data type: .txt file

Link: https://doi.org/10.3897/phytokeys.220.96922.suppl5

Supplementary material 6

Combined chloroplast multiple-sequence alignment

Authors: Stuart D. Desjardins, John P. Bailey, Baowei Zhang, Kai Zhao, Trude Schwarzacher

Data type: .txt file

Link: https://doi.org/10.3897/phytokeys.220.96922.suppl6

Supplementary material 7

Total evidence multiple-sequence alignment

Authors: Stuart D. Desjardins, John P. Bailey, Baowei Zhang, Kai Zhao, Trude Schwarzacher

Data type: .txt file

Link: https://doi.org/10.3897/phytokeys.220.96922.suppl7

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The molecular phylogenetic position of *Harpagocarpus* (Polygonaceae) sheds new light on the infrageneric classification of *Fagopyrum*

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Abstract

In the context of the molecular phylogeny of Polygonaceae, the phylogenetic positions of most genera and their relationships have been resolved. However, the monotypic genus *Harpagocarpus* has never been included in any published molecular phylogenetic studies. In the present study, we adopt a two-step approach to confirm the phylogenetic placement of *Harpagocarpus* using two datasets: (1) a concatenated dataset of three chloroplast DNA (cpDNA) regions (*matK*, *rbcL* and *trnL-F*) for Polygonaceae and (2) a combined cpDNA dataset of five sequences (*accD*, *matK*, *psbA-trnH*, *rbcL* and *trnL-F*) for *Fagopyrum*. Our analyses confirm the previous hypothesis based on morphological, anatomical and palynological investigations that *Harpagocarpus* is congeneric with *Fagopyrum* and further reveal that *H. snowdenii* (≡ *F. snowdenii*) is sister to the woody buckwheat *F. tibeticum*. Within *Fagopyrum*, three highly supported clades were discovered and the first sectional classification was proposed to accommodate them: sect. *Fagopyrum* comprises the two domesticated common buckwheat (*F. esculentum* and *F. tataricum*) and their wild relatives (*F. esculentum* subsp. *ancestrale*, *F. homotropicum* and *F. dibotrys*) which are characterised by having large corymbose inflorescences and achenes greatly exceeding the perianth; sect. *Tibeticum*, including *F. snowdenii* and *F. tibeticum*, is characterised by the achene having appurtenances along the ribs, greatly exceeding the perianth and the

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* These authors contributed equally to this work.

perianth accrescent in fruit; sect. *Urophyllum* contains all other species of which the achenes were completely enclosed in the perianth. This study is very helpful to understand the phylogeny of the *Fagopyrum* and sheds light on the future study of taxonomy, biogeography, diversification and character evolution of the genus.

Keywords

buckwheat, *cp*DNA, Fagopyreae, morphology, new section

Introduction

Polygonaceae, a family of the flowering plants known as the buckwheat family, can be easily distinguished by its ocrea, orthotropous ovules, trigonal (typically) achenes and quincuncial aestivation (Judd et al. 2007) and is found in almost all ecosystems (Sanchez et al. 2009). Numerous molecular phylogenetic analyses (e.g. Cuénoud et al. (2002); Schäferhoff et al. (2009); Moore et al. (2010); Yang et al. (2015); Walker et al. (2018); Yao et al. (2019); Li et al. (2021)) have provided strong evidence for the monophyly of Polygonaceae and the family's membership in the FTPP clade of the order Caryophyllales, which also includes the Plumbaginaceae, Polygonaceae, Tamaricaceae and Frankeniaceae, has been securely supported (e.g. Cuénoud et al. (2002); Brockington et al. (2009); Walker et al. (2018)). Since the first large-scale molecular phylogenetic reconstruction of the Polygonaceae in 2003 (Lamb-Frye and Kron 2003), the infrafamilial relationships have gradually been resolved in subsequent studies (e.g. Kim and Donoghue (2008a, b); Kim et al. (2008); Sanchez and Kron (2008, 2009, 2011); Galasso et al. (2009); Sanchez et al. (2009, 2011); Burke et al. (2010); Tavakkoli et al. (2010, 2015); Yurtseva et al. (2010, 2016); Schuster et al. (2011a, b, 2015); Kempton (2012)) and its classification at subfamilial and tribal levels has been significantly improved (Sanchez and Kron 2008; Galasso et al. 2009; Sanchez et al. 2009, 2011; Schuster et al. 2011b, 2015). The majority of genera have been included in previous molecular phylogenetics and their monophyly and circumscription were validated, but a few genera were re-circumscribed, such as *Atraphaxis* L., *Koenigia* L., *Polygonum* L., *Ruprechtia* C.A.Mey. etc. As a result, some new genera were erected, i.e. *Duma* T.M.Schuster (Schuster et al. 2011b), *Salta* Adr.Sanchez and *Magoniella* Adr.Sanchez (Sanchez and Kron 2011), *Bactria* O.V.Yurtseva & E.V.Mavrodiev (Yurtseva et al. 2016), *Persepolium* O.V.Yurtseva & E.V.Mavrodiev (Yurtseva et al. 2017) and several old genera have been reduced, for example, *Aconogonon* (Meisn.) Rchb., *Rubrivena* M.Král and *Emex* Neck. ex Campd. (Schuster et al. 2015), *Parapteropyrum* A.J.Li (Sanchez et al. 2011), *Polygonella* Michx. (Schuster et al. 2011a) etc. However, due to a dearth of materials or insufficient molecular data to date, the systematic positions of two resistant genera, *Harpagocarpus* Hutch. & Dandy and *Eskemukerjea* Malick & Sengupta, have not yet been thoroughly evaluated in molecular analyses (Schuster et al. 2015).

The genus *Harpagocarpus* was established on the basis of its distinct fruit morphology (Hutchinson and Dandy 1926) and contains the sole species, *H. snowdenii* Hutch. & Dandy, which was originally recorded only in Uganda, but now has been reported from Kenya, Tanzania, Rwanda and Cameroon (Ayodele 2003). Jacques-Félix (1946) described *Fagopyrum ciliatum* Jacq.-Fél. from Cameroon, but according to Graham (1958), it is merely a synonym of *H. snowdenii*. Due to its unique appurtenances growing along the achene ribs, which are long purple setae with the radially arranged retrorse barbs at the tip of each seta (Fig. 1), *H. snowdenii* is a distinctive species in Polygonaceae (Hutchinson and Dandy 1926).

In the protologue, *Harpagocarpus* was morphologically compared to *Polygonum* L. and *Fagopyrum* Mill., but it was thought to resemble the latter considerably more on its broad cotyledons, large and obviously exerted fruits and the shape and venation of the leaves (Hutchinson and Dandy 1926). On the basis of anatomical similarities, Haraldson (1978) hypothesised that *Harpagocarpus* may be closely related to *Fallopia* Adans. However, Ronse Decraene and Akeroyd (1988) argued against this hypothesis and pointed out that *Harpagocarpus* and *Fagopyrum* share considerable similarity in the morphology of floral characteristics. Hong (1988) further reduced *Harpagocarpus* to a synonym of *Fagopyrum* and proposed the new combination *F. snowdenii* (Hutch. & Dandy) S.P.Hong for *H. snowdenii* after concluding from additional palynological research. Though this treatment has been followed in some literature (e.g. Brandbyge (1993); Friis and Vollesen (1998); Sanchez et al. (2011); de Klerk et al. (2015)), it was, nonetheless, recommended that molecular data be used to confirm the phylogenetic position of *Harpagocarpus* (Schuster et al. 2015).

In the present study, we obtained a few precious pieces of leaf materials of *H. snowdenii* from the specimen *Marshall A.R. WK 374* (detailed information available from: http://legacy.tropicos.org/image/100427626), which provided us an invaluable opportunity to investigate the phylogenetic position of *Harpagocarpus*, based on additional molecular data. We adopted two steps of phylogenetic analyses to infer the generic and specific affinities of *H. snowdenii*. Firstly, we used three chloroplast DNA (cpDNA) markers (*matK*, *rbcL* and *trnL-F*) to present the backbone phylogeny of Polygonaceae and affirmed the position of *Harpagocarpus* in *Fagopyrum*. Subsequently, based on five cpDNA regions (*accD*, *matK*, *psbA-trnH*, *rbcL* and *trnL-F*), we further reconstructed the phylogeny of *Fagopyrum* and clarified the accurate specific relationships of *F. snowdenii* within *Fagopyrum*.

Materials and methods

Taxon sampling, choice of markers and datasets

We employed *matK*, *rbcL* and *trnL-F* sequences, which have been extensively used in previous studies (e.g. Lamb-Frye and Kron (2003); Sanchez and Kron (2008); Sanchez et al. (2009, 2011); Burke et al. (2010); Schuster et al. (2015)), to generate a concatenated cpDNA dataset (D1) for reconstructing the backbone phylogeny of Polygonaceae. The ingroup taxa were selected from the entire family to cover all recognised tribal clades (Sanchez et al. 2011; Kempton 2012; Schuster et al. 2015) with at least one representative of each genus. A total of 37 genera and 77 species were sampled. *Plumbago auriculata* Lam. from Plumbaginaceae, which is the sister family of Polygonaceae (Yao et al. 2019;

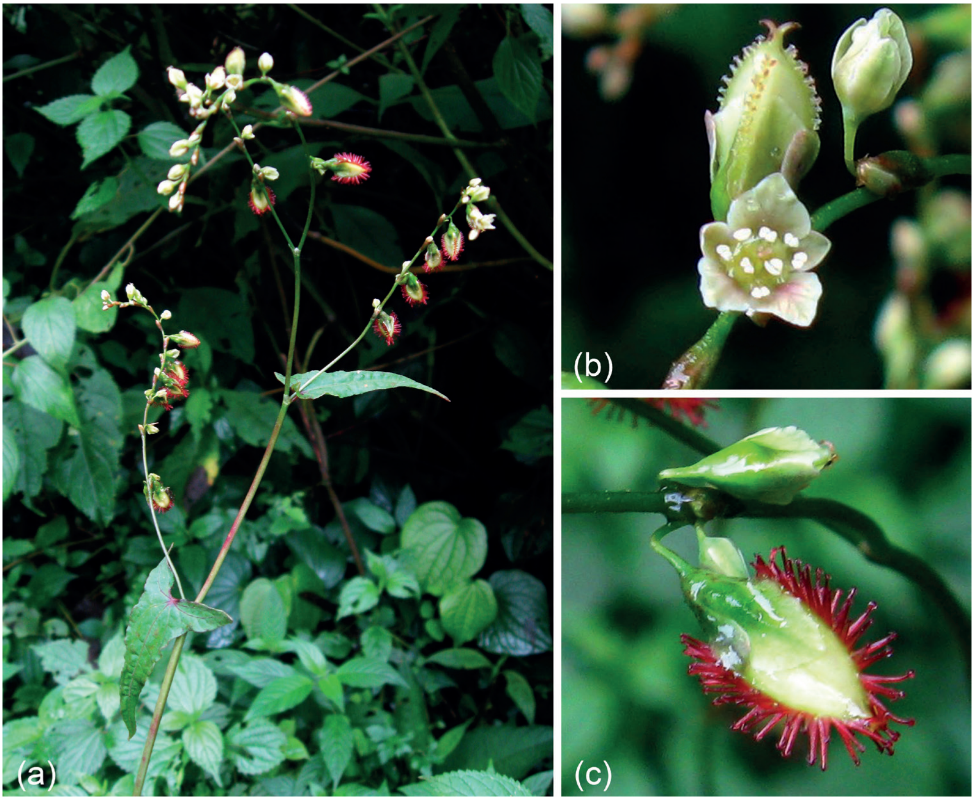

Figure 1. Morphology of *Harpagocarpus snowdenii* Hutch. & Dandy **a** ovate-triangular leaf blades and raceme-like inflorescences **b** an opened flower and a young fruit with minute papillae **c** a mature fruit with long purple setae. (Photographer: Vincent Droissart).

Li et al. 2021), was selected as the outgroup taxon. The source publications or voucher information for all sequences were listed in Suppl. material 1: table S1.

As the analyses of the D1 dataset demonstrated that *Harpagocarpus* is nested within *Fagopyrum*, we designed another dataset (D2) using five cpDNA regions (*accD*, *matK*, *psbA-trnH*, *rbcL* and *trnL-F*), with an expanded sampling of *Fagopyrum* aiming for a more accurate placement of *H. snowdenii* (= *F. snowdenii*). The ingroups of D2 dataset included 33 taxa of *Fagopyrum* covering most of the recognised species in the genus and the outgroup taxon was set as *Pteroxygonum giraldii* Damm. et Diels according to the results presented in Schuster et al. (2015). Voucher information and GenBank accession numbers for taxa used in the D2 dataset are provided in Suppl. material 1: table S2.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from fresh or silica gel dried leaves following the manufacturer's specifications of the DNEasy Plant Mini Kit (Qiagen, Valencia, CA, USA). After extraction, the DNA was resuspended in double-distilled water and kept at -40 °C for polymerase chain reaction (PCR). The PCR reactions and amplification protocol followed Schuster et al. (2011a). The amplified products were purified using a PCR Product Purification Kit (Shanghai SBS, Biotech Ltd., China). Sequencing reactions were conducted with the forward and reverse PCR primers using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, U.K.) with an ABI PRISM 3730 automatic DNA sequencer (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China). Both strands of the DNA were sequenced with overlapping regions to ensure that each base was unambiguous. Electropherograms were assembled and consensus sequences were generated with Geneious Prime 2022.0.2 platform.

Phylogenetic analysis

Sequencher version 5.4.6 (Gene Codes Corporation 2021) was used to evaluate chromatograms for base confirmation and editing contiguous sequences. All DNA sequences were initially aligned using Clustal X version 2.1 (Larkin et al. 2007) and adjusted manually in BioEdit Sequence Alignment Editor version 7.2.1 (Hall 1999).

Phylogenetic analyses were conducted, based on the combined cpDNA dataset D1 and D2. The cpDNA regions were supposedly safe to be combined in phylogenetic analyses (Olmstead and Sweere 1994) because the plastid genome is mostly uniparentally inherited (Soltis and Soltis 1998). The datasets were analysed separately using the methods of Maximum Likelihood (ML) and Bayesian Inference (BI).

ML and BI analyses were carried out using RAxML-HPC2 version 8.2.9 (Stamatakis 2014) and MrBayes version 3.2.2 (Ronquist et al. 2012) as implemented on the CIPRES Science Gateway (Miller et al. 2010), respectively. The ML analysis was performed under the GTRGAMMA model with the bootstrap iterations (-# | -N) set to 1000. The BI analysis was executed with most of the default parameters, but manually setting the following: the best substitution types (Nst) and rate distribution models (rates) that were determined by the jModelTest version 2.1.7 (Darriba et al. 2012), sampling one tree every 3000 generations for 100 million generations, stop early if the convergence diagnostic falls below the stop value 0.001 and show tree probabilities on the 50% majority-rule consensus tree with simple output format.

Results

Phylogenetic analyses of Polygonaceae

The concatenated cpDNA dataset D1 has 78 aligned sequences and comprises 4167 characters (1585 bp for *matK*, 1432 bp for *rbcL* and 1150 bp *trnL-F*, respectively), of which 1756 are variable (42.14%) and 1181 are parsimony-informative (28.34%). The ML and BI analyses, based on dataset D1, generated nearly identical topologies (Suppl. material 1: figs S1, S2); therefore, only the ML tree is presented, with ML bootstrap (BS) and posterior probabilities (PP) values marked on each branch, respectively (Fig. 2).

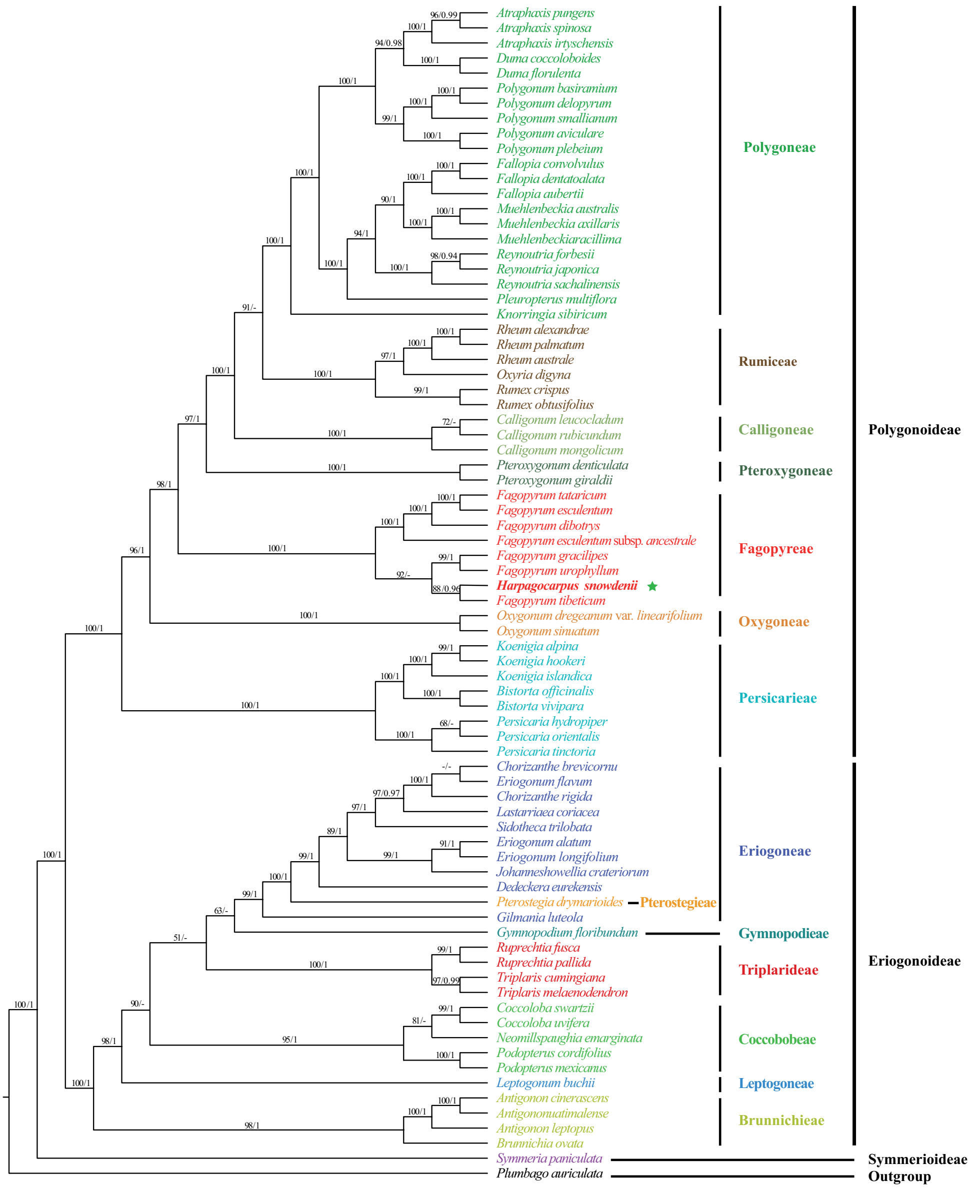

Figure 2. Maximum Likelihood phylogram of Polygonaceae as inferred from analysis of the combined cpDNA dataset of *matK*, *rbcL* and *trnL-F*. Support values ≥ 50% BS or 0.90 PP are displayed above the branches, respectively. The tribal classification of Eriogonoideae followed Sanchez and Kron (2008) and Kempton (2012) and that of Polygoniodeae followed Sanchez et al. (2011) and Schuster et al. (2015). The green star indicates the position of *Harpagocarpus snowdenii*.

The ingroup (Polygonaceae) is well supported as monophyletic (Fig. 2; BS = 100%, PP = 1.00; all support values follow this order hereafter). Within Polygonaceae, the first branch, represented by *Symmeria paniculata* Benth., is Symmerioideae which is sister to a large clade comprising Eriogonoideae and Polygonoideae. Within Eriogonoideae, six tribes are recovered with Brunnichieae emerging as the first divergent clade and then subsequently followed by Leptogoneae, Coccobobeae, Triplarideae, Gymnopodieae and Eriogoneae+Pterostegieae. *Pterostegia drymarioides* Fisch. & C.A.Mey. of Pterostegieae is shown to be nested within Eriogoneae in our analyses. In Polygonoideae, all seven tribes are fully supported as monophyletic (Fig. 2) with Persicarieae, Oxygoneae, Fagopyreae, Pteroxygoneae, Calligoneae and Rumiceae successively sister to the rest. With the inclusion of *Harpagocarpus*, *Fagopyrum* obtained high support values (Fig. 2; 100, 1.00).

Phylogenetic analyses of *Fagopyrum*

The combined dataset D2 has 31 aligned sequences and comprises 6378 characters (1425 bp for *accD*, 2278 bp for *matK*, 513 bp for *psbA-trnH*, 1278 bp for *rbcL* and 883 bp for *trnL-F*), of which 735 are variable (11.52%) and 428 are parsimony-informative (6.71%). ML and BI trees generated from the D2 dataset yielded similar topologies (Suppl. material 1: figs S3, S4); thus, only the ML tree is shown (Fig. 3). In both of the analyses, the monophyly of *Fagopyrum* was strongly supported and three monophyletic subclades were recovered: the first subclade comprises *F. esculentum* Moench, *F. esculentum* subsp. *ancestrale* Ohnishi, *F. homotropicum* Ohnishi, *F. tataricum* (L.) Gaertn. and *F. dibotrys* (D.Don) H.Hara (100, 1.00), the second one is formed by *F. snowdenii* (≡ *Harpagocarpus snowdenii*) and *F. tibeticum* (A.J.Li) Adr.Sanchez & Jan.M.Burke (90, 0.99) and the third includes the remaining taxa of the genus.

Discussion

Phylogenetic placement of *Harpagocarpus* in Polygonaceae

After 20 years of molecular reconstruction of Polygonaceae (e.g. Kim and Donoghue (2008a); Sanchez and Kron (2008, 2009, 2011); Galasso et al. (2009); Sanchez et al. (2009, 2011); Burke et al. (2010); Tavakkoli et al. (2010, 2015); Yurtseva et al. (2010, 2016, 2017); Schuster et al. (2011a, b, 2015); Tian et al. (2011); Kempton (2012); Fan et al. (2021)), only a few recalcitrant genera, such as *Harpagocarpus* and *Eskemukerjea*, have not been included in molecular analyses and their phylogenetic positions are still unresolved. *Harpagocarpus* is distinct from all other genera of Polygonaceae in having a remarkable fruit that has long setae growing along the ribs with the radially arranged retrorse barbs at the tip of each seta (Hutchinson and Dandy 1926) (Fig. 1). The current study is the first to include the genus *Harpagocarpus* in molecular phylogenetics and it demonstrates in all analyses, based on the cpDNA datasets D1 and D2, that *H. snowdenii*, the sole species of the genus, is deeply nested within *Fagopyrum* (Figs 2, 3). The additional molecular evidence undoubtedly confirms the previous hypothesis that *Harpagocarpus* and *Fagopyrum* are congeneric in respect of

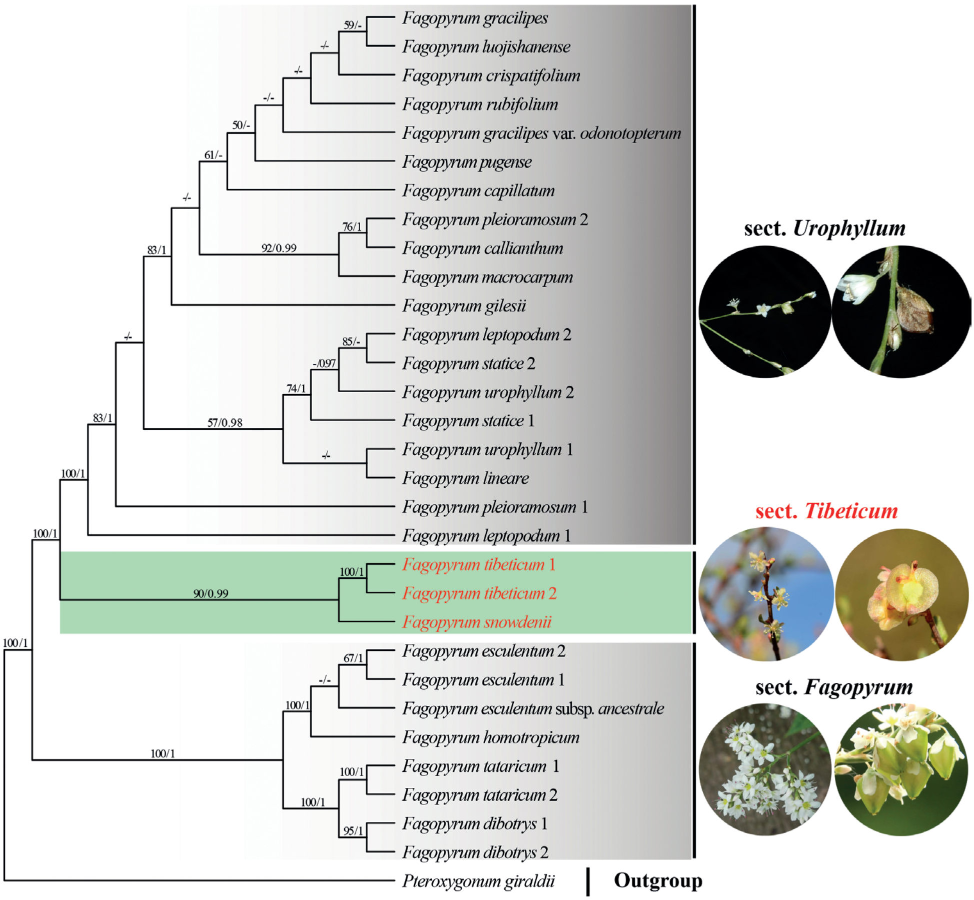

Figure 3. Maximum Likelihood phylogram of *Fagopyrum* as inferred from analysis of the combined cpDNA dataset of *accD*, *matK*, *psbA-trnH*, *rbcL* and *trnL-F*. Support values ≥ 50% BS or 0.90 PP are displayed above the branches, respectively. The two black boxes covered the *cymosum* group and the *urophyllum* group as defined in Yasui and Ohnishi (1998a), respectively. The green box indicates the third group, namely the *tibeticum* group, as revealed in the present study. The representative photos in the circles showing the inflorescences and the fruits of *urophyllum* group, *tibeticum* group and *cymosum* group are *F. urophyllum*, *F. tibeticum* and *F. dibotrys*, respectively. All photos were taken by Bo Li.

morphological, anatomical and palynological investigations (Hong 1988; Ronse Decraene and Akeroyd 1988; Hong et al. 1998).

Morphologically, *H. snowdenii* has sagittate to ovate-triangular leaf blades, seven palmate veins, large and clearly exerted fruits from the persistent tepals and broad cotyledons, which are very similar to those traits presented in *Fagopyrum* species (Hutchinson and Dandy 1926). Anatomical studies showed that *H. snowdenii* and *Fagopyrum* species share a series of similar floral characteristics, such as nectaries which are present as receptacular mamillae behind the stamens, inner stamens which are always linked with two lateral nectaries and cells of the inner tepal epidermis which are rectangular to elongated (Hong 1988; Ronse Decraene and Akeroyd 1988). Palynologically, Ronse Decraene and Akeroyd (1988) emphasised that they observed an identical pollen structure between *Harpagocarpus* and *Fagopyrum* and Hong (1988) further noted that it is hardly possible to find any pollen morphological differences between *Harpagocarpus* and the species of *Fagopyrum*. The pollen of *Fagopyrum* is ovate, tricolpate with narrow furrows and a reticulate surface pattern. The pollen grains of *Harpagocarpus* are slightly smaller than those of *Fagopyrum* species, but they undoubtedly belong to the same pollen type (Hong 1988; Ronse Decraene and Akeroyd 1988). Considering this evidence, Ronse Decraene and Akeroyd (1988) suggested that *H. snowdenii* should probably be included within *Fagopyrum*, perhaps as a separate section and Hong (1988) formally combined *H. snowdenii* as *F. snowdenii*.

It is noteworthy to point out that our molecular analyses not only supported the amalgamation of *Harpagocarpus* with *Fagopyrum*, but also clarified the accurate specific relationships of *F. snowdenii* within *Fagopyrum*, which was stably supported to be a sister of *F. tibeticum* using cpDNA sequences (Figs 2, 3). *F. tibeticum* was originally described in the monotypic genus *Parapteropyrum* A.J.Li as *P. tibeticum* A.J.Li, which is a shrub, endemic to the central Qinghai-Tibetan Plateau of China and is characterised by having fascicled leaves, terminal raceme-like inflorescences, five unequally lobed tepals with the outer two smaller, perianth persistent and accrescent in fruit, three free styles with capitate stigmas and trigonous achenes with broad wings along ribs (Li 1981). *P. tibeticum* was considered to be most similar to *Pteropyrum* Jaub. & Spach in gross morphology (Li 1981), but surprisingly tested to be a member of *Fagopyrum* in molecular phylogenetic studies (Sanchez et al. 2009; Tavakkoli et al. 2010; Tian et al. 2011) and, thus, formally combined in *Fagopyrum* as *F. tibeticum* (Sanchez et al. 2011). The origin of the woody *F. tibeticum* was thought to be a consequence of the large-scale uplift of the Qinghai-Tibetan Plateau which not only promoted continental species radiation, but also the secondary feature of woodiness in a few herbaceous lineages in response to strong selection pressures (Tian et al. 2011).

The inclusion of *F. tibeticum* in *Fagopyrum* has updated our knowledge of morphology in the genus, but now, the sister relationships between *F. snowdenii* and *F. tibeticum*, revealed in our molecular analyses, would not only further expand the morphological variation of *Fagopyrum*, but also shed light on the thinking of the biogeographical origin of the genus, because *F. snowdenii* is the only species of *Fagopyrum* distributed in Africa, while all other congeneric taxa occur mainly in East Asia. Jacques-Félix (1946) suggested that *Fagopyrum* perhaps entered Africa via a Middle Asian pathway during the Quaternary-periglacial period, just like other genera with both Afromontane and Central Asian representatives, such as *Cicer* L. and *Colutea* L. (Chapman and White 1970). However, de Klerk et al. (2015) stated that long-distance transport of pollen grains of *F. snowdenii* from Asia to Africa seems unlikely, but alternatively, they found out there are indications from pollen and macrofossils that a wild *Fagopyrum* ancestor may have been widespread in western Eurasia during the Late Tertiary and the Pleistocene Ice-Ages and became extinct afterwards. *F. snowdenii* may represent the only surviving African lineage that split from the wild widespread *Fagopyrum* ancestor.

Infrageneric relationships within *Fagopyrum*

Fagopyrum is a small genus comprised of ca. 25 species according to the most updated classification (Ohsako and Li 2020). The genus is economically important and well known for containing two domesticated common buckwheat, i.e. *F. esculentum* and *F. tataricum* which have been widely cultivated in Australia, Asia, Europe and North America for producing gluten-free grains (Li and Hong 2003). Geographically, most of the wild species of *Fagopyrum* are mainly distributed in mountainous regions of southwest China, a few are endemic to the south-eastern edge of the Qinghai-Tibetan Plateau (Ohnishi and Matsuoka 1996; Ohnishi 1998; Li and Hong 2003) and only the *F. snowdenii* confirmed in the present study is occurring in Africa (Hutchinson and Dandy 1926; Ayodele 2003). Eastern Tibet to western Sichuan of China was indicated to be the birthplace of the two cultivated common buckwheat in the AFLP (amplified fragment length polymorphism) analysis (Konishi et al. 2005). Taxonomically, *Fagopyrum* was separated from the large and heterogenous Linnaeus's genus *Polygonum* L. (Miller 1754) and has long been treated as a section of *Polygonum* (e.g. Meisner (1856); Samuelsson (1929); Steward (1930)) or considered to be an independent genus, but closely related to *Polygonum* (e.g. Dammer (1894); Gross (1913); Hedberg (1946); Haraldson (1978); Ronse Decraene and Akeroyd (1988)). In the context of the molecular phylogeny of Polygonaceae, *Fagopyrum* was not only supported as a monophyletic genus, but also indicated to represent an isolated tribal clade in the subfamily Polygonoideae (Sanchez et al. 2011; Schuster et al. 2015). Morphologically and anatomically, *Fagopyrum* could be distinguished from other genera of Polygonoideae by having large conduplicate cotyledons and/or embryos in the central region in achene (Dammer 1894; Gross 1913; Nakai 1926; Chapman and White 1970; Sanchez et al. 2011).

Within *Fagopyrum*, two groups have been recognised in classical taxonomy, based on the morphology of inflorescence and the achene size: one group was mainly represented by *F. cymosum* (Trevir.) Meisn. (= *F. dibotrys*), *F. esculentum* and *F. tataricum* and characterised by having corymbose inflorescences with many branching and dense flowers and the achene greatly exceeding the perianth, while the other group is composed of other species (including *F. urophyllum* (Bureau & Franch.) H.Gross) having raceme-like inflorescences with sparse flowers and the achene completely enclosed in perianth (Gross 1913; Roberty and Vautier 1964; Ohnishi and Matsuoka 1996) (Fig. 3). These two groups are mostly concordant with the *cymosum* group and the *urophyllum* group defined by Yasui and Ohnishi (1998a) in molecular phylogenetic analyses using DNA sequences of the nuclear internal transcribed spacer (nrITS) and cpDNA region *rbcL-accD*. Other molecular studies, no matter using isozyme variability and RFLP (Ohnishi and Matsuoka 1996), cpDNA sequences (Yasui et al. 1998; Ohsako et al. 2001; Jin et al. 2018), nuclear genes or regions (Yasui and Ohnishi 1998b; Nishimoto et al. 2003) and complete plastomes (Fan et al. 2021; Li et al. 2022), all clearly indicated that the *cymosum* group and the *urophyllum* group are both monophyletic clades.

In our present analyses, the above-mentioned two clades were recovered too, but the third clade, formed by *F. snowdenii* and *F. tibeticum*, was discovered, which is sister to the 'Urophyllum' clade (Fig. 3). We failed to generate any nuclear sequences from the specimen sample of *F. snowdenii*; thus, we could not test the sister relationships between *F. snowdenii* and *F. tibeticum*, as well as the sister relationships between *F. snowdenii* +*F. tibeticum* clade and the 'Urophyllum' clade in nuclear analysis. However, when only *F. tibeticum* was included in the ITS analysis, the topology of the phylogenetic tree is similar to that yielded from the combined cpDNA dataset, in which *F. tibeticum* is sister to the 'Urophyllum' clade clade (Tian et al. 2011). Considering the sister relationships between *F. snowdenii* and *F. tibeticum* could be additionally supported by morphological and palynological evidence, such as raceme-like inflorescences, unequal tepals with the outer two smaller, perianth accrescent in fruit, large achenes greatly exceeding the perianth, special appurtenances (either wings or setae) growing along the fruit ribs and smaller pollen grains than the other *Fagopyrum* species (Hutchinson and Dandy 1929; Ronse Decraene and Akeroyd 1988; Hong 1995), we believe that *F. snowdenii* and *F. tibeticum* represent a separate clade in *Fagopyrum*. Future analyses, based on more comprehensive sampling and using nuclear sequences data, may further confirm or update the infrageneric relationships of *Fagopyrum* as inferred in this study. As far as the current results are concerned, a sectional classification for *Fagopyrum* is here proposed, based on the differentiation of gross morphology in the three clades, which is the first infrageneric classification of the genus.

Taxonomic treatment

***Fagopyrum* Mill., Gard. Dict. Abr.**

Fagopyrum Mill., Gard. Dict. Abr., ed. 4, 495. 1754 [≡ *Polygonum* sect. *Fagopyrum* (Mill.) Meisn., Monogr. Polyg. 43, 61. 1826.] – Type: *Fagopyrum esculentum* Moench (≡ *Polygonum fagopyrum* L.).

= *Harpagocarpus* Hutch. & Dandy, Bull. Misc. Inform. Kew. 364. 1926 – Type: *Harpagocarpus snowdenii* Hutch. & Dandy [≡ *Fagopyrum snowdenii* (Hutch. & Dandy) S.P.Hong].

= *Parapteropyrum* A.J.Li, Acta Phytotax. Sin. 19: 330. 1981 – Type: *Parapteropyrum tibeticum* A.J.Li [≡ *Fagopyrum tibeticum* (A.J.Li) Adr.Sanchez & Jan.Burke].

Fagopyrum* sect. *Fagopyrum

Type. *Fagopyrum esculentum* Moench. (≡ *Polygonum fagopyrum* L.).

Diagnosis. This section is characterised by having large corymbose inflorescences with many branches and dense flowers and large achenes greatly exceeding the persistent perianth.

Species. *F. dibotrys*, *F. esculentum* subsp. *esculentum*, *F. esculentum* subsp. *ancestrale*, *F. homotropicum* and *F. tataricum*.

Distribution. Bhutan, India, Myanmar, Nepal, Pakistan, Thailand and Vietnam of southern and south-eastern Asia and southern and south-western China.

Fagopyrum **sect.** ***Tibeticum*** **Bo Li & M.L.Zhou, sect. nov.**

urn:lsid:ipni.org:names:77315008-1

Type. *Fagopyrum tibeticum* (A.J.Li) Adr. Sanchez & Jan. Burke (≡ *Parapteropyrum tibeticum* A.J.Li).

Diagnosis. The new section is characterised by having raceme-like inflorescences with sparse flowers, large achenes with appurtenances (wings or setae) along the ribs and greatly exceeding the perianth and persistent perianth accrescent in fruit.

Species. *F. snowdenii* and *F. tibeticum*.

Distribution. Cameroon, Kenya, Rwanda, Tanzania and Uganda of Africa (*F. snowdenii*) and Tibet of south-western China (*F. tibeticum*).

Fagopyrum **sect.** ***Urophyllum*** **Bo Li & M.L.Zhou, sect. nov.**

urn:lsid:ipni.org:names:77315009-1

Type. *Fagopyrum urophyllum* (Bureau & Franch.) H.Gross (≡ *Polygonum urophyllum* Bureau & Franch.).

Diagnosis. This new section is characterised by having raceme-like, spicate, capitate or paniculate inflorescences with mostly sparse or rarely dense flowers and achenes completely enclosed in the persistent perianth.

Species. *F. callianthum* Ohnishi, *F. capillatum* Ohnishi, *F. caudatum* (Sam.) A.J.Li, *F. crispatifolium* J.L.Liu, *F. densovillosum* J.L.Liu, *F. gilesii* (Hemsl.) Hedberg, *F. gracilipedoides* Ohsako & Ohnishi, *F. gracilipes* (Hemsl.) Dammer, *F. jinshaense* Ohsako & Ohnishi, *F. leptopodum* (Diels) Hedberg var. *leptopodum*, *F. leptopodum* var. *grossii* (Lévl.) Lauener & D.K.Ferguson, *F. lineare* (Sam.) Haraldson, *F. longistylum* M.L.Zhou & Y.Tang, *F. longzhoushanense* J.R.Shao, *F. luojishanense* J.R.Shao, *F. macrocarpum* Ohsako & Ohnishi, *F. pleioramosum* Ohnishi, *F. pugense* Y.Tang, *F. qiangcai* D.Q.Bai, *F. rubifolium* Ohsako & Ohnishi, *F. statice* H.Gross, *F. urophyllum* (Bureau & Franch.) H.Gross, *F. wenchuanense* J.R.Shao.

Distribution. Guizhou, Sichuan and Yunnan Provinces of southwest China.

Identification keys to three sections of *Fagopyrum*

1	Achenes completely enclosed in the perianth	**sect. *Urophyllum***
–	Achenes greatly exceeding the perianth	**2**
2	Raceme-like inflorescences with sparse flowers and achenes having appurtenances (wings or setae) along the ribs	**sect. *Tibeticum***
–	Corymbose inflorescences with dense flowers and achenes without appurtenances	**sect. *Fagopyrum***

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Supplementary material 1

Supplementary information

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Data type: tables, figures (Pdf file)

Explanation note: Taxa, GenBank accession numbers of DNA sequences with their vouchers or source of publication used in the molecualr dataset and phtlogenetic trees generated from BI and ML analyses.

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An updated classification of the Brassicaceae (Cruciferae)

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Abstract

Based on recent achievements in phylogenetic studies of the Brassicaceae, a novel infrafamilial classification is proposed that includes major improvements at the subfamilial and supertribal levels. Herein, the family is subdivided into two subfamilies, Aethionemoideae (subfam. nov.) and Brassicoideae. The Brassicoideae, with 57 of the 58 tribes of Brassicaceae, are further partitioned into five supertribes, including the previously recognized Brassicodae and the newly established Arabodae, Camelinodae, Heliophilodae, and Hesperodae. Additional tribus-level contributions include descriptions of the newly recognized Arabidopsideae, Asperuginoideae, Hemilophieae, Schrenkielleae, and resurrection of the Chamireae and Subularieae. Further detailed comments on 17 tribes in need of clarifications are provided.

Keywords
classification, subfamily, supertribe, taxonomy, tribe

Introduction

Rapid advances in our understanding of phylogenetic relationships among taxa are driving the development of modern classification schemes that accurately reflect current knowledge. Brassicaceae (Cruciferae) is a relatively large family, currently comprising ca. 4140 species (original data), for which various classification systems have been proposed, including influential historical classifications contributed by de Candolle (1821), Hayek (1911), Schulz (1936), and Janchen (1942). The first infrafamilial classification for the Brassicaceae based on molecular phylogenetic data, proposed by Al-Shehbaz et al. (2006), included 25 tribes but no higher taxonomic units. The phylogenetic findings available at the time were based on relatively few species (e.g., ~ 100 spp.) and lacked clarity regarding the limits and relationships among the inferred major lineages (referred as to I, II, and III by Beilstein et al. 2006). Since then, numerous additional taxa have been included in phylogenetic studies and the amount, quality, and reliability of phylogenetic data has increased tremendously. This has led to the discussion of numerous informal evolutionary lineages (Huang et al. 2016; Nikolov et al. 2019) and the recognition of more than 50 tribes (e.g., Hohmann et al. 2015; Huang et al. 2020). Hence, there is an obvious need to codify the current well-supported understanding of Brassicaceae relationships (e.g., Nikolov et al. (2019), Walden et al. (2020), and especially Hendriks et al. (2022)) into an updated classification scheme that can now include robust subfamilial and supertribal groups.

Taxonomy

Brassicaceae Burnett, Outlines Bot.: 854, 1093, 1123. Feb 1835, nom. cons., nom. alt.; Cruciferae Juss., Gen. Pl.: 237. 4 Aug 1789, nom. cons.

Type. *Brassica* L.

Distribution. Cosmopolitan, centered in temperate regions of the Northern Hemisphere.

1. Subfamilial division

All phylogenetic studies over the past two and a half decades identify *Aethionema* W.T. Aiton as sister to all other Brassicaceae, which supports the recognition of two highly unequal subfamilies, the new unigeneric Aethionemoideae with 58 species and the much bigger Brassicoideae, comprising the other 98.6% of species and the rest of the generic and tribal diversity of the family.

Aethionemoideae D.A.German, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & Al-Shehbaz, subfam. nov.
urn:lsid:ipni.org:names:77315165-1

Type. *Aethionema* W.T. Aiton

Description. Trichomes and multicellular glands absent. Leaves entire, articulate at base. Fruits silicles, angustiseptate, bilocular, few-seeded, dehiscent, or unilocular, one-seeded, indehiscent; sometimes both types present. Most common $x = 11, 12$.

Distribution. Primarily SW Asia, especially Turkey, Iran & Transcaucasia.

Tribes. Aethionemeae Al-Shehbaz, Beilstein & E.A. Kellogg.

Note. For many species of *Aethionema* a 3-nerved petal claw has been described (e.g., Hedge 1965). Further studies are needed to verify whether this is a feature present in all members of *Aethionema* and whether it is unique to the genus (and then diagnostic for the subfamily).

Brassicoideae Prantl, Text-book Bot.: 255. 1880 ('Brassiceae').

Type. *Brassica* L.

Description. Trichomes (simple and/or variously branched) and multicellular glands absent or present. Leaves entire to variously dissected, simple or compound, not articulate at base. Fruits various in compression, dehiscence, length to width ratio, number of seeds (one to > 100), etc. Base chromosome numbers various; the lowest $x = 4$.

Distribution. Same as the whole family.

2. Supertribal division

Brassicoideae is subdivided into the following five supertribes corresponding to the main evolutionary lineages discussed in detail by Hendriks et al. (2022).

Arabodae D.A.German, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & Al-Shehbaz, supertrib. nov.
urn:lsid:ipni.org:names:77315210-1

Type. *Arabis* L.

Description. Trichomes present, mainly branched (exclusively or in combination with simple); multicellular glands absent. Leaves predominantly undivided or slightly divided, auriculate at base or not. Most common $x = 8$.

Distribution. Mainly Northern Hemisphere (predominantly Holarctis of Eurasia, also of N America and Africa), S America (Andes).

Tribes. Arabideae DC., Alysseae DC., Asperuginoideae trib. nov., Stevenieae Al-Shehbaz, D.A.German & M.Koch.

Notes. Corresponds to evolutionary lineage IV of Nikolov et al. (2019) and Hendriks et al. (2022) or lineage D of Huang et al. (2016). Limits of this supertribe are not yet fully understood due to discordance in positions of tribes and their taxa in the nuclear vs. plastid phylogenies of Hendriks et al. (2022). It might be eventually restricted to Arabideae, while Alysseae and possibly Asperuginoideae would better be recognized as a separate supertribe, Alyssodae. Proper placement of Stevenieae also needs further clarification due to its grouping within Camelinodae lineage in chloroplast phylogenies (Walden et al. 2020; Hendriks et al. 2022).

Brassicodae V.E. Avet. in Biol. Zhurn. Armenii 43: 602. 1990 ('Brassicidinae').

Type. *Brassica* L.

Syn. Sisymbriodae V.E. Avet., Thelypodiodae V.E. Avet.

Description. Trichomes absent or simple, rarely branched; multicellular glands absent. Leaves predominantly undivided or slightly divided, rarely much divided, often auriculate at base. Most common $x = 7$.

Distribution. Mainly Northern Hemisphere (Holarctis of Eurasia, N America and Africa), to a lesser degree C and S America.

Tribes. Aphragmeae D.A.German & Al-Shehbaz, Brassiceae DC. [incl. Bivonaeeae M.A. Koch & Warwick], Calepineae Horan., Coluteocarpeae V.I. Dorof., Conringieae D.A. German & Al-Shehbaz, Eutremeae Al-Shehbaz, Beilstein & E.A. Kellogg, Fourraeeae Al-Shehbaz, M.A. Koch, R. Karl & D.A.German, Isatideae DC., Kernereae Al-Shehbaz, Warwick, Mumm. & M.A. Koch, Plagiolobeae Khosravi & Eslami-Farouji, Schrenkielleae trib. nov., Sisymbrieae DC., Thelypodieae Prantl, Thlaspideae DC.

Notes. Corresponds to evolutionary lineage II introduced by Beilstein et al. (2006) and subsequently modified by Franzke et al. (2011) to become known as "expanded lineage II", or lineage B of Huang et al. (2016). Cochlearieae reveals relationship with Brassicodae in nuclear-based phylogeny, though it groups with "rogue" tribes of Heliophilodae in plastid trees (details in Hendriks et al. 2022). Its supertribal assignment is therefore yet unclear.

Camelinodae D.A.German, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & Al-Shehbaz, supertrib. nov.

urn:lsid:ipni.org:names:77315211-1

Type. *Camelina* Crantz

Description. Trichomes usually present, simple and/or branched; multicellular glands absent. Leaves not or variously divided to compound, auriculate at base or not. Base numbers various, most common $x = 6, 7, 8$.

Distribution. Represented by native taxa at all continents except Antarctica; most diverse in Holarctis of Eurasia and N America.

Tribes. Alyssopsideae Al-Shehbaz, Warwick, Mumm. & M.A. Koch, Arabidopsideae trib. nov., Boechereae Al-Shehbaz, Beilstein & E.A. Kellogg, Camelineae DC., Cardamineae Dumort., Crucihimalayeae D.A.German & Al-Shehbaz, Descurainieae Al-Shehbaz, Beilstein & E.A. Kellogg, Erysimeae Dumort., Halimolobeae Al-Shehbaz, Beilstein & E.A. Kellogg, Hemilophieae trib. nov., Lepidieae DC., Malcolmieae Al-Shehbaz & Warwick, Microlepidieae Al-Shehbaz, Warwick, Mumm. & M.A. Koch, Oreophytoneae Al-Shehbaz, Warwick, Mumm. & M.A. Koch, Physarieae B.L. Rob., Smelowskieae Al-Shehbaz, Beilstein & E.A. Kellogg, Turritideae Buchenau, Yinshanieae Al-Shehbaz, Warwick, Mumm. & M.A. Koch.

Notes. Corresponds to evolutionary lineage I of Beilstein et al. (2006) and subsequent studies, or lineage A of Huang et al. (2016). Two genera of Camelinodae, *Chrysochamela* Boiss. and *Pseudoarabidopsis* Al-Shehbaz, O'Kane & R.A. Price, both excluded from Camelineae (see discussion below) are currently unassigned to a tribe.

Heliophilodae D.A.German, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & Al-Shehbaz, supertrib. nov.

urn:lsid:ipni.org:names:77315212-1

Type. *Heliophila* L.

Description. Trichomes absent or simple, rarely branched; multicellular glands absent. Leaves mainly not or slightly divided, rarely much divided to compound, usually not auriculate at base. Base numbers are various due to post-polyploid diploidization – 12 tribes have originated through whole-genome duplications (data lacking for Hillielleae).

Distribution. Well-represented in both Hemispheres; Eurasia (mainly SW Asia & S Europe), N, Tropical & S Africa, C & S America, New Zealand.

Tribes. Anastaticeae DC., Asteae Al-Shehbaz, Warwick, Mumm. & M.A. Koch [incl. Scoliaxoneae Al-Shehbaz & Warwick], Biscutelleae Dumort., Chamireae Sond., Cremolobeae R. Br., Eudemeae Al-Shehbaz, Warwick, Mumm. & M.A. Koch, Heliophileae DC., Hillielleae H.L. Chen, T. Deng, J.P. Yue, Al-Shehbaz & H. Sun, Iberideae Webb & Berthel., Megacarpaeeae Kamelin ex D.A.German, Notothlaspideae Al-Shehbaz, Warwick, Mumm. & M.A. Koch, Schizopetaleae R. Br. ex Barnéoud, Subularieae DC.

Notes. This group corresponds to evolutionary lineage V of Nikolov et al. (2019) and Hendriks et al. (2022). Anastaticeae, Biscutelleae, Hillielleae, Iberideae, and Megacarpaeeae are tentatively assigned to Heliophilodae due to their partially resolved phylogenetic position (grouping with others only in nuclear-based trees; see Hendriks et al. (2022) for details and discussion). Eventually, these five tribes may be recognized as a separate supertribe, e.g., Anastaticodae, based on the most speciose tribe among them. In the latter case, Heliophilodae would become unique among supertribes being almost completely restricted to the Southern Hemisphere.

Hesperodae D.A.German, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & Al-Shehbaz, supertrib. nov.
urn:lsid:ipni.org:names:77315213-1

Type. *Hesperis* L.

Description. Trichomes usually present, simple and/or branched; multicellular glands often present. Leaves normally little divided, nearly never auriculate at base. Most common $x = 7$.

Distribution. Native to Eurasia (predominantly temperate and dry subtropical Asia).

Tribes. Anchonieae DC., Buniadeae DC., Chorisporeae C.A. Mey., Dontostemoneae Al-Shehbaz & Warwick, Euclidieae DC., Hesperideae Prantl, Shehbazieae D.A.German.

Note. Corresponds to evolutionary lineage III of Beilstein et al. (2006) and subsequent studies, or lineage E of Huang et al. (2016).

3. New tribal adjustments

Updates at the tribal level include recognition of additional six tribes, of which four are newly described and another two are resurrected. Tribal names are followed in parenthesis by numbers of genera and species.

3a. Tribal assignment of *Arabidopsis*

Huang et al. (2016) were the first to show that *Arabidopsis thaliana* (L.) Heynh. and *A. lyrata* (L.) O'Kane & Al-Shehbaz formed a clade unrelated to the core Camelineae representatives *Capsella rubella* Reut., *Catolobus pendulus* (L.) Al-Shehbaz, and *Camelina sativa* (L.) Crantz. Nikolov et al. (2019) obtained the same results using the same taxa minus *Catolobus* (C.A. Mey.) Al-Shehbaz. Their findings are fully supported by Hendriks et al. (2022). As a result, *Arabidopsis* (DC.) Heynh. is placed in its own tribe.

Arabidopsideae Al-Shehbaz, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & D.A.German, trib. nov. (1: 18)
urn:lsid:ipni.org:names:77315214-1

Type. *Arabidopsis* (DC.) Heynh.

Description. Herbs, annual or perennial. Trichomes simple, mixed with stalked 1–3(or 4)-forked. Multicellular glands absent. Cauline leaves petiolate to subsessile and cuneate to attenuate at base, not auriculate. Racemes ebracteate, often elongated in fruit. Flowers actinomorphic; sepals ascending to spreading, base of lateral pair slightly saccate or not; petals white, pink, or purple; claw obscurely differentiated from blade or distinct; filaments unappendaged, wingless; pollen 3-colpate; ovules 15–80 per ovary. Fruits siliques, linear, terete or latiseptate, unsegmented; styles obsolete or to 1 mm long; stigma entire. Seeds uniseriate; cotyledons accumbent or rarely incumbent. *x* = 5 and 8.

Distribution. Eurasia, Africa, North America.

Notes. Arabidopsideae is distinguished from the Camelineae by the lack of stellate and dendritic trichomes, though both also have simple and stalked forked trichomes, by having petiolate or subsessile cauline leaves not auriculate at base, by the lack of yellow flowers, 15–80 ovules per ovary, silique fruits, and accumbent or rarely incumbent cotyledons. By contrast, the Camelineae usually have some stellate or dendritic trichomes, always sessile and auriculate to sagittate cauline leaves, usually yellow flowers, though white to pink flowers occur just as in the Arabidopsideae, 2–40 ovules per ovary, silicle or rarely silique fruits, and incumbent or rarely accumbent cotyledons.

3b. *Asperuginoides*

There has been no agreement among various authors about the tribal assignment of monospecific *Asperuginoides* Rauschert. For example, Khosravi et al. (2009) indicated a close relationship to the Cochlearieae, whereas German et al. (2009) and Warwick et al. (2010) showed no affinity to any tribe. It was listed as an unplaced genus by Al-Shehbaz (2012). More recently, Nikolov et al. (2019) and Hendriks et al. (2022) identified a sister relationship to the Alysseae, but Španiel et al. (2015) excluded it from the tribe. Furthermore, the plastome data by Walden et al. (2020) did not support that nor indicated any relationship to the 50+ tribes. Given the current data, it appears that the best solution is to place this anomalous genus in its own tribe.

Asperuginoideae Al-Shehbaz, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & D.A.German, trib. nov. (1: 1)

urn:lsid:ipni.org:names:77315215-1

Type. *Asperuginoides* Rauschert

Description. Herbs annual. Trichomes stalked, stellate or substellate, 4–6-rayed, these mixed with glochidate ones on fruit. Multicellular glands absent. Cauline leaves petiolate, not auriculate. Racemes bracteate throughout, usually elongated in fruit, with strongly recurved fruiting pedicels. Flowers actinomorphic; sepals ascending, base of lateral pair not saccate; petals white, claw undifferentiated from blade; filaments slender at base, unappendaged; pollen 3-colpate; ovules 2 per ovary, apical. Fruits dehiscent silicles, suborbicular, latiseptate, unsegmented, wingless, with long-stalked, setose, stiff trichomes glochidiate at apex; septum complete or absent; style distinct; stigma entire. Seeds aseriate, broadly winged; cotyledons accumbent. *x* = 16.

Distribution. Afghanistan, Armenia, Iran, Kazakhstan, Kyrgyzstan, Pakistan, Tajikistan, Turkey, Turkmenistan, Uzbekistan.

3c. *Chamira*

Although the tribe Chamireae was first recognized by Sonder (1846) and later accepted by Schulz (1936), it has not been widely recognized since, and *Chamira* Thunb. was listed as unplaced in Al-Shehbaz (2012). The findings of Hendriks et al. (2022) agree with those of Mummenhoff et al. (2005), Mandáková et al. (2012), Nikolov et al. (2019), Walden et al. (2020), and Dogan et al. (2021) that *Chamira* and *Heliophila* are closely related genera that do not belong to the same tribe, and the former has been used as the outgroup for phylogenetic and genomic studies of the latter. A tribal description comparable to that of other tribes is provided below.

Chamireae Sond. in Abh. Naturwiss. Verein Hamburg 1: 267. 1846. (1: 1)

Type. *Chamira* Thunb.

Description. Herbs, annual. Trichomes absent. Leaves sessile or short petiolate, not auriculate at base, lowest pair opposite, representing persistent cotyledons and main photosynthetic part of plant, to 25 cm wide, cauline leaves alternate, much smaller, sometimes fail to develop. Racemes ebracteate, elongated in fruit. Sepals connivent, dimorphic, median (outer) pair not saccate at base, lateral pair with a distinct spur 1–2.5 mm long; petals white, with well-differentiated claw; filaments unappendaged; pollen 3-colpate; ovules 2–8 per ovary. Fruits siliques, dehiscent, terete to sublatiseptate, unsegmented; styles distinct; stigma entire. Seeds uniseriate; cotyledons longitudinally folded and margins deeply folded within. $x = 19$.

Distribution. *Chamira circaeoides* (L. f.) Zahlbr. is endemic to the Western Cape of South Africa.

3d. *Dipoma* and *Hemilophia*

Dipoma Franch. was first studied by Warwick et al. (2010) who did not assign it to any tribe, and together with *Hemilophia* Franch., they were listed as unplaced in Al-Shehbaz (2012). Nikolov et al. (2019) showed the two genera form a monophyletic clade unrelated to any tribe and suggested their placement in a new tribe. However, plastome data by Walden et al. (2020) showed *Dipoma* to be affiliated with the Crucihimalayeae and not with *Hemilophia*. The results from the nuclear genome of Hendriks et al. (2022) fully agree with those of Nikolov et al. (2019), and the new tribe Hemilophieae is proposed here to accommodate both genera, leaving incongruent chloroplast and nuclear-based phylogenies.

Hemilophieae Al-Shehbaz, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & D.A.German, trib. nov. (2: 7)

urn:lsid:ipni.org:names:77315216-1

Type. *Hemilophia* Franch.

Description. Herbs rhizomatous perennials. Trichomes simple, malpighiaceous, sometime short-stalked forked. Multicellular glands absent. Cauline leaves petiolate to subsessile and cuneate to attenuate at base, not auriculate. Racemes bracteate throughout, elongated or not in fruit. Flowers actinomorphic; sepals ascending to spreading, base of lateral pair not saccate; petals white, pink, or purple; claw obscurely differentiated from blade or distinct; filaments slender or dilated at base and sometimes strongly appendaged; pollen 3-colpate; ovules 2 or 4 per ovary, apical. Fruits dehiscent silicles, oblong to ovoid, terete or slightly angustiseptate, unsegmented, wingless or with narrow wings or crests; septum complete or absent; styles distinct, cylindrical or conical; stigma entire. Seeds aseriate; cotyledons accumbent. Base numbers various.

Distribution. Endemic to China (Sichuan and Yunnan).

Note. The tribe includes narrowly distributed monospecific *Dipoma* and *Hemilophia* (6 spp.).

3e. *Idahoa* and *Subularia*

Beilstein et al. (2006, 2008) studied two samples of *Idahoa* A. Nelson & J.F. Macbr. and their position was unresolved in a polytomy that included *Asta* Klotzsch ex O.E. Schulz and Cremolobeae (*Cremolobus* DC. and *Menonvillea* DC.). Couvreur et al. (2010) sampled only *Subularia* L. but it was oddly placed in the Isatideae. By contrast, the family-wide phylogenetic study of Warwick et al. (2010) was the first that dealt with both *Idahoa* and *Subularia*. The former was sister clade to *Petrocallis* W.T. Aiton and together they were sister to *Subularia*. That clade was sister to many taxa of various tribes. These early studies did not resolve the relationship of both genera, and Al-Shehbaz (2012) listed both genera as unplaced.

The first clear relationship of *Idahoa* and *Subularia* to other tribes was given in Nikolov et al. (2019). The two genera formed a monophyletic group sister to a clade of *Asta* and *Scoliaxon* Payson (Asteae), which was sister to the South American CES clade of Salariato et al. (2016): *Cremolobus* (Cremolobeae), *Brayopsis* Gilg & Muschl. (Eudemeae), and *Schizopetalon* Sims (Schizopetaleae). The findings of Walden et al. (2020) and Dogan et al. (2022) were basically similar in terms of the entire complex of tribes except for minor differences in the position of *Idahoa* and *Subularia* relative to the other tribes. The findings of Hendriks et al. (2022) are basically the same except for the unexpected position of *Teesdalia* W.T. Aiton (Iberideae) between *Subularia* and *Idahoa*, and further studies should resolve such a relationship. Regardless of the slight differences in the most recent plastid vs. nuclear family-wide phylogenies, it is evident that these two genera should be placed in one tribe, and the name Subularieae was validly proposed over two centuries ago.

Subularieae DC., Mém. Mus. Hist. Nat. 7(1): 257. 20 Apr 1821. (2: 3)

Type. *Subularia* L.

Description. Herbs scapose annuals. Trichomes absent. Multicellular glands absent. All leaves in a basal rosette, sessile or petiolate, cauline leaves absent. Racemes ebracteate throughout and elongated or not in fruit, or flowers solitary on long pedicels originating from center of rosette. Flowers actinomorphic; sepals spreading or ascending, base of lateral pair not saccate; petals white, claw obscure or undifferentiated from blade; filaments slender at base; pollen 3-colpate; ovules 4–18. Fruits dehiscent, unsegmented silicles, orbicular and strongly latiseptate or obovoid to ellipsoid and slightly angustiseptate; septum complete; styles minute or absent; stigma entire. Seeds biseriate, broadly winged and accumbent, or wingless and incumbent. x = 14 and 15.

Distribution. The tribe includes monospecific *Idahoa* (NW USA and Canadian British Columbia) and two aquatic or littoral species of *Subularia*, of which *S. monticola* A. Braun ex Schweinf. is restricted to tropical East Africa, and *S. aquatica* L. is distributed in northern North America (subsp. *americana* G.A. Mulligan & Calder) and temperate Eurasia (subsp. *aquatica*).

3f. *Schrenkiella*

This monospecific genus was based on *Diplotaxis parvula* Schrenk, a species that fluctuated between unrelated genera solely on morphological grounds. It was first shown by German et al. (2009) to occupy an isolated position among Asian Brassicaceae and was subsequently recognized by German and Al-Shehbaz (2010) as a monospecific genus that was not placed in any tribe. It was shown by Huang et al. (2016) to form a basal clade to that including *Sisymbrium* L. and six genera of the Brassiceae. The first robust position of *Schrenkiella* was shown by Walden et al. (2020) and fully supported by Hendriks et al. (2022). It is sister to a clade including the Fourraeeae and sister clade including the Brassiceae and Isatideae plus Sisymbrieae and Thelypodieae. The isolated position of monophyletic *Schrenkiella* strongly supports its placement in its own tribe.

Schrenkielleae Al-Shehbaz, Hendriks, M.Koch, F.Lens, Lysak, C.D.Bailey, Mumm. & D.A.German, trib. nov. (1: 1)

urn:lsid:ipni.org:names:77315217-1

Type. *Schrenkiella* D.A.German & Al-Shehbaz

Description. Herbs annual, glaucous. Trichomes absent. Multicellular glands absent. Cauline leaves petiolate to subsessile, fleshy, cuneate at base, not auriculate. Racemes ebracteate, elongated in fruit, rachis strongly flexuous. Flowers actinomorphic; sepals suberect, base of lateral pair not saccate; petals absent, rarely present, white, subequaling sepals; claw obsolete; filaments slender, unappendaged; pollen 3-colpate; ovules 24–50 per ovary. Fruits dehiscent siliques, linear, latiseptate, unsegmented; septum complete; styles distinct; stigma entire. Seeds biseriate; cotyledons incumbent. x = 7.

Distribution. *Schrenkiella parvula* (Schrenk) D.A.German & Al-Shehbaz is sporadically distributed in Armenia, Azerbaijan, Iran, Kazakhstan, Russia, Turkey, Turkmenistan, and Uzbekistan.

4. Further tribal comments

The following alphabetical tribal discussions are based on the phylogenies of Hendriks et al. (2022), along with comparison of the recent family-wide phylogenies of Nikolov et al. (2019), Walden et al. (2020), and few earlier ones. Generic limits and species number closely follow BrassiBase (Kiefer et al. 2014) with some updating. As above, tribal names are followed in parenthesis by numbers of genera and species, and those that showed no conflict with previous phylogenies are not discussed here. They include Anastaticeae (13: 67), Aphragmeae (1: 13), Biscutelleae (5: 74), Boechereae (9: 125), Buniadeae (1: 2), Calepineae (3: 9), Cardamineae (14: 344), Chorisporeae (4: 56), Cochlearieae (2: 29), Coluteocarpeae (1–12: 130), Crucihimalayeae (3: 15), Erysimeae (1: 274), Euclidieae (30: 155), Eutremeae (1: 44), Halimolobeae (5: 39), Heliophileae (1: 105), Hesperideae (1: 52), Isatideae (5: 99), Kernereae (3: 3), Lepidieae (1: 268), Malcolmieae (1: 6), Megacarpaeeae (2: 11), Microlepidieae (15: 57), Notothlaspideae (1: 3), Oreophytoneae (2: 7), Physarieae (7: 133), Schizopetaleae (4: 21), Shehbazieae (1: 1), Sisymbrieae (1: 49), Smelowskieae (1: 25), Stevenieae (2: 10), Thelypodieae (34: 235), Thlaspideae (13: 39), Turritideae (1: 2), and Yinshanieae (1: 4).

Aethionemeae (1: 58). The tribe is distributed primarily in SW Asia and the Mediterranean region, with the center of greatest diversity located in Turkey, in which 23 of the 40 species are endemic. All previous molecular studies have supported the tribal position as a sister clade to the rest of the Brassicaceae recognized above at subfamilial level.

Alysseae (24: 282). The tribe is almost exclusively distributed in Eurasia, with several native species in North Africa and one in North America. The largest and most complex genera are *Alyssum* L. and *Odontarrhena* C.A. Mey. ex Ledeb. with about 114 and 91 species, respectively. The tribe has recently been revised by Španiel et al. (2015), and its database AlyBase (www.alysseae.sav.sk; Španiel et al. 2015) should be consulted for further data and updates. All except *Brachypus* Ledeb. (1 sp.), *Galitzkya* V.V. Botschantz. (3 spp.), and *Takhtajaniella* V.E. Avet. (1 sp.) are included in the phylogeny by Hendriks et al. (2022).

Alyssopsideae (4: 9). A small Asian tribe distributed predominantly in Afghanistan, Azerbaijan, Iran, Tajikistan, and Turkmenistan. It is monophyletic in Hendriks et al. (2022) and a sister clade to *Chrysochamela* and together are sister to *Pseudoarabidopsis*. These two genera belong to paraphyletic Camelineae III and together are sister to the Turritideae (2 spp.). The sister relationship of *Pesudoarabidopsis* to the Turritideae was demonstrated earlier by Walden et al. (2020) who showed that their clade is distinct from the Camelineae including the generic type *Camelina*. It is clear that these taxa do not belong to the Camelineae s. str. (Hendriks et al. 2022), but further studies are needed to explore whether they are well supported within Alyssopsideae.

Anchonieae (9: 75). Except for several species of *Matthiola* W.T. Aiton in Europe, the tribe is distributed primarily in SW and C Asia, and Africa. Only monospecific *Eremoblastus* Botsch. is not covered in Hendriks et al. (2022). The generic type, *Anchonium* DC., has recently been reduced to synonymy of the earlier-published *Sterigmostemum* M. Bieb. (German and Al-Shehbaz 2017). The tribe is characterized by the presence of multicellular-multiseriate glands, though apparently these structures were independently lost in *Veselskya* Opiz (1 sp.), one species of *Sterigmostemum*, and some species of *Matthiola* (ca. 56 spp.). Such glands are also found in the related tribes Chorisporeae and Dontostemoneae.

Arabideae (18: 559). The tribe is the largest and most complex in the family. It includes ten monospecific genera, and *Draba* L. (ca. 410 spp.), *Arabis* L. (ca. 100 spp.), and *Aubrieta* Adans. (23 spp.) are the most species rich ones. The tribe has been the focal topic for the Koch lab (Heidelberg University) for about three decades and despite carving nearly a dozen segregates into several tribes, *Arabis* still needs further focus and taxonomic adjustments are under consideration (see Koch et al. 2022 for references).

Asteae (2: 2). The findings of Hendriks et al. (2022) strongly justify merging the Mexican monospecific tribe Scoliaxoneae with the earlier published Asteae. That clade is most closely related to the South American CES clade *sensu* Salariato et al. (2016). These findings are in full agreement with those of Walden et al. (2020), but not closely related to the European Kernereae, a tribe more closely related to the Cochlearieae, Conringieae, and Coluteocarpeae in Hendriks et al. (2022).

Brassiceae (53: 243). The tribe has been recognized by all authors since it was established by de Candolle (1821). With the exception of a few genera (e.g., *Ammosperma* Hook.f., *Bivonaea* DC., *Horwoodia* Turrill, and *Pseuderucaria* O.E. Schulz), the plants have conduplicate cotyledons and/or segmented (heteroarthrocarpous) fruits. All except four genera (*Cordylocarpus* Desf., *Fezia* Pit. ex Batt., *Muricaria* Desv., and *Rytidocarpus* Coss.) were included in Hendriks et al. (2022). Unlike the findings of Walden et al. (2020) based on chloroplast data, *Bivonaea* was placed as sister to the tribe Fourraeeae.

Monophyly of *Brassica* is established based on most recent molecular phylogenies (e.g., Hendriks et al. 2022). About a dozen species of *Brassica* have been transferred to *Guenthera* Andrz., but monophyly of the latter with additional species needs to be established. Two other genera of the tribe, *Diplotaxis* DC. and *Erucastrum* C. Presl, remain artificially delimited, and similar studies are needed to accurately define their boundaries.

Camelineae (4: 16). As shown by Hendriks et al. (2022), the Camelineae as hitherto accepted are paraphyletic, of which Camelineae I includes *Camelina* (8 spp.), *Capsella* Medik. (5 spp.), *Catolobus* (1 sp.), and *Neslia* Desv. (2 spp.). Camelineae III is discussed above in connection with the Alyssopsideae. Finally, Camelineae II includes only *Arabidopsis*, which is shown in Hendriks et al. (2022) and some earlier studies to form a distinct clade from the rest of the Camelineae and recognized above in its own tribe. With the exclusion of Camelineae II and III, the tribal description of Camelineae s.str. is updated below:

Herbs, annual or perennial. Trichomes stalked or sessile, stellate, dendritic, or forked, sometimes mixed with simple ones. Multicellular glands absent. Cauline leaves sessile, mostly entire, auriculate or sagittate at base. Racemes ebracteate, often elongated in fruit. Flowers actinomorphic; sepals erect to spreading, lateral pair often not saccate at base; petals white, yellow, orange, pink, or purple, often with a distinct claw; filaments unappendaged, wingless; pollen 3-colpate; ovules 2–40 per ovary. Fruits silicles or siliques, dehiscent or indehiscent, latiseptate, terete, or angustiseptate, unsegmented; styles often distinct; stigma entire or rarely 2-lobed. Seeds biseriate, uniseriate, or aseriate; cotyledons incumbent or rarely accumbent.

Conringieae (1: 3) vs. **Plagiolobeae** (1: 5). The Conringieae *sensu* Al-Shehbaz (2012) was broadly delimited to encompass a heterogenous assembly of the genera *Conringia* (6 spp.) and *Zuvanda* (3 spp.). The findings of Hendriks et al. (2022) agree with those of Walden et al. (2020) and Nikolov et al. (2019) in that the Conringieae s.l. is not monophyletic. Based on the molecular findings and re-evaluation of morphology in light of those studies, one species, *C. planisiliqua*, was assigned to the genus *Iljinskaea* (Al-Shehbaz et al. 2021) of the Isatideae, *Zuvanda* and three species of *Conringia* are currently recognized as five species of *Plagioloba* of the tribe Plagiolobeae (German 2021; German 2022; Khosravi et al. 2022), and the remaining three species of *Corningia* are retained in the genus. The Conringieae differs from the Plagiolobeae by having 4- to 8-angled (vs. terete) fruits and entire (vs. slightly to prominently 2-lobed) stigmas with connivent (or sometimes decurrent) lobes.

Cremolobeae (4: 32). As currently recognized (Salariato et al. 2016; Salariato et al. 2020), the tribe includes the genera *Aimara* Salariato & Al-Shehbaz (1 sp.), *Cremolobus* (5 spp.), *Menonvillea* (24 spp.), and *Yunkia* Salariato & Al-Shehbaz (2 spp.). Hendriks et al. (2022) included five species of the tribe that belong to the first three genera, and their findings support the monophyly of the tribe, as did the above studies of Salariato et al. (2016, 2020). However, Walden et al. (2020) showed that *Menonvillea* did not fall with the rest of the tribe, and further studies are definitely needed (see tribe Eudemeae below).

Descurainieae (6: 48). Except for the monospecific Patagonian *Trichotolinum* O.E. Schulz, which has not yet been included in any phylogenetic studies, the position of other five genera in Hendriks et al. (2022) agrees with earlier studies.

Dontostemoneae (2: 14). Position of Dontostemoneae, Chorisporeae, and their intertribal hybrid Shehbazieae are in full agreement with the initial findings by German and Friesen (2014) and Walden et al. (2020). In contrast to these consistent findings, Liu et al. (2021) probably erroneously considered *Shehbazia* D.A. German as member of the paraphyletic Chorisporeae.

Eudemeae (9: 40). Hendriks et al's. (2022) sampling of five species of five genera supports the monophyly of this tribe. Together with the other exclusively South American tribes, Cremolobeae (see above) and Schizopetaleae of the CES clade *sensu* Salariato et al. (2016) and North American Asteae, the group forms a monophyletic New World clade. Such generic relationship was first observed by Walden et al. (2020) who demonstrated that *Menonvillea* falls outside the Cremolobeae. Salariato et al. (2022)

showed that *Alshehbazia* Salariato & Zuloaga (3 spp.), *Aschersoniodoxa* Gilg & Muschl. (3 spp.), *Gongylis* Theophr. ex Molinari & Sánchez Och. (1 sp.), *Onuris* Phil. (5 spp.), and *Xerodraba* Skottsb. (5 spp.) are monophyletic, whereas *Brayopsis* (9 spp.), *Dactylocardamum* Al-Shehbaz (2 spp.), *Eudema* Humb. & Bonpl. (4 spp.), and *Stenodraba* O.E. Schulz (8 spp.) are polyphyletic. Clearly, the entire complex is much in need of further studies based on extensive sampling of most species of the entire complex.

Fourraeeae (2: 3) This tribe has recently been established by Koch et al. (2022) to accommodate three species previously assigned to *Arabis*. Those authors discussed previously published extensive molecular studies that did not support the placement of those species within *Arabis*. The group includes the European *Fourraea alpina* (L.) Greuter & Burdet and two Moroccan species assigned to the new genus *Hurkaea* Al-Shehbaz, M.A. Koch, R. Karl & D.A. German. The data of Hendriks et al. (2022) strongly support the recognition of this tribe.

Hillielleae (1: 11). The recently established Hillielleae was previously part of the Yinshanieae, but Chen et al. (2016) clearly showed that the two tribes are distantly related. Walden et al. (2020) confirmed the findings of Chen et al. and demonstrated that the Hillielleae is sister to a clade containing the Iberideae and Megacarpaeeae but remotely related to the Biscutelleae. However, Hendriks et al. (2022) showed that the Hillielleae is sister to the clade including the last three tribes and together are sister to the Anastaticeae.

Iberideae (2: 30). The tribe includes the primarily European *Iberis* L. (27 spp.) and *Teesdalia* (3 spp.). Only Warwick et al. (2010) included *Teesdalia* in their studies and showed it to form a sister clade to *Iberis* and thus placed both genera in the tribe Iberideae. In Hendriks et al. (2022), two species of *Teesdalia* and one of *Iberis* were sampled and the results showed them to be remotely related. Clearly a better sampling of *Iberis* ought to be done to check whether or not the two genera can be maintained in one tribe.

Concluding remarks

The taxonomic framework presented here reflects a growing body of phylogenetic knowledge derived from continual advances in the sampling of species, broader representation of major groups, and the extensive sampling of genomic regions needed to help robustly resolve relationships across scales (Hendriks et al. 2022). The consistent nature of those findings suggest that this classification is a considerable advance over previously available formal classifications. However, we are fully aware that further accumulation of phylogenetic data will result in additions and modifications to our understanding of relationships among a minority of Brassicaceae. Most importantly, elements of phylogenetic uncertainty, illustrated by the presence of a few “jumpy clades” and discordance between nuclear and plastid phylogenies, highlight both the need to continue to resolve Brassicaceae relationships and regions of “the family tree” that are likely to experience and require future taxonomic modifications.

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