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A common *Bistorta* was misidentified as a novel species in *Fagopyrum* (Polygonaceae): the confirmation of the taxonomic identify of *F. hailuogouense* by morphological and molecular evidences

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Abstract

A comprehensive phylogenetic analysis of the subfamily Polygonoideae (Polygonaceae) indicated that *Fagopyrum hailuogouense* is a member of *Bistorta*. Morphological investigations also confirmed that *F. hailuogouense* is identical to *B. pergracilis* which is a widely distributed species in E-Asia. *F. hailuogouense* is here proposed as a synonym (new synonymy) of *Bistorta pergracilis*. A lectotype for the name *Bistorta pergracilis* is designated here on a specimen preserved at K (iso lectotype at NY).

Key words: Fagopyreae, molecular phylogenetics, morphology, Persicarieae, taxonomy

Introduction

Fagopyrum Miller (1754: without page), an economically important genus in the knotweed family, is well known by containing two domesticated common buckwheat in China, i.e. *F. esculentum* Moench (1794: 290), and *F. tataricum* (Linnaeus 1753: 364) Gaertner (1790: 182). The genus is common around the regions of the SW-China and adjacent areas (Ohnishi & Matsuoka 1996, Li 1998, Ohnishi 1998). Over the past 100 years, 10 to 18 wild *Fagopyrum* species were gradually recognized by generations of Polygonaceae taxonomists (see e.g., Gross 1913, Steward 1930, Li 1998, Ohnishi 1998, Chen 1999, Ohsako *et al.* 2002, Li *et al.* 2003). However, the species number dramatically exploded in the recent ten years, with the addition of 10 new taxa described by a Chinese research group. All of these taxa were collected from Sichuan, one of the southwestern provinces of China, including *F. polychromifolium* A.H.Wang, J.L.Liu & P.Yang in Xia *et al.* (2007: 11), *F. crispatifolium* J.L.Liu in Liu *et al.* (2008a: 930), *F. densivillosum* J.L.Liu in Liu *et al.* (2008b: 531), *F. pugense* T.Yu in Tang *et al.* (2010: 239), *F. qiangcai* D.Q.Bai in Shao *et al.* (2011: 256), *F. wenchuanense* J.R.Shao in Shao *et al.* (2011: 258), *F. densivillosum* var. *pterocarpum* J.L.Liu & X.J.Li in Li *et al.* (2012: 816), *F. hailuogouense* J.R.Shao, M.L.Zhou & Q.Zhang in Zhou *et al.* (2015: 222), *F. luojishanense* J.R.Shao in Hou *et al.* (2015: 22), and *F. longzhoushanense* J.R.Shao in Wang *et al.* (2017: 76). Six of these taxa (*F. crispatifolium*, *F. densivillosum* var. *densivillosum*, *F. densivillosum* var. *pterocarpum*, *F. longzhoushanense*, *F. luojishanense*, and *F. pugense*) were reported not only from the same county, Puge County of Liangshan Prefecture, but also from the same mountain, Luojishan Mountain, and all of them were compared with the same species, *F. gracilipes* (Hemsley in Forbes & Hemsley 1891: 340) Dammer ex Diels (1900: 315). Other three taxa (*F. qiangcai*, *F. polychromifolium*, and *F. wenchuanense*) were described from Aba Prefecture. The last one—*F. hailuogouense*—was collected from Luding County, Sichuan, China, and was considered to be similar to *F. cymosum* (Treviranus 1826: 177) Meisner (1832: 63). In the protologue, Zhou *et al.* (2015) noted that *F. hailuogouense* is characterized by having a succulent rhizome with congested nodes and numerous adventitious roots. Such a rhizome has never before been reported in *Fagopyrum*, but can be easily found in another genus, *Bistorta* (Linnaeus 1753: 360) Scopoli (1754: 24).

Moreover, Zhou *et al.* (2015) did not provide any line drawing or photos of living plants to show the morphology of the "new species", making a direct recognition of the taxa difficult, but we found out such illustrations from two master's theses (Zheng 2012, Zhang 2013), which were guided by Prof. Jirong Shao who is one of the corresponding

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authors of Zhou *et al.* (2015). In these illustrations (Fig. 1), *F. hailuogouense* bears several basal leaves with long petioles, 1–2 unbranched terrestrial stems with terminal and axillary spicate inflorescences, and a few shortly petiolate to sessile clasping cauline leaves. These traits show apparently that *F. hailuogouense* is not a *Fagopyrum*, but a *Bistorta*. Based on our taxonomic experiences in Polygonaceae, we further found that *F. hailuogouense* is nearly identical to *B. pergracilis* (Hemsley in Forbes & Hemsley 1891: 344) Gross (1913: 16) (Fig. 2), a widely distributed species in East Asia.

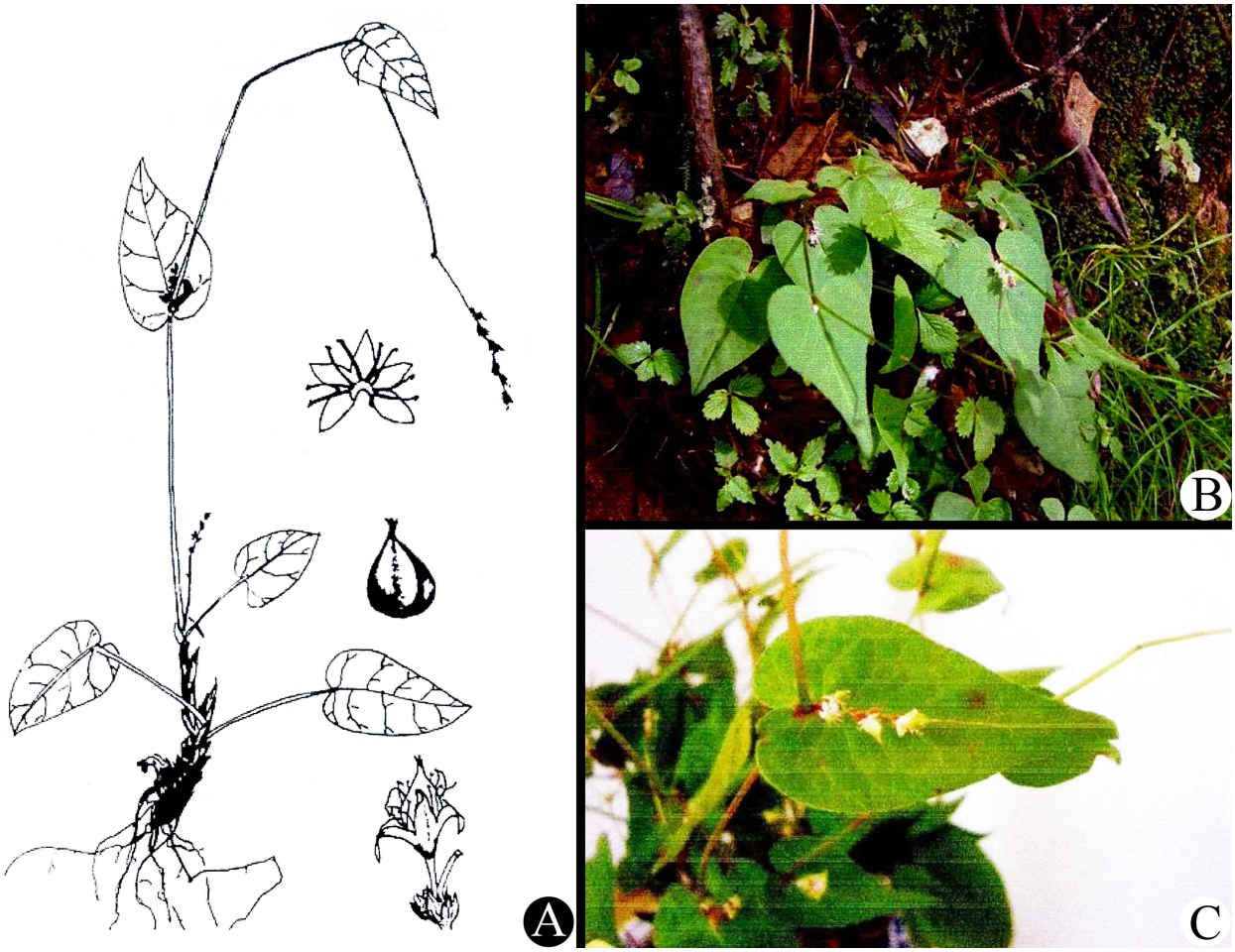

FIGURE 1. Morphology of *Fagopyrum hailuogouense*. A. line-drawing illustration (from Zhang 2013); B. leaves and inflorescences (from Zheng 2012).

Futhermore, both Zheng (2012) and Zhang (2013) conducted molecular phylogenetic analysis for *F. hailuogouense* and they concluded that it is sister to the rest of *Fagopyrum*. However, Zheng (2012) only selected one non-*Fagopyrum* species as outgroup, *Fallopia multiflora* (Thunberg 1784: 379) Haraldson (1978: 77) [now treated as *Pleuropterus multiflorus* (Thunb.) Nakai (1914: 264)], while Zhang (2013) did not include any other taxa from related genera. Based on such insufficient sampling, it is even difficult to infer whether *F. hailuogouense* is a *Fagopyrum*, but both Zheng (2012) and Zhang (2013) made a questionable conclusion that *F. hailuogouense* represents "the most primitive species in *Fagopyrum*" and had its "own unique evolutionary path". After including *F. hailuogouense* in a more comprehensive phylogenetic analysis of the subfamily Polygonoideae, we confirm that *F. hailuogouense* is a member of *Bistorta* and herein clarify it.

Materials and methods

Morphological observations

Both herbarium specimens (mainly from IBSC, JXAU, KUN, LBG, NAS, and PE. For herbarium acronyms see Index Herbariorum: http://sweetgum.nybg.org/ih) and living plants of *Fagopyrum* and *Bistorta* were examined. High

resolution images of the type materials of *B. pergracilis* (held at K and NY) were consulted on JSTOR Global Plants (http://about.jstor.org/, accessed 10 November 2017), and the type specimen held at K (barcode no. K000830498) were examined in the herbarium under a stereo dissecting microscope.

Molecular datasets and phylogenetic analyses

Three cpDNA makers, *matK*, *rbcL* and *trnL-F*, were employed to reconstruct the phylogeny of Polygonoideae, because they have been successfully used in previous studies (Galasso *et al.* 2009, Sanchez *et al.* 2009, Sanchez *et al.* 2011, Schuster *et al.* 2015). According to Schuster *et al.* (2015), Oxygoneae is sister to the rest of Polygonoideae, thus two representatives from this tribe were selected as outgroups, while 50 taxa from other six tribes, Calligoneae, Fagopyreae, Persicarieae, Polygoneae, Pteroxygoneae and Rumiceae, were sampled as ingroups. Taxa and GenBank accession numbers were listed in Table 1 (for *Fagopyrum hailuogouense* data came also from the isotype at SAU).

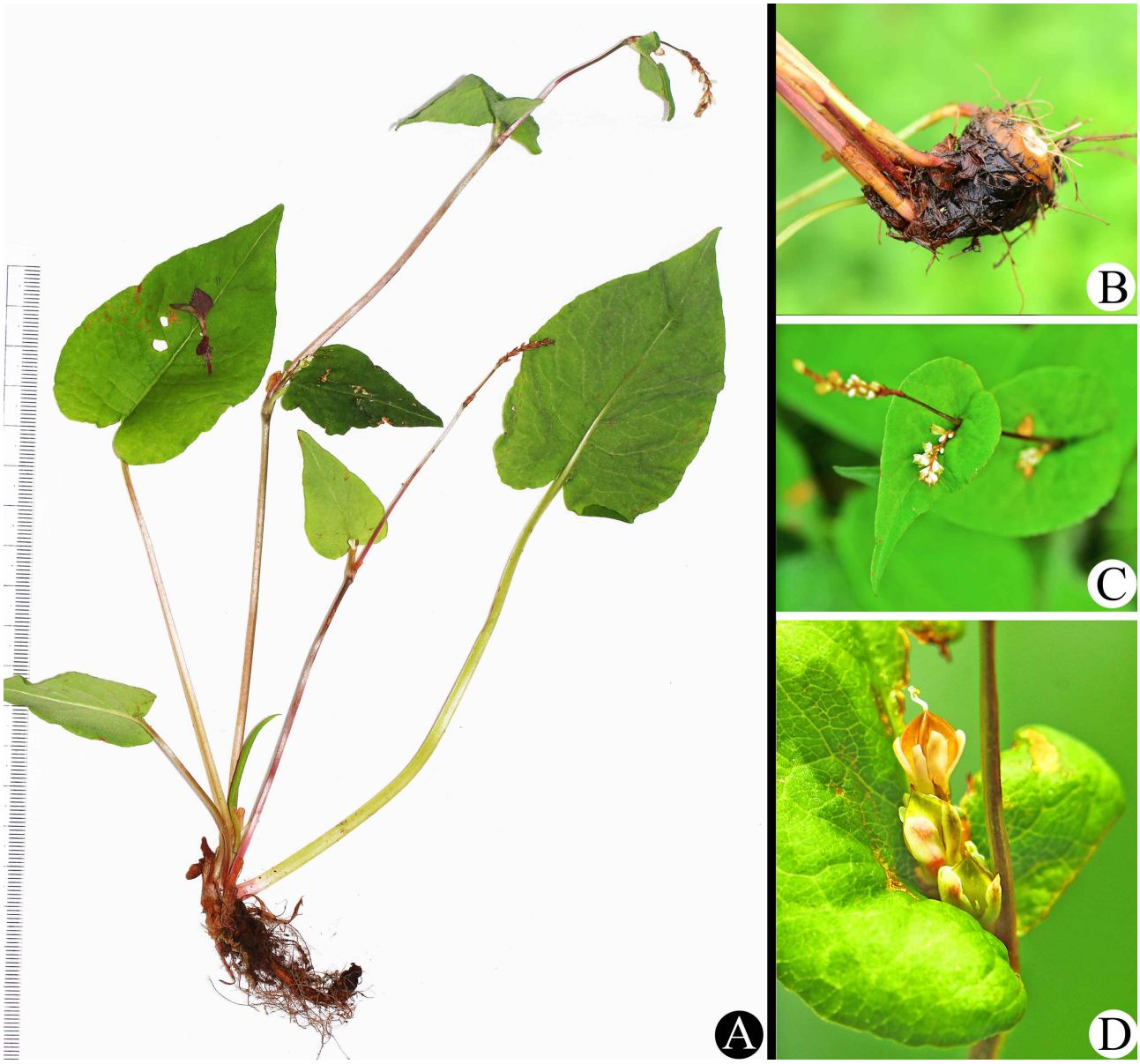

FIGURE 2. Morphology of *Bistorta pergracilis*. A. habit; B. rhizome; C. sessile and clasping cauline leaves, and terminal and axillary spicate inflorescences; D. axillary infructescence with achenes exceeding from persistent perianth. A, D photographed by Bo Li, and B, C provided by Xing-xing Zhu.

TABLE 1. Taxa and GenBank accession numbers of the subfamily Polygonoideae that were used in the present study.

Taxa	*matK*	*rbcL*	*trnL-F*
Bistorta amplexicaulis	EF438014	JN234952	JN235033
Bistorta macrophylla	JF955593	HQ435351	JN235071
Bistorta officinalis	AF204859	FM883607	KR537728
Bistorta paleacea	-	JN234987	KF586490
Bistorta plumosa	KC474180	KC482106	GQ244642
Bistorta sinomontana	-	JF943523	JN235032
Bistorta suffulta	-	JN234990	JN235073
Bistorta vivipara	EU840456	EU840288	EU024776
Calligonum roborowskii	JX259347	JQ009270	JQ009288
Fagopyrum callianthum	AB026329	AB000302	-
Fagopyrum capillatum	AB026321	AB000303	-
Fagopyrum crispatifolium	JF829975	-	-
Fagopyrum cymosum	AB026335	AB093065	-
Fagopyrum dibotrys	GQ434135	JN234983	JN235065
Fagopyrum esculentum	JF829981	EU254477	EU024792
Fagopyrum gilesii	AB086257	AB056689	-
Fagopyrum gracilipes	AB026319	AB000311	EU024787
Fagopyrum hailuogouense	JQ807586	-	-
Fagopyrum leptopodum	JF829978	AB000313	-
Fagopyrum lineare	AB026333	AB000314	-
Fagopyrum macrocarpum	AB026327	AB056687	-
Fagopyrum pleioramosum	AB026324	AB000315	-
Fagopyrum pugense	JF829976	-	-
Fagopyrum rubifolium	AB026322	AB056686	-
Fagopyrum statice	AB026313	AB000317	-
Fagopyrum tataricum	JF829984	D86287	HQ843172
Fagopyrum tibeticum	-	JQ009278	JQ009296
Fagopyrum urophyllum	AB026330	D86288	-
Fagopyrum wenchuanense	JF829982	-	-
Koenigia alpina	HM357917	FM883602	KR537738
Koenigia forrestii	AY042605	AF297144	JN235043
Koenigia hookeri	KT280246	EU840289	EU840541
Koenigia islandica	EU840455	EU840287	KF541257
Koenigia mollis	GQ206190	EF653764	EF653790
Muehlenbeckia complexa	HM851072	HM850184	JF831305
Oxygonum dregeanum	JN161150	-	JN161140
Oxygonum sinuatum	KR734898	KR736460	KR537752
Oxyria digyna	KC475052	KM360910	JN235068
Persicaria hydropiper	HM357924	EF653780	JN235045
Persicaria perfoliata	HM357916	HM357890	FJ627264
Persicaria posumbu	GU266606	EF653778	JN235044
Persicaria runcinata	AY042627	AF297124	EF653795
Persicaria sagittata	EF438018	EF653773	EF653799
Pleuropterus multiflorus	EF159150	HM357901	EU024777
Polygonum arenastrum	JN895284	HE963604	KR537759
Polygonum aviculare	AB976686	HM850273	JN235060
Polygonum plebeium	-	HQ435348	EU109598
Pteropyrum aucheri	GQ206205	GQ206227	AB542791
Pteroxygonum denticulatum	HM357915	HM357889	HQ843149
Pteroxygonum giraldii	GU373528	GQ206230	EU402464
Rheum australe	KF906711	EU840309	AY566459
Rumex japonicum	GQ434138	EU554021	AB817486

The symbol "-" indicates missing data.

Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) methods. BI analysis was executed using MrBayes version 3.2.2 (Ronquist *et al.* 2012) on the CIPRES Science Gateway (Miller *et al.* 2010) with the default parameters. The best substitution types (Nst) and rate distribution models (rates) were determined by the Akaike information criterion (AIC) using Model Test v.3.7 (Posada & Crandall 1998) with the hierarchical likelihood ratio tests. Four Markov chain Monte Carlo (MCMC) chains were run, each beginning with a random tree and sampling one tree every 100 generations for 20,000,000 generations.ML analyses were performed on the web server RAxML Black Box (Stamatakis *et al.* 2008). Before each submission, the "Maximum likelihood search" and "Estimate proportion of invariable sites" options were selected, with a total of 1,000 bootstrap replicates performed.

Results

BI and ML analyses of separate data sets (*matK*, *rbcL*, and *trnL-F*) did not yield fully resolved gene trees for the subfamily Polygonoideae, but *Fagopyrum hailuogouense* deeply nested in the genus *Bistorta* in both BI and ML trees generated from *matK* matrix. Based on the combined dataset, BI and ML analyses yielded very similar topologies, hence the 50% majority-rule consensus tree from the BI analysis is showed here (Fig.3). The monophyly of Calligoneae, Fagopyreae, Persicarieae, Polygoneae, Pteroxygoneae and Rumiceae were well supported, and their relathionships are consistent with those presented in Sanchez *et al.* (2011) and Schuster *et al.* (2015). *Fagopyrum* was highly supported as a monophyletic group without including *F. hailuogouense* (Bayesian posterior probability = 1.00, ML bootstrap percentage = 100), while the inclusion of *F. hailuogouense* in *Bistorta* was also well supported (PP = 1.00, ML-BP= 99).

Morphological observations in *Bistorta* show that *F. hailuogouense* can not be distinguished from *B. pergracilis* (Fig. 1 & 2). They both have ovate basal leaves with cordate bases, cordate cauline leaves with short petioles and uppermost sessile and clasping, and slender, interrupted, spicate inflorescences. Additionally, their achenes are both smooth and shiny, and slightly exceeding from persistent perianth.

Taxonomic treatment

Bistorta pergracilis (Hemsl.) Gross (1913: 16) (Figs. 1 & 2) ≡ *Polygonum pergracile* Hemsl. in Forbes & Hemsley (1891: 344)

Type (lectotype, here designated):—CHINA. Sichuan: Mount Omei, December 1887, *E.Faber 839* (K000830498! [image available at http://specimens.kew.org/herbarium/K000830498], isolectotype NY00323895!).

= *Fagopyrum hailuogouense* J.R.Shao, M.L.Zhou & Qian Zhang in Zhou et al. (2015: 222), *syn. nov.*

Type:—CHINA. Sichuan: Moxi Ancient Town, Luding County, Tibetan Autonomous Prefecture of Garze, in thick growth of weeds & maize fields on slopes, 3100–3300 m, 24 August 2011, *Shao, Zhou & Zhang 4325* (holotype SAU!, isotype SAU!).

Typification of *Polygonum pergracilis*:—Hemsley in Forbes & Hemsley (1891: 344) reported, after the diagnosis of *P. pergracile*, "Szechuen: summit of mount Omei (Faber!). Herb. Kew." as the type in the protologue. According to *International Code of Nomenclature* (McNeill *et al.* 2012) and the considerations by McNeill (2014), despite we found only one specimen (at K) collected by Faber (barcode no. K000830498), we cannot be sure that it is that used by Forbes & Hemsley (l.c.) to describe the species and it is not considered here as the holotype of *P. pergracile* since Hemsley had not designated it as the holotype. Thus we here designate the Kew specimen as the lectotype of *P. pergracile* in accordance with Art. 9.2 of ICN. We also traced a duplicat of the lectotype at NY (isolectotype).

Note:—*Bistorta pergracilis* is an easily distinguishable species that frequently occurs in mountain regions of E-Asia from 1,200 m to 4,000 m a.s.l., and grows always on grassy slopes, near wet valleys, and under subtropical evergreen broad-leaved forests. *Fagopyrum hailuogouense* represents merely an ordinary population of *B. pergracilis* in Sichaun Province, but it was not only misidentified as a *Fagopyrum*, but also successfully published as a "new species". Such a mistake should have been avoided since Zheng (2012) and Zhang (2013) have obtained sequences from the sample. It is an easy and convenient method to roughly identify the unknown taxa by running the nucleotide blast program in the National Center for Biotechnology Information. However, based on such a fake new species, two master's theses and a peer-reviewed publication have been produced.

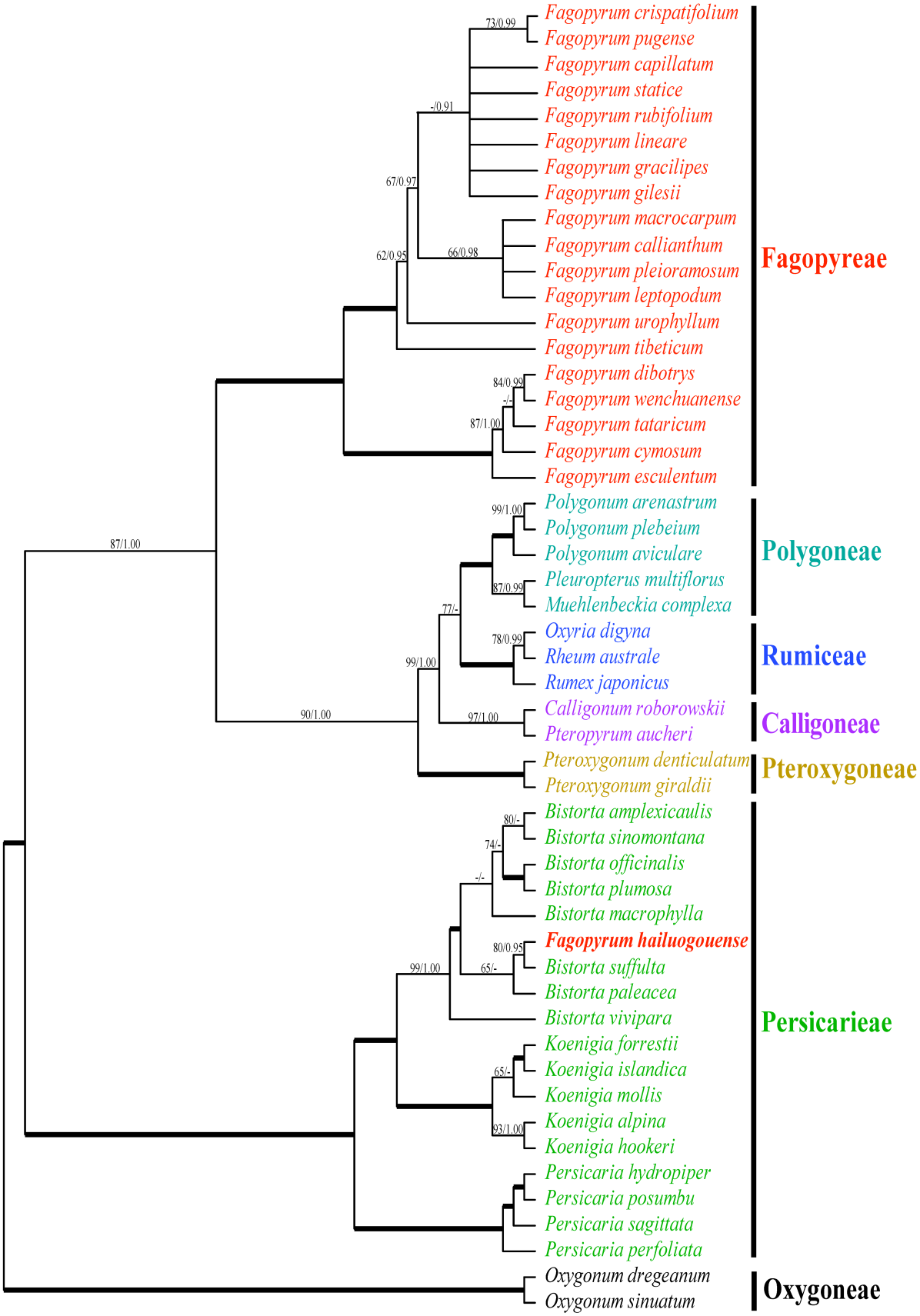

FIGURE 3. The Bayesian 50% majority-rule consensus tree of subfamily Polygonoideae based on combined *matK+rbcL+trnL-F* dataset. Support values displayed on the branches are Bayesian posterior probabilities / ML bootstrap percentages. A dash "–" indicates support values of less than 0.90 in BI or 50% in ML, respectively, while bold lines indicate PP = 1.00 and ML-BP = 100, simultaneously. Tribes recognized by Schuster *et al.* (2015) are distinguished by different colors, and *Fagopyrum hailuogouense* was marked in red bold font.

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