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Molecular phylogeny of tribe Atraphaxideae (Polygonaceae) evidenced from five cpDNA genes

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Abstract: Traditionally, *Atraphaxis*, *Calligonum*, *Pteropyrum* and *Parapteropyrum* are included in the tribe Atraphxideae. Recently, sequence data has revealed that this tribe is not monophyletic. The structure of the tribe was examined by adding more taxa and sequences to clarify the congruence between morphology and molecular phylogeny, the systematic placements of four genera in Polygonaceae, as well as the infra-generic relationships of *Atraphaxis* and *Calligonum* within Atraphaxideae. Five chloroplast genes, *atp*B-*rbc*L, *psb*A-*trn*H, *trn*L–*trn*F, *psb*K-*psb*I, and *rbc*L of *Atraphaxis*, *Calligonum*, *Pteropyrum*, and *Parapteropyrum* were sequenced. The non-monophyly of Atraphaxideae was confirmed. *Atraphaxis* and *Calligonum*, respectively, formed a monophyletic group that was well supported. *Calligonum* is closely related to *Pteropyrum*; *Atraphaxis* is sister to *Polygonum* s. str.; and *Parapteropyrum* is allied with *Fagopyrum*. Although the morphology suggested the four genera should form a tribe, the molecular data indicated Atraphaxideae was not one monophyletic group. The clades identified within *Atraphaxis* corresponded well with the current sectional classification based on morphological features. As for *Calligonum, Medusa* was identified as a non-monophyletic section.

Keywords: tribe Atraphaxideae; *Atraphaxis*; *Calligonum*; chloroplast genes; monophyly

Polygonaceae, in general, has two recognized subfamilies, Polygonoideae and Eriogonoideae (Jaretzky, 1925; Haraldson, 1978; Brandbyge, 1993; Heywood *et al*., 2007; Sanchez *et al*., 2011). Subfamily Polygonoideae has five tribes (Haraldson, 1978; Brandbyge, 1993; Sanchez and Kron, 2008), however, the tribal classification within this subfamily is under dispute. Tribe Atraphaxideae was proposed by Dammer (1893) to include the genera *Atraphaxis*, *Calligonum*, and *Pteropyrum*. Afterward, these three genera were treated as subtribe Atraphaxidinae (Jaretzky, 1925; Hong, 1995) or subfamily Calligonoideae (Hong, 1995); Haraldson (1978) and Brandbyge (1993) placed them in the tribe Polygoneae. Chinese researchers established the new Atraphaxideae genus (*Parapteropyrum*) from Tibet, China. At present, the tribe Atraphaxideae, according to the most widely accepted taxonomy, consists of *Atraphaxis*, *Calligonum*, *Pteropyrum*, and *Parapteropyrum* (Li *et al*., 1998; Takhtajan, 2009). These four genera are all shrubs, usually have five petals and a 3-coporate aperture. Most species of the tribe occur over an area including Central and Western Asia, westward to North Africa and Southeast Europe, and eastward to East Asia (Bao and Li, 1993; Li *et al*., 2003).

Bao and Li (1993) proposed a tribal classification system and postulated an evolutionary framework for Atraphaxideae. Based on the evolution of morphological characters as well as pollen and embryo, *Atraphaxis* was thought to be the most primitive genus. Jaretzky (1925) and Haraldson (1978) hypothe sized that *Atraphaxis* and *Pteropyrum* are related, but Hong (1995) presented evidences from the pollen of the four genera to show that *Parapteropyrum* is very similar to

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Pteropyrum and *Atraphaxis* is different from the other three genera. After comparing thirty morphological characters in *Atraphaxis*, *Calligonum* and *Pteropyrum*, Tavakkoli *et al.* (2008) suggested that *Calligonum* and *Pteropyrum* are closely related.

The karyotype of the tribe provides useful taxonomic data. Jaretzky (1928) considered the basic chromosome number to be n=11 in the Polygonaceae, with other values being derived from it. Recently, Tian *et al*. (2009) reported the chromosome karyotypes of two *Atraphaxis* species and *Parapteropyrum*. *Parapteropyrum* was found to be tetraploid (2x=48). Therefore, a notable difference among three of the genera within the tribe (*Pteropyrum* was not examined) was revealed by basic chromosome numbers, n=9 for *Calligonum*, n=11 for *Atraphaxis*, and, as Tian *et al*. (2009) suggested, n=12 for *Parapteropyrum*. *Calligonum* also had polyploidy, including tetraploids and triploids (Mao *et al*., 1983). Most *Atraphaxis* species are diploid. Thus, the aneuploid evolution and polyploidy of the three genera studied provide a complicated background for the systematic and evolutionary history of Atraphaxideae, in combination with molecular phylogeny (Tian *et al*., 2009).

According to recent molecular phylogenies, *Atraphaxis* is related to the genera of the currently recognized tribe Polygoneae (Lamb-Frye and Kron, 2003; Sanchez and Kron, 2008; Sanchez and Kron, 2009; Sanchez *et al*., 2009; Tavakkoli *et al*., 2010). *Calligonum* and *Pteropyrum* form a clade, along with *Pteroxygonum*, but are distant from *Atraphaxis* (Sanchez *et al*., 2009, Tavakkoli *et al*., 2010). Meanwhile, Sanchez *et al*. (2009) using cpDNA and ITS sequence data showed that Atraphaxideae was not monophyletic, however, *Parapteropyrum* was absent from their chloroplast data. Tavakkoli *et al*. (2010) again demonstrated the polyphyly of Atraphaxideae using ITS and *trn*L-*trn*F sequence data by including four Atraphaxideae genera and focusing on several *Calligonum* and *Pteropyrum* species in Iran.

The previous molecular phylogenies, mainly focusing on higher taxonomic levels, have omitted *Parapteropyrum*, and have lacked adequate *Atraphaxis* samples. Considering the disagreement over relationships in Atraphaxideae, we attempted to explore the phylogeny of Atraphaxideae by sampling more taxa and sequences, particularly to (1) further test the monophyly of Atraphaxideae; (2) investigate the diversification of the four Atraphaxideae genera; and (3) present a preliminary molecular phylogeny of *Atraphaxis* and *Calligonum* in China.

1 Materials and methods

1.1 Taxon sampling

We sampled eight species from *Atraphaxis*, nine from *Calligonum*, and one from *Parapteropyrum* and *Pteropyrum*, respectively (Table 1). All of them, except *Pteropyrum* (obtained from the LE herbarium), were sampled in China. The leaf materials were dried with silica gel from the botanical garden or field.

1.2 DNA sequencing and alignment

Total genomic DNA was extracted using the CTAB method (Doyle and Doyle, 1987). The polymerase chain reaction (PCR) was used for double stranded DNA amplification. Each 25 μL reaction contained 0.25 μL of Ex Taq (2.5 u/μL), 2.5 μL of 10× Ex Taq buffer (Mg^{2+} concentration of 25 mM), 2.0 μL of dNTP mix (at 2.5 mM concentration for each dNTP), 1 μL of each, forward and reverse primers at 5 μmol/μL. The following primers were used: *trn*L-*trn*F (Taberlet *et al*., 1991), *atp*B-*rbc*L (Janssens *et al*., 2006), *psb*AF (Sang *et al*., 1997) and *trn*HR (Tate and Simpson, 2003) for the *psb*A-*trn*H IGS, *psb*K (5′-TTAGCCTTTGTTTGGCAAG-3′) and *psb*I (5′-AGAGTTTGAGAGTAAGCAT-3′) provided by Kim Ki-Joong for the IGS between *psb*K and *psb*I, 1FS (Lamb-Frye and Kron, 2003) and *rbc*L-1460R (5′-TTTAGTAAAAGATTGGGCCGAG-3′) for *rbc*L. For PCR amplifications, predenaturation was first conducted at 94°C for 3 min, followed by 30 cycles of (1) denaturation at 94°C for 30 s, (2) annealing at 48°C–54°C for 30 s, and (3) extension at 72°C for 1 min. At the end of the cycles, a final extension was used at 72°C for 10 min. PCR products were purified using the PEG precipitation procedure (Johnson and Soltis, 1995). Sequencing reactions were performed by Beijing Sanbo Biological Engineering Technology and Service Corporation, China. Sequences were aligned using CLUSTAL X software (Thompson *et al*., 1997),

Table 1 Voucher information for the 19 species of Atraphaxideae

Genera	Section	Species	Voucher	Source	GenBank Accession Nos. (*atp*B-*rbc*L, *psb*K-I, *psb*A-*trn*H, *rbc*L, *trn*L-*trn*F)				
Atraphaxis L.	*Tragpyrum*	*A. bracteata* A. Los.	M.L. Zhang 0811 (XJBI)	TBG, Xinjiang, China	JQ009204	JQ009242	JQ009223	JQ009261	JQ009279
		A. jrtyschensis Yang et Han	M.Z. Chen 0821 (XJBI)	MBG, Gansu, China	JQ009208	JQ009246	JQ009227	JQ009265	JQ009283
		A. manshurica Kitag.	M.Z. Chen 0822 (XJBI)	Lanzhou, Gansu, China	JQ009211	JQ009249	JQ009230	JQ009268	JQ009286
		A. virgata (Regel) Krassn.	B.R. Pan 0881 (XJBI)	Tuoli, Xinjiang, China	JQ009209	JQ009247	JQ009228	JQ009266	JQ009284
		A. pungens (Bieb.) Jaub. et Spach	M.L. Zhang 0812 (XJBI)	TBG, Xinjiang, China	JQ009210	JQ009248	JQ009229	JQ009267	JQ009285
		A. spinosa L.	M.Z. Chen 0823 (XJBI)	MBG, Gansu, China	JQ009207	JQ009245	JQ009226	JQ009264	JQ009282
	Atraphaxis	*A. compacta* Ledeb.	Y.X. Sun 0806 (XJBI)	Urumqi, Xinjiang, China	JQ009206	JQ009244	JQ009225	JQ009263	JQ009281
		A. replicate Lam.	B.R. Pan 0871 (XJBI)	Altai, Xinjiang, China	JQ009205	JQ009243	JQ009224	JQ009262	JQ009280
	Calliphysa (Fisch. et Mey.) Endl.	*C. junceum* (Fisch. et Mey.) Litv.	M.L. Zhang 0844	TBG, Xinjiang, China	JQ009214	JQ009252	JQ009233	JQ009271	JQ009289
		C. aphyllum (Pall.) Gürke	M.L. Zhang 0841	TBG, Xinjiang, China	JQ009215	JQ009253	JQ009234	JQ009272	JQ009290
	Pterococcus (Pall.) Endl.	*C. leucocladum* (Schrenk) Bge.	M.L. Zhang 0845	TBG, Xinjiang, China	JQ009218	JQ009256	JQ009237	JQ009275	JQ009293
		C. rubicundum Bge.	M.L. Zhang 0848	TBG, Xinjiang, China	JQ009212	JQ009250	JQ009231	JQ009269	JQ009287
Calligonum L.	*Calligonum*	*C. densum* Borszcz.	M.L. Zhang 0843	TBG, Xinjiang, China	JQ009216	JQ009254	JQ009235	JQ009273	JQ009291
		C. arborescens Litv.	M.L. Zhang 0842	TBG, Xinjiang, China	JQ009219	JQ009257	JQ009238	JQ009276	JQ009294
	Medusa Sosk. et Alexandr.	*C. mongolicum* Turcz	M.L. Zhang 0846	TBG, Xinjiang, China	JQ009220	JQ009258	JQ009239	JQ009277	JQ009295
		C. roborowskii A. Los.	M.L. Zhang 0847	TBG, Xinjiang, China	JQ009213	JQ009251	JQ009232	JQ009270	JQ009288
		C. zaidamense A. Los.	M.L. Zhang 0849	TBG, Xinjiang, China	JQ009217	JQ009255	JQ009236	JQ009274	JQ009292
Parapteropyrum A. J. Li		*P. tibeticum* A. J. Li	Z.Z. Zhou 0801	Jiacha,Tibet, China	JQ009221	JQ009259	JQ009240	JQ009278	JQ009296
Pteropyrum Jaub. & Spach.		*P. aucherii* Jaub. et Spach	A.L. Ashirova, F. Kerimova & al. s.n. (LE)	Kaakhnishsky, Turcoman (LE)	JQ009222	JQ009260	JQ009241		

Note: TBG, Turpan Botonical Garden; MBG, Minqin Botonical Garden; LE, Herbarium of Vascular Plants, Komarov Botanical Institute of Russian Academy of Sciences

and then adjusted by hand. All gaps were treated as missing characters. Finally, the *rbc*L dataset and five-gene dataset of *atp*B-*rbc*L, *psb*A-*trn*H, *trn*L-*trn*F, *psb*K-*psb*I and *rbc*L were combined and used for phylogenetic analyses.

1.3 Phylogenetic analyses

Sequences of coding regions, such as *rbc*L, were conserved in sequence length, and alignments were straightforward. In contrast, sequences of noncoding regions showed length variation and it was necessary to introduce indels in the alignment. We did not include indel information in our phylogenetic analyses. The phylogenetic analyses (Maximum Parsimony, Maximum Likelihood and Bayesian Inference) of *rbc*L and 5-gene datasets, respectively, were conducted using PAUP* 4.0b10 (Swofford, 2002) and MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Maximum parsimony searches were performed using heuristic search methods: tree-bisection-reconnection (TBR), branches collapsed (creating polytomies) if the maximum branch length was zero, and all characters weighed equally. The analyses were repeated 100 times with a random order of sequence addition in an attempt to sample multiple islands of the most parsimonious trees. Bootstrap analyses (Felsenstein, 1985) under MP analyses were performed to assess the relative support of the branches. Heuristic search settings identical to those above were used to estimate bootstrap values (BS) with 1,000 replicates. For searching the likelihood tree, the same MP parameters were used with PAUP*. For ML analyses, Modeltest 3.6 (Posada and Crandall, 1998) was used, and the nucleotide substitution model GTR+G+I was generated. Bayesian analyses were conducted using MrBayes, version 3.0b4 (Huelsenbeck and Ronquist, 2001; Huelsenbeck and Rannala, 2004). Four chains were run (Markov Chain Monte Carlo), beginning with a random tree and saving a tree every 100 generations, for one million generations.

The incongruence length difference (ILD) test (Farris *et al*., 1994, 1995) for the combined data set of five genes was implemented in PAUP*.

2 Results

2.1 Aligned DNA sequences

The aligned sequence information for each gene marker is presented in Table 2. The five-gene data set was not significantly incongruent based on the ILD tests ($P = 0.28$).

2.2 Phylogenetic analyses

2.2.1 *rbc*L analysis

Four genera of Atraphaxideae were placed in an analysis mostly containing genera of Polygonoideae to investigate their general position in Polygonoideae.

The four genera of Atraphaxideae were found in a large clade (Fig. 1), and within this clade, three subclades (A–C) were discovered. Clade A corresponded to *Calligonum* and *Pteropyrum* alone; clade B contained *Parapteropyrum*, *Fagopyrum* and the genera of the currently recognized tribe Rumiceae. *Parapteropyrum* and *Fagopyrum* were closely related (MP/PP=99/1.00). The third (clade C) included *Atraphaxis*, *Polygonum* s. str., *Polygonella* and *Fallopia*, and was also strongly supported (MP/PP=100/1.00). *Atraphaxis* was strongly supported (MP/PP = 82/1.00) as a sister to the clade including *Polygonum* s. str. and Polygoneae.

There were slight differences in the topologies re covered by MP, ML and Bayesian analyses. As sister to *Calligonum*, *Pteropyrum* received low support in

Table 2 Data set and tree statistics from separate maximum parsimony analyses of *rbc*L and combined 5 gene

Genic region	Aligned seq. length (bp)	No. of variable sites	No. of PIS[a]	No. of MP[b] trees	Length of MP trees	Consistency index (CI)	CI (excl. invariant sites)	Retention index (RI)
*rbc*L	1,380	385	243	99	710	0.6252	0.5281	0.7997
5-cpDNA	4,439	630	235	9	745	0.9163	0.8108	0.9559

Note: [a]parsimony-informative sites; [b]most parsimonious

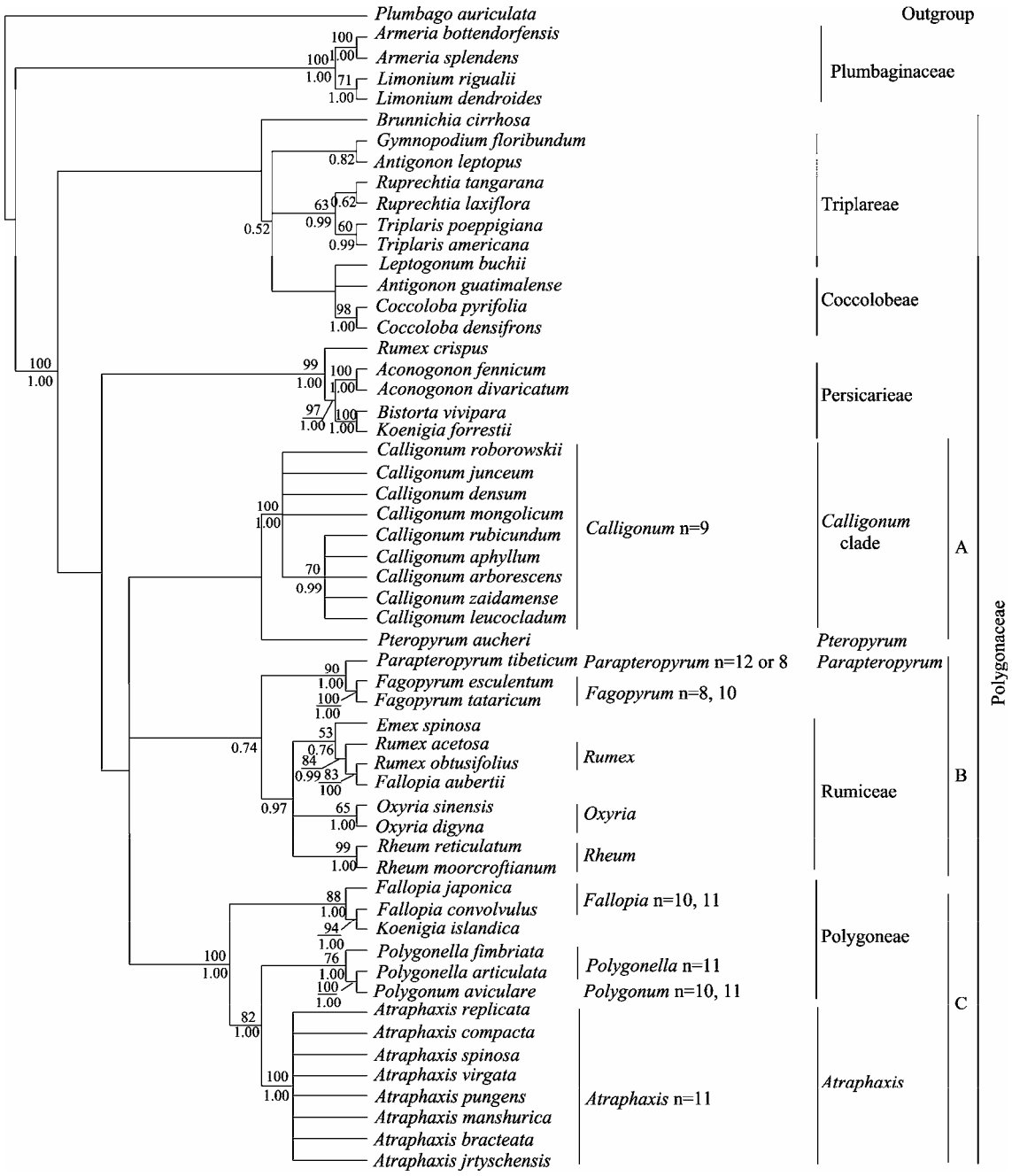

Fig. 1 Topology resulting from maximum likelihood analysis of *rbc*L data set using Garli (GTR+G+I). Bootstrap support values (> 50%) are indicated above the branches; posterior probabilities (> 0.5) are presented below the branches.

MP and Bayesian analysis (Fig. 1).

2.2.2 Five-gene analysis

The monophyly of *Atraphaxis* and *Calligonum* were both well supported. Within *Atraphaxis*, a clade formed by *A. spinosa*, *A. compacta* and *A. replicata* was strongly supported (Fig. 2). In addition, *A. spinosa* was most closely related to *A. compacta*, with a poor MP but high Bayesian support (MP/PP=65/1.00). The relationships among the remaining species were less resolved except for a poorly supported (MP/PP=60/0.68) clade that included *A. virgata*, *A. pungens* and *A. manshurica*. Within *Calligonum*, there was a clade with strong Bayesian support (PP=0.94), including *C. aphyllum*, *C. arborescens*, *C. leucocla-*

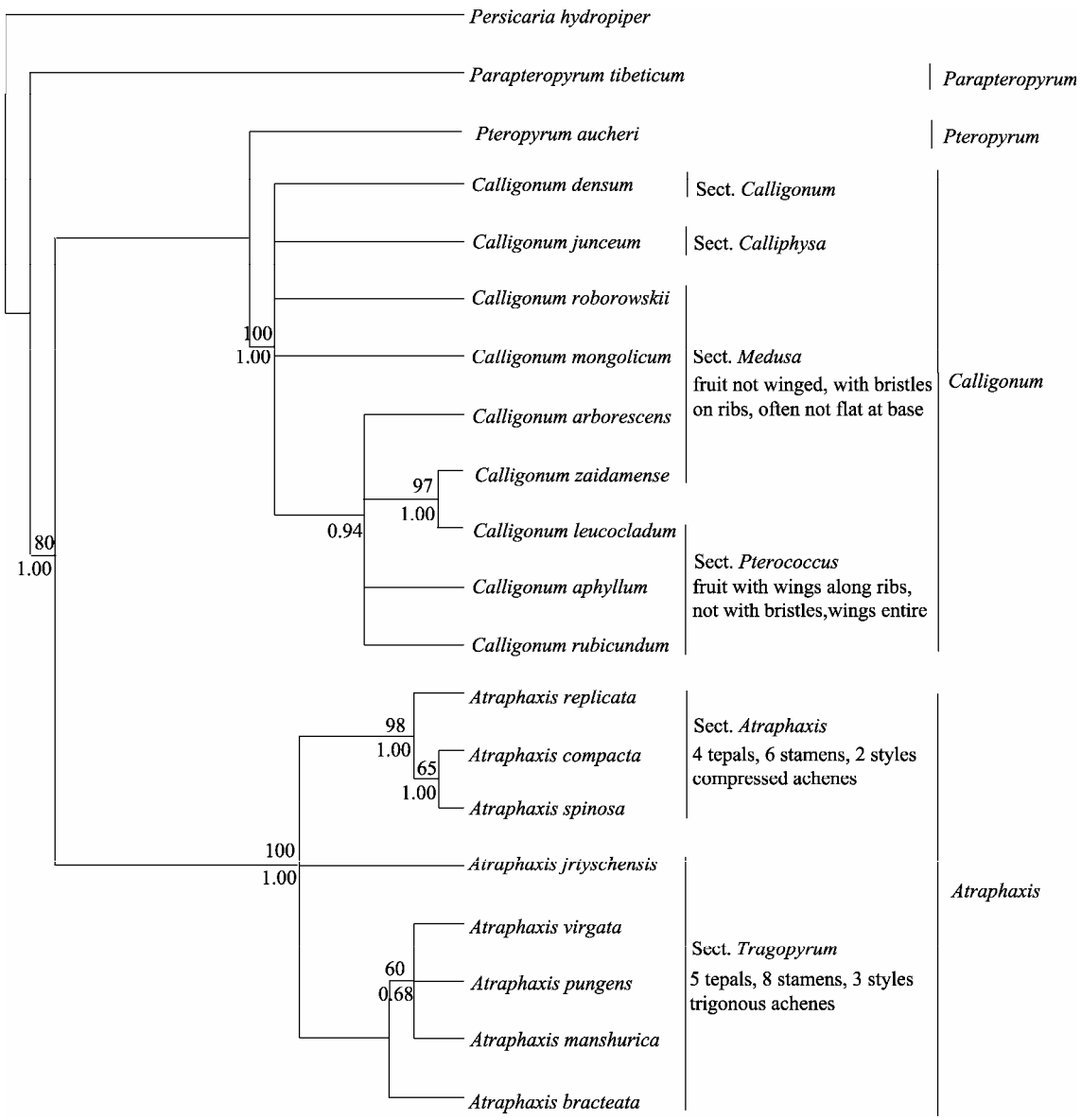

Fig. 2 Topology resulting from maximum likelihood analysis for the combined data set of 5-cpDNA genes (*atp*B-*rbc*L, *psb*A, *psb*K-I, *rbc*L, and *trn*L–F) using Garli (GTR+G+I). Bootstrap support values of maximum parsimony (MP) > 50% or posterior probability support Bayesian inference (PP) > 0.5 are indicated above and below the branches, respectively.

dum, *C. rubicundum*, and *C. zaidamense*. And *C. leucocladum* and *C. zaidamense* form a sister pair (MP/PP=97/1.00).

3 Discussion and conclusion

3.1 Non-monophyly of Atraphaxideae

Since the four genera in Atraphaxideae were placed in three separate clades in each of the analyses, Atraphaxideae in the phylogenetic tree of this study (Fig. 1) was clearly shown to be not monophyletic. This confirms previous molecular phylogenies (Sanchez *et al*., 2009; Tavakkoli *et al*., 2010) and the inferences from chromosome karyotypes (Tian *et al*., 2009), but it is contradicted by traditional morphological taxonomy, as mentioned above.

Recent molecular phylogenies (Sanchez *et al*., 2009; Tavakkoli *et al*., 2010; Sanchez *et al*., 2011) have shown that the four genera of Atraphaxideae did not form a monophyletic group, and only *Calligonum* and *Pteropyrum* were closely united in a single clade. However, few *Atraphaxis* species were sampled in the

previous studies. Sanchez *et al*. (2009) and Tavakkoli *et al*. (2010) each sampled only two species. This is obviously insufficient for this genus, which includes about twenty-five species. Furthermore, in an ITS tree (Sanchez *et al*., 2009; Fig. 2), two *Atraphaxis* species did not form a clade. This suggests that it is necessary to sample more taxa and more genes to obtain a robust molecular phylogeny. The present paper attempted to complement sufficient *Atraphaxis* samples with an increased sampling of their genes to detect relationships within *Atraphaxis* and among Atraphaxideae. Eight species were sampled from *Atraphaxis* collected in China, and five cpDNA sequences were conducted.

3.1.1 Placement of *Atraphaxis*

Atraphaxis is shown to be related to *Polygonum* s. str. and *Polygonella* (Fig. 1). This coincides with the classification systems of Haraldson (1978) and Brandbyge (1993). Both included *Atraphaxis*, *Polygonum* s. str. and *Polygonella* in the tribe Polygoneae. Our results agreed with previous molecular systematic studies (Lamb-Frye and Kron, 2003; Sanchez and Kron, 2008; Sanchez and Kron, 2009; Sanchez *et al*., 2009; Tavakkoli *et al*., 2010). Interestingly, *Atraphaxis*, sister to *Polygonella* and *Polygonum* s. str., had the same basic chromosome number, n=11 (*Polygonum* n=11 or 10) (Tian *et al*., 2009). Therefore, combined molecular, karyological and morphological data confirm that these three genera can be classified into one group.

3.1.2 Placement of *Parapteropyrum*

Parapteropyrum and *Fagopyrum* have a close relationship in the result tree (Fig. 1). This relationship was first noticed by Sanchez *et al*. (2009), and then by Tavakkoli *et al*. (2010). *Parapteropyrum*, a monotypic genus, is endemic to a narrow region of Tibet. Its diagnostic characters are an acute apex in the achene, calyx with entire wings in the fruit (wings have no break and do not divide into two), and inflorescences in the racemes. These characters are different from that of *Pteropyrum*, both genera at first was thought to be closely related in morphology. However, this morphological similarity could not obtain support from molecular evidence.

Fagopyrum is usually classified in Persicarieae (Ronse Decraene and Akeroyd, 1988; Brandbyge, 1993), and is distinguished from putatively related genera by the character of thick folded cotyledons lying in the center of the achene (Nakai, 1926; Ohnishi, 1998). This genus has been claimed to be closely related to *Fallopia* or *Persicaria* (Ronse Decraene and Akeroyd, 1988), but the morphological similarity between *Fagopyrum* and *Fallopia* or *Persicaria* was not confirmed by molecular evidence.

Although there are some differences between *Parapteropyrum* (shrub, unequal tepals) and *Fagopyrum* (herb, equal tepals), some similarities between them include: simple leaves, petiolate; ocrea membranous, oblique; bisexual flowers; perianth persistent, five-parted; stamens eight; styles three; stigmas capitate; achenes trigonous; perianth persistent, five-parted; and exine microreticulate. These characters of general morphology and pollen coincided with the molecular phylogeny. In other words, both morphology and molecular phylogeny provided convincing evidence for a close relationship between *Parapteropyrum* and *Fagopyrum*. As a result, it is likely that *Parapteropyrum* should be interpreted as being a hexaploid based on n=8, as found in *Fagopyrum* (Fig. 1), rather than a tetraploid based on n=12, as suggested previously by Tian *et al*. (2009).

3.1.3 Placement of *Calligonum* and *Pteropyrum*

A relationship between *Calligonum* and *Pteropyrum* had been suggested based on morphology (Tavakkoli *et al*., 2008) and molecular evidence (Sanchez *et al*., 2009; Tavakkoli *et al*., 2010). The topologies of our *rbc*L tree revealed a similar result, though not with high MP and Bayesian support (Fig. 1). Considering the weak relationship found between *Pteropyrum* and *Calligonum*, we prefer to suggest the similarities between *Pteropyrum* and *Calligonum* probably to be a convergence.

In the classification systems of Haraldson (1978) and Brandbyge (1993), the four Atraphaxideae genera plus *Fagopyrum*, *Fallopia* (*Fallopia* was absent in Polygoneae), *Oxygonum*, *Polygonum* s. str., *Polygonella* and *Reynoutria* were included in the tribe Polygoneae. This treatment for the tribe Polygoneae can not be supported by the present study, since the members of this tribe do not form a single clade and are scattered throughout subfamily Polygonoideae (Fig. 1).

3.2 Infrageneric relationships in *Atrapahxis* and *Calligonum*

Since only one of four to five species of *Pteropyrum* was sampled and *Parapteropyrum* is monotypic, we pay attention to the infra-generic relationships of *Atraphaxis* and *Calligonum* respectively as follows. The monophyly of both genera respectively was well supported (Figs. 1, 2).

3.2.1 Infrageneric relationships in *Atraphaxis*

There are three sections recognized within *Atraphaxis* (Lovelius, 1978; Bao and Li, 1993). The Chinese *Ataphaxis* species are divided into two sections (Bao and Li, 1993; Li *et al*., 2003), i.e., section *Atraphaxis* and section *Tragopyrum*. The species *A. compacta*, *A. spinosa*, *A. canescens* and *A. replicata* (variety of *A. spinosa*) were placed into section *Atraphaxis*, distinguished by four tepals, six stamens, two styles and lenticularly compressed achenes. The other species, characterized by five tepals, eight stamens, three styles and trigonous achenes, were members of section *Tragopyrum*. As shown in Fig. 2 (five-gene tree), our results, on the whole, coincide with this current sectional classification. The clade formed by *A. compacta*, *A. spinosa* and *A. replicata*, and belonging to section *Atraphaxis*, was strongly supported (MP/PP = 98/100) (note that *A. replicata* is sometimes treated as a variety of *A. spinosa*). *A. jrtyschensis* was not included a weak supported clade made up of four species, *A. virgata*, *A. pungens*, *A. manshurica* and *A. bracteata* in section *Tragopyrum* (Fig. 2). Section *Atraphaxis* and section *Tragopyrum* differ in morphological aspect, including tepal number, stamen number, style number and achene shape (Fig. 2), the present molecular phylogenetic tree is congruent with their dissimilarities.

3.2.2 Infra-generic relationships in *Calligonum*

According to Mao *et al*. (1983), Mabberley (1990), Bao and Li (1993), Li *et al*. (1998), Li *et al*. (2003) and Tavakkoli *et al*. (2008), *Calligonum* possesses 30–80 species. It is a xeromorphic plant, and is distributed from northern Africa and southern Europe to Central Asia, including northwestern China as well as northeastern China. Three to four sections have been recognized in *Calligonum*: *Calliphysa*, *Calligonum*, *Medusa* and *Pterococcus* (Pavlov, 1936; Mao *et al*., 1983; Li *et al*., 2003) but Rechinger and Schiman-Czaika (1968) and Tavakkoli *et al*. (2008) did not recognize section *Medusa*.

Recently, Tavakkoli *et al*. (2008) presented a cladistic analysis of 18 species using 30 morphological characters. The results revealed that *Calligonum* was monophyletic and composed of two clades: one including the winged fruit species (section *Pterococcus*), and the other the bristled fruit taxa (section *Calligonum*). We sampled nine species that represented all four sections. The five-gene tree (Fig. 2) provided an identical topology for *Calligonum*, which was illustrated as absolutely monophyletic with high support MP/PP=100/1.00. However, none of the sections was monophyletic. A possible monophyletic clade was section *Pterococcus* with Bayesian support PP=94 (in the five-gene tree), however, since *C. arborescens* and *C. zaidamense* of section *Medusa* are nested within section *Pterococcus*, this destroyed the monophyly of section *Medusa*. It is notable that *C. zaidamense* from section *Medusa* and *C. leucocladum* from section *Pterococcus* show a close relationship with high support MP/PP=97/1.00. Together with the results of the morphological cladistic study (Tavakkoli *et al*., 2008) in which *C. arborescens* of section *Medusa* is nested in section *Calligonum*, these results shown the necessity of carrying out a comprehensive study of *Calligonum* with much increased sampling.

3.3 Incongruence among morphology, chromosome and molecular phylogeny in Atraphaxideae evolution

Concerning the evolution of the tribe, different opinions have been proposed in the light of the evidence, mainly from morphology, pollen, embryo and chromosome karyotype. In addition to the evolutionary trends for Atraphaxideae, Calligonum was proposed as the least derived genus. Based on a comprehensive evolutionary trend analysis of embryo, pollen and morphology, Bao and Li (1993) suggested that *Atraphaxis* is the most primitive in Atraphaxideae, and within *Atraphaxis*, section *Tragopyrum* is more primitive than section *Atraphaxis*. In *Calligonum*, in terms of chromosome numbers and anatomy of young branches, Mao *et al*. (1983) considered section *Calliphysa*, identifying *C. junceum* as the most primitive, and section *Medusa* as the most advanced, but there

are many different opinions (Mao *et al*., 1983). Chromosome basic numbers have been treated as an important evolutionary feature in Polygonaceae, n=11 being considered the most likely primitive number and others being hypothesized to have derived from it (Jaretzky, 1928). Maekawa (1964) considered n=14 to be the original basic number in the family Polygonaceae, leading to a descending aneuploid series. Tian *et al*. (2009) also suggested that aneuploid evolution played an important role in the early diversification of the Atraphaxideae.

Even though we have broadly sampled the outgroup and sequenced five genes, we could not sufficiently discuss the Atraphaxideae evolution from molecular phylogeny since we were unable to determine which taxon was primitive or advanced in the phylogenetic trees. In other words, we could not find the evolutionary trend from molecular phylogeny, like a chromosome or one consistent with it. From the *rbc*L tree (Fig. 1), the scattered Atraphaxideae clades are paraphyletic, thus the most primitive taxon could not be ascertained. Similarly, within *Atraphaxis*, sections *Atraphaxis* and *Tragopyrum* were paraphyletic, so we could not judge which was primitive or advanced. Moreover, we found some contradictions between the general morphological taxonomic inference and molecular phylogeny. For instance, *Parapteropyrum*, a tetraploid or hexaploid species with narrow distribution in the Tibetan Plateau, should obviously be considered advanced in view of insights from the general morphology and chromosome base number. However, in the phylogenetic tree (five-gene tree, Fig. 2), it is derived from an ancestor of many taxa and seems primitive. To discuss the evolution of Atraphaxideae, much molecular work is needed in the future.

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Appendix

Sequence data downloaded from the Genbank

***rbc*L**—*Aconogonon divaricatum* L., FM883603;
Aconogonon xfennicum Reiersen, FM883604;
Antigonon guatimalense Meisn., FJ154449;
Antigonon leptopus Hook. & Arn., AF297146;
Armeria bottendorfensis A. Schulz, Z97640;
Armeria splendens (Lag. & Rodr.) Webb, Y16908;
Bistorta vivipara (L.) S.F. Gray, EU840288;
Brunnichia cirrhosa Gaertn., AF297136;
Coccoloba densifrons Mart. ex Meisn., AF297138;
Coccoloba pyrifolia Desf., Z97647;
Emex spinosai (L.) Gampdera, AF297142;
Fagopyrum esculentum Moench, EU840292;
Fagopyrum tataricum (L.) Gaertn., D86287;
Fallopia aubertii (L. Henry) Houlub, EU840324;
Fallopia convolvulus (L.) Löve, FM883612;
Fallopia japonica (Houtt.) Dcne., AF297131;
Gymnopodium floribundum Rolfe, GQ206220;
Koenigia forrestii (Diels) Mesicek & Sojak., AF297144;
Koenigia islandica L., EF653763;
Leptogonum buchii Urb., GQ206223;
Limonium rigualii M.B., Z97645;
Limonium dendroides Svent., Z97644;
Oxyria digyna (L.) Hill., EU840291;
Oxyria sinensis Hemsl., AF297148;
Persicaria hydropiper f. ciliare Domin, FM883629;
Plumbago auriculata Lam., EU002283;
Polygonella articulate (L.) Meisn., EF653760;
Polygonella fimbriata (Elliot) Horton, AF297132;
Polygonum aviculare L., AF297127;
Pteropyrum aucheri Jaub. et Spach, GQ206227;
Rheum moorcroftianum Royle, EU840300;
Rheum reticulatum A. Los., EU840299;
Rumex acetosa L., AY395559;
Rumex crispus L., EU840290;
Rumex obtusifolius L., AF297126;
Ruprechtia laxiflora Meisn., EF437987;
Ruprechtia tangarana Standl., GQ206233;
Triplaris americana L., Y16910;
Triplaris poeppigiana Weddell., AF297137.

***psb*K-*psb*I**—*Persicaria hydropiper* f. ciliare Domin, EU749803.

***trn*L-*trn*F**—*Persicaria hydropiper* f. ciliare Domin, EF653806.

***psb*A-*trn*H**—*Persicaria hydropiper* f. ciliare Domin, EF653754.