Phylogenetic Position of *Polygonum bungeanum* in *Polygonum* L.s.lat. (Polygonaceae) as Evidenced from nrDNA ITS, cpDNA *atp*B-*rbc*L and *trn*L-F Sequences

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Abstract: *Polygonum bungeanum* is an annual herb with erect or ascending stems, retrorse prickles, lanceolate or narrowly elliptic leaf blades and spicate inflorescences, whose distribution ranges from northeastern and northern China to Japan, Korea and Russia (Far East). In the present study, the phylogenetic position of *Polygonum bungeanum* in *Polygonum* L.s.lat. was assessed using internal transcribed spacer (nrDNA *ITS*) from nuclear ribosomal DNA, chloroplast (cp) DNA *atpB-rbcL* and *trnL-F* sequence data. The results of maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses of these data showed that there were no differences in the topological structures of the phylogenetic trees and the cpDNA sequences, especially with the combined sequence data, which were more appropriate than the *ITS* single sequence data for the analyses. It was suggested that sect. *Persicaria* and sect. *Echinocaulon* should be raised to genus *Persicaria* and genus *Tracaulon*, respectively, and that *P. bungeanum* should be placed in the genus *Persicaria*, changing its name to *Persicaria bungeanum* Nakai ex Mori. Our study provides evidence in support of Li Anjen's theory that the genus *Antenoron* should be treated as an independent genus, and that the three genera may be closely related sister groups.
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1. Introduction

The genus *Polygonum* L.s.lat., which has approximately 230 species distributed from northeastern and northern China to Japan, Korea and Russia (Far East), consists of perennial herbs and a few subshrubs or small shrubs. The plants' stems are erect, prostrate or ascending, usually with conspicuously swollen nodes. All of them have membranous tubular ocreas (Li *et al*., 2003).

The genus *Polygonum* in the broad sense is generally assigned to a variety of distinct genera, such as *Polygonum* s.s., *Persicaria*, *Aconogonon* and *Bistorta* (Gross, 1913; Hedberg, 1946; Haraldson, 1978). Steward subdivided *Polygonum* into several sections, including *Avicularia*, *Bistorta*, *Persicaria*, *Cephalophilon*, *Tiniaria*, *Aconogonon*, *Fagopyrum* and *Echincaulon*, based on their fruits, perianths, inflorescences and ocreas (Steward, 1930). In Flora of China (Li *et al*., 2003), the genera *Fagopyrum*, *Antenoron*, *Reynourtria* and *Fallopia* were treated as separate genera, and the genus *Polygonum* was subdivided into six sections, including *Polygonum* sect. *Polygonum*, *Polygonum* sect. *Bistorta*, *Polygonum* sect. *Persicaria*, *Polygonum* sect. *Cephalophilon*, *Polygonum* sect. *Aconogonon* and *Polygonum* sect. *Echincaulon*.

Ronse Decraene & Akeroyd treated *Polygonum* sect. *Cephalophilon* and *Polygonum* sect. *Echinocaulon* as components of an expanded *Persicaria* sect. *Persicaria* (Ronse Decraene & Akeroyd, 1988). *Persicaria* Mill. is comprised of the sections *Amblygonum* Meisn., *Cephalophilon* Meisn., *Echinocaulon* Meisn., *Persicaria* Meisn. and *Tovara* Adans. The sections of *Persicaria* Mill. assigned to this genus show similarities in their pollen grains which, in all investigated species, are spherical and have a more or less coarse reticulum (Hedberg, 1946).

The section *Echinocaulon* Meisn. was raised to a genus with the name *Tracaulon*, and this classification has been maintained by a majority of later American authors. When studying only North American material, this change appears to be very reasonable. However, when material from East Asia is included, there is no distinct boundary between the sections *Echinocaulon* and *Persicaria*. Both sections have exactly the same *Persicaria*–type pollen (Hedberg, 1946; Zhang & Zhou, 1988). The resemblance of these two sections had been pointed out before by Gross in 1913 (Gross, 1913).

The plants of *Polygonum* sect. *Persicaria* (Miller) Meisner (Li *et al*., 2003) have no prickles and their leaves are generally narrowly elliptical to ovate, not hastate nor sagittate. Furthermore, the prostrate or climbing plants of *Polygonum* sect. *Echinocaulon* Meisner, which have hastate to sagittate leaves and

capitate to shortly paniculate inflorescences (Li *et al*., 2003), often have retrorse prickles on their stems and petioles. So, *P*. sect. *Persicaria* should be distinct from *P*. sect. *Echinocaulon* based on morphology.

Polygonum bungeanum Turcz. was assigned to *P*. sect. *Echinocaulon* by Li (Li *et al*., 2003); however, it shares more morphological characteristics with *P*. sect. *Persicaria*, such as erect or ascending stems, lanceolate or narrowly elliptical leaf blades and spicate inflorescences, with the exception that it is retrorsely prickly. Thus, its systematic position should be further investigated.

Molecular phylogenetic studies have successfully provided references for the systematic positions of generic and infrageneric classifications. Many documents have reported that nuclear ribosomal DNA ITS (nrDNA *ITS*, or *ITS* for short) and chloroplast DNA (cpDNA) *atpB-rbcL* and *trnL-trnF* sequences were valuable markers for the phylogenetic and evolutionary inferences of species (Sun *et al*., 2008; Morton, 2009; Sanchez *et al*., 2009; Xu *et al*., 2009; Cecchi *et al*., 2010; Kumagai *et al*., 2010; Liu *et al*., 2010; Martinez *et al*., 2010; Rua *et al*., 2010; Wang *et al*., 2010)**.**

The objectives in this study were as the followings: (1) to provide a more comprehensive understanding of the relationship between *P*. sect. *Persicaria* and *P*. sect. *Echinocaulon;* (2) to understand the systematic position of *Polygonum bungeanum* Turcz. in the genus *Polygonum* L.s.lat.; (3) to understand which one in *ITS* or cpDNA sequences is a more appropriate approach for phylogenetic infrageneric analyses in Polygonaceae; and (4) to determine the most suitable method for the reconstruction of phylogenetic trees among maximum parsimony (MP), maximum likelihood (ML) or Bayesian inference (BI) methods.

2. Material and Methods

2.1. Plants. — A total of 32 species of the family Polygonaceae from China, including eight species of *Polygonum* sect. *Echinocaulon*, 11 species of *Polygonum* sect. *Persicaria* were used in this study. One species of *Polygonum* sect. *Polygonum*, two species of *Polygonum* sect. *Bistorta*, two species of *Polygonum* sect. *Cephalophilon*, two species of *Polygonum* sect. *Aconogonon*, one species of genus *Antenoron***,** one species of genus *Reynoutria*, two species of genus *Fallopia* and two species of genus *Fagopyrum* species were used for reference (Table 1). *Polygonum lichiangense* W.W. Smith, *Polygonum sibiricum* Laxm. and *Polygonum chinense* L. were used as outgroups. Species and sections were sampled according to the classification by Li & Bao (Li & al., 2003). All of the accessions were collected from the field by Prof. Zhong-ze Zhou, Yun-jiang Min, Pan Gao, Chun Yue, *et al*. (Anhui University, China). The voucher species and plant materials were stored at Anhui University, China (ANU).

2.2. DNA extraction, amplification and sequencing. — Total DNA was extracted from silica gel-dried leaf materials (Table 1), following the modified CTAB procedure (Doyle & Doyle, 1987). Fragments of the nr DNA *ITS* region (including *ITS1*, 5.8S and *ITS2*) and the chloroplast DNA (cpDNA) *atpB-rbcL* and *trnL-trnF* sequences were amplified with the following universal primer pairs: (1) for *ITS*, *P18S* (5'-CGT AAC AAG GTT TCC GTA GGT GAA G-3') and *P26S* (TTA TTG ATA TGC TTA AAC TCA GCG GG-3') (Sun & *al*., 2008); (2) for *atpB-rbcL*, *atpB* (5' –ACA TCK ART ACK GGA CCA ATA A-3') and *rbcL*(5' –AAC ACC AGC TTT RAA TCC AA-3') (Chiang *et al*., 1998); and (3) for *trnL-trnF*, 5' *trnL*(UAA) (5' –CGA AAT CGG TAG ACG CTA CG-3') and *trnF*(GAA) (5' –ATT TGA ACT GGT GAC ACG AG-3') (Taberlet *et al*., 1991), respectively. The primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd., China. Every 50-μL PCR reaction mixture included 1 μL template DNA, 1 μL 20 μM of each primer, 0.5 μL Taq DNA polymerase (5 U/μL), 5 μL l0 × PCR buffer (with Mg^{2+}), 1 μL dNTPs (10 mM) and 40.5 μL H_2O. PCR reactions were carried out respectively using the following procedures: (1) for *ITS*, an initial denaturation at 95 ℃ for 4 min., followed by 39 cycles of denaturation at 95 ℃ for 1 min., annealing at 52 ℃ for 1 min. and extension at 72 ℃ for 1 min., and a final extension at 72 ℃ for 7 min.; (2) for *atpB-rbcL*, an initial denaturation at 94 ℃ for 4 min., followed by 39 cycles of denaturation at 94 ℃ for 45s., annealing at 49 ℃ for 45s and extension at 72 ℃ for 75s, and a final extension at 72 ℃ for 10 min; and (3) for *trnL- trnF*, an initial denaturation at 95 ℃ for 5 min., followed by 39 cycles of denaturation at 95 ℃ for 1 min., annealing at 56 ℃ for 30s and extension at 72 ℃ for 1 min., and a final extension at 72 ℃ for 7 min. PCR products were purified on 1% agarose gels using the QIAquick® Purification Kit and directly sequenced on an ABI 3770 Automated DNA Sequencer (Shanghai Sangon Biological Engineering Technology and Service Co., Ltd., China). Sequencing primers were the same as those used for the previous PCR reaction and were used singly in forward and reverse reactions.

2.3. Sequence comparisons and phylogenetic analyses. — A BLAST analysis was carried out on all of the sequences (http://blast.ncbi.nlm.nih.gov /Blast.cgi) to confirm that they originated from Polygonaceae plants. The forward and reverse sequences were initially assembled and aligned, using the Geneious 4.8.3 software package (Biomatters Ltd., http://www.geneious.com/), and then manually adjusted as necessary.

All chloroplast sequences, including *atpB-rbcL*

and *trnL-trnF*, were concatenated directly to the cpDNA dataset (*atpB-rbcL* + *trnL-trnF*), and then the cpDNA and *ITS* sequences were concatenated to create another combined dataset (*atpB-rbcL* + *trnL-trnF* + nrDNA *ITS*). The cpDNA and nrDNA *ITS* markers were each analyzed separately and the combined data matrices were also assayed by maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI), respectively.

Parsimony and maximum likelihood analyses were carried out with PAUP* version 4.0b10 (Swofford, 2003).

Under MP, all characteristics were treated as unordered and were equally weighted for the heuristic searches. Gaps were treated as missing, and multistate taxa were classified as uncertain. Each heuristic search used 1,000 random sequence addition replicates, stepwise addition, MulTrees and tree-bisection-reconnection (TBR) branch swapping. MP bootstrap percentages were calculated from 1,000 bootstrap replicates, with MulTrees deactivated and a single random addition tree as the starting point.

The substitution models for maximum likelihood were chosen using an Akaike Information Criterion (AIC) of the models implemented in Modeltest 3.7 (Table 2). ML bootstraps were carried out for the heuristic searches with 1,000 bootstrap replicates. The parameter settings corresponded to the GTR+G mode (or GTR+G+I mode, ITS). Starting tree(s) were obtained via stepwise addition and random sequence addition with 10 replicates, with one tree held at each step during the stepwise addition and TBR branch swapping.

Bayesian inference analysis was conducted using the MrBayes version 3.1.2 plug-in, (Huelsenbeck & Ronquist, 2001) for the Geneious 4.8.3 software package. All of parameters used were default settings for calculating every matrix, as described below. The nucleotide substitution model was HKY85, the model for among-site rate variation was gamma and the number of rate categories for the gamma distribution was 4. Markov Chain Monte-Carlo (MCMC) settings included a chain length of 1,100,000, a subsampling frequency of 200, four heated chains, a burn-in length of 100,000 and a heated chain temperature of 0.2.

3. Results

3.1. Phylogeny analysis using *ITS*, *atpB-rbcL*, *trnL-trnF* and combined datasets. — Incongruence length difference tests confirmed that there was no significant difference between the phylogenetic signals of the chloroplast (*atpB-rbcL*, *trnL-trnF*) and nuclear (*ITS*) datasets (P = 0.817). Slight topological differences between individual phylogenetic analyses of chloroplast and nuclear sequence data did not show minimal bootstrap support (> 50%) or significant Bayesian posterior probabilities (≥ 0.95); thus, we report results from phylogenetic analyses of combined chloroplast datasets or combined chloroplast and nuclear datasets (Baird *et al*., 2010).

The details of the nrDNA *ITS*, *atpB-rbcL*, *trnL-trnF*, *atpB-rbcL+trnL-trnF* and *atpB-rbcL +trnL-trnF*+nrDNA *ITS* DNA dataset matrices, including the characteristics and statistics from the maximum parsimony and selected best-fit models for maximum likelihood analyses by AIC in Modeltest 3.7, are described in Table 2. The gaps were always treated as missing.

Two cpDNA and nrDNA *ITS* markers were analyzed separately, and then the two data matrices were combined (Baird *et al*., 2010). The phylogenetic trees based on maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI), respectively, were constructed, using each marker. The results showed that similar topologies with different bootstrap (BS) and posterior probability (PP) values were generated by MP, ML and BI. The trees derived using two cpDNA-combined matrices and three combined marker datasets are shown in Figs. 1--2, but the trees derived using each individual cpDNA or nrDNA *ITS* marker are not shown.

The results of the phylogenetic analyses showed that: (1) there were no differences in the topological constructions of the phylogenetic trees generated by MP, ML and BI; (2) the cpDNA sequences, especially the combined sequence data, were more appropriate than the *ITS* single sequence data for phylogenetic infrageneric analyses in Polygonaceae, providing a more reasonable topology structure and better percentage support values on the phylogram; (3) evidence obtained from the molecular data does not support the traditional systematic position of *Polygonum bungeanum* in section *Echinocaulon et al*., 2003), but rather in section *Persicaria*, based on morphological characteristics; (4) all of the phylogenetic trees (MP, ML and BI) support the conclusion that section *Echinocaulon* and section *Persicaria* form a monophyletic group with relatively high bootstrap and posterior probability (ML-BS /MP-BS = 100%, BI-PP = 1.0) support values (Figures.1--2), validating the viewpoints of Hedberg (Hedberg, 1946; Zhang & Zhou, 1988) and Gross (Gross, 1913).

4. Discussions

The phylogenetic analysis performed in this study using molecular data suggested that sect. *Echinocaulon* and sect. *Persicaria* were sister groups with close affinities. Although Li Anjen (Li *et al*., 2003) placed *P. bungeanum* within its own section instead of in sect. *Persicaria* because of the retrorse prickles on the stems, our molecular datasets robustly supported *P.*

bungeanum being moved to sect. *Persicaria*. The results of the present study showed that *P*. sect. *Persicaria* and *P*. sect. *Echinocaulon* were monophyletic, as shown in clades A and B on Figures. 1--2. However, *P. bungeanum* did not nest within clade A, which was entirely represented by *P*. sect. *Echinocaulon* (Li *et al*., 2003). Instead, it was well-nested within clade B, which was sister to group A (*P*. sect. *Echinocaulon*) (Nie *et al*., 2009; Baird & al., 2010). Compared to the other species in this study, the members of clades A and B and *Antenoron filiforme* var. *neofiliforme* were the most closely related, as they were nested within the same clade from the base. Even the genus *Antenoron* was more closely related to sect. *Persicaria*, since the representative of the genus *Antenoron filiforme* var. *neofiliforme* was nested within the same subclade and was sister to group A (sect. *Echinocaulon*), with MLBS/MPBS/BIBS = 100/100/ 1.0 (Figures. 1--2).

The morphological characteristics of sect. *Persicaria*, such as the presence of prickles, stem morphology, leaf forms and inflorescence styles, are obviously different from that of sect. *Echinocaulon et al*., 2003). However, both sect. *Echinocaulon* and sect. *Persicaria* have morphologically similar *Persicaria*–type pollen (Hedberg, 1946). The pollen of the genus *Antenoron* was obviously different from the Persicaria type, being more similar to the Tovara type (Zhang & Zhou, 1988).

Polygonum bungeanum Turcz. possesses more *Persicaria*-like morphological characteristics, such as erect or ascending stems, lanceolate or narrowly elliptical leaf blades and spicate inflorescences. Thus, it should be assigned to sect. *Echinocaulon* rather than sect. *Persicaria*. We hypothesized that the prickles on the stems are a variation resulting from long-term adaptation to cold growth environments, since their distribution ranges from Gansu, Hebei, Heilongjiang, Jiangsu, Jilin, Liaoning, Nei Mongol, Ningxia, Shandong and Shanxi in China to Japan, Korea and Russia (Far East) (Li *et al*., 2003; Wu *et al*., 2003).

5. Conclusion

In consideration of the above analysis, our study suggested that sect. *Persicaria* and sect. *Echinocaulon* should be raised to genera in agreement with the findings of Hedberg (Hedberg, 1946), i.e., the genus *Persicaria* and the genus *Tracaulon*, respectively, as well as *P. bungeanum*, should be placed within the genus *Persicaria*, so its name should be *Persicaria bungeanum* Nakai ex Mori. Our results were also in accordance with those of Li Anjen (Li *et al*., 2003), who treated the genus *Antenoron* as an independent genus and suggested that the three genera should be considered to be the closest related sister groups.

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Table 1. Species of Polygonaceae sampled in analyses, with voucher information and Genbank numbers (ANU = Anhui University; 18 accessions with sign * were obtained from GenBank, and the remaining 78 accessions were new)

Taxa	Locality	Collector(s); Number (Herbarium)	GenBank No.		
			*atp*B-*rbc*L	*trn*L-*trn*F	nrDNA ITS
Polygonum perfoliatum L.	Tiantangzhai, Anhui	Min Y. J. 09067 ANU	HQ843098	HQ843151	DQ372904*
Polygonum thunbergii Sieb. et Zucc.	Xiaokeng, Anhui	Zhou Z. Z. 09064 ANU	HQ843099	HQ843152	HQ843128
Polygonum senticosum (Meisn.) Franch. et Sav.	Xiaokeng, Anhui	Zhou Z. Z. 09058 ANU	HQ843117	EU024791*	HQ843141
Polygonum darrisii Lévl.	Xiaokeng, Anhui	Zhou Z. Z. 09060 ANU	HQ843104	HQ843156	HQ843133
Polygonum dissitiflorum Hemsl.	Xiaokeng, Anhui	Zhou Z. Z. 09065 ANU	HQ843120	HQ843168	HQ843147
Polygonum sieboldii Meisn.	Dali, Yunnan	Min Y. J. & Zhou Z. Z. 09012 ANU	HQ843118	EU109603*	DQ006031*
Polygonum bungeanum Turcz.	Qipanshan, Liaoning	Zhou Z. Z. 08082 ANU	HQ843106	HQ843158	HQ843134
Polygonum hastato-sagittatum Mak.	Xiaokeng, Anhui	Zhou Z. Z. 09063 ANU	HQ843119	HQ843167	HQ843142
Polygonum amphibium L.	Lijiang, Yunnan	Min Y. J. & Zhou Z. Z. 09016 ANU	HQ843113	EF653803*	EF653700*
Polygonum japonicum Meisn.	Dali, Yunnan	Min Y. J. & Zhou Z. Z. 09006 ANU	HQ843109	HQ843162	DQ406634*
Polygonum viscosum Buch.-Ham. ex D. Don	Hefei, Anhui	Min Y. J. 09068 ANU	HQ843116	HQ843166	HQ843140
Polygonum persicaria L.	Xiaokeng, Anhui	Zhou Z. Z. 09057 ANU	HQ843111	EU024781*	HQ843137
Polygonum viscoferum Mak. var. *rubstum* Mak.	Xiaokeng, Anhui	Zhou Z. Z. 09056 ANU	HQ843112	HQ843163	HQ843138
Polygonum lapathifolium L.	Baihe, Jilin	Zhou Z. Z. 08085 ANU	HQ843105	HQ843157	DQ631412*
Polygonum orientale L.	Shenyang, Liaoning	Zhou Z. Z. 08081 ANU	HQ843103	HQ843155	HQ843132
Polygonum hydropiper L.	Lijiang, Yunnan	Min Y. J. & Zhou Z. Z. 09015 ANU	HQ843114	HQ843164	U51275*
Polygonum pubescens Blume	Xiaokeng, Anhui	Zhou Z. Z. 09059 ANU	HQ843115	HQ843165	HQ843139
Polygonum posumbu Buch.-Ham. ex D. Don	Xiaokeng, Anhui	Z. Z. Zhou 09059 ANU	HQ843110	EU024778*	EF653701*
Polygonum longisetum De Br.	Baihe, Jilin	Zhou Z. Z. 08086 ANU	HQ843107	HQ843159	HQ843135
Polygonum lichiangense W. W. Smith	Lijiang, Yunnan	Min Y. J. & Zhou Z. Z.09025 ANU	HQ843121	HQ843169	HQ843144
Polygonum sibiricum Laxm.	Shangri-La, Yunnan	Min Y. J. & Zhou Z. Z. 09030 ANU	HQ843095	HQ843148	HQ843125
Polygonum chinense L.	Kunming, Yunnan	Zhou Z. Z. 08072 ANU	DQ539664*	HQ843150	FJ648806*
Polygonum microcephalum var. *sphaerocephalum* H. Hara	Emeishan, Sichuan	Min Y. J. & Zhou Z. Z. 09055 ANU	HQ843123	HQ843171	HQ843145
Polygonum aviculare L.	Shangri-La, Yunnan	Min Y. J. & Zhou Z. Z.09033 ANU	DQ539667*	HQ843161	DQ406624*
Polygonum subscaposum Diels	Lijiang, Yunnan	Min Y. J. & Zhou Z. Z. 09022 ANU	HQ843122	HQ843170	HQ843143
Polygonum macrophyllum var. *stenophyllum* (Meisner)A. J. Li	Mangkang, Xizang	Min Y. J. & Zhou Z. Z. 09048 ANU	HQ843108	HQ843160	HQ843136
Fallopia denticulata (C. C. Huang) J. Holub	Kunming, Yunnan	Zhou Z. Z. 08073 ANU	HQ843096	HQ843149	HQ843126
Fallopia dentate-alata (F. Schm.) Holub	Shenyang, Liaoning	Zhou Z. Z. 08083 ANU	HQ843102	EU024785*	HQ843131
Reynoutria japonica Houtt.	Tiantangzhai, Anhui	Min Y. J. 09066 ANU	HQ843097	EU024786*	HQ843127
Antenoron filiforme var. *neofiliforme* (Nakai) A. J. Li	Tiantangzhai, Anhui	Min Y. J. 09124 ANU	HQ843100	HQ843153	HQ843129
Fagopyrum tataricum (L.) Gaertn.	Shangri-La, Yunnan	Min Y. J. & Zhou Z. Z.09031 ANU	HQ843124	HQ843172	HQ843146
Fagopyrum caudatum (Sam.) A. J. Li	Kunming, Yunnan	Min Y. J. & Zhou Z. Z. 08121 ANU	HQ843101	HQ843154	HQ843130

Table 2. Details of the different datasets used in this analysis (The gaps were treated as missing; *atp*B-*rbc*L + *trn*L-*trn*F and *atp*B-*rbc*L + *trn*L-*trn*F + nrDNA ITS means that two or three markers, respectively, were combined into a simple dataset. Best-fit model means were selected for likelihood by AIC in Modeltest 3.7.)

Dataset	nrDNA ITS	*atp*B-*rbc*L	*trn*L-*trn*F	*atp*B-*rbc*L + *trn*L-*trn*F	*atp*B-*rbc*L + *trn*L-*trn*F + nrDNA ITS
Total characteristics	675	906	1020	1926	2601
Average (G+C) content (%)	63.8	29.9	34.3	32.1	40.6
Variable characteristics (%)	542 (80.3)	483 (53.3)	411 (40.3)	894 (46.5)	1436 (55.2)
Parsimony-informative characteristics for MP (%)	299 (44.3)	232 (25.6)	234 (22.9)	466 (24.2)	765 (29.4)
Tree length	1472	782	654	1441	3021
Consistency index (CI)	0.6250	0.8095	0.8073	0.8057	0.5811
Retention index (RI)	0.6415	0.8261	0.8540	0.8372	0.7018
Best-fit model	GTR+G	TVM+G	K81uf+G	K81uf+G	GTR+G

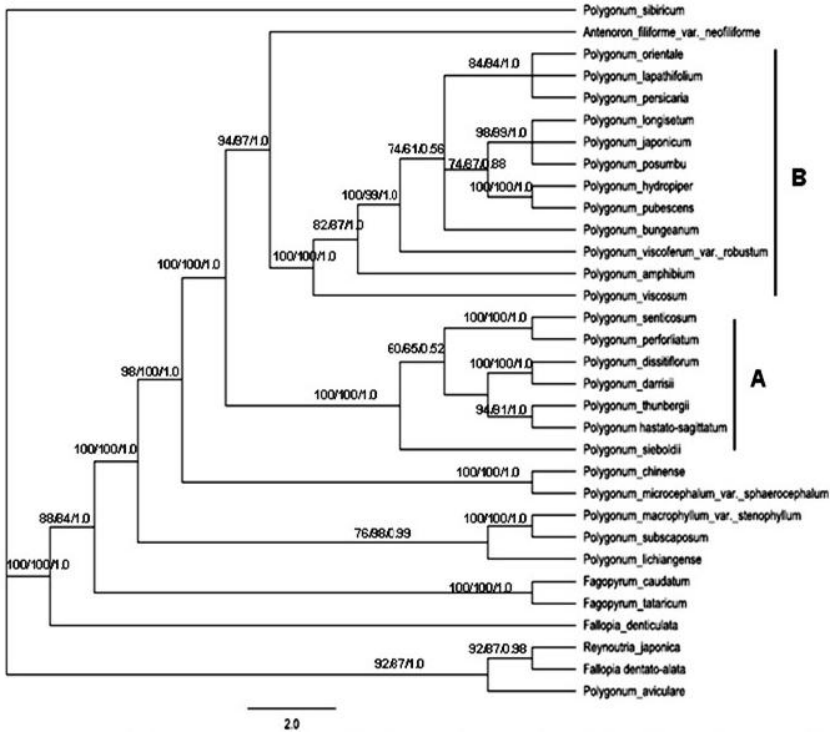

Figure 1. Phylogram of the best maximum likelihood (ML) tree as determined by cpDNA *atp*B-*rbc*L and *trn*L-*trn*F combined sequence datasets. All branches have support indices of 100/100/1.0 (maximum likelihood [ML] bootstrap percentage / maximum parsimony [MP] bootstrap percentage / Bayesian inference posterior probability).

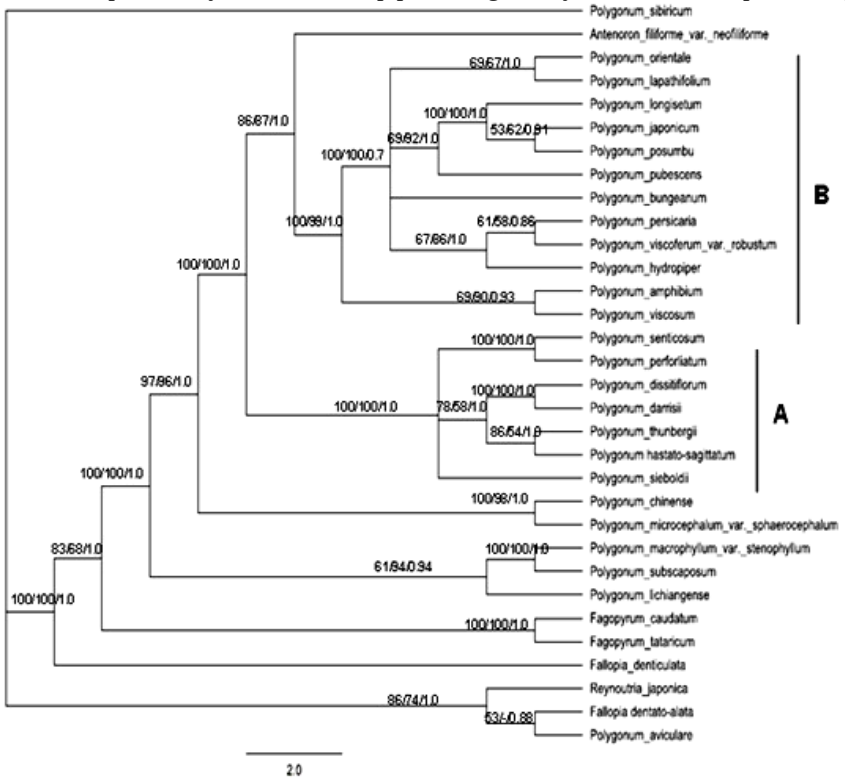

Figure 2. Phylogram of the best maximum likelihood (ML) tree as determined by ITS, cpDNA atpB-rbcL and trnL-trnF combined sequence datasets. All branches have support indices of 100/100/1.0 (maximum likelihood bootstrap percentage [MLBS] / maximum parsimony bootstrap percentage [MPBS] / Bayesian inference posterior probability [BIPP]).