

***Caelestium*, genus novum (*Polygonaceae*, *Polygoneae*): evidence based on the results of molecular phylogenetic analyses of tribe *Polygoneae*, established with consideration of the secondary structure of the ITS nrDNA regions**

***Caelestium*, genus novum (*Polygonaceae*, *Polygoneae*): обоснование, представленное по результатам молекулярно-филогенетических анализов трибы *Polygoneae*, предпринятых с учетом вторичной структуры локусов ITS1 и ITS2 ядерной рибосомной ДНК**

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Abstract. The genus *Bactria*, recently described to house *Atraphaxis ovczinnikovii* from the Pamirs and *Bactria lazkovii* from the Central Tien Shan, may also include *Polygonum tianschanicum* from the Eastern Tien Shan. In order to re-circumscribe *Bactria* and to clarify the place of *Polygonum tianschanicum* in tribe *Polygoneae*, we performed Maximum Likelihood and Bayesian analyses of combined regions of the plastid genome and ITS1–2 regions of nrDNA for 58 species of tribe *Polygoneae*, with special attention to the secondary structure of pre-rRNA of the ITS1 and ITS2 loci. In all our analyses, *Bactria lazkovii* and *Polygonum tianschanicum* formed a highly supported clade, sister to *Bactria ovczinnikovii* in the plastid trees, but separate from the latter, as well as from the remaining genera in the ITS-based trees. Details of the secondary structure of pre-rRNA of the ITS1 and ITS2 loci also confirmed the close relationships of *Bactria lazkovii* and *Polygonum tianschanicum*, quite different from *Bactria ovczinnikovii*. Based on the molecular analyses, details of the secondary structure of pre-rRNA of the ITS1 and ITS2 loci, fine morphological distinctions, and distributional data, we propose the new genus *Caelestium* Yurtseva et Mavrodiev, presumably of hybrid origin, to include *C. lazkovii* and *C. tianschanicum*.

Keywords: *Polygoneae*, *Atraphaxis*, *Bactria*, *Caelestium*, new genus, incongruence, molecular analyses, pseudogene, *rpl32–trnL*^(UAG), secondary structure of pre-rRNA, *trnL–trnF*, Pamirs, Tien Shan.

Аннотация. Род *Bactria*, недавно установленный по результатам молекулярного и морфологического исследования трибы *Polygoneae* для *Atraphaxis ovczinnikovii* с Южного Памира и *Bactria lazkovii* с Центрального Тянь-Шаня, может включать также морфологически близкий *Polygonum tianschanicum*, редкий эндемик Восточного Тянь-Шаня, который ранее не был исследован с молекулярно-филогенетической точки зрения. Чтобы уточнить состав рода *Bactria* и выяснить филогенетическое положение *Polygonum tianschanicum* в трибе *Polygoneae*, мы выполнили молекулярно-филогенетические анализы (метод максимального правдоподобия и Байесов анализ) объединенных участков пластидного генома и участка ITS ядерной рибосомной ДНК для 58 видов *Polygoneae*, с особым вниманием ко вторичной структуре пре-рРНК локусов ITS1 и ITS2. Во всех наших анализах *Bactria lazkovii* и *Polygonum tianschanicum* образовали хорошо поддержанную кладу, сестринскую к *Bactria ovczinnikovii* в пластидных деревьях, но обособленную от последнего вида (как и от прочих родов *Polygoneae*) в анализе ITS. Детали вторичной структуры пре-рРНК локусов ITS1 и ITS2 также показали тесное родство *Bactria lazkovii* и *Polygonum tianschanicum*, равно как и их существенные отличия от *Bactria ovczinnikovii*. Результаты молекулярных анализов пластидных и ядерных участков, различия вторичной структуры пре-рРНК локусов ITS1 и ITS2, морфологические отличия и географическое распространение позволяют выделить новый для науки род *Caelestium* Yurtseva et Mavrodiev. Род имеет предположительно гибридогенное происхождение и включает два узколокальных эндемика Тянь-Шаня: *C. lazkovii* (= *Bactria lazkovii*) из Кыргызстана и *C. tianschanicum* (= *Polygonum tianschanicum*) из

Северо-Восточного Китая. Полученные результаты обсуждаются в контексте уточненного состава трибы *Polygoneae*, который определяется на основании интерпретации результатов молекулярно-филогенетических анализов.

Ключевые слова: *Polygoneae*, *Atraphaxis*, *Bactria*, *Caelestium*, новый род, неконгруэнтность, молекулярный анализ, псевдоген, *rpl32-trnL^{UAG}*, вторичная структура пре-рРНК, *trnL-trnF*, Памир, Тянь-Шань.

Introduction

Atraphaxis L. and *Polygonum* L. established by C. Linnaeus (1753) are perhaps the most problematic taxa of tribe *Polygoneae* (*Polygonaceae* Juss., *Polygonoideae*); their relationships and circumscriptions are still not exactly clarified, despite several attempts (Ronse De Craene et al., 2004; Yurtseva et al., 2010, 2012a, b, 2016, 2017; Schuster et al., 2011b, 2015). S. Tavakkoli et al. (2015) recently transferred *Polygonum* sect. *Avicularia* Meisn. “§” *Spinescentia* Boiss. (Boissier, 1879: 1027) to *Atraphaxis* as *A.* sect. *Polygonoides* S. Tavakkoli, Kaz. Osaloo et Mozaff., but O. V. Yurtseva et al. (2017) accepted it as the genus *Persepolium* Yurtseva et Mavrodiev, which is sister to *Atraphaxis*. A new genus *Bactria* Yurtseva et Mavrodiev was established recently to include *Atraphaxis ovczinnikovii* (Czukav.) Yurtseva from the Pamirs (Yurtseva et al., 2014: 43), originally described in *Polygonum* (Czukavina, 1962: 64), and *Bactria lazkovii* Yurtseva et Mavrodiev from the Tien Shan (Yurtseva et al., 2016: 43). These two species formed a monophyletic *Bactria* sister to the clade (*Atraphaxis* s. str. + *Persepolium*) in plastid trees, but showed paraphyly in ITS-based trees (Yurtseva et al., 2016), which requires further research and reappraisal.

Polygonum tianschanicum Chang Y. Yang (Yang, 1983) from Xinjiang, China, strongly resembles *Bactria lazkovii* in its habit, perianth, fruit and pollen morphology, and at the first approach may be treated as part of *Bactria*. We fully agree with C. Y. Yang (2010) who correctly considered *Polygonum popovii* Borodina (1989: 104) from China as a synonym of *P. tianschanicum*. Based solely on perianth and pollen morphology, *P. popovii* was recently transferred to *Atraphaxis* as *A. popovii* (Borodina) Yurtseva (Yurtseva et al., 2014). In this study we aimed to clarify the phylogenetic placement of *Polygonum tianschanicum* within *Polygoneae*.

Because *P. tianschanicum* and *Bactria ovczinnikovii* (Czukav.) Yurtseva et Mavrodiev were originally described in *Polygonum*, in our analyses we used selected members of all intrageneric taxa currently accepted in the genus numbering ca. 130 species. Traditionally, four “main” sections were recognized in *Polygonum* in a “moderately narrow” circumscription (e. g., Dammer, 1893; Hedberg, 1946; Haraldson, 1978), namely: *P.* sect. *Polygonum* (= *Avicularia*) with ca. 100 species (Meisner, 1826: 43, 85), *P.* sect. *Tephis* (Adans.) Meisn.

(Meisner, 1857: 84) with two species, *P.* sect. *Pseudomollia* Boiss. (Boissier, 1879: 1027) with two species, and *P.* sect. *Duravia* S. Watson (1873: 665) with ca. 20 species. N. N. Tzvelev (1987: 78) added *P.* sect. *Plebeja* Tzvelev for *P. plebeium* R. Br. *Polygonella* Michx. (Michaux, 1803: 240) was traditionally accepted as a separate genus numbering 11 species (e. g., Meisner, 1857; Bentham, Hooker, 1880; Dammer, 1893; Horton, 1963; Freeman, 2005), but today it is also treated as part of *Polygonum* (Ronse De Craene et al., 2004; Schuster et al., 2011b, 2015). Hereinafter we attributed all the listed sections of *Polygonum* and *Polygonella* to the genus *Polygonum* s. l. in its “moderately narrow” circumscription, and treat the Old World sections as *Polygonum* s. str.

As a result of Maximum Parsimony (MP) analysis based on 51 morphological characters of 25 species of *Polygonum a priori* accepted in a wide circumscription, L.-P. Ronse De Craene et al. (2004) recognized *P.* sect. *Polygonum* with subsect. *Polygonum* and subsect. *Tephis* (Adans.) Ronse Decr. et S. P. Hong; *P.* sect. *Pseudomollia*; and *P.* sect. *Duravia* with subsect. *Duravia* (S. Watson) Ronse Decr. et S. P. Hong and subsect. *Polygonella* (Michx.) Ronse Decr. et S. P. Hong.

Our phylogenetic analyses of nrDNA ITS1–2 data of *Polygonum* s. l. (Yurtseva et al., 2010, 2012b) confirmed two major clades combining, first, mostly Eurasian species of *P.* sect. *Polygonum* (incl. *P.* sect. *Pseudomollia*); second, the North American *P.* sect. *Duravia* and *P.* sect. *Polygonella*. These results were generally confirmed by T. M. Schuster et al. (2011b, 2015).

Our preliminary study of the ITS sequence alignment of *Polygoneae* (Yurtseva et al., 2010, 2016) showed that many taxa of *Polygoneae* have extensive indels in the ITS1 and ITS2 regions, which cause some difficulties in the alignment of the sequence data. These difficulties, however, can be overcome by comparing the secondary structures of pre-rRNA of the rDNA loci, since it is a way to align sequence regions more precisely and to clarify the relations of organisms of different taxonomic ranks more accurately (e. g., Denduangboripant, Cronk, 2001; Gottschling et al., 2001; Goertzen et al., 2003; Wang et al., 2007; Tippery, Les, 2008; Milyutina et al., 2010; Edger et al., 2014; Giudicelli et al., 2017).

Despite intensive modern molecular-phylogenetic studies of tribe *Polygoneae* (Schuster et al., 2011a, b, 2015), not much attention has been paid to the incongruences of the plastid- and ITS-based trees. Sometimes

the ITS sequence data were just merged with the plastid ones after exclusion of poorly aligned regions (e. g., Schuster et al., 2015), that obscured the difference in phylogenetic signals.

The main goals of our present study were: (1) to update the ITS phylogeny of tribe *Polygoneae*, but with special attention to the secondary structure of pre-RNA of the ITS1 and ITS2 regions, which has never been done before; (2) to compare the ITS and plastid topologies in more detail; (3) to clarify the circumscriptions of the genera *Bactria* and *Polygonum* s. l., with special attention to the pre-RNA secondary structure of the ITS1 and ITS2 regions.

Material and methods

Morphological study

The morphological study was based on the type and verified specimens of *Bactria ovczinnikovii*, *B. lazkovii*, and *Polygonum tianschanicum* (= *P. popovii*) deposited in the Herbaria of the Komarov Botanical Institute of RAS, St. Petersburg, Russia (LE) and Lomonosov Moscow State University, Moscow, Russia (MW).

Sampling for molecular study

Our study involved selected members of the taxa of *Polygoneae* analyzed by T. M. Schuster et al. (2011b), although they took *Polygonella* as part of *Polygonum* s. l.: *Atraphaxis* (10 species), *Duma* T. M. Schust. (3), *Fallopia* Adans. (3), *Polygonella* (8), *Polygonum* sect. *Polygonum* (11), *P. sect. Duravia* (6), *P. sect. Pseudomollia* (1), and *P. sect. Plebeja* (1), *Muehlenbeckia* Meisn. (2), *Reynoutria* Houtt. (2), and *Knorringia* (Czukav.) Tzvelev (1). The list of taxa was supplemented by members of *Persepolium* (5), *Bactria* (2), *Polygonum tianschanicum* (1), and *P. afromontanum* Greenway (1), one of two members of *Polygonum* sect. *Tephis*. *Oxyria digyna* Hill (*Rumiceae*) was selected as an outgroup (Sanchez et al., 2011; Schuster et al., 2011a, b). The names of taxa and GenBank sequences (<http://www.ncbi.nlm.nih.gov>) are presented in Appendix (see the journal's website: www.binran.ru/journals/novitates/).

In total, 31 sequences of the cpDNA *rpl32-trnL*^(UAG) region representing 30 species were analysed: one sequence was generated for this study, 13 sequences were obtained from our previous studies (Yurtseva et al., 2016), and 17 sequences were downloaded from GenBank. In total, 48 sequences of the cpDNA *trnL* intron^(UAA) + *trnL-trnF* IGS region were analysed: two sequences were elaborated for this study, 14 sequences were obtained from our previous studies, and the remaining ones were downloaded from GenBank. Finally, the combined plastid alignment includes 60 accessions of 58 species of *Polygoneae*.

The same set of taxa (60 accessions of 58 species) was used for the analysis of nrDNA ITS1–5.8S–ITS2 regions. In total, 60 ITS sequences have been analysed; two of them were sequenced for this study, 31 sequences have been obtained earlier (Yurtseva et al., 2010, 2012b), and 27 sequences were downloaded from GenBank.

DNA isolation, amplification and DNA sequencing

Methods of DNA extractions and amplification, as well as the strategies of the phylogenetic analyses were described in O. V. Yurtseva et al. (2016). The nrDNA ITS region for most of the taxa was amplified using external primers and, in some cases, internal primers (Table 1). Due to the substantial problems with reading of heterogeneous nrDNA ITS sequences of *Bactria lazkovii*, in this study we designed various primers as well as PCR profiles. Specifically, we used internal primers ITS2-BaN, ITS2-BaPSE, ITS3-BaN and ITS3-BaPSE and combined them with external primers ITS1, E1128f for amplification of the 18S–ITS1 region, and with B, R2, R3, R4, R5 for amplification of the ITS2–26S region. In the case of *Polygonum tianschanicum*, we used the RCA technique (Brockington et al., 2008) to improve the quality of weak DNA extraction.

Identification of putative pseudogenes

To distinguish the putative pseudogenic ITS region, we examined nucleotide divergence, insertion-deletion events, the secondary structures themselves, as well as the methylation-induced substitution patterns (e. g., Buckler, Holtsford, 1996; Buckler et al., 1997; Mayol, Rosselloó, 2001; Hughes et al., 2002; Bailey et al., 2003; Harpke, Peterson, 2006; Bayly, Ladiges, 2007; Ochieng et al., 2007; Zheng et al., 2008). The indel events and nucleotide substitutions in the 5.8S region were used as a primary criterion, while the lower GC-content, the destruction of the secondary structure, and pattern of substitutions of the ITS1–5.8S–ITS2 region were used as additional criteria for ITS pseudogene identification. Pairwise comparisons used to detect pseudogenic sequences were conducted separately for the regions with different levels of variation (ITS1, 5.8S, ITS2), as C. D. Bailey et al. (2003) recommended. The nucleotide composition of the sequences and *p*-distance were calculated using MEGA V.6.0 (Tamura et al., 2013) using Tamura three-parameter distance (Kimura, 1980) with a gap treated as a complete deletion.

Strategies of sequence alignment

All sequences have been edited using BioEdit 7.0 (Hall, 1999). As before (Yurtseva et al., 2016), the combined *trnL-trnF* and *rpl32-trnL*^(UAG) sequences were

Table 1. Primers used for amplification of the ITS1–5.8S–ITS2 region, partial sequencing of the 18S and 26S nrDNA, *trnL* intron + *trnL*–*trnF* IGS cpDNA, and *rp32*–*trnL*^(UAG) IGS cpDNA

Locus	Forward primer			Reverse primer		
	Name	Sequence 5' to 3'	Source	Name	Sequence 5' to 3'	Source
ITS1–2	ITS1	TCC GTA GGT GAA CCT GCG	White et al., 1990	ITSB	GAT ATG CTT AAA CTC AGC CG	Milyutina et al., 2010
ITS1–2	ITS3	GCA TCG ATG AAG AAC GCA GC	White et al., 1990	ITS2	GCT GCG TTC TTC ATC GAT GC	White et al., 1990
ITS1–2	NNC-18S10	AGG AGA AGT CGT AAC AAG	Wen, Zimmer, 1996	C26A	GTT TCT TTT CCT CCG CT	Wen, Zimmer, 1996
18S–ITS1	E1128f	AAA CTT AAA GGA ATT GAC G	Ishii et al., 2015	ITS2-BaN ITS2-BaPSE	CGT TCT TCA TCG ATG CGA GAG C TAT TCT TGA TCG TCG CAG AGT	this paper
ITS2–26S	ITS3-BaN ITS3-BaPSE	GCT CTC GCA TCG ATG AAG AAC G ACT CTC GCA CGA TCA AGA ATA	this paper	R2	TAC TTG T(C/T)(G/C/T) CTA TCG	Van der Auwera et al., 1994
ITS2–2S	ITS3-BaN ITS3-BaPSE	GCT CTC GCA TCG ATG AAG AAC G ACT CTC GCA CGA TCA AGA ATA	this paper	R3	CTC CTT GGT CCG TGT TTC AAG ACG G	Van der Auwera et al., 1994
ITS2–26S	ITS3-BaN ITS3-BaPSE	GCT CTC GCA TCG ATG AAG AAC G ACT CTC GCA CGA TCA AGA ATA	this paper	R4	GCT ATC CTG AGG GAA ACT TCG G	Van der Auwera et al., 1994
ITS2–26S	ITS3-BaN ITS3-BaPSE	GCT CTC GCA TCG ATG AAG AAC G ACT CTC GCA CGA TCA AGA ATA	this paper	R5	GTT GTT ACA CAC TCC TTA GCG GA	Van der Auwera et al., 1994
<i>trnL</i> intron	<i>trnL</i> ^(UAA) (c)	CGA AAT CCG TAG ACG CTA CG	Taberlet et al., 1991	<i>trnL</i> ^(UAA) (d)	GGGATAGAGGGACTTGAAC	Taberlet et al., 1991
<i>trnL</i> – <i>trnF</i> IGS	<i>trnL</i> ^(UAA) (e)	GGT TCA AGT CCC TCT ATC CC	Taberlet et al., 1991	<i>trnF</i> ^(GAA) (f)	ATT TGA ACT GGT GAC ACG AG	Taberlet et al., 1991
<i>rp32</i> – <i>trnL</i> ^(UAG) IGS	<i>rp32</i> F	CAG TTC CAA AAA AAC GTA CTT C	Shaw et al., 2007	<i>trnL</i> ^(UAG)	CTG CTT CCT AAG AGC AGC GT	Shaw et al., 2007

aligned using MAFFT (Katoh et al., 2002; Katoh, Standley, 2013), and then adjusted manually. We followed MAFFT's L-INS-i alignment strategy (Yurtseva et al., 2016), with the default settings for gap opening penalty and offset value strategy (Katoh et al., 2002; Katoh, Standley, 2013).

Because the explored ITS1 and ITS2 loci contained GC-rich repeats, forming several helices in the secondary structure, we used two identical ITS data sets aligned with two different alignment strategies: MAFFT's L-INS-i alignment strategy mentioned above, and accurate manual adjustment of the MAFFT-aligned matrix according to the general architecture of the helices recognized in the secondary structure of pre-rRNA of the ITS1 and ITS2 loci (see below).

Modelling of the secondary structure of pre-rRNA of the ITS regions

We modelled the secondary structure of pre-rRNA of the ITS1 and ITS2 loci using program MFOLD (Zuker, 2003), version 3.6 (<http://mfold.rna.albany.edu/>). The 5' and 3' ends of the ITS1 and ITS2 regions were determined using the available ITS sequences of *Polygonaceae* from GenBank. For each sequence, the optimal structure and a set of suboptimal ones were determined. The choice of the secondary structure model relied on the analysis of several species for each genus. Generally, the thermodynamically optimal model was chosen. However, in a few cases (*Polygonum minimum* S. Watson, *Duma florulenta* (Meisn.) T. M. Schust., *Polygonella basiramea* (Small) G. L. Nesom et V. M. Bates) the subsequent analysis considered thermodynamically suboptimal models, which bore the most resemblance to the models of closely related taxa.

Results

Combined plastid topology

The Maximum Likelihood (ML) and Bayesian (BI) analyses (Figs. 1; 2) resulted in similar topologies. *Knorringia sibirica* (Laxm.) Tzvelev is a sister to the remaining members of *Polygoneae*, which are subdivided in two major clades. The first one (the RFM-clade sensu Schuster et al., 2011b) includes monophyletic *Reynoutria*, *Fallopia*, and *Muehlenbeckia*. The sister clade (the APD-clade sensu Schuster et al., 2011b), includes *Polygonum* s. l. and a highly supported clade (*Bactria* (*Atraphaxis* + *Persepolium*)). The *Duma* clade is a non-supported sister of the latter.

Polygonum s. l. (as outlined above) is monophyletic and highly supported. It combines the highly supported New World clade with subclades *Polygonella*

and *Polygonum* sect. *Duravia*, and the highly supported Old World clade *Polygonum* s. str. with two subclades. Within *Polygonum* s. str., the subclade which includes *P. aviculare* L., the nomenclatural type of the section, was recognized as *P. sect. Polygonum*. Besides the species of this section distributed worldwide, it includes also *P. molliiforme* (the type of *P. sect. Pseudomollia*) from Iran and Tajikistan, and *P. plebeium* (the type of *P. sect. Plebeja*) distributed in Southeast Asia, Australasia and Africa. The sister subclade includes *P. afromontanum* (one of two members of *P. sect. Tephis*) from East Africa, which is nested among the remaining members of *P. sect. Polygonum* distributed in Central Asia, so this subclade was recognized here as *P. sect. Tephis*.

The strongly supported (ML/BI = 0.86/0.99) clade *Bactria* including *B. ovczinnikovii* is a sister of a highly supported subclade (*Polygonum tianschanicum* plus *Bactria lazkovii*), which is recognized here as the new genus *Caelestium*.

Bactria lazkovii differs from *B. ovczinnikovii* by having 43 nucleotide substitutions, 6 one-nucleotide and 8 larger (5–28 bp) indels on the combined 3-loci plastid alignment 1218 bp long. The *p*-distance between *Caelestium* (*Bactria lazkovii* and *Polygonum tianschanicum*) and other genera varies from 9.3 % (for *Bactria ovczinnikovii*) to 16.5 % (for *Fallopia*), and these values are the maximum among the estimates of total difference over sequence pairs between genera compared in tribe *Polygoneae* (Table 2). *Bactria lazkovii* differs from *Polygonum tianschanicum* with 9 one-nucleotide substitutions, 3 one-nucleotide and 3 non-single nucleotide (4 and 28 nt long) indels of the combined 3-loci plastid alignment 1324 bp long. For this group, the within-group average *p*-distance is 14.9 %.

Table 2. The number of differences per site over Sequence Pairs between selected genera of *Polygoneae* based on 21 sequences of combined plastid regions (*trnL* intron + *trnL*–*trnF* IGS and *rpl32*–*trnL*^(UAG) IGS), excluding all positions that contain gaps and missing data. A total of 1218 positions were analyzed in MEGA 6.0 (Tamura et al., 2013)

	1	2	3	4	5	6
[1] <i>Fallopia</i>						
[2] <i>Reynoutria</i>	0.080					
[3] <i>Muehlenbeckia</i>	0.067	0.037				
[4] <i>Atraphaxis</i>	0.104	0.060	0.057			
[5] <i>Bactria</i>	0.097	0.052	0.053	0.029		
[6] <i>Caelestium</i>	0.165	0.127	0.125	0.107	0.093	
[7] <i>Polygonum</i> s. l.	0.107	0.091	0.085	0.093	0.085	0.161

ITS topology based on the MAFFT alignment strategy

The ML and BI analyses of the MAFFT-based ITS matrix demonstrated that *Knorringia sibirica* is a sister to the rest of *Polygoneae*, which contain several highly supported clades, although their relationships are not well resolved (Figs. 3; 4).

Both ML and BI analyses confirmed the RFM-clade, including *Reynoutria*, *Fallopia*, and *Muehlenbeckia*, but *Fallopia* is monophyletic only on the BI tree. *Duma* appeared as part of the RFM-clade (ML tree in Fig. 3), or as part of the APD-clade, including *Atraphaxis*, *Polygonum*, and *Duma* (BI tree in Fig. 4); however, the position of *Duma* is not supported in either case.

Polygonum s. l. is monophyletic, but non-supported on the BI tree (Fig. 4), and paraphyletic on the ML tree (Fig. 3). It includes the same highly supported Old World clade *Polygonum* s. str. and the highly supported New World clade with subclades *Polygonella* and *Polygonum* sect. *Duravia*. The Old World clade *Polygonum* s. str. combines two subclades. Like in plastid trees, the subclade *P. sect. Polygonum* unites part of *P. sect. Polygonum* (incl. *P. aviculare*), and the subclade *P. sect. Tephis* unites *P. afromontanum* (*P. sect. Tephis*) and the rest of *P. sect. Polygonum* from Central Asia. *Polygonum molliiforme* (*P. sect. Pseudomollia*) and *P. plebeium* (*P. sect. Plebeja*) fall into the latter subclade, in contrast to their positions in *P. sect. Polygonum* on the plastid trees (Figs. 1; 2).

Atraphaxis and *Persepolium* are highly supported, although their combined clade is not supported. *Bactria* is polyphyletic: *B. ovczinnikovii* is a highly supported sister of the clade (*Atraphaxis* + *Persepolium*), while the highly supported subclade *Caelestium* (*Bactria lazkovii* + *Polygonum tianschanicum*) is nested in *Polygonum* s. l. on the ML tree (Fig. 3), or joins *Polygonum* s. l. on the BI tree without support (Fig. 4).

ITS topologies based on the MAFFT alignment, manually adjusted according to pre-rRNA secondary structure of the ITS1 and ITS2 loci of *Polygoneae*

The ML and BI analyses based on the MAFFT alignment, manually adjusted according to the secondary structure of pre-rRNA of the ITS1 and ITS2 loci of *Polygoneae* (the secondary-structure based alignment, SSBA hereinafter) show that *Polygoneae* include the same several clades (Figs. 5; 6), with their relationships being not fully resolved, as in the previous analyses (Figs. 3; 4).

Polygonum s. l. is polyphyletic. The clade *Polygonum* s. str. is nested separately from the North American clade (*Polygonella* + *Polygonum* sect. *Duravia*); the latter is part of the grade that also includes the RFM-clade and *Duma*. *Polygonella* and *Polygonum* sect. *Du-*

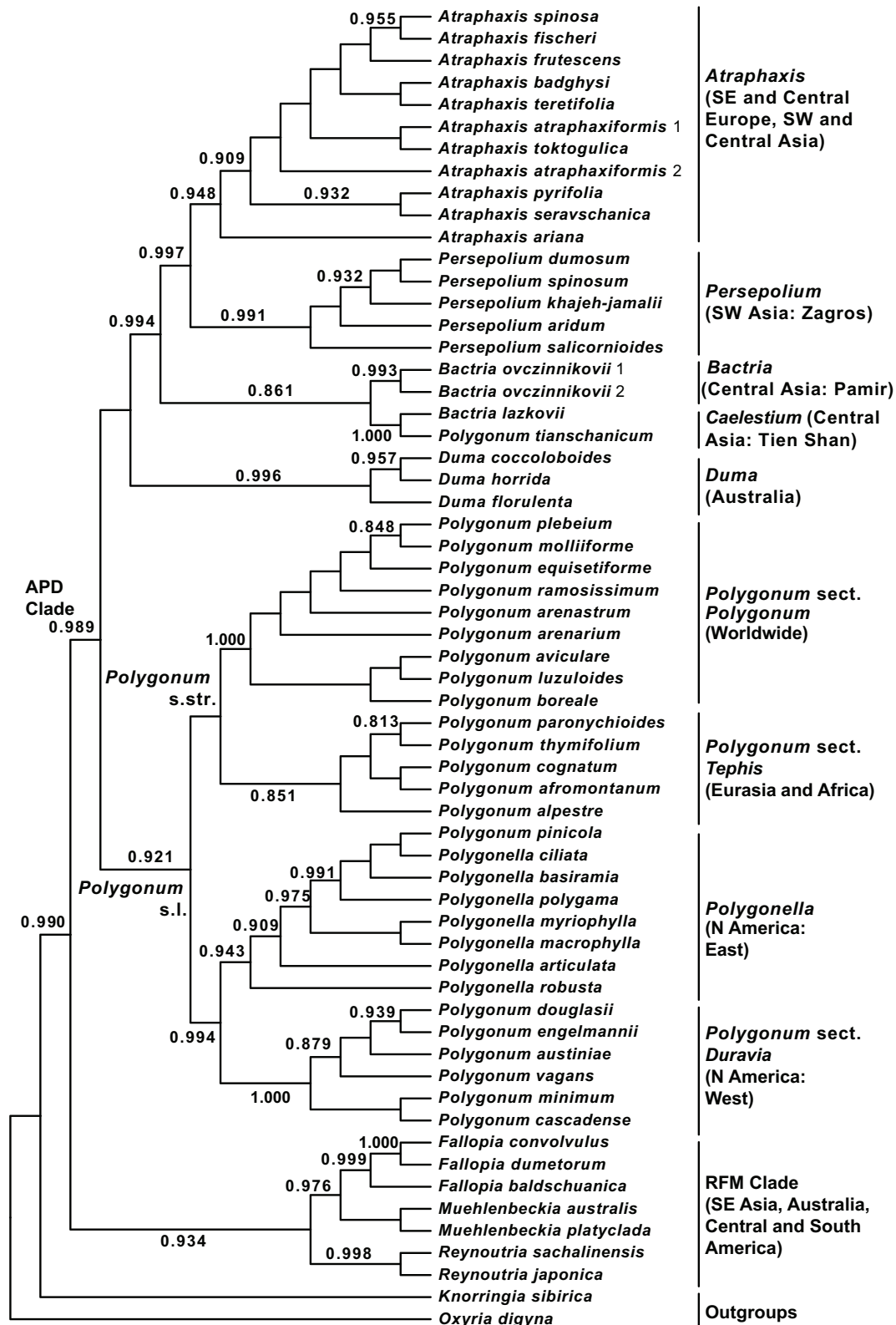


Fig. 1. ML phylogeny of *Polygoneae* based on the MAFFT-alignment of the plastid dataset: *trnL* intron + *trnL*–*trnF* IGS and *rpl32*–*trnL*^(UAG) IGS (log likelihood: –11015.339832). Numbers above or below branches indicate the aLRT support values equal to or greater than 0.8.

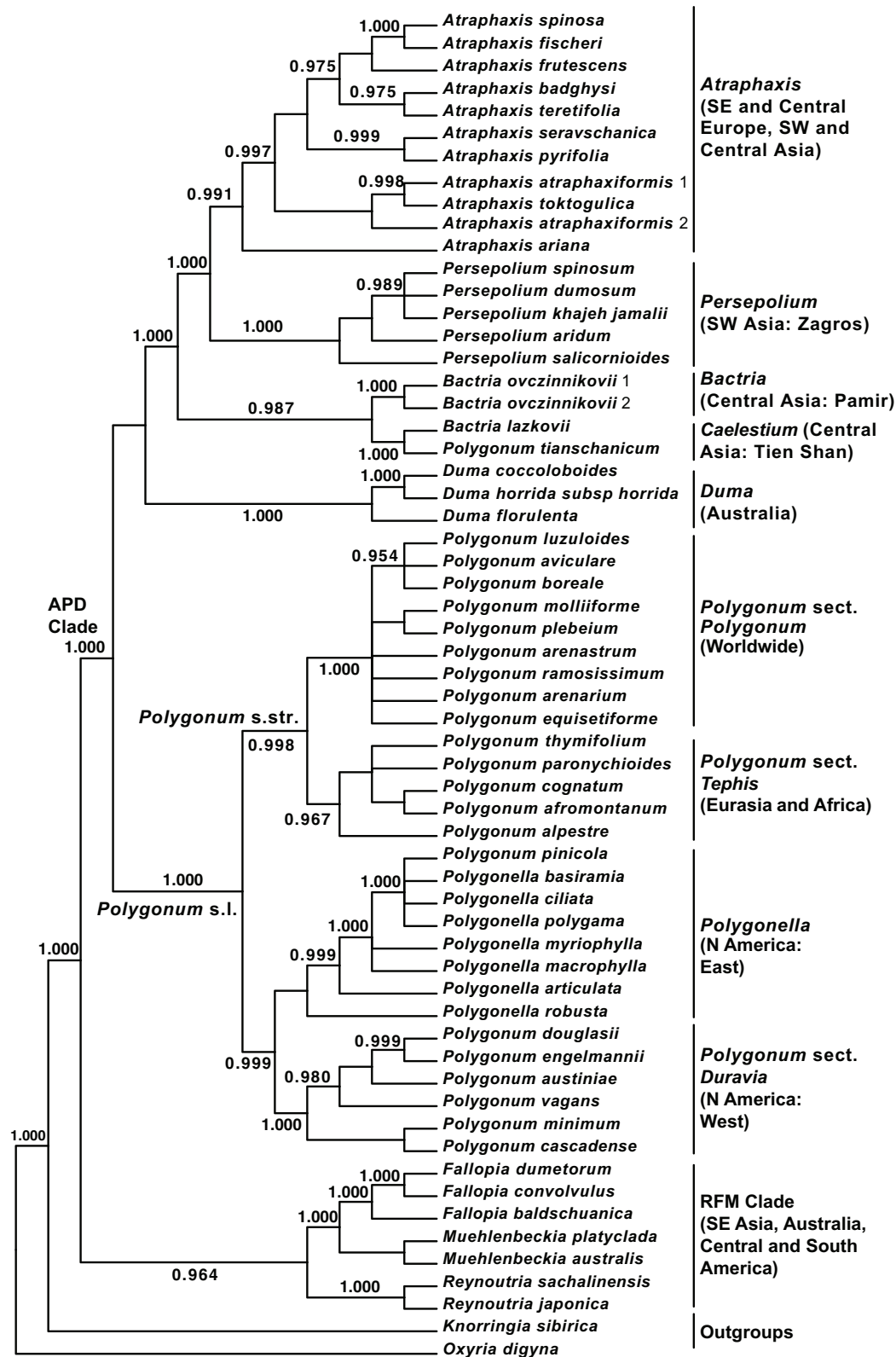


Fig. 2. Bayesian phylogeny of *Polygoneae* based on the MAFFT-alignment of the plastid dataset: *trnL* intron + *trnL*–*trnF* IGS and *rpl32*–*trnL*^(UAG) IGS. Numbers above branches indicate the posterior probabilities from BI analysis equal to or greater than 0.9.

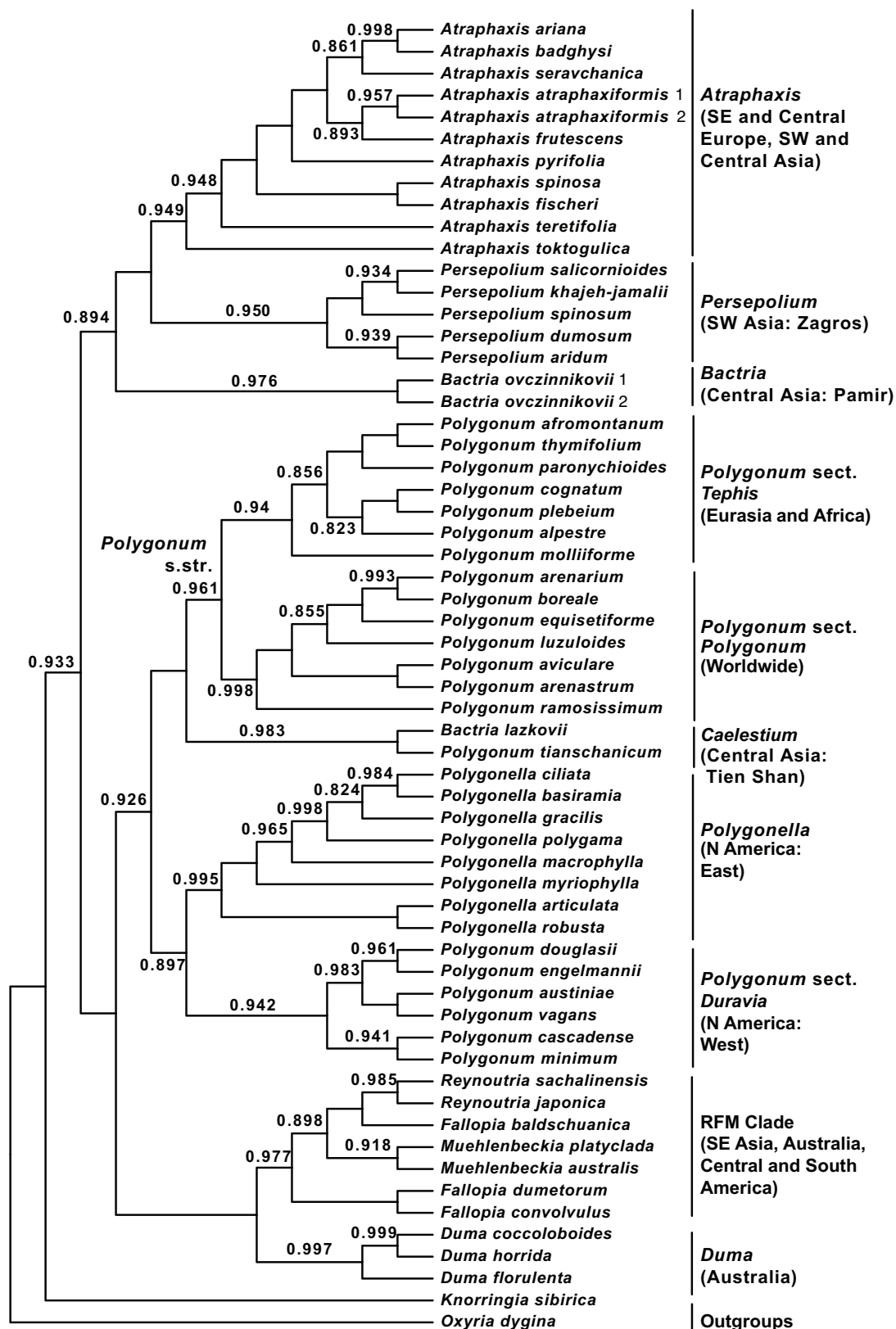


Fig. 3. ML phylogeny of *Polygoneae* based on the MAFFT-alignment of the ITS1–2 loci nrDNA (log likelihood: –6641.832866). Numbers above or below branches indicate the aLRT support values equal to or greater than 0.8.

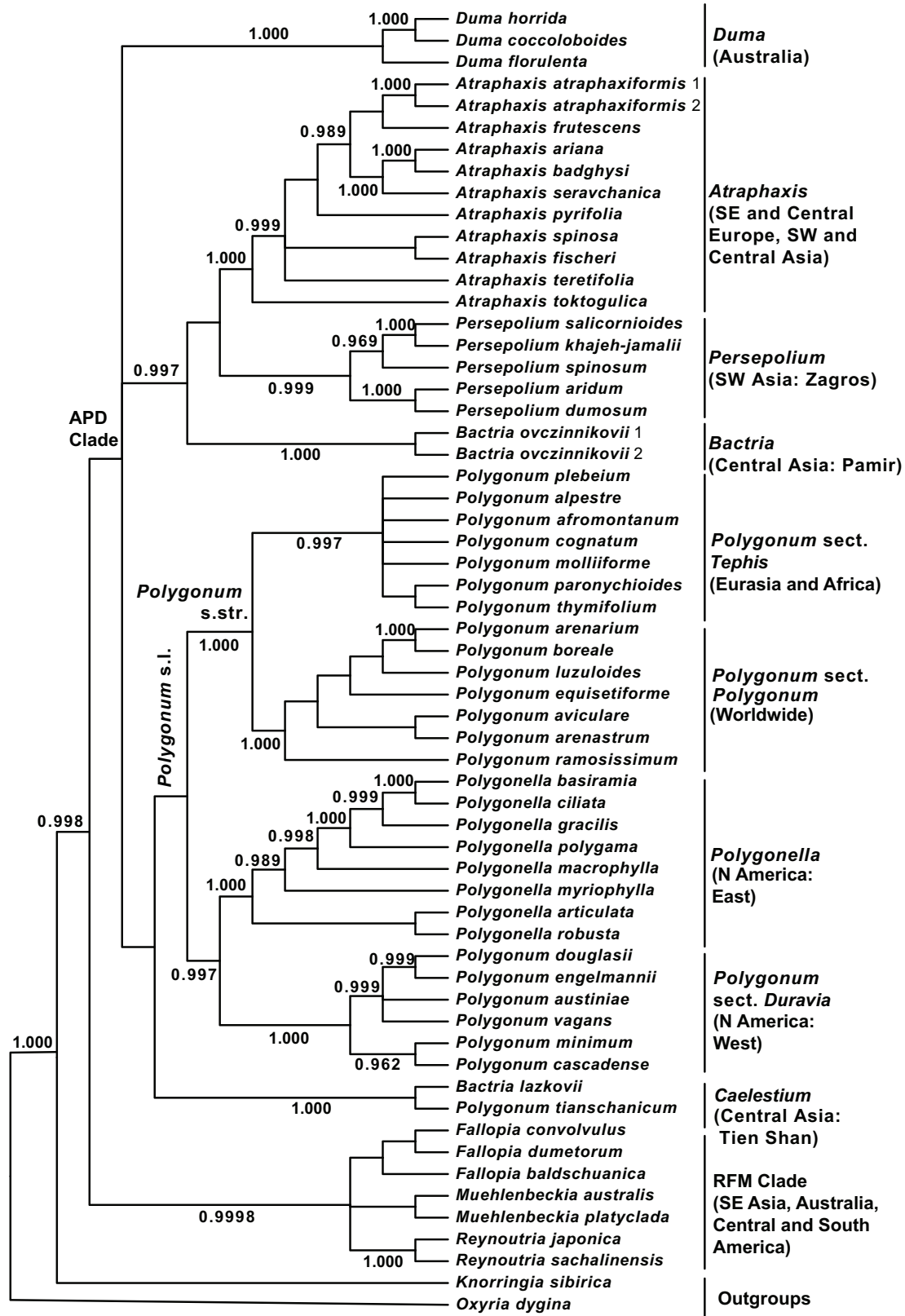


Fig. 4. Bayesian phylogeny of *Polygoneae* based on the MAFFT-alignment of the ITS1–2 loci nrDNA. Numbers above or below branches indicate posterior probabilities from BI analysis equal to or greater than 0.9.

ravia are both monophyletic and highly supported. The clade *Polygonum* s. str. is highly supported only in ML analysis (Fig. 5). It combines two strongly supported subclades. The first includes the members of *P.* sect. *Polygonum* (incl. *P. aviculare*) distributed worldwide. The second subclade recognized as *P.* sect. *Tephis* combines *P. afromontanum* (*P.* sect. *Tephis*) and the members of *P.* sect. *Polygonum* from Central Asia. *Polygonum molliiforme* (*P.* sect. *Pseudomollia*) and *P. plebeium* (*P.* sect. *Plebeja*) appear again in the same subclade, in contrast to their positions in the subclade *P.* sect. *Polygonum* on the plastid trees (Figs. 1; 2).

The clades *Atraphaxis* and *Persepolium* are highly supported, however, the clade (*Atraphaxis* + *Persepolium*) has no support. *Bactria* is clearly polyphyletic (Figs. 5; 6). Only on the BI tree it is associated with *Atraphaxis* and *Persepolium* (Fig. 6). The position of *Caelestium* is not resolved, but in either case it is not combined with any genus of *Polygoneae*, in contrast to the sistership with *Bactria ovczinnikovii* on the plastid trees.

Locus ITS1 and its pre-rRNA secondary structure

Since the phylogenetic analysis based on ITS region did not show a resolved topology and the positions of some taxa were not determined, we compared the pre-rRNA secondary structure of their ITS1 and ITS2 loci.

The pre-rRNA secondary structure of the ITS1 has a central loop with several inner and outer helices formed by inverted repeats with high GC content. Maximum (five) outer helices are present in pre-rRNA secondary structure of the ITS1 of *Reynoutria* (Fig. 7: A), *Fallopia*, *Muehlenbeckia*, *Duma* (Fig. 7: G), *Atraphaxis ariana* (Grig.) T. M. Schust. et Reveal (Fig. 7: B) (and also *A. badghysi* Kult., *A. radkanensis* S. Tavakkoli, Kaz. Osaloo et Mozaff. absent from the analyses). Most species of *Atraphaxis* (Fig. 7: C), as well as *Persepolium* and *Bactria ovczinnikovii* (Fig. 7: D), have helix II, but lack helix III. In contrast, *Bactria lazkovii* and *Polygonum tianschanicum* recognized here as the members of the new genus *Caelestium*, and the members of *Polygonum* s. l. lack helix II, but have helix III (Fig. 7: E, F, H, I).

The outer helix I (12 nt) is formed by 5'-CUGC-3' and 5'-GCAG-3' inverted repeats.

The outer helix II (22–41 nt) is variable, but present only in part of *Polygoneae* (Fig. 8; Table 3). *Atraphaxis ariana*, *Persepolium*, and *Bactria ovczinnikovii* have a similar length (32–37 nt) and composition of helix II (Fig. 8: A–D). In all of these taxa, the conserved part of the stem is formed by inverted repeats (5'-GGCGGGGG-3' and 5'-UCCCCCCC-3') shown in a frame in Fig. 8. The differences are in several one-nucleotide substitutions and one-nucleotide deletions. *Persepolium* (Fig. 8: A) and some of *Atraphaxis* species

(Fig. 8: D) have additional GC pairs at the base of the stem. *Duma florulenta* (Fig. 8: E) has the shortest helix II (22 nt). It has the conserved part of the stem as short as that in *Reynoutria* and *Muehlenbeckia*, but this conserved part is even shorter in *Fallopia* (Table 3). The members of the RFM-clade have the longest helix II (41 nt) due to additional GC-rich inverted repeats in the apical part of the stem, but lack base pairs in the proximal part (Fig. 8: F).

The outer helix III (17–25 nt) (Fig. 9; Table 3) has a stem formed by inverted repeats 5'-GCCCCGGG-(nN)-CCCCGGGC-3'. *Reynoutria*, *Muehlenbeckia*, *Fallopia*, and *Duma* have the identical composition of helix III (Fig. 9: A). The same, or almost the same, composition of helix III was found in some species of *Polygonella* and in the *Polygonum* species, which fall within the subclade *Polygonum* sect. *Tephis* on the ITS-based trees and grow in Central Asia and Africa (Fig. 9: E, H; Table 3): *P. molliiforme* (*P.* sect. *Pseudomollia*), *P. plebeium* (*P.* sect. *Plebeja*), *P. afromontanum* (*P.* sect. *Tephis*), *P. cognatum* Meisn., *P. thymifolium* Jaub. et Spach (among other Central Asian members of *P.* sect. *Polygonum*).

The members of *Polygonum* sect. *Duravia* (*P. minimum* S. Watson, *P. douglasii* Greene) and *P.* sect. *Polygonum* (*P. aviculare*, *P. boreale* Small) have 1–2 extra base pairs in the outer position and lack one of nucleotides in the right arm of helix III (Fig. 9: F, G; Table 3). Besides, the members of *P.* sect. *Polygonum* (*P. aviculare*, *P. boreale*) have the largest terminal loop (Fig. 9: F) of helix III.

Atraphaxis ariana (and also *A. badghysi*, *A. radkanensis*) have an extra base pair (A–U) in the outer position (Fig. 9: B) of helix III. *Polygonum tianschanicum* and *Bactria lazkovii* share the shortest helix III (17 nt), which lacks one base pair (C–C) in the stem (Fig. 9: C–D), and can be produced from helix III of any taxon. Both have 5 nucleotides in the terminal loop, like some species of *Polygonum* s. str. (e. g., *P. plebeium*, *P. boreale*) and *Polygonella* (Table 3).

The outer helix IV (22–23 nt) is the most conserved (Table 3). The stem is normally formed by 9 bp. The motif GGCTY-(4–7n)-GYGYCAAGGAA noted by J. S. Liu and C. L. Schardl (1994) as conserved across plants is present in helix IV as 5'-GGCGC-(GGA(U/C)U)-GCGCCAAGGAC-3' in all members of *Polygoneae*. Although the composition of helix IV is almost the same in most of *Polygoneae*, one-nucleotide substitutions are present in some non-related taxa. Some members of *Polygonella* and *Polygonum* sect. *Duravia* (*P. minimum*) lack one nucleotide in the left arm of the stem. *Duma florulenta* (Meisn.) T. M. Schust. and *Polygonum douglasii* have an extra base pair in the stem, but smaller terminal loop.

Outer helix V is the most variable in *Polygoneae* (36–45 nt) (Table 3). *Duma* is close to the members of the RFM-clade by the structure of helix V. The North American *Polygonella* and *Polygonum* sect. *Duravia* slightly differ from each other and from the Old World *Polygonum* s. str. in the structure of helix V. The same applies to the members of the subclades *P.* sect. *Tephis* and *P.* sect. *Polygonum* (Table 3) as they appeared on the ITS-based trees (Table 3; Figs. 3–6). *Atraphaxis*, *Bactria ovczinnikovii* and *Persepolium* share the length (40 nt) and structure of helix V, though some species of *Atraphaxis* have extra base pairs in the inner position. *Bactria lazkovii* and *Polygonum tianschanicum* have unique structure of helix V, distinct in the proximal part of the stem.

To summarize, *Bactria ovczinnikovii*, *Persepolium*, and most of *Atraphaxis* species have the same set of helices of similar length in the pre-rRNA secondary structure of the ITS1 (Table 3). *Polygonum* sect. *Duravia* and *Polygonella* differ from the rest of *Polygonum* s. l. by the length and structure of helix V. The subclades *P.* sect. *Polygonum* and *P.* sect. *Tephis*, as they appeared in the results of the ITS analyses, differ in the length and structure of helices III and V. *Duma* is close to the members of the RFM-clade in the whole secondary structure of pre-rRNA of the ITS1, and in the structure of helices II–V. *Polygonum tianschanicum* and *Bactria lazkovii* resemble *Polygonum* s. l. in the general pre-rRNA secondary structure of the ITS1 locus (Fig. 7: E, F, H, I), but differ in having the unique structures of helices III and V.

Locus ITS2 and its pre-rRNA secondary structure

The pre-rRNA secondary structure of the ITS2 region has a central loop and four outer helices (Fig. 10). Helices I and II have a common stem, and helices III and IV are branched from the central loop. Helices I and IV are the most variable, while helices II and III are more conserved (Table 4).

The outer helix I 30–43 nt long includes two conserved inverted repeats in the stem base (5'-CGCCCC-3' and 5'-GGGGCG-3', 5'-CCCCC-3' and 5'-GGGGGG-3'), but is rather polymorphic in the apical part (Table 4). It is shortest in the RFM-clade and *Duma*, in some members of *Polygonum* sect. *Duravia* and *Polygonella*, e. g., *P. robusta* (Small) G. L. Nesom et V. M. Bates (= *Thysanella robusta* Small) (30–32 nt), and *Polygonum tianschanicum* (33 nt). Helix I is longest in *P. afromontanum*, *P. cognatum* (*P.* sect. *Tephis*), and *Bactria lazkovii* (43 nt). In some aspects, the structure of helix I of *B. lazkovii* and *Polygonum tianschanicum* is similar to that of *Polygonella* (Table 4). In contrast, *Atraphaxis*, *Persepolium* and *Bactria ovczinnikovii* share the distinct length (38–40 nt) and structure of helix I.

The outer helix II has a very conserved basal part of the stem 12 bp long, which is formed by inverted repeats (5'-GGCCCCCGUGC-3' and 5'-G(C/U)GC-GGCCGGCC-3'), but the apical part of the stem and the loop vary in size and composition (Table 4). For example, *Atraphaxis* has additional base pairs flanking the terminal loop. *Persepolium* and *Bactria ovczinnikovii* have a very similar composition of helix II, but *Persepolium* lacks one nucleotide in the left arm and has two extra base pairs in the inner position. *Bactria lazkovii* and *Polygonum tianschanicum* have identical structure and length of helix II (38 nt), different from that of *Bactria ovczinnikovii*, with one extra base pair in the apical part of the stem and a compensatory substitution A→U→A in the outer position. *Polygonella* and *Polygonum* sect. *Duravia* are close to *Duma* and the members of the RFM-clade in the structure of helix II. The members of *Polygonum* s. str. demonstrate some differences, e. g., *P. molliiforme* (*P.* sect. *Pseudomollia*) has a much longer apical part of the stem.

The outer helix III is the longest (61–82 nt) and rather variable. The positions of inner loops in the stem distinguish the taxa of *Polygoneae* from each other. *Polygonella* differs from the rest of *Polygonum* s. l. in the structure of helix III. The sections of *Polygonum* s. l. demonstrate slight, but definite distinctions. *Polygonum molliiforme* (*P.* sect. *Pseudomollia*) and *P. plebeium* (*P.* sect. *Plebeja*) have a distinct proximal part of helix III, but are close to the members of *P.* sect. *Tephis* in the apical part of the stem. *Polygonum* sect. *Tephis* shares the proximal part with *P.* sect. *Polygonum*. *Bactria lazkovii* and *Polygonum tianschanicum* are close to *Atraphaxis*, *Persepolium*, and *Bactria ovczinnikovii* in the structure of helix III (Table 4).

The outer helix IV is most variable; it strongly changes its secondary structure depending on thermodynamic conditions and is not reliable for comparing.

In summary, *Duma* is the most close to the members of the RFM-clade by the structure of helices I, II, and III. The New World *Polygonella* and *Polygonum* sect. *Duravia* demonstrate slight, but definite differences in the structure of helices I and III. The same applies to the subclades of the Old World *Polygonum* s. str. *Bactria ovczinnikovii*, *Atraphaxis*, and *Persepolium* have similar structure of helices I and III, but *Atraphaxis* has longer helix II. *Bactria lazkovii* and *Polygonum tianschanicum* (the genus *Caelestium*) have unique structure of helix II and share the structure of helix III with *Bactria ovczinnikovii*, *Atraphaxis* and *Persepolium*.

The “anomalous” ITS sequences of *Bactria lazkovii* and their pre-rRNA secondary structure

As a result of applying multiple sets of primers and PCR profiles, besides the putatively functional ITS(A),

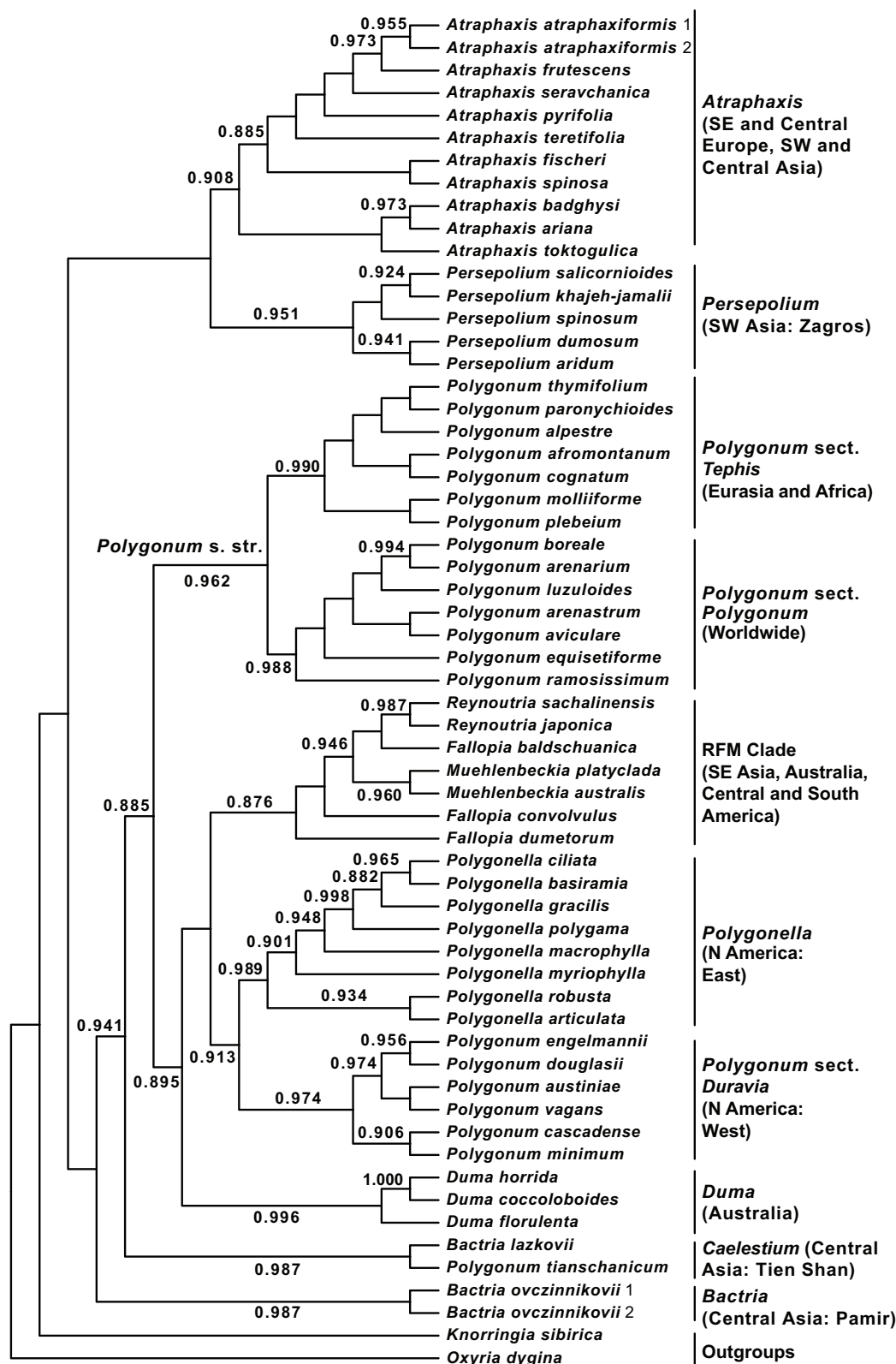


Fig. 5. ML phylogeny of *Polygoneae* based on the pre-rRNA secondary structure on the related alignment of the ITS1 and ITS2 loci nrDNA (log likelihood: -6493.537870). Numbers above or below branches indicate the aLRT support values equal to or greater than 0.8.

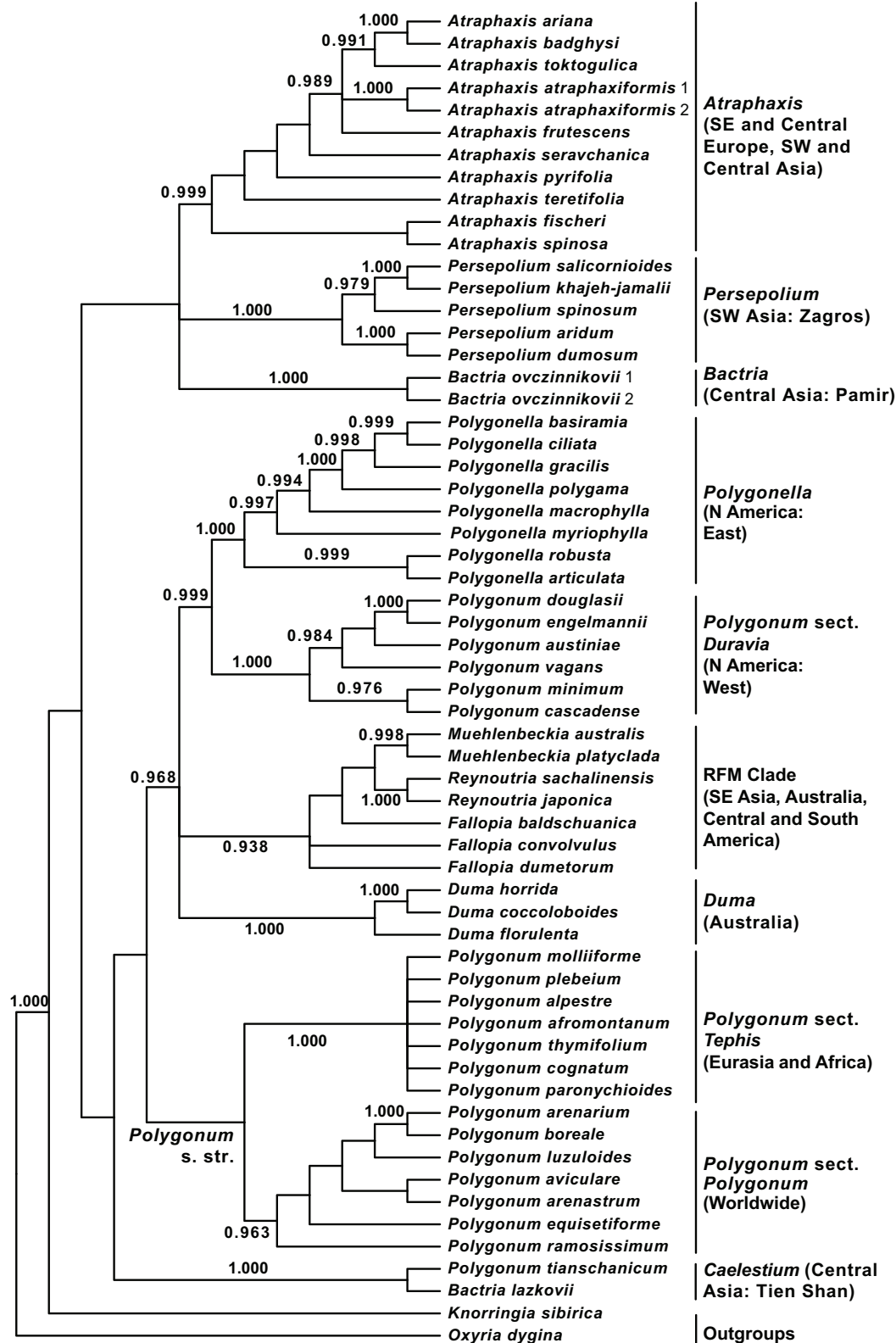


Fig. 6. Bayesian phylogeny of *Polygoneae* based on the pre-rRNA secondary structure and on the related alignment of the ITS1–2 loci nrDNA. Numbers above or below branches indicate posterior probabilities from BI analysis equal to or greater than 0.9.

the putatively pseudogenic ITS(B) was amplified from the same sample of *Bactria lazkovii*. External primers NNC-18S10 and C26 resulted in amplification of mostly heterogeneous ITS sequences. The most of combinations of selective internal primers for functional ITS (ITS2-BaN and ITS3-BaN) with universal external primers (Table 1), resulted in successful selective amplification of the fragments, including 3'-end of the 18S region, the ITS1–5.8S–ITS2 region, and 5'-end of the 26S region. Combinations of selective internal primer ITS2-BaPSE with external primers ITS1, E1128f were used for amplification of the 18S–ITS3–5.8S region, and combinations of selective internal primer ITS3-BaPSE with B, R2, R3, R4, R5 were used for amplification of the 5.8S–ITS2–26S region (Table 1), however only the combinations ITS1 with B, and ITS3-BaPSE with B and R2 resulted in successful amplification of the 5.8S–ITS2–26S region for the pseudogenic ITS.

In total, four identical sequences of putatively functional ITS(A) and five sequences of the highly variable pseudogenic ITS(B) (excluded from the phylogenetic analyses but used for further comparisons of the secondary structures) were successfully obtained.

The putatively pseudogenic ITS(B) of *Bactria lazkovii* differs from the functional ITS(A) by the presence of 75 nucleotide substitutions, mainly transitions G→A (36 %) and C→T (33.3%) on the ITS1–2 region. As we originally estimated, on average, the GC-content of the ITS1–2 region in *Polygoneae* is relatively high (65.5–70.1 %), especially in *Fallopia*, *Reynoutria*, and *Muehlenbeckia*. The pseudogenic ITS(B) of *Bactria lazkovii* has a lower GC-content (59.4 %) than its functional ITS(A) (68.8 %), or the ITS of other genera of *Polygoneae*, except for the ITS of *Polygonella*, which also has low (62.0 %) GC-content.

The functional ITS(A) of *Bactria lazkovii* shows 5.8–17.9 % range of *p*-distance from other genera on the ITS1–5.8S–ITS2 region (ITS1, 6.8–27.0 %; ITS2, 6.5–28.3 %; 5.8S, 0.8–3.2 %). These values correspond to the average level (5.3–22.9 %) of intergeneric divergence in *Polygoneae* on the same region (ITS1, 7.0–53.6 %; ITS2, 8.9–25.0 %; 5.8S, 0.3–3.5 %).

The pseudogenic ITS(B) of *Bactria lazkovii* has a much higher range of *p*-distance on the ITS1–2: 17.7–33.9 % (ITS1, 20.7–51.1 %; ITS2, 19.0–47.3 %; 5.8S, 10.5–15.6 %) from other genera of *Polygoneae*, and 17.9 % (ITS1, 17.6 %; ITS2, 23.2 %; 5.8S, 11.6 %) from the functional ITS(A) of *B. lazkovii*.

For the 5.8S gene, the range of *p*-distance between the functional ITS(A) of *Bactria lazkovii* and the ITS of other genera of *Polygoneae* (0.8–3.2 %) is comparable with the range of intergeneric (0.3–3.5 %) divergence in *Polygoneae*. In contrast, the pseudogenic ITS(B) of

Bactria lazkovii has a much higher level of intergeneric *p*-distance (10.5–15.6 %) for the 5.8S gene, which differs from the functional ITS(A) by 18 nucleotide substitutions and two one-nucleotide deletions.

In *Bactria lazkovii*, the pre-rRNA secondary structure of the pseudogenic ITS1(B) does not form helix I, but forms helices II–V (Fig. 11: A; Table 3). In contrast to the functional ITS1(A) of the same sample (Fig. 7: E), its helix II (Fig. 11: A) is unique in *Polygoneae*: its basal conserved part is as short as in *Duma*, *Reynoutria*, and *Muehlenbeckia* (Fig. 8: F), but its apical part is as long as that of *Atraphaxis*, *Persepolium*, and *Bactria ovczinnikovii* (Fig. 8: A–D). Helix III of the ITS1 has 5 nucleotides in the terminal loop of both functional and pseudogenic variants, but the pseudogenic ITS1(B) has an extra nucleotide in the stem (18 vs 17), that can be viewed as a transitional state to its functional variant, which lacks the whole bp (Fig. 9: C, D; Table 3). In the pseudogenic ITS1(B), helix IV has an extra nucleotide in the stem (24 vs 23 nt), helix V is shorter (38 vs 40 nt) than that in the functional ITS1(A), but can be produced from the latter (Fig. 11: A; Table 3). Thus, the pre-rRNA secondary structure of the pseudogenic ITS1(B) of *B. lazkovii* can be produced from its functional ITS1(A), but has additional helix II unique in *Polygoneae*.

The pseudogenic ITS2 of *B. lazkovii* has the same four helices in the pre-rRNA secondary structure, like the ITS2 of the rest of *Polygoneae*, but due to numerous substitutions, the pseudogenic ITS2 of *B. lazkovii* differs in the size and composition of the helices from its own functional ITS2 (Fig. 11: B), these helices are absent from Table 4.

Discussion

Incongruence of the plastid and ITS trees and paraphyly of *Bactria* s. l.

Both BI and ML trees based on the ITS data (Figs. 3–6) and on the combined plastid matrix (Figs. 1; 2) recovered the same eight highly supported clades: the RFM-clade named by T. M. Schuster et al. (2011b), including *Reynoutria*, *Fallopia*, and *Muehlenbeckia*; the clade *Duma*; the clade *Polygonum* s. str.; the clade (*Polygonella* + *Polygonum* sect. *Duravina*); the clade *Atraphaxis*; the clade *Persepolium*; the clade *Bactria ovczinnikovii* (two accessions of the same species); and the clade *Caelestium* (*Bactria lazkovii* + *Polygonum tianschanicum*).

Our ML and BI plastid trees (Figs. 1; 2) are similar to those obtained by Schuster et al. (2011b, 2015) for the combined plastid plus ITS sequence data matrix, and in general agree with our previous plastid trees (Yurtseva et al., 2016). Our plastid data confirmed the RFM-clade and the APD-clade revealed by Schuster et al. (2011b, 2015).

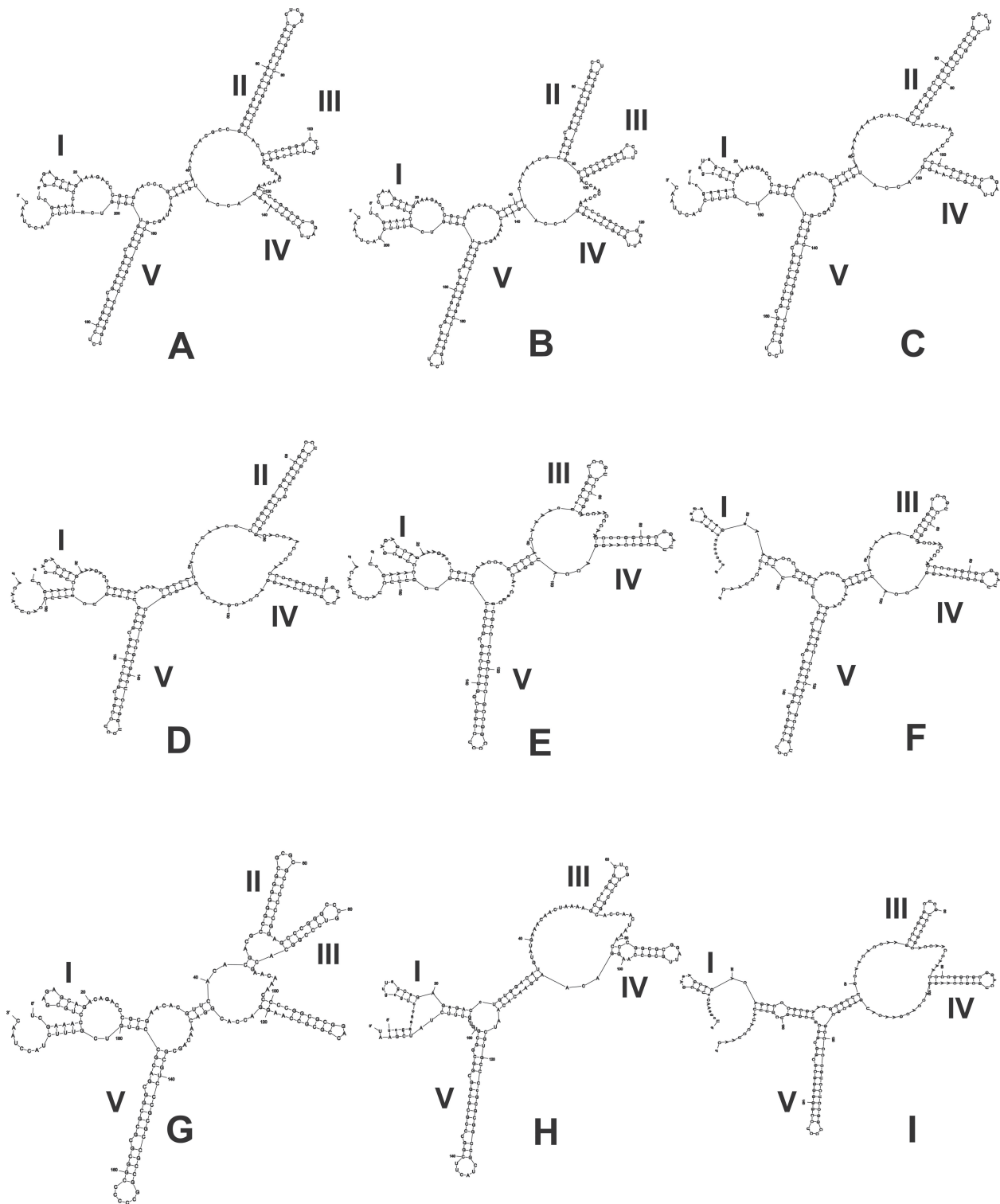


Fig. 7. Pre-rRNA secondary structure of the ITS1 locus for some genera of tribe *Polygoneae*.

A – *Reynoutria sachalinensis*; B – *Atraphaxis ariana*; C – *A. pyrifolia*; D – *Bactria ovczinnikovii*; E – *Caelestium lazkovii*; F – *C. tianschanicum*; G – *Duma florulenta*; H – *Polygonella polygama*; I – *Polygonum afromontanum*. I–V – outer helices.

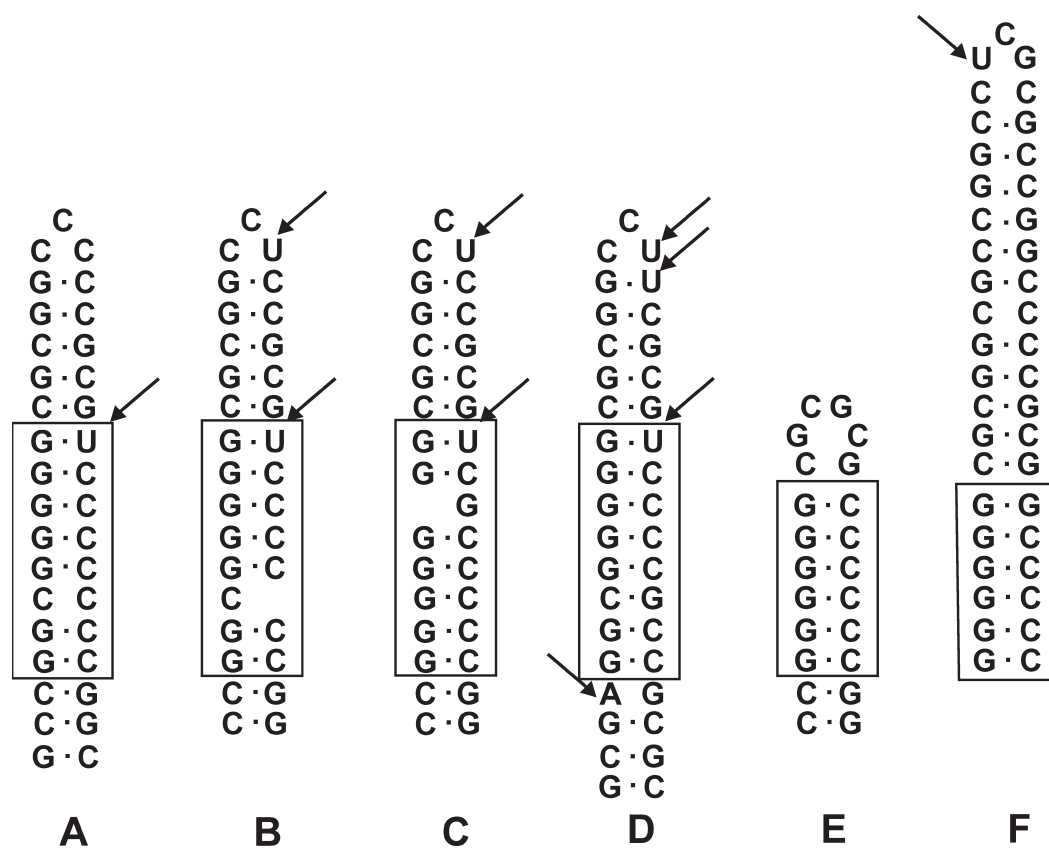


Fig. 8. Outer helix II of the pre-rRNA secondary structure of the ITS1 locus for some genera of tribe *Polygoneae*. A – *Persepolium salicornioides*; B – *Atraphaxis ariana*; C – *Bactria ovczinnikovii*; D – *Atraphaxis pyrifolia*; E – *Duma florulenta*; F – *Reynoutria sachalinensis*. The stem is formed by conserved poly-G and poly-C repeats (in frames). Arrows indicate nucleotide substitutions.

The APD-clade recovered by Schuster et al. (2011b, 2015), was confirmed only by the analyses of combined plastid data (Figs. 1; 2). It combines *Atraphaxis*, *Duma*, and *Polygonum* s. l. On the plastid trees, *Duma* is a weakly supported sister of the clade ((*Atraphaxis* + *Persepolium*) *Bactria*) (Figs. 1; 2). It is associated with the RFM-clade in the analyses of MAFFT-aligned matrix of the ITS (Figs. 3; 4), and forms a weak grade with the RFM-clade, *Polygonella* and *Polygonum* sect. *Duravina* on the ITS SSBA-based ML and BI trees (Figs. 5; 6), that agrees with the similarity of the pre-RNA secondary structure of the ITS1 and ITS2 (Tables 3, 4).

All variants of analyses confirmed the clade combining *Atraphaxis* s. str., *Persepolium*, and *Bactria ovczinnikovii*, which corresponds to the *Atraphaxis* s. l. broadly treated by S. Tavakkoli et al. (2015). The highly supported clade that includes *Atraphaxis* and *Persepolium* constantly appears on our previous (Yurtseva et al., 2016: 8, fig. 2) and current plastid trees (Figs. 1; 2), and on morphology-based trees (Mavrodiev, Yurtseva, 2017; Yurtseva et al., 2017), but it is not supported on

the ITS-based trees (Figs. 3–6, see also Yurtseva et al., 2016: 7, fig. 1). Similarly, the clade ((*Atraphaxis* + *Persepolium*) + *Bactria* s. l.), circumscribed as *Atraphaxis* s. l. (Tavakkoli et al., 2015), is supported on the plastid trees (Figs. 1; 2), but not supported on the ITS ISSB-based trees (Figs. 5; 6). That argues for generic independence of *Atraphaxis*, *Persepolium* and *Bactria*, although these taxa demonstrate great similarity in the pre-RNA secondary structure of the ITS1 and ITS2 (Tables 3, 4).

As we have mentioned above, and as we found before (Yurtseva et al., 2016), *Bactria* is monophyletic on the plastid trees (Figs. 1; 2), but non-monophyletic on all the ITS-based trees (Figs. 3–6). Although *Bactria ovczinnikovii* is associated with *Atraphaxis* and *Persepolium* on the plastid trees (Figs. 1; 2) and on most of the ITS-based trees, the clade *Caelestium* (*Bactria lazkovii* + *Polygonum tianschanicum*) is separate from *Bactria ovczinnikovii* in all the ITS-based trees (Figs. 3–6).

According to the trees that resulted from the analysis of the MAFFT-aligned matrix of the ITS region (Figs. 3; 4), the clade *Caelestium* might be interpreted

Table 3. Helices II–V of the pre-rRNA secondary structure of the ITS1 locus in tribe *Polygoneae*

Taxon	Species	Helix II			Helix III			Helix IV			Helix V					
		6	12	5	1	3	3	4	2	2	2	5	5	3	–	loop
<i>Reynoutria</i>	<i>R. sachalinensis</i>															
<i>Muehlenbeckia</i>	<i>M. platyclada</i>	6	12	5	1	3	3	4	2	2	2	5	5	3	–	3
<i>Fallopia</i>	<i>F. baldschuanica</i>	–	5	12/13	2	3	3	4	2	2	2	5	5	2	–	4
<i>Duma</i>	<i>D. florulenta</i>	2	6	1	4	1	3	3	4	2	2	5+1	5–2	3	–	4+2
<i>Polygonella</i>	<i>P. polygama</i>	–	–	–	1	3	3	4	2	1/2	5	5	5	3	0/1	3
	<i>P. gracilis</i>	–	–	–	1	3/2	3	5	2	1/2	5	5	5	2	0/1	3
<i>Polygonum</i>	<i>P. articulata</i>	–	–	–	?	?	?	?	2	2	2	5	5	2	0/1	4
	<i>P. minimum</i>	–	–	–	1	3/2	3+2	4	2	1/2	5	5	5	5	0/1	4
<i>P. sect. Duracia</i>	<i>P. douglasii</i>	–	–	–	1	3/2	3+1	4	2	2	2	5+1	5–2	5	0/1	4
<i>P. sect. Pseudomollia</i>	<i>P. molliiforme</i>	–	–	–	1	3	3	4	2	2	2	5	5	3	–	4
<i>P. sect. Plebeja</i>	<i>P. plebeium</i>	–	–	–	1	3/4	3	5	2	2	2	5	5	3	–	4
<i>P. sect. Tephis</i>	<i>P. afromontanum</i>	–	–	–	1	3	3	4	2	2	2	5	5	3	–	4
	<i>P. cognatum</i>	–	–	–	1	3	3	4	2	2	2	5	5	3	–	4
<i>P. sect. Polygonum</i>	<i>P. thymifolium</i>	–	–	–	1	3	3	4	2	2	2	5	5	2	–	4
	<i>P. aciculare</i>	–	–	–	1	3/2	3+2	5+3	2	2	2	5	5	3	–	4
<i>Caelestium</i>	<i>P. boreale</i>	–	–	–	1	3/2	3+2	5	2	2	2	5	5	3	–	4
	<i>C. tianschanicum</i>	–	–	–	1	2	3	5	2	2	2	5	5	3	0/1	4
<i>Bactria</i>	<i>C. lazkovii</i> (A)	–	–	–	1	2	3	5	2	2	2	5	5	3	–	4
	<i>C. lazkovii</i> (B)	1	6	5	3	1	3/2	3	5	2	2/3	5	5	3	–	4+2
<i>Persepolium</i>	<i>B. oeczimmikovii</i>	2	7/8	5	3	–	–	–	2	2	2	5	5	2	–	4
<i>Atraphaxis</i>	<i>P. salicornioides</i>	3	8	5–1	3+2	–	–	–	2	2	2	5	5	2	–	4
	<i>A. ariana</i>	2	8/7	5	3	1	3	3+1	4	2	2	5	5	3	–	4
	<i>A. frutescens</i>	5	8	5	3	–	–	–	2	2	2	5	5	3	–	4
	<i>A. pyrifolia</i>	4	8	5–1	3+2	–	–	–	2	2	2	5	5	3	0/1	4

Note. The nucleotides in the terminal loop of a helix are designated in bold. The rest numbers designate the number of base pairs in the stem; 0/1 – the number of nucleotides in the left/right arm of the helix.

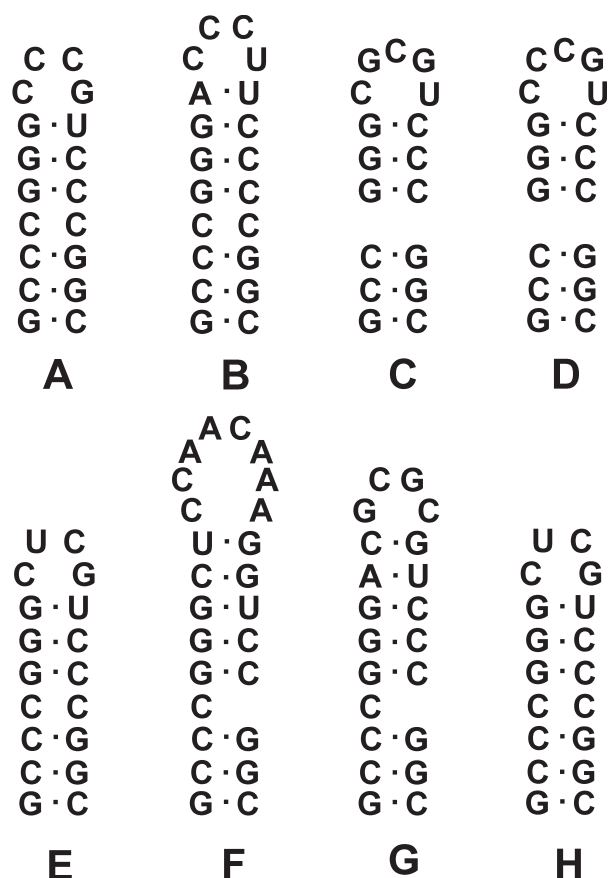


Fig. 9. Outer helix III of the pre-rRNA secondary structure of the ITS1 locus for some genera of *Polygoneae*.

A — *Reynoutria sachalinensis* and *Duma florulenta*; B — *Atraphaxis ariana*; C — *Caelestium lazkovii*; D — *C. tianschanicum*; E — *Polygonum afromontanum*; F — *P. aviculare*; G — *P. douglasii*; H — *Polygonella polygama*.

as a result of hybridization of *Bactria* with any lineage from *Polygonum* s. l. However, the ITS SSBA-based trees show the position of the clade *Caelestium* as a non-supported sister of the group that combines the RFM-clade, *Polygonum* s. l., and *Duma*. The clade *Caelestium* is either grouped with the RFM-clade, *Duma* and the North American *Polygonella* and *Polygonum* sect. *Duraviva* (ML analysis, Fig. 5), or is weakly associated with the same taxa and all the clades of *Polygonum* s. l. (BI analysis, Fig. 6). Thus, the ITS secondary structure-based reconstructions provide even less information about the nature of *Caelestium*.

Such incongruence used to be technically interpreted as a result of ancient reticulation events, rapid radiation and “incomplete lineage sorting”, higher divergence rate of the ITS1–2 regions in comparison to coding plastid regions, or other reasons (Rieseberg, Soltis, 1991; Doyle, 1992; Baldwin et al., 1995; Soltis,

Kuzoff, 1995; Wendel, Doyle, 1998; Holder et al., 2001; Álvarez, Wendel, 2003; Albach, Chase, 2004; Orthia et al., 2005; Fehrer et al., 2007; Degnan, Rosenberg, 2009; Mallet et al., 2016).

But why different alignment strategies of the same ITS matrix resulted in so different topologies? The pre-rRNA secondary structure of the ITS1 and ITS2 loci clear up the reasons of these distinctions.

Alignment strategies and the pre-rRNA secondary structure of the ITS1 and ITS2 loci of *Polygoneae*

Both BI and ML trees based on the ITS SSBA (Figs. 5; 6) and on the “raw” MAFFT (Figs. 3; 4) alignment of the ITS region recovered the same eight highly supported clades listed above, although their relationships are not resolved. Similar unresolved topologies were obtained by T. M. Schuster et al. (2015) from the analyses of the ITS data set, or the combined plastid and nuclear data sets after deletion of poorly aligned regions. Due to the low support of the basal nodes (Figs. 3–6), the trees based on different alignment strategies may be tentatively treated as “softly” incongruent (Wendel, Doyle, 1998).

The ML tree based on the MAFFT-alignment of the ITS data set is almost congruent to the ML plastid tree and agrees with our previous results based on the same alignment strategy (Yurtseva et al., 2016), or the results by Schuster et al. (2011b, 2015) obtained for the combined plastid and nuclear data sets. Although the SSBA-based ML tree (Fig. 5) is generally incongruent to the ML plastid tree (Fig. 1), the ITS SSBA-based ML tree (Fig. 5) has a higher value of log likelihood than the MAFFT-based ML tree (Fig. 3) (–6493.537870 (SSBA) vs –6641.832866 (MAFFT)).

Our thorough investigation of the ITS pre-rRNA secondary structure of various taxa of *Polygoneae* (Figs. 7–10; Tables 3, 4) showed that MAFFT, if applied to the raw sequences of the ITS1 regions lacking either helix II, or helix III, simply aligned: the left arms of helix II with the left arms of helix III; the right arms of helix II with the right arms of helix III. Next, in the MAFFT alignment, for the taxa that have a full set of helices on the ITS1 (*Reynoutria*, *Fallopia*, *Muehlenbeckia*, *Duma*, *Atraphaxis ariana*), the region of central loop is partly homologized with the proximal part of the left arm of helix II (in *Persepolium*, *Bactria ovczinnikovii*, most of *Atraphaxis* species), or helix III (in *Polygonum* s. l., *Bactria lazkovii*, *Polygonum tianschanicum*). Therefore, paradoxically, the strong congruence of the ITS MAFFT-alignment based trees (Figs. 3; 4) and the plastid trees (Figs. 1; 2) (see also Yurtseva et al., 2016) may be viewed as an artifact of the MAFFT alignment strategy, if applied to the regions with extensive indels.

Table 4. Helices I–III of the pre-rRNA secondary structure of the ITS2 locus in tribe *Polygoneae*

Taxon	Species	Helix I			Helix II			Helix III										loop	
		1+6	2	5	4	12	2	4	4, 0/1, 3	3/4	4	5/4	9	2/0	5	1/0	4	4	4
<i>Reynoutria</i>	<i>R. sachalinensis</i>	6	2/3	5	4	12	1	6	2, 0/1, 6	0/1	4	5/4	8	5/0	–	–	8	6	6
<i>Muehlenbeckia</i>	<i>M. platyclada</i>	6	2	5	4	12	1	6	2, 0/1, 3	1/2	6	5/4	8	5/0	–	–	8	6	6
<i>Fallopia</i>	<i>F. baldschuanica</i>	6	2	5	4	12	2	4	2, 0/1, 3	1/2	6	3/2	10	2/0	4	2/1	4	4	3
<i>Duma</i>	<i>D. florulenta</i>	1+6	2	5	6	12	2	4	2, 0/1, 3	1/2	6	3/2	12	3/3	6	–	–	6	6
	<i>D. horrida</i>	1+6	0/1	7/6	6	12	2	4	4	0/1	4	5/4	7	4/2	5	2/1	4	4	3
	<i>P. americana</i>	1+6	0/1	6	5	12	2	4	4	0/3	6	5/4	7	4/2	5	2/1	4	4	3
<i>Polygonella</i>	<i>P. polygama</i>	1+6	1/2	5	5	12	2	4	–	–	7	1/0	7	4/2	5	2/1	4	4	3
	<i>P. basiramea</i>	1+6	1/2	5	4	12	2	4	2	0/1	12	1/2	9	4/2	5	2/1	4	4	3
	<i>P. robusta</i>	6	2	6	8	12	2	4	6	0/1	4	5/4	9	2/0	5	2/1	4	4	3
<i>Polygonum</i>	<i>P. minimum</i>	6	0/1	5	5	12	2–1	4+2	–	–	4	5/4	9	2/0	5	2/1	4	4	3
<i>P. sect. Duracia</i>	<i>P. douglasii</i>	6+5	0/1	8	5	12	7/6	4	–	–	10, 0/1, 2	2/0	9	2/0	5	2/1	4	4	3
<i>P. sect. Pseudomalitia</i>	<i>P. molliforme</i>	6	1/2	6	5	12	3–1	4+2	–	–	10	1/0	9	2/0	5	2/1	4	4	3
<i>P. sect. Plebeja</i>	<i>P. plebeium</i>	6	4/4	9	5	1+12	3–1	4+2	2, 0/1, 3	1/2	7, 0/1, 2	2/0	9	2/0	5	2/1	4	4	3
	<i>P. afromontanum</i>	6	2/3	9	10	12	3–1	4+2	2, 0/1, 3	1/2	8, 0/1, 2	2/0	9	2/0	5	2/1	4	4	3
<i>P. sect. Tephis</i>	<i>P. cognatum</i>	6	4/2	7	4	12	3	4	2, 0/1, 3	1/2	7, 0/1, 2	2/0	9	2/0	5	2/1	4	4	3
	<i>P. thymifolium</i>	6	1/2	6	4	12	3	4	2, 0/1, 3	1/2	7, 0/1, 2	2/0	9	3/1	4	2/1	4	4	3
<i>P. sect. Polygonum</i>	<i>P. aviculare</i>	6	1/2	7	3	12	3	4	2, 0/1, 3	1/2	5, 0/1, 2	2/0	9	3/1	4	2/1	4	4	3
	<i>P. boreale</i>	1+6	1/2	6	4	12	5a	4	2	0/1	6, 0/1, 4	5/4	9	2/0	5	2/1	4	4	3
<i>Caelestium</i>	<i>C. tianschanicum</i>	1+6	1/2	6+4	4–1	12	5a	4	2	0/1	6, 0/1, 4	5/4	9	2/3	2	0/1	4	4	8
	<i>C. lazkovii</i> (A)	6	2/3	6/7+2	4	12	4b	4	2	0/1	6, 0/1, 4	5/4	9	2/3	2	0/1	4	4	8
<i>Bactria</i>	<i>B. ovczimikozii</i>	6	3/3	7+2	4	2+11/12	4b	4	2	0/1	6, 0/1, 4	4/2	10/11	2/3	2	0/1	4	4	8
<i>Persepolium</i>	<i>P. salicornioides</i>	6	3/3	7+2	4	12	7/8	9	3	0/1	5, 0/1, 4	5/4	9	2/3	2	0/1	4	4	8
	<i>A. ariana</i>	6	2/3	7/8+2	4	12	7/8	5	3	0/1	5, 0/1, 4	5/4	9	2/0	5	2/1	4	4	3
<i>Atraphaxis</i>	<i>A. frutescens</i>	6	2/3	7/8+2	4	12	7/8	5	3	0/1	6, 0/1, 4	5/4	9	2/0	5	2/1	4	4	3
	<i>A. pyrifolia</i>	6	2/3	7/8+2	4	12	7/8	5	3	1	6, 0/1, 4	5/4	9	2/0	5	2/1	4	4	5

Note. The nucleotides in the terminal loop of a helix are designated in bold. The rest numbers designate the number of base pairs in the stem; 0/1 – the number of nucleotides in the left/right arm of the helix. The letters a and b in Helix II designate different substitutions in the apical part of the stem.

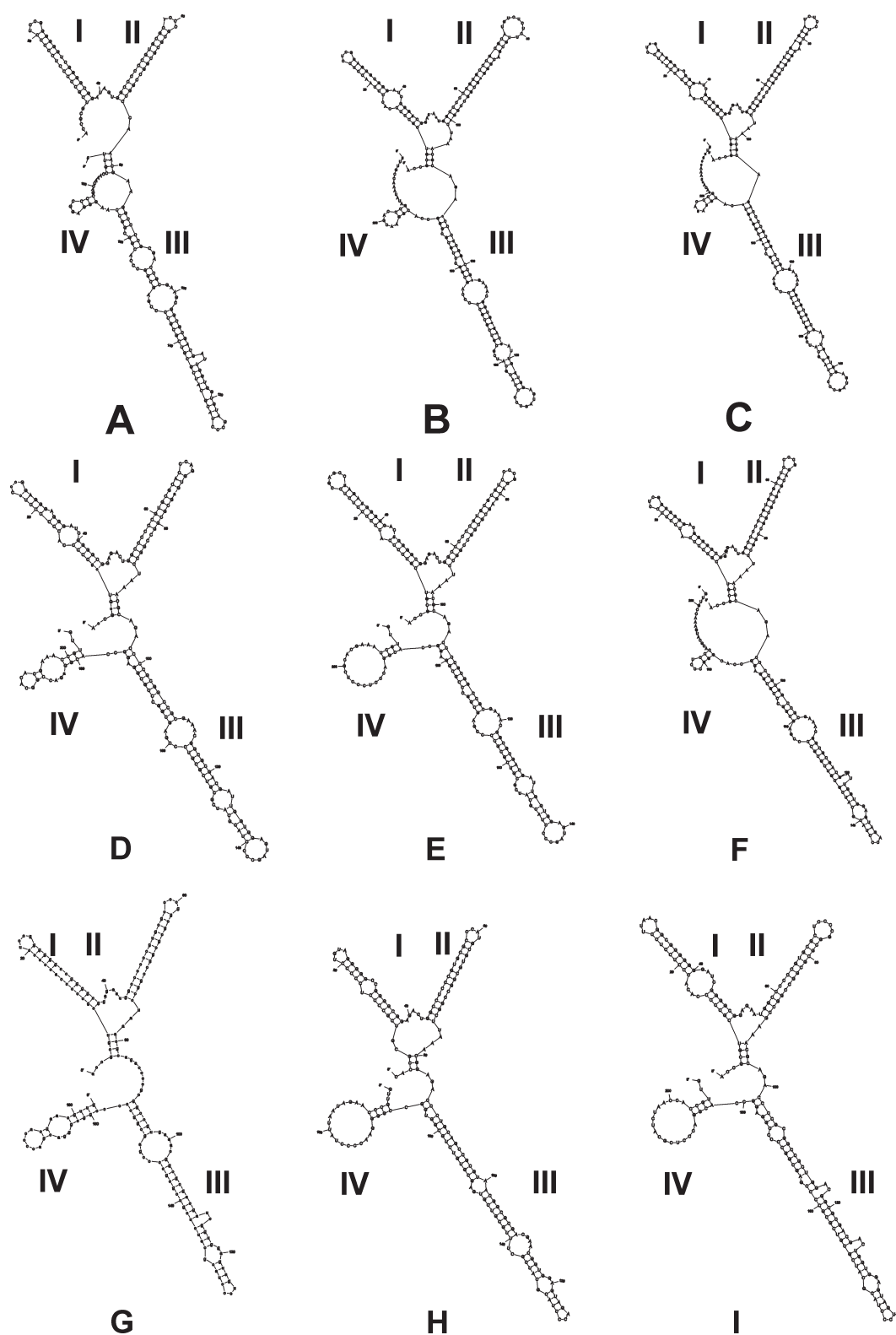


Fig. 10. Pre-rRNA secondary structure of the ITS2 locus for some genera of tribe *Polygoneae*.

A – *Reynoutria sachalinensis*; B – *Atraphaxis ariana*; C – *A. pyrifolia*; D – *Bactria ovczinnikovii*; E – *Caelestium lazkovii*; F – *C. tianschanicum*; G – *Duma florulenta*; H – *Thysanella robusta*; I – *Polygonum afromontanum*. I–IV – outer helices.

Paraphyly of *Bactria* on the ITS-based trees is not an artifact

Paraphyly of *Bactria*, which included *B. ovczinnikovii* and *B. lazkovii*, has been shown in our previous phylogenetic analyses of the ITS matrix, which were based on MAFFT alignment strategy (Yurtseva et al., 2016), and was confirmed in this study. Keeping in mind the monophyly of the same genus on the plastid-based tree, earlier we interpreted paraphyly of *Bactria* (Yurtseva et al., 2016) as a result of reticulation of one of two species, or as a putative artifact of the ITS sequence data, such as pseudogenisation (Baldwin et al., 1995; Álvarez, Wendel, 2003; Feliner, Rosselló, 2007; Poczar, Hyvönen, 2010). The addition of the new non-pseudogenic ITS sequence of *Polygonum tianschanicum* to the ITS matrix clearly revealed the close relationship of *Bactria lazkovii* and *Polygonum tianschanicum* by the ITS and the separate position of their common clade from *Bactria ovczinnikovii* in all the ITS-based trees (Figs. 3–6).

The detailed examination of the secondary structure of pre-rRNA of the ITS1 locus of *Polygoneae* confirmed the close relationship of *Bactria lazkovii* and *Polygonum tianschanicum*, as well as the strong contrast with *Bactria ovczinnikovii*. By the whole pre-rRNA secondary structure of the ITS1 locus, *B. lazkovii* and *Polygonum tianschanicum* are close to *Polygonum* s. l. (incl. *Polygonella*) (Fig. 7: E, F, G, H), that agrees with the results of ML analysis of the MAFFT-aligned ITS data set, but they differ from *Polygonum* s. l. in their unique structure of helices III and V (Fig. 7: C, D; Table 3). For the ITS2 locus, *Bactria lazkovii* and *Polygonum tianschanicum* share the structure (but not a composition) of helix I with some species of *Polygonum* s. l., but have a unique structure of helix II, and share the structure of helix III with *Bactria ovczinnikovii*, *Atraphaxis* and *Persepolium*.

In contrast, *Bactria ovczinnikovii* is close to *Persepolium* and most of *Atraphaxis* species (except species having a full set of helices), by the pre-rRNA secondary structure of the ITS1 and ITS2 loci and the composition of shared helices (Fig. 7: B–D, 10; Tables 3, 4).

Thus, the ITS regions of *Bactria lazkovii* and *Polygonum tianschanicum*, on the one side, and that of *Bactria ovczinnikovii*, on the other, are rather different in terms of both first and secondary structures. Despite the fact that *Bactria lazkovii* and *Polygonum tianschanicum* resemble *Polygonum* s. l. in the entire pre-rRNA secondary structure of the ITS1, the structure of the individual helices on the ITS1 and ITS2 does not allow this group to be attributed to any subclade of *Polygonum* s. l. or *Bactria*.

The most likely explanation for the incongruent positions of *Caelestium* in the plastid and ITS SSBA-based trees and the difference in the pre-rRNA secondary

structure of the ITS1 and ITS2 loci is that this group appeared as a result of reticulation of *Bactria* as a maternal taxon and an unknown taxon, which served as a putative donor of the ITS array(s) in the distant past, before its splitting into *B. lazkovii* and *Polygonum tianschanicum*. The donor of the ITS is probably extinct or missing from the analyses. However, due to a lack of exact knowledge of the past, this explanation, even if it seems simple, remains speculative.

The pseudogenic ITS is a primary state for *Bactria lazkovii*?

The pseudogenic ITS1–2 of *Bactria lazkovii* detected in this study along with the functional one, is characterised by abundant transitions and indels, including the 5.8S gene, a low GC-content and a partly destroyed pre-rRNA secondary structure of the ITS2 locus. The pre-rRNA secondary structure of pseudogenic ITS1 locus partly matches that of its functional ITS, but has helix II. That can be considered a pseudogenic paralog of the functional ITS1–2, which preserved the primary state of pre-rRNA secondary structure of ITS1 locus with a full set of helices on the ITS1. Otherwise, it can be considered as ITS obtained as a result of hybridization of *B. lazkovii* with an unknown taxon that served as a donor of additional ITS. In any case, the pseudogenic ITS1–2 of *B. lazkovii* differs from the functional ITS1–2 of *Bactria ovczinnikovii* in terms of both the first and secondary structures and cannot be considered as the primary state for the latter.

A full set of helices on the pre-rRNA secondary structure of the ITS1 was apparently a primary state not only for *Bactria lazkovii*, but for other taxa of *Polygoneae*. For example, the members of *Reynoutria*, *Fallopia*, *Muehlenbeckia*, *Duma*, some *Atraphaxis* (*A. ariana*, and also *A. badgysi*, *A. radkanensis*) have a full set of outer helices (five in total) on the ITS1. It is obvious that the same helices have been lost independently in different genera of *Polygoneae*. The members of *Polygonum* s. l., *P. tianschanicum*, and *Bactria lazkovii* have lost helix II on the functional ITS1. In contrast, most species of *Atraphaxis*, *Persepolium*, and *Bactria ovczinnikovii* have lost helix III on the functional ITS1 (Fig. 7; Table 3). The species of *Atraphaxis* with a full set of helices on the ITS1, or lacking helix III, have similar structure and composition of shared helices, that cannot be said about *Bactria ovczinnikovii*, on the one hand, and *B. lazkovii* and *Polygonum tianschanicum*, on the other hand.

Our study confirmed that the pre-rRNA secondary structure of the ITS1 and ITS2 is a helpful tool for optimizing alignment and for making full use of the phylogenetic information of DNA sequences. This in itself is a

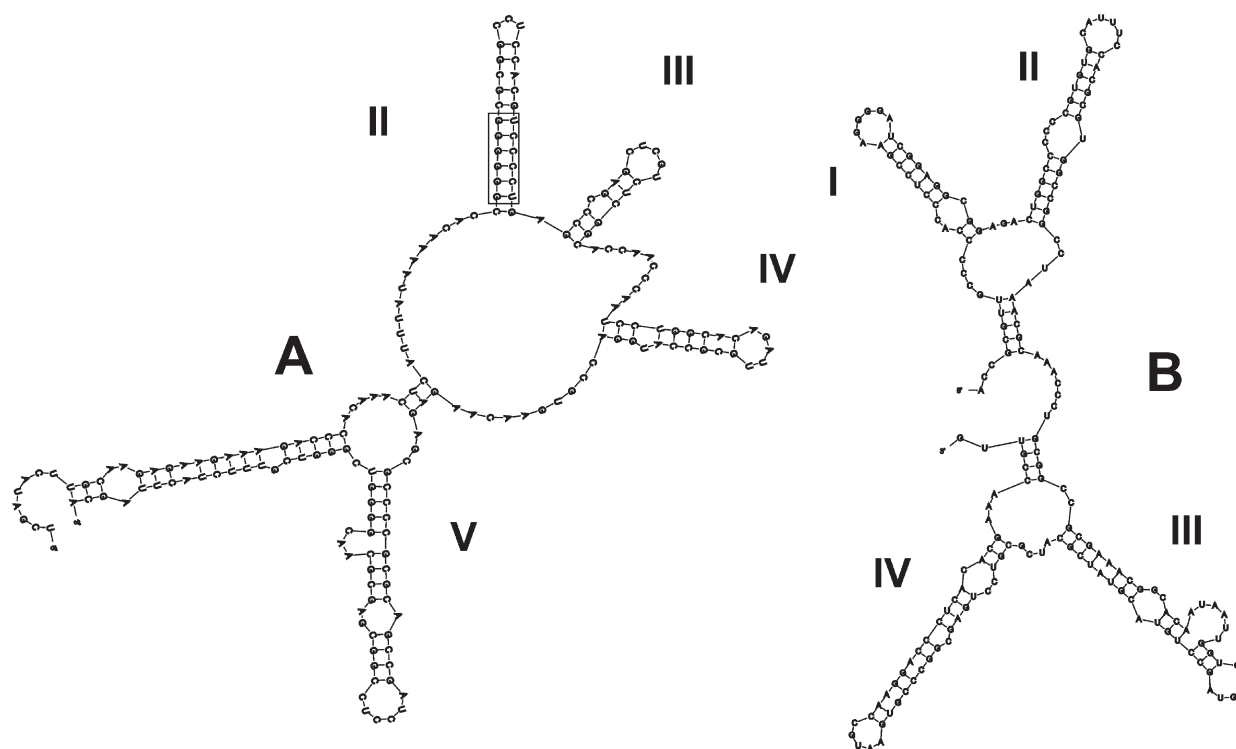


Fig. 11. Pre-rRNA secondary structure of the ITS1 (A) and ITS2 (B) loci of the pseudogenic ribotype B of *Caelestium lazkovii*.

fairly useful taxonomic character (Mai, Coleman, 1997; Gottschling et al., 2001; Wang et al., 2007; Tippery, Les, 2008; Giudicelli et al., 2017).

On ranks and circumscriptions

In general, we completely agree that any historical explanations (for example, the models of speciation, current reconstructions of geological histories, putative problems with the climates that may have existed in the past, even the discussions regarding the fossil records and molecular dating) that seem to be hypothetical or assumption-based by definition, should not be confounded with the criteria used to distinguish taxa (Wheeler, Platnick, 2000).

At the present stage of molecular-based studies, genera and other taxa of higher ranks can and should be congruent with a phylogenetic pattern. Many authors (Backlund, Bremer, 1998; Entwisle, Weston, 2005; Humphreys, Linder, 2009; Barraclough, Humphreys, 2015) provide the principles of phylogenetic classification and propose the criteria for distinguishing the taxa. Among them, monophyly of a taxon is a basic principle (e. g., Stevens, 1985; Backlund, Bremer, 1998; Entwisle, Weston, 2005). B. E. Pfeil and M. D. Crisp (2005) agree that taxa should be monophyletic and discuss

the problem of paraphyletic groups which perpetuate a classification, that obscures information and reduces predictive power. A. Backlund and K. Bremer (1998) also recommend minimising nomenclatural change and maximising its stability, providing phylogenetic information, and easing identification of the taxa. However, strictly speaking, there are no formal criteria that would clearly define the rank of a taxon, and the definition of a genus is sometimes even more arbitrary than the definition of a species.

Backlund and Bremer (1998) and P. F. Stevens (2001, 2002; see also Bentham, 1861) would not recommend recognising a monotypic genus if it is a sister of a larger genus. They believe that if a newly discovered taxon is the sister to an existing named genus, this does not mean that a new genus is needed for the newly described taxon. However, if the new taxon makes the genus not-monophyletic, the latter cannot be adopted in a broad circumscription (Stevens, 2001).

Thereby, the maintenance of paraphyletic *Bactria*, as this genus appeared on the ITS trees (Yurtseva et al., 2016 and this study) would be simply incorrect. The same is true for *Polygonum* s. l., which demonstrates monophyly on the plastid trees (Figs. 1; 2) but is polyphyletic on the ITS SSBA-based trees (Figs. 5; 6).

The split of the most inclusive genus *Bactria* into two small monophyletic genera (monotypic *Bactria* with a single species *Bactria ovczinnikovii* and *Caelestium* including *Bactria lazkovii* and *Polygonum tianschanicum*) is a decision alternative to the single genus *Bactria* Yurtseva et Mavrodiev s. l. (Yurtseva et al., 2016) with three species. As already discussed, the clear sistership of the clade (*Bactria lazkovii* + *Polygonum tianschanicum*) and *B. ovczinnikovii* on the plastid trees does not contradict the separation of *B. lazkovii* and *Polygonum tianschanicum* from *Bactria ovczinnikovii* as distinct genera. The sistership of two taxa in itself provides absolutely no analytical reason for lumping these two taxa into one taxon (for example, due to the principle of monophyly), especially where there are clear morphological and other differences between sister taxa. The flowering plants and the gymnosperms are an extreme example (Soltis et al., 2018). Another example is the recently described genus *Pseudoziziphus* Hauenschild (*Rhamnaceae*), which appeared as a sister of the genus *Condalia* Cav. (Hauenschild et al., 2016), which is confirmed by a clear difference in morphology and biogeography. The papers of E. V. Mavrodiev et al. (2014), M. B. Crespo et al. (2015) and S. L. Low et al. (2018) contain similar examples.

As a result of the genus-rank separation of *Caelestium*, we obtain the monotypic *Bactria* for *B. ovczinnikovii*, which was also viewed as part of a widely treated *Atraphaxis* s. l. (Tavakkoli et al., 2015), including also *Persepolium* and *Bactria* s. l. The similarity of the pre-RNA secondary structure of the ITS1 and ITS2 supports the opinion by S. Tavakkoli et al. (2015). But the weak support of their common clade on the ML and BI ITS SSBA-based trees and the low support of the clade (*Atraphaxis* + *Persepolium*) in all the ITS-based trees, argue against their lumping. Sound distinctions in the morphology of the shoots, ochreas, and pollen (Yurtseva et al., 2016, 2017), as well as distinct exocarp anatomy (Yurtseva et al., 2019, in this issue) provide evidences in favor of distinguishing these genera.

Some authors consider plastid markers to be more significant than the ITS, in which case we should support the genus *Bactria* with three species. As we have demonstrated, *Caelestium* (*Bactria lazkovii* and *Polygonum tianschanicum*) shows a maximum total difference over sequence pairs from other genera of *Polygoneae* (Table 2). For example, *Bactria lazkovii* differs from *B. ovczinnikovii* by one-nucleotide substitutions in 43 sites and by numerous indels in 3-loci plastid alignment 1218 bp long. These results provide strong evidence for the separation of *B. lazkovii* and *Polygonum tianschanicum* from *Bactria ovczinnikovii*. Strong molecular distinctions of *B. lazkovii* and *Polygonum tianschanicum* give no rea-

son for considering them a single species. They differ by substitutions in 10 sites and two indels (1 and 9 bp long) on the ITS region, and by substitutions in 7 sites and by an indel 28 bp long on the combined plastid region.

Since W. Hennig (1966), the notion that sister taxa need to have equal rank is common, as reviewed by G. Giribet et al. (2016). Thus, *Bactria ovczinnikovii* and *Caelestium* (*Bactria lazkovii* and *Polygonum tianschanicum*) can be accepted at the rank of genus. A small number of species in these genera is the result of their ancient origin and reduced diversity, which follows from their highly isolated local distribution in the old mountain systems of the Pamirs and Tien Shan.

Morphology and distribution of *Bactria ovczinnikovii*, *B. lazkovii* and *Polygonum tianschanicum*

The morphology of the taxa is given in detail in O. V. Yurtseva et al. (2019, in this issue) and is only briefly summarized here. All three species, *Bactria ovczinnikovii*, *B. lazkovii* and *Polygonum tianschanicum*, are dwarf shrubs 10–30 cm tall with leathery leaf blades (but of different shapes). The combinations of morphological traits that differentiate *Caelestium* (*Bactria lazkovii* and *Polygonum tianschanicum*) from *Bactria ovczinnikovii* are summarized in Table 5 (see also Yurtseva et al., 2016). The perianth of all the taxa is divided for $\frac{4}{5}$ – $\frac{9}{10}$ of its length in 5 (rarely 6) equal tepals, which bear papillae at the edges (Fig. 12). The perianth of *B. ovczinnikovii* resembles that of *Persepolium*, and the perianth of *Bactria lazkovii* and *Polygonum tianschanicum* resembles that of *Polygonum* s. str.: *Bactria*

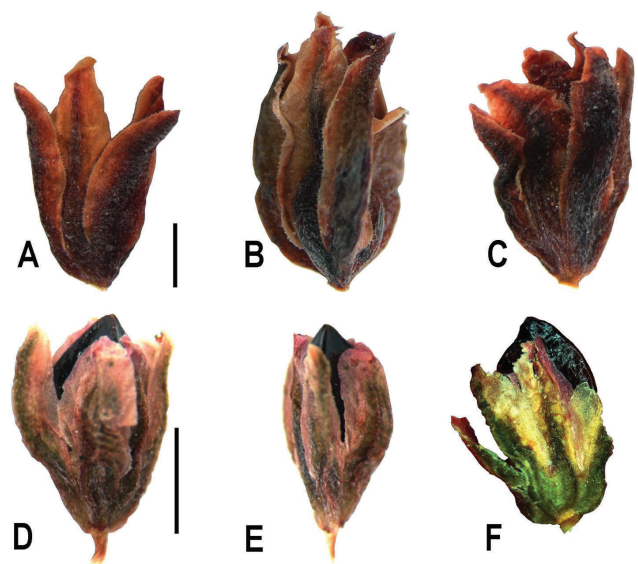


Fig. 12. Perianths.

A–C — *Bactria ovczinnikovii*; D–E — *Caelestium lazkovii*; F — *C. tianschanicum*. Scale bars — 1 mm.

Table 5. Morphological differences of *Bactria* and *Caelestium*

Morphological traits		<i>Bactria</i> (<i>B. ovczinnikovii</i>)	<i>Caelestium</i> (<i>C. lazkovii</i> , <i>C. tianschanicum</i>)
Perianth tepals	texture	sepaloid	petaloid
	shape	lanceolate	broadly ovate
	apex	acuminate	obtuse
	color	greenish-purple	pinkish-white
Fruit	ribs	sharp	obtuse
	faces	concave, matt	flat, glossy
Styles	shape	linear below stigmas	linear for the whole length
	connation	connate at base	free from base
Stigmas	shape	mini-capitate	inconspicuous

ovczinnikovii has sepaloid tepals of the perianth, while they are petaloid in *B. lazkovii* and *Polygonum tianschanicum*. All three taxa have perianth glabrous outside and bearing papillae only at the tepals edges, which we have never seen in *Polygonum* s. str.

All three species have the microreticulate-foveolate sporoderm ornamentation (Yurtseva et al., 2014, 2016), which is quite different from any type of sporoderm ornamentation observed in *Polygonum* s. l. (Hedberg, 1946; Hong et al., 2005): psilate, micropunctate, microspinulose (palynotype *Avicularia*); rugulate or foveolate with microspinules around the colpi, semitectate-reticulate at mesocolpia (palynotype *Duravia*); psilate around the colpi, verrucate at $\frac{1}{3}$ of mesocolpia and poles (palynotype *Pseudomollia*).

Bactria ovczinnikovii and *B. lazkovii* demonstrate a striking contrast in the structure of the exocarp cells of their pericarp, which differ in the shape and size of the lumen and pore channels, in the details of their walls, which is combined with different shapes of fruits, styles, and tepals (Yurtseva et al., 2019, in this issue).

The distributional ranges of three species are very distant and divided by the high mountain ranges of the Pamirs and Tien Shan. *Bactria ovczinnikovii* is a local endemic of the Southwestern Pamir, where it grows in several populations on the right bank of the Pyandj River (Tajikistan, Khatlon reg., Shuroabad and Hamadony districts). It is part of the Turkestanian Province of the Western Asian Subregion, the flora of which is related to the Armeno-Iranian flora in the west and to the Central Asian and northwestern Himalayan flora in the east and numbers no less than 50 endemic genera and hundreds of endemic species (Takhtajan, 1986).

Bactria lazkovii and *Polygonum tianschanicum* are endemics of two different regions of the Central Asian Subregion (Takhtajan, 1986). *B. lazkovii* was collected in a single locality in the Inner Tien Shan (Kyrgyzstan, Naryn reg., Zhumgal distr.), part of the Central Tien Shanian Province. The flora of this province includes

ca. 1800 species and only one endemic genus *Tianschan-iella* B. Fedtsch. ex Popov (Takhtajan, 1986), though G. A. Lazkov and A. R. Umralina (2015) listed numerous endemic or subendemic genera of flowering plants in the flora of Kyrgyzstan. *Polygonum tianschanicum* is a local endemic of the Dzungaro-Tien Shanian Province of the Central Asian Subregion, which contains about 50 endemic species and several endemic genera (Takhtajan, 1986).

The Pamir and Tien Shan Mountains are similar in their floristic composition, but consistently have been treated as separate biogeographical entities (e. g., Koro-rov, 1962; Takhtajan, 1986). As V. N. Pavlov (1980) summarized, ca. 70 % of the species are common for both floras; however, the number of endemic genera is very high in the Pamir and Tien Shan regions (Nowak, Nobis, 2010; Nowak et al., 2011; Safarov, 2013; Lazkov, Umralina, 2015). In short, the floras of the Pamirs and Tien Shan are closely related, but are quite different in their composition. Similarly, *Bactria ovczinnikovii* and *Caelestium* are closely related, but differ greatly in morphology and molecular markers studied.

Morphology, distributions, and intrageneric taxa recognized in *Polygonum* s. l.

In our current analyses, not only *Bactria*, but also *Polygonum* s. l. are monophyletic on the plastid trees (Figs. 1; 2), but non-monophyletic on ML and BI trees based on the ITS SSBA matrix (Figs. 5; 6), that is in agreement with ITS-based topologies obtained by T. M. Schuster et al. (2015). Thus, we would like to describe a more general context of our current re-circumscription of the genus *Polygonum* s. l.

On the ITS SSBA-based trees, the North American clade (*Polygonella* + *Polygonum* sect. *Duravia*) is grouped with the members of the RFM-clade and *Duma* distributed in Southeast Asia, Australasia and South America, but separately from the clade *Polygonum* s. str. (Figs. 5; 6), which combines the species distrib-

uted mostly in Central Asia and the species distributed worldwide in the regions with temperate climate.

Our results have shown that the North American *Polygonella* and *Polygonum* sect. *Duravia* differ from *Polygonum* s. str. (as well as from the rest of *Polygoneae*) in the composition of helix V of the pre-rRNA secondary structure of the ITS1 (Table 3) and of the most conserved helix III (Mai, Coleman, 1997; Coleman, 2003, 2007) of the pre-rRNA secondary structure of the ITS2 (Table 4). In addition, *Polygonella* differs from *Polygonum* sect. *Duravia* in the structure of helix III on the ITS1 and helix III on the ITS2.

Polygonella and *Polygonum* sect. *Duravia* have a fairly distinct palynotype *Duravia* (Hedberg, 1946; Hong et al., 2005). MP analysis of morphological data (Ronse De Craene et al., 2004) also combined *Polygonum* sect. *Duravia* with *Polygonella* in a clade sister to the rest of *Polygonum* s. l. In contrast to *P. sect. Polygonum* and *P. sect. Tephis*, both having petioles with 4–6 bundles and collenchyma in four strands, *P. sect. Duravia* and *Polygonella* have the petioles with 1–3 bundles and poorly developed collenchyma (Haraldson, 1978). In addition, *Polygonella* differs from the rest of *Polygonum* s. l. in having internodal branching, strongly reduced vascularization of the flower, abruptly dilated inner filaments, solitary flowers at the nodes, and conspicuous scarious bracts (Watson, 1873; Horton, 1963; Ronse De Craene et al., 2004).

The strong geographic isolation of the North American *Polygonella* and *Polygonum* sect. *Duravia* from *Polygonum* s. str., which is distributed mostly in the Old World, and clear differences in their morphology are consistent with the separate positions of these taxa on the ML and BI ITS SSBA-based trees (Figs. 5; 6), that makes the inclusion of *Polygonella* and *Polygonum* sect. *Duravia* into the broadly defined genus *Polygonum* s. l. (Ronse De Craene et al., 2004; Schuster et al., 2011b, 2015) at least questionable. Thus, our current phylogenetic results are consistent with the narrow circumscription of *Polygonella*, which was previously considered as a distinct genus (Meisner, 1826, 1857; Benthams, Hooker, 1880; Dammer, 1893), and *Duravia* (S. Watson) Greene (1904: 23).

Two highly supported subclades of the clade *Polygonum* s. str. which were recognized here as *P. sect. Polygonum* (incl. *P. aviculare*) and *P. sect. Tephis* (incl. *P. afromontanum*), are special in their distribution and ecology. The subclade *P. sect. Polygonum* combines mostly mesophytic or mesoxerophytic species distributed worldwide in regions with a temperate climate, as pioneer species on sea or river banks, on the shores of estuaries, irrigation canals and temporary streams, as well as in disturbed steppes, urban and ruderal places.

Our results have shown that the subclades, which correspond *P. sect. Polygonum* (incl. *P. aviculare*) and *P. sect. Tephis*, as they appeared on the ITS-based trees, differ from each other in the composition of helix III and helix V of the pre-rRNA secondary structure of the ITS1 (Table 3) and have slight differences in the composition of the most conserved helix III (Mai, Coleman, 1997; Coleman, 2003, 2007) of the pre-rRNA secondary structure of the ITS2 (Table 4).

Our results have shown the place of *P. afromontanum*, one of two members of *P. sect. Tephis* from Africa, in the subclade which includes the members of *P. sect. Polygonum* distributed mostly in Southwest and Central Asia and named here “*P. sect. Tephis*” (Komarov, 1936; Hara et al., 1982; Li et al., 2003).

M. Adanson (1763: 276) described the genus *Tephis* Adans. for *Atraphaxis undulata* L. (Linnaeus, 1753: 333), which presently is known as *Polygonum undulatum* (L.) P. J. Bergius (1767) from South Africa and has a tetramerous perianth, dimerous ovary and short internodes. *Polygonum* sect. *Tephis* (Adans.) Meisn. was established by C. F. Meisner (1857) and accepted by G. Benthams and J. D. Hooker (1880), U. Dammer (1893), R. Jaretsky (1925), G. Roberty and S. Vautier (1964). *P. afromontanum* was added to this section as sharing the characteristics of stem and petiole anatomy (Haraldson, 1978), perianth, and ovary (Ronse De Craene, Akeroid, 1988). Both species have palynotype Avicularia with densely microspinulose ornamentation of sporoderm (Hong et al., 2005), peculiar also for *P. sect. Polygonum*.

The current position of *P. afromontanum* does not contradict the position of *P. undulatum* in the clade *Polygonum* s. l. as a sister to the group including *P. aviculare* in *rbcL* phylogeny (Galasso et al., 2009). Also it does not contradict the results of MP analysis based on morphology (Ronse De Craene et al., 2004), which demonstrated the sisterhood of subsections *Polygonum* and *Tephis* within *P. sect. Polygonum*. The latter, in turn, appeared as a sister to *P. sect. Duravia* (incl. *Polygonella*). It is likely that we can apply the name “*Polygonum* sect. *Tephis*” to the whole subclade of Afro-Asian *Polygonum* species (Figs. 5–10), but the nomenclatural decision is premature until analyses of *P. undulatum*, the nomenclatural type of *P. sect. Tephis*.

Our results confirmed positions of *Polygonum molliiforme* (the nomenclatural type of *P. sect. Pseudomollia*) and *P. plebeium* (the nomenclatural type of *P. sect. Plebeja*) in the subclade “*P. sect. Tephis*” in the ITS-based trees, that was demonstrated earlier (Yurtseva et al., 2010; Schuster et al., 2011b). *Polygonum molliiforme* and *P. plebeium* share the structure of helices IV and V of the pre-rRNA secondary structure of the ITS1, but

P. plebeium differs from the rest members of “*P. sect. Tephis*” by the structure of helix III. Both species have also distinct structure of helices I, II, and III of the pre-rRNA secondary structure of the ITS2, *P. molliiforme* having the longest helices.

Our results have shown incongruent positions of *P. molliiforme* and *P. plebeium* on the plastid and ITS trees. Both species fall into the subclade “*P. sect. Tephis*” in our current ITS-based topologies (Figs. 3–6), but fall in the sister subclade *P. sect. Polygonum* on the plastid trees (Figs. 1; 2). The incongruent placement of *P. molliiforme* and *P. plebeium* in two sister subclades might be a result of introgression, or caused by other reasons (Soltis, Kuzoff, 1995; Rieseberg, Soltis, 1991; Rieseberg et al., 1996; Mallet et al., 2016), which makes the taxonomy of *Polygonum molliiforme* and *P. plebeium* questionable. Extensive analyses of additional plastid markers and a wider range of species are necessary to clarify the reticulation in *Polygonum* s. str., which was shown also with ISSR and RAPD analyses (Yurtseva et al., 2006; Voylokov et al., 2009).

Conclusions

1. Based on the results of molecular phylogenetic analyses of combined plastid data and ITS data, on the details of the pre-rRNA secondary structure of the ITS1 and ITS2 loci, morphological and distributional data, we strongly recommend accepting the clade (*Bactria lazkovii* + *Polygonum tianschanicum*) at generic rank. This clade is presumably a result of ancient hybridization of any taxon related to *Bactria* and an unknown taxon, and demonstrates the role of hybridization in the generic origin in tribe *Polygoneae*.

2. The new genus *Caelestium* Yurtseva et Mavrodiev is established here to include two species (*C. lazkovii* and *C. tianschanicum*). This genus is endemic to the Tien Shan Mountains (Central Asia).

3. Based on the standard molecular, as well as morphological and distributional data, the broad circumscription of the genus *Polygonum* as including *Duravia* and *Polygonella* seems questionable. Our current results agree with the narrow treatment of *Polygonella*, *Duravia*, and *Polygonum* s. str. A larger set of species and additional markers are necessary to clarify the relationships within *Polygonum* s. str.

Taxonomic treatment

Key to the genera *Atraphaxis*, *Bactria*, *Caelestium*, *Duravia*, *Persepolium*, *Polygonum* s. str., and *Polygonella*

1. Branches adnate to stems, appearing to arise internodally 1. *Polygonella*.

- + Branches not adnate to stems, not appearing to arise internodally 2.
2. Annual or perennial herbs 3.
- + Shrubs or subshrubs 4.
3. Stems rounded, finely 8–16-ribbed; leaf blade venation pinnate, secondary veins conspicuous; worldwide 2. *Polygonum* s. str. (in part).
- + Stems quadrangular in cross section, ribs obscure or absent; leaf blade venation parallel, secondary veins not conspicuous; mostly Western North America 3. *Duravia*.
4. Perianth tube long, filiform; perianth tepals 4–5, unequal; 2–3 inner tepals strongly accrescent in fruiting 4. *Atraphaxis* (in part).
- + Perianth tube short, funnel-shaped; perianth tepals 5, equal, non accrescent in fruiting 6.
6. Ochreas tubular, later lacerate in two aristate-subulate lacinulas and finely serrate-incised middle portion 4. *Atraphaxis* (in part).
- + Ochreas tubular-lanceolate, later lacerate in two, four and more lacinulas 7.
7. Leaf blades linear-lanceolate, deciduous in fruiting; perianth totally shortly velutinous puberulent outside; Zagros 5. *Persepolium*.
- + Leaf blades ovate or lanceolate, preserved in fruiting; perianth glabrous or papillate either only on the tube, or only on the tepal margins 8.
8. Perianth glabrous, or papillose on tube; SW and Central Asia 2. *Polygonum* s. str. (in part).
- + Perianth glabrous, bear papillae on the tepal margins; Pamirs, Tien Shan 9.
9. Perianth tepals sepeloid, lanceolate or oblong-elliptical; purple with narrow pinkish margin; three outer tepals keeled and cucullate; tube funnel-shaped; fruit with distinct ribs and concave matt faces; styles fused at base in a stub; stigmas globular; SW Pamir 6. *Bactria*.
- + Perianth tepals petaloid, broadly ovate or oblong-elliptical; green with wide pinkish-white margin; three outer tepals almost flat; tube cup-shaped or sacciform; fruit with obtuse ribs and flat glossy faces; styles free from base, linear for their whole length; stigmas invisible; Tien Shan 7. *Caelestium*.

Bactria Yurtseva et Mavrodiev, 2016, PeerJ, 4 (e1977): 42, p. p., quoad *B. ovczinnikovii*. ≡ *Atraphaxis* sect. *Ovczinnikovia* Yurtseva ex S. Tavakkoli, 2015, Pl. Syst. Evol. 301 (4): 1167. — Type: *Bactria ovczinnikovii* (Czukav.) Yurtseva et Mavrodiev.

Bactria ovczinnikovii (Czukav.) Yurtseva et Mavrodiev, 2016, PeerJ, 4 (e1977): 43. ≡ *Polygonum ovczinnikovii* Czukav. 1962, Izv. Akad. Nauk Tadzhiksk. SSR, Otd. Est. Nauk, 2 (9): 64; ead. 1968, Fl. Tadzhiksk. SSR, 3: 250; ead. 1971, Consp. Fl. Asiae Mediae, 2: 208. ≡ *Atraphaxis ovczinnikovii* (Czukav.) Yurtseva, 2014, Pl. Syst. Evol. 300 (4): 763. — Holotype: “Tajikistan [Khatlon Region, Shuroabad Distr.], the right bank of the Pyandj River to the N of the village Bag [Bog], at

gravely ridges at the Schpilau River, 1100 m a. s. l., 1 VI 1960, S. Yunusov, № 1586" (LE: LE 01065511!; iso-type – TAD).

Ic.: Fig. 12: A–C. — For published figures see Czukavina, 1962: fig. 1; Czukavina, 1968: tab. 44, figs. 2–6; Yurtseva et al., 2014: figs. 1: A–C; 4: A–C; Yurtseva et al., 2016: figs. 3: B; 4; 5; 16: A; 19: A, B; suppl. 10; Yurtseva et al., 2017: 161, figs. 6: B, C; 9: A, B; 14: A–C; 15: K; 17: F.

Dwarf shrub. Woody shoots divaricately branched, covered with gray bark, fibrously disintegrated. Annual shoots leafy, shortly puberulent. Leaves thick, broadly ovate, suddenly narrowed to a petiole, joined with articulation. Ochreas lanceolate-tubular, later bilacrate or bidentate. Thyrses terminal, leafy, with 3–7 clusters of 1–2(3) flowers. Perianth campanulate, divided to $\frac{5}{6}$ – $\frac{8}{10}$ in 5(6) equal lanceolate or oblong-elliptical sepaloid tepals. Three outer tepals keeled and cucullate, papillate on the margins; tube funnel-shaped. Stamens 8(9). Styles 3, fused at base in a stub, stigmas globular. Fruit ovoid, trigonous, ribs distinct, faces concave, dull. Pollen tricolporate, oblong-spheroidal to spheroidal (P/E = 1.1–1.4), elliptical in equatorial view, rounded-trilobed in polar view, sporoderm ornamentation microreticulate.

Distribution and ecology. Endemic to the Southwestern Pamir (Tajikistan), grows on the right bank of the Pyandj River (Tajikistan, Khatlon reg., Shuroabad and Hamadoni distr.). Records in the adjacent part of Afghanistan can be expected. Rocky and gravelly slopes in the belt of forest-steppe, ca. 600 m a. s. l. Fl. V–VI, fr. VI.

Additional specimens examined. **South Tajikistan, Khatlon Region.** *Shuroabad District*: Mts in environs of the vill. Bag [Bog] near the Pyandj River, right bank of the Shpilau River, lower reaches, creek bed on the mountainside, 1 VI 1960, G. N. Nepli (LE!); mts in environs of the vill. Bag near the Pyandj River, red sandstones on the right bank of the Shpilau River, 1 VI 1960, V. Botschantzev, T. Egorova, № 777 (LE!); mts in environs of the vill. Bag near the Pyandj River, red and gray sandstones on the left bank of the Aarzy-Su River, 2 VI 1960, *idem*, № 814 (LE!); right bank of the Pyandj River between Bog and Bakhorak, at south slope, 1100 m a. s. l., 2 VI 1960, S. Yunusov, № 1624 (LE!, TAD); right bank of the Pyandj River between Bog and Bakhorak, 29 V 1961, S. Yunusov, G. Kinzikaeva (LE!); mouth of the Surkh-Alam River, red-gray sandstone, at gravelly-stone ridges, 580 m a. s. l., 25 VII 2013, U. Ukrainskaya et al., № 12 (MW!); *Moskovsky District* [now Hamadoni Distr.]: gorge Suleyman-Dara, near the village Sarygor, gravelly slope, 31 V 1961, G. Kinzikaeva (LE!) (more in Czukavina, 1962, 1968).

Caelestium Yurtseva et Mavrodiev, gen. nov.

Type: *Caelestium lazkovii* (Yurtseva et Mavrodiev) Yurtseva et Mavrodiev.

Dwarf shrub. Woody shoots divaricately branched, covered with gray bark, fibrously disintegrated. Annual shoots leafy, shortly puberulent. Leaves oblong-elliptical, gradually narrowed to a petiole and articulated. Ochreas lanceolate-tubular, later bilacrate or bidentate. Thyrses terminal, leafy, with 3–7 clusters of 1–2(3) flowers. Perianth campanulate, divided to $\frac{4}{5}$ – $\frac{9}{10}$ into 5 equal petaloid tepals. Tepals oblong-elliptical or broadly ovate, almost flat, papillate on the margins; tube cup-shaped or sacciform. Stamens 8. Styles 3, free from base, linear for their whole length, stigmas inconspicuous. Fruit ovoid, trigonous, with obtuse ribs and almost flat faces, glossy. Pollen tricolporate, oblong-spheroidal to spheroidal (P/E = 1.1–1.4), elliptical in equatorial view, rounded-trilobed in polar view, sporoderm ornamentation microreticulate to foveolate-perforate.

Affinity. *Caelestium* resembles *Bactria* (in our revised circumscription) in the shrubby habit, lanceolate-bilacrate ochreas, campanulate perianth papillate on the tepal margins, and microreticulate to foveolate-perforate ornamentation of the sporoderm (Yurtseva et al., 2016). The petaloid tepals, cup-shaped or sacciform perianth tube, fruits with obtuse ribs and flat faces, and free linear styles differentiate *Caelestium* from *Bactria*, the latter having sepaloid tepals, funnel-shaped perianth tube, fruits with distinct ribs and concave faces, and styles connate at the base forming a stub, with small capitate stigmas.

Caelestium resembles *Polygonum* s. str. in having a campanulate perianth with five equal-sized tepals, and in habit. However, *Polygonum* s. str. differs by lanceolate-tubular ochreas with 4–18 veins, deeply lacerate-fimbriate (Komarov, 1936; Brandbyge, 1993) and psilate, micropunctate, microspinulose sporoderm ornamentation (Hedberg, 1946; Hong et al., 2005). *Polygonum* s. str. lacks papillae on the tepal edges, though some species (e. g., *P. thymifolium* Jaub. et Spach, *P. biaristatum* Aitch. et Hemsl.) bear papillae on the perianth tube (Yurtseva, pers. obs.).

Distribution. Locally distributed in the Inner and Eastern Tien Shan.

Etymology. The name is originated from “caelestis” (= heavenly, divinus).

Key to identification of species

1. Perianth divided for $\frac{4}{5}$ – $\frac{5}{6}$ of its length; tepals obtuse; leaf blades 7–10 × 2.5–3 mm, bright green, oblong-elliptical or oblanceolate, acuminate; margin revolute; fruits 2.5–3 × 1.8–2 mm; annual shoots reddish-brown 1. *C. lazkovii*.
- + Perianth divided for $\frac{8}{10}$ – $\frac{9}{10}$ of its length; tepals obtuse or slightly acuminate; leaf blades 7–8(15) × 3–5 mm, dark green, oblong-elliptical, almost spatulate, obtuse; margin flat; fruits 3–4 × 2–3 mm; annual shoots creamy or pale-gray 2. *C. tianschanicum*.

1. **Caelestium lazkovii** (Yurtseva et Mavrodiev) Yurtseva et Mavrodiev, comb. nova. $\equiv$ *Bactria lazkovii* Yurtseva et Mavrodiev, 2016, PeerJ, 4 (e1977): 43. — Holotype: “Kyrgyzstan, Naryn Region: Zhungal Distr., Kavak-Too Ridge, 5 km N of the village Sary-Bulun, on rocks, 7 VII 2006, G. Lazkov, № 24” (MW0595509!; isotypes — B, LE).

Ic.: Fig. 12: D, E. — For published figures see Yurtseva et al., 2016: figs. 3: A; 6; 16: B; 19: K, L; suppl. 9.

Distribution and ecology. Kyrgyzstan, the Inner Tien Shan, a single location on the southern slopes of the Kavak-Too Ridge. Rocky slopes at elevation ca. 1500 m a. s. l. Fl. VI–VII, fr. VII–VIII.

2. **Caelestium tianschanicum** (Chang Y. Yang) Yurtseva et Mavrodiev, comb. nova. $\equiv$ *Polygonum tianschanicum* Chang Y. Yang, 1983, J. Aug. 1st Agric. Coll. Xinjiang, 4: 55; id. 2010, Sylva Xinjiangensis: 114. — Type: “China, Xinjiang, Hejing Xian, Balguntay, in promontoriis et declivibus montibus. Alt. 1400–1500 m. 15 VI 1981. Chang Y. Yang, Bing Wang, № 810295, № 810290” (syntypes — XJA).

= *Polygonum popovii* Borodina, 1989, Pl. As. Centr. 9: 104; A. J. Li et al. 1998, Fl. Reipubl. Popularis Sin. 25(1): 7; A. J. Li et al. 2003, Fl. China, 5: 283. $\equiv$ *Atraphaxis popovii* (Borodina) Yurtseva, 2014, Pl. Syst. Evol. 300 (4): 763. — *Polygonum vacciniifolium* Popov, in sched., A. K. Skvortsov, in sched.; unpubl., non *P. vacciniifolium* Wall. ex Meisn. 1832. — Holotype: China, Xinjiang, “Кашгария, юго-восточная часть Тянь-Шаня, между Кучей и Курлей, горы у сел. Ишма, в скалах, 20 VIII 1929, М. Г. Попов, № 609 [Kashgaria, SE part of Tien Shan, between Kucha and Kurla, mountains at Ishma village, in rocks, 20 VIII 1929, M. G. Popov, № 609]” (LE: LE 01013264!; isotype — LE 01013263!).

Ic.: Fig. 12: F. — For published figures see Yurtseva et al., 2014: fig. 4: L, M.

Distribution and ecology. China (Xinjiang-Uyghur autonomous region), southern macro-slope of the Eastern Tien Shan. Mountain slopes at elevations of 1400–2600 m a. s. l. (Li et al., 2003). Fl. VI–VII, fr. VII–VIII.

Additional specimen examined. **China, Xinjiang, Dzungaria:** declive siccum in via ab Urumchi [Ürümqi] ad Karashar (на дороге от Урумчи до Карашар), 2300 m. a. s. l., 22 VII 1958, Li, Chu, № 6223 (LE 01013265! — paratype of *P. popovii* Borodina, Fbr. 1988). Specimens cited by A. E. Borodina (1989): Tien Shan, the upper reaches of the river Algoi, 1800–2400 m a. s. l., 12 IX 1879, A. Regel; 30 km to East of Ürümqi, 1000 m a. s. l., on the slope, 21 VI 1958, Li, Chu, № 7373; the mountain road from Bartu to the timber mill in Khomot, 2160 m a. s. l., 3 VIII 1958, Li, Chu, № 6980; the Hanga river valley, 25 km NW of the village Balintoy (on

the road from Korashar [Karashar] to Yuldus), 1 VIII 1958, Yunatov, Yuan, № 175 (all in LE).

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Supplementary material (Appendix) to the article is available on the journal website (www.binran.ru/journals/novitates/).

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