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THE MOLECULAR-PHYLOGENETIC STUDY OF *PETROSIMONIA* SPECIES OF CHENOPODIACEAE JUSS. FAMILY

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To reconstruct phylogeny and verify the monophyly of Petrosimonia genus, a total of 10 species representing in Euroasia were sampled, with analysis based on ITS1,2 nrDNA using maximum parsimony and Bayesian inference methods. Our molecular evidence provides strong support for the following: 1) P. nigdeensis, P. triandra 2) P. squarrosa, P. glauca and P. glaucescens 3) P. monandra, P. oppositifolia. P. litwinowii, P. brachiata and P. sibirica are nested with subclades with week support or separated from subclades in basal position. Single PCR NTS of 5S nrDNA product were confirmed for P. monandra, P. oppositifolia, P. glaucescens and P. squarrosa. Two PCR NTS products were confirmed for P. brachiata and P. sibirica and three products for P. litwinowii. The P. brachiata, P. sibirica and P. litwinowii are hybridogenic species with homogenic ITS1,2 nrDNA sequences. The homogenic sequences used for molecular-phylogeny do not reflect of correct (real) position of species on molecular trees.

Key words: Chenopodiaceae, Petrosimonia, molecular phylogeny, NTS and ITS1,2 nuclear ribosomal DNA, genome homogenization, systematics.

Chenopodiaceae Juss. Family comprises ca. 102 and ca. 1500 species, mainly native to arid, saline and alkaline regions. Chenopodiaceae arisen on littoral sand is widely distributed on the territories freed from the epicontinental Tethys ocean. A.A. Bunge (1880) distinguished 10 independent centers of origin and diversity of halophytic flora. These territories show the tendency to expansion in recent years as result of climate aridisation. Chenopodiaceae species have played an important role in vegetation, wind control, soil fixation and water conservation in deserts. For the man, Chenopodiaceae is a valuable source for pasture, fodder, food and as technical plants. *Petrosimonia* species of Chenopodiaceae are autumn fodder for camels and cattle, and are recommended for cultivation on the salty soils. In addition, attention to the representatives of this family is due to the opening of their single cell C4-photosynthesis [7 21], which makes them the model systems for studying evolution of photosynthesis Kranz paradigm [2]. In the latest classification of *Petrosimonia*, the position of some species is not satisfactory.

ITS1, ITS2 nrDNA markers which are most oftenly used for molecular-phylogenetic analysis [3, 18, 24] display polymorphism within the same species pointing to their hybrid origin. In this case hybrid taxa containing ITS1, ITS2 fragments or their parts are excluded

from analysis and studied by means of other approaches. The polymorphic sequences ITS1, ITS2 nrDNA are homogenized as result of concerted evolution [6]. This process reduces effectiveness of ITS1, ITS2 nrDNA use for studying taxa phylogenetic relationships and determination of their systematic position.

The use of chloroplast markers, which do not have high level of polymorphism, reconstructs evolution. However, the molecular markers have been reported to be used for hybridisation detection. This method is more preferable and provides more accurate data on the saved information about these events in the genome. We prefer to use the NTS 5S nrDNA marker which is conservative and various enough for species level, besides, it saves information about hybridisation events. The NTS polymorphism is studied for *Beta* [19], *Chenopodium* [14], *Populus* [15], *Triticum* [5], *Vitis* [8] genera.

The aim of this study is in comparative analysis of the data based on ITS1, ITS2 45S nrDNA and NTS 5S nrDNA sequences as main phylogenetic markers for taxonomic and systematic classifications of difficult *Petrosimonia* genus with homogenic ITS1, ITS2 45S nrDNA.

Materials and methods

1. Plant sampling.

Eight species of *Petrosimonia* and one species of *Ofaiston monandrum* from Chenopodaceae family were sampled for this study (Table 1).

Table 1

List of taxa sampled, vouchers and collectors, NTS bands number and ITS1, ITS2 polymorphism

№	Species	Source and collectors	NTS bands number	ITS1,2 status
1	*Petrosimonia monandra* (Pallas) Bunge	Russia, Saratovskaya obl., Novouzenskiy r-n, koshara Togus-Molokan. Dry steppe. T.A. Feodorova. 27.08.2008 y. Latitude 50,15306, longitude 48,37383. (MW)	1	Not polymorphic
2	*P. triandra* (Pallas) Simonk	Russia, Saratovskaya obl., Novouzenskiy r-n, selo Pigary. Dry steppe. T.A. Feodorova. 25.08.2008 y. Latitude 51,4421, longitude 49,67922. (MW)	1 minor, 1 major	Not polymorphic
3	*P. glauca* Bunge	Zakaspiyskaya obl. Vannovskoe v Shculy. Road side. D. Litvinov. 19.09.1898 y. (MW)	1	Not polymorphic
4	*P. oppositifolia* Litv.	Russia, Volgogradskaya obl., Pallasovskiy r-n, ozero Elton. Solt steppe. T.M. Lysenko. 13.08.07 y. (MW)	1	Not polymorphic

Окончание табл. 1

№	Species	Source and collectors	NTS bands number	ITS1,2 status
5	*P. litwinowii* Korsh.	Russia, Saratovskaya obl., Novouzenskiy r-n, koshara Togus-Molokan. Dry steppe. T.A. Feodorova. 27.08.2008 y. Latitude 50,15306, longitude 48,37383. (MW)	2 major, 1 minor	Not polymorphic
6	*P. brachiata* Bunge	Kustanayskaya obl., Ubaganskiy r-n, sovchoz imeny Щербакова. N. Pavlov. 04.08.1956 y. (MW)	1 major, 1 minor	Not polymorphic
7	*P. glaucescens* Iljin	Kazakhstan, Semiresche, Semipalatinskaya obl., south Aktogay. M.N. Lomonosova, A.P. Suchorukov. 25.09.2000 y. (MW)	1	Not polymorphic
8	*P. squarrosa* Bunge	Midlle Asia, Zautguzskie Kara-Kumy. S.V. Viktorov. 07.07.1953 y. (MW)	1	Not polymorphic
9	*Ofaiston monandrum* (Pallas) Moq.	Russia, Astrakhanskaya obl., ozero Baskunchak. Solt bank. T.A. Feodorova. 23.08. 2006 y.	—	Not polymorphic

2. Molecular-phylogenetic analysis.

DNA isolation, PCR, purification, sequencing.

Isolation of total DNA of 9 species followed the manufacturer's protocol with using Diatom DNA Prep 100 Kit (Izogen Lab., Moscow) from dry material. The ITS1, ITS2 and NTS regions were amplified with primers:

ITSL: 5'-TCGTAACAAGGTTTCCGTAGGTG-3';

ITS4: 5'–TCCTCCGCTTATTGATATGC-3' (White et al., 1990);

NTS 5S1: 5'-GGATGGGTGACCTCCCGGGAAGTCC-3';

NTS 5S2: 5'-CGCTTAACTGCGGAGTTCTGATGGG-3' [8]. PCR amplification followed in the manufacturer's protocol by Encyclo PCR Kit (Eurogen, Moscow).

The ITS1, ITS2 PCR products were electrophoresed using a 0.8 % agarose gel in a 0.5x TBE (pH 8.3) buffer and NTS PCR products were electrophoresed using a 1.5% agarose gel in a 0.5x TAE buffer, stained with ethidium bromide to confirm a single product, and purified using the Purification DNA Kit (Tsitokin, Saint-Petersburg). The sequencing was performed with an ABI Prism 3730 Genetic Analyzer (Centre of collective using "Genome", V.A. Engelgart Institution of Molecular Biology RAN).

We combined our data with the ITS1, ITS2 *Petrosimonia* (EF453458, AY489194, HM131642, HM131640, EF453456, HM131641, EF453457) data from NCBI GenBank previously published [1, 22]. *Ofaiston monandrum* is a sister clade to *Petrosimonia* genus and we used *Ofaiston* as outgroup, as was pointed out earlier [22].

Phylogenetic analyses.

Automated DNA sequencing chromatograms were proofed, edited, and contigs were assembled using Chromas 4.6 и Bioedit [11]. The clade *Petrosimonia+Ofaiston* is a fragment of Salsoleae tree with 200 sequences. The matrix result was then checked by eye for necessary minor correction to the alignment.

The Maximum Parsimony (MP) and Bayesian inference (BA) analyses were employed for phylogenetic analysis of the dataset. MP analysis were performed using PAUP 4.0b8 [20]. The Bayesian analysis were performed using MrBayes 3.1.2 [13, 17]. For Bayesian analysis the appropriate model of DNA substitution «SYM+I+G» was estimated using MrModelTest [16] and information criteria Akaike. Clade support was estimated using 1000 heuristic bootstrap replicates [9, 12].

Results and discussion

Internal transcribed spacers 1 and 2 (*ITS1* and ITS2) from *Petrosimonia* species (*P. monandra*, *P. oppositifolia*, *P. triandra*, *P. litwinowii*) and *Ofaiston monandrum* were amplified and sequenced. These sequences included few polymorphic nucleotides and consensus sequences were made and used in construction of phylogenetic trees. The trees were constructed by means of Maximum Parsimony and Bayes methods. The previously found sequences of ITS1 and ITS2 from other *Petrosimonia* species were also used in analysis. As a result three clusters have been identified. The clusters comply with Maximum Parsimony and Bayes trees, but statistical support is different: 1) *P. nigdeensis* and *P. triandra* (bootstrap support is 100%), 2) *P. squarrosa*, *P. glauca* and *P. glaucescens* (bootstrap support is 52%), 3) *P. monandra* and *P. oppositifolia* (bootstrap support is 58%). *P. litwinowii*, *P. brachiata* and *P. sibirica* locate in basal positions (in Maximum Parsimony tree) or grouped with the clusters (in Bayes tree) but bootstrap support is low (Fig. 1, 2).

Non-transcribed spacers (NTS) of 5S rDNA from 7 *Petrosimonia* species were amplified. Major 310 bp fragment was identified in *P. monandra*, *P. oppositifolia*. Major 320 bp fragment and minor 240 bp fragments were observed in *P. triandra*. Two major 300 and 110 bp fragments were detected in *P. brachiata* and *P. litwinowii*, but one additional 350 bp fragment was identified in *P. litwinowii* lane. These results suggest, that *P. brachiata* and *P. litwinowii* are hybridogenic species and *P. glaucescens* is the most likely parent (Fig. 3).

Likely *P. monandra*, *P. oppositifolia*, *P. glaucescens* and *P. squarrosa* are ancient stable species with different single NTS fragments and non-polymorphic ITS1, ITS2.

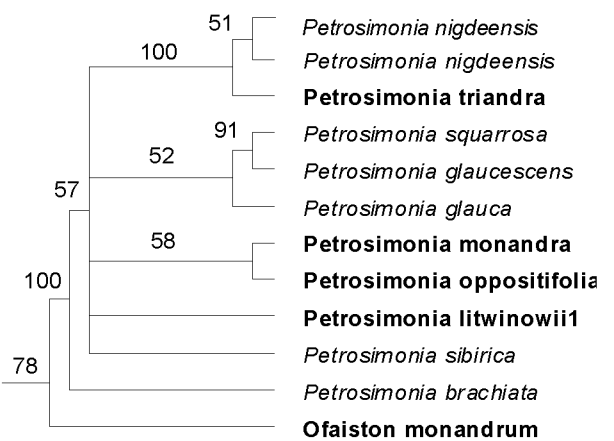

Fig. 1. Fragment of the maximum parsimony tree for *Salsoloideae* subgenus, *Caroxyloneae* tribe (*Petrosimonia* genus) (Feodorova, 2012). Bootstrap support values are indicated on branches. Data from GenBank is allocated by italics

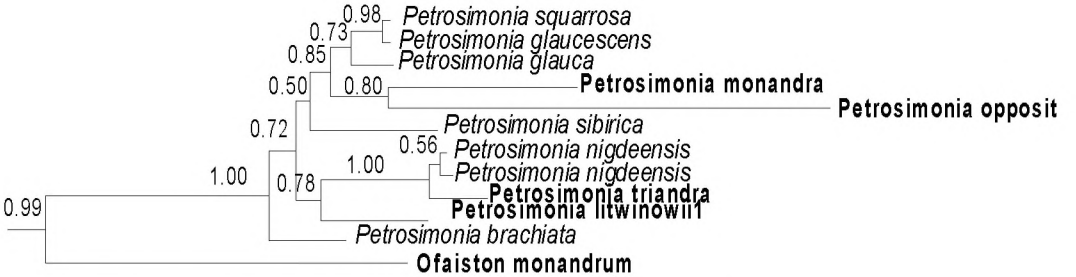

Fig. 2. Fragment of the Bayes tree for *Salsoloideae* subgenus, *Caroxyloneae* tribe (*Petrosimonia* genus). Posteriori probability values are indicated on branches. Data from GenBank is allocated by italics

P. triandra, P. brachiata and *P. litwinowii* are younger paleohybrids with two or three NTS fragments and homogenized ITS1, ITS2.

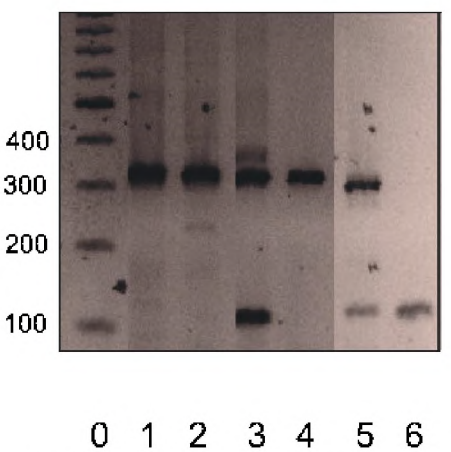

Fig. 3. Results of the NTS 5S rDNA amplification in *Petrosimonia* species: 1 – *P. monandra*, 2 – *P. triandra*, 3 – *P. litwinowii*, 4 – *P. oppositifolia*, 5 – *P. brachiata*, 6 – *P. glaucescens*

Basal non-clustered position in phylogenetic tree and non-polymorphic ITS1, ITS2 of *P. brachiata*, *P. sibirica* and *P. litwinowii* indirectly points to their hybrid origin. This fact is also confirmed by results of NTS analysis that indicated more than one NTS fragment in these species.

Thus, the taxonomic status of the *Petrosimonia* species may be adjusted. Section *Brachyphyllon* includes *P. brachiata*. Section *Synandra* Iljin includes *P. glauca*. This species has the most limited habitat. It is found only in the Caucasus, Iran and Central Asia. However, the related species (*P. glaucescens* and *P. squarrosa*) are found from China (Dzungaria, Hinggan) to the Lower Volga region and the Caucasus. Composition of this group does not coincide with the composition of section, since its type species (*P. oppositifolia*) is included in the second group, which includes *P. monandra* from Petrosimonia section. This section combines species with wide habitats (from China (Dzungaria and Hinggan) and southwestern Siberia to the Black Sea). *P. oppositifolia* was also noted in the Balkans. Third group includes *P. triandra* (*Triandra* Section) *and P. nigdeensis* (this species is discribed in Central Turkey). Species of this group also have a wide area (from the Dzungaria, Kashgar, Mongolia and western Siberia, to Saratov and Rostov regions in the north and Iran in the south). Thus, the position of *P. sibirica* and *P. litwinowii* can not be established, perhaps they form two independent sections.

Conclusions

As a result it can be concluded that species with basal low bootstrap position in clades may have non-polymorphic ITS1, ITS2, which are homogenized after hybridization, wherein ITS1, ITS2 analysis leads to false results. In these cases the phylo-

genetic tree analysis must be accompanied by the study of other markers (for example, NTS 5S rDNA).

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МОЛЕКУЛЯРНО-ФИЛОГЕНЕТИЧЕСКОЕ ИЗУЧЕНИЕ ПРЕДСТАВИТЕЛЕЙ РОДА *PETROSIMONIA* СЕМЕЙСТВА CHENOPODIACEAE JUSS.

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Исследование молекулярной филогении 10 видов рода Petrosimonia с использованием маркеров ITS1,2 ярДНК, показало его монофилию. Часть видов кластеризуются в три группы, другие виды не группируются с выявленными кластерами и занимают базальное положение. Нетранскрибируемые межгенные спейсеры (NTS) 5S ярДНК были амплифицированы у 7 видов Petrosimonia. Виды, образующие кластеры, имеют один фрагмент NTS. Виды с несколькими фрагментами NTS, группирующиеся с другими видами с низкой статистической поддержкой или занимающие базальное положение в кладе, имеют гибридное происхождение, но уже гомогенизированные последовательности ITS1,2 ярДНК. Использование этих последовательностей для филогении не отражает реального филогенетического положения исследуемых видов и приводит к неправильным реконструкциям эволюции таксонов.

Ключевые слова: Chenopodiaceae, Petrosimonia, молекулярная филогения, NTS и ITS1,2 ядерной рибосомальной ДНК, гомогенизация генома, систематика

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