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RECONSTRUCTION OF PHYLOGENY OF THE GENUS *RHODODENDRON* L. FROM RUSSIA BASED ON THE MOLECULAR GENETIC DATA

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Abstract

As a result of the analysis performed, a genealogical network representing evolutionary relations between *Rhododendron* L. species was obtained. Phylogeny of *Rhododendron* species reconstructed on the basis of ITS1-ITS2 sequence corresponds to anatomical-morphological classification of D. Chamberlain (1996).

Key words: anatomical-morphological classification, evolutionary relations, genealogical network.

Introduction

One of the tasks of plant systematics is construction of a natural classification pattern of a taxon; which should reflect evolutionary history. Data on DNA nucleotide composition are best suited for analyzing micro-changes of the species. ITS1-ITS2 (internal transcribed spacer 1, gene 5.8S ribosomal RNA, internal transcribed spacer 2) section of nuclear DNA of *Rhododendron* L. species growing in Russia was studied. Based on data on nucleotide sequences and statistical analysis, one of alternative classification schemes was justified.

Materials and Methods

Material for molecular genetic analysis was taken from living plants cultivated in Central Siberian Botanical Garden, SB RAS and those brought from the natural habitats. Isolation of total DNA was performed from the leaves dried in silica gel with the help of Diamond DNA Kit (ABT Llc., Russia) by the directions of the firm-manufacturer. ITS1-ITS2 locus was amplified by N. Friesen's modified method (2007) with the use of direct primer - 5'-AAGGTTTCCGTAGGTGAAC-3' – and reverse primer – 5'-TATGCTTAAACTCAGCGGG-3' (Desfeux and Lejeune 1996). For amplification reaction the following PCR

mixture was used: 31,5 μL of H_2O, 2 μL of DNA, 5 μL of 10x buffer, 5 μL of 25 mM $MgCl_2$, 2 μL of each 10 mM primer, 2 μL of 20 mM dNTPs. 0,2 μL (5 u / μL) Taq polymerase of was added directly to PCR mixture (so called "hot start") after the first cycle of warming up, which made it possible to increase yield of amplification, to get rid of non-specific annealing of primers and formation of primer dimmers (Real time PCR 2009).

Amplification was carried out as follows: 1 cycle: 95°C for 3 min; 35 cycles: 95°C – 20 sec, 56°C – 30 sec, 72°C – 80 sec; closing stage: 72°C – 10 min., cooling at 4°C.

Before sequencing, the amplified product was cleaned by NucleoSpin® Extract II Kit (Macherey-Nagel) following the instructions of the manufacturer.

Sequencing was carried out using automatic sequencer ABI Prism 3130xl. For the first time the sequence of the studied section was obtined for *Rh. dauricum* L., *Rh. ledebourii* Pojark., *Rh. parvifolium* Adams, *Rh. smirnowii* Trautv., *Rh. adamsii* Rehd. (Tuva population) = *Rh. burjaticum* Malyschev (Malyshev 1961), *Rh. sichotense* Pojark, *Rh. fauriei* Franch., *Rh. caucasicum* Pall., *Rh. japonicum* (A. Gray) Suring, and *Calluna vulgaris* L. The rest of the sequences used in the analysis were taken from GenBank, NCBI. Accession Numbers of used sequences: HM854157, HM854158, HM854159, HM854160, HM854161, HM854162, HM854163, HM854164, HM854165, HM854166, HM854167, X97415, AF393409, AF393412, X96814, AF404816, X97426, AF393418, X96806, AF393413.

Results and Discussion

A low rate of variability of ITS1-ITS2 was determined for the species studied. A cluster analysis of nucleotide sequences was performed by ME method (minimum evolution) in MEGA 4.1 (Kumar et al. 2008) and a bootstrap-consensus tree was constructed. Nucleotide sequences were aligned in a combined manner – automatic by ClustalW with correction "by hand". The total aligned length of leveling was 638 nucleotide pairs. There were 10000 replications of the bootstrap test. As an out group was taken *Calluna vulgaris* L. which demonstrated the closest relationship with *Rhododendron* species on the base of the analysis of nucleotide sequences by BLAST (NCBI). As a result, a reconstruction of phylogeny of ITS1-ITS2 sequences reflecting evolutionary relationship between *Rhododendron* species was obtained (Fig. 1). It is worth noting that cladograms constructed by MP method (maximum parsimony) had similar topology and bootstrap support of clades.

The phylogenetic tree resulting of Rhododendron species corresponded to the anatomical-morphological classification accepted in West Europe (Chamberlain 1996), as opposed to alternative classification by the Russian botanists (Alexandrova 1975, Koropachinskiy and Vstovskaya 2002).

Rh. adamsii and *Rh. parvifolium* manifested close relationship, and therefore, it is doubtful to assign them to different sections: *Pogonanthum* and *Rhododendron*, respectively.

A low degree of differentiation among *Rh. mucronulatum*, *Rh. dauricum*, *Rh. ledebourii* and *Rh. sichotense* is observed in the subgenus *Rhododendron*, section *Rhododendron* and subsection

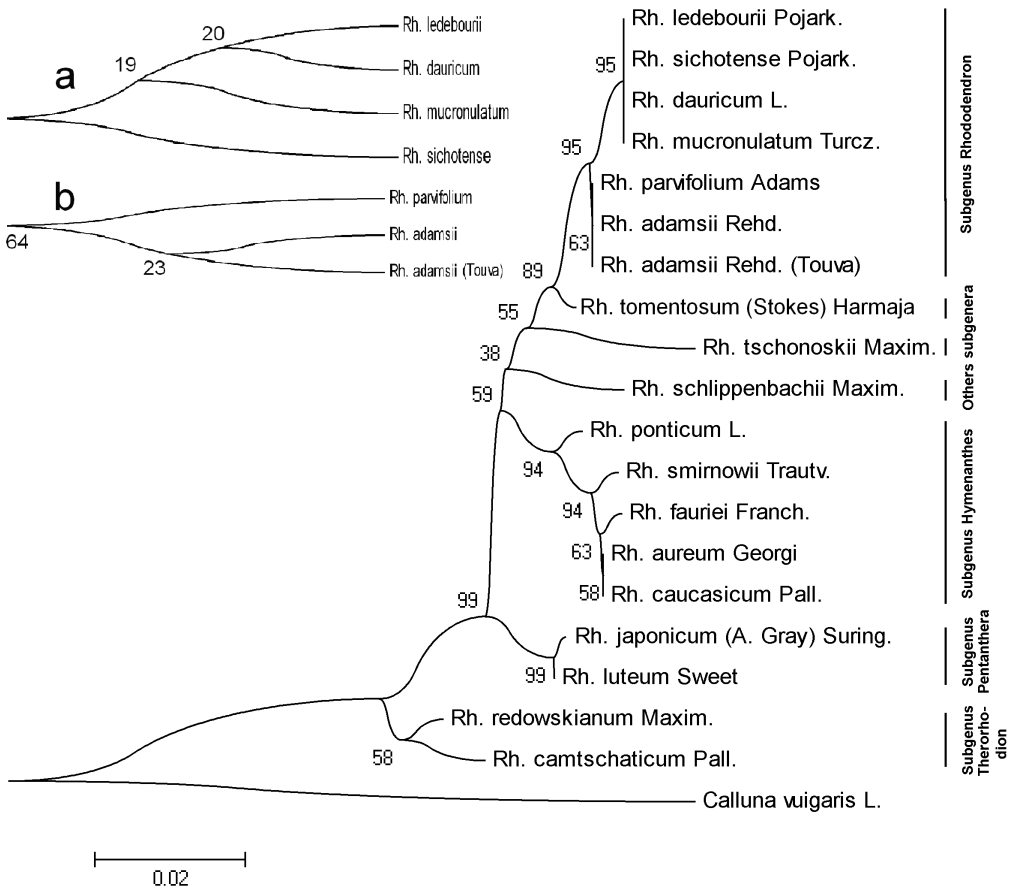

Fig. 1. ME-cladogram of *Rhododendron* L. representatives based on ITS1-5.8S-ITS2 sequences.

Rhodorastra (according to Chamberlain), which suggests recent isolation of these species (Fig. 1a). Moreover, low levels of support are evident in this clade, which does not allow establishing true phylogenetic relationships.

It is impossible to place *Rh. schlippenbachii* Maxim. and *Rh. tschonoskii* Maxim. in the phylogenetic diagram with confidence as the clade formed by these species has a low level of bootstrap support. Concerning these two species, we should follow the point of view of Alexandrova (1975) who separated them into independent taxa of subgeneric rank.

Noteworthy is a clear-cut distinction of the species of the subgenus *Therorodion*: *Rh. redowskianum* and *Rh. camtschaticum*, which were considered by some researchers to be the same species: *Rh. redowskianum* as a high-

mountain ecological form of *Rh. camtschaticum* (Alexandrova 1975).

Independence of the genus *Ledum* L. is doubtful. *Ledum palustre* groups in the center of the tree of the genus *Rhododendron*, consequently, it belongs to this genus, which was already noted in Chamberlain's classification where this species was called *Rh. tomentosum* (Stokes) Harmaja.

Conclusion

Thus phylogeny of *Rhododendron* L. species reconstructed on the basis of ITS1-ITS2 – sequence corresponds to anatomical-morphological classification of Chamberlain (1996) with small additions. The reconstruction of phylogeny based on ITS1-ITS2 sequences is only possible for wall isolated *Rhododendron* L. species. To establish isolation of species in the Section *Rhododendron*, it is necessary to conduct population genetic research based on the methods of screening the whole genome (ISSR, RAPD or AFLP).

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