ISSN 1022-7954, Russian Journal of Genetics, 2008, Vol. 44, No. 3, pp. 360–364. © Pleiades Publishing, Inc., 2008.
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SHORT COMMUNICATIONS

RAPD Analysis of Genome Polymorphism in the Family Lemnaceae

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Received April 17, 2007

Abstract—The multilocus RAPD analysis of intergeneric, inter- and intraspecific nuclear genome polymorphism was used for the first time to assess intergeneric, interspecific, and intraspecific polymorphism in Lemnaceae growing on the territory of Russia. The origin of the chosen accessions overlapped with the natural range of duckweeds in Russia. Seventy-five Lemnaceae accessions representing eight species (*L. minor, L. gibba, L. turionifera, L. japonica, L. trisulca, L. aequinoctialis, S. polyrhiza*, and *L. punctata*) from three genera (*Lemna, Spirodela*, and *Landoltia*), were analyzed. The highest variability levels were revealed in *L. minor* accessions (0.03–0.20). Species *L. trisulca* and *S. polyrhiza* were characterized by values of genetic distance 0.01–0.18 and 0.03–0.16, respectively. The lowest polymorphism levels were detected for *L. turionifera* (0.01–0.11). The dendrogram based on RAPD data showed that *L. aequinoctialis* was the most genetically distant species of the genus *Lemna.* Accessions of species *L. turionifera* and *L. japonica*, as well as *L. minor* and *L. gibba*, did not form separate species-specific subclusters; rather, they fell into clusters with *L. japonica*/*L. turionifera* and *L. minor*/*L. gibba.* Accessions of the genera *Spirodela* and *Landoltia* formed two separate clusters combined into one group.

DOI: 10.1134/S1022795408030198

The Lemnaceae includes the smallest flowering plants, whose vegetative and generative organs are characterized by a substantial reduction. Extremely small size and the lack of clearly differentiated vegetative organs place the constraints on the number of taxon-specific characters. In this respect, this family is ascribed to one of most complicated flowering plants in terms of systematics [1]. The existing classifications differentially interpret the species and genus composition of Lemnaceae [2]. According to one of the latest classifications, this family includes five genera: *Lemna* L., *Spirodela* Schleiden, *Landoltia* Les & Crawford, *Woffiella* Hegelmaier, and *Wolffia* Horkel ex Schleiden [3]. At present, there are several problems concerning the species and even genera affiliation within Lemnaceae, which is confirmed by the fact that species *S. punctata* earlier belonging to genus *Spirodela* is now isolated as the genus *Landoltia* [3].

The substantial reduction of vegetative organs typical for representatives of this family also hampers evaluation of potential genetic diversity and reconstruction of phylogenetic relationships within the family when based only on morphological characters. The employment of molecular methods for genome analysis is relevant, including studies of nucleotide polymorphism of genome and plastome sequences.

To date, molecular methods, such as RAPD and AFLP, are most frequently used to detect the levels of genomic polymorphism, to establish the taxonomic status of individual accessions, and to define phylogenetic relationships among various taxa [4].

The biodiversity of Lemnaceae has been poorly studied by molecular methods. Previously, analysis of variability of chloroplast DNA sequences was conducted, and based on the data obtained, Lemnaceae phylogenetic relationships were established [2]. However, intraspecific polymorphism was not characterized in this study, because each species was represented only by a single accession. In addition, analysis of the nuclear genome and evaluation of polymorphism at different taxonomic levels, including the intraspecfic level, has not yet been conducted in Lemnaceae.

The objective of this work was to detect genetic differences of Lemna accessions by multilocus RAPD analysis and with consideration of the results to evaluate the levels of inter-, intraspecific, and intergeneric variation of the nuclear genome in members of Lemnaceae species and genera from the territory of Russia.

Previously, we have constructed a collection consisting of 256 Lemnaceae accessions collected in 119 growth localities. To cover the range of each species to the highest extent, 75 accessions were selected from the collection. According to morphological data and geographical position along with preliminary molecular data (N.N. Ryzhova, unpublished), these accessions completely reflect genomic variation and biodiversity of eight species belonging to three Lemnaceae genera. This set included *L. minor* (the most widespread in the territory of Russia), 24 accessions; *L. gibba*, 6; *L. turionifera*, 5; *L. japonica*, 3; *L. trisulca*, 16; *L. aequinoctialis*, 3; *S. polyrhiza*, 16, and *L. punctata,* 2 (Table 1). *Pistia stratiotes* (family Araceae related to Lem-

Table 1. Analyzed accessions of the family Lemnaceae

Species/Accession	Sampling locality
Genus *Lemna*	
L. minor 4.4	Serpukhov, Moscow oblast
L. minor 5.6	Yekaterinburg
L. minor 7.1	Tyunzyry, Chuvashia
L. minor 7.4	Tyunzyry, Chuvashia
L. minor 8.4	Peat-hog, Chuvashia
L. minor 9.1	Kanash, Chuvashia
L. minor 9.2	Kanash, Chuvashia
L. minor 10.1	Cheboksary, Chuvashia
L. minor 10.2	Cheboksary, Chuvashia
L. minor 10.6	Cheboksary, Chuvashia
L. minor 12.3	Vladimir
L. minor 13.1	Vladimir
L. minor 14.1	Vladimir
L. minor 16.1	Tula
L. minor 17.5	Tula
L. minor 18.1	Vageningen, Netherlands
L. minor 29.1	Vologda
L. minor 35.1	Barnaul
L. minor 36	Mikhailov, Ryazan oblast
L. minor 37.2	Novosibirsk
L. minor 38	Novokuznetsk, Kemerovo oblast
L. minor 41	Bryn', Kaluga oblast
L. minor 43.1	Village Alferovo, Gorno-Altaisk
L. minor 45.1	Voronezh
L. gibba 1.1	Khot'kovo, Moscow oblast'
L. gibba 2.1	Sergiev Posad, Moscow oblast'
L. gibba 2.3	Sergiev Posad, Moscow oblast'
L. gibba 5.1	Volgograd
L. gibba 6.1	Volgograd
L. gibba 7.1	Voronezh
L. turionifera 4	Blagoveshchensk
L. turionifera 5.3	Novosibirsk
L. turionifera 6.1	Kosmodem'yansk, Chuvashia
L. turionifera 7.3	Blagoveshchensk
L. turionifera 8.1	Kosh-Agach, Republic Altai
L. japonica 1.3	Village Tolstovka, Amur oblast
L. japonica 2.5	Village Volkovo, Amur oblast
L. japonica 3	Nizhnii Tsasuchei, Chita oblast
L. aequinoctialis 1	Moscow oblast, fish breeding economy
L. aequinoctialis 2	GBC, Moscow
L. aequinoctialis 5.4	GBC, St. Petersburg
L. trisulca 1	Moscow, Bitza park
L. trisulca 2	Volkhov, Leningrad oblast
L. trisulca 3	Skhodnya, Moscow oblast
L. trisulca 4.1	Moscow
L. trisulca 6.6	Cheboksary, Chuvashia
L. trisulca 7.1	Vladimir
L. trisulca 8	Tambovka, Amur oblast
L. trisulca 9.2	Village Volkovo, Amur oblast
L. trisulca 11.1	Lake Balan'kul', Khakassia
L. trisulca 12	Vologda
L. trisulca 13	Novosibirsk
L. trisulca 14.1	Voronezh
L. trisulca 14.3	Voronezh
L. trisulca 14.4	Voronezh
L. trisulca 15	Novosibirsk
L. trisulca 16.1	Tambovka, Amur oblast
Genus *Spirodela*	
S. polyrhiza 1	Skhodnya, Moscow oblast
S. polyrhiza 3.2	Lake Svetloe, Chuvashia
S. polyrhiza 4.8	Cheboksary, Chuvashia
S. polyrhiza 7.7	Vladimir
S. polyrhiza 9	Tambovka, Amur oblast
S. polyrhiza 10	Village Gil'chinovka, Amur oblast
S. polyrhiza 11.6	Village Volkovo, Amur oblast
S. polyrhiza 13	Vageningen, Netherlands
S. polyrhiza 14.9	Blagoveshchensk
S. polyrhiza 15.1	Sergiev Posad, Moscow oblast
S. polyrhiza 16.3	Bryn', Kaluga oblast
S. polyrhiza 17.3	Kaluga
S. polyrhiza 18.2	Mogilev, Belarus
S. polyrhiza 19	Gomel', Belarus
S. polyrhiza 21	Dmitrov, Moscow oblast
S. polyrhiza 22	Novosibirsk
Genus *Landoltia*	
L. punctata 1.2	GBC, St. Petersburg
L. punctata 2.1	GBC, St. Petersburg

Table 2. Genetic distances (*GD*) in members of the family Lemnaceae calculated from the RAPD data

Intergeneric distances	
Spirodela–Landoltia	0.21–0.29
Spirodela–Lemna	0.24–0.38
Landoltia–Lemna	0.25–0.36
Interspecific distances	
L. minor/L. gibba–L. turionifera/L. japonica	0.14–0.31
L. minor/L. gibba–L. trisulca	0.24–0.35
L. minor/L. gibba–L. aequinoctialis	0.29–0.37
L. minor/L. gibba–S. polyrhiza	0.27–0.38
L. minor/L. gibba–L. punctata	0.26–0.33
L. turionifera/L. japonica–L. trisulca	0.20–0.29
L. turionifera/L. japonica–L. aequinoctialis	0.29–0.34
L. turionifera/L. japonica–S. polyrhiza	0.24–0.36
L. turionifera/L. japonica–L. punctata	0.27–0.32
L. trisulca–L. aequinoctialis	0.27–0.37
L. trisulca–S. polyrhiza	0.26–0.35
L. trisulca–L. punctata	0.30–0.36
L. aequinoctialis–S. polyrhiza	0.34–0.39
L. aequinoctialis–L. punctata	0.30–0.32
Intraspecific distances	
L. minor/L. gibba	0.03–0.20
L. turionifera/L. japonica	0.01–0.11
L. aequinoctialis	0.03–0.10
L. trisulca	0.01–0.18
S. polyrhiza	0.03–0.16

naceae), which is also an aquatic plant characterized by reduction of vegetative organs, served as an outgroup.

Total plant DNA was isolated according to standard protocol [5] with a additional purification of samples with phenol–chloroform mixture. We used eight standard oligonucleotide RAPD primers (Operon Technologies, Alameda, California, United States) that had been previously chosen from 15 primers and proved most informative: OPK6, OPK8, OPK10, OPN1, OPN8, OPN14, OPH9, and OPD12. For polymerase chain reaction, sets of reagents from Dialat-LTD (Moscow) were used. DNA amplification was conducted by a standard method [6].

Amplification products were fractionated by electrophoresis in a 1.7% agarose gel in 1 × TBE buffer followed by staining with ethidium bromide. Statistical analysis was conducted only for distinct, reproducible fragments.

Statistical analysis was conducted using the STATISTICA 6.0 and TREECON computer packages [7]. The level of genomic variation was estimated using genetic distance (*GD*). Intergeneric, inter- and intraspecific genetic distances were determined using Jacquard's coefficient. For further cluster analysis, UPGMA was used [8]. Bootstrap indices (BI) were calculated for 1000 replications.

RAPD analysis of the genome in 75 Lemnaceae accessions conducted with the eight primers yielded 242 RAPD fragments, 201 (83%) of which were polymorphic.

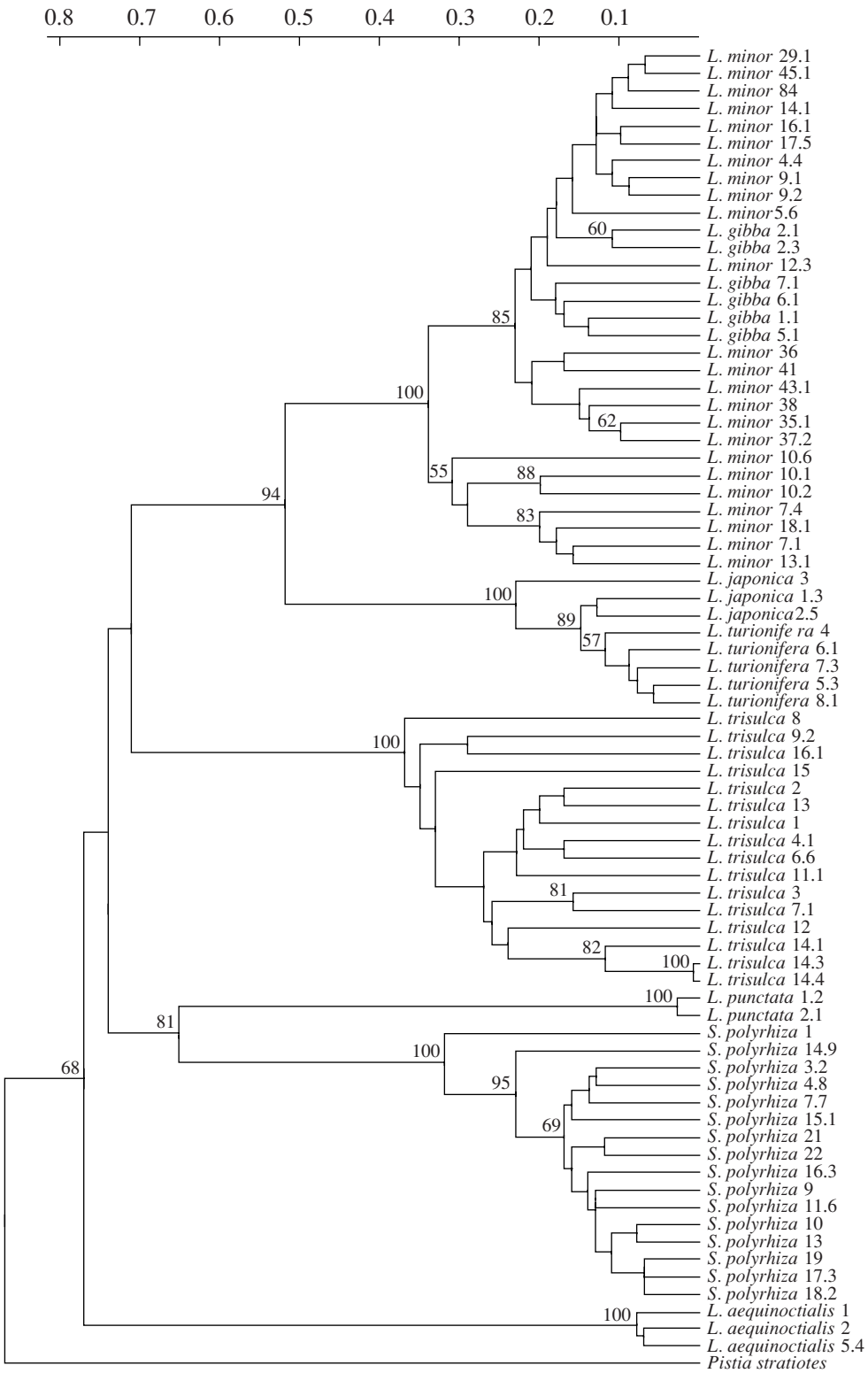
Genus- and species-specific RAPD fragments of Lemnaceae were identified together with fragments characteristis of individual plants. Note that the total number of genus-specific fragments was 10, and the number 46 was found for species-specific fragments (among them, 14 fragments in *L. minor*, 12 in *L. trisulca*, 9 in *L. turionifera*, 11 in *S. polyrhiza*). Additionally, 19 fragments typical for RAPD spectra of individual accessions were identified. Note that we failed to identify specific fragments in *L. japonica* and *L. gibba*. Spesies-specificity of spectra in accessions of *L. japonica* did not differ from that in *L. turionifera* as well as accessions of *L. gibba* did not differ from those of *L. minor.* They were considered as complexes of species *L. japonica/L. turionifera* and *L. minor/L. gibba* in further work.

The results of the conducted RAPD analysis were used to evaluate intraspecific, interspecific, and intergeneric variation of the nuclear genome and the similarity of genomes of various Lemnaceae taxa (Table 2).

We found that genetic differences in the species of the genus *Lemna* ranged from 0.01 (*L. turionifera*) to 0.20 (*L. minor*) and that the highest level of polymorphism was in accessions of *L. minor* (0.03–0.20). The least polymorphic of these variants were *L. japonica* and *L. turionifera* (0.01–011). Species *L. trisulca* and *S. polyrhiza* had similar values of genetic distances: 0.01–0.18 and 0.03–0.16, respectively.

Interspecific variation of the genus *Lemna* was, on average, not higher than 0.37. In accordance with calculated coefficients of genetic difference, the most closely related species were *L. turionifera–L. minor* (Table 1). Interestingly, the species *L. trisulca* differing from other Lemnaceae species had, as demonstrated by molecular data, high similarity in morphology to *L. turionifera* and less similarity to *L. minor* (Table 2), whereas we expected to observe equal distance of them from both *L. turionifera* and *L. minor* accessions. As shown by RAPD analysis, species *L. aequinoctialis* unexpectedly proved to be the most genetically distant member of the genus, the difference values being 0.27–0.37 for *L. aequinoctialis–L. trisulca*; 0.29–0.37 for *L. aequinoctialis–L. minor*; and 0.29–0.34 for *L. aequinoctialis–L. turionifera.*

The level of detected intergeneric polymorphism only slightly differed from values of interspecific variation of genus *Lemna*, being 0.24–0.38, 0.21–0.29, and 0.25–0.36 in pairs *Spirodela–Lemna*, *Spirodela–Landoltia*, and *Landoltia–Lemna*, respectively. These data reveal similarity between genera *Landoltia* and *Spirodela* (*Landoltia* being isolated from *Spirodela*) [3]. It is of interest that variability analysis of sequences of genes *rbcL, matK* and introns *trnK, rpl16* [2], or spacer *trnL–trnF* [9]



Dendrogram of genetic differences between the Lemnaceae accessions.

of chloroplast DNA revealed a high similarity of genera *Landoltia* and *Lemna.* On the basis of these data, it may be assumed that *Landoltia* is a sister group for *Lemna*, whereas *Spirodela* forms a single cluster.

The dendrogram based on the RAPD data clearly showed three major clusters (figure). The first cluster included variants of all *Lemna* species, except for members of *L. aequinoctialis,* which formed an independent

cluster (IB 68%). In turn, the cluster that combines the remaining *Lemna* species had two subclusters. All *L. trisulca* accessions fell into one subcluster (IB 100%). The second subcluster included accessions of species *L. minor*/*L. gibba* and *L. japonica*/*L. turionifera.* Within this subcluster, a division into two separate groups is observed: morphologically related and poorly distinguished species *L. minor* and *L. gibba* form one branching clade (IB 97%). The inclusion of *L. gibba* accessions into the clade generated by representatives of species *L. minor* confirms the similarity of their genomes and, additionally, may point to the ambiguous definition of species boundaries of *L. minor* and *L. gibba*, which is supported by both morphological and biochemical data [2]. Representatives of morphologically similar species *L. japonica* and *L. turionifera* form the second clade (IB 100%), and one of these species, namely, *L. japonica* may have a hybrid origin, whereas *L. turionifera* is considered a putative parental variant [10].

Note that the clustering pattern of *Lemna* species revealed on the dendrogram constructed using molecular analysis is, in general, similar to the clustering shown by analysis of sequences of the chloroplast genome or based on morphological and biochemical results [2]. The only exception is species *L. trisulca* that, according to Les et al. [2], constituted a single subcluster with *L. japonica* and *L. turionifera.* The dendrogram (figure) shows that accessions of genus *Landoltia* form a distinct clade within a single cluster with members of *Spirodela*, and this confirms the similarity of genomes in these two genera.

Cluster analysis failed to reveal intraspecific differentiation connected with the geographic distribution of the Lemnaceae accessions. Apparently, this may be conditioned by the absence of territorial isolation due to possible dispersal of Lemnaceae along river beds and with migrating water birds.

Thus, we conducted molecular analysis of the Lemnaceae nuclear genome for the first time and established the levels of intergeneric, interspecific, and interpopulation genome polymorphism in 75 Lemnaceae accessions and, in addition, evaluated the degree of similarity between genomes of various taxons.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (grant no. 05-04-4945a).

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