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SHORT COMMUNICATIONS

Natural Hybridization of Two Mussel Species *Dreissena polymorpha* (Pallas, 1771) and *Dreissena bugensis* (Andrusov, 1897)

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Abstracts—Dreissenids display a high diversity of shell morphology, and it is frequently difficult to ascribe some individuals from mixed populations to one of the two species, *Dreissena polymorpha* (Pallas, 1771) or *D. bugensis* (Andrusov, 1897). Presumably, such individuals may be interspecific hybrids. We have analyzed species-specific allozyme loci of the typical representatives of these two mussel species and putative interspecific hybrids. A natural interspecific hybrid between *D. polymorpha* and *D. bugensis* was discovered for the first time by genetic methods. It has been demonstrated that *D. bugensis* was a maternal parent.

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The rapid expansion of the area of the Quagga mussel (*Dreissena bugensis*), which started in the 1990s, covered the freshwater bodies of both Europe and North America (Zhuravel', 1967; Antonov, 1993; Spidle et al., 1994; Kharchenko, 1995; Rosenberg, Ludansky, 1995; Orlova et al., 2003, 2004). With invasion into the aquatic bodies inhabited by *D. polymorpha*, *D. bugensis* commenced forming joint communities with *D. polymorpha*; moreover, the reproduction periods of both species partially overlap.

Dreissenids display a high diversity of shell morphology (Karpevich, 1955; Antonov, 1997), and this considerably hinders the study of mixed *D. polymorpha* and *D. bugensis* populations, because they frequently contain individuals without distinct specific characteristics of either species. In particular, such individuals have been described in the mixed populations of the Uglich and Rybinsk dam reservoirs, belonging to the basin of the upper Volga River, and interpreted as putative interspecific hybrids (Orlova et al., 2003).

On the other hand, genetic methods (analysis of DNA and isozymes) have demonstrated a considerable genetic distance between these species and characterized them as valid species (Spidle et al., 1994; Stepien et al., 1999).

A specialized genetic study of mixed populations of the two mussel species in the Great Lakes in North America failed to discover any interspecific hybrids (Spidle et al., 1995), and the experiment on their hybridization gave ambiguous results. The obtained hybrid larvae died; however, it is unclear whether this mortality resulted from the genetic incompatibility of these two mussel species or inappropriate conditions for larva cultivation (Nichols, Black, 1994).

The goal of this work was to clarify the possibility of a hybrid origin of atypical morphs in mixed populations of two mussel species in the Rybinsk dam reservoir using biochemical and molecular genetic methods.

MATERIAL AND METHODS

To find the hybrids by genetic methods, we analyzed the mollusks from mixed *D. polymorpha* and *D. bugensis* populations from the Volzhskii pool of the Rybinsk dam reservoir gathered at the Glebovo (lat. 58°00′ N, long 38°26′ E) and Koprino (lat. 58°05′ N, 38°18′ E) biological stations.

All studied individuals formed three distinct morphological groups according to the shape of the shell valves, namely, typical *D. polymorpha*, typical *D. bugensis*, and individuals with atypical morphotypes, which were regarded as potential interspecific hybrids (Fig. 1).

The following morphological traits were characteristic of the putative hybrids: a distinct keel in the anterior third of the shell, which is characteristic of *D. polymorpha*, whereas in putative hybrids it was located closer to the back side; in the remaining part of the shell, the keel is rounded and located closer to the middle of the valve, which is the trait characteristic of *D. bugensis* (Fig. 1).

In all, 37 mussel individuals were assayed according to allozyme patterns and the restriction fragment

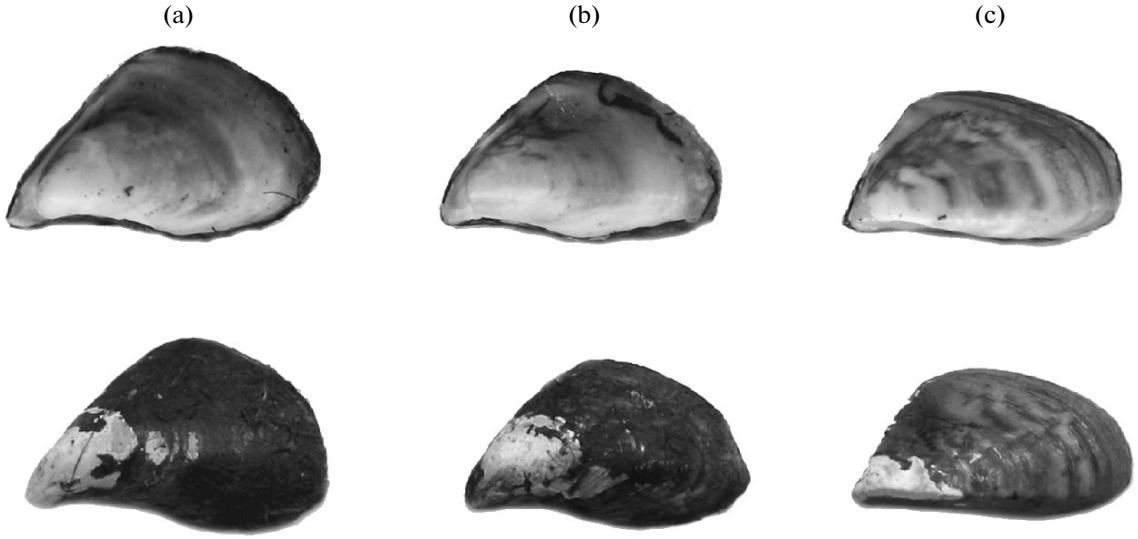

Fig. 1. Shell valves of dreissenids: (a) *D. bugensis*; (b) hybrid *D. bugensis* × *D. polymorpha*; and (c) *D. polymorpha*.

length polymorphism (RFLP) of the COI locus in mitochondrial DNA (mtDNA). Among them, 10 individuals belonged to typical *D. polymorpha*; 11 mussels, to typical *D. bugensis*; and 16 displayed the atypical morphotype.

The collected material was stored in a freezer at a temperature of –70°C; mussel gill tissues were mainly used for allozyme analysis. Electrophoresis of enzymes was conducted in 12.5% starch gel using a morpholine–citrate buffer system pH 6.8 (Clayton, Tretiak, 1972) or 7.5% polyacrylamide gel using Tris–EDTA–borate buffer (Peacock et al., 1965). Histochemical staining was performed according to Aebersold et al. (1987).

Allozyme loci that could be species-specific for *D. polymorpha* and *D. bugensis* according to the available data (May, Marsden, 1992; Spidle et al., 1994; Andreeva et al., 2001) and loci with the allelic frequencies significantly differing in these two species were used: total soluble protein (*GP**), malate dehydrogenase (*MDH-1** and *MDH-2**), phosphoglucomutase (*PGM**), 6-phosphogluconate dehydrogenase (*6-PGD**), malic enzyme (*MEP-2**), and esterase (*EST-2**).

When determining the maternal species, which was especially important in the case of putative hybrids, we used RFLP analysis of the locus COI in mtDNA. It was demonstrated earlier that the nucleotide sequence of a 619-bp fragment of this locus differed by 16–17% in these two mussel species (Baldwin, 1996).

To detect diagnostic restriction sites, we compared the nucleotide sequences of *D. polymorpha* (AF510510) and *D. bugensis* (DQ840132), extracted from GenBank. To confirm the diagnostic significance of the *Dra I* and *Rsa I* restriction sites, we used BLAST (NCBI, GenBank) software to analyze the polymorphism of all COI nucleotide sequences for these mussel species available in GenBank, namely, AF120663, AF474404, AF479636, AF492005, AF510508–AF510510, AM48975–AM748977, AM748985, AM748986, AM748988–AM748990, AM748997, AM748999, AM749001, DPU47653, DQ840121–DQ840125, EF414493–EF414495, EU484431–EU484435, and EU484437–EU484456 for *D. polymorpha* and AF096765, AF479637, AF495877, AF510504, DBU47650, DBU47651, DQ840132, EF080861, EF080862, EU484436, EU604834, and EU651840 for *D. bugensis*.

DNA was isolated by phenol–chloroform extraction from frozen mussel tissues. The following primers were used to amplify the fragment containing *Dra I* and *Rsa I* restriction sites, diagnostic for these two species: 5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al., 1994).

The synthesis was conducted in a Tertsik (DNK-Tekhnologiya, Moscow, Russia) amplifier in 25 μl of the amplification buffer (Fermentas, Lithuania) containing 10 mM Tris–HCl (pH 8.8); 50 mmol KCl, 1.5–2 mmol $MgCl_2$, and 0.08% Nonidet P40 (according to the data of the manufacturer). The amplification mixture contained 100–300 ng of total cellular DNA (the DNA concentration was estimated spectrophotometrically), 10 pmol of each primer, 200 nmol of each deoxyribonucleotide, and 0.5–1 U of *Taq* polymerase (Bionem, Moscow, Russia). The amplification scheme comprised initial denaturation for 4 min at 95°C; 35 cycles of 50 s at 95°C, 50 s at 56°C, and 1 min at 72°C; and final elongation for 10 min at 72°C.

The resulting fragment was hydrolyzed in the buffers recommended by the manufacturer of the corresponding restriction endonucleases (Promega, United

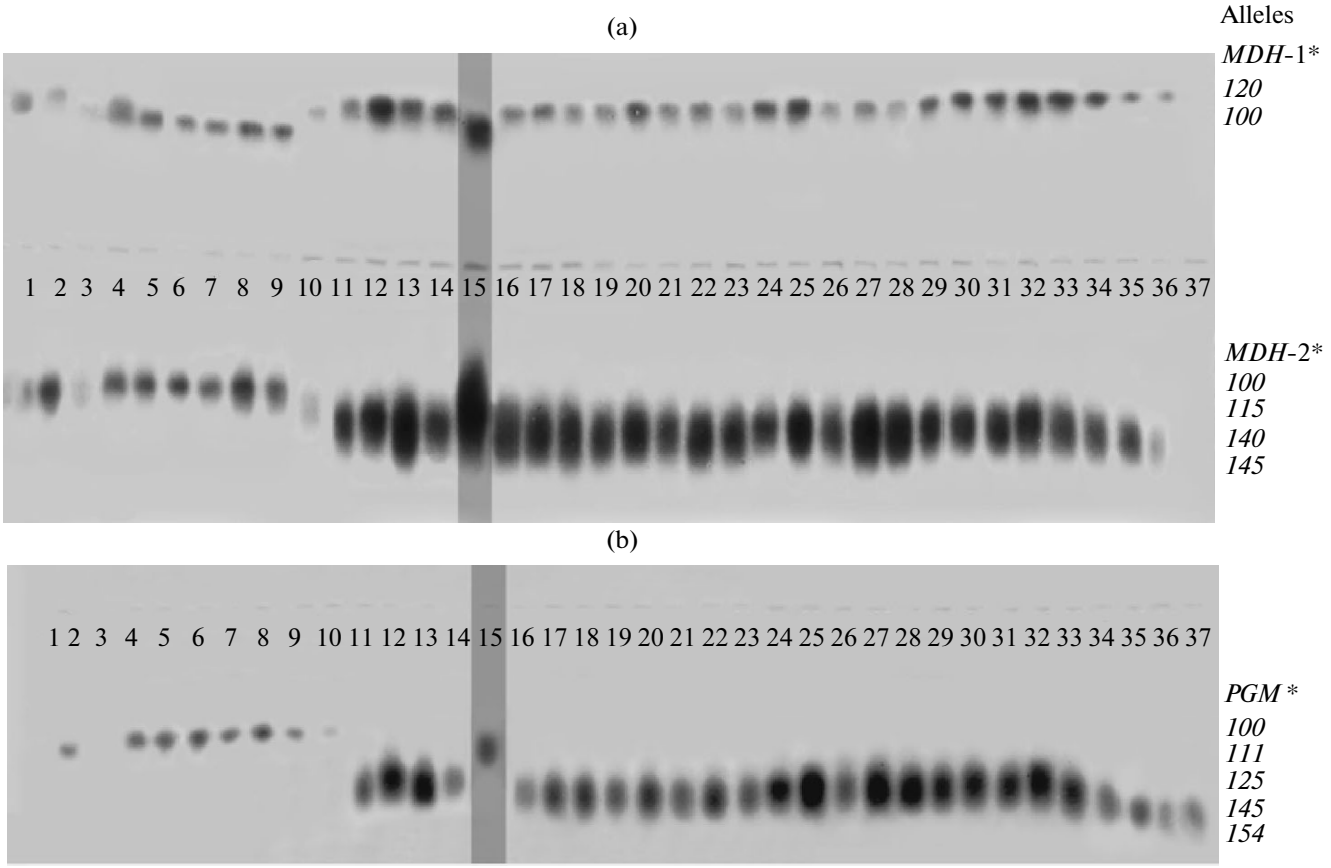

Fig. 2. Alleles of the isozyme loci of (a) malate dehydrogenase (*MDH-1** and *MDH-2**) and (b) phosphoglucomutase (*PGM**) in populations of (lanes 1–10) *D. polymorpha*, (27–37) *D. bugensis*, and (11–26) their putative hybrids.

States). The restriction fragments were analyzed in 2% agarose gel using the Tris–acetate buffer (40 mmol Tris–acetate and 2 mmol EDTA, pH 8). Double-stranded markers with a length step of 50 bp in the range of 50 to 800 bp with an additional 1800-bp fragment (50 bp DNA Step Ladder, Promega) were used as DNA length markers. Gels were stained with ethidium bromide (0.5 μg/ml) and photographed in ultraviolet light ($\lambda = 312$ nm) with a DC 290 Zoom Digital Camera (Kodak, United States).

RESULTS

All the analyzed allozyme loci were polymorphic at least in one of the mussel species. On the other hand, species-specific alleles were detected only for the loci *MDH-2** and *PGM**, which allowed them to be used for identification of hybrids. *D. polymorpha* was monomorphic for the locus represented by the allelic variant *MDH-2*100*, whereas this locus of *D. bugensis* displayed three alleles: *MDH-2*115, MDH-2*140,* and *MDH-2*145*. These species carry different alleles of the *PGM** locus, namely, *D. polymorpha*, *PGM*100* and *PGM*111* and *D. bugensis*, *PGM*125* and *PGM*145*.

Among the putative hybrids, the combination of allozyme loci indicating a hybrid origin was found in only one individual. This individual was heterozygous at the loci *MDH-2*100/115* and *PGM*100/125* (Fig. 2). The remaining loci appeared noninformative for detection of hybrids in the mussel populations of the Rybinsk dam reservoir; however, the data for these loci did not contradict the conclusion reached. The remaining 15 specimens of putative hybrids displayed only the allelic variants characteristic of *D. bugensis* or common for both species (Fig. 3).

RFLP analysis of the fragment of the mitochondrial gene COI demonstrated that *D. polymorpha* was the maternal species for the individual identified as a hybrid by allozyme analysis. Analysis of mtDNA of the remaining individuals from the sample of putative hybrids confirmed that they belonged to *D. bugensis* (Fig. 3).

DISCUSSION

Discovery of an individual with an undoubtedly hybrid nature in the sample from a mixed population of two mussel species is direct evidence that *D. bugensis* and *D. bugensis* can hybridize under natural condi-

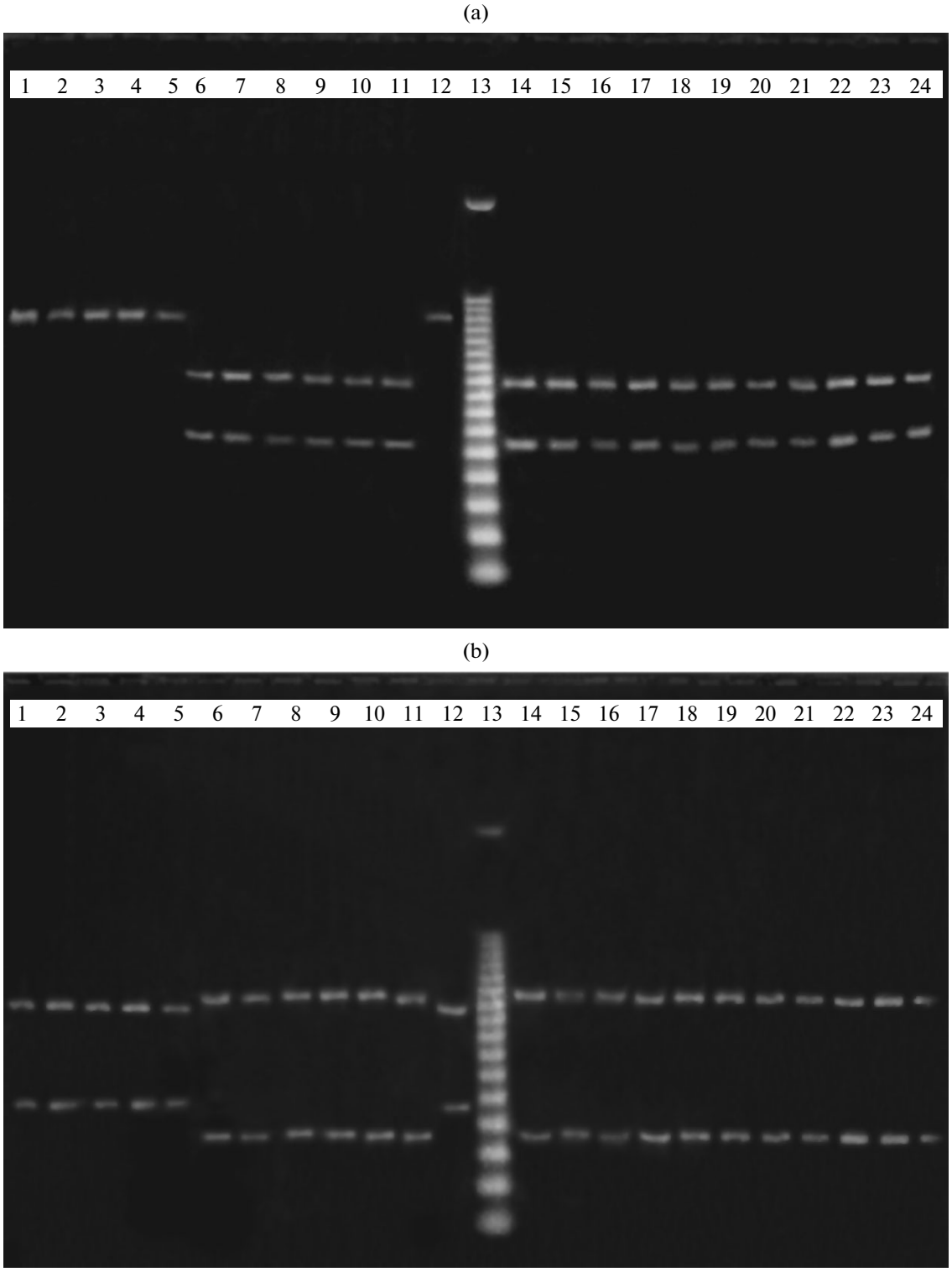

Fig. 3. Restriction pattern of the PCR products hydrolyzed with the restriction endonucleases (a) *Dra*I and (b) *Rsa*I: (lanes 1–5) *D. polymorpha*; (6–12 and 14–19) putative hybrids *D. bugensis* ×*D. polymorpha*; (20–24) *D. bugensis*; and (13) 50 bp DNA Step Ladder (Fermentas).

tions as well as of the viability of such hybrids. On the other hand, the morphological criteria appeared not completely valid for detection of hybrid individuals, because only one individual of the 16 specimens displaying a distinctly intermediate shell valve form was actually hybrid. This fact can be a result of insufficient knowledge about the intraspecific morphological variation, especially for *D. bugensis*.

It was earlier demonstrated that the alleles of *6-PGD** and β-*EST-2** loci widely present in *D. bugensis* were considerably more frequent in the *D. polymorpha* individuals from the mixed populations of these two mussel species as compared with the monospecific populations of this species. In addition, a polymorphism in the β-*EST-2** locus, similar to the polymorphism observed in *D. polymorpha*, was detected in *D. bugensis* (Andreeva et al., 2001). These data together with our evidence on the existence of intraspecific hybrids suggest that introgression of genes of one species into the genome of the other species is one of the consequences of hybridization between Quagga and zebra mussels.

Both hybridization in the first generation and subsequent introgression, as a rule, lead to an increase not only in genetic variation, but also in morphological variation (Mair, 1968; Verspoor, Hammar, 1991). It is known that during introgressive hybridization, the population size of one species can decrease or parental forms can be replaced with hybrids if the viability of hybrid individuals is not inferior to the viability of the parental species (Rhymer, Simberloff, 1996). Since hybrids can be adapted both better and worse to the local conditions as compared with the parental taxa (Burke, Arnold, 2001), it is likely that a gradual replacement of *D. polymorpha* in mixed populations can be connected not only with competitive advantages of *D. bugensis* but also with their hybridization.

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