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IDENTIFICATION OF GENDER-ASSOCIATED MITOCHONDRIAL HAPLOTYPES IN *ANODONTA ANATINA* (BIVALVIA: UNIONIDAE)

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ABSTRACT: Doubly uniparental inheritance (DUI) of mitochondrial DNA, different from maternal inheritance, is known to occur in five bivalve families (Mytilidae, Veneridae, Unionidae, Margaritiferidae, Hyriidae). DUI involves two types of mitochondrial DNA: F type, inherited from the mother and M type, inherited from the father. Females have only F type mtDNA, males have both types, M type being located in their gonads, F type in their somatic tissues. Among freshwater bivalves known to show DUI, only *Anodonta woodiana* occurs in Poland. The aim of this study was to ascertain if DUI occurred in another native bivalve, *Anodonta anatina*, based on DNA sequence of mitochondrial gene of cytochrome oxidase subunit I (*cox1*). M haplotype was found in male gonads, and F haplotype in somatic tissues of both sexes. Seven sequences were obtained for each F and M haplotypes, 625–709 base pairs long. Variation of 0.2 and 0.3% was found within F and M *cox1* sequences, respectively, and 29–32% variation between them.

KEY WORDS: mitochondrial DNA, *cox1*, F and M haplotypes, *Anodonta anatina*, Unionidae

INTRODUCTION

As a rule, in animals mitochondrial DNA (mtDNA) is inherited from the mother (SMI, Standard Maternal Inheritance). A different mode of inheritance of mtDNA, called doubly uniparental inheritance (DUI) was described in some marine (Mytilidae, Veneridae) and freshwater (Unionidae, Margaritiferidae, Hyriidae) bivalves, for the first time in the 1990s (SKIBINSKI et al. 1994a, ZOUROS et al. 1994a, WALKER et al. 2006). In DUI mode of inheritance females transmit their mtDNA (F haplotype) to both daughters and sons, whereas males transmit male mtDNA (M haplotype) only to their sons, and it is located in the gonads. The separate tissue localisation leads to independent evolution of the two lineages of mtDNA and their increasing divergence. The fact that DUI has been found to occur in phylogenetically remote families suggests that the phenomenon may be widespread among bivalves.

The origins of DUI are probably ancient; analyses of mitochondrial F and M types in freshwater bivalves suggest that they have evolved separately for at least 100 mln years, and DUI has been functioning in Unionidae for at least 200 mln years (HOEH et al. 2002). The DUI phenomenon is probably even older. Considering fossil record of Unionidae and Margaritiferidae, its age is estimated as 450 mln years (CUROLE & KOCHER 2002). There are two hypotheses explaining DUI in marine and freshwater bivalves (HOEH et al. 1996, 2002). One postulates that DUI has evolved independently in three lineages leading to unionids, *Mytilus* and *Geukensia*. According to the other, DUI has evolved once in an ancestral bivalve lineage and was later modified or lost in some derived lineages.

The mechanism of DUI has been well studied in five species of Mytilidae, however among venerids it has been described only for *Tapes philippinarum* based on the gene 16S rRNA (HOEH et al. 1996, 1997, 2002, QUESADA et al. 1999, PASSAMONTI & SCALI 2001, ZBAWICKA et al. 2003). Among the over 600 species of freshwater Unionoida DUI has been detected based on single genes (*cox1, cox2* or cytochrome b) in ca. 40 species representing mainly Unionidae (CUROLE & KOCHER 2002, HOEH et al. 2002, MOCK et al. 2004,

WALKER et al. 2006, BRETON et al. 2007). DUI has also been described in Margaritiferidae and Hyriidae based on 1–3 species (WALKER et al. 2006).

Of the freshwater bivalves for which DUI had been described only *Anodonta woodiana* occurred in Poland. In the case of the remaining seven native unionids and dreissenids DUI had not been detected or described which did not exclude its occurrence; what more, it could be expected because of the close relationship with species in which DUI had been observed.

A. anatina is genetically poorly studied in spite of its common occurrence in Europe. The GenBank contains 15 reports on known sequences in the species, pertaining to nuclear genes (18S, 5.8S, 28S), nuclear non-coding fragments (ITS1 and ITS2) and two mitochondrial genes (16S and *cox1*). Among the eight sequences of *cox1* gene, seven were provided by M. SOROKA and pertain to Polish specimens of the species, one comes from a Swedish population (DQ060168, KÄLLERSJÖ et al. 2005).

The objective of this study was to search for DUI through identification of male and female haplotypes of mitochondrial gene *cox1* (M and F types, respectively) in a freshwater bivalve *Anodonta anatina*. Another aim was an estimate of genetic variation within the F and M types and between them, based on the obtained results and on data available from the GenBank.

MATERIAL AND METHODS

The material included 12 specimens of *Anodonta anatina* (Linnaeus, 1758) collected in five localities in Poland in 2002–2007: Western Pomerania (Lake Wicko, rivers Odra and Brda), Kaszuby (Lake Wdzydze) and Central Poland (Grabia River). The number of analysed specimens per site ranged from one to four; a total of four females and eight males were analysed.

Sex of the bivalves was determined based on wet preparations from the gonads, examined in light microscope. The presence of large oocytes indicated female sex of the specimen, their absence indicated a male. Moreover, the gills of females were inspected for glochidia, which are present during the summer and autumn.

Table 1. Charactersitics of F and M sequences obtained for *cox1* gene in *A. anatina*. Numbers in parentheses following accession numbers refer to the number of individuals studied

F-haplotypes

GenBank accession number	Length of sequence in base pairs	Polymorphic site in 461bp	Sampling sites
AF494102 (3)	625	C	Brda River Grabia River
EF440347 (1)	647	C	Lake Wdzydze
EF440346 (3)	709	T	Odra River Lake Wdzydze

M-haplotypes

GenBank accession number	Length of sequence in base pairs	Polymorphic sites		Sampling sites
		116bp	674bp	
AF462071 (1)	647	C	–	Lake Wicko
EF440348 (3)	709	C	A	Lake Wdzydze Odra River
EU252510 (2)	653	T	A	Lake Wdzydze
EU252509 (1)	709	C	G	Odra River

Total DNA was isolated from the gills and gonads of the males and females using the standard phenol/chloroform method. For DNA isolation from male and female gonads a small quantity of material was taken from the microscope slide, following examination of the slide and determining sex. PCR was carried out in order to detect F (somatic tissues) and M (male gonads) haplotypes for the region of the mitochondrial cytochrome oxidase subunit I (*cox1*) gene, using LCO1490 and HCO2198 universal primers (FOLMER et al. 1994). Details of DNA extraction and conditions of PCR have been described by SOROKA & GRYGIEŃCZO-RAŹNIEWSKA (2005). Following 1.5% agarose gel electrophoresis, the products of *cox1* gene amplification were viewed under UV light. The results were saved and the sizes of the PCR products were analysed with BioCapt and Bio1D programs (Vilbert Lourmat, France), respectively.

The PCR product sequencing was carried out in Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University in Poznań (Poland), using genetic analyser ABI Prism 3130XL (Applied Biosystems) and sequencing kit BigDye Terminator v3.1 chemistry. The obtained sequences were submitted to the GenBank, their accession numbers are presented in Table 1.

A comparative analysis of the obtained sequences was carried out using DNAMAN 5.2.9 software (Lynnon Corporation, Canada). Also one sequence available in the GenBank for *A. anatina* (accession number DQ060168) was used in the analyses. In the molecular analyses, the parameters of genetic similarity and distances of the compared sequences were estimated according to the observed variation and Kimura's two-parameter model (KIMURA 1980).

RESULTS

Analysis of somatic tissues of three females and four males yielded seven female sequences of *cox1* gene (F haplotypes). The sequences were from 625 to 709 bp long (Table 1). Two haplotypes were distinguished, differing in one C/T substitution in position 461. Haplotype with base C (AF494102 and EF440347 differing only in the sequence length) was more frequent (0.571) and was present in four specimens from three sites (rivers Grabia, Brda and Lake Wdzydze). The second haplotype with base T (EF440346) was found in three specimens, with the frequency of 0.429, in two sites (Odra River and Lake Wdzydze). The two sequences showed genetic variation of 0.2% and 0.3–0.5%, compared to the Swedish specimen (Table 2).

Seven male sequences of gene *cox1* (M haplotypes) were obtained as a result of amplification and sequencing of DNA from gonads of seven males; they were 647–709 bp long. Three different haplotypes were distinguished based on two polymorphic positions: 116 and 674, where substitutions C/T and A/G, respectively, were observed (Table 1). Haplotypes

Table 2. Matrix of distances between specimens of *A. anatina* for *cox1* gene using the observed genetic variation method (above diagonal) and Kimura's two-parameter model (below diagonal)

	M haplotypes				F haplotypes			
	1	2	3	4	5	6	7	8*
1. AF462071	0	0.000	0.000	0.002	0.320	0.318	0.311	0.315
2. EF440348	0.000	0	0.001	0.002	0.320	0.291	0.294	0.315
3. EU252509	0.000	0.001	0	0.003	0.320	0.292	0.295	0.315
4. EU252510	0.002	0.002	0.003	0	0.315	0.296	0.294	0.312
5. AF494102	0.420	0.420	0.420	0.411	0	0.002	0.000	0.003
6. EF440346	0.417	0.369	0.372	0.378	0.002	0	0.002	0.005
7. EF440347	0.404	0.374	0.377	0.374	0.000	0.002	0	0.003
8. DQ060168*	0.411	0.411	0.411	0.406	0.003	0.005	0.003	0

*sequences obtained from GenBank

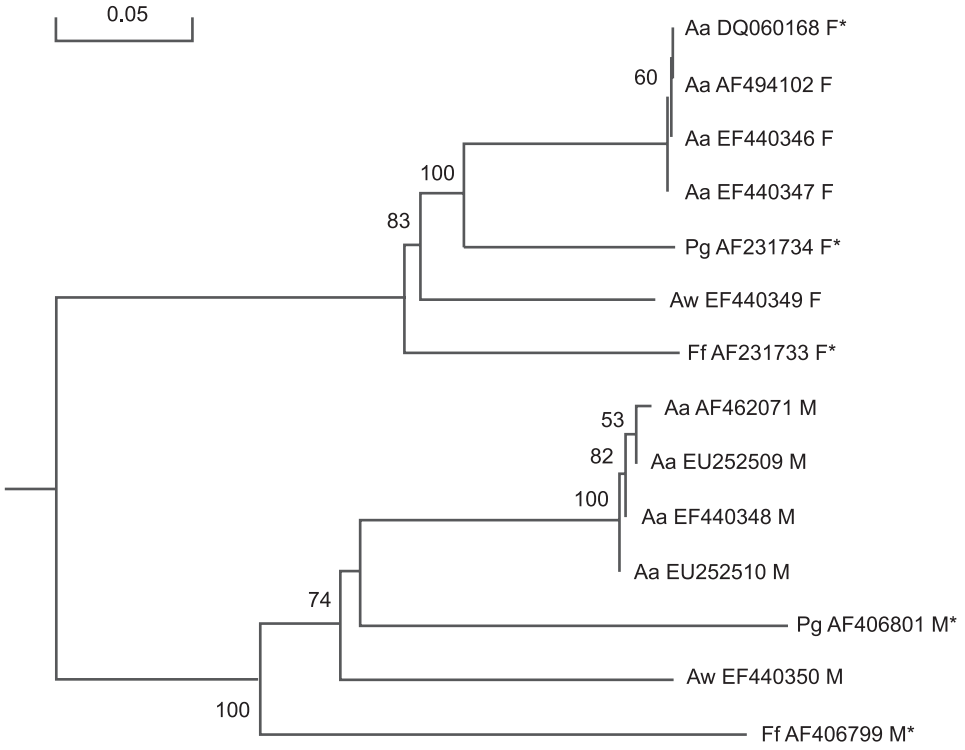

Fig. 1. Maximum Likelihood tree with bootstrap values (%); frequencies < 50% not shown; Aa – *Anodonta anatina*, Aw – *A. woodiana* (author's other studies), Ff – *Fusconaia flava*, Pg – *Pyganodon grandis*, * sequences obtained from GenBank

AF462071 and EF440348, differring only in sequence length, were found in four males from lakes Wicko and Wdzydze, and the Odra River. Haplotypes EU252510 and EU252509 were found twice (Lake Wdzydze) and once (Odra River), respectively. The genetic variation of these three male haplotypes was up to 0.3% (Table 2).

Polymorphic positions observed in F and M haplotypes were different. Genetic variation between these haplotypes in *A. anatina* was 29.1–32.0% in the case of observed variation and 36.9–42.0% using Kimura's two-parameter model (Table 2). In the phylogenetic analysis male and female haplotypes formed separate clades (Fig. 1).

DISCUSSION

Discovering heteroplasmy and DUI inheritance of mitochondrial DNA in marine bivalves of the genus *Mytilus* triggered search for similar phenomena in freshwater bivalves (SKIBINSKI et al. 1994a, b, ZOUROS et al. 1994a, b, BRETON et al. 2007). The first freshwater species with gender-associated mitochondrial DNA lineages were *Pyganodon grandis*, *P. fragilis* and *Fusconaia flava* of the family Unionidae (LIU et al. 1996a, b, HOEH et al. 1996). At present, despite detection of DUI in over 40 species of Unionoida (Unionidae, Margaritiferidae and Hyriidae), the taxonomic distribution of the phenomenon is still unclear and poorly studied (WALKER et al. 2006). Many researchers of freshwater bivalves use somatic tissues as the source of DNA; when studying mtDNA they obtain only F haplotype and thus neglect the possibility of identification of M haplotype and detection of DUI (STEPIEN et al. 1999, GRAF & Ó FOIGHIL 2000, GIRIBET & WHEELER 2002, LEE & Ó FOIGHIL 2004, THERRIAULT et al. 2004, ARAUJO et al. 2005). Isolating DNA from only somatic tissues (gills, foot, hepatopancreas) is not significant when studying nuclear genes, but during analyses of mitochondrial genes it precludes solving the problem of inheritance of mitochondrial DNA, characterisics of possible M haplotypes and comparison of M and F sequences.

In this study M haplotypes and thus DUI were found for the first time in another freshwater unionid, *Anodonta anatina*. In order to obtain male mtDNA, DNA was isolated from male gonads in the spring and summer. At that time large oocytes are visible in female gonads which makes it possible to correctly determine the sex of specimens.

Though DUI occurs in both marine (Mytilidae, Veneridae) and freshwater (Unionoida) bivalves, the character of the phenomenon differs between the two groups. In marine *Mytilus* the genetic variation between types F and M ranges from 2% to 21%. There is also evidence for recombination and masculinisation (a female-transmitted mtDNA reverses its role and becomes transmitted paternally) and thus in phylogenetic analyses the two molecules of mtDNA do not form distinct clades (HOEH et al. 1996, 1997, 2002, QUESADA et al. 1999, BURZYŃSKI et al. 2003, BRETON et al. 2007). Furthermore, besides male gonads, M haplotype has been detected, in small quantities, also in male somatic tissues and few tissues of females of such speies as *M. edulis, M. trossulus* and *Tapes philippinarum* (GARRIDO-RAMOS et al. 1998, DALZIEL & STEWART 2002, PASSAMONTI et al. 2003).

In freshwater bivalves (Bivalvia: Unionoida) the observed differences between F and M types are much higher and range from 28% to 34%, at their smaller internal variation compared to the genus *Mytilus*. Freshwater bivalves show no recombination and masculinisation and thus in phylogenetic analyses yield separate F and M clades which can be used independently for phylogenetic inferences. The existing literature reports on the occurrence of M type only in male gonads of freshwater bivalves (HOEH et al. 2002, MOCK et al. 2004, SOROKA 2005).

The present results, based on a few individuals from Polish populations of *A. anatina*, revealed a 0.2% variation within the sequence of the fragment of *cox1* gene for F-haplotype and 0.3–0.5% variation, compared to the Swedish specimen. M haplotypes showed a slightly higher variation, of up to 0.3%. Male and female sequences of *cox1* gene showed polymorphism in different positions and 29–32% genetic variation using observed variation (37–42% for Kimura's two-parameter model) (Table 2). Besides, sequences of the two haplotypes clearly form separate F and M clades (Fig. 1), confirmed also by literature data (HOEH et al. 1996, 2002). Though the genetic variation of sex-dependent lineages within the fragment of *cox1* gene in *A. anatina* is very high, it is still characteristic for bivalves in which DUI has been detected and described (HOEH et al. 1996, 1997, 2002, SOROKA 2008).

Variation of F haplotypes of *cox1* gene between *A. anatina*, *A. cygnea* and *Pseudanodonta complanata* described in the literature is 11.3–13.9% (KÄLLERSJÖ et al. 2005). There are few direct literature data on the intraspecific variation in various species of freshwater bivalves. Comparison of sequences of the fragment of *cox1* gene in *P. grandis* available from the GenBank (AF2314734F, AF406801M and AF156504) showed, like in *A. anatina*, a 0.2% variation in F haplotype which is within the range characteristic for DUI in freshwater bivalves. A distinctly higher intraspecific variation within *cox1* gene has been observed in *Dreissena polymorpha* – up to 1.1% (THERRIAULT et al. 2004).

Another freshwater unionid, *A. woodiana*, showed no variation among the Polish specimens for F

haplotypes of *cox1* gene and 6.8% compared to Japanese specimens. Variation between F and M haplotypes in that species reached 34% and 46% using observed genetic variation and Kimura's two-parameter model, respectively (SOROKA 2005, 2008).

Variation in mitochondrial haplotypes in *P. grandis* described by LIU et al. (1996a, b) is up to 0.5% and 12% within F and M haplotypes, respectively, and from 6.1 to 8.9% between F and M. The latter value is smaller than described earlier for unionids since it was obtained with restriction analysis of the whole mitochondrial molecule (mtDNA RFLP), and not with sequencing of the fragment of *cox1* gene. The technique however reflects the general tendency of higher rate of nucleotide substitution within M compared to F genome in unionids (SKIBINSKI et al. 1994a, HOEH et al. 2002, MOCK et al. 2004, ZOUROS et al. 2004a).

The results of this study confirm the occurrence of DUI, specify the level of genetic variation of sex-dependent mitochondrial genomes in *A. anatina* and at least partly fill the gap in the knowledge of DUI in freshwater bivalves. The taxonomic distribution of the phenomenon still requires more extensive studies, considering several genes and precise isolation of DNA from somatic tissues and gonads of males and females of the largest possible number of species.

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REFERENCES

ARAUJO R., GOMEZ I., MACHORDOM A. 2005. The identity and biology of *Unio mancus* Lamarck, 1819 (=*U. elongatulus*) (Bivalvia: Unionidae) in the Iberian Peninsula. J. Moll. Stud. 71: 25–31.

BRETON S., BEAUPRE H. D., STEWART D. T., HOEH W. R., BLIER P. U. 2007. The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough? Trends Genet. 23: 465–474.

BURZYŃSKI A., ZBAWICKA M., SKIBINSKI D. O. F., WENNE R. 2003. Evidence for recombination of mtDNA in the marine mussel *Mytilus trossulus* from the Baltic. Mol. Biol. Evol. 20: 388–392.

CUROLE J. P., KOCHER T. D. 2002. Ancient sex-specific extension of the cytochrome c oxidase II gene in Bivalves and the fidelity of Double-Uniparental Inheritance. Mol. Biol. Evol. 19: 323–1328.

DALZIEL A. C., STEWART D. T. 2002. Tissue-specific expression of male-transmitted mitochondrial DNA and its implications for rates of molecular evolution in *Mytilus* mussels (Bivalvia: Mytilidae). Genome 45: 348–355.

FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

GARRIDO-RAMOS M. A., STEWART B., SOUTHERLAND B., ZOUROS E. 1998. The distribution of male-transmitted and female-transmitted mitochondrial DNA types in somatic tissues of blue mussels: implication for the operation of doubly uniparental inheritance of mitochondrial DNA. Genome 41: 818–824.

GIRIBET G., WHEELER W. 2002. On bivalve phylogeny: a high-level analysis of the Bivalvia (Mollusca) based on combined morphology and DNA sequence data. Invertebr. Biol. 121: 271–324.

GRAF D. L., Ó FOIGHIL D. 2000. The evolution of brooding characters among the freshwater pearly mussels (Bivalvia: Unionoidea) of North America. J. Moll. Stud. 66: 157–170.

HOEH W. R., STEWART D. T., SUTHERLAND B. W., ZOUROS E. 1996. Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). Evolution 50: 2276–2286.

HOEH W. R., STEWART D. T., SAAVEDRA C., SUTHERLAND B. W., ZOUROS E. 1997. Phylogenetic evidence for role-reversals of gender-associated mitochondrial DNA in *Mytilus* (Bivalvia: Mytilidae). Mol. Biol. Evol. 14: 959–967.

HOEH W. R., STEWART D. T., GUTTMAN S. I. 2002. High fidelity of mitochondrial genome transmission under the doubly uniparental model of inheritance in freshwater mussels (Bivalvia: Unionoidea). Evolution 56: 2252–2261.

KÄLLERSJÖ M., VON PROSCHWITZ T., LUNDBERG S., EELDENÄS P., ERSÉUS CH. 2005. Evaluation of ITS rDNA as a complement to mitochondrial gene sequences for phylogenetic studies in freshwater mussels: an example using Unionidae from north-western Europe. Zool. Scr. 34: 415–424.

KIMURA M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16**:** 111–120.

LEE T., Ó FOIGHIL D. 2004. Hidden Floridian biodiversity: mitochondrial and nuclear gene trees reveal four cryptic species within the scorched mussel, *Brachidontes exustus*, species complex. Mol. Ecol. 13: 3527–3542.

LIU H-P., MITTON J. B., HERRMANN S. J. 1996a. Genetic differentiation in and managment recommendations for the freshwater mussel, *Pyganodon grandis* (Say, 1829). Am. Malacol. Bull. 13: 117–124.

LIU H-P., MITTON J. B., WU S-K. 1996b. Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the

freshwater mussel, *Anodonta grandis grandis*. Evolution 50: 952-957.

MOCK K. E., BRIM-BOX J. C., MILLER M. P., DOWNING M. E., HOEH W. R. 2004. Genetic diversity and divergence among freshwater mussel (Anodonta) populations in the Bonneville Basin of Utah. Mol. Ecol. 13: 1085–1098.

PASSAMONTI M., SCALI V. 2001. Gender-associated mitochondrial DNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca Bivalvia). Curr. Genet. 39: 117–124.

PASSAMONTI M., BOORE J. L., SCALI V. 2003. Molecular evolution and recombination in gender-associated mitochondrial DNAs of the manila clam *Tapes philippinarum*. Genetics 164: 603–611.

QUESADA H., WENNE R., SKIBINSKI D. O. F. 1999. Interspecies transfer of female mitochondrial DNA is coupled with role-reversal and departure from neutrality in the mussel *Mytilus trossulus*. Mol. Biol. Evol. 16: 655–665.

SKIBINSKI D. O. F., GALLAGHER C., BEYNON C. M. 1994a. Sex-limited mitochondrial DNA in the marine mussel *Mytilus edulis*. Genetics 138: 801–809.

SKIBINSKI D. O. F., GALLAGHER C., BEYNON C. M. 1994b. Mitochondrial-DNA inheritance. Nature 368: 817–818.

SOROKA M. 2005. Genetic variability among freshwater mussel *Anodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) populations recently introduced in Poland. Zool. Sci. 22: 1137–1144.

SOROKA M. 2008. Doubly uniparental inheritance of mitochondrial DNA in freshwater bivalve *Anodonta woodiana* (Bivalvia: Unionidae). Folia Biol. 56: 91–95.

SOROKA M., GRYGIEŃCZO-RAŹNIEWSKA E. 2005. Mitochondrial DNA-based diagnostic molecular markers for freshwater bivalves. Folia Malacol. 13: 145–152.

STEPIEN C. A., HUBERS A. N., SKIDMORE J. L. 2002. Diagnostic genetic markers and evolutionary relationships among invasive dreissenoid and corbiculoid bivalves in North America: phylogenetic signal from mitochondrial 16S rDNA. Mol. Phylogenet. Evol. 13: 31–49.

THERRIAULT T. W., DOCKER M. F., ORLOVA M. I., HEATH D. D., MACISAAC H. J. 2004. Molecular resolution of the family Dreissenidae (Mollusca: Bivalvia) with emphasis on Ponto-Caspian species, including first report of *Mytilopsis leucophaeata* in the Black Sea basin. Mol. Phylogenet. Evol. 30: 479–489.

WALKER J. M., CUROLE, J. P., WADE D. E., CHAPMAN E. G., BOGAN A. E., WATTERS G. T., HOEH W. R. 2006. Taxonomic distribution and phylogenetic utility of gender-associated mitochondrial genomes in the Unionoida (Bivalvia). Malacologia 48: 265–282.

ZBAWICKA M., SKIBINSKI D. O. F., WENNE R. 2003. Doubly uniparental transmission of mitochondria DNA length variants in the mussel *Mytilus trossulus*. Marine Biology 142: 455–460.

ZOUROS E., BALL A. O., SAAVEDRA C., FREEMAN K. R. 1994a. An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. Proc. Natl. Acad. Sci. USA 91: 7463–7467.

ZOUROS E., BALL A. O., SAAVEDRA C., FREEMAN K. R. 1994b. Mitochondrial-DNA inheritance. Nature 368: 818.

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