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GENETIC AND MORPHOLOGICAL VARIABILITY AND DIFFERENTIATION OF FRESHWATER MUSSELS (BIVAVIA, UNIONIDAE, ANODONTINAE) IN UKRAINE

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Genetic and Morphological Variability and Differentiation of Mussels (Bivavia, Unionidae, Anodontinae) in Ukraine. **Mezhzherin S. V., Yanovich L. M., Zhalay E. I., Vasilieva L. A., Pampura M. M**. — The study of allozymes variation and sequence analysis of two mitochondrial genes supports the concept that there are four species of subfamily Anodontinae in the Eastern European fauna, three of them; *Anodonta cygnea* Linnaeus, 1758; *A. anatina* Linnaeus, 1758; *Pseudanodonta complanata* (Rossmässler, 1835) are indigenous and one is invasive (*Sinanodonta woodiana* Lea, 1834). This paper analyzes morphological diagnostic features of which some can be used in identification of these species.

K e y w o r d s: Anodontinae, freshwater mussels, allozymes, sequence analysis, mtDNA, morphometry, variability.

Генетическая и морфологическая изменчивость и дифференциация беззубок (Bivavia, Unionidae, Anodontinae) в Украине. **Межжерин С. В., Янович Л. Н., Жалай Е. И., Васильева Л. А., Пампура М. М**. — Проведенное исследование на уровне аллозимов и секвенирования последовательностей двух митохондриальных генов подтверждает концепцию, согласно которой в восточноевропейской фауне присутствуют четыре вида этого подсемейства, три из которых: *Anodonta cygnea* Linnaeus, 1758*; A. anatina* Linnaeus, 1758*; Pseudanodonta complanata* (Rossmässler, 1835), аборигенные и один — адвентивный (*Sinanodonta woodiana* Lea, 1834). Проанализированы морфологические диагностические признаки, часть из которых может быть использована при определении этих видов.

К л ю ч е в ы е с л о в а: Anodontinae, беззубки, аллозимы, секвенирование, мтДНК, морфометрия, изменчивость.

Introduction

Subfamily Anodontinae is one of the groups of European freshwater molluscs with confused taxonomy. In 1930–1950, six species of the genus *Anodonta* were recognized in the Ukrainian waters (Zhadin, 1938, 1952). Within some of them a number of sub-species and forms were considered. In 1970–1980, the Soviet malacologists supervised by Ya. I. Starobogatov (Starobogatov, 1970, 1971, 1977; Shikov, Zatravkin, 1991) made systematic revision based on the characters of shell shape narrowing the range of the natural habitat of the species. The status of species was given to many subspecies and forms, and systematic ranks of higher categories were raised accordingly. Finally, the fauna of Ukraine accounted three genera with 10 species. The results of this revision were recognized by some investigators (Moskvicheva, 1973; Akramovskiy, 1976; Stadnichenko, 1984), but were not supported by others (Dyduch-Falniowska, Koziol, 1989; Piechoki, Dyduch-Falniowska, 1993; Nagel et al., 1998; Glöer, Meier-Brook, 1998; Juřičková et al., 2001; Kornyushin, 2002). The latter, in fact, consider three polytypic species proposed by Starobogatov as synonyms of three polymorphic species: *Anodonta* = *Anodonta cygnea*; *Colletopterum* = *A. anatina*; *Pseudanodonta* = *P. complanata*.

The experimental genetic research such as analysis of electrophoretic spectra of muscle proteins (Kodolova, Logvinenko, 1974; Kodolova 1977; Logvinenko Kodolova, 1983), allozymes (Nagel et al., 1998) in general confirm views of those who supports the presence in Europe of three mussel species with wide range. Karyological studies gave another argument in favour of evolutionarily conservatism of mussels (Melnichenko, 2001). They showed the constant karyotype (2n = 38; NF = 76) for the members of this subfamily.

However, taxonomy of this group remains quite controversial, and in the recent "Key to freshwater invertebrates of Russia *neighbouring territories*" (2004), the system of Ya. I. Starbogatov is represented, so confrontation of scientific schools persists. Therefore, we expect the further researches based on genetic and morphological analysis of the population's variability can resolve this protracted dispute.

Material and methods

The allozyme and morphological analyses of populations were made on the basis of mussels' series, and the species in each of them were identified according to the systematic presentation (Piechocki, Dyduch-Falniowska 1993; Glöer, Meier-Brook, 1998; Kornyushin, 2002). This material was collected in major river systems of Ukraine (fig. 1) and included *A. anatina* (9 samples, 231 spec.), *A. cygnea* (7 samples, 110 spec.), *P. complanata* (9 samples, 81 spec.) and one sample of invasive species *Sinanodonta woodiana* (34 spec.). Some specimens from different geographic populations and different species by Starobogatov's system were used for sequence analysis of two mitochondrial genes fragments (table 1).

The morphometric analysis was performed on genetically pre-labelled series according to the scheme of measurements developed for this group (Stadnichenko, 1984; Glöer, Meier-Brook, 1998). Shell measurements were the following: length (L), height (H), tip umbonal height (H_1), wing height (H_2), front length (L_1), distance between the tip umbonal and the most distant point of the bottom edge (L_2), convex (S), front valve thickness (T).

Also, some qualitative characteristics (Stadnychenko 1984; Bogatov, Saenko, 1996) reflecting features in colour of organs and structure of the umbo of shell were used.

Allozyme multiloci analysis was performed in polyacrylamide gel in Tris-borate EDTA Na_2 buffer continuous system with pH 8.5 (Peacock et al., 1965).

Genomic DNA was isolated by the standard protocol using a set of DNA–EXTRAN–2 (Synthol Cat No. EX–511). Partial sequences of two mitochondrial genes — cytochrome oxidase subunit I (COI) 572 bp and 16S rRNA gene, 471 bp in length, were used for comparative analysis.

Corresponding sequences were amplified with primers LCO1490 5'–GGTCAACAAAT-CATAAAGATATTGG–3' and COI–H 5'–TCAGGGTGACCAAAAAATCA–3' for COI (Machordom et al.,

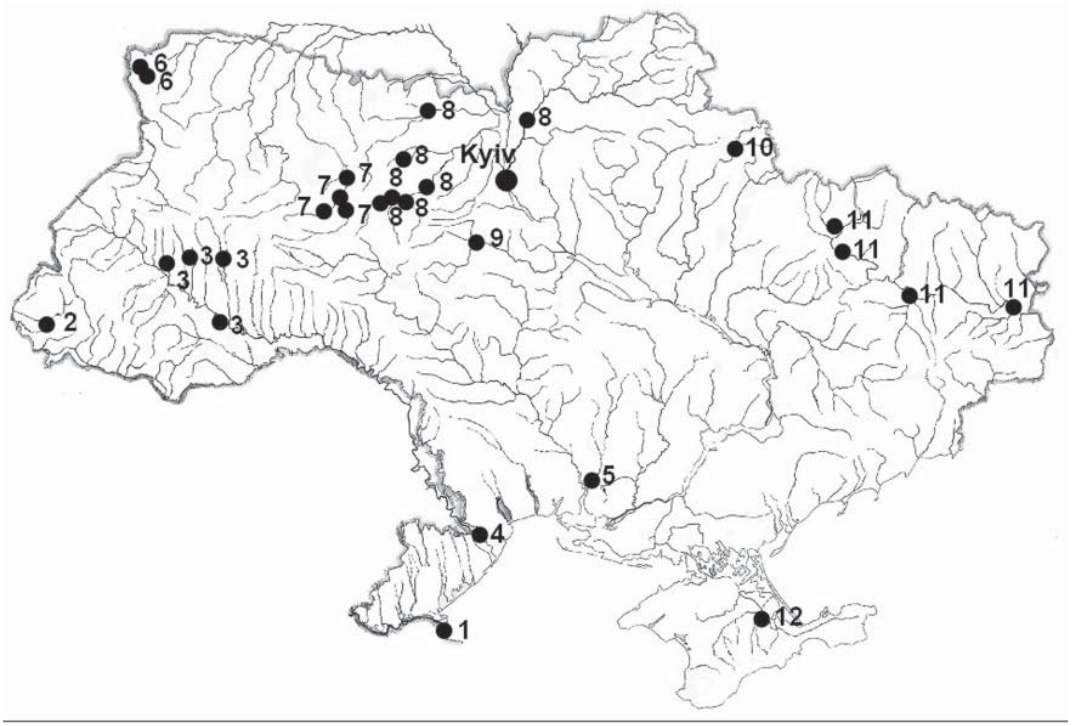


Fig. 1. Main places of material collection: 1 — r. Danube, 2 — r. Tysa, 3 — Upper Dniester, 4 — Lower Dniester, 5 — r. Ingul, 6 — r. Western Bug, 7 — r. Prypyat, 8 — Upper Dnipro, 9 — r. Ros, 10 — r. Psel, 11 — r. Siversky Donets, 12 — r. Salgyr.

Рис. 1. Локализация основных мест сбора материала: 1 — Нижний Дунай, 2 — бассейн р. Тисы, 3 — бассейн Верхний Днестр, 4 — Нижний Днестр, 5 — р. Ингул, 6 — бассейн р. Западный Буг, 7 — бассейн р. Припять, 8 — бассейн Верхнего Днепра, 9 — р. Рось, 10 — левые притоки Днепра (реки Десна, Сейм и Псёл), 11 — р. Северский Донец, 12 — р. Салгир.

Table 1. Places of collection specimens for sequence analysis and geographic coordinates of these places, also numbers of these specimens in gene bank

Таблица 1. Места сбора особей для секвенирования и географические координаты этих мест, а также номера особей в генетическом банке

n	Species according to the system of Ya. I. Starobogatov (1977)	Site of collection	Longitude	Latitude	ID COI	ID 16s
1	*Colletopterum piscinalis*	r. Salgir, Crimea	34°45′42″	45°27′32″	JQ253883	JQ253859
2	*C. piscinale*	r. Dniester, vil. Mayaki (Lower Dniester)	30°16′23″	46°24′43″	JQ253884	JQ253860
3	*C. subcirculare plattenicum*	r. Dniester, vil. Mayaki (Lower Dniester)	30°16′23″	46°24′43″	JQ253885	JQ253861
4	*A. stagnalis*	r. Derevychka, vil. Velyki Derevychi	27°36′18″	49°58′04″	JQ253886	JQ253862
5	*A. zellensis*	r. Teterev, Zhytomyr town	28°39′51″	50°14′32″	JQ253887	JQ253863
6	*A. (Pseudanodonta) rossmaessleri*	r. Danube, Vylkovo town (Lower Danube)	29°35′42″	45°23′58″	JQ253888	JQ253864
7	*P. kletti*	r. Slutch, Baranivka town	27°40′7″	50°17′38″	JQ253889	JQ253865
8	*P. elongata tanousi*	r. Uzh, vil. Tartak	28°55′6″	51°04′51″	JQ253890	JQ253866
9	*P. complanata complanata*	r. Siversky Donets, Stanychno-Luhanske town	39°28′25″	48°40′15″	JQ253891	JQ253867
10	*P. complanata complanata*	r. Latorytsa, vil. Chabanivka	22°43′00″	48°28′60″	JQ253892	JQ253868
11	*Sinanodonta woodiana*	r. Danube, Vylkovo town (Lower Danube)	29°35′42″	45°23′58″	JQ253893	JQ253869
12	*S. woodiana*	r. Latorytsa, vil. Chabanivka	22°43′00″	48°28′60″	JQ253894	JQ253870

2003); 16sar 5'–CGACTGTTTAACAAAAACAT–3' and 16sbr 5'–CCGTTCTGAACTCAG CTCATGT–3' for 16S (Lydeard et al., 1996). The reaction of COI fragment amplification was carried out according to the following cyclogram: 95 °C for 5 min followed by 35 cycles — 95 °C for 30 seconds, 50 °C for 60 seconds, 72 °C for 60 seconds. For amplification of the 16S gene the same program was used with the exception of reduced by 40 °C temperature of primers annealing. The reaction mixture contained 10μl 2.5-fold reaction mixture (Synthol Cat. No. M–428), 10 pcM of primer and 50 ng of genomic DNA each in total reaction volume of 25 ml.

After enzymatic treatment with exonuclease mixture I (Fermentas Cat. No. EN0581) and alkaline phosphatase (SibEnzyme Cat. No. E328), fragments were sequenced in two directions on genetic analyzer ABI 3130xl (Applied Biosystems). After proofreading and alignment, resulting sequences were listed in GenBank, ID (UIN) are shown in table 1.

Statistical processing was performed using the software Statistica V.6, PHYLIP–3.69, TreeView 1.6.6.

Results and discussion

Allozyme analysis

Electrophoretic analysis of five enzyme systems in extracts of foot muscles and liver tissues allowed us to identify 10 loci, for some of which the interspecific variability is characteristic, and for another ones — intraspecific variability (table 2). In general, the level of genetic polymorphism in the four species studied was low. The highest values of heterozygosity were observed in *P. complanata* — 0.025, in *A. cygnea* it was significantly lower — 0.013, and 0.008 in *A. anatina*, while in *S. woodiana* monomorphism by loci studied was identified. At the same time, expected levels of heterozygosity by polymorphic loci did not differ from those observed. Similar low polymorphism was found in Western European populations of those species (Nagel, 1996) and this may be a specific pattern.

The genetic differentiation of mussels was highly significant (fig. 2). The average genetic distance by M. Nei (1972) between the studied Anodontinae species was two times higher than those among Unioninae (Mezhzherin et al., 2011); D = 0.69 without *S. woodiana*, and with this species D = 0.85. The average genetic distance (D) between populations of *A. cygnea* and *A. anatina* was 0.50, and *P. complanata* diverged from them at D = 0.79,

supporting its placement into a separate genus. Within the latter species, two subclusters are clearly visible directed to western and eastern populations, respectively.

We should state the reliable reproductive isolation between species, and this fact is supported by the absence of heterozygous genotypes by loci diagnostic for these species.

Within every species, "species" in the sense Ya. I. Starobogatov can be isolated by the nuances in shell shape (table 2). Comparison of allele frequencies in these samples revealed no allozyme differences between specimens of such "species".

The geographical differentiation of *P. complanata* populations with different frequencies of *Mdh*–1 alleles is of interest. In populations of the Danube basin, on the one hand, and those from the Siversky Donets River, on the other hand, alternative alleles are fixed. At the same time, in Dniester and Dnipro basins there are populations with intermediate allele frequencies (fig. 3). Such a distribution of allele frequencies gives reason to believe that there are two genetically alternative groups of populations in Ukraine — western and eastern, and between them there is the area of intermediate frequencies of *Mdh*-1 alleles identifying the eastern and western groups of populations. Usually such distribution pattern is explained by the presence of two vicarious species connected by the wide zone of gene introgressions (Arnold, 1996; Yanchukov et al., 2006).

Analysis of mitochondrial genes fragments

Phenogramma of genetic relationships between representatives of different species and populations (fig. 4) is based on the generalized matrix of genetic distances calculated by nucleotide substitutions of two mitochondrial genes fragments. It clearly identifies four clusters corresponding to four species. The average genetic distance between them estimated by nucleotide substitutions was about 0.115, and excluding an adventive species

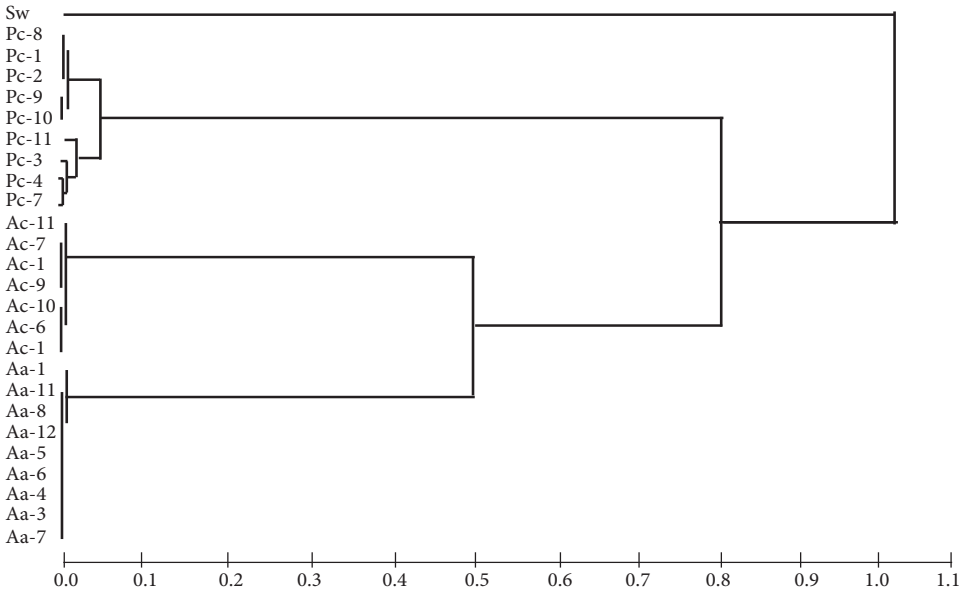


Fig. 2. UPGMA phenogram of genetic distances (Nei, 1972) between mussel species and populations.

Note. Aa — *A. anatina*, Ac — *A. cygnea*, Pc — *P. complanata*, Sw — *S. woodiana*. Riwer systems: 1 — Danube, 2 — r. Tysa, 3 — Upper Dniester, 4 — Lower Dniester, 5 — r. Ingul, 6 — r. Western Bug, 7 — r. Prypyat, 8 — Upper Dnipro, 9 — r. Ros, 10 — r. Psel, 11 — r. Siversky Donets, 12 — r. Salgyr.

Рис. 2. UPGMA — фенограма генетических дистанций (Nei, 1972) между видами и популяциями двустворчатых моллюсков.

Примечание. Aa — *A. anatina*, Ac — *A. cygnea,* Pc — *P. complanata*, Sw — *S. woodiana*. Речные системы: 1 — Дунай, 2 — р. Тиса, 3 — Верхний Днестр, 4 — Нижний Днестр, 5 — р. Ингул, 6 — р. Западный Буг, 7 — р. Припять, 8 — р. Верхний Днепр, 9 — р. Рось, 10 — р. Псёл, 11 — р. Северский Донец, 12 — р. Салгир.

Table 2. Allozyme variability in different species (*A. cygnea, A. anatina, P. complanata and S. woodiana*) and forms in the subfamily Anodontinae

Таблица 2. Аллозимная изменчивость у разных видов (*A. cygnea, A. anatina, P. complanata и S. woodiana*) и форм в подсемействе Anodontinae

Locus	Allele	*A. cygnea*			*A. anatina*		*P. complanata*			*S. woodiana*
		«cygnea»	*«zellensis»*	*«stagnalis»*	*«ponderosum»*	*«piscinales»*	*«complanata»*	*«elongata»*	*«kletti»*	
Mdh-1A	100	1	1	1	1	1				
	110						0.30	0.45	0.43	
	120						0.70	0.55	0.57	
	140									1
Mdh-1B	100				1	1				
	0	1	1	1			1	1	1	1
Pgm-1	98				0.02	0.01				
	99				0.93	0.94				
	100	0.99	0.98	1						
	102									1
	103				0.05	0.05				
	105	0.01	0.01							
	106		0.01							
	108						0.99	1	1	
	112						0.01			
Es-3	95						1	1	1	
	98									1
	100	1	1	1	1	1				
Es-1	98	1	1	1						
	100				1	1				
	102									1
	104						1	1	1	
Es-2	100				1	1				
	104	1	1	1						
	106						1	1	1	
	110									1
Sod	80									1
	100	1	1	1	1	1	1	1	1	

Note. Loci *Aat*-1, *Es*-4, *Es*-5 showed no variation under such electrophoretic conditions.

S. woodiana, which, as expected, was clustered separately, it was found to be 0.100. Within *A. anatina* representatives, the genetic distance was 0.020. Between specimens of *P. complanata* which are cosidered as different species by Starobogatov, it was significantly lower than 0.010; within *A. cygnea*, where, by Starobogatov, we can also distinguish two species, *A. stagnalis* and *A. zellensis*, it was very small: 0.001. Between specimens of invasive species *S. woodiana* representing geographically isolated populations of the Lower Danube and Latoritsa rivers, no nucleotide substitutions were found at all. This proves very low individual genetic variability between the populations of this East Asian invader.

Especially interesting in the aspect of this work is the situation with the differentiation within *P. complanata*. Paradoxically, the maximum differences were found between

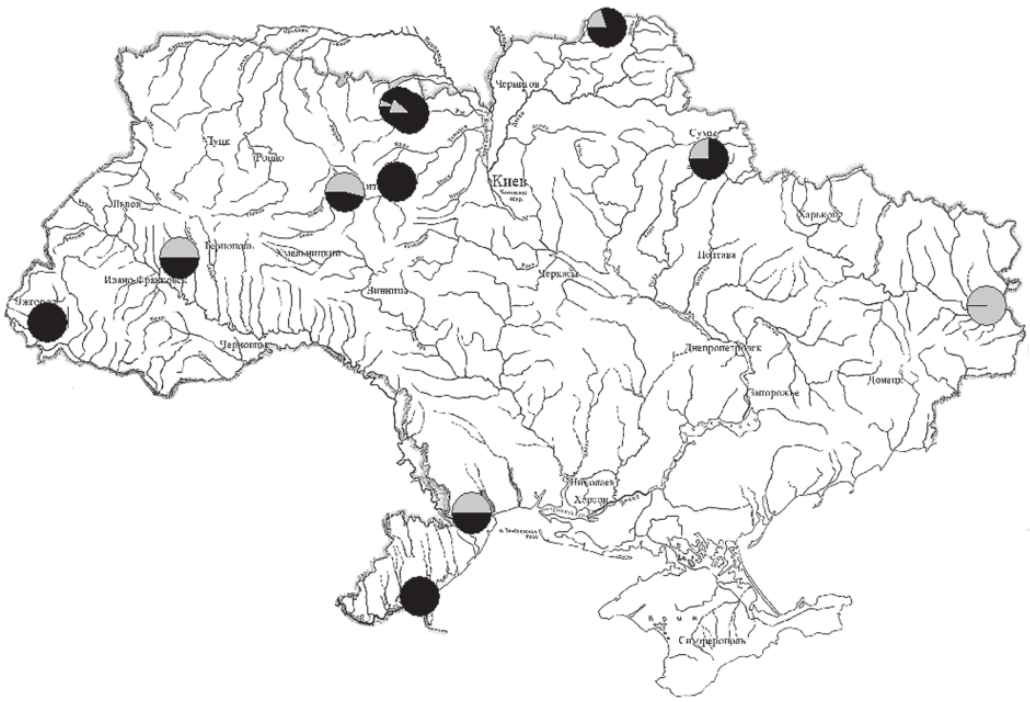


Fig. 3. Geographic variation of *Mdh*-1 locus in *P. complanata* populations in Ukraine (*Mdh*-1^{110} — filled with black, *Mdh*-1^{120} — filled with grey).

Рис. 3. Географическая изменчивость локуса *Mdh*-1 в популяциях *P. complanata* в пределах Украины (*Mdh*-1^{110} — заполнение чёрным цветом, *Mdh*-1^{120} — серым).

specimens of *P. complanata* "species" in the narrow sense, but collected in distant river basins (the Danube and Siversky Donets), whereas nucleotide substitutions among specimens of different "species" (*P. kletti, P. elongata*) from the Upper Dnipro catchment basin were much less. This confirms the results obtained by allozyme analysis showing that the geographic factor in genetic differentiation is significantly higher than genetic differences between conchologic species as Ya. I. Starobogatov understood it.

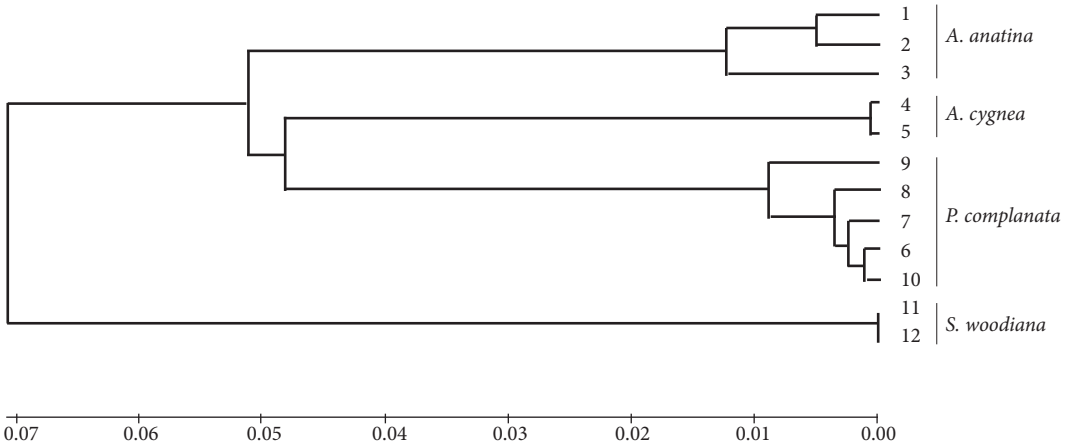


Fig. 4. UPGMA phenogram of genetic distances (Tamura et al., 2004, 2011) between specimens of four Anodontinae species made by homologous sequences of two homologous loci (COI and 16S).

Рис. 4. UPGMA фенограмма генетических дистанций (Tamura et al., 2004, 2011), построенная по гомологичным последовательностям двух локусов (COI и 16S) между отдельными особями четырёх видов Anodontinae.

Morphometric and structural shell features

Despite the highly significant differences in the mean values of the shell measurements in the four freshwater mussel species studied (table 3), none of them was unambiguously identifiable. This results from the overlapping limits of variation for each of them. The low resolution of individual characters is confirmed by the discriminant analysis (table 4). In this case, the average level of identifiability for specific index was only 57.8 % of correctly identified specimens.

Identification by combined index was higher — about 80 % (table 5). Moreover, none of the species can be identified by 100 % as shows the overlapping of dispersion areas of specimens from these four species in the space of the two first components (fig. 5). The lowest identifiability degree is in *A. cygnea* specimens.

Undoubted diagnostic significance has umbonal sculpture, distinctive characters and easily recognizable in specimens of those four species (fig. 6). *A. cygnea* has thin concentric lines, *A. anatina* — wavy ones, top of *P. complanata* shows 2–3 rows of small humps placed on the slightly thickened elevations, while in *S. woodiana* slightly wavy rough elevations in apical sculpture are placed at a distance one from another. However, using of this feature is also limited, because the structure of the apical part of the shell is often damaged, especially in the older age groups, making the use of this feature impossible.

Structure and coloration of internal organs

The structure of inhalant siphons was previously proposed as a parameter reliably separating mussel species (Bogatov, Saenko, 1996; Saenko, 2007; Saenko et al., 2009). Analysis of their variability in a series of specimens the species of which were previously identified by allozymes, confirmed its diagnostic value. It should be noted that due to the complexity of all methods of analysis of thin internal structures, in practice such an approach is of a little use. Interspecific differences in shape, size and pappilae arrangement are obvious even visually (fig. 7). This is confirmed by the quantitative analysis made by three siphons measurements. In this case, the average discrimination estimated by

Table 3. Statistical data on the variability of the main shell measurements in four Anodontinae species: mean (M) and standard error (m), variability limits (lim), and number of samples (N)

Таблица 3. Статистические показатели изменчивости основных индексов раковины четырёх видов Anodontinae: средние значения (M) и их стандартная ошибка (m), пределы (lim) изменчивости, а также объёмы (N) выборок

Measurement		*A. anatina*	*A. cygnea*	*P. complanata*	*S. woodiana*
H/L	M ± m	0.55 ± 0.0027	0.53 ± 0.0031	0.49 ± 0.0036	0.65 ± 0.0079
	lim	0.45–0.68	0.45–0.61	0.41–0.60	0.56–0.77
T/L	M ± m	0.01 ± 0.0002	0.008 ± 0.0002	0.013 ± 0.0004	0.015 ± 0.0012
	lim	0.0046–0.0203	0.0028–0.0141	0.006–0.021	0.006–0.035
H_1/L	M ± m	0.52 ± 0.002	0.51 ± 0.004	0.45 ± 0.004	0.60 ± 0.008
	lim	0.40–0.63	0.44–0.59	0.37–0.54	0.52–0.74
H_2/L	M ± m	0.58 ± 0.004	0.53 ± 0.003	0.53 ± 0.004	0.67 ± 0.01
	lim	0.45–0.76	0.47–0.64	0.43–0.64	0.54–0.78
S/L	M ± m	0.33 ± 0.002	0.33 ± 0.003	0.26 ± 0.006	0.38 ± 0.005
	lim	0.24–0.41	0.26–0.42	0.15–0.45	0.32–0.44
L_1/L	M ± m	0.25 ± 0.002	0.29 ± 0.005	0.21 ± 0.003	0.30 ± 0.005
	lim	0.18–0.34	0.20–0.54	0.16–0.29	0.23–0.37
L_2/L	M ± m	0.60 ± 0.003	0.56 ± 0.003	0.57 ± 0.005	0.70 ± 0.007
	lim	0.48–0.72	0.48–0.67	0.49–0.68	0.62–0.77
N		231	106	75	34

Table 4. Discriminatory power of individual shell parameters as a percentage of correctly identified specimens of Anodontinae species

Таблица 4. Дискриминационная способность отдельных индексов раковины, выраженная в процентах правильно идентифицированных особей видов Anodontinae

Species	Shell measurements						
	L_1/L	L_2/L	H/L	H_1/L	H_2/L	S/L	T/L
A. cygnea	37.1	38.8	0.0	0.0	37.1	0.0	18.1
A. anatina	90.1	82.8	90.1	91.4	80.2	96.6	95.7
P. complanata	17.3	0.0	46.7	61.3	0.0	69.3	2.7
S. woodiana	0.0	52.9	73.5	55.9	50.0	20.6	26.5
Totally	57.9	55.8	58.9	60.6	53.8	61.9	55.6

Table 5. The results of discriminant analysis by the following shell measurements: the proportion of correctly identified specimens for each species (%), and the absolute number of specimens assigned to each of the four Anodontinae species

Таблица 5. Результаты дискриминантного анализа по совокупности индексов раковины: доля правильно определённых особей каждого вида (%), а также абсолютное количество особей, отнесённых к каждому из четырёх видов Anodontinae

Species	%	*A. cygnea*	*A. anatina*	*P. complanata*	*S. woodiana*
A. cygnea	60.37	64	39	3	0
A. anatina	86.14	19	199	6	7
P. complanata	86.66	2	7	65	1
S. woodiana	85.29	0	5	0	29
Totally	80.04	85	250	74	37

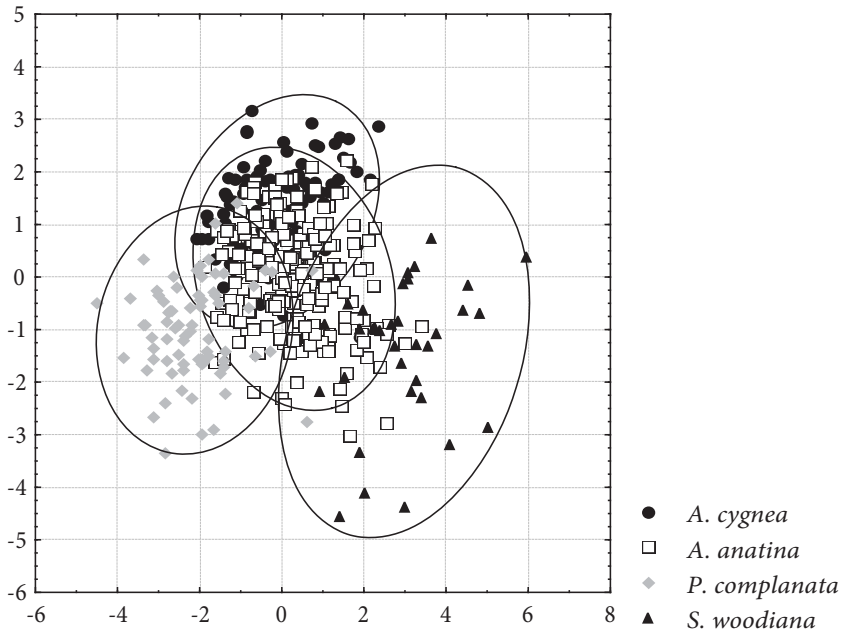


Fig. 5. The distribution of mussels from four species in the space of canonical variables made by conchological characters.

Note. Extrapolation of specimens distribution on probability level is $p < 0.05$.

Рис. 5. Распределение особей четырёх видов беззубок в пространстве канонических переменных, построенных по конхиологическим признакам.

Примечание. Экстраполяция распределения особей проведена на уровне вероятности $p < 0,05$.

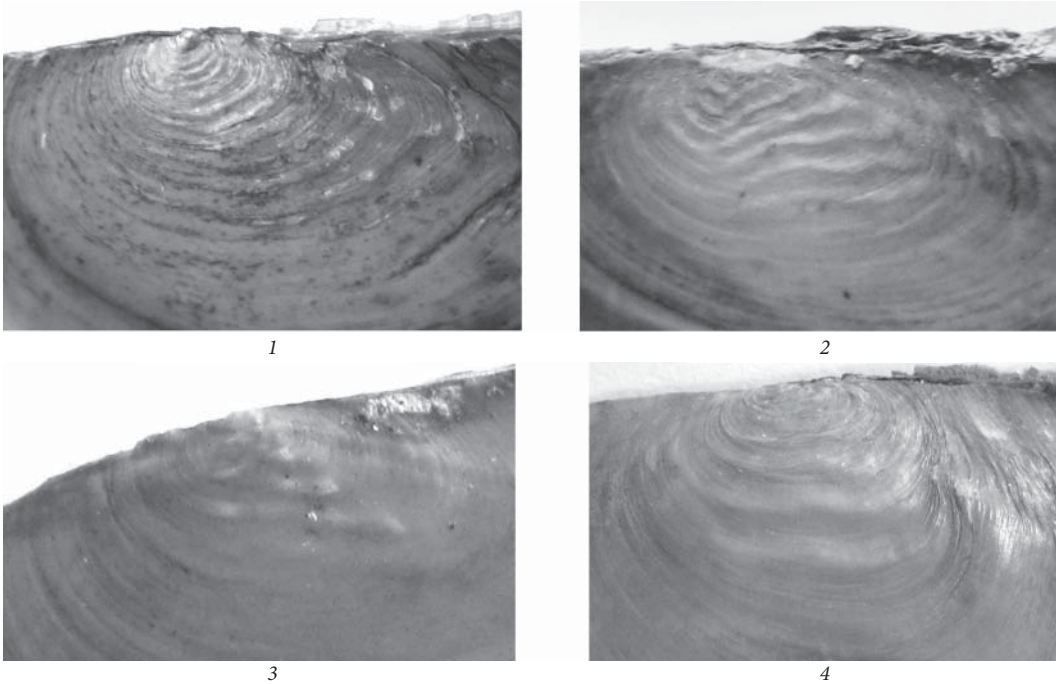
the percentage of correctly identified specimens of four species is 82 % with the largest identifiability in *A. cygnea* specimens.

Another possible parameter for separation of the mussel species is leg colour. Indeed, *A. anatina* is clearly separated by leg colour; it is characterized by bodily and light-brown colour, whereas *A. cygnea* and *P. complanata* have yellow and orange colour of different shades (table 7).

Discussion

Previously published material on allozyme differentiation of Unioninae (Mezhzherin et al., 2011) and similar materials in this paper on Anodontinae from the Central Europe suggest that the genetic divergence within Anodontinae at least two times greater than that in Unioninae. The average genetic distance between species *Unio crassus* Philipsson, 1788, *U. tumidus* Philipsson, 1788, *U. pictorum* Linnaeus, 1758 was $D_{Nei} = 0.29$ whereas in three European representatives of Anodontinae it was more than two times higher: $D_{Nei} = 0.69$. Differences in the degree of genetic differentiation between the species of these two subfamilies were also reported in other studies of allozyme variability (Nagel, Badino, 2001; Nagel et al., 1996, 1998). These results, however, were not confirmed by sequence analysis. In Anodontinae, the average genetic distance by nucleotide substitution was $D_{Nei} = 0.1002 \pm 0.0015$, and in Unioninae $D_{Nei} = 0.097 \pm 0.002$ (Mezhzherin et al., 2013), so there are no differences. Therefore, for allozymes we can state that the group Anodontinae is much older than Unioninae while the analysis of mitochondrial gene sequences indicates that the age of divergence is the same.

However, the possible differences in the age of Anodontinae and Unioninae had no effect on the number of taxa on species level. While V. I. Zhadin (1938, 1952) recognised at least six species in Eastern Europe, Ya. I. Starobogatov with followers found 10 species (Starobogatov, 1970). The genetic criteria supported the presence of only three species (*A. anatina, A. cygnea, P. complanata*), as many as in the European Unioninae.



1 *2* *3* *4*

Fig. 6. The umbonal structure of the shell in molluscs from subfamily Anodontinae: *1 — A. cygnea*; *2 — A. anatina*; *3 — P. complanata*; *4 — S. woodiana.*

Рис. 6. Структура вершины раковины моллюсков подсемейства Anodontinae: *1 — A. cygnea*; *2 — A. anatina*; *3 — P. complanata*; *4 — S. woodiana.*

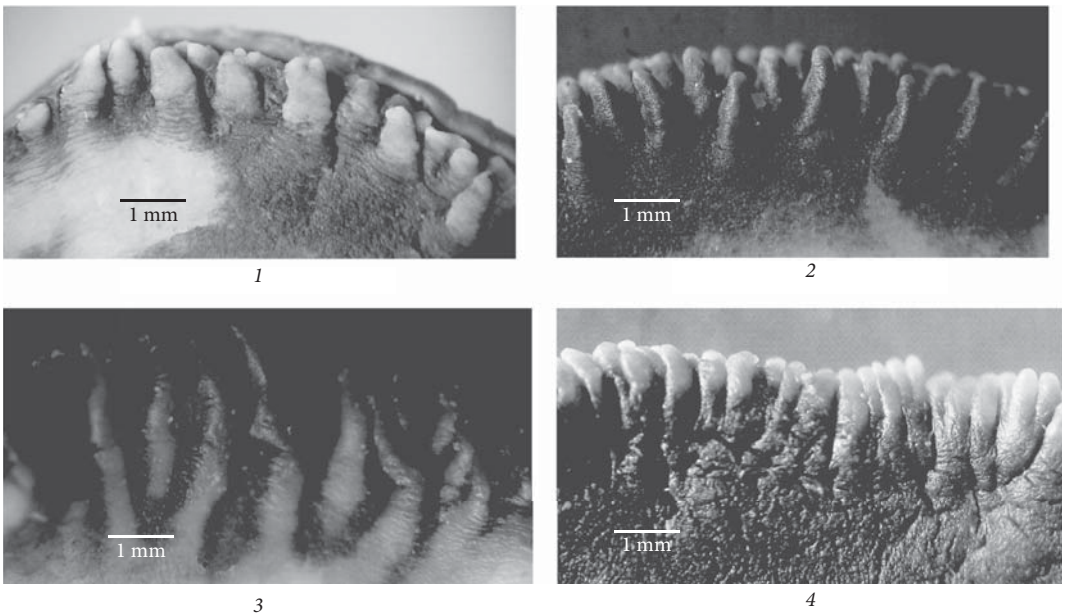


Fig. 7. Micrographs of the outer edge of the inhalant siphon in mussels: *1 — A. cygnea* (r. Derevychka); *2 — A. anatina* (Lyutsymer lake); *3 — P. complanata* (r. Uzh); *4 — S. woodiana* (r. Danube).

Рис. 7. Микрофотографии наружного края вводных сифонов у беззубок: *1 — A. cygnea* (р. Деревичка); *2 — A. anatina* (оз. Люцимер); *3 — P. complanata* (р. Уж); *4 — S. woodiana* (р. Дунай).

Table 6. The results of discriminant analysis of inhalant siphons in Anodontinae

Таблица 6. Результаты дискриминантного анализа признаков вводных сифонов Anodontinae

Species	%	*A. cygnea*	*A. anatina*	*P. complanata*	*S. woodiana*
A. cygnea	100	30	0	0	0
A. anatina	86.67	0	26	4	0
P. complanata	76.67	0	6	23	1
S. woodiana	46.67	0	0	8	7
Totally	81.9	30	32	35	8

Note. Three measurements used: height of papilla, width of papilla in its base, distance between the first row papillae.

At allozyme polymorphism, populations of Anodontinae are more conservative genetically than Unioninae, and genetic differentiation estimated from nucleotide substitutions in specimens of distant geographic populations is not different in the members of the subfamily. The average genetic distances in the three species of Unioninae were $D_{Nei} = 0.016 \pm 0.002$, and in Anodontinae $D_{Nei} = 0.012 \pm 0.002$. However, morphological variability in Anodontinae is much higher than that in Unioninae. The latter fact is reflected in the discriminant analysis. So, an average level of identifiability of conchological characters for three European species from the genus *Unio* is 94 % (Mezhzherin et al., 2011). In Anodontinae, including even genetically very distant *S. woodiana*, by the same characters this level is only 80 % due to the high individual variability of shell parameters shown in the analysis of variance of the same parameters. For example, variances ($\sigma^2 \times 10^4$) of H/L index in three species of Unioninae species varies from 5.81 to 9.52 (Mezhzherin et al., 2011), while in Anodontinae — from 9.49 to 17.36, and L_1/L index in the first group is 7.63–12.8, and 7.43–26.25 in the second group, the same trend is also seen for L_2/L index: 8.1–9.64 in mussels and 11.43–22.73 in Unioninae. The situation is the same for other indices. We can suggest that different levels of morphological variability in members of these

Table 7. Distribution of specimens (%) from subfamily Anodontinae by variations in leg colour

Таблица 7. Распределение особей (%) подсемейства Anodontinae по вариантам окраски ноги

Sign	Parameter variations	*A. cygnea* (N = 110)	*A. anatina* (N = 231)	*P. complanata* (N = 81)	*S. woodiana* (N = 34)
Leg colour	light yellow	0	0	0	100
	yellow	20	0	14.8	0
	body	0	47.2	0	0
	light brown	0	52.8	0	0
	orange	80	0	0	0
	brown- orange	0	0	85.2	0

two subfamilies should be found not only in their specific population-genetic structure or in the environment, but in the different evolutionary age of species and most likely in peculiarities of morphogenesis, more stable in Unioninae.

Therefore, this study shows the presence of only three species native for Ukraine: *A. cygnea, A. anatina, P. complanata*, and one invasive species — *S. woodiana*. This is recognized by the most modern taxonomists. However, the data on genetic differentiation call the correctness of the taxonomic division on generic level in question. While *P. complanata* is clearly separated from two others on generic level of divergence in allozyme analysis, according to data of the mtDNA sequencing, all three native species are equidistant.

We may suggest further systematic innovations in giving the status of allospecies to geographical forms with unique allelic variants, and the status of superspecies to classical forms, respectively. This, above all, applies to *P. complanata* with highly differentiated populations in different river basins while the borders between them are fuzzed by the gene flow and introgressive hybridization.

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