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Karyological Features of the Genus *Planorbarius* (Gastropoda, Pulmonata, Bulinidae) of the Ukranian Fauna

D. A. Garbar and A. V. Garbar

Franko State University, ul. Bol'shaya Berdichevskaya 40, Zhitomir, 10008, Ukraine
E-mail: garbar@zu.edu.ua

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Abstract—The absence of significant distinctions between the species of the genus *Planorbarius* in the narrow sense (*P. corneus*, *P. banaticus*, *P. purpura*, and *P. grandis*) has been established. All investigated species had identical chromosomal formulas ($2n = 30m + 6sm = 36$) and fundamental numbers (FN = 72). Reproducible distinctions between them were not found by total complement length (TCL), relative length of chromosomes (RL), and centromeric indexes. The species selected on the basis of genetic marking differed clearly by centromeric index of chromosome 12, which confirms the allospecies frame of *P.corneus* s. lato.

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INTRODUCTION

The first karyological investigations of the genus *Planorbarius* were performed in the middle of the 20th century. However, the results were rather contradictory. For example, for *P. corneus* (Linné, 1758), different numbers of chromosomes in the diploid set were reported: $2n = 36$ [1, 2] and $2n = 34$ [3].

Botke [4] investigated the chromosomal set of this species in more detail. According to his data, the karyotype of *P. corneus* consists of 36 monocentric chromosomes, which gradually decrease in size, with a median or submedian location of the centromere. The limited amount of the investigated material (7 metaphase plates with a high degree of chromosome spiralization) did not permit a more comprehensive description of the karyotype of this species. Note that in all the listed papers, the species *P. corneus* was taken in the broad sense (as a single representative of the genus), and most modern malacologists hold these viewpoints [5–10].

However, some researchers, primarily Russians, are inclined to assign species status to numerous morphological forms. They distinguish from five to eight sympatric species [11–13] within the genus *Planorbarius*. For example, for the Ukrainian fauna Stadnichenko [14] distinguishes five species: *P. corneus* (Linné, 1758), *P. banaticus* (Lang, 1856), *P. purpura* (O.F. Müller, 1774), *P. grandis* (Dunker, 1856), and *P. stenostoma* (Bourguignat in Servain, 1881). Maksimova attempted to study the karyological features of the species of the genus *Planorbarius* in the narrow sense [12]. She investigated the chromosomal sets of four species (*P. corneus*, *P. purpura*, *P. grandis*, and *P. adelosius*). For karyological analysis, chromosomes primarily at the stage of prophase and metaphase of the second meiotic division were used. It was found that the number of chromosomes in a haploid set varied widely ($n = 15–20$), although $n = 18$ was dominant. The author concluded that the instability of the number of chromosomes in various cells of the gonad was related to the presence of additional chromosomes. Therefore, the use of specific features of the karyotype in the systematics of this group is rather troublesome.

The results of our biochemical gene marking [15] unambiguously prove the conspecifics of *P. corneus*, *P. banaticus*, *P. purpura*, and *P. grandis* that are mentioned by some authors for the Ukrainian fauna. Comparison of the variation of allozyme markers and morphological traits indicated that the isolation distance is far more significant that the differentiation for symbiotopically dwelling species. Moreover, the significance of the genetic distinctions for mollusks from the northeastern and eastern populations from the central and western Ukrainian demonstrates the possibility of the allospecies frame of *P.corneus* s.l.

In this connection, we performed a comparative-karyological analysis of four species distinguished on the basis of a morphological approach (*P. corneus*, *P. banaticus*, *P. purpura*, and *P. grandis*), as well as of two allospecies distinguished on the basis of gene marking in order to determine the level of karyological differences between them.

MATERIAL AND METHODS

The material for karyological investigations was collected by the authors over Ukrainian territory from 2002 to 2005 (Table 1).

Specimens of chromosomes were prepared from gonad tissues using methods that have been successfully used for the study of the karyotypes of other groups of mollusks [16, 7]. The animals were injected with 0.02% colchicine 16–17 h before dissection.

Table 1. Material used for karyological studies

Taxon by morphological criteria	Localization	Taxon by genetic markers	Studied*	
			specimens	metaphase plates
P. corneus (Linné, 1758)	Chernigov, lake	Eastern	31	11
	Zhitomir, Teterev River	Western	20	7
P. banaticus (Lang, 1856)	Zhitomir, Teterev River	Western	16	4
	Zhitomir oblast, Singury village, pond	Western	5	1
	Chernigov, lake	Eastern	16	12
	Ternopol oblast, Podgaitsy city, Koropets River	Western	10	2
P. purpura (O.F. Müller, 1774)	Zhitomir, Teterev River	Western	35	13
	Chernigov, lake	Eastern	5	1
	Suma oblast, Pechishche village, Sula River	Eastern	9	2
	Sumy, Lake Durova	Eastern	11	2
P. grandis (Dunker, 1856)	Odessa oblast, Kiliya city, Dunabe River	Western	12	2
	Zhitomir, Teterev River	Western	28	13

Notes: * no metaphase plates were obtained from some specimens.

Gonad fractions were minced and subjected to hypotonic treatment for 15–20 min in distilled water. Material was fixed in a mixture of 96% ethanol and glacial acetic acid in a 3 : 1 ratio. A cellular suspension was prepared by maceration of the material in a mixture of glacial acetic acid and 60% lactic acid (30 : 1), after which it was dropped onto dry slides warmed to 50°C, using a capillary pipet. The dried specimens were stained for 10 min in a 10% Romanovskii solution of azur-eosine prepared on 0.01 M phosphate buffer (pH 6,8). The preparations were passed through xylol and embedded into Canadian balsam. The analysis of preparations was performed using a Mikmed microscope with 10×90 magnification. Metaphase plates ($2n$) with a satisfactory scatter of chromosomes and an equal degree of spiralization were selected for subsequent photography and measurement. On the basis of measurements, the total complement length (TCL), relative length of chromosomes (RL – total length of a chromosomal pair/TCL $\times$ 100%) and centromeric index (Ci, the length of a short shoulder/chromosomal length) were determined. The morphological type of chromosomes was determined according to Levan's classification [18]. The fundamental number (FN) was determined as the number of chromosomal shoulders in a diploid set. The linear parameters of chromosomes were processed by methods of variation statistics (STATISTICA 6.0, Microsoft Excel 2003).

RESULTS AND DISCUSSION

Description of karyotypes. *Planorbarius "corneus"*, the diploid set ($2n$), consists of 36 chromosomes. The TCL = 156.56 ± 5.91 μm. The chromosomes gradually decrease in size from the 1st to the 18th pair. Their relative length varies from 8.42 (the 1st pair) to 3.69% (18th pair). The morphological characteristic of the karyotype are as follows: the 2nd, 14th, and 17th pairs of chromosomes are represented by submetacentrics; the remaining pairs are metacentric. The chromosomal formula is $2n = 30m + 6sm = 36$. The fundamental number FN = 72 (Figs. 1.1 and 2.1).

Planorbarius "banaticus", the diploid set ($2n$), consists of 36 chromosomes. TCL = 167.96 ± 0.92 μm. The chromosomes gradually decrease in size from the 1st to the 18th pair. Their relative length varies from 8.41 (the 1st pair) to 3.48% (the 18th pair). The morphological characteristic of the karyotype are as follows: the 2nd, 14th, and 17th pairs of chromosomes are represented by submetacentrics; the remaining pairs are metacentric. The chromosomal formula is $2n = 30m + 6sm = 36$. The fundamental number FN = 72 (Figs. 1.3 and 2.3).

Planorbarius "grandis", the diploid set ($2n$), consists of 36 chromosomes. The TCL = 166.06 ± 0.76 μm. The chromosomes gradually decrease in size from the 1st to the 18th pair. Their relative length varies from 8.63 (the 1st pair) to 3.385 (the 18th pair). The morphological characteristic of the karyotype are as follows: the 2nd, 14th, and 17th pairs of chromosomes are represented by submetacentrics; the remaining pairs are metacentric. The chromosomal formula is $2n = 30m + 6sm = 36$. The fundamental number FN = 72 (Figs. 1.4 and 2.4).

Eastern form (Chernigov city, lake), a diploid set ($2n$), consists of 36 chromosomes. TCL = 164.40 = 1.60 μm. The chromosomes gradually decrease in size from the 1st to the 18th pair. Their relative length varies from 8.24 (the 1st pair) to 3.89% (the 18th pair). The morphological characteristic of the karyotype: 2nd,

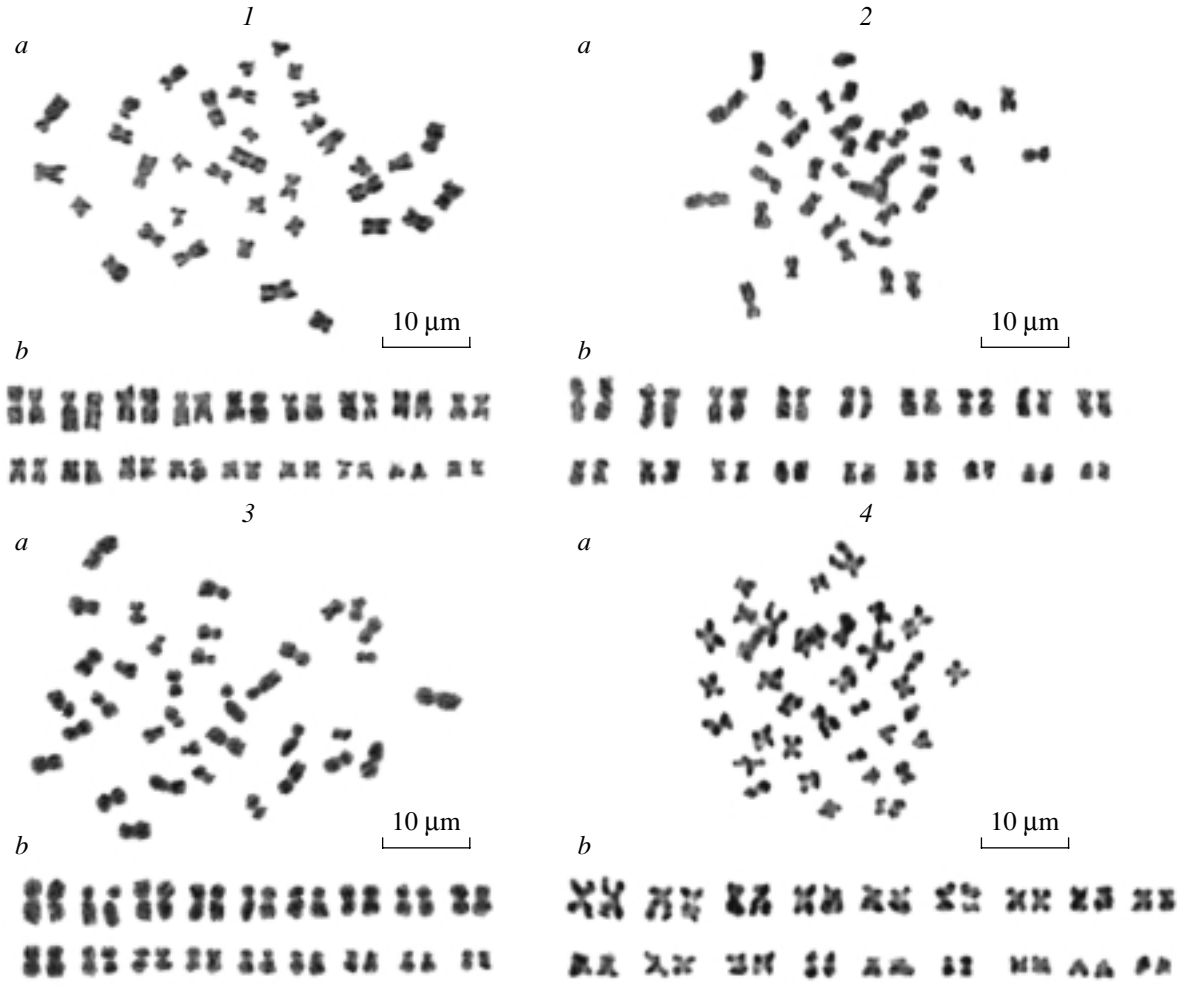

Fig. 1. Chromosomes of mollusks of the genus *Planorbarius*: (1) *P. "corneus"*; (2) *P. "banaticus"*; (3) *P. "purpura"*; (4) *P. "grandis"*; (*a*) mitotic metaphase (2@n); (*b*) karyogram.

14th, and 17th pairs of chromosomes are represented by submetacentrics; the remaining pairs are metacentric. The chromosomal formula $2n = 30m = 6sm = 36$. The fundamental number FN = 72 (Figs. 1.1 2, and 2.5).

Western form (Zhitomir city, the Teterev River), the diploid set ($2n$), consists of 36 chromosomes. TCL = 164.86 ± 2.52 μm. The chromosomes gradually decrease in size from the 1st to the 18th pair. Their relative length varies from 8.33 (the 1st par) to 3.32% (the 18th pair). The morphological characteristic of the karyotype are as follows: the 2nd, 14th, and 17th pairs of chromosomes are represented by submetacentrics; the remaining pairs are metacentric. The chromosomal formula is $2n = 30m + 6sm = 36$. The fundamental number FN = 72 (Figs. 1.3, 4, and 2.6).

Comparative analysis of karyotypes. The centromeric index (Ci) turned out to be the most variable index of the karyotype (Figs. 3.3 and 3.4). Some differences were found between the four traditional species separated by morphological characters (dispersion analysis, LSD test). For instance, *O. "banaticus"* significantly differed from *P. "purpura"* by the centromeric index of the 1st pair of chromosomes ($P = 0.01$), and *P. "grandis"* from *P. "purpura"* by the centromeric index of the 4th pair ($P = 0.03$).

The appearance of changes in the morphology of some pairs of chromosomes is more frequently related to their rearrangements (inversions, translocations, divisions, etc.). When this rearrangement occurs in some pair of chromosomes in one of the species, it should then significantly differ in the centromeric index of this pair from all the remaining species, whose karyotypes were not changed. In case of four of the investigated species, this pattern was not observed; therefore, the revealed differences in centromeric indexes between some pairs of species are insufficient for their discrimination.

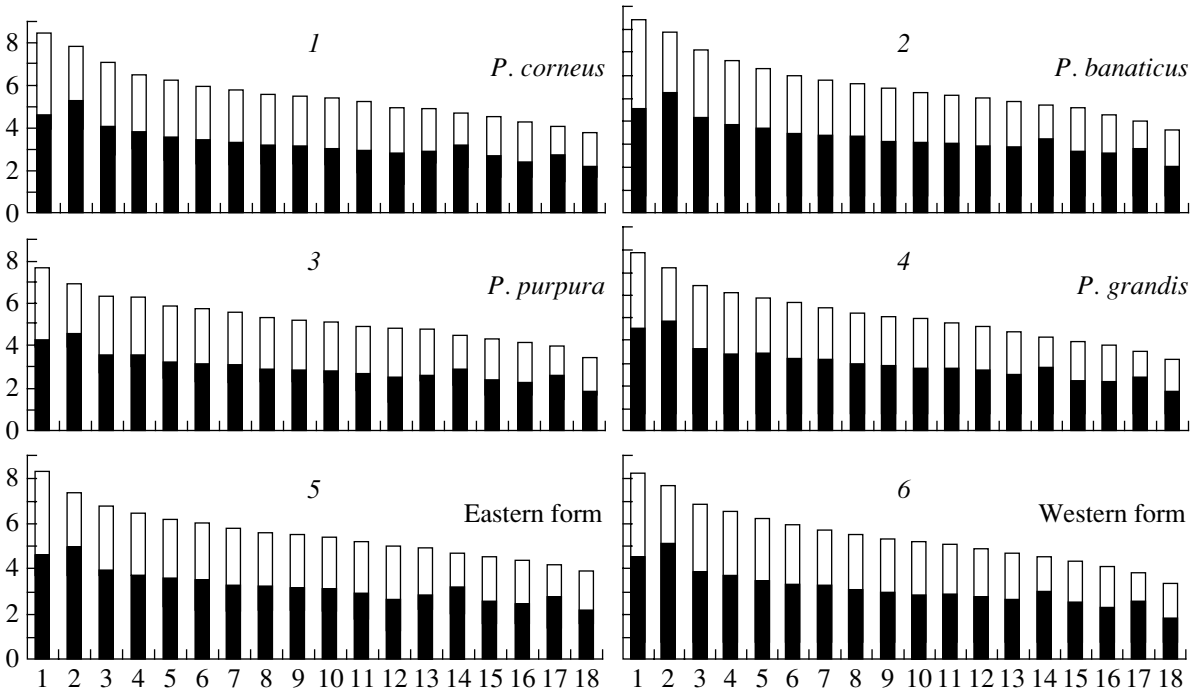

Fig. 2. Idiograms of karyotypes of mollusks of the genus *Planorbarius*: on the vertical axis is the relative length of chromosomes (RL%); on the horizontal axis, numbers of chromosome pairs.

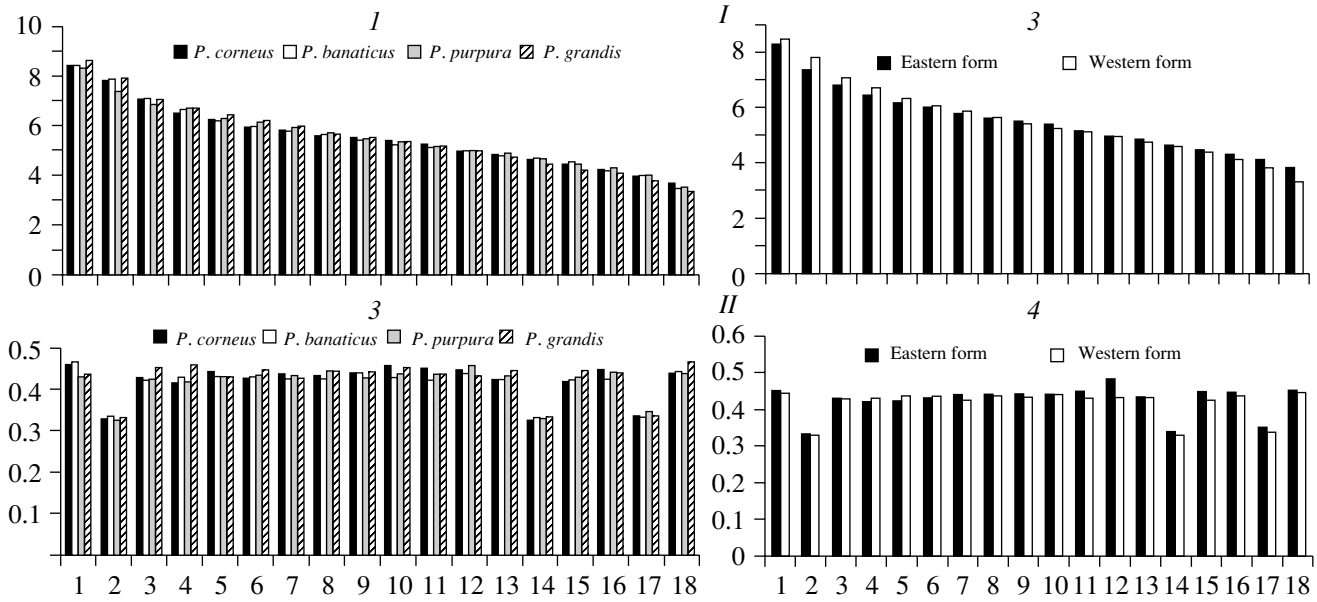

Fig. 3. Relative length of chromosomes (RL%) (I) and centromeric indexes (Ci) (II) of the studied species of the genus *Planorbarius*.

Comparison using the centromeric indexes of the allospecies distinguished on the basis of biochemical genetic marking indicated the presence of more significant differences between them than for the four traditional species. The two mentioned allospecies differ significantly by the centromeric indexes of the 12 (P = 0.0001) and the 15th (P = 0.02) pairs of chromosomes. While in case of the 15th chromosomal pair, the level of significance of the differences is comparable to that between traditional species, in case of the 12th pair, the

Table 2. The reliability of discrimination (%) by centromeric indexes of allospecies of the genus *Planorbius* separated according to results of biochemical genetic marking

Allospecies	%	Eastern	Western
Eastern	94.44	17	1
Western	96.77	1	30
Total	95.91	18	31

significance of these differences is higher by an order of magnitude. Mollusks from the northeast and east of Ukraine are characterized by a centromeric index of the 12 pair of 0.48 ± 0.01, while for mollusks from the center and west of Ukraine, this index is slightly lower, 0.43 ± 0.01.

The results of discriminant analysis (Table 2) demonstrated that these allospecies are differentiated at the 96% level. Although the samples are rather close on the scatter diagram, they are, nevertheless, rather distinctly isolated (Fig. 4). Hence, most specimens of these allospecies may be differentiated by centromeric indexes.

Thus, some karyological characters of the species of the genus *Planorbarius* may be used for differentiation purposes. However, most parameters of their karyotypes exhibit considerable similarity. For instance, the investigated allospecies have 36 chromosomes in the diploid set, which agrees with the previous data [1, 2, 4]. The chromosomal number $2n = 34$ established for *P. corneus* [3] was apparently determined erroneously. In addition, the allospecies of *Planorbius* have identical chromosomal formulas and fundamental numbers (FN). No significant differences were revealed either by the relative length of chromosomes (RL) (Figs. 3.1 and 2) and the length of the diploid set (TCL). Hence, the karyological characters in this group may be also used in grouping. The considerable similarity in the chromosome sets of the allospecies testifies to their close phylogenetic relation and an inconsequential role of chromosomal rearrangements in the processes of species formation within the genus *Planorbarius*.

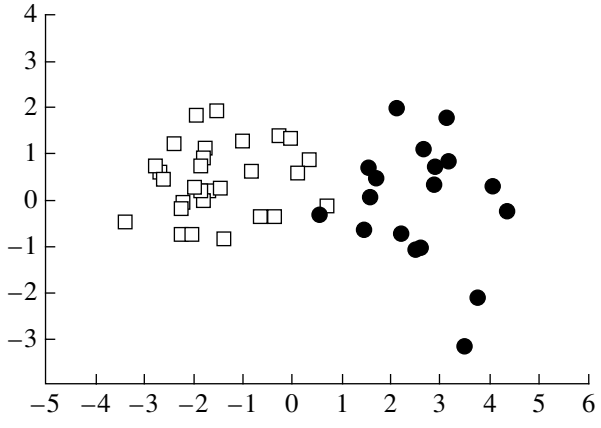

Fig. 4. Distribution of studied specimens of allospecies of *Planorbarius* in the field of the first two discriminant functions: along the horizontal axis is function 1; on the vertical axis, function 2; ● eastern form; □ western form.

CONCLUSIONS

As a result of the comparative analysis of karyotypes of the genus *Planorbarius*, an absence of significant distinctions was revealed between the species in the narrow sense distinguished by morphological characters (*P. "corneus"*, *P. "banaticus"*, *P. "purpura"*, and *P. "grandis"*). All the investigated species had identical chromosomal formulas ($2n = 30m + 6sm = 36$) and fundamental numbers (FN = 72). As well, no significant distinctions were found between them by total complement length (TCL), relative length of chromosomes (RL), and centromeric indexes (Ci).

However, allospecies distinguished on the basis of genetic marking are well differentiated by the centromeric index of the 12th pair of chromosomes, which is a marker for them. Thus, the karyological data favor the idea that *P. corneus* is a polytypic species (superspecies) represented by a series of replacing allospecies. If the genus *Planorbius* is regarded in these terms, the karyotype acquires definite differentiating importance, and several of its parameters (primarily the centromeric indexes) may be useful for future taxonomic and cytogenetic studies of this group.

REFERENCES

1. Le Calvez, J. and Certain, P., Données cardiologiques sur quelques pulmones basommatophores, *C. R. Acad. Sci. Paris*, 1950, no. 231, p. 794.
2. Burch, J.B., The Chromosomes of *Planorbarius corneus* (Linnaeus), with a Discussion on the Value of Chromosome Numbers in Snails Systematic, *Basteria*, 1961, vol. 25, no. 4/5, p. 45.
3. Azavedo, J.F. and Concalves, M.M., Ensaios sobre o estuda da numeracao chromosomica de algumas espeices de moluscos de aqua doce, *Anais Inst. Med. Trop.*, 1956, vol. 13, p. 569.
4. Bottke, W., Heterochromatin in a Pulmonate Snail *Planorbarius corneus*, *Caryologia*, 1982, vol. 35, no. 4, p. 443.
5. Zhadin, V.I., *Molluski presnykh i solonovatykh vod SSSR* (Mollusks of Fresh and Brackish Waters of the USSR), Moscow: Akad. Nauk SSSR, 1952.
6. Baker, F.C., The Molluskan Family Planorbidae, Urbana: Univ. Illinois, 1945.
7. Hudenbick, B., Phylogeny in the Planorbidae, *Trans. Zool. Soc. London*, 1955, vol. 28, no. 6, p. 453.
8. McMillan, N.F., The Range of *Planorbarius corneus* (L.) in the British Isles, *J. Conch.*, 1955, vol. 20, p. 63.
9. Falkner, G., Obrdlik, P., and Falkner, M., Mollusques continentaux de France, Paris, 2002.

10. Glöer, P., Die Sübwassergastropoden Nord- und Mitteleuropas Bestimmungsschlüssel, Lebensweise, Verbreitung, Conch Books, 2002.
11. Krivosheina, L.B. and Starobogatov, Ya.I., Composition and Zoogeographic Characteristic of Freshwater Malacofauna of the Mountain Part of the Upper Irtysh Basin, *Zool. Zh.*, 1973, vol. 22, no. 3, p. 348.
12. Maksimova, T.I., Morphological and Genetic Analyses of Mollusks of the Family Bulinidae (Gastropoda, Pulmonata) of the Fauna of Russia and Adjacent Territories, *Cand. Sci. (Biol.) Dissertation*, St. Petersburg, 1995.
13. *Opredelitel' presnovodnykh bespovoznochnykh Rossii i sopredel'nykh territorii, t. 6: Molluski, Polikhety, Nemertiny* (Key to Freshwater Invertebrates of Russia and Adjacent Territories, vol. 6: Mollusks, Polychaetes, Nemertines), Tsalolikhin, S.Ya, Ed., St. Petersburg: Nauka, 2004.
14. Stadnichenko, A.P., Lymnaea-Like Mollusks, in *Fauna Ukrainy* (Fauna of Ukraine), Kiev: Nauk. Dumka, 1990, vol. 29.
15. Mezhzherin, S.V., Garbar, D.A., and Garbar, A.V., Systematic Structure of the Complex *Planorbius corneus* (Linaeus, 1758) s.l.: Analysis of Allozyme Markers and Morphometric Characters, *Vestn. Zool.*, 2005, vol. 39, no. 6, p. 11.
16. Thiriot-Quievreux, C., Chromosome Studies in Pelagic Opistobranch Mollusks, *Can. J. Zool.*, 1988, no. 66, p. 1460.
17. Garbar, A.V., Karyotype *Lymnaea auricularia* (Gastropoda, Pulmonata, Lymnaeidae) from Central Poles'e, *Vestn. Zool.*, 1998, vol. 32, no. 5/6, p. 137.
18. Levan, A., Fredga, K., and Sandberg, A., Nomenclature for Centromeric Position on Chromosomes, *Hereditas*, 1964, no. 52, p. 201.

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