

# Freshwater mollusks from Neogene-Quaternary Dniester and Prut riverine deposits as indicator paleoenvironments: chemical composition of shells and its palaeoecological interpretation

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## Abstract

The respective environments in two ancient rivers were studied using geochemical methods with paleogeographic reconstructions of fossil material represented by shells of freshwater bivalve mollusks. The studied outcrops are located in the basins of the Dniester and Prut rivers. Materials were collected from two Pliocene (Brînza, Giurgiuleşti) outcrops and the ages of the second group of localities (Sucleia, Slobozia Mare, Gura Bîcului) are from Middle to Late Pleistocene. The determination of the taxonomic position was carried out using standard malacological methods. Geochemical data were used for the environmental reconstruction and included stable isotope ratio and trace element compositions in subfossil freshwater bivalve shell (Bivalvia: Unionoida). Key indicators of paleoenvironments show changes in water temperature. Changes in the taxonomic composition of bivalve mollusk assemblages also occurred. In the present study, increasing the water temperature caused a change of stenobiont species of bivalve mollusks to eurybiont species. Eutrophication of watercourses, caused by anthropogenic pollution and climate change in the direction of warming, has led to the replacement of pearl mussels of the family Margaritiferidae by more eurybiont species of the family Unionidae in the benthic communities of European oligotrophic rivers. These processes are similar to those deduced for the Neogene-Quaternary watercourses of the Prut and Dniester basins, but they proceed at a much faster pace. They are processes of eutrophication of watercourses – the main factor leading to the catastrophically rapid modern reduction of the ranges of pearl mussel.

## Keywords

freshwater bivalve mollusks, elemental composition, stable isotope ratio, paleoclimate, Neogene-Quaternary riverine deposits

## Introduction

The Neogene-Quaternary riverine deposits of the Russian Plain are the focus of attention for geologists, geographers, and paleontologists. In the literature (e.g., Matoshko et al. 2004), it is noted that they have a role as natural archives which make it possible to reconstruct the environment of the past. Further, the application of the methods of modern paleogeography to various fossil materials is currently particularly relevant. For example, mollusks, as filtering organisms, are a source of information about the environment in ancient rivers. Fossil shells of mollusks are widely used in paleogeographic studies (Yan et al. 2014) and hydroclimatic reconstructions (Geist et al. 2005, Schöne et al. 2007). Various methods have been developed to obtain information on environmental conditions in the historical and geological past. Among them, geochemical methods are quite effective and represent the analysis of ratios of stable oxygen isotopes (Goodwin et al. 2003) and carbon isotopes (Fritz and Poplawski 1974, Latal et al. 2004), and an analysis of the content of trace elements in the carbonate of shells (Bolotov et al. 2015). Techniques such as laser ablation, for example, allow the detection of changes in hydroclimatic conditions in a waterbody during the life of a mollusk, based on an analysis of the layers in the shell cut (Schöne et al. 2010). Lower resolution methods are used to reconstruct paleoecological conditions of the geological past. There are numerous examples of such usage of geochemical data (Jass et al. 2002, Kieniewicz and Smith 2007, Wang et al. 2016). Based on this, an application of geochemical methods to fossil shells of freshwater bivalve mollusks from Neogene-Quaternary localities in the south of the Russian Plain was undertaken in this research. The main goal of this work was the reconstruction of some parameters of the aquatic environment in the Pliocene and Pleistocene biotopes in the Prut and Dniester river basins.

## Materials and methods

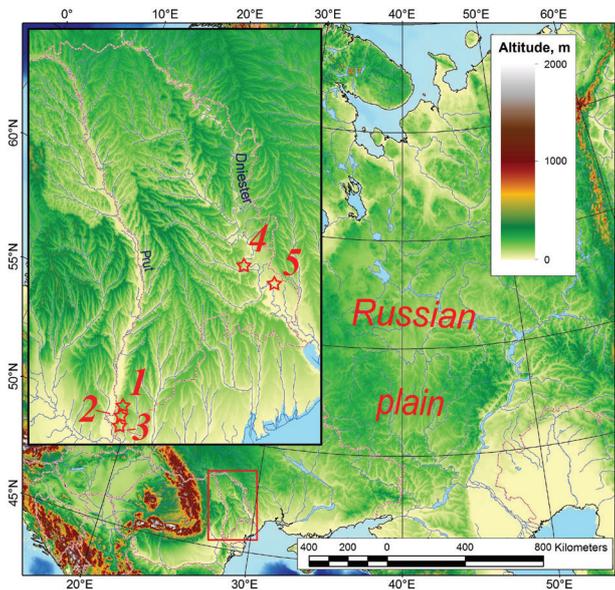
The studied outcrops are located in the basins of the Dniester and Prut rivers (Fig. 1). Their descriptions are presented in Table 1. The names of the localities are given with respect to nearby settlements. A detailed description of the studied localities, outcrops and their geological ages are developed in Vangengeym et al. (2005), Yan-shin (1989), Adamenko (1986), Titov (2008), and Tesakov (2004). In those studies, materials were collected from two Pliocene (Brînza, Giurgiulești) outcrops and the ages of the second group of localities (Sucleia, Slobozia Mare, Gura Bîcului) are from Middle to Late Pleistocene.

The materials for the study were subfossil shells of freshwater bivalve mollusks, belonging to the genera *Margaritifera* and *Unio* (Fig. 2).

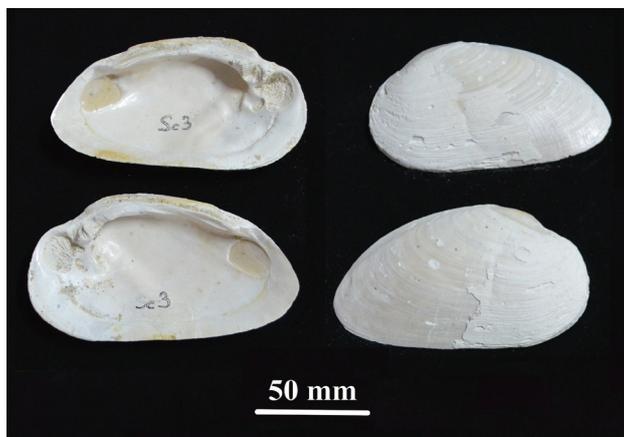
The collecting of mollusk shells during the fieldwork consisted of clearing of an outcrop in the locality, and layer-by-layer descriptions of the sediments of the entire section. Before collecting any fossil materials, their respective positions in the sediments and relation to each other along the strike (by area), and along the outcrop, were recorded (Lavrov and Kupman 1989). The collecting of subfossil materials was carried out from 1 to 20 May 2013.

The determination of the taxonomic position was carried out in accordance with the methods described in Chepalyga (1967) and Araujo et al. (2009). For chemical analyses, the shell fragments from the umbo area were pre-ground in an agate mortar.

Concentrations of Mg, Ca, Fe in the carbonate of subfossil mollusk shells were determined using atomic emission spectrometry (AES) with inductively coupled plasma (ICP, iCAP-6500, Thermo Scientific, USA). Concentrations of Mn, Sr, Ba in the shell material were determined using inductively coupled plasma mass spectrometry (ICP-MS, X-7,



**Fig. 1.** Location map of the field study areas. 1 – Brînza, 2 – Slobozia Mare, 3 – Giurgiuleşti, 4 – Gura Bicului, 5 – Sucleia



**Fig. 2.** Subfossil shell of freshwater bivalve mollusk *Mar-garitifera (Pseudunio) spp.*

Thermo Elemental, USA) (Karandashev et al. 2016, Shevchenko et al. 2017).

The isotope composition of carbon and oxygen in the shell material was determined at the Geological Institute of the Siberian Branch of the Russian Academy of Sciences (Ulan-Ude, The Republic of Buryatia, Russia) using the Finningan MAT253 mass spectrometer (ThermoScientific). The measurements were carried out in the form of CO<sub>2</sub> in the mode of a continuous flow of helium using the GasBench mass spectrometer configuration. International standards NBS-18 and NBS-19 were used to calculate δ<sup>13</sup>C and δ<sup>18</sup>O. The accuracy of the obtained values was monitored by taking regular measurements of the Russian standards MSA-7, MSA-8 (Far East Geological Institute at the Far Eastern Branch of the Russian Academy of Sciences, FEGI FEB RAS). The repeatability of the obtained values was ± 0.2 ‰.

The Kruskal-Wallis H test was used for the measurement of the differences between the content of chemical elements in fossil mollusk shells, and these data were ranked. Ranking consisted of the transition from the quantitative values of the concentrations of chemical elements to the ranks, which were subsequently compared with each other. To identify statistically significant differences in the content of chemical elements between two sample groups of different ages, the nonparametric Mann-Whitney U test was used. Cluster analysis of geochemical data was carried out to classify the localities in accordance with redox conditions in ancient biotopes. Statistical data processing was performed using StatSoft, Inc. (2011), STATISTICA (data analysis software system), version 10.

**Table 1.** List of studied localities

Nº	Locality	District, country	Coordinates	River basin	Hosting deposits
1	Brînza	Cahul District, Moldova	45°39'26" N 28°10'24" E	Prut	Yellowish-orange ferruginized sand and subjacent layer braun pebble
2	Slobozia Mare	Cahul District, Moldova	45°36'24" N 28°9'56" E	Prut	Braun pebble with ferruginized sand
3	Giurgiuleşti	Cahul District, Moldova	45°29'3" N 28°11'0" E	Prut	Yellowish friable sand with clay's lens and inclusions small pebble
4	Gura Bicului	Anenii Noi District, Moldova	46°57'4" N 29°27'13" E	Dniester	Coarse pebble with sand's interbeds
5	Sucleia	Slobozia District of Transnistria, Moldova	46°49'59" N 29°42'6" E	Dniester	Smalls light brown pebble with gray sand and underlying coarse pebbles' layer

## Results

The chemical characteristics of the carbonate of the studied shells are presented in Supplementary material 1, 2 (Tables S1, S2). High concentrations of Fe, Mn, Sr were measured. The mean concentrations of Mn in the materials of the shells are from  $214.9 \pm 42.3$  mg/kg to  $434.7 \pm 71.5$  mg/kg, the average amounts of Fe and Sr are from  $60.5 \pm 19.8$  mg/kg to  $868.5 \pm 233.4$  mg/kg and from  $537 \pm 47.8$  mg/kg to  $2001.5 \pm 172.2$  mg/kg, respectively. These are typical indicators for the elemental composition of freshwater riverine bivalve mollusk shells. The measured  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are in the ranges from  $-12.1$  ‰ to  $-10.35$  ‰ for stable isotope of carbon-13 and from  $20.44$  ‰ to  $22.41$  ‰ for stable isotope of oxygen-18. Such values of these indicators are not abnormal for such material.

## Discussion

For reconstruction of the redox conditions in ancient river biotopes, changes in the Mn/Fe ratios in fossil shells from the studied sites were analyzed (Fig. 3). This geochemical indicator shows that the highest concentrations of oxygen in water are found in paleobiotopes, which have been reconstructed based on data obtained from the Sucleia and Gura Bicului outcrops. Coarse deposits, represented by pebbles and gravel, interbedded with coarse sand were investigated. The lowest values of Mn/Fe are characteristic of the location of Giurgiulești and Brinza. Here, a layer of a pebble, bordering on ferruginized sand, has less thickness, than in the outcrops of Sucleia and Gura Bicului.

Based on the results obtained by comparing individual locations using the Kruskal-Wallis H test

**Table S1.** Elemental composition of the subfossil bivalve shells from studied outcrops

Locality	Mollusk's taxa	Number of samples	Mn, ppm	Fe, ppm	Ba, ppm	Sr, ppm	Mg, ppm	Ca, ppm
Brinza	Margaritifera	5	$282.9 \pm 25.2$	$868.5 \pm 233.4$	$65.2 \pm 7.5$	$537.0 \pm 47.8$	$44.5 \pm 3.9$	$388555.0 \pm 4696.0$
Slobozia Mare	Unio	5	$231.9 \pm 51.8$	$258.5 \pm 86.6$	$54.1 \pm 4.9$	$687.0 \pm 33.1$	$71.6 \pm 16.9$	$387862.4 \pm 5707.7$
Giurgiulești	Margaritifera	5	$369.2 \pm 108.2$	$681.7 \pm 396.8$	$51.6 \pm 7.7$	$654.1 \pm 108.1$	$140.9 \pm 88.1$	$396040.8 \pm 3750.2$
Gura Bicului	Unio	5	$434.7 \pm 71.5$	$127.5 \pm 72.8$	$43.6 \pm 7.0$	$1220.9 \pm 70.6$	$16.9 \pm 4.9$	$389267.9 \pm 4960.2$
Sucleia	Margaritifera	5	$214.9 \pm 42.3$	$60.5 \pm 19.8$	$176.9 \pm 49.3$	$2001.5 \pm 172.2$	$38.3 \pm 2.3$	$401224.8 \pm 2871.1$

**Table S2.** Stable isotope ratio  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in the subfossil bivalve shells from studied outcrops

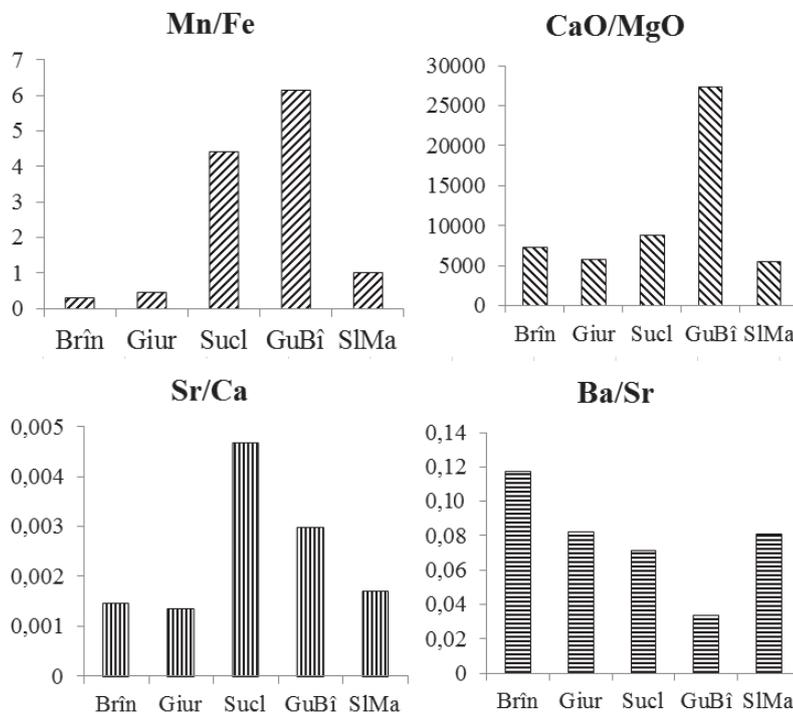
Locality	Mollusk's taxa	Number of samples	$\delta^{13}\text{C}$ , ‰ (PDB)	$\delta^{18}\text{O}$ , ‰ (VSMOW)
Brinza	Margaritifera	5	$-12.1 \pm 0.38$	$22.41 \pm 0.11$
Slobozia Mare	Unio	5	$-11.54 \pm 0.37$	$21.84 \pm 0.04$
Giurgiulești	Margaritifera	5	$-12.16 \pm 0.36$	$21.97 \pm 0.18$
Gura Bicului	Unio	5	$-10.35 \pm 0.48$	$20.44 \pm 0.02$
Sucleia	Margaritifera	5	$-10.42 \pm 0.47$	$21.39 \pm 0.11$

( $p=0.022$ ), groups of Pliocene and Pleistocene sections were compared using the Mann-Whitney U test. Statistically significant differences in the Mn/Fe ratios were found ( $p=0.024$ ). The average rank is higher in the Pleistocene outcrops.

The geochemical indicator CaO/MgO is directly related to temperature (Lukashev 1972). Its use, in being applied to the fossil shells under consideration, allowed us to compare the temperature conditions in which the malacocenoses developed. To contrast the indicator values in subfossil shells from five different localities, we used the Kruskal-Wallis H test, which

allowed us to reveal statistically significant differences between them ( $p=0.009$ ). The warmest conditions were reconstructed for the Gura Bicului locality, and there we found mollusk taphocenoses with the largest number of species, including those belonging to the genus *Unio*. The lowest values of the CaO/MgO ratio were obtained for outcrops located in the Prut river basin. There were no statistically significant differences in the values of this indicator between the Pleistocene and Pliocene localities ( $p=0.079$ ).

The Ba/Sr indicator has an inverse correlation with water temperature. According to this indicator, its



**Fig. 3.** Change of median values of the studied geochemical indicators in the period from Pliocene to Late Pleistocene in subfossil shells of freshwater mollusks (Bivalvia: Unionoida): Brîn – Brînza, Giur – Giurgiulești, Sucl – Sucleia, GuBî – Gura Bîcului, SI Ma – Slobozia Mare

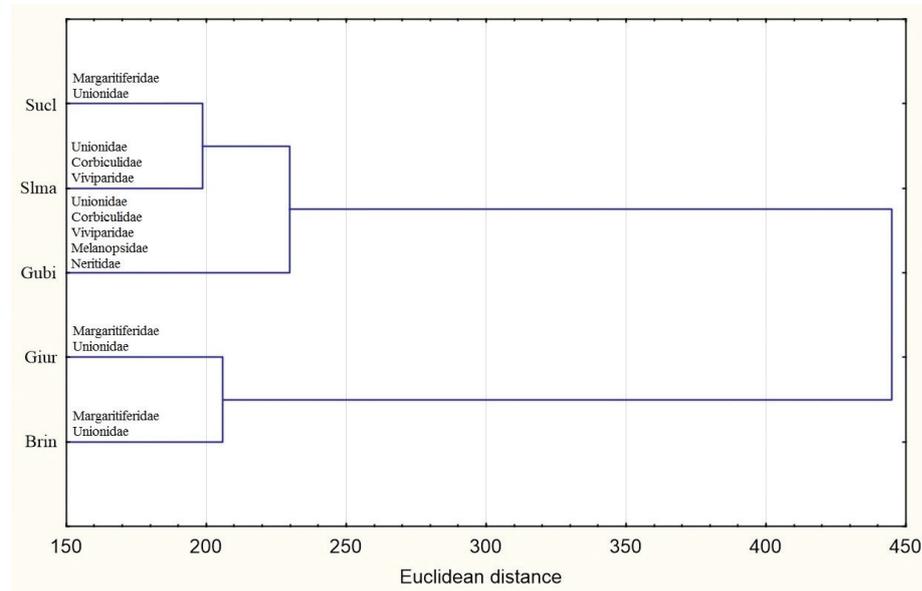
highest values are measured for shells from the Brînza outcrop. The warmest conditions of sedimentation existed in paleobiotopes reconstructed from materials from the Gura Bîcului outcrops. It is noteworthy that they are of a young age. Using the Kruskal-Wallis H test, the difference in Ba/Sr index values was proved between samples from certain localities ( $p=0.003$ ) and using Mann-Whitney U criteria, the difference in Ba/Sr index values was proved between Pliocene and Pleistocene biotopes ( $p=0.006$ ). Warmer hydroclimatic conditions existed in the Pleistocene ecosystems.

Data on the depth of the rivers in paleobiotopes were obtained on the basis of the Sr/Ca ratio. According to our data, the Dniester River sections reconstructed along the Sucleia and Gura Bîcului outcrops had the greatest depth; the shallowest depth was observed in the locations of Brînza and Giurgiulești, respectively (Kruskal-Wallis H test,  $p=0.001$ ). Using the Mann-Whitney U test allowed us to conclude that there are statistically significant differences between the samples of Pliocene and Pleistocene shells

( $p=0.002$ ). The greatest depths of the considered sections of the rivers were in the Pleistocene.

The  $\delta^{13}\text{C}$  variations are significantly different in the shells from the five outcrops ( $p=0.021$ ) under study. The highest values of this indicator are peculiar to Pleistocene localities, and in Pliocene shells the values are lower ( $p=0.003$ ). The variation in  $\delta^{13}\text{C}$  values over time indicates a change in the trophic status of river ecosystems.

The  $\delta^{18}\text{O}$  values, measured in the material of mollusk shells, are inversely correlated with the water temperature in which the mollusk lived, and based on this indicator, it is possible to obtain some idea about the change of hydroclimate in ancient rivers (Kelemen et al. 2017). In our case, statistically significant differences exist both between samples from certain outcrops ( $p=0.0005$ ) and between groups of different ages ( $p=0.0005$ ). Warmer environments were specified for the Pleistocene paleobiotopes that were reconstructed from data from the Gura Bîcului and Sucleia sites.



**Fig. 4.** Cluster analysis diagram based on the data on Fe, Mn concentrations and isotopic ratios  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in the carbonate of shells from localities of different age with information about the taxonomic composition of subfossil mollusk taphocenoses: Brin – Brînza, Giur – Giurgiulești, Sucl – Sucleia, Gubi – Gura Bicului, Slma – Slobozia Mare

Figure 4 shows the change in the taxonomic composition of the fossil mollusk taphocenoses in Pliocene and Pleistocene sediments of the Dniester and Prut rivers that were dependent on the paleoenvironments in ancient biotopes. To assess the hydroclimatic situation, data on variations of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  and concentrations of Mn and Fe in fossil shells were used here.

Two reconstructed Pliocene biotopes in localities in the Prut river basin (Giurgiulești, Brînza) were distinguished by a relatively low content of organic matter, as well as a low water temperature during the period of sedimentation. Using the Fe/Mn ratio, the biotope in which the mollusks lived can be characterized as a shallow section of a river with a sandy bottom. The result of the combination of these factors was the development of Margaritiferid assemblages in particular populations in which the species of pearl mussels *Margaritifera flabelliformis* dominated.

The Pleistocene sites of Sucleia and Gura Bicului have a layer of coarse-grained sediments with numerous mollusk shells in the outcrop. High values of the Mn/Fe ratio indicate high oxygen-enriched waters during sedimentation, which led to the development of the Gastropoda and Bivalvia assemblages that were

found here. Using the materials obtained from Slobozia-Mare, we reconstructed the meso-eutrophic environment of the watercourse and compared it with the low Mn/Fe ratio of Sucleia, which indicates low oxygen concentrations in the waters of the ancient river, and this explains the of freshwater pearl mussels in taphocenoses and their depleted species composition.

The elemental composition changes of the studied mollusk shells are consistent with the granulometric composition of the sediments, characteristic of certain paleoecological conditions. On the basis of geochemical indicators, such as the ratio Ba/Sr and  $\delta^{18}\text{O}$ , we note that there was an increase in water temperature during the development of the river ecosystem. It is likely that this led to the complete displacement of freshwater pearl mussels, living in waters with low organic content, to bivalve mollusks from family Unionidae, which can successfully live in eutrophic water.

Summarizing the data obtained on the studied indicators of palaeoecological conditions, we conclude that in benthic assemblages of oligotrophic rivers in the Neogene-Quaternary river basins of Prut and Dniester rivers, stenobiont species of pearl mussels from family Margaritiferidae were the domi-

nant freshwater mussel group up to the Middle Pleistocene. Pearl mussel shells are massively represented in the Pliocene and Middle Pleistocene alluvial sediments that were studied. However, with climate change and the eutrophication of watercourses, the role of pearl mussels in these communities declined, and the pearl mussels were replaced by more eurybiontic mollusks species from family Unionidae which are, currently, the only large bivalve mollusks in the Dniester and Prut river basins.

Modern global eutrophication of watercourses, caused by anthropogenic pollution and climate change in the direction of warming (Fritts et al. 2017, Lundquist et al. 2019), leads to the replacement of pearl mussels of the family Margaritiferidae by more eurybiont species of the family Unionidae in the benthic communities of European oligotrophic rivers. These processes are similar to those deduced for the Neogene-Quaternary watercourses of the Prut and Dniester basins, but proceed at a much faster pace. The processes of eutrophication of watercourses are the main factor leading to the catastrophically rapid modern reduction of the ranges of pearl mussel. The data obtained in this study indicate the need to develop pan-European programs on the problem of eutrophication of oligotrophic rivers, otherwise, there is a risk of extinction of the remaining populations of pearl mussels in Europe (Bolotov et al. 2018).

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## Conclusion

Based on geochemical indicators of environments, it was revealed by the results of the present study that an increase in water temperature in the studied ancient biotopes was accompanied by eutrophication of watercourses. At the same time, there was a change in the taxonomic composition of the bivalve mollusk assemblages in ancient rivers. The status of the primary oligotrophic rivers Dniester and Prut over long-term periods (several million years) changed significantly up to them being organic-enriched eutrophic watercourses. Further studies of fluvial ‘archives’, paleoecology of freshwater mussels, and Neogene-Quaternary paleogeography of the southwestern part of the Russian Plain should be focused on while using new methods of dating and environmental reconstruction of Pliocene and Pleistocene fossils and deposits.

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