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Research Article

Subfossil freshwater bivalve mollusk shells present data on stratigraphy of Dniester and Prut riverine deposits

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Abstract

The article provides information on the outcrops of the Neogene-Quaternary riverine sediments of the North-Western Black Sea coastal area. A description of five outcrops of fluvial deposits located on the territory of the modern basins of the Dniester and Prut rivers is given. Based on the granulometric composition of the sediments and the presence in them of the fossil shells of freshwater bivalve mollusks (Bivalvia: Unionoida), an assumption has been made about the characteristics of the ancient river ecosystems of the Dniester and Prut on different Pliocene and Pleistocene time sections. A review of the data in the body of literature on the geological age of the studied outcrops was undertaken. The localities considered provide information on the sedimentation conditions in this region from the Pliocene to the Late Pleistocene. Previously, various approaches were used for dating such rocks and for determining the stratigraphic position of fossil material found in them. The article considers the method of amino acids racemization as one of the approaches. Racemization allows to obtain new data on the stratigraphy of Neogene-Quaternary riverine sediments and to solve the problem of their dating. The sampling principles of carbonate material for the analysis of amino acids from the mollusk shells and features of sample preparation are described. Five amino acids were used: aspartic acid (Asp), glutamic acid (Glu), leucine (Leu), phenylalanine (Phe) and isoleucine (Ile). The D/L ratios were analyzed (amino acid racemization (or AAR) is the interconversion of amino acids from one chiral form (the L – (laevo) amino acids which are the building blocks of proteins) to a mixture of L- and D- (dextro) forms. The extent of racemization is measured by the ratio of D/L isomers (it increases as a function of time and temperature and can be used for geochronology) from the same group of fossils (genera), which were preserved under similar environmental conditions, inorganic geochemistry and thermal histories. Based on the obtained values, it can be concluded that the analysis of the racemization of amino acids is useful for determining the geological age of fossil shells of freshwater bivalve mollusks, but there are limitations regarding age. In the shells of the Pliocene sites, the values of some amino acids are close to one, indicating that the racemization took place. D/L values in shells from Pleistocene localities allowed the determination of their stratigraphic position.

Keywords

subfossil shells, freshwater bivalve mollusks, aminostratigraphy, riverine deposits, geological age

Introduction

Determining the age of Pliocene and Pleistocene riverine sediments is often problematic due to various reasons, including the age limitations of some dating methods or the inability to apply the method to a specific material. Numerous works are devoted to the geological characteristics of the sediments of the rivers of the Russian Plain which affect, among other things, the region of the North-Western Black Sea region (Chepalyga 1967, Mihailescu and Markova 1992, Bilinkis 2004, Matoshko et al. 2009). Matoshko et al. (2004) describe the history of formation and provide information on the stratigraphy and paleontology of sediments of the Dniester, Dnieper, Don and Volga rivers. They consist of alluvial suites, each of which has unique lithofacial characteristics. The correlation given by the respective authors is based on the elevations of layers of marine transgressions, glaciations, as well as the biostratigraphy of mammals and mollusks from river sediments. The oldest Pliocene alluvium is preserved in the form of numerous small fragments in the highest parts of the present-day Dniester-Prut interfluve. However, the Upper Pliocene and Quaternary riverine sediments of the Dniester and other Carpathian rivers are represented by the down cutting and filling of terraced steps, features that are easily distinguishable on the sides of their deeply incised valleys.

In this paper, we tested the method of amino acid racemization in relation to subfossil shells of bivalve mollusks found in uneven-aged sediments of the Dniester and Prut rivers. The territory of these riverine basins is interesting in terms of paleogeography, because in the Pliocene and Pleistocene riverine sediments there is often found fossil material represented by the bones of various animals, mollusk shells and many other artifacts (Adamenko 1996). This material can be used to reconstruct paleoenvironments in different geological periods and to determine the age of the enclosing sediments. The use of new dating methods, such as the analysis of racemization of amino acids, can solve some problems of the stratigraphic division of heterochronous riverine sediments in the region. This approach to dating riverine deposits in the studied area may have an advantage when comparing with other dating methods for the determination of a geological age of the sediments. Its ranges of age determination make it possible to clarify information about the stratigraphy of the riverine deposits in this region. The mollusks were chosen by us as the material for amino acids racemization dating, because in this case the sample preparation proved to be very simple. The use of subfossil shells of bivalve mollusks allows solving the problems of stratigraphic dissection of both continental and marine sediments. There are examples of dating such a material using the amino acids method and even from locations in the Russian High Arctic (Mangerud et al. 2008), although in such conditions the set of amino acids used to determine the geological age is very limited. Hence, the problem of dating based on amino acid racemization is also important for the Arctic region.

The aim of this work was to study the D/L ratio of some amino acids (amino acid racemization (or AAR) which is the interconversion of amino acids from one chiral form (the L – (laevo) amino acids which are the building blocks of proteins) to a mixture of L- and D- (dextro) forms. The extent of racemization is measured by the ratio of D/L isomers and increases as a function of time and temperature, and can be used for geochronology (Murray-Wallace 1995) and comparing these values with each other, as well as comparing these results with published data on the numerical and relative geological age of sediments in the studied localities.

Materials and methods

Geological settings of studied localities

The collecting of the subfossil shells of freshwater bivalve mollusks was made from five outcrops, located on the territory of the Dniester and Prut riverine basins (Fig. 1). Their descriptions are shown in the Appendix Table A1 and photos are presented in Appendix Figures A1–A5.

The Brînza outcrop is located between the Brînza village and the Văleni village, between the Prut river and the highway passing here. It is a sand and gravel pit in which the river sediments of the end of the Early Pliocene are outcropping (Vangengeym et al. 2005, Table 1). For collecting subfossil freshwater bivalve shells we chose a small area in the central part of the quarry. The outcrop is a large geological formation composed of light yellow, sometimes yellowish-orange sand, which is divided by a layer of brown pebbles of slightly less than one meter in thickness. In terms of the riverine conditions, this layer is marked with a fairly powerful flow which was able to carry such material. Subfossil shells of *Margaritifera* sp., which are abundantly represented in sediments, are assigned to be this layer.

The Giurgiulești outcrop is located in a small sand pit to the west of the Giurgiulești village. Riverine deposits are outcropped here. The geological age of this locality may vary widely, for example in the Late Pliocene age of the outcrop located near Reni village (Tesakov 2004, Titov 2008). The layers we studied here may be aged from the Late Pliocene to the Early Pleistocene (David and Obada 2004). Large and rather fragile shells of freshwater pearl mussels (*Margaritifera* sp.) and bivalve mollusks from family Unionidae were found by us in a layer of light yellowish-brown sand with lenses of clay and including a small pebble layer.

The Sucleia locality is a famous outcrop, where one can find subfossil mollusk shells belonging to the Tiraspol faunal complex (Chepalyga 1967). It is located to the east of the village and is a quarry under development in which Middle Pleistocene riverine

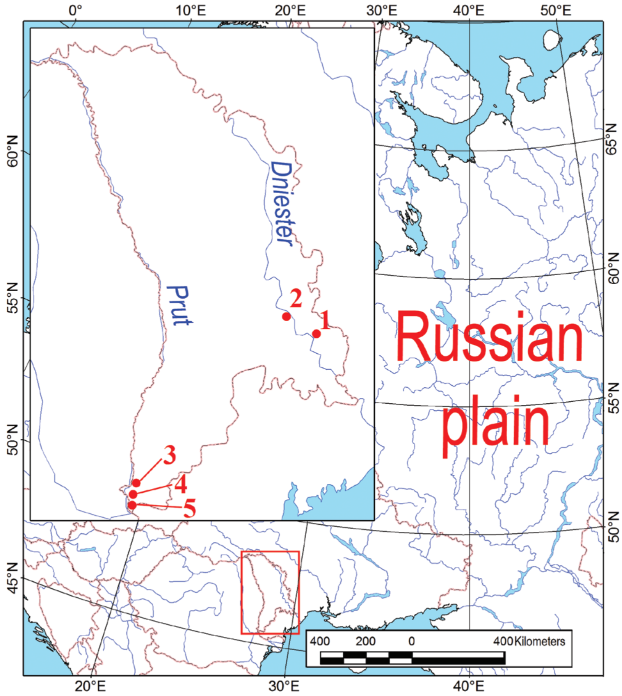

Fig. 1. Location map of the field study areas: 1 – Sucleia, 2 – Gura Bîcului, 3 – Brînza, 4 – Slobozia Mare, 5 – Giurgiulești

Table 1. Published data on the numerical age of sediments with freshwater bivalve shells in the studied localities

Locality	Geological age (dating's method)	Geological epoch	Reference
Brînza	3855–4045 ka (biometric + paleomagnetic)	Early Pliocene	Vangengeym et al. 2005
Sucleia	710–800 ka (thermoluminescent)	Middle Pleistocene	Yanshin 1989, Zubakov and Borzenkova 1990
Gura Bîcului	140±35 ka (thermoluminescent)	Middle Pleistocene	Adamenko 1986

sediments are represented by light-yellow sand with a pebble layer. Freshwater pearl mussel shells belonging to the genus *Pseudunio* were found here in the pebble layer with light brown sand.

The Gura Bîcului outcrop is located in the north of the village near the Dniester River. The composition of the sediments is in many ways similar to the Middle Pleistocene locality of Sucleia. These are sands with a pebble layer, and those include numerous bivalve shells belonging to the genus *Unio*. In addition, there are shells of freshwater Gastropoda and small bivalve shells from genus *Corbicula* that are present in abundance. Such a composition of sediments and the species composition indicates a younger age for this locality in comparison with the outcrop at Sucleia.

In the ravine, located north of the village of Slobozia Mare, sediments are exposed, represented by a pebble layer with sand, and including numerous mollusk shells (*Unio* sp.). Loamy rocks are located in the lower part of this outcrop. According to the species composition of mollusks and the composition of the deposits, Taphocenoses in the locality of Slobozia Mare are similar to the modern fauna of the Prut River. Modern mollusks of the family Unionidae inhabit the watercourses of this area on clay grounds and the clay rocks in the section lie below the layers of sand containing fossil bivalves.

Samples

Samples for amino acid racemization dating were collected from the bivalve shells *Unio* and *Margaritifera* that were recovered from different sites in Moldova (Fig. 2). A list of analytical samples with nomenclature and laboratory numbers are presented in Table 2.

The fieldwork

During the fieldwork, a layered description of ancient alluvial deposits from top to bottom and the collecting of subfossil material together with the fixing of its position in the outcrop were undertaken. The fieldwork was carried out during May 2013.

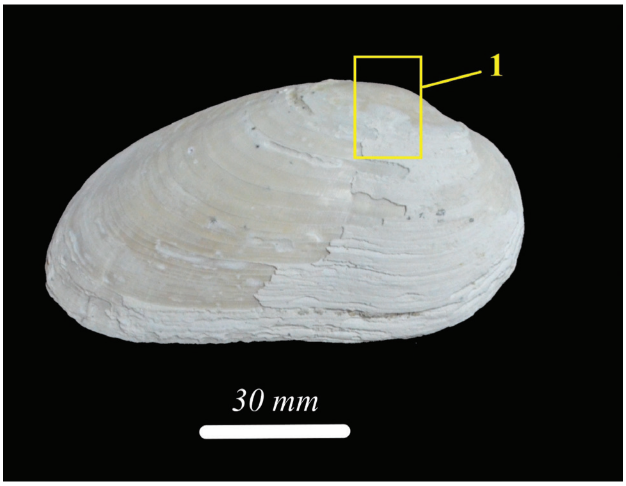

Fig. 2. Materials sampling scheme for analysis: 1 – The area from which the shell's fragments were cut for analysis

Amino acid racemization dating

Ten (10) *Unio* and *Margaritifera* shells (analytical samples) were taken from each site (a total of 50 samples). The use of monogeneric samples taxonomically reduces the controlled variability in D/L ratios (Murray-Wallace 1995, Murray-Wallace and Goede 1995) but these two are similar. In the laboratory, shells were carefully sonicated and cleaned with water to remove sediment. Peripheral parts, approximately 20–30%, were removed after chemical cleaning of the sample with 2N HCl.

As with several studies (Haugen and Sejrup 1992, Wehmiller 1993, Torres et al. 1994) that reported intra-shell variation of D/L ratios depending on the part of the carapace from which the sample is recovered, we recovered the analytical samples at the umbo in order to reduce the sample error (Murray-Wallace 1995). Afterwards, small pieces from this part of the shell (5–37 mg) were selected for amino acid racemization analysis.

Amino acid concentrations and ratios were quantified using HPLC following the sample preparation protocol described in Kaufman and Manley (1998) and Kaufman (2000). This procedure involves hydrolysis, which was performed under N_2 atmosphere in 7 μl of 6M HCl for 20 h at 100 °C. The hydrolysates were evaporated to dryness in vacuum, and then rehydrat-

Table 2. List of analytical samples

Nomenclature	Locality	Analytical samples
GUBI	Gura Bîcului	LEB 12161- LEB 12170
SLMA	Slobozia Mare	LEB 12171- LEB 12174, LEB 12185 - LEB 12190
GIUR	Giurgiulești	LEB 12175 - LEB 12184
SUCL	Sucleia	LEB 13191 - LEB 13195, LEB 13206 - LEB 13210
BRIN	Brînza	LEB 13211-LEB 13220

ed in 7 μl 0.01 M HCl with 1.5 mM sodium azide and 0.03 mM L-*homo*-arginine (internal standard).

Samples were injected into an Agilent HPLC-1100, equipped with a fluorescence detector. Excitation and emission wavelengths were programmed at 335 nm and 445 nm, respectively. A Hypersil BDS C18 reverse-phase column (5 μm; 250 × 4 mm i.d.) was used for the analysis.

The derivatization takes place before injection by mixing the sample (2 μl) with the pre-column derivatization reagent (2.2 μl), which comprised 260 mM isobutyryl-L-cysteine (chiral thiol) and 170 mM o-phtaldialdehyde, dissolved in 1.0 M potassium borate buffer solution at pH 10.4. Eluent A consisted of 23 mM sodium acetate with 1.5 mM sodium azide and 1.3 micro M EDTA, adjusted to pH 6.00 with 10 M sodium hydroxide and 10% acetic acid. Eluent B was HPLC-grade methanol and eluent C consisted of HPLC-grade acetonitrile. A linear gradient was performed at 1.0 ml/min and 25 °C, from 95% eluent A and 5% eluent B upon injection to 76.6 eluent A, 23% eluent B, and 0.4% eluent C at 31 min.

According to Kaufman (2000), the analysis of more than one amino acid provides largely redundant information on the sample age. Thus, to establish the aminostratigraphy of the studied sites, we used the aspartic acid (Asp), glutamic acid (Glu), leucine (Leu), phenylalanine (Phe) and isoleucine (Ile) contents of *Unio* and *Margaritifera* shells.

To compare the D/L values in the shells from localities of different ages, the Wilcoxon signed-rank test was used. Statistical data processing was performed using StatSoft, Inc. software (2011), STATISTICA (data analysis software system), version 10.

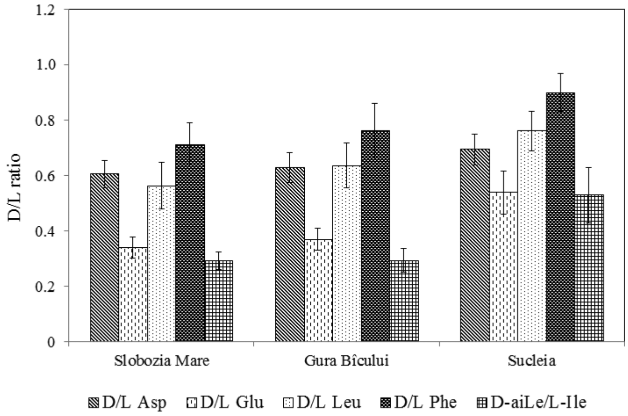

Fig. 3. Mean D/L-values of amino acids for Pleistocene localities: Asp – aspartic acid, Glu – glutamic acid, Leu – leucine, Phe – phenylalanine, Ile – isoleucine

Results

We obtained mean D/L-values for each of the 5 locations. The values for aspartic acid were from 0.606±0.054 to 0.773±0.042, and for glutamic acid were from 0.340±0.040 to 0.919±0.067. For leucine, phenylalanine and isoleucine, D/L-values greater than 1 are marked. The results obtained allow us to compare the relative geological age of the sediments containing subfossil shells. The average values of five amino acid ratios for the Pleistocene outcrops do not exceed one, which is important for the interpretation of these results in the geochronological aspect.

Table 3. Mean amino acid racemization ratios obtained in *Unio* and *Margaritifera* shells from studied sites

Locality	N	D/L Asp	D/L Glu	D/L Leu	D/L Phe	D-aiLe/L-Ile
GUBI	10	0.630 ± 0.049	0.370 ± 0.037	0.636 ± 0.084	0.763 ± 0.080	0.293 ± 0.032
SLMA	10	0.606 ± 0.054	0.340 ± 0.040	0.563 ± 0.081	0.711 ± 0.098	0.293 ± 0.042
GIUR	10	0.771 ± 0.055	0.899 ± 0.077	0.957 ± 0.072	1.243 ± 0.068	1.059 ± 0.100
SUCL	10	0.695 ± 0.050	0.539 ± 0.056	0.762 ± 0.051	0.900 ± 0.068	0.530 ± 0.057
BRIN	10	0.773 ± 0.042	0.919 ± 0.067	1.008 ± 0.087	1.202 ± 0.079	1.014 ± 0.090

Notes: N – number of individuals analysed (analytical samples).

Discussion

Aminostratigraphy consists in placing sets of geological, paleontological or archaeological localities in a stratigraphical order according to the measured D/L ratios from the same group of fossils (genera), which were preserved under similar environmental conditions, inorganic geochemistry and thermal histories. Accordingly, each group of levels with the same amino acid racemization values constitutes an almost-isochronous event.

The amino acid racemization/epimerization ratios (Table 3) can be used for a relative dating of these sites: Giurgiuleşti and Brînza are the oldest localities, with D/L values of some amino acids close to 1, indicating that the equilibrium of the amino acid racemization took place, and cannot be used for numerical dating purposes. This is consistent with the age of these locations given in the works of Vangengeym et al. (2005), Tesakov (2004), and Titov (2008).

The age of the Pleistocene localities was interpreted based on the racemization rate of amino acids in subfossil shells, as described in what follows. Slobozia Mare is the youngest locality, as the D/L values in the shells from this site are the lowest. Mihailescu and Markova (1992) present the relative geological age of a series of paleontological sites located along the coast of Prut River. Here, there are layers with freshwater mollusk shells, including freshwater bivalve shells from the genus *Unio*. It may have a geological age near the borderline of Middle and Late Pleistocene according those authors. The Gura Bîcului locality is a bit older than the Slobozia Mare freshwater layer, although D/L values do differ very little. This is evidenced by higher D/L values for the aspartic acid, glutamic acid, leucine, and phenylalanine. The dating of the layers exposed here is given in Adamenko (1986) and according to that author the age of subfossil shells is 140±35 thousand years (the age was determined by using the thermoluminescent method). The layer with *Pseudunio* shells in the Sucleia outcrop is clearly older than Slobozia Mare and Gura Bîcului, because D/L values of all amino acids, except the aspartic acid, are significantly higher for shells from this locality (Fig. 3). This result is consistent with the ideas concerning the Middle Pleistocene age of this paleontological site (Table 1, Chepalyga 1967, Yanshin 1989), which are also based on the results of thermolumeniscence dating (Zubakov and Borzenkova 1990).

Pairwise comparison of these three localities across the D/L ratios and across all five amino acids using the Wilcoxon signed-rank test, allow us to conclude that the D/L ratios are statistically significantly different between Slobozia Mare and Sucleia ($p=0{,}042$) and between Gura Bîcului and Sucleia ($p=0{,}043$). When comparing the Slobozia Mare and Gura Bîcului localities, no statistically significant differences were found for these indicators ($p> 0.05$).

Dating of Middle and Late Pleistocene paleontological sites with mollusk fauna using the amino acid racemization method is a promising area of paleogeographical research on the territory of the Russian plain. Many paleontological sites located in the southern part of the Russian plain provide the necessary material for such studies.

Conclusion

A comparison of the results of the analysis of amino acid racemization in the subfossil shells of freshwater bivalve mollusks with the numerical age of riverine sediments, shows that the determinated geological age of shells using D/L ratio of five amino acids can reach up to 800 thousand years. This is consistent with modern concepts of time constraints which use the method of aminostratigraphy. Further studies of the fluvial deposits of Dniester and Prut rivers should aim to form databases on amino acid racemization in subfossil mollusk shells, because this may be useful for the stratigraphic division of sedimentary strata.

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Appendix

Table A1. List of studied localities.

No	Name of locality, geographical location and coordinates	Outcrop's description
1	Brînza (Cahul District, Moldova, 45°39'26"N, 28°10'24"E, Fig. A1)	Layer thickness in studied area is 7.5 m, 0–100 cm – soil layer; 100–150 cm – light–yellow sand; 150–300 cm – yellowish–orange ferruginized sand with mollusks' shells (*Margaritifera* sp.); 300–380 cm – braun pebble with ferruginized sand, this layer includes numerous mollusks' shell (*Margaritifera* sp.); 380–500 cm – light brown sand with including of pebble; 500–750 cm – light–yellow sand.
2	Giurgiulești (Cahul District, Moldova, 45°29'3"N, 28°11'0"E, Fig. A2)	Layer thickness in studied area is 4 m, 0–80 cm – soil layer; 80–100 cm – yellow sand; 100–115 cm – gray sand; 115–125 cm – dark grey sand; carbonate including; 125–140 cm – light dark grey loam; 140–152 cm – pebble and dark grey sand 152–402 cm – light yellowish–brown sand with lenses of clay and including small pebble, numerous mollusks' shell (*Margaritifera* sp., *Unio* sp.)
3	Sucleia (Slobozia District of Transnistria, Moldova, 46°49'59"N, 29°42'6"E, Fig. A3)	Layer thickness in studied area is 4.6 m. 0–40 cm – light–yellow sand with small pebble; 40–290 cm – Smalls light brown pebble with gray sand and bivalve mollusk's shells (*Margaritifera* (*Pseudunio*) sp.); 290–370 cm – coarse pebble with numerous bivalve mollusks' shells (*Margaritifera (Pseudunio)* sp.); 370–460 cm – pebble with light brown sand with bivalve mollusks' shells (*Margaritifera* (*Pseudunio*) sp.).
4	Gura Bîcului (Anenii Noi District, Moldova, 46°57'4"N, 29°27'13"E, Fig. A4)	Layer thickness in studied area is 6.5 m. 0–100 cm – soil layer; 100–550 cm– yellowish–gray sand with clay's lenses 550–650 cm – coarse pebble with sand's interbeds, numerous mollusks' shells (*Unio* sp., *Corbicula* sp.).
5	Slobozia Mare (Cahul District, Moldova, 45°36'24"N, 28°9'56"E, Fig. A5)	Layer thickness in studied area is 8.7 m. 0–50 cm – soil layer; 50–300 cm – gray sand with horizontal layered; 300–500 cm – braun pebble with ferruginized sand, numerous mollusks' shells (*Unio* sp.); 500–550 cm – ferruginized sand; 550–570 cm – pebble, mollusks shells (*Valvata* sp.); 570–870 cm – light–gray clay.

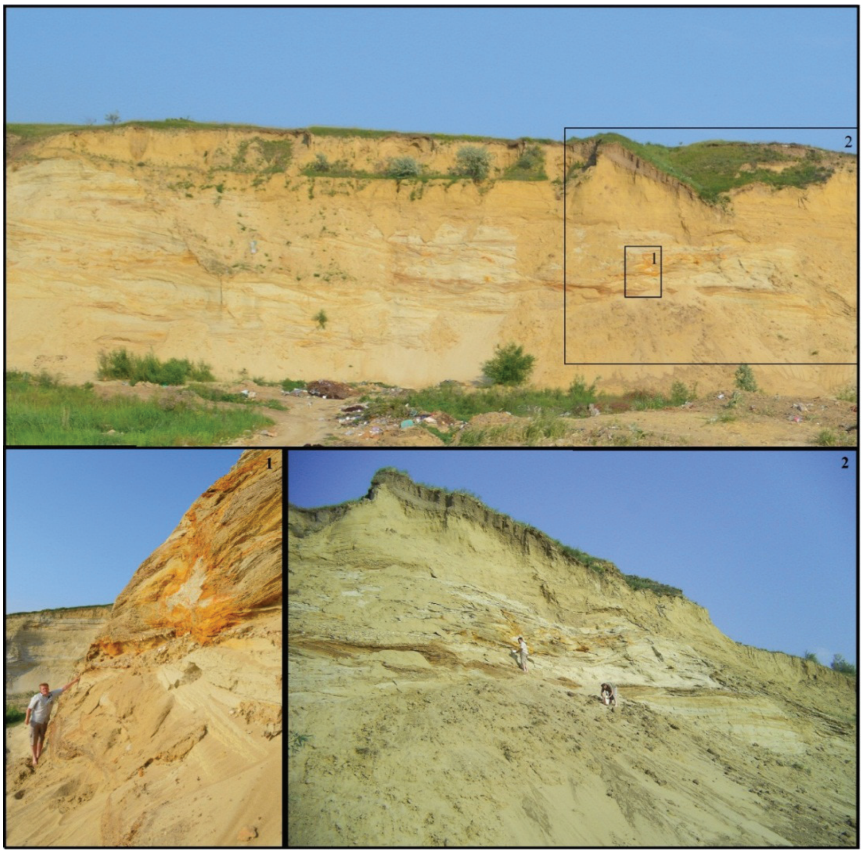

Fig. A1. Brînza (Cahul District, Moldova)

Fig. A2. Giurgiuleşti (Cahul District, Moldova)

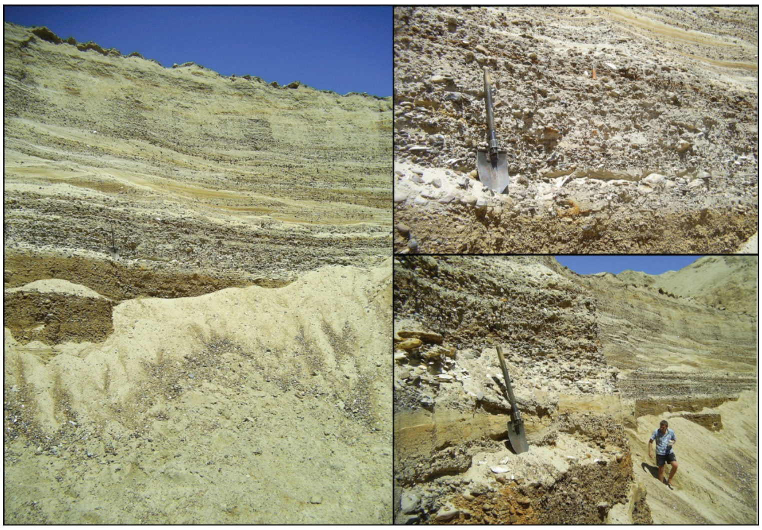

Fig. A3. Sucleia (Slobozia District of Transnistria, Moldova)

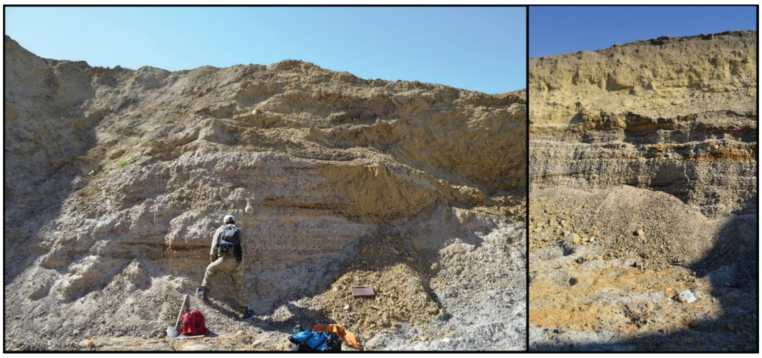

Fig. A4. Gura Bîcului (Anenii Noi District, Moldova)

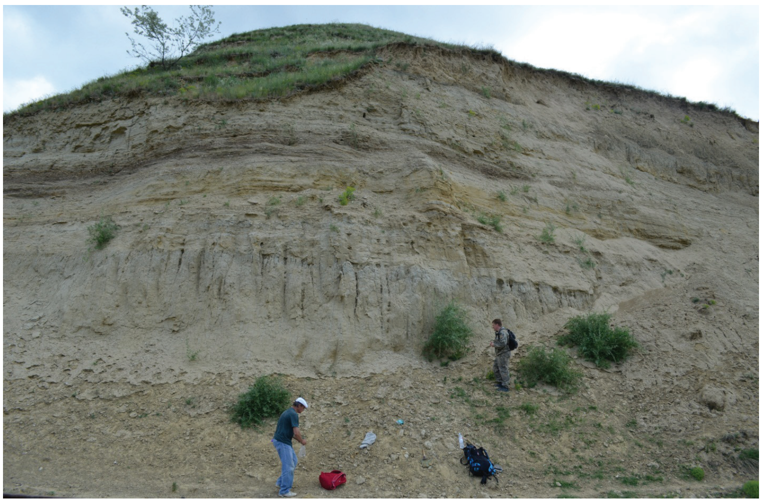

Fig. A5. Slobozia Mare (Cahul District, Moldova)