First fossil record of Early Sarmatian didemnid ascidian spicules (Tunicata) from Moldova

Keywords: Ascidiae, Didemnidae, sea squirts, Sarmatian, Moldova
ABSTRACT

In the present study, numerous ascidian spicules are reported for the first time from the Lower Sarmatian Darabani-Mitoc Clays of Costești, north-western Moldova. The biological interpretation of the studied sclerites allowed the distinguishing of at least four genera (*Polysyncraton, Lissoclinum, Tridemnum, Didemnum*) within the Didemnidae family. Three other morphological types of spicules were classified as indeterminable didemnids. Most of the studied spicules are morphologically similar to spicules of recent shallow-water taxa from different parts of the world. The greater size of the studied spicules, compared to that of present-day didemnids, may suggest favourable physicochemical conditions within the Sarmatian Sea. The presence of these rather stenohaline tunicates that prefer normal salinity seems to confirm the latest hypotheses regarding mixo-mesohaline conditions (18–30 ‰) during the Early Sarmatian.

PLAIN LANGUAGE SUMMARY

For the first time the skeletal elements (spicules) of sessile filter feeding tunicates called sea squirts (or ascidians) were found in 13 million-year-old sediments of Moldova. When assigned the spicules to Recent taxa instead of using parataxonomical nomenclature, we were able to reconstruct that they all belong to family Didemnidae. Moreover, we were able to recognize four genera within this family: *Polysyncraton, Lissoclinum, Tridemnum*, and *Didemnum*. All the recognized spicules resemble the spicules of present-day extremely shallow-water ascidians inhabiting depths not greater than 20 m. The presence of these rather stenohaline tunicates that prefer normal salinity seems to confirm the latest hypotheses regarding mixo-mesohaline conditions during the Early Sarmatian. Moreover, the high concentrations of calcium carbonate in the Sarmatian seawater allowed ascidians to build spicules of greater size.
INTRODUCTION

Ascidians, also called “sea squirts” are sessile marine tunicates. These filter-feeder colonial or solitary organisms inhabit both shallow-water and deep-sea environments. They are salinity-sensitive, and, despite being able to live in mangroves, harbors, river mouths or even marine lakes (Monniot and Monniot, 2008; Monniot, 2009), they prefer normal-salinity habitats (Monniot et al., 1991).

Some ascidian taxa are characterized by the bearing of mineral spicules (the Didemnidae, Pyuridae, Polyclinidae, and, partly, Polycitoridae and Styelidae families), which are contained mainly in the tunic. The spicules of Ascidiacea are quite frequent in the carbonate sediments of recent seas (e.g., the Great Barrier Reef and the Bahamas).

However, there is a relatively scarce fossil record of ascidians, despite their fossils being known from the Lower Cambrian (Shu et al., 2001; Chen et al., 2003). Moreover, the fossil spicules of Ascidiacea are rarely described (Brookfield, 1988), for various reasons. One of them is that the ascidian spicules are often ignored and miss-assigned (e.g., Deflandre-Rigaud, 1956; Messenger et al., 2005). Also, the use of the parataxonomical approach is more frequent in terms of fossil ascidian sclerite nomenclature. Yet, the parataxonomical assignment does not connect the spicules with the living species bearing the spicules, thus makes the findings of fossil ascidians worthless to modern biological sciences (e.g., Perch-Nielsen, 1988; Varol and Houghton, 1996; Varol, 2006; Cachão and Conceição, 2006; Jerković and Ćorić, 2006; Sagular, 2009).

The fossil record of disassociated ascidian spicules goes back to the Mesozoic. They were described, for instance, from the Arabian Peninsula (Varol, 2006) and Mexico (Bonet and Benveniste-Velasquez, 1971; Buge and Monniot, 1972). There are also records of Cenozoic spicules of Ascidiacea from Australia (Wei, 1993; Łukowiak, 2012) and France (Durand 1948, 1955; Deflandre and Deflandre-Rigaud, 1956; Deflandre-Rigaud, 1968; Monniot and
Buge, 1971). The fossil sclerites of these tunicates were mentioned from Turkey (Ćorić et al., 2012), the Celtic Sea, the Mediterranean, the Coral Sea, Great Britain, the Atlantic, the NW Pacific, and Germany, as well (for more details and references, see Varol and Houghton, 1996).

There is, however, no fossil record of these tunicates from the area of Moldova. Likewise, from the area of the Moldavian Platform (Romania), fossil ascidian spicules have, so far, been mentioned only by Brânzilă and Chira (2005). The authors described some spicules of these tunicates, as well as thoracospheres (Dinophyceae) and calcareous nannofossils from the Middle Miocene of Romania, but without attempts to biologically assign the studied spicules. Also, Chira and Malacu (2008) noted the presence of ascidians in the Miocene sediments of Romania, yet, in this case, the spicules were assigned to the parataxonomic species Microascidites vulgaris.

Spicules play a secondary role in ascidian taxonomy, as recent Ascidiacea are classified based mainly on soft body elements (Kott, 2001). Despite the substantial intraspecific variations in the size and shape of spicules (see e.g., Hirose et al., 2010), in some cases, they are taxa-specific and, therefore, potentially valuable features for ascidian taxonomy, which can be used for taxonomical assignment (e.g., Monniot et al., 1991; Kott, 2001; 2004a, b). Moreover, in fossils, spicules are often the only hard element of the ascidian body that remains as proof of the organism’s previous existence. Nevertheless, we must be aware of the limitations of taxonomical assignment basing exclusively on spicules.

MATERIAL AND METHODS
The samples were collected from Lower Sarmatian [(sensu lato) (upper Middle Miocene)] clays cropping out on the left side of the Prut River, near the “Stânca – Costești” barrier lake (WGS 84, N 47°51’25,9’’, E 27°14’58,1’’), in north-western Moldova.

We analyzed three samples approximately 100 g in weight. The samples were crushed and weathered by means of water immersion. The material obtained was then dried, and the residue was passed through three sieves (0.466 mm, 0.263 mm, and 0.122 mm, respectively), being separated into four fractions for an easier analysis of microfossils. All the studied spicules were identified in the fraction finer than 0.122 mm. Then, the ascidians spicules were handpicked using a Carl Zeiss Jena SM XX binocular stereo microscope. In the samples analyzed, we found approximately 200 very well-preserved spicules. The spicules were photographed under a Vega/Tescan SEM microscope within the Faculty of Biology of the “Alexandru Ioan Cuza” University of Iași, Romania.

The material investigated is deposited in the Original Paleontological Collections Museum of the “Alexandru Ioan Cuza” University of Iași, Romania, under the inventory number 17070–1907.

**GEOLOGICAL SETTING**

In the Miocene, the studied area was a part of Eastern Paratethys, a large shallow sea that stretched from the region north of the Alps to current Central Asia. This part of the basins was infilled with fine clastic sediments (Rögl and Steininger, 1984; Pisera, 1996), accompanied in this area by “Bioherms with Serpula” (Ionesi and Ionesi, 1981, 1982). All these sediments extend, as Medobory Hills, from Ukraine into Moldova over an area up to 40 kilometers in width and 200 kilometers in length (Figure 1) (Studencka and Jasionowski, 2011; Bliuc and Malai, 2012; Peryt and Jasionowski, 2012).
The Early Sarmatian age of these sediments was postulated by Simionescu (1902) and has also been confirmed by more recent studies [Paghida-Trelea (1969), Ionesi and Ionesi (1981, 1982), and Brânzilă (1999)].

The samples containing the ascidians spicules were collected from the “Darabani–Mitoc Clays” at an altitude between 122–125 meters, approximately three to five meters above the “Bioherms with Serpula” (Figure 2). The clays are ashy-grey and can be compacted or laminated. Moreover, some fine intercalation of sand can be observed. In these sediments numerous fragments of bivalves, including Inaequicostata inopinata (Grischevich), were found. Moreover, the presence of foraminifera, i.e., Quinqueloculina akneriana (d’Orbigny), Pseudotriloculina consobrina consobrina (d’ Orbigny), P. consobrina nitens (Reuss), Articulina problema (Bogdanowich), Cycloforina predcarpatica (Serova), and Elphidiella serena (Venglinski), otolites, statolites of Mysidae, and dinoflagellate piritizated cysts, has also been noted.

**SYSTEMATIC PALEONTOLOGY**

Class ASCIDIACEA Nielsen, 1995

Order APLOUSOBRANCHIA Lahille, 1886

Family DIEMNIDAE Giard, 1872

Genus POLYSYNCRATON Nott, 1892

POLYSYNCRATON sp. A

Figure 3.1, 3.2

**Material.** Two spicules (1707/35.1; 1708/49.3).
**Description of spicules.** Spherical spicules, up to 100 μm in diameter, with 10–12 lobe-like rays in transverse section. The numerous short, rounded and nipple-like lobes are of equal length. They are moderately crowded, with an insignificant space between the lobes. The height of the lobe is slightly greater than its diameter at the base. The surface of the spicule is rather smooth, with some unevenness in the transition zone between the lobes.

**Remarks.** The spicules are similar to those of the recent genus *Polysyncraton* Nott, 1892. There are at least four species of *Polysyncraton* described recently from the adjacent Mediterranean, namely *P. lacazei* Giard, 1872, *P. bilobatum* Lafargue, 1968, *P. cantenese* (Brément, 1913), and *P. haranti* Lafargue, 1975, which possess spicules of similar morphology [Ascidiacea World Database (AWDb)]. *Polysyncraton galaxum* Kott, 2004c is also characterized as having similar spicules. However, the spicules belonging to the latter display a smaller number of rays (Kott, 2004c, figure 14G). Moreover, this species has been described only from around Australia (Kott, 2004c). The same is true for *Polysyncraton linere* Kott, 2004b (Kott, 2004b, figure 4), which bears spicules up to 70 μm in diameter, with nine to 11 short rounded projections evenly placed on the spicule central mass. However, once again, this species has, so far, been noted only from Australian waters (AWDb).

There are also some *Didemnum* species that exhibit morphologically similar spicules. One example is *Didemnum albidum* (Verill, 1871) (see e.g., Van Name, 1945, figure 33 and Marks, 1996, figure 7). However, its spicules are quite large, measuring about 400–650 μm, while the studied spicules are only 100 μm in diameter. Likewise, their outgrowths are more conical. *Didemnum albidum* has been noted from Arctic regions and the northern parts of the eastern North American coast (Van Name, 1945), as well as from European waters (AWDb). The other didemnid species bearing similar sclerites is *Tridemnum strangulatum* (Ritter, 1901). In this species, the spicules are also equipped with rounded projections, but, compared to the studied spicules, where each outgrowth is separated from the rest by a space, in *T.*
The considerable resemblance to the spicules of several Polysyncraton species allows the assignation of the studied spicules to Polysyncraton.

Comparable spicules with rounded knob-like rays were noted by Łukowiak (2012) from the late Eocene of southeastern Australia, and described as Polysyncraton-like spicules (compare with Łukowiak, 2012, figure 3.9, 10). Furthermore, Varol and Houghton (1996) illustrated similar spicules from the Late Pleistocene of the Red Sea (see plate 2, figure 5). However, this sclerite was assigned to Bonetia quasitruncata in the parataxonomic system. Monniot and Buge (1971) described spicules of comparable morphology from the Eocene of France (see plate B, figure F). They were also assigned to Micrascidites vulgaris based on the parataxonomical nomenclature.

**Occurrence.** Darabani–Mitoc Clays, Costești, north-western Moldova.

**Family** DIEMNIDAE Giard, 1872  
**Genus** POLYSYNCRATON Nott, 1892  
**POLYSYNCRATON sp. B**  
Figure 3.3, 3.4

**Material.** Three spicules (1709/49.12; 1710/49.6; 1711/49.32).

**Description of spicules.** Spherical spicules 70 to 90 μm in diameter, with 10 to 12 equally-sized rays in transverse optical section. The rays are short and conical. The height of each ray is equal to its diameter at the base. The rays are spread across the spicule surface because they are set on polygonal concavities. Nevertheless, the polygonal bases are moderate and not well-developed.
Remarks. These spicules resemble the sclerites of the didemnid genus *Polysyncraton*, e.g., *P. scorteum* Kott, 2001, which bears spherical spicules up to 90 µm in diameter, with short rounded-to-conical rays protruding from the surface (compare with Kott, 2007, figure 7E). However, in *P. scorteum*, the number of rays is greater (about 15–17). Moreover, this species currently inhabits only the shallow waters of Tasmania and Australia (Kott, 2007).

The spicules of another shallow-water species, *Polysyncraton doboense* (Sluiter, 1913), are characterized by conical rays that are separated from one another (see Kott, 1981, figure 18D). Nevertheless, they are only 50 µm in diameter, and the number of rays is slightly larger (15) than in the studied species. *Polysyncraton doboense* lives in the reef waters of the Fiji Islands (Kott, 1981).


Family DIDEMNIIDAE Giard, 1872
Genus LISSOCLINUM Verill, 1871

LISSOCLINUM sp.

Figure 3.5–3.9

Material. Seventeen spicules (1712/49.37; 1713/49.21; 1714/48.14; 1715/49.7; 1716/49.26; 1717/49.16; 1718/48.5; 1719/49.1; 1720/49.15; 1721/48.12; 1722/49.38; 1723/49.5; 1724/49.42;1725/ 49.8; 1726/49.22; 1727/49.36; 1728/31).

Description of spicules. Spherical spicules about 90–100 µm in diameter, with conical to slightly rounded rays of variable number. The rays grow from polygonal flattened to concave pedestals/bases. The polygons do not always possess rays, and, in some cases, the “empty” polygons are detached from the ones equipped with rays. In some sclerites, the polygonal bases form a uniform surface, while in others the pedestals are separated by concavities.
Because of the “blank” polygons, the number of spicule rays varies from eight to 12 in traverse section within this morphospecies.

**Remarks.** The described sclerites, with very specific polygonal ray bases, can be found in various species of *Lissoclinum*, such as *L. diversum* Kott, 2004c (see Kott, 2004c, figure 21B) – a species currently inhabiting the eastern Indian Ocean (AWDb). However, next to spicules with conical rays protruding from the polygonal bases, globular to burr-like ones, with rod-like to club-shaped rays, appear in this species. *Lissoclinummn fragile* (Van Name, 1902), which is a cosmopolitan species noted in locations such as Panama, S Africa, the Gulf of Mexico, the Azores, and the western part of the Central Atlantic (AWDb), also possesses spicules that resemble those described here (compare e.g., with Lafarque and Laubier, 1980, figure 7A). The sclerites of *L. fragile* display rays developed to a lesser degree, however. Furthermore, the polygons are clearly separated from one another, forming separated columns. The same is true for some sclerites of *L. bistratum* (Sluiter, 1905) (see e.g., Monniot et al., 1991, figure 1), known from Djibouti and the Indo-West Pacific, and *L. timorense* (Kott, 2001, figure 178D, E), noted from the Central Indo-Pacific (AWDb).

The studied spicules also resemble those of *Polycitorella mariae* (Polycitoridae; see e.g., Monniot et al., 1991, plate 77, figure 1). The spicules of this polycitorid are also equipped with polygonal bases. At the center of the bases there are some low bumps. However, these bases are detached from one another, and the granular bumps are less ray-like than in the studied spicules. Moreover, the spicules are much smaller, reaching a diameter of only about 25 µm.

**Occurrence.** Darabani–Mitoc Clays, Costești, north-western Moldova.
TRIDIDEMNUM sp. A

Figure 3.10


Description of spicules. Stellate spicule about 80 µm in diameter, with 10 rays in optical transverse section. The rays are harsh, conical to nipple-shaped, with slightly rounded tips. The diameter of the ray base is slightly smaller than the ray height. The rays are sparsely placed on the spicule surface, and grow from smooth polygonal flattened-to-concave bases.

Remarks. The sclerites resemble those of the didemnid genus Trididemnum. For example, in T. cereum Giard, 1872, the spicules exhibit high rounded rays and well-defined space between the rays (compare with Turon, 1986, figure 16). This species is known from, among other localities, the Mediterranean Sea (AWDb). However, the sclerites of T. cereum are two times smaller than the one described, and there are no polygonal bases in which the rays are set. The same is true for T. cyclops Michaelsen, 1921 and T. paracyclops Kott, 1980, where the sclerites are of similar form, but only about 50 µm in diameter (compare with Kott, 2001, figure 174D and H; respectively). Both of these species are known only from waters around Australia (AWDb).


Family DIDEMNIDAE Giard, 1872
Genus TRIDIDEMNUM Della Valle, 1881

TRIDIDEMNUM sp. B

Figure 3.11, 3.12

Material. Four spicules (1730/49.13; 1731/49.2; 1732/49.40; 1733/48.10).
Description of spicules. Stellate spicules 75 μm in diameter, with 10 to 12 equal-sized rays in transverse section. The rays are conical, with rounded tips. The height of the rays is slightly greater than their diameter at the base. The rays are evenly spread across the spicule surface. The discrete space between the rays is developed as delicate concave bases.

Remarks. Similar spicules can be found in some species of Trididemnum, such as Trididemnum paracyclops Kott, 1980. Although the sclerites of this didemnid are slightly smaller (reaching only about 50 μm in diameter), they display other features, such as conical rays with a space between them, that make them similar to the described spicules (compare with Kott, 1980, plate 4, figure 2A, B). The spicules of the species Trididemnum strigosum Kott, 1980 (plate 3.3), which inhabits the shallow waters of the Philippines, also resemble the described sclerites. They are similar in size (50–80 μm), and equipped with high, dispersedly-arranged conical rays with rounded tips. However, the number of rays is smaller: only seven in transverse section.

Some of the spicules of Trididemnum savignii (Herdman, 1886) are also very similar, with rounded projections. Their height is slightly greater than their diameter at the base. The diameter (40–100 μm) of the spicules seems to be comparable with that of the described spicules, as well (see Kott, 1981, figure 33A). Nevertheless, the central mass in the spicules of T. savignii is more granular, and the rays are more crowded. This species is known from the entire Circum-Equatorial zone (AWDb).


Family DIEMNIDAE Giard, 1872
Genus TRIDIDEMNUM Della Valle, 1881
TRIDIDEMNUM sp. C

Figure 3.13
**Material.** Two spicules (1734/49.17; 1735/49.39).

**Description of spicules.** Uneven globular spicules 80–100 μm in diameter, with irregularly-arranged conical projections. The projections display rounded tips. They are usually slightly longer than the diameter of their base, and they may fuse with the neighboring projections, as well. Between them there are numerous smaller coarse surfaces. They are irregularly arranged, of unequal size, and lower than the surrounding rays.

**Remarks.** These globular spicules bear a resemblance to those of *Trididemnum maratuae* Monniot and Monniot, 2008 (figure 30B). Morphologically, they are nearly the same, with irregularly-arranged unequal rays and a coarse surface in-between the rays. However, the spicules of *T. maratuae* are twice as small as the fossil ones. This species is presently known from the Danau Haji Buang marine lake of Indonesia (Monniot and Monniot, 2008).

The other didemnid species with comparable spicules is *Trididemnum farrago* Kott, 2004a. In this case, the spicules are not identical (they possess more conical rays), but their size range is similar to that of the studied spicules (Kott, 2007, figure 10H). This species currently inhabits SE Asia (Kott, 2004a). The sclerites of *Trididemnum maragogi* Rocha, 2002 are similar in both morphology and size (Rocha, 2002, figure 2). This species is noted only from the Western South Atlantic (AWDb).

There are other species of *Trididemnum* that bears spicules with similar features, as well, such as *T. cereum* Giard, 1872 (Lafargue and Laubier, 1980, plate 1, figure A), *T. timorense* (compare with figure 10 in Hirose et al., 2010), and *T. inarmatum* (Drasche, 1883) (Lafargue and Laubier, 1980, plate 1, figure G).

Spicules of comparable morphology, such as *Micrascidites vulgaris*, from the Eocene of France were illustrated by Monniot and Buge (1971, plate B, figure D) using the parataxonomical nomenclature.

Family DIDEMNIDAE Giard, 1872
Genus TRIDIDEMNUM Della Valle, 1881
TRIDIDEMNUM sp. D
Figure 3.14

Material. Single spicule (1736/49.30).

Description of spicules. Stellate spicule 100 µm in diameter, with eight to 10 short conical rays in optical transverse section. The rays are low and the diameter of the ray base is slightly larger than the ray height. Sometimes, the rays are knitted and semi-detached. They may also be separated one from one another by a granular central mass that sometimes forms isolated plates of irregular shape. The granular plates may overlap the base of some rays, or fluently transform into the rays.

Remarks. The spicules described are comparable in shape, surface features, and number of rays to Didemnum lissoclinum Kott, 2001. However, the characteristics of the spicules of D. lissoclinum are based mostly on their photograph (see figure 19E in Kott, 2004b), rather than on the description provided by Kott (which is very short and concise). The resemblance of the studied spicule to those of D. lissoclinum might, however, be coincidental. Apart from this, the spicules of this species are smaller, and the species is currently noted only from Australia (AWDb).

The spicules of Trididemnum cereum (Giard, 1872) (see Turon, 1986, figure 16) also resemble the described fossil spicules. Their characteristics, as provided by Turon, namely the anomalies of the rays, the coarseness of the space between the rays, and the anomalies present in the spicule structure, as well as the pictures of the spicules, are very similar to those of the
fossil sclerites. However, the size of the spicule (about 40 µm) is two times smaller than that of the described spicule (100 µm). On the other hand, this species inhabits areas adjacent to the study area: the Mediterranean Sea, the N Atlantic Ocean, and the United Kingdom (AWDb).

The appearance of the surface, the shape, and the number of rays are almost identical to those exhibited by *Trididemnum alexi* Lambert, 2003, which is currently noted from Washington, the USA and British Columbia, Canada (compare with Lambert, 2003, figure 1). However, in this case, as well, spicule size (up to 30 µm) considerably differs from that of the described sclerites.

Nevertheless, other features, along with the resemblance to several trididemnids, allow the assignment of this spicule to *Trididemnum*.

**Occurrence**. Darabani–Mitoc Clays, Costești, north-western Moldova.

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**Family** DIDEMNIDAE Giard, 1872

**Genus** DIDEMNUM Savigny, 1816

**DIDEMNUM sp. A**

Figure 3.15

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**Material**. Single spicule (1737/48.11).

**Description of spicules**. Globular, spherical spicules about 90 µm in diameter, with 14–16 projections in optical transverse section. The projections are coarse and low – the diameter of the base is slightly larger than the projection height. They are placed unevenly across the spicule surface. Some of them seem to be tightly connected to others, fusing into one structure, while the others are more easily distinguishable.
Remarks. There are some spicules in *Didemnum moseleyi* Kott, 2007 that are globular, with surfaces covered with tightly-arranged, low, coarse projections (Kott, 2007, figure 9C). In the case of *D. moseleyi*, however, the projections are more differentiated, having diverse diameters of the bases. Likewise, the spicules of *D. moseleyi* are smaller and measure only 50 µm.

In *D. elongatum*, the spicules are also characterized by coarse, harsh surfaces, but the projections are higher and better defined. However, apart from the appearance of the surface, these two species have in common the compact arrangement of the projections, with no visible space between the rods (compare with Kott, 2001, figure 169B).

Also, among the didemnid ascidians from the tropical western Pacific, there are species that exhibit very similar spicules, e.g., *Didemnum ahu* Monniot and Monniot, 1987, *D. lacustre* Monniot and Monniot, 2008, and, especially, *Didemnum mekeald* Monniot and Monniot, 2008 (compare with Monniot and Monniot, 2008, figures 5B, 7 and 9, respectively). The latter is characterized by having, next to rod-like sclerites, smaller ones with low granular projections. Nevertheless, these spicules are smaller, up to about 40 µm in diameter.


Family DIDEMNIDAE Giard, 1872

Genus DIDEMNUM Savigny, 1816

**DIDEMNUM sp. B**

Figure 3.16

Material. Single spicule (1738/49.33).

Description of spicules. Stellate spicule 90–100 µm in diameter. It possess 10 conical rays in optical transverse section. The length of the ray base is slightly smaller than its height. The
rays are round in transverse section, and their surface is rough and fibrous. Some of the ray-tips are not entirely developed. No space between the rays is noticeable.

Remarks. The described spicules are similar in morphology to the spicules of *Didemnum coccineum* [(Drasche, 1883), described by Mèdioni in 1970 as *Didemnum posidoniae*; see Mèdioni, 1970, figure 2D]. Nevertheless, the spicules of *D. coccineum* are smaller and not more than 50 µm in diameter. Furthermore, the number of rays in transverse section also seems to be lower. Currently, this species inhabits the Mediterranean Sea and N Atlantic waters (AWDb).

The other didemnid species which bear morphologically-similar spicules is *Didemnum candidum* Savigny, 1816. This cosmopolitan species is characterized by spicules with about 10 coarse conical rays in transverse section (see Mèdioni, 1970, figure 1C). However, in this case, as well, the size of the spicules is smaller than the size of the fossil spicules, as they reach only about 50 µm in transverse section. The sclerites of *D. helgolandicum* also bear a resemblance to the described spicules (compare with Monniot, 1970; plate 1C), but they are two times smaller.

Moreover, there are certain species within the genus *Polysyncraton* that possess similar sclerites, e.g., *P. bilobatum* Lafargue, 1968 and *P. lacazei* (Giard, 1872) (Monniot 1970; plate 2A, D, respectively).

Morphologically similar, yet smaller, spicules, exhibiting densely-packed cylindrical rays with conical points, were described from the late Eocene of southeastern Australia by Łukowiak (2012, figure 3.15) and classified as didemnid spicules.


Family DIEMNIDAE Giard, 1872

DIEMNIDAE gen. et sp. indet. A
Material. Four spicules (1739/31.4; 1740/31.5; 1741/31.6; 1742/49.24).

Description of spicules. Spherical spicules 90 to 125 μm in diameter. Ten to 12 rays in transverse optical section. Short and conical rays are of identical size. The ray tips are pointed. The height of the ray is equal to its diameter at the base. The transitional zone between the rays is smooth or slightly rough.

Remarks. Comparable spicules appear in some didemnids, e.g., *Didemnum obscurum* Monniot, 1969. In this species, the spicules are equipped with about 12 conical rays in transverse optical section, but they are much smaller, reaching only about 30 μm in diameter. Likewise, in *D. obscurum*, the space between the rays is better developed (compare with Monniot, 1970, plate 1, figure 6). The spicules of *Didemnum fragum* Kott, 2001 also bear a resemblance to the described fossil sclerites, but they are smaller, reaching about 60 μm in diameter (Kott, 2001, figure 169H).

There are also other didemnid species with similar spicules, e.g., *Leptoclinides cucurbitus* Kott, 2004b. The spicules display 11–13 pointed rays in optical transverse section and a similar size (90 μm in diameter; see Kott 2004b, figure 1B). However, the space between the rays is better defined.

Because of the lack of very specific characteristics in these spicules, as well as the presence of similar sclerites in many different groups, it is difficult to assign these spicules to precise didemnid genera.


Family DIDEMNIDAE Giard, 1872

DIDEMNIDAE gen. et sp. indet. B
Material. Single spicule (1743/49.4).

Description of spicules. Stellate spicule about 100 µm in diameter, with 10 rays in optical transverse section. The rays are smooth and conical, and possess pointed tips. The height of the ray is equal or slightly smaller than the diameter of the ray base. The rays are of unequal size, sparsely arranged across the spicule surface, and set on subtle-polygonal to rounded-concave bases. In some cases, the bases are not well-developed.

Remarks. The spicules belonging to *Trididemnum criastum* (Kott, 2001, figure 175H) are up to 90 µm in diameter, and possess smooth conical (or chisel-like) rays and a well-developed central mass, which makes them similar to the described sclerites. However, the rays in *T. criastum* sometimes bifurcate. This species is currently noted from a considerable depth of 128–676 m off Tasmania (Kott, 2001). Likewise, the spicules of *Trididemnum savignii* (Herdman, 1886) possess stellate spicules with eight moderately-high rays and a well-developed space between the rays. However, in contrast to the studied fossil spicules, there are no polygonal bases between the rays (see Monniot, 1970, plate 1, figure 1). Certain didemnid species also possess spicules with conical, moderately-high rays, separated from one another by a central mass. One example is *Didemnum bicolor* Vasseur, 1969, which is characterized by comparable spicules, the number of rays occasionally being nine or higher (see Kott, 2001, figure 168G). *Didemnum macrosiphonium* Kott, 2001 displays the same number of conical rays (Kott, 2001, figure 168H). Still, the spicules of both species are much smaller than the described ones, measuring only about 30 µm in diameter. These two ascidians are present in the Australian shallow-water zone (Kott, 2001).


Description of spicules. Stellate spicule 80–90 µm in diameter. It possesses 12–14 conical rays in optical transverse section. The length of the ray base is equal or slightly smaller than the ray height. The rays are round in transverse section, and their surface is smooth. A minute space is visible between the rays.

Remarks. Spicules of similar morphology, namely stellate, with 12–14 low conical rays, are quite common in many didemnid groups, e.g., in the genera Leptoclinides (L. latus Monniot 1983, L. confirmatus Kott, 2001), Didemnum (D. ligulum Monniot, 1983, D. mutabile Monniot and Monniot, 1987), and Trididemnum (e.g., T. cyclops Michaelsen, 1921). However, in all of these cases, the spicules are much smaller than the studied ones. The very general character of these sclerites does not allow the assignment to specific didemnid groups. Comparable spicules were already described by Varol and Houghton (1996) from the Late Pleistocene of the Red Sea (see plate 2, figure 8). However, this sclerite was assigned to Bonetia brevis in a parataxonomic system.


DISCUSSION

Most of the studied spicules resemble those of modern didemnids that inhabit waters not exceeding twenty meters in depth (e.g., Polysyncraton galaxum, P. scorteum, P. doboense, Trididemnum strigosum, Didemnum bicolor, and D. macrosiphonium).
It is hypothesized that, during the Sarmatian, brackish conditions were dominant in the studied area due to the partial isolation of the Paratethys basin (see Papp et al., 1974, Rögl and Steininger, 1984). On the other hand, Pisera (1995, 1996) and Koubová and Hudáčková (2010) showed that salinity in the Paratethys varied during the Sarmatian, from brackish through normal saline to hypersaline. Brestenská (1974) established a salinity value from 18 to 25 ‰ for the Pannonian Basin System, while Iljina et al. (1976) and Nevesskaja et al. (1986) determined a value of 14 ‰ for the Early Sarmatian in the Eastern Parathetys. It was recently suggested that the salinity of the Early Sarmatian of the Paratethys was of mixo-mesohaline character, and that the basin was sometimes connected to the Mediterranean (Paramonova, 1995; Iljina, 2000; Popov et al., 2004, 2005; Nevesskaja et al., 2006). These results were questioned by Piller and Harzhauser (2005), who reconsidered the brackish character of the Sarmatian and postulated normal marine conditions for some parts of the Sarmatian Sea during the time span. Studies carried out by Studencka and Jasionowski (2011) based on the bivalve fauna dominated by species of the genus *Obsoletiforma* pointed to mixo-mesohaline conditions, with a salinity ranging between 18 and 30 ‰ in this area during the Early Sarmatian.

Mostly because of the lack of ionic and osmotic regulation, salinity is an important environmental determinant in ascidians (Goodbody, 1974; Prosser, 1973). These tunicates are stenohaline and prefer rather high salinity – above 25 ‰ (Millar, 1971; Toop and Wheatly, 1993; Gröner et al., 2011). Only a limited number of taxa can tolerate low salinity (e.g., Lambert, 2005; Monniot and Monniot, 2008). Likewise, only some ascidians tolerate a wide salinity range; e.g., *Didemnum vexillum* and *D. listerianum* (e.g., Brunetti et al., 1988; Lambert, 2009; Gröner et al., 2011).

It is worth mentioning that, despite the morphological resemblance to spicules of modern ascidian taxa, in almost all cases, the fossil spicules displayed a considerably greater size than
that of their recent counterparts. This might be due to different physicochemical properties of the Sarmatian Sea. The high alkalinity facilitated calcium carbonate precipitation (Pisera, 1995, 1996; Jasionowski, et al. 2003; Jasionowski, 2006) and thus, allowed ascidians to build spicules of greater sizes. On the other hand, spicule formation in Didemnidae does not rely exclusively on physicochemical processes (Lafargue and Kniprath, 1978).

According to the present study, the presence of the spicules of these rather normal-salinity organisms shows that the salinity might have been of a mixo-mesohaline nature. This interpretation of mixo-mesohalinity of the Sarmatian Sea during the Early Sarmatian.

CONCLUSIONS

In the Early Sarmatian sediments of Moldova, an abundant assemblage ($n > 200$) of didemnid ascidian spicules was found. Among the described spicules, at least twelve taxa were described. They were assigned to four different recent didemnid genera (*Polysyncraton*, *Trididemnum*, *Didemnum*, and *Lissoclinum*). Apart from this, three other types of spicules were catalogued as indeterminable didemnids. The material was studied from the biological rather than parataxonomical perspective, which allowed us to assign the ascidian spicules to recent taxa. Also, due to this approach, we were able to reconstruct their environmental preferences; e.g., the water depth they might have inhabited. Most of the spicules resemble those of present-day didemnids inhabiting waters that do not exceed twenty meters in depth. The considerably greater size of the studied spicules if compare with their recent counterparts, may be due to the higher concentrations of calcium carbonate in the seawater. The presence of these rather stenohaline animals, that prefer normal salinity, supports the hypothesis of mixo-mesohaline conditions in the Sarmatian Sea.
REFERENCES


Cachão, M. and Conceição Freitas, M. 2006. Holocene calcareous nannofossils from the Mira Core (SW Portugal): A Sunspot Proxy on a Coastal Paleo-Ria?. Abstract Volume of
the 11\textsuperscript{th} International Nannoplankton Association Conference, Lincoln, Nebraska, 22-24 pp.


Simionescu, I. 1902. Geological constitution of the Prut River shoreline from the northern part of Moldova. The Publishing of Romanian Academy, Bucharest.


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Figure captions:

**FIGURE 1.** 1. Map of the Middle Miocene deposits in the Carpathian Foreddeep Basin showing the distribution of reef deposits; modified after Peryt and Jasionowski (2012); 2, distribution of the Badenian and Sarmatian reefs from the left and right side of the Prut River, respectively (after Bliuc and Malai, 2012).

**FIGURE 2.** Litostratigraphic column of the Costești area, with the location of the samples in which the ascidians spicules were found.

**FIGURE 3.** Scanning electron microphotographs of the Lower Sarmatian spicules of the didemnid ascidians from Moldova. 1, Spicule of *Polysyncraton* sp. A. 2, Spicule of *Polysyncraton* sp. B. 3–9, Spicules of *Lissoclinum* sp. 10, Spicule of *Trididemnum* sp. A. 11, 12, Spicules of *Trididemnum* sp. B. 13, Spicule of *Trididemnum* sp. C. 14, Spicule of *Trididemnum* sp. D. 15, Spicule of *Didemnum* sp. A. 16, Spicule of *Didemnum* sp. B. 17, 18, Spicules of Didemnidae gen. et sp. indet. 19, Spicule of Didemnidae gen. et sp. indet. B. 20, Spicule of Didemnidae gen. et sp. indet. C.