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Microsculpture of *Pisidium casertanum* (POLI, 1791) and some related species and forms (Bivalvia: Sphaeriidae)

With 6 Figures and 1 Table

RAFAEL ARAUJO[1] & ALEXEI V. KORNIUSHIN[2]

Abstract. Density of pores of the shell observed in five populations of *Pisidium casertanum* var. *globulare* CLESSIN taken from the geographically distant areas (Ukraine, European Russia, Siberia and Kyrgyzstan) was significantly higher than that in typical form of the species. Clear difference was found in all cases when both forms occurred in the same habitat, which makes possible to suppose taxonomic value of the pores pattern. These data are supported by comparison of hinge characters and agree with the anatomical differences found earlier. Data for other species of *Pisidium*, which often occur together with the two *P. casertanum* forms, are provided for comparison.

Kurzfassung. **Mikroskulptur von *Pisidium casertanum* (POLI, 1791) und einiger verwandter Arten und Formen (Bivalvia: Sphaeriidae).** - Die festgestellte Porendichte der Schale bei fünf Populationen von *Pisidium casertanum* var. *globulare* CLESSIN von geographisch entfernten Herkünften (Ukraine, europäisches Rußland, Sibirien und Kirgisistan) war signifikant höher als diejenige der typischen Form der Art. Ein klarer Unterschied wurde in allen jenen Fällen festgestellt, bei denen beide Formen im gleichen Habitat vorkommen. Das weist auf den taxonomischen Wert des Porenmusters hin. Diese Befunde werden unterstützt durch einen Vergleich der Merkmale der Schloßleiste und stimmen mit anatomischen Befunden, welche schon früher gemacht wurden, überein. Angaben zu anderen *Pisidium*-Arten, welche oft mit den zwei Formen von *P. casertanum* gemeinsam vorkommen, werden zum Vergleich gegeben.

Introduction

In many bivalve families the inner surface of the shell against the mantle is marked by minute pores that are the openings of tubules. Since the review by DYDUCH (1983), characters of pores penetrating shells of sphaeriid clams have been discussed in the taxonomic literature. KUIPER & HINZ (1983) applied the data on pore density to distinguish the new species *Pisidium meierbrooki* KUIPER & HINZ, 1983 from the widely distributed *P. casertanum* (POLI, 1791). ADLER & FIECHTNER (1992) have shown considerable variability in the above-mentioned character and its correlation with ecological factors. ARAUJO (1993) studied the microsculptural variability of two different Spanish populations of *P. henslowanum* (SHEPPARD, 1823). He also demonstrated clear differences in patterns of porosity among species occuring in Portugal (ARAUJO, 1998). Comparative data on the pores density and structure for some taxa occuring in Ukraine were published by KIRITSHUK & STADNICHENKO (1996). For many species, the data published by different authors agree, but some

Addresses of the authors:
[1] Museo Nacional de Ciencias Naturales (C.S.I.C.), José Gutiérrez Abascal 2, 28006 Madrid (Spain). E-mail: mcnra2f@fresno.csic.es
[2] I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, ul. B. Khmelnitzky 15, 252601 Kiev (Ukraine)

discrepancies also occur. One may conclude from this short review that pore characters must have taxonomic value. However, their variability should be taken into account. The most reliable data can be obtained when comparing the species taken from the same habitat.

The species considered here, namely *P. casertanum*, is the most variable in the genus. At least two distinct conchological forms are widely distributed in Europe (ELLIS, 1978; PIECHOCKI, 1989): var. *ponderosum* STELFOX, 1918 and var. *globulare* CLESSIN, 1873. The shell microstructure of the former, which occurs in large lakes and rivers, was investigated by DYDUCH (1983). The second form, inhabiting bogs and wet meadows, has remained poorly studied in this respect until now.

KORNIUSHIN (1992, 1996) has shown that populations of *casertanum*-like *Pisidium* sampled in bogs were distinguishable by their large outer demibranch. Later on, differences in the pore density between this form and the typical *P. casertanum* were reported (IZZATULLAEV & KORNIUSHIN, 1993). While investigating the type materials of *P. globulare* CLESSIN, 1873 deposited at the Senckenberg Museum, Frankfurt/M., clear remnants of the large outer demibranch were found (KORNIUSHIN, 1996). Thus, the identity of the anatomically distinct form considered here, *P. casertanum* var. *globulare* is confirmed.

The aim of this study is to demonstrate conchological differences, especially microsculptural ones between *P. casertanum s. str.* and *P. casertanum* var. *globulare* sampled in different geographical areas. We also studied *Pisidium personatum* MALM, 1855 and *P. obtusale* (LAMARCK, 1818), which are evidently related to *P. casertanum* and similar in some characters to var. *globulare* (KORNIUSHIN, 1996), in order to get more clear view on relationship between the species and forms of *P. casertanum* group. The question of species distinctness of *P. globulare* as well as that of some other species distinguished in Russian publications (IZZATULLAEV & KORNIUSHIN, 1993; KORNIUSHIN, 1996) is beyond the scope of this paper.

Material and methods

For this study we took five samples of *P. casertanum* var. *globulare* from different countries and localities. Each sample originally included specimens fixed with ethanol and thus all specimens were checked for anatomical characters. In two samples the *globulare* form occurred together with the typical *P. casertanum* and in one sample with *P. obtusale*. Several samples of the typical *P. casertanum*, *P. personatum* and *P. obtusale* were also taken for comparison. Voucher specimens are deposited in collections of the Museo Nacional de Ciencias Naturales in Madrid, Spain.

It follows the list of the samples with the number of study specimens (when there is more than one valve, they come always from different specimens):

P. casertanum typical form
A lake near the Black Lake (Lago Negro), Lérida, Spain. (25, 7, 1991). 2 valves.
The Louro River, Porriño, Pontevedra, Spain. (28, 5, 1986). 1 valve.
A small lake in the valley of the Yana River, Nizhneyansk, Russia (Eastern Siberia). (2, 8, 1988). 3 valves.
A small pond near Kanev, Ukraine. (25, 5, 1985). 3 valves.

P. casertanum var. *globulare*
A swamp near Kiev, Ukraine. (5, 5, 1985). 3 valves.
A small pond near Kanev, Ukraine. (25, 5, 1985). 1 valve.
A small lake in the valley of the Yana River, Nizhneyansk, Russia (East Siberia). (2, 8, 1988). 3 valves.
Swamps near Bryansk, Russia. (1988). 2 valves.
The Suusamyr River, Kyrgyzstan. (4, 7, 1985). 3 valves.

P. personatum
Somolinos Lake, Guadalajara, Spain. (30, 4, 1989). 2 valves.
The Louro River, Eidos, Pontevedra, Spain. (28, 5, 1986). 1 valve.
Springs in the Berezinskiy Reserve, the Vitebsk region, Belarus (= Byelorussia). (27, 6, 1987). 2 valves.

Species	N	Internal pores, mean and range	Author
P. casertanum (Spain)	3	5.3 ± 1.5 (4-7)	ARAUJO (1995)
P. casertanum (Portugal)	1	10	ARAUJO (1997)
P. casertanum (Siberia)	3	very few	This paper
P. casertanum (Ukraine)	3	max. 5	This paper
P. casertanum (Pooled)*	5	6.2 ± 2.3	This paper
P. casertanum form *globulare* (Ukraine)	4	17.0 ± 1.0 (16-20)	This paper
P. casertanum form *globulare* (Bryansk)	2	24-25	This paper
P. casertanum form *globulare* (Siberia)	3	12.3 ± 1.5 (11-14)	This paper
P. casertanum form *globulare* (Kyrgyzstan)	3	19.7 ± 5.9 (13-24)	This paper
P. casertanum form *globulare* (Pooled)	12	18 ± 5	This paper
P. personatum (Spain)	3	very few - 5-10	ARAUJO (1995)
P. personatum (Portugal)	1	13	ARAUJO (1997)
P. personatum (Belarus)	2	7-10	This paper
P. personatum (Pooled)*	5	19.0 ± 3.1	This paper
P. obtusale (Russia)	3	12.7 ± 3.2 (9-15)	This paper
P. obtusale (Kyrgyzstan)	3	15.7 ± 1.2 (15-17)	This paper
P. obtusale (Pooled)	6	14.2 ± 2.7	This paper

Table 1: Pore density in the studied specimens.
* In *P. casertanum* and *P. personatum* only the specimens that had enough pores for evaluation (counting) were included in the file for statistical processing.

P. obtusale
The Suusamyr River, Kyrgyzstan. (4, 7, 1985). 2 valves.
A swamp near Yelnya, the Smolensk region, Russia. (24, 8, 1988). 3 valves.

To study the pores and microsculpture, organic material from the shells was dissolved in 5 % commercial chlorox (sodium hypochlorite) for 17-48 hours at room temperature. Then,

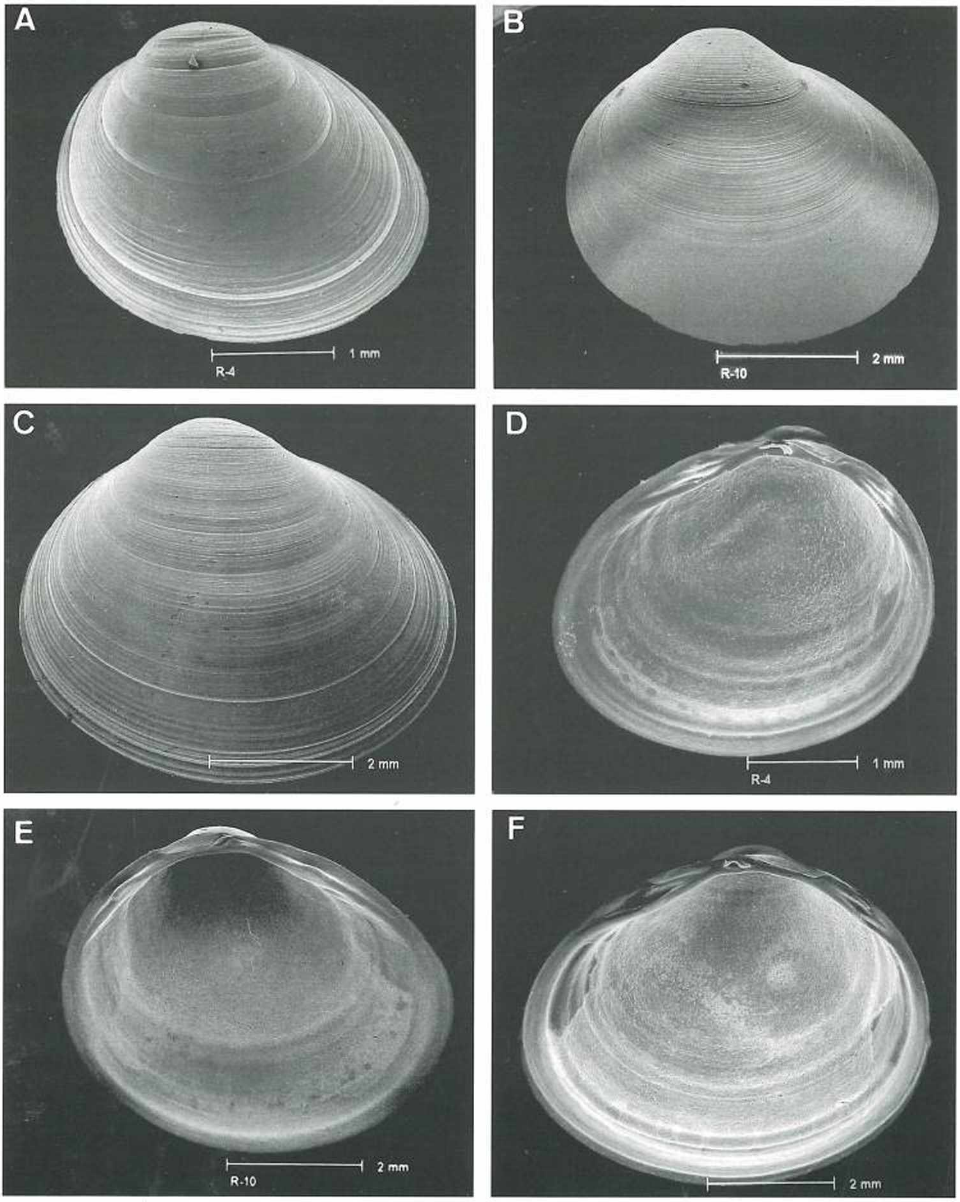

Fig. 1: Typical form of *Pisidium casertanum*. Right valve from **A.** Siberia, **B.** Ukraine, **C.** Spain. Interior view of **D.** Right valve (Siberia). **E.** Left valve (Ukraine). **F.** Right valve (Spain).

the shells were cleaned in distilled water, dried at 50 °C and coated with gold (20 nm thick) in a Bio-Rad SC515 sputter coating unit. SEM observations were made using a Philips XL20 Scanning Electron Microscope at accelerating voltages of 20-30 kv.

The number of pores is always counted in the dissoconch in a flat (not concave) area of 100×100 μm (= 10,000 μm^2) below the umbo and in the zone where they are more abundant so the number is the maximum found.

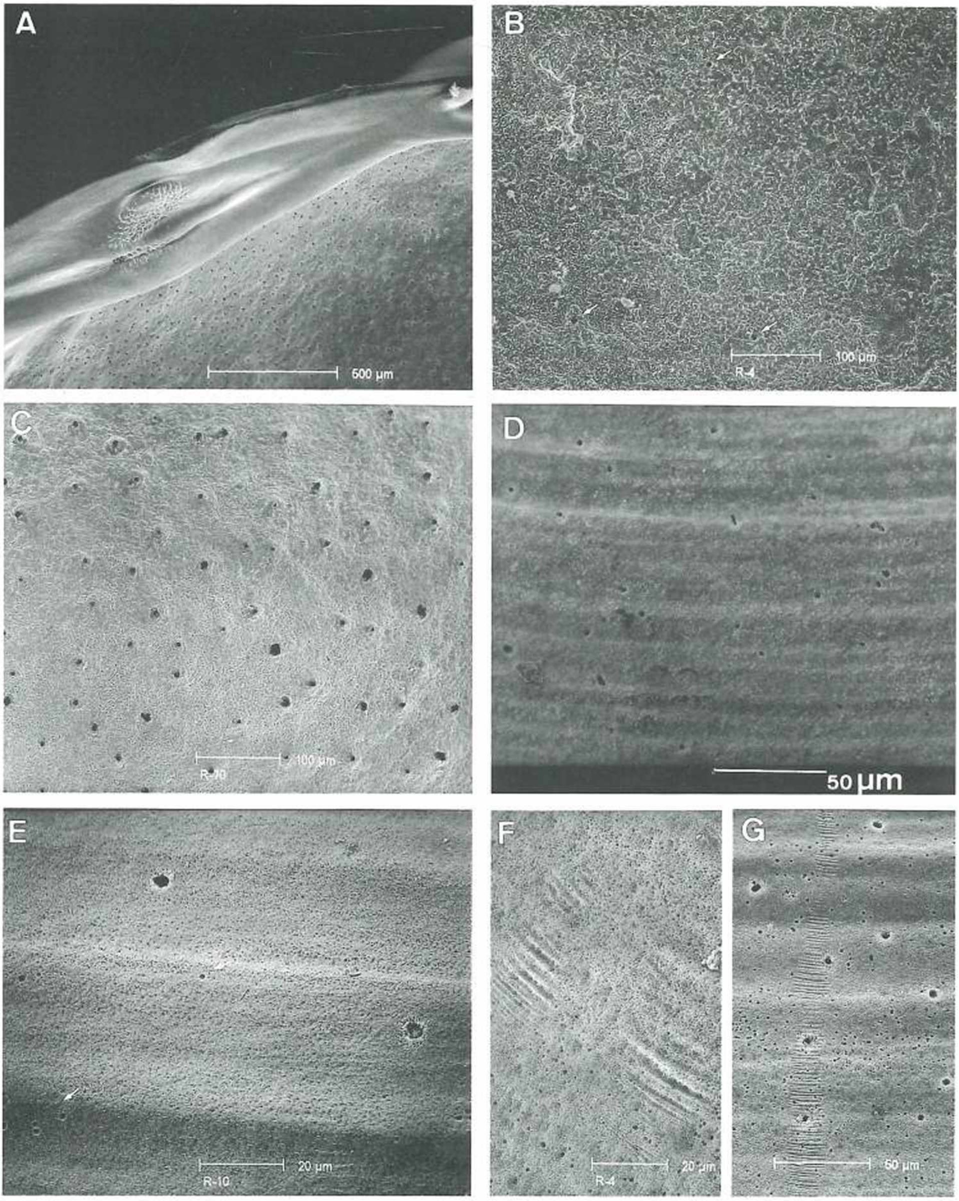

Fig. 2: Microsculpture of the typical form of *Pisidium casertanum*. **A.** Bumps in the anterior lateral teeth of the right valve (Spain). **B.** Internal pores (arrows) (Siberia). **C.** Internal pores (Ukraine). **D.** External pores (Spain). **E.** External pseudopores (arrows) (Ukraine). **F.** External ribs (Siberia). **G.** External ribs (Spain).

Besides the internal pores, we paid attention to the other characters of the shell surface: external pores (the opening of the tubules on the external surface of the shell) and pseudopores (excavations on the external surface of the shell), fine concentric ribs and the form and structure of the hinge teeth.

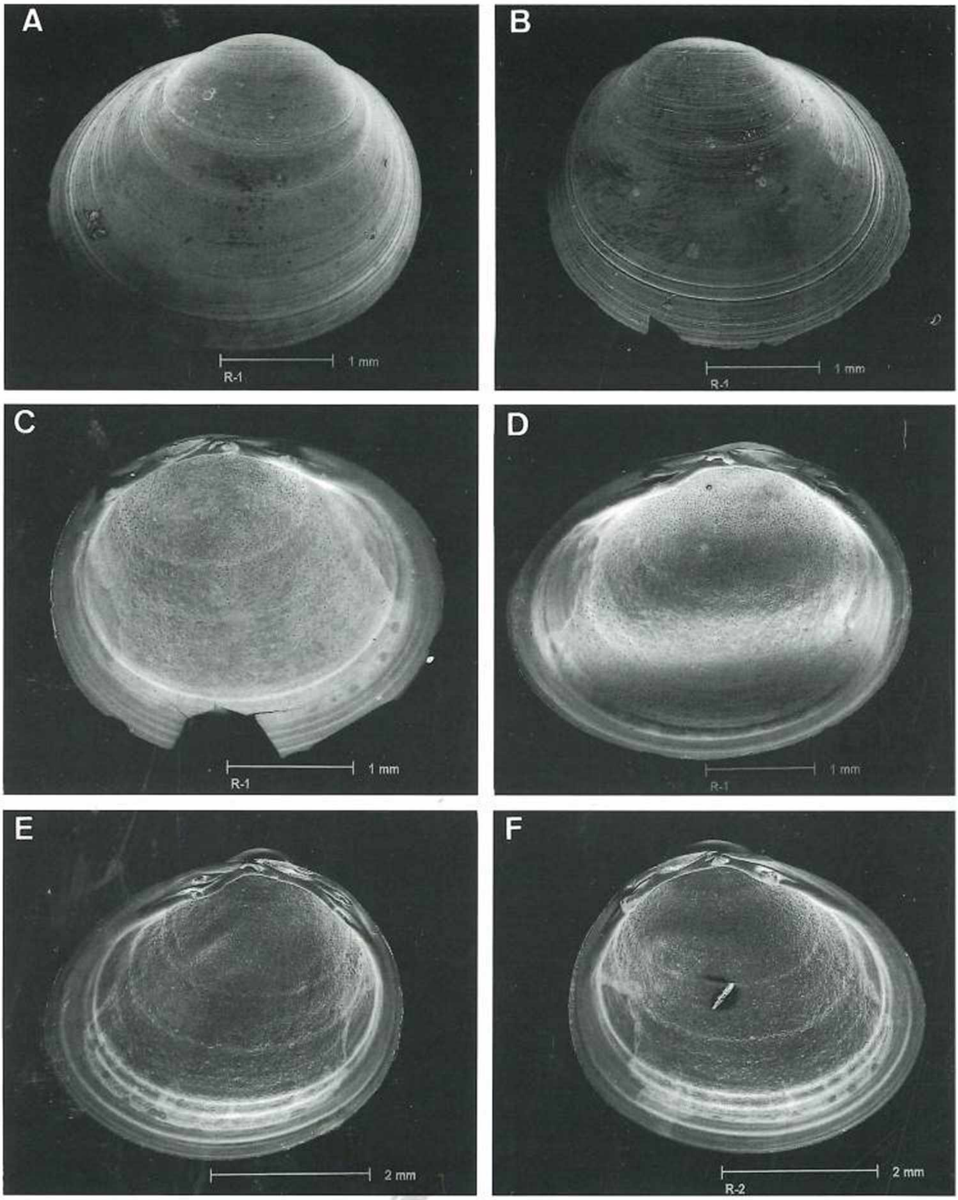

Fig. 3: *Pisidium casertanum globulare* form. Specimens from Ukraine **A.** Left valve. **B.** Right valve. Interior views **C.** Left valve. **D.** Right valve. Specimens from Siberia. **E.** Right valve. **F.** Left valve.

Results

Here we present descriptions of shell form and microsculptural peculiarities in the study species and varieties. The number of internal pores is summarized in Table 1, both from our own results and from the bibliography.

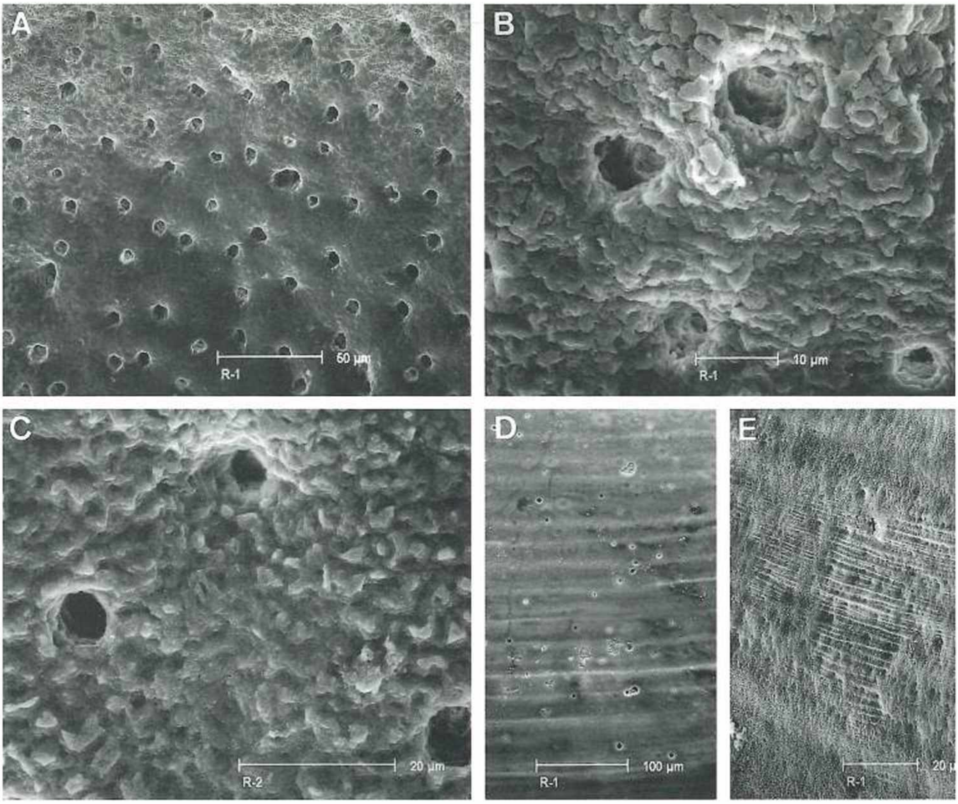

Fig. 4: Microsculpture of the *globulare* form of *Pisidium casertanum*. **A.** Internal pores (Ukraine). **B.** Detail of A. **C.** Detail of the internal pores of a juvenile specimen (Siberia). **D.** External pores (Ukraine). **E.** External ribs (Ukraine).

Pisidium casertanum typical form

All study shells corresponded in their form to the published descriptions of *P. casertanum* (ELLIS, 1978; PIECHOCKI, 1989) (Fig. 1A, B, C). The hinge was characterized by relatively long lateral teeth (Fig. 1D, E, F). The presence of microscopic bumps in the lateral teeth was very clear in Spanish specimens (Fig. 2A) and less developed in those of Ukraine and Siberia.

Internal pores were scarce (Fig. 2B, C) and in all study valves their density did not exceed 7 per 10,000 μm^2 (Table 1). External pores were present in Spanish specimens (Fig. 2D) but absent in those from Siberia and Ukraine. Small circular excavations (pseudopores) not perforating the shell (Fig. 2E) and a microsculpture of very fine parallel ribs arranged in radial bands (Fig. 2F, G) were noticed on the external surface of all specimens.

Pisidium casertanum var. *globulare*

The shells of this form were characterized by the relatively broad umbo (Fig. 3A, B). The hinge was distinguishable by very short lateral teeth (Fig. 3C, D, E, F), resembling that of *P. obtusale*. This peculiarity was the most obvious in left valves, where both the anterior and posterior laterals terminated abruptly. The microscopic bumps of the teeth are practically absent.

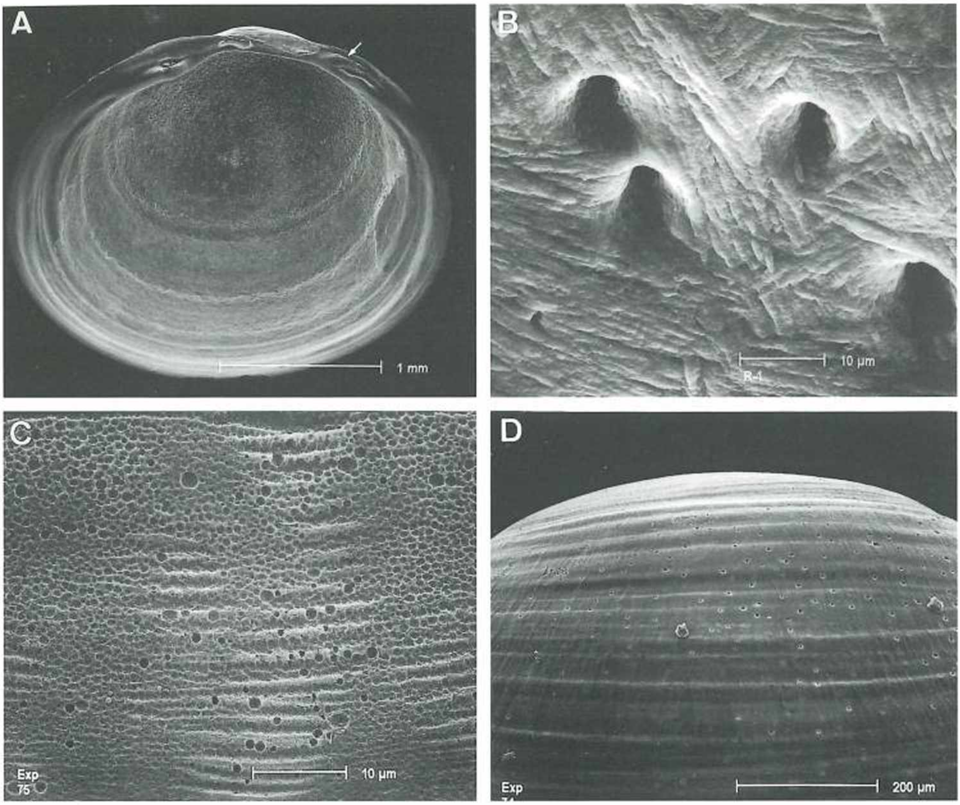

Fig. 5: *Pisidium personatum*. **A.** Interior view of right valve (Spain). The arrow indicates the callus. **B.** Detail of internal pores (Belarus). **C.** External pseudopores and ribs (Spain). **D.** External pores (Spain).

In all study samples, high internal pore density was observed. Usually more than 20 pores per 10,000 μm^2 were found (Fig. 4A, B, C). Only in the Arctic population were these values 11 to 14 (Table 1). However, in the latter case, pore density in the *globulare* form was considerably higher than in the typical *P. casertanum* from the same habitat. Such a situation occured in the sample taken from the small pool near Kanev, Ukraine, which also included the *globulare* form besides the typical form (Table 1). In young specimens the number of pores was usually lower than in adults, but material was not sufficient for statistical treatment.

We only studied the external surface of the specimens from Kiev, Ukraine, finding external pores in only one of the two valves studied (Fig. 4D) and microsculpture of ribs in both (Fig. 4E).

Pisidium personatum

Shells of this species can be recognized by the weak hinge with thin teeth and presence of the callus (Fig. 5A).

The density of pores on the inner surface varied. Up to 10 pores per 10,000 μm^2 were found in specimens from Belarus (Fig. 5B) and in some of the Spanish specimens (Table 1). In other specimens from Spain, collected at the same time and in the same locality as one spe-

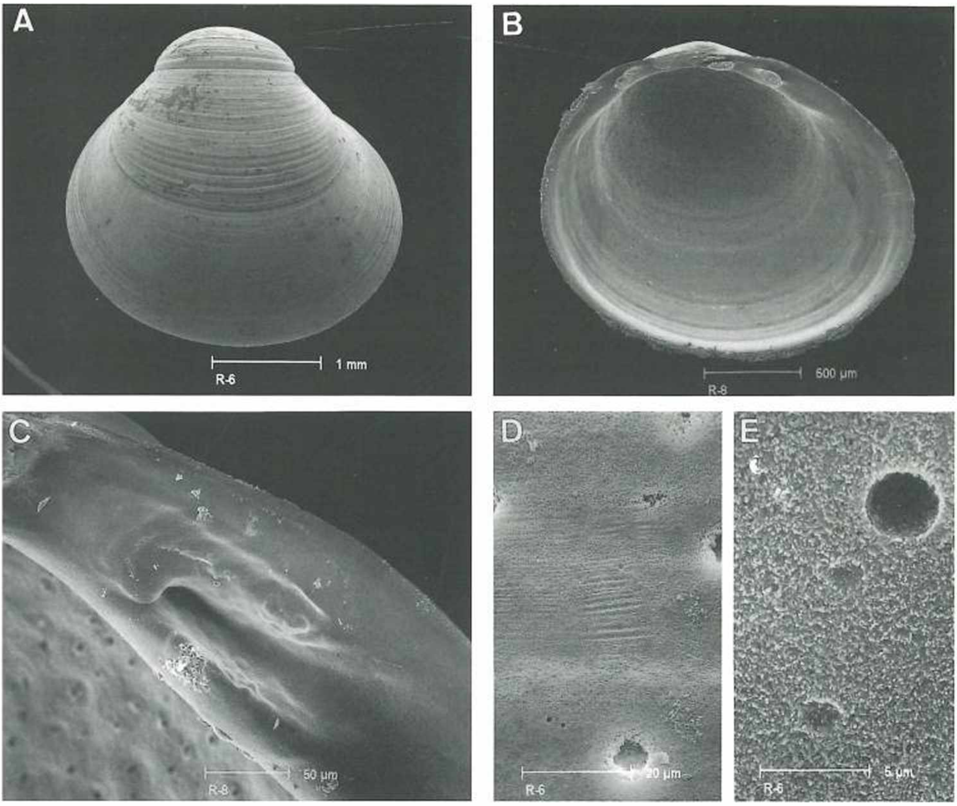

Fig. 6: *Pisidium obtusale*. **A.** Right valve (Kyrgyzstan). **B.** Internal view of left valve (Russia). **C.** Detail of the pseudocallus in the posterior lateral teeth of right valve (Russia). **D.** External pseudopores and ribs (Kyrgyzstan). **E.** Detail of the pseudopores of D.

cimen with five pores, they were so scarce that their density could not be evaluated. Such a condition was also found in the typical form of *P. casertanum* from Siberia.

All study specimens presented microsculpture of ribs and pseudopores on the external surface (Fig. 5C), but true external pores were only present in the Spanish specimens (Fig. 5D). The microscopic bumps were seen with a variable intensity in the teeth of the Spanish specimens.

Pisidium obtusale

The shell was very tumid and with broad umbones (Fig. 6A). The hinge of the study specimens had typical cardinal teeth (Fig. 6B) and a well developed pseudocallus (Fig. 6C).

The density of internal pores was almost as high as in *P. casertanum* var. *globulare*: 9 to 17 per 10,000 μm^2 (Table 1). In the sample from Kyrgyzstan containing *P. obtusale* with *P. casertanum* var. *globulare*, the latter had considerably higher pore density.

No external pores were observed, but all specimens present rib microsculpture (Fig. 6D) and the pseudopores (Fig. 6E). The lateral teeth presented more or less marked microscopic bumps (Fig. 6C).

The lots taken for SEM studies included a few specimens, but the total number for each species was enough to evaluate standard errors. T-test confirms distinctness of *P. caserta-*

num var. *globulare* from the typical *P. casertanum* and *P. personatum* ($p < 0.05$). Alongside pores density, the forms of *P. casertanum* differ in hinge characters, namely in length of lateral teeth. The difference between *P. obtusale* and *P. personatum* and that between *P. obtusale* and *P. casertanum* var. *globulare* were not significant. However, these species are well distinguished by the hinge characters. Similarity of *P. casertanum* var. *globulare* and *P. obtusale* in high pores density and shortened lateral teeth is also notable. However, pseudocallus was never found in the former.

Discussion

Heterogeneity of microsculptural characters in *P. casertanum s. l.* was first demonstrated by DYDUCH (1983). Basing her argument on these characters, this author proposed taxonomic heterogeneity of the species. The high pore density found in *P. casertanum* var. *globulare* provides new evidence for this statement. Despite the small numbers of specimens in each lot studied, the reliability of our data is supported by the wide geographic range of the material and clear differences found in all cases of sympatry.

KIRITSHUK & STADNICHENKO (1996) reported the differences in porosity between several "small species" distinguished within *P. casertanum s. l.* In those of the *Roseana* group which according to the approach accepted here should be identified as *P. casertanum* var. *globulare* average pores density was higher (1556+27 to 1665+21 pores/mm^{-2}) than in *Euglesa s. str.* group corresponding to the typical *P. casertanum* (1229+83 to 1465+63). The figures are different from those provided here probably because of less accurate method of counting (under low magnification of the light microscope). It is also important that all populations mentioned in the cited paper were allopatric.

The authors reporting scarcity of pores in *P. casertanum* (ADLER & FIECHTNER, 1992; ARAUJO, 1995, 1998) almost certainly dealt with the typical form of the species. Our data on pore density in *P. personatum* agree to a great extent with those of ARAUJO (1998) for the species in Portugal (Table 1) and KIRITSHUK & STADNICHENKO (1996) in Ukraine. Real high pore density in *P. obtusale* is reported for the first time. KIRITSHUK & STADNICHENKO (1996) provide lower figures for this species (maximum 1136 pores/mm^{-2}).

Extensive porosity found in *P. casertanum* var. *globulare* may be associated with its ecological preferences. According to PIECHOCKI (1989) and to our own observations, it inhabits water bodies with some shortage of oxygen supply (usually swamps). The role of shell pores for respiration was supposed by ADLER & FIECHTNER (1992) and the numerous pores as well as the large outer demibranch (KORNIUSHIN, 1996) may ensure more intensive oxygen uptake. High pore density found in *P. obtusale*, which also inhabits swamps, is rather obvious in this respect. A summary of the hypotheses concerning pore function may be found in ARAUJO *et al.* (1994).

On the other hand, differences in pores density as well as those in hinge characters between the two forms of *P. casertanum* taken from the same habitat show that these characters could be genetically determined. This fact points to the species distinctness of *P. globulare*, supposed by KORNIUSHIN (1996). Final decision on the status of this species is to be made after settling nomenclatorical questions.

Scarcity of pores in young specimens in comparison with adults observed in *P. casertanum* var. *globulare* agree with data of ARAUJO (1995) on *P. amnicum* (MÜLLER, 1774). These differences might be explained by development of pores in the course of shell growth after calcification, as shown for superfamily Arcoida (WALLER, 1980), and *Corbicula fluminea* by ARAUJO *et al.* (1994). The data supporting possibility of secondary development of pores in sphaeriids were provided by ADLER & FIECHTNER (1992) and ARAUJO (1998).

Our results indicate the significance of the data on inner pore density for taxonomic research in Sphaeriidae. The taxonomic value of pseudopores, fine concentric ribs and the microsculpture of lateral teeth is still not clear. In addition to the species described here, those structures were found in Portuguese specimens of *P. casertanum*, *P. personatum*, *P. nitidum* JENNYNS, 1832 and *P. milium* HELD, 1836 (ARAUJO, 1998) and in two Spanish populations of *P. henslowanum* (ARAUJO, 1993), but their presence seems not to be constant.

Acknowledgements

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