

**SEM MORPHOLOGICAL ANALYSIS OF FERTILIZED CYSTS
IN THE GENUS *BRANCHIPUS* (BRANCHIOPODA, ANOSTRACA) –
CAN CYST MORPHOLOGY BE USED AS A RELIABLE TAXONOMIC CRITERION?**

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Abstract — In order to illuminate taxonomic significance of cyst variability (as a part of total morphological variability) in the genus *Branchipus*, cysts (fertilized eggs) originating from southern parts of the Pannonian Plain are described on the basis of scanning electron microscopy (SEM) observations. The present study sets out to: 1) improve knowledge on use of egg morphology as a reliable taxonomic criterion in the genus *Branchipus*; 2) discuss egg variability in Pannonian populations; and 3) briefly discuss possible adaptive implications of egg morphology from the evolutionary point of view.

Key words: Branchiopoda, Anostraca, *Branchipus*, SEM, cyst morphology, taxonomy

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INTRODUCTION

In the second half of the 20th century, the external morphology and size and internal structure of resting eggs in branchiopods were studied by several authors. As regards the tertiary egg shell, varied functions have been attributed to it (Dumont and Negrea, 2002). The tertiary eggshell is known to be a useful outer protective cover, particularly during the drought periods. Cysts are known to be drought-resistant (they can hatch after long periods of dry storage). After pools refill, the well-sculptured shell, which represents a relatively large lamellar surface, is exposed to osmotic pressure of the aquatic environment, making the floating eggs buoyant (Brendonck and De Meester, 2003). According to some authors, cyst ornamentation can also be considered as a defense mode against egg predation (Dumont et al., 2002).

However, the complex architecture of the tertiary envelope should be considered not only as a character that allows a higher survival rate in ephemeral habitats, but also as one that could be useful in taxonomy. Previously conducted SEM surveys of the taxonomical value of cyst morphology in large bran-

chiopods indicated that many species show stability of egg pattern and egg diameter in their wider zone of occurrence (Europe and/or the Mediterranean) (Thiéry and Gasc, 1991). On the other hand, we have seen many opinions claiming that if only the egg morphology pattern has been taken into account, some anostracan species (including those in the genus *Branchipus*) cannot be distinguished beyond any doubt (Mura, 1986; Thiéry et al., 1995; Dumont and Negrea, 2002).

In order to illuminate the taxonomic significance of morphological variability of the cysts, a series of fertilized eggs originating from southern parts of the Pannonian Plain are herein described on the basis of scanning electron microscopy (SEM) observations. In addition to more precise defining of cysts morphology, the present study puts forward a model that can better reflect taxonomic relationships of lower rank within the genus.

MATERIAL AND METHODS

The investigated area lies mostly in southern parts of the Pannonian Plain, administratively belonging to the Vojvodina Province (Northern Serbia). Egg

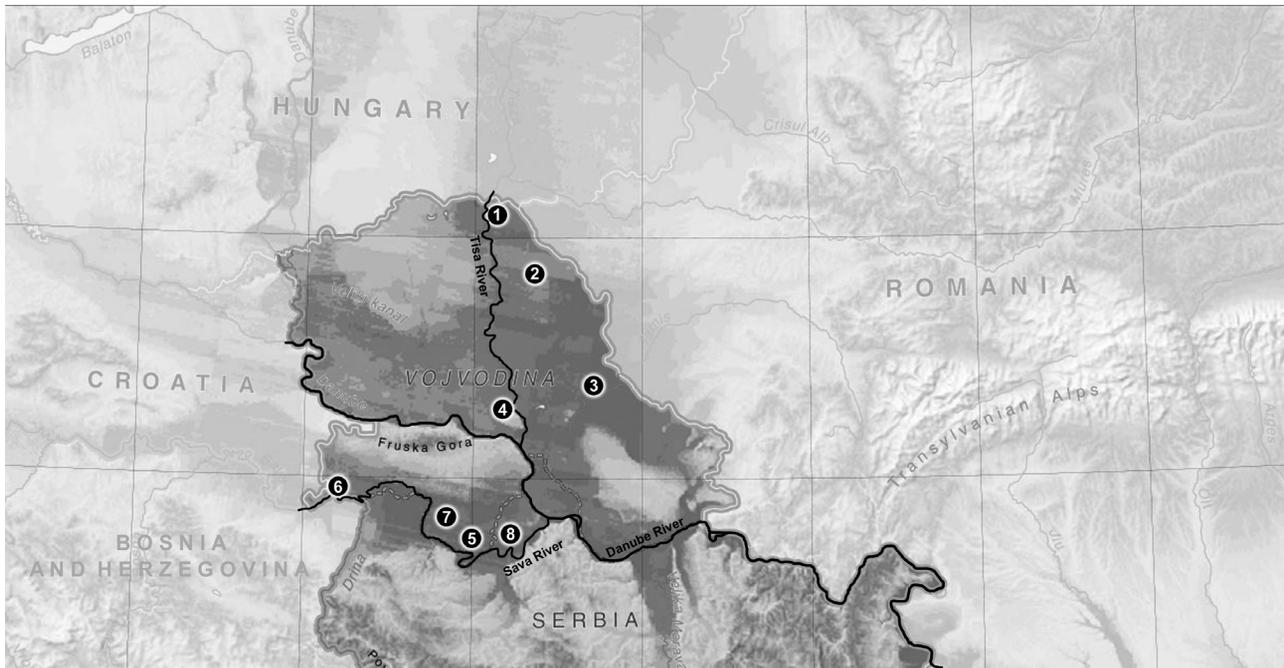


Fig. 1. The investigated area. (1) Banatsko Aranđelovo; (2) Banatski Monoštor; (3) Sutjeska; (4) Titel-Mošorin; (5) Progar; (6) Jamena; (7) Tovarnik; (8) Ogar.

pattern diversity among populations in different parts of the province was examined at the following localities: Banatsko Aranđelovo (1), Banatski Monoštor (2), Sutjeska (3), a locality between the villages of Titel and Mošorin (4), Progar (5), Jamena (6), Tovarnik, (7) and Ogar (8) (Fig. 1).

For SEM analysis, material was prepared according to Mura (1986). Cysts removed from brood pouches of several females were rinsed in distilled water, oven-dried, mounted on stubs, and gold-coated. Cysts were observed and photographed under a JEOL JSM 6460LV scanning electron microscope.

RESULTS

All populations examined show a constant pattern in egg morphology (Fig. 2): “equatorial” ridges appear in number six. They form a circular belt in the middle of the cyst surface, running parallel with the medial axis of the cyst. “Joining” ridges are thinner, paired ridges. They run horizontally, joining two neighboring equatorial ridges. “Meridional” ridges are also thin. They run vertically, starting from each equatorial edge and stretching towards

the cyst poles. Eggs have two confluence points on the egg poles. Exemplary eggs from different localities are presented in Fig. 3. Brief descriptions of the cysts are summarized in Table 1.

The surface pattern study

Locality 1 (Banatsko Aranđelovo)

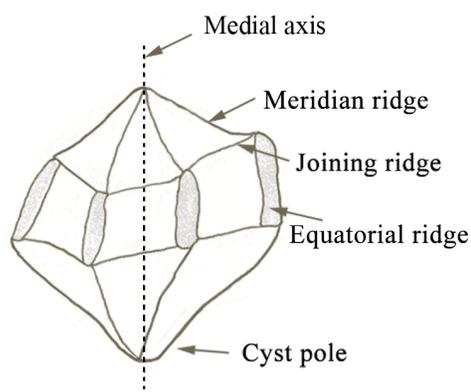
Equatorial ridges high, broad, with well-defined ends, parallel with each other. Joining ridges high, long, rounded, and S-shaped. Meridional ridges similar to equatorial ones. They form many irregular, deep depressions on the egg surface. In addition, joining and equatorial shell ridges reach each other, forming bow-like depressions. The cyst poles are broad and widely rounded. Eggs are hexagonal, with a very wrinkled appearance.

Locality 2 (Banatski Monoštor)

Equatorial ridges somewhat shorter and thinner than at the previous locality. Joining ridges thin, slightly S-shaped (they also form bow-like depressions). Meridional ridges almost straight and also thin. They stretch to form prominent cyst poles.

Table 1. Brief descriptions of cysts of the genus *Branchipus* from southern parts of the Pannonian Plain.

No	Locality	Cyst poles	Ridges	Depressions
1.	Banatsko Arandelovo	Broad, widely rounded	Rounded	Irregular and bow-like, deep
2.	Banatski Monoštor	Prominent	Rounded	Irregular and bow-like, deep
3.	Sutjeska	Prominent	Sharp and thin	Polygonal, deep
4.	Titel-Mošorin	Broad, widely rounded	Sharp and thin	Irregular and bow-like, deep
5.	Progar	Prominent	Smoothly rounded, thin	Irregular, shallow
6.	Jamena	Prominent	Rounded, thin	Triangular and rectangular, deep
7.	Tovarnik	Prominent	Rounded, thin	Irregular, deep
8.	Ogar	Broad, widely rounded	Rounded, thin	Irregular and bow-like, deep

**Fig. 2.** Main characters defining the surface pattern of fertilized cysts in the genus *Branchipus* (model).

There are many deep depressions on the egg surface.

Locality 3 (Sutjeska)

There are numerous thin and sharp lamellar shell ridges on the egg surface. Ridges arise almost perpendicularly from the egg surface. They are similar to one another, arranged almost regularly (some of the margins come into contact at an angle of almost

90°). Fields (depressions) between the wrinkles are relatively deep, well-defined, and polygonal. Cyst poles are well differentiated.

Locality 4 (Titel-Mošorin)

Equatorial ridges thin, sharp, and relatively short. Joining ridges have a “broken” appearance, in some places forming a V. Meridional ridges also sharp and thin. They form widely rounded and well differentiated cyst poles. Cysts are hexagonal, with a very wrinkled, almost cylindrical appearance. Deep depressions within the ridges are triangular or polygonal. Sometimes joining ridges are situated close to one another, forming (with the equatorial ones) deep and sharp bow-like depressions.

Locality 5 (Progar)

All of the surface ridges have a “gentle” appearance, rising gradually. Equatorial ridges very enlarged and rounded, with two well-differentiated, spherical endings. They run at an angle of about 30° to the medial axis of the cyst. Joining ridges are short, discreet, and rounded. Meridional ones are considerably enlarged and slightly bent. They are conflu-

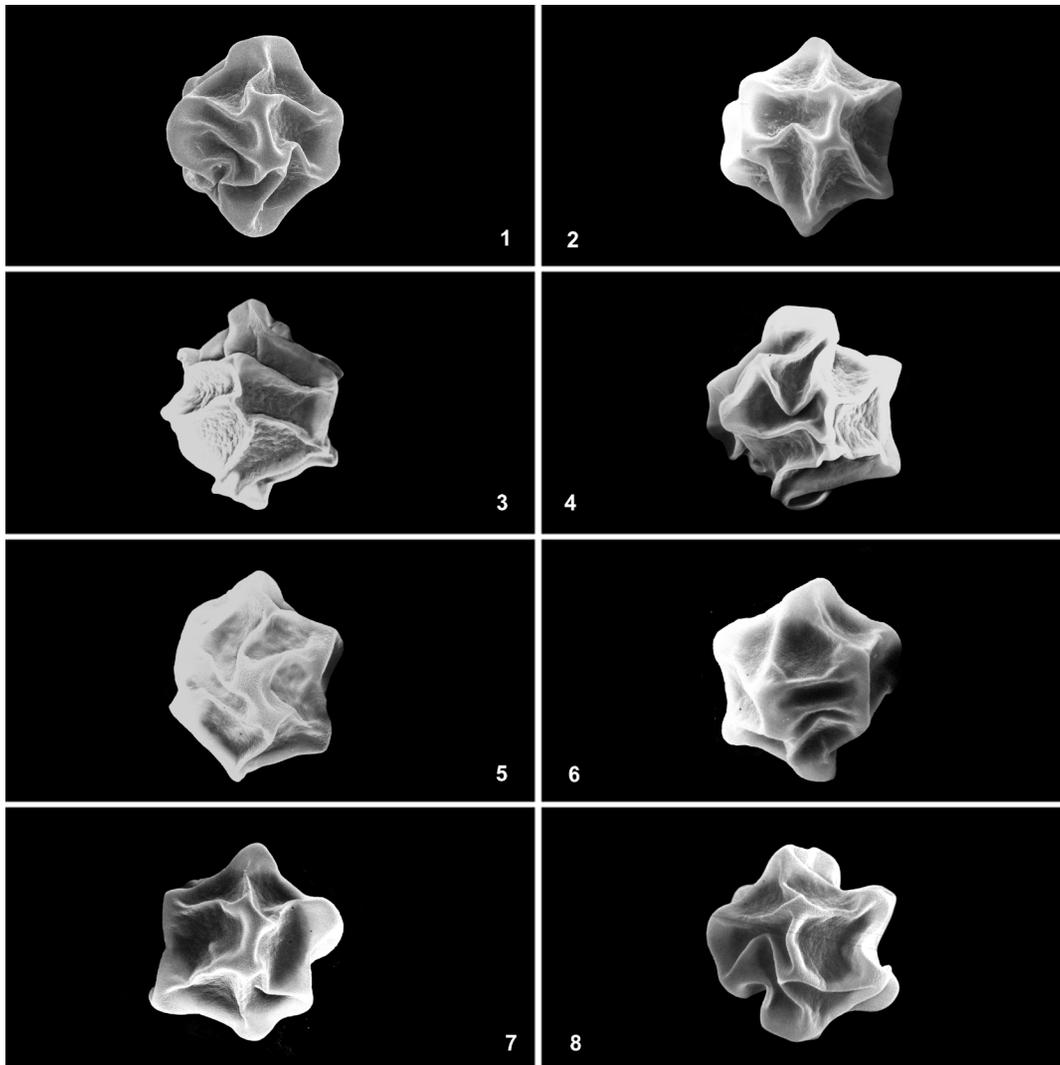


Fig. 3. Diversity of *Branchipus* cysts from southern parts of the Pannonian Plain. (1) Banatsko Arandjelovo; (2) Banatski Monoštor; (3) Sutjeska; (4) Titel-Mošorin; (5) Progar (6) Jamena; (7) Tovarnik; (8) Ogar.

ent, forming prominent cyst poles. Consequently, the cyst has a somewhat elongated and “twisted” appearance. The egg surface has an unevenly edged, undulate pattern with the presence of relatively shallow, irregular fields.

Locality 6 (Jamena)

Equatorial ridges relatively short. Joining ridges straight and parallel. Meridional ridges short, very similar to the joining ones. Cyst poles prominent. All the smoothly rounded ridges delineate triangular and rectangle fields.

Locality 7 (Tovarnik)

Equatorial ridges well differentiated. Joining ridges thin, rounded, with a somewhat “broken” appearance. Meridional ridges short, forming well-differentiated cyst poles. Shell depressions irregular and deep.

Locality 8 (Ogar)

Cysts have a very wrinkled appearance. Equatorial ridges thin, high, somewhat curved. Joining ridges somewhat similar to the equatorial ones. They form

deep and sharp bow-like depressions. Meridional ridges also curved, forming broad, widely rounded cyst poles. Eggs have a very folded appearance, with irregularly shaped cells surrounded by distinctly high ridges.

DISCUSSION

In 80% of European branchiopod taxa, the egg surface pattern and size are the two main valid parameters for species identification (Thiéry et al., 1995). In the genus *Branchipus* Schaeffer, 1766 fertilized eggs (cysts) are considered as generic, with a complex architecture. Cysts were mainly defined as unevenly edged or “folded”, “wrinkled”, and “angulated”, with a polygonal network of alternating reliefs and depressions (Mura, 1986, 1992; De Walsche et al., 1991; Thiéry et al., 1995).

Our study showed the presence of a well-defined eggshell pattern that can be useful as a tool in species identification. The main characters defining all analyzed cysts are: (1) a hexagonal ground plane; (2) the presence of three different types of shell ridges through the egg surface (“equatorial”, “joining”, and “meridional”); (3) the presence of different shaped depressions delineated by the ridges; and (4) the presence of two confluence points on the egg poles, forming the „tops“ of resting eggs.

From the point of view of the model presented in this study, *Branchipus* eggs originating from southern parts of the Pannonian Plain are characterized by a well-developed ornamentation with rather deep fields. Due to similar topography, climatological peculiarity, and historical biogeography of habitats, the cyst morphologies in different Pannonian populations overlap to a certain extent. However, certain variability in the size and appearance of shell wrinkles (ridges), as well as in the shape and depth of surface (fields) within these wrinkles, is evident among populations within the studied geographical range. It is supposed that the some of these differences are of no taxonomic value and can be attributed to variability within field populations. A recent study on egg morphology in different species of the genus *Chirocephalus* Prévost, 1803 supports such an opinion. Mura (2001) reported a high frequency of diverging cyst appearance in *Chirocephalus diapha-*

nus carinatus (Daday, 1910) from Sutjeska (the same locality as in the present study). Similar variations of cyst morphology have also been confirmed in a large number of different *Chirocephalus* populations from the Balkans (Mura et al., 2002).

On the other hand, differences of egg morphology exhibited by some populations in the present SEM analysis suggest the possibility that the Pannonian Plain area could be a contact zone between the ranges of the “western” species *Branchipus schaefferi* Fischer, 1834 and the “eastern” species *Branchipus intermedius* Orghidan, 1947 in Europe. Most of the analyzed eggs are somewhat similar to the cysts of *B. schaefferi*, with prominent cyst poles and more or less irregularly shaped depressions (Mura, 1986; Thiéry and Gasc, 1991; Thiéry et al., 1995). However, cysts from some localities resemble *B. intermedius* eggs (with broad, widely rounded cyst poles and irregularly shaped bow-like depressions), which was also noted elsewhere in the region (Cvetković-Miličić and Petrov, 2007). Earlier investigations of large branchiopods (Petrov and Marinček, 1991) likewise pointed to the occurrence of local *Branchipus* populations that morphologically differ from *B. schaefferi*. The authors speculated that these populations could be transitional ones between *B. schaefferi* and *B. intermedius*. In addition to this, recent results of multivariate analyses showed a statistically significant separation of geographic groups (1), (4), and (8) from others in the area of the Pannonian Plain, the separation of these groups indicating a certain level of difference in these populations (Miličić, 2007).

Compatibility between the shape and height of tertiary shell wrinkles and between the shape and depth of fields within the wrinkles is also evident. These congruencies can be interpreted from an evolutionary and adaptive perspective: alternating reliefs and depressions on the cyst surface allow better packing of the cysts in the female brood pouches (like a three-dimensional puzzle). Brendonck et al. (1992) stated that efficient egg packing requires less energy in the process of formation of the clutch. Furthermore, it increases fecundity and allows a higher survival rate in ponds subjected to severe droughts with very short wet periods.

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SEM МОРФОЛОШКА АНАЛИЗА ОПЛОЂЕНИХ ЈАЈА РОДА BRANCHIPUS (BRANCHIOPODA, ANOSTRACA) – МОЖЕ ЛИ СЕ МОРФОЛОГИЈА ЦИСТИ КОРИСТИТИ КАО ПОУЗДАН ТАКСОНОМСКИ КРИТЕРИЈУМ?

ДРАГАНА МИЛИЧИЋ и БРИГИТА ПЕТРОВ

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У циљу расветљавања таксономског значаја варијабилности оплођених јаја (цисти) као дела укупне морфолошке варијабилности у оквиру рода *Branchipus*, цисте пореклом из јужних делова Панонске низије анализирани су на основу

посматрања на скенинг електронском микроскопу (SEM). Циљ ове студије био је унапређивање знања о коришћењу морфологије оплођених јаја као поузданог таксономског критеријума, анализа варијабилности цисти панонских популација

и разматрање адаптивног значаја сложене морфологије цисти јединки рода *Branchipus*. Евидентна је компатибилност облика и висине набора терцијарне опне оплођених цисти са обликом и дубином поља која ти набори окружују. То омогућава ефикасније паковање цисти у оквиру јајне кесе. Овакав начин паковања обезбеђује мањи утросак енергије и повећава фекундитет и стопу пре-

живљавања у условима смењивања суше са повременим и веома кратким влажним периодима на станишту. Разлике у морфологији оплођених јаја у оквиру ове анализе сугеришу могућност да би подручје Панонске низије могло бити контакт-зона распрострањења претежно „западне“ врсте *Branchipus schaefferi* и претежно „источне“ врсте, *Branchipus intermedius* у Европи.