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Cyst morphology of large branchiopod crustaceans (Anostraca, Notostraca, Laevicaudata, Spinicaudata) in western Poland

MICHAŁ JAN CZYŻ[1,2], PAWEŁ WOLIŃSKI[1] and BARTŁOMIEJ GOŁDYN[3]

[1] Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland
[2]Research Centre of Quarantine, Invasive and Genetically Modified Organisms, Institute of Plant Protection, National Research Institute, Węgorka 20, 60-318 Poznań
[3] Department of General Zoology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland
Corresponding author: Michał Jan Czyż, mczyz@iorpib.poznan.pl

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Abstract: The morphology of resting eggs was studied in Polish populations of 7 large branchiopod species: *Branchipus schaefferi*, *Chirocephalus shadini*, *Eubranchipus grubii* (Anostraca), *Lepidurus apus*, *Triops cancriformis* (Notostraca), *Lynceus brachyurus* (Laevicaudata), and *Cyzicus tetracerus* (Spinicaudata). Scanning electron microscope photographs for each species are also provided. In some species (especially *E. grubii*), the ornamentation and size of cysts from Polish populations significantly differ from literature data for populations from other parts of Europe. We also present a key to determination of the studied species based on cyst morphology and discuss whether those traits are useful in surveys of new localities of endangered large branchiopods.

Keywords: dormant eggs, temporary waters, fairy shrimps, tadpole shrimps, clam shrimps, *Branchipus schaefferi*, *Chirocephalus shadini*, *Eubranchipus grubii*, *Lepidurus apus*, *Triops cancriformis*, *Lynceus brachyurus*, *Cyzicus tetracerus*

INTRODUCTION

Invertebrates inhabiting temporary bodies of water use several strategies enabling their populations to survive drought (Williams 2006). One of the strategies is formation of dormant egg banks in the sediments. Large branchiopods are classic examples of animals utilizing this strategy (Brendonck & De Meester 2003; Vanshoenwinkel et al. 2010).

Large branchiopods form a paraphyletic group, consisting of 4 crustacean orders: fairy shrimps (Anostraca), tadpole shrimps (Notostraca), and clam shrimps (Spinicaudata and Laevicaudata). Members of this group inhabit almost exclusively temporary waters of various kinds. Since adult individuals are not able to survive drying, only the resting eggs (also called cysts) deposited in the sediments sustain the population during the following inundation periods (Kraus et al. 2004). Inside the cysts, diapausing embryos are encapsulated by a thick shell made of lipoproteins, mucopolysaccharides and other substances (see Fryer 1996 and the references therein). They can survive several years of drought and are also resistant to other harsh environmental conditions, like extreme temperatures, UV radiation, and vacuum (e.g. Brendonck & De Meester 2003; Clegg & Campagna 2006; Shckorbatov 2010).

Large branchiopods are regarded as a globally endangered group (Brendonck et al. 2007) and there is an increasing need for surveys aiming to map their biodiversity and distribution. Since surface morphology of their cysts is considered to be species-specific (Mura & Thiery 1986; Thiery & Gasc 1991), determination of cysts extracted from the sediments is a promising tool for such surveys (Boven et al. 2008). That is why knowledge on variability in cyst ornamentation and biometry becomes essential. In some species, data on cyst morphology are very scarce and refer to single populations or even single measurements (e.g. Thiery & Gasc 1991; Petkovski 1995). Studies comparing the data from different geographic regions, populations, and species are crucial for judging the utility of this trait for taxonomic and ecological research (Hill & Shepard 1997; Thiery et al. 1997; Mura 2001).

For now, there is an almost complete lack of data on the morphology of large branchiopod cysts from Central Europe (excluding Slovakia, Thiery et al. 1997). The present paper provides new data on cyst morphology in 7 species occurring in western Poland, compared to the literature data from other European localities, and a short key for species determination basing on cyst morphology.

MATERIAL AND METHODS

Resting eggs of 7 large branchiopod species were used in this research: the anostracans *Branchipus schaefferi* Fischer, 1834, *Chirocephalus shadini* (Smirnov, 1928), and *Eubranchipus grubii* (Dybowski, 1860); the notostracans *Lepidurus apus* (Linnaeus, 1758) and *Triops cancriformis* (Bosc, 1801); the leavicaudatan *Lynceus brachyurus* O. F. Müller, 1776; and the spinicaudatan *Cyzicus tetracerus* (Krynicki, 1830).

Adult specimens were collected from puddles in an inactive part of the Poznań-Biedrusko military area (localities of *B. schaefferi* and *T. cancriformis*) and from field ponds in Kaźmierz near Poznań in western Poland (for details on the habitats, see Gołdyn et al. 2007, 2012) and determined following Brtek (1962). Specimens of *Chirocephalus schadini* and *Cyzicus tetracerus* came from a single locality, whereas for the remaining species, animals from at least 3 populations were collected. The animals were kept in 20-l aquariums and fed with green algae from a culture. After a week, the cysts were collected from the bottom of the aquariums. The cysts of *Lepidurus apus* and *T. cancriformis* were also taken from egg sacks of preserved females.

Resting eggs were kept in formaldehyde (10%) and then fixed in a bath of KAAD (30% kerosene, 60% absolute ethanol, 5% acetic acid, 5% dioxane) for 20 h (Thiery & Gasc 1991). This preparation preserves the natural shape of the tissues (Tommasini

& Scanabissi 1989). In general, 20 randomly chosen eggs of each species were dehydrated by critical point drying with CO_2, next coated with gold, and then observed using a Hitachi S3000N scanning electron microscope (SEM).

The data on cyst diameter in Polish populations were compared to the results known from the literature using the following equation:

$$D\% = (d_{min2} - d_{max1}) / d_{max1} \times 100\%,$$

where: D% = difference between the data sets; d_{min2} = minimum diameter from the population with larger cysts; d_{max1} = maximum diameter from the population with smaller cysts.

Some of the cited papers lack numerical data on cyst diameter. In such cases, we followed Thiery et al. (1995), where the diameters were measured from published calibrated photographs.

RESULTS

In most cases the shape, ornamentation, and size of cysts was species-specific in the dataset studied. Cysts irregular in shape occurred only in *Branchipus schaefferi* (Fig. 1a). The examined notostracans had spherical resting eggs with a smooth surface (Fig. 1d, e). In *E. grubii*, the surface was covered with ridges (Fig. 1c), visible even under a small magnification in a stereomicroscope. In *Chirocephalus shadini*, the ornamentation could be seen only under a SEM microscope and the cysts were covered with a labyrinth of folds, resembling the convoluted surface of a human brain (Fig. 1b). Cysts of *Lynceus brachyurus* were covered with angular depressions (Fig. 1f), differing in shape and number.

An unexpected number of cysts in all species except *Eubranchipus grubii* were deformed during fixation. The most vulnerable where those of *Cyzicus tetracerus* (11 of 20 deformed and all with a damaged outer envelope). In general, resting eggs of Spinicaudata have a thin tertiary envelope, which can be easily destroyed, so KAAD is necessary for their preservation (Thomasini & Scanabissi 1989). In our case, even a bath of 20 h in KAAD did not preserve the outer envelope in *C. tetracerus*, so we were not able to observe the surface ornamentation. However, according to Thiery & Gasc (1991) and Thiery et al. (1997), cysts in this species are spherical with a smooth surface.

Among the species studied, the smallest cysts (Table 1) were those of *L. brachyurus* (diameter 110–129 µm) and *C. tetracerus* (139–155 µm), whereas the biggest cysts occurred in *Triops cancriformis* (394–424 µm), *E. grubii* (399–431 µm), and *Lepidurus apus* (520–630 µm).

A simple key for determination of the species occurring in western Poland basing on their cyst morphology can be constructed:

1	Cyst shape spherical	2
1*	Cyst shape irregular	*Branchipus shaefferi*
2	Surface smooth	3
2*	Surface ornamented	5
3	Diameter <150 µm	4

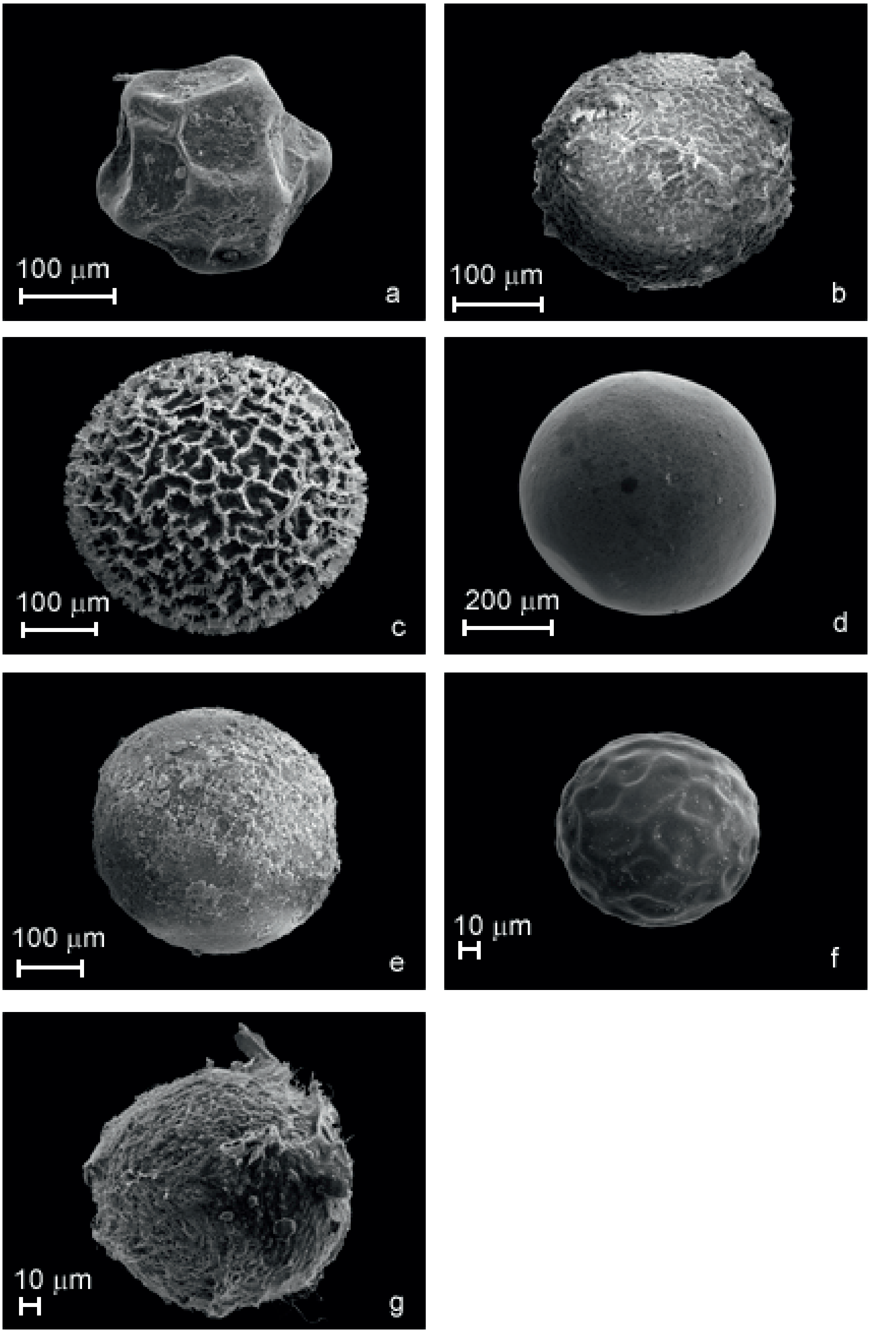

Fig. 1. Resting eggs of large branchiopods occurring in western Poland: (a) *Branchipus schaefferi*; (b) *Chirocephalus shadini*; (c) *Eubranchipus grubii*; (d) *Lepidurus apus*; (e) *Triops cancriformis*; (f) *Lynceus brachyurus*; (g) *Cyzicus tetracerus*

Table 1. Morphology and measurements of the studied cysts from Polish populations of large branchiopods

	Shape	Surface ornamentation	Diameter (µm)				
			mean	min	max	SD	*N*
Branchipus schaefferi	Irregular	no ornamentation	253.25	238.4	257.6	6.12	10
Chirocephalus shadini	spherical	folded	288.60	264.6	314.4	15.80	7
Eubranchipus grubii	spherical	ridges	414.05	398.6	431.0	8.14	20
Lepidurus apus	spherical	no ornamentation	599.36	521.6	633.7	31.64	10
Triops cancriformis	spherical	no ornamentation	410.75	394.4	423.9	10.40	10
Lynceus brachyurus	spherical	angular depressions	117.79	109.7	128.7	4.60	16
Cyzicus tetracerus	spherical	no ornamentation (?)	148.08	138.9	155.3	5.26	9

3*	Diameter >230 µm	6
4	Surface with angular depressions	*Lynceus brachyurus*
4*	Surface without angular depressions	*Cyzicus tetracerus*
5	Surface with ridges	*Eubranchipus grubii*
5*	Surface without ridges but folded	*Chirocephalus shadini*
6	Diameter <430 µm	*Triops cancriformis*
6*	Diameter >500 µm	*Lepidurus apus*

DISCUSSION

In most of the species, our observations on cyst ornamentation do not differ remarkably from those described for other European populations (Thiery & Gasc 1991; Thiery et al. 1997; Nagorskaja et al. 1998; Mura 2001). Only in *Eubranchipus grubii*, cysts from Moravian and Slovakian populations have much lower ridges, forming a thinner network (compare Fig. 1c in the present paper and Fig. 14 in Thiery & Gasc 1991). Similar differences have been reported for *Chirocephalus diaphanus* from Italy (Mura 1992), where mountain populations had higher ridges than lowland ones.

In the studied populations of 4 species, cyst diameters deviated from the known values from other parts of their ranges (Fig. 2). The greatest differences are noticeable in *E. grubii*, where our measurements gave much higher values than all those reported in the literature: 13% more than in Slovakian populations (Thiery at al. 1997), 21% more than in Germany (Buchholz 1864), 33% more than in Moravia (Thiery & Gasc 1991), and 28% more than in an unknown locality studied by Mura (1992). In *Lepidurus apus*, our results line up with those of Mathias (1937) from an unknown locality and Reau (1908) from France, but cysts from the remaining 8 populations studied in the past (Arnoult 1951; Alonso & Alcaraz 1984; Alonso 1985; Thiery & Gasc 1991; Thiery et al. 1997) were more than 10% smaller than those described in our study. For *B. schaefferi*, our results differ much from those reported from Germany (Kupka 1940), where cysts were 22% smaller, and from Saudi Arabia (Thiery 1995) and Italy (Mura 1986), where they were 24% larger and similar to the remaining 8 populations studied with this respect (Mathias 1937; Gilchrist 1978; Alonso 1985; Mura & Thiery 1986; Thiery et al. 1997). In the case of *T. cancriformis*, diameters of Polish cysts line up with those from 2 French localities (Mathias 1937; Thiery & Gasc 1991) and were 10–16% larger than those reported from 5 other populations studied (Thomasini & Scanabissi 1989; Thiery 1995; Thiery et al. 1997).

Intraspecific differences in the size of resting eggs in large branchiopods are suggested to depend on geographical latitude (Thiery & Gasc 1991) and environmental factors, specific to a given locality (Belk 1977; Mura 1992). In the first case, in colder climates, it is more efficient to allocate more resources in production of bigger cysts to assure better survival of freshly hatched larvae. In the second case, small amounts of big cysts are produced when the conditions are more predictable (*K*-strategy); otherwise it is more efficient to lay numerous small eggs (*r*-strategy). We assume that the geographical explanation of the observed differences applies to our findings, since the populations sampled for the present research belong to the northernmost of the studied so far with respect to cyst morphology. The observed differences may also indicate that at least some of the species studied are as a matter of fact complexes of cryptic species. Genetic and molecular methods should be used to test this hypothesis.

In addition to the 7 species compared in this study, 5 other large branchiopod species have been reported from other parts of Poland (Zwolski 1956) and one more is likely to occur in the country. Cysts of *Branchinecta paludosa* (known from the Tatra Mts.) are spherical, with a network of very low ridges, which make them similar to the surface of *E. grubii* cysts. In *Chirocephalus diaphanus* (2 localities, in eastern and northern Poland), cysts are covered with a network of high ridges, also resembling those of *E. grubii*. Cysts of the remaining 4 species are not similar to any of the species described in the present paper. In *Chirocephalus josephinae* (3 localities, in central Poland, all before 1939) they are covered with bumps, in *Streptocephalus torvicornis* (2 localities, in central and southern Poland) they have a strongly folded surface, while in *Limnadia lenticularis* (several localities across the country), they are non-spherical, biconvex, with a surface covered with ridges. In contrast, cysts of *Tansymasix stagnalis* (known from all the adjacent countries but not reported from Poland so far) have a unique lenticular shape (all descriptions based on Thiery et al. 1997).

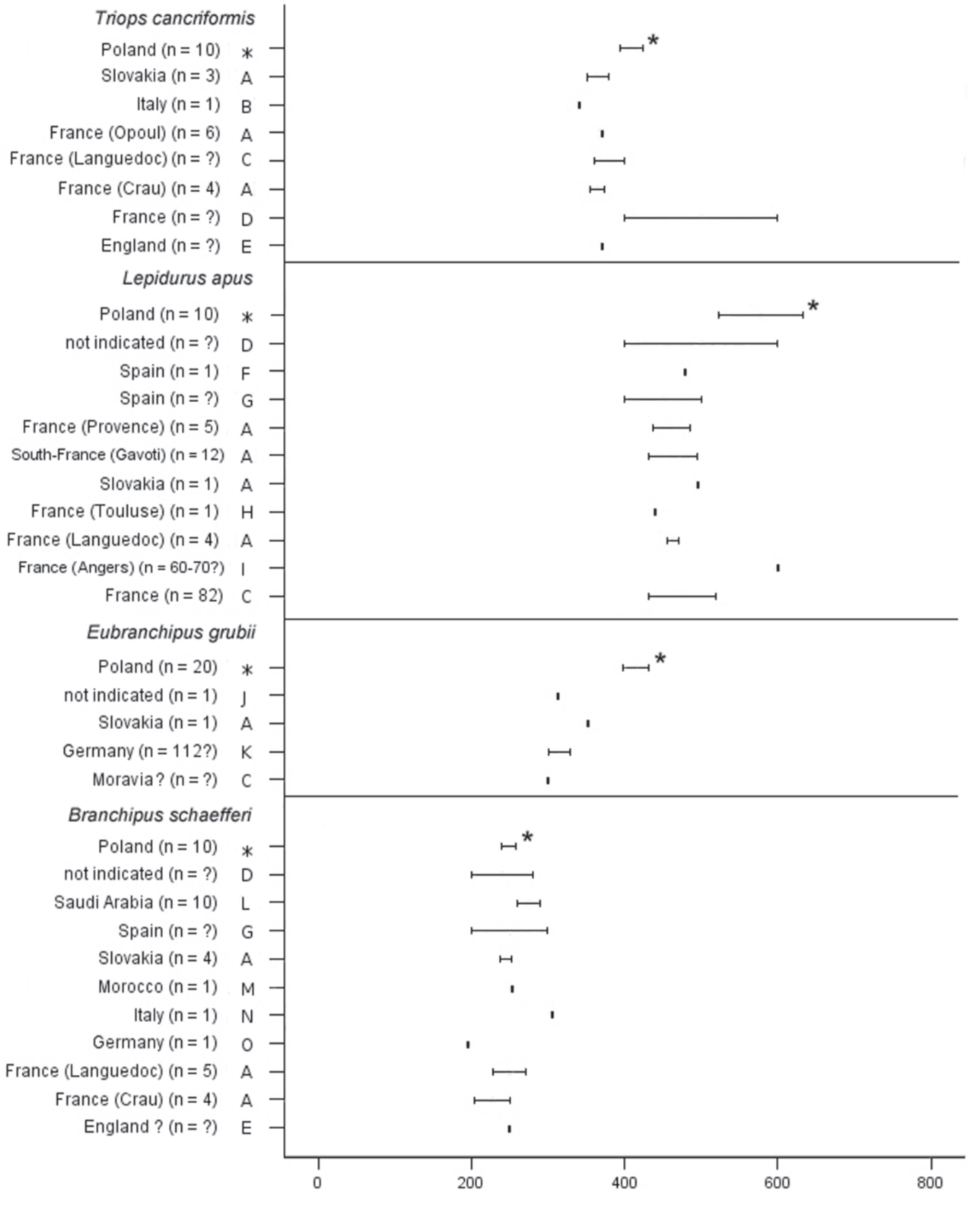

Fig. 2. Comparison of large branchiopod cyst diameter in this study (asterisks) with literature data. Letters = references to the papers cited: A = Thiéry et al. 1995, ,B = Tommasini et al. 1989, C = Thiéry & Gasc 1991, D = Mathias 1937, E = Gilchrist 1978, F = Alonso & Alcaraz 1984, G = Alonso 1985, H = Arnoult 1951, I = Reau De La Gaignonnière 1908, J = Mura 1992, K = Buchholz 1864, L = Thiéry 1995, M = Mura & Thiery 1986, N = Mura 1986, O = Kupka 1940. Only 4 species, where the differences were the most visible, are shown; *N* = number of cysts measured; all measurements in µm

Taking into account our results, we disagree with the statement that cyst morphology and diameter could be sufficient traits for species separation on a global scale (Thiery & Gasc 1991; Thiery et al. 1997). We agree that even within one species, cyst diameter and morphology in populations from different parts of the species range vary so much that these traits could be insufficient for determination below the level of genus or group of species (Mura 1986, 1991, 2001).

On the other hand, surveys in search for new localities of globally endangered large branchiopods are greatly impeded by the fact that occurrence of particular species is often restricted to a very short time. In such cases, data obtained on the basis of cysts present in the sediments are a valuable supplement to the observations of adult specimens. The presented results show that on a scale of a particular region (here: western Poland), relatively precise determination of species using the morphology of cysts obtained from the sediments of potential habitats is possible. It must be preceded, however, with examination of resting egg variability within the particular part of the species range. Cysts determined according to the keys created for a given region could be considered as a clue for subsequent, more detailed exploration of a particular locality. Without verification in adult specimens collected in nature or reared from the sediments in the laboratory, determination based solely on cyst morphology should be treated with caution.

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