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Intraspecific morphological diversity of anostracan resting eggs: *Chirocephalus ruffoi* Cottarelli & Mura, 1984 as a study case

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The extent of intraspecific morphological variability in the egg shells of anostracans, never previously assessed, was investigated in the 13 known *Chirocephalus ruffoi* populations, a species endemic to Italy. Twelve sampling sites were chosen in the Northern Apennines, with distances between neighbouring sampling sites ranging from a minimum of 23 m to a maximum of 63 km. Another site was located in the Calabrian Apennines at a distance between 643 and 703 km from the previous set of sampling points. The SEM (scanning electron microscopy) observations of 300 cysts per population revealed the existence of great morphological diversity for most of the analysed populations. Up to 12 different morphotypes were described, with highly variable frequencies among the populations. Outer and inner cyst diameters and height of the "ridges" ornamenting cyst surface were calculated on a randomly selected sub-sample of 391 cysts. Statistically significant differences among populations were found for all the measured characters. No relationships between either occurrence of morphotypes or morphometric profiles and characteristics of sampling sites were found. In addition, the amount of morphological variability of the cysts did not seem to depend on the distances between sites. The results of this study are discussed in the light of the debate on the taxonomic value of the anostracan cyst ornamentation and the factors that can be invoked to explain the presence of high intraspecific morphological diversity.

Key words: Chirocephalidae, *Chirocephalus ruffoi*, intraspecific morphological variability, scanning electron microscope, Northern Apennines, Calabrian Apennines.

INTRODUCTION

It is now accepted that the morphology of the "resting eggs" (also called cysts) is not always a reliable character in delineating species in Anostraca, in contrast to the conclusions drawn from previous scanning electron microscopy (SEM) studies (e.g. Cottarelli & Mura, 1984; Mura, 1986, 1991a, 1992a, 2001; Mura & Thiéry, 1986; Thiéry, 1986; Thiéry & Gasc, 1991; Hamer & Appleton, 1993; Thiéry & Fugate, 1994; Thiéry *et al*., 1995; Rabet & Thiéry, 1996; Belk *et al*., 1998; Thiéry & Champeau, 1998). This is due to the fact that a number of fairy shrimp species do not present constant cyst patterns. Many examples for the presence of a more or less marked intraspecific variability are available in the literature. The first observations showing significant differences in the height and development of the ridges ornamenting the cyst surface, depending on their geographical origin, were performed on *Chirocephalus diaphanus* populations from Italy (Mura, 1992b). About the same period, the existence of different "egg types" in the Streptocephalidae was reported when examining the cyst morphology of African populations of *Streptocephalus torvicornis torvicornis*, *S. vitreus*, *S. cafer*, *S. indistinctus*, and *S. macrourus*, among others (Brendonck *et al*., 1992; Brendonck & Coomans, 1994a, b).

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The previous studies concluded that the egg morphology does not seem to be free from ambiguities and, consequently, it is of limited taxonomic value. Similar results were obtained when analysing different cyst patterns in populations of *Drepanosurus hankoi* and *Chirocephalus josephinae* from several localities of Belarus (Nagorskaya *et al*., 1998). More recently, even more striking interpopulation differences in "egg" morphology were revealed from observations on a number of *Chirocephalus diaphanus carinatus* populations from the Balkans, with morphological patterns in ridges, spines, and bulges overlapping those found in three other *Chirocephalus* species from neighbouring areas (Mura *et al*., 2002).

Morphological variation has been recorded not only at the interpopulation but also at the individual level. For example, diverging egg morphologies were observed within the same clutch of a single individual of *Streptocephalus papillatus* (Brendonck & Coomans, 1994a), *Chirocephalus josephinae* (Nagorskaya *et al*., 1998), *S. torvicornis*, and *S. rubricaudatus* (Beladjal & Mertens, 2003). However, it should be noted that these studies were often based on the analysis of preserved museum specimens.

The aim of the present study was to gain more information on the degree of cyst variability in 13 populations of *Chirocephalus ruffoi*, a fairy shrimp species endemic to Italy with a rather fragmentary and disjunct distribution (Cottarelli & Mura, 1984; Rebecchi *et al*., 1990; Mura & Rossetti, 2002).

MATERIALS AND METHODS

Study sites and sampling

Figure 1 shows the distribution of the 13 localities from which anostracan samples were obtained. All sampling sites are in protected areas above the tree line, at altitudes ranging from 1595 to 1971 m a.s.l. Lago Scaffaiolo (SCF) is a permanent pond (surface area 11700 m^2, maximum depth 2.4 m) fed by rainfall and melting snow, without permanent surface inflows or outflows. The remaining sites are small and shallow temporary pools. Information on their geographic characteristics and distances is provided in Tables 1 and 2, respectively. The Piani del Pollino (PPO), in the Calabrian-Lucan Apennines, is the only studied pool not located in the Northern Apennines. Its distance from other sampling sites is between 643 and 703 km. Site codes used in the text are listed in Table 1.

Sampling was performed in summer 2003 and then repeated in 2005 and 2006. Anostracans were collected with a plankton net (15 cm in diameter, 125 μm mesh size). When present, an average of 20 gravid females per site were individually isolated in Falcon tubes to avoid loss or mixing of the eggs and brought

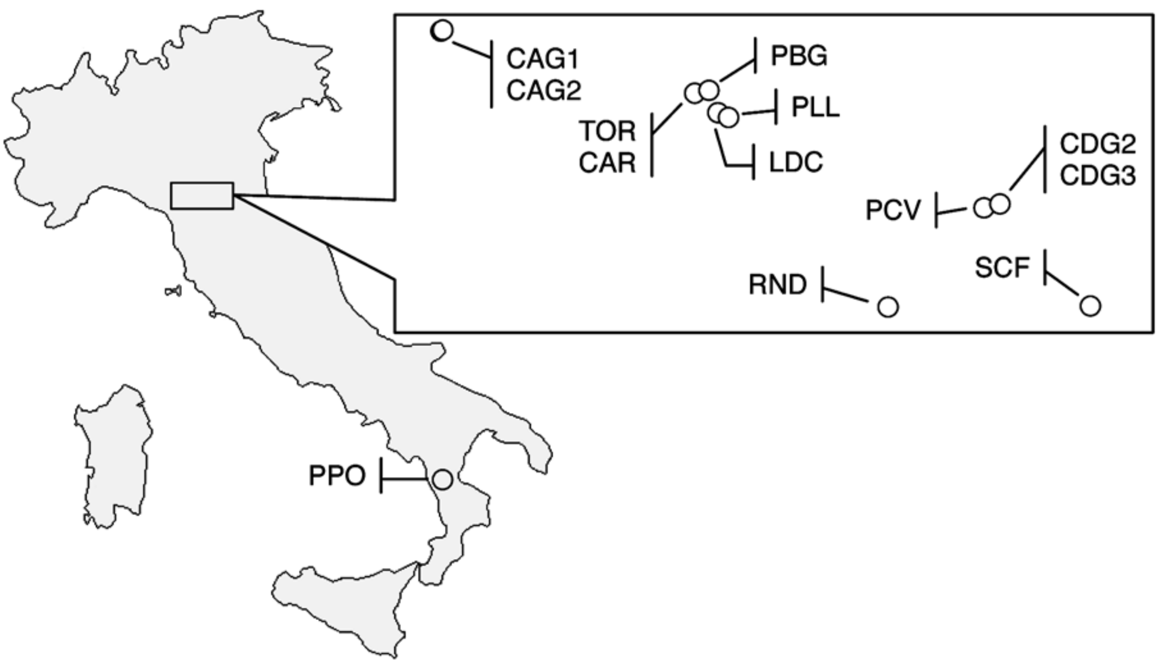

FIG. 1. Map of Italy and location of the study pools. Codes as in Table 1.

TABLE 1. Geographic characteristics of the pools included in this study

Code	Site name	m a.s.l.	N latitude	E longitude
CAG1	Cagnin 1	1605	44° 21′ 25″	10° 05′ 30″
CAG2	Cagnin 2	1595	44° 21′ 29″	10° 05′ 32″
CAR	Caricatore	1610	44° 18′ 08″	10° 22′ 23″
TOR	Torbiera 1 Prati Sara	1615	44° 18′ 08″	10° 22′ 24″
PBG	Piano Bagioletto	1754	44° 18′ 15″	10° 23′ 18″
LDC	Lagadello del Cusna	1971	44° 17′ 07″	10° 23′ 56″
PLL	Piella	1877	44° 16′ 51″	10° 24′ 35″
RND	Rondinaio	1776	44° 07′ 14″	10° 35′ 04″
PCV	Pian Cavallaro	1788	44° 12′ 06″	10° 41′ 36″
CDG2	Cresta di Gallo 2	1749	44° 12′ 17″	10° 42′ 40″
CDG3	Cresta di Gallo 3	1748	44° 12′ 16″	10° 42′ 40″
SCF	Lago Scaffaiolo	1775	44° 07′ 05″	10° 48′ 33″
PPO	Piani del Pollino	1781	39° 55′ 03″	16° 09′ 02″

TABLE 2. Distances (m) between pools located in the Northern Apennines (pool codes as in Table 1)

	CAG1	CAG2	CAR	TOR	PBG	LDC	PLL	RND	PCV	CDG2	CDG3	SCF
CAG1	–											
CAG2	131	–										
CAR	23246	23236	–									
TOR	23268	23258	23	–								
PBG	24371	24358	1238	332	–							
LDC	25762	25759	2792	2615	2261	–						
PLL	26738	26736	3770	3292	3104	996	–					
RND	47687	47718	26569	27919	25953	23797	22864	–				
PCV	51014	51014	27899	15124	26876	25273	24288	12068	–			
CDG2	52253	52251	29090	14607	28046	26496	25516	13312	1473	–		
CDG3	52267	52266	29106	14660	28062	26510	25530	13288	1467	37	–	
SCF	63151	63163	40410	27976	39475	37685	36690	17456	13125	12412	12382	–

alive to the laboratory where females were kept in culture until deposition.

SEM studies: morphology and morphometrics

The morphology of the resting eggs was analysed by examining samples of 300 cysts (100 cysts per population per year of sampling) from randomly collected clutches of freshly deposited cysts. The resulting cysts were harvested, rinsed in distilled water and carefully checked under a stereomicroscope to visually assess their condition.

The material was mounted on stubs, gold-coated (see Mura, 1992a for a detailed description of SEM cyst preparation), and photographed using an EVO LEO 040 electron microscope. All SEM observations were performed exactly under the same light conditions in order to avoid undesired effects (distortions) on the images to be analyzed.

The images obtained were used to characterize cyst morphotypes. In a sub-sample of 391 randomly selected images (from a minimun of 20 to a maximum of 35 per population), outer and inner cyst diameters and height of the "ridges" ornamenting cyst surface were calculated using as reference the scale appearing on the SEM photographs. We considered as outer diameter (od) the cyst diameter including ornamentations, whereas with inner diameter (id) we referred to the cyst surface only. The height of the "ridges" (hr) was measured by considering the peripheral area of the egg and excluding the centre, i.e. where the ridges are perpendicular to the image.

Statistics

Distributions of cyst measurements (od, id, and hr) were tested for normality using the Shapiro-Wilk test for each population. Because this test showed that in most cases the data were not normally distributed, the Kruskal-Wallis non-parametric test was used to compare the medians between populations, followed by *post-hoc* Mann-Whitney tests for pairwise comparisons to determine which paired samples were significantly different ($p < 0.05$ Bonferroni-corrected for multiple comparisons, $\alpha = 0.00064$). All statistical analyses were performed using the program PAST ver. 1.06 (Hammer *et al*., 2001).

RESULTS

As first described in Cottarelli & Mura (1984), the resting eggs of *C. ruffoi* have a spiny ("hedgehog") appearance, due to the presence on their surface of thin curved jagged crests (flanges) ending in several single, bifurcate or trifurcate spines (see Fig. d in Cottarelli & Mura, 1984). However, when considered in detail, the eggs show marked variation not only in the height and the pattern of flanges, but also in the extent of intersections at their base, their density (number of crests per surface unit), the width of the irregular polygonal fields delimited on the egg surface, and the ratio between crests and egg diameter. Based on these characters, 12 morphotypes were identified and described as follows (Figs 2-13).

Morphotype 1 (Fig. 2): almost no crests or very low crests; dense single or multiple short spines, emerging from the cyst surface.

Morphotype 2 (Fig. 3): similar to type 1, but sparse short spines emerging from the surface.

Morphotype 3 (Fig. 4): spiny appearance; very densely interconnected low flanges ending in long, mostly bifurcate or trifurcate spines; irregular polygonal fields not very evident.

Morphotype 4 (Fig. 5): "lace-like" appearance; thin, densely interconnected jagged flanges ending in long spines, delineating expanded, more regular polygonal fields; inner diameter large compared with the total diameter.

Morphotype 5 (Fig. 6): similar to morphotype 4, but smaller and scarcely delineated polygonal fields; less jagged crests ending in shorter spines; inner diameter large with respect to the total diameter.

Morphotype 6 (Fig. 7): conspicuous spiny appearance; very long, serrated ridges (compared with the inner diameter of the egg), interconnected and ending in spines of irregular height, plus some very long single spines.

Morphotype 7 (Fig. 8): "Chinese lantern" aspect; highly developed interconnected crests of almost constant height, ending in a rather jagged edge; well evident, irregular polygonal fields.

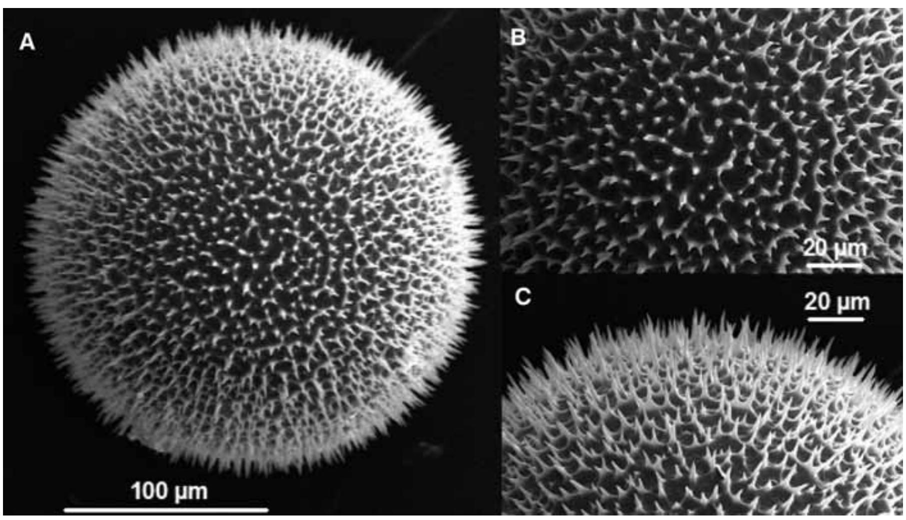

FIG. 2. Morphotype 1: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

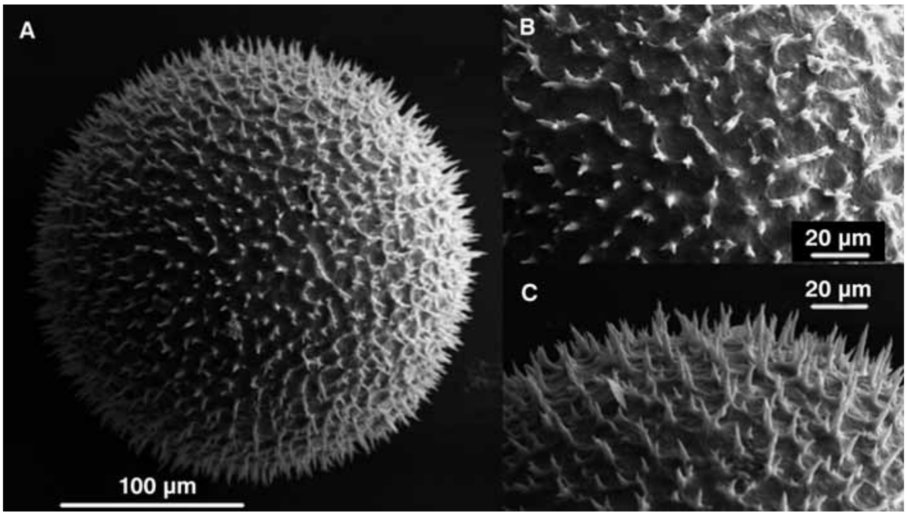

FIG. 3. Morphotype 2: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

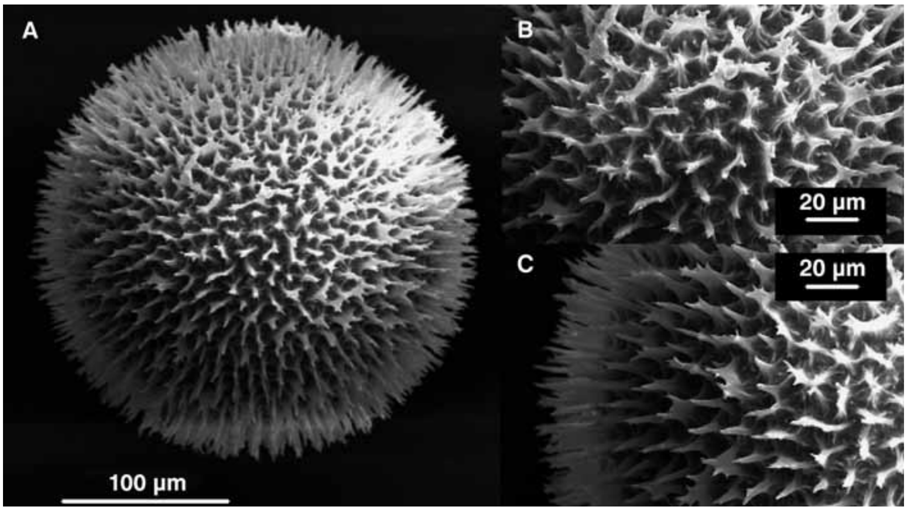

FIG. 4. Morphotype 3: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

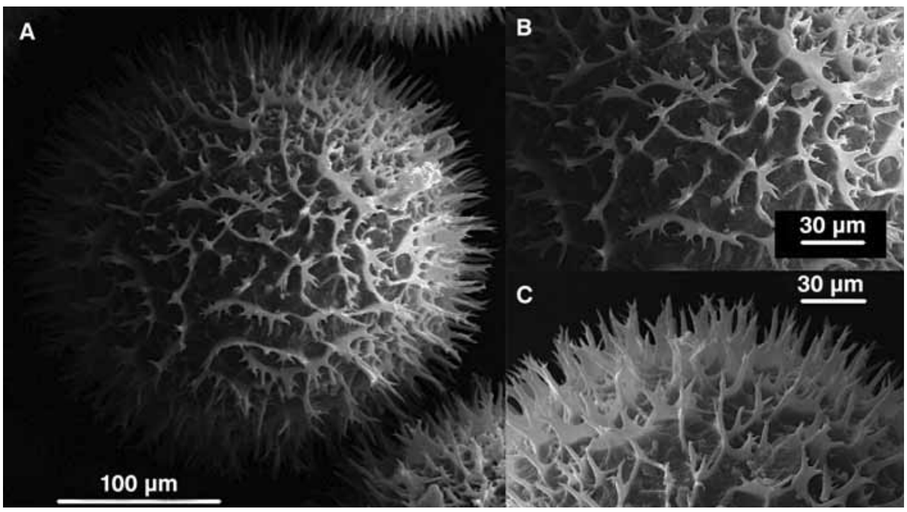

FIG. 5. Morphotype 4: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

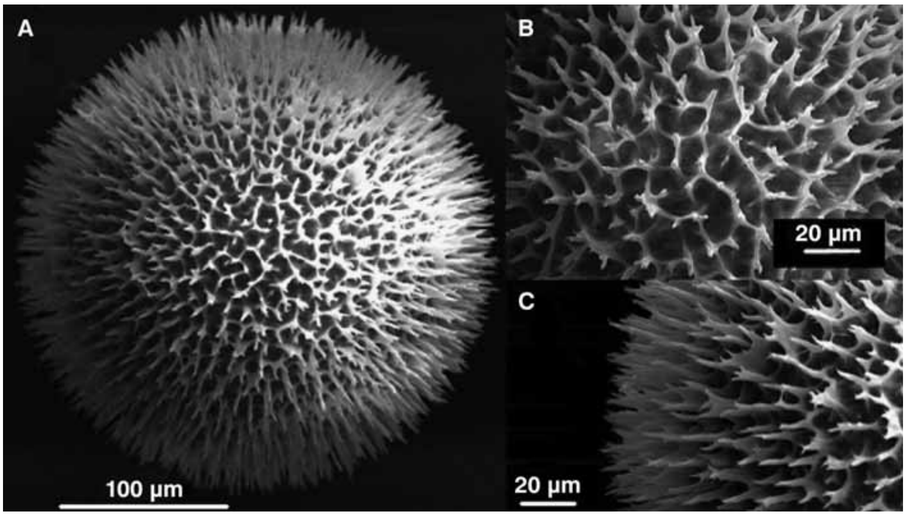

FIG. 6. Morphotype 5: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

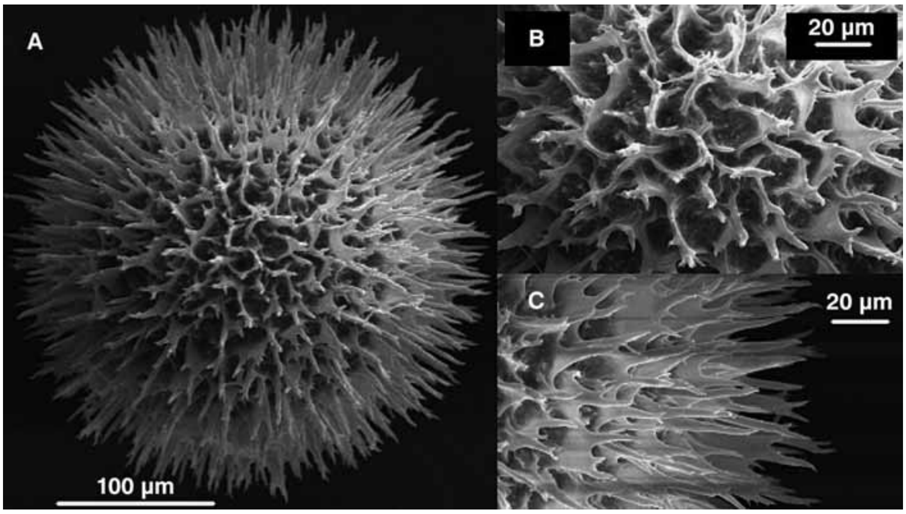

FIG. 7. Morphotype 6: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

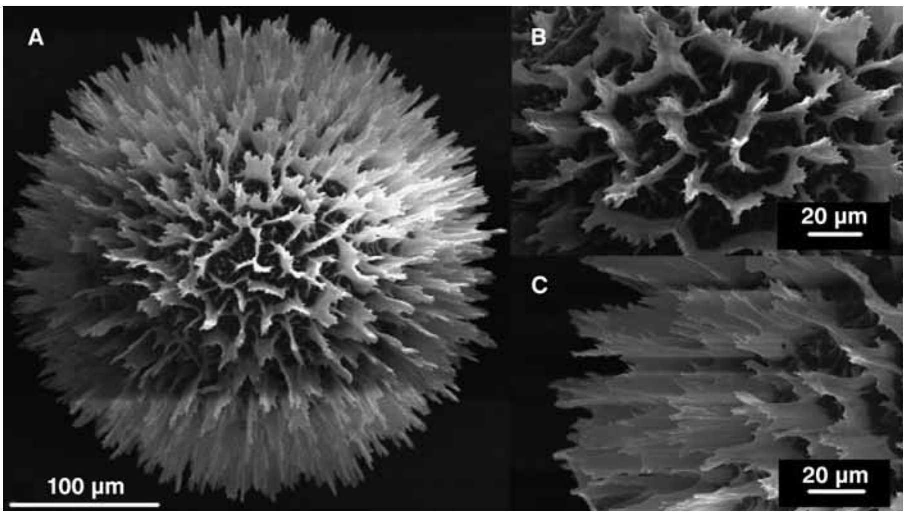

FIG. 8. Morphotype 7: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

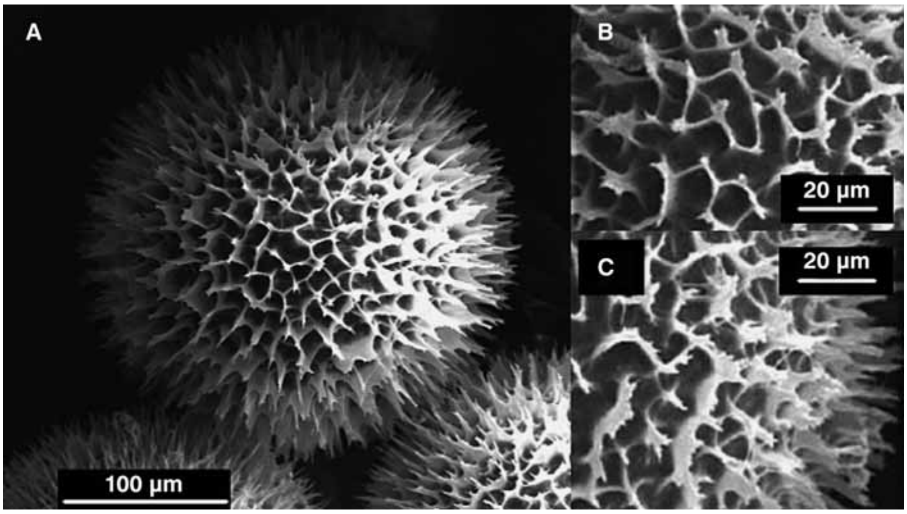

FIG. 9. Morphotype 8: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

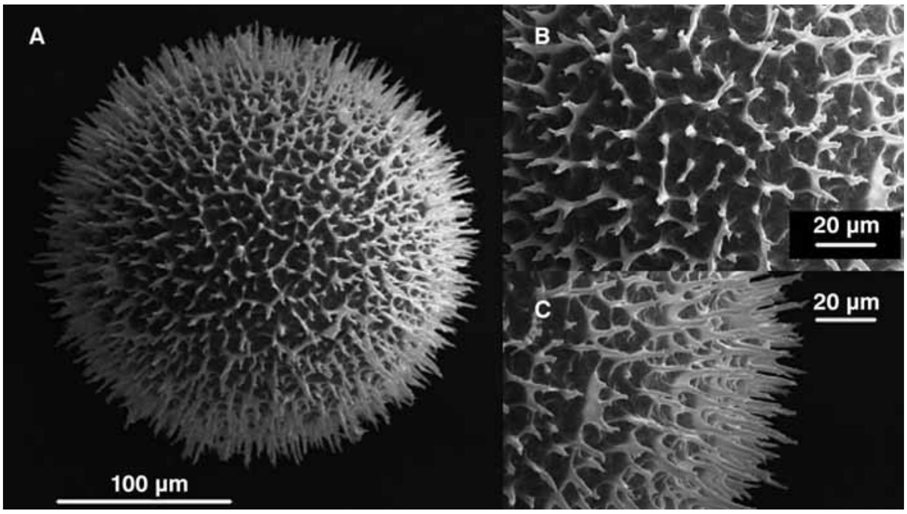

FIG. 10. Morphotype 9: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

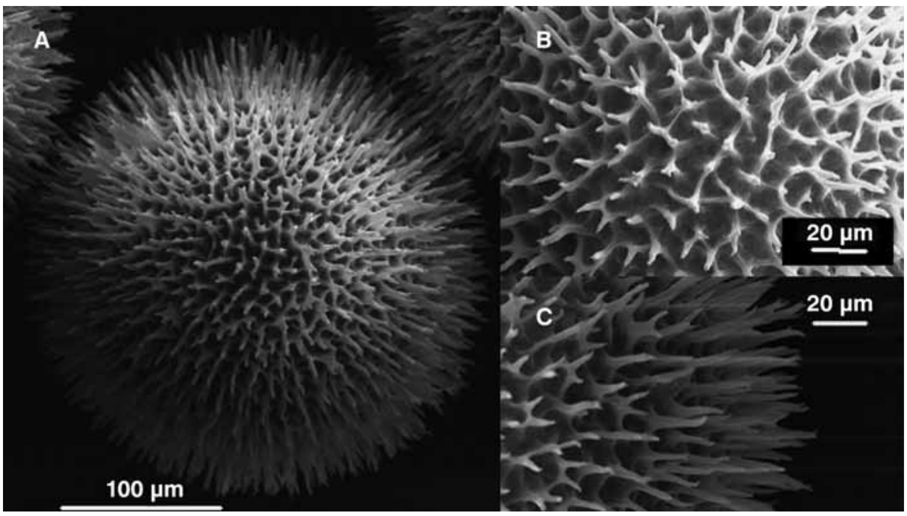

FIG. 11. Morphotype 10: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

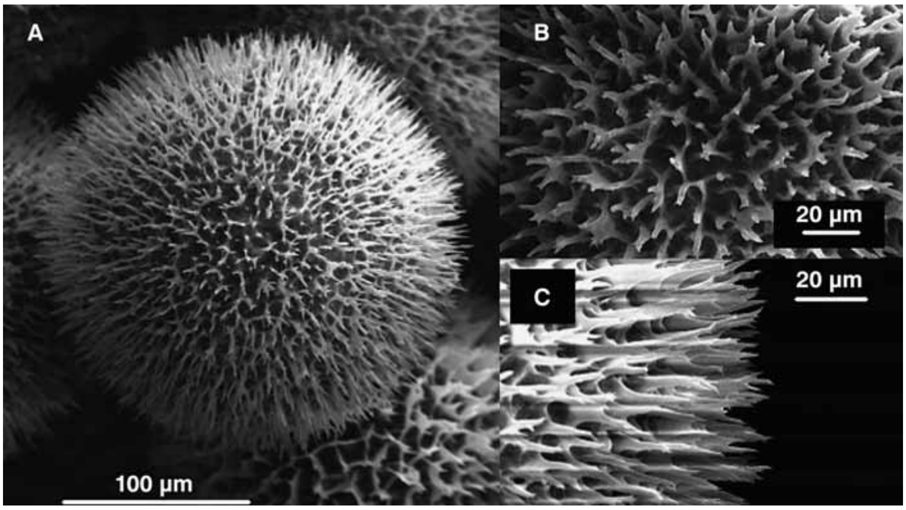

FIG. 12. Morphotype 11: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

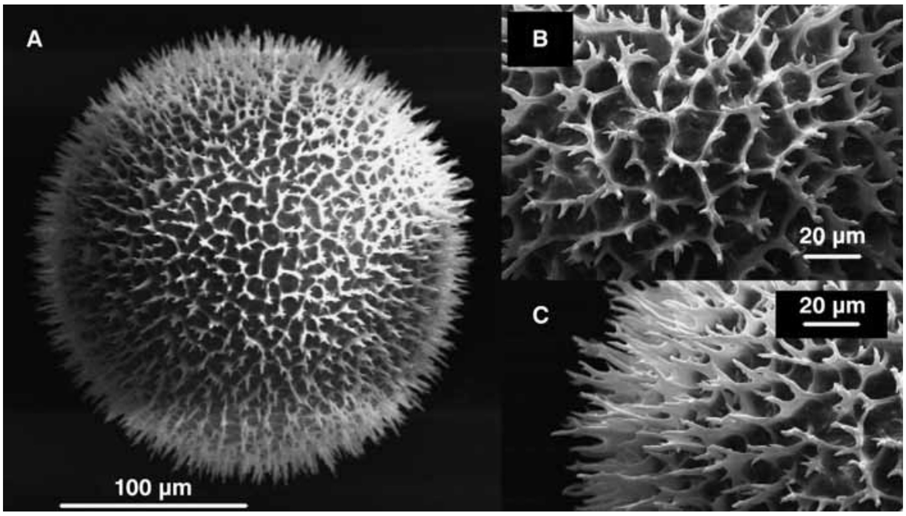

FIG. 13. Morphotype 12: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

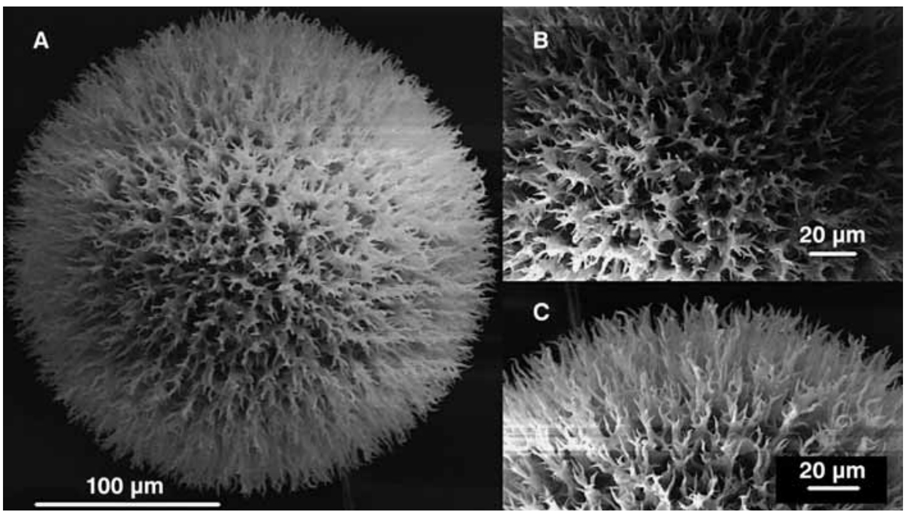

FIG. 14. Rare morphotype found in a single cyst of *Chirocephalus ruffoi* from CAG2: egg *in toto* (A), detail of the central area (B), detail of the peripheral area (C).

Morphotype 8 (Fig. 9): honeycomb pattern; inner diameter small compared with the total diameter; well-defined polygonal fields.

Morphotype 9 (Fig. 10): similar to morphotype 1, but more sparsely interconnected flanges terminating in slightly longer, single or bifurcate spines.

Morphotype 10 (Fig. 11): similar to morphotype 3, but more dense ridges ending in higher spines.

Morphotype 11 (Fig. 12): pattern intermediate between morphotypes 4 and 5, with a marked spiny aspect; thin jagged crests more dense than in morphotype 4 but looser than in morphotype 5.

Morphotype 12 (Fig. 13): similar to morphotype 4, but with low, thin jagged crests, ending in short spines.

An additional morphotype, first illustrated here (Fig. 14), has only been found in a single cyst from CAG2 during a previous sampling campaign.

Table 3 summarizes the frequency of the identified morphotypes in the 13 populations examined. It can be seen that none of the morphotypes was found in all populations. Some populations, e.g. LDC (one morphotype, M1) or CAR and PPO (two morphotypes) were relatively homogeneous, whereas others, as CAG2 (five morphotypes) or CAG1, PLL, and SCF (four morphotypes), exhibited a much higher variability in cyst morphology. Morphotypes 5 and 11 were by far the most frequent ones, occurring in ten and seven populations and accounting for 50 and 10% of the total cysts, respectively. Other morphotypes, for example morphotypes 2 (1% of total cysts, only in SCF) and 7 (1.1% of total cysts, in PBG and PLL), were rather rare.

The results of the measurements of inner and outer diameter and height of the ridges ornamenting the cyst surface are reported in Table 4 and Figure 15. The Kruskal-Wallis test for medians revealed statistically significant differences ($p < 0.01$) among populations for all the measured characters. The cysts of the PBG population had the largest size of outer diameter, whereas those of PCV and LDC were characterized by the smallest values. The *post-hoc* pairwise comparisons revealed that PBG was the only population significantly different from all other populations. The most marked intrapopulation variability was observed in PPO and RND; on the contrary, the range was markedly narrower in CAG1, CAR, and TOR. The largest inner diameters were found in TOR, CAR, CDG2, PLL, and SCF; the highest variances were seen in CDG2 and PBG. As it is obvious from Figure 15, inner diameters showed less interpopulation variability than outer diameters and height of the spines. Only 17 out of 78 *post-hoc* pairwise comparisons were significant, and 10 pairs contained the TOR population. Finally, PLL and PCV were characterized by the lowest ridges, whereas PBG, LDC, and CDG3 showed the highest ridges; the maximum variability was observed in PBG and PPO. Regarding outer diameters, PBG was the only population significantly different

TABLE 3. Frequency (%) of the different morphotypes (M1-M12) in the 13 *Chirocephalus ruffoi* populations examined in this study

	CAG1	CAG2	CAR	CDG2	CDG3	LDC	PBG	PCV	PLL	PPO	RND	SCF	TOR
M1		7				100		59	31				
M2												14	
M3	12	12		8								6	
M4					9							12	
M5	48	37	86	12	54				1	80	87	68	78
M6							57						
M7							18		5				
M8								25			7		
M9	33	24											
M10		20	14						63				
M11	7			80			25	16		20	6		22
M12					37								

from all others; the same is true for the height of spines.

No relationship between occurrence of morphotypes and characteristics of sampling sites (e.g. altitude and hydroperiod) was found. Also, the amount of morphological variability of the cysts did not seem to depend on the distances among sites (Table 2, Fig. 1). It can be noted that pairs of distant pools (for example CAG1 and CDG3 or CAG1 and SCF) often share a higher number of morphotypes than closer ones (for example, CAR and TOR or LDC and PLL). A similar lack of relationship was also evident when comparing the morphometric characteristics of the cysts and the linear distances of the sampling sites (Tables 2 and 4).

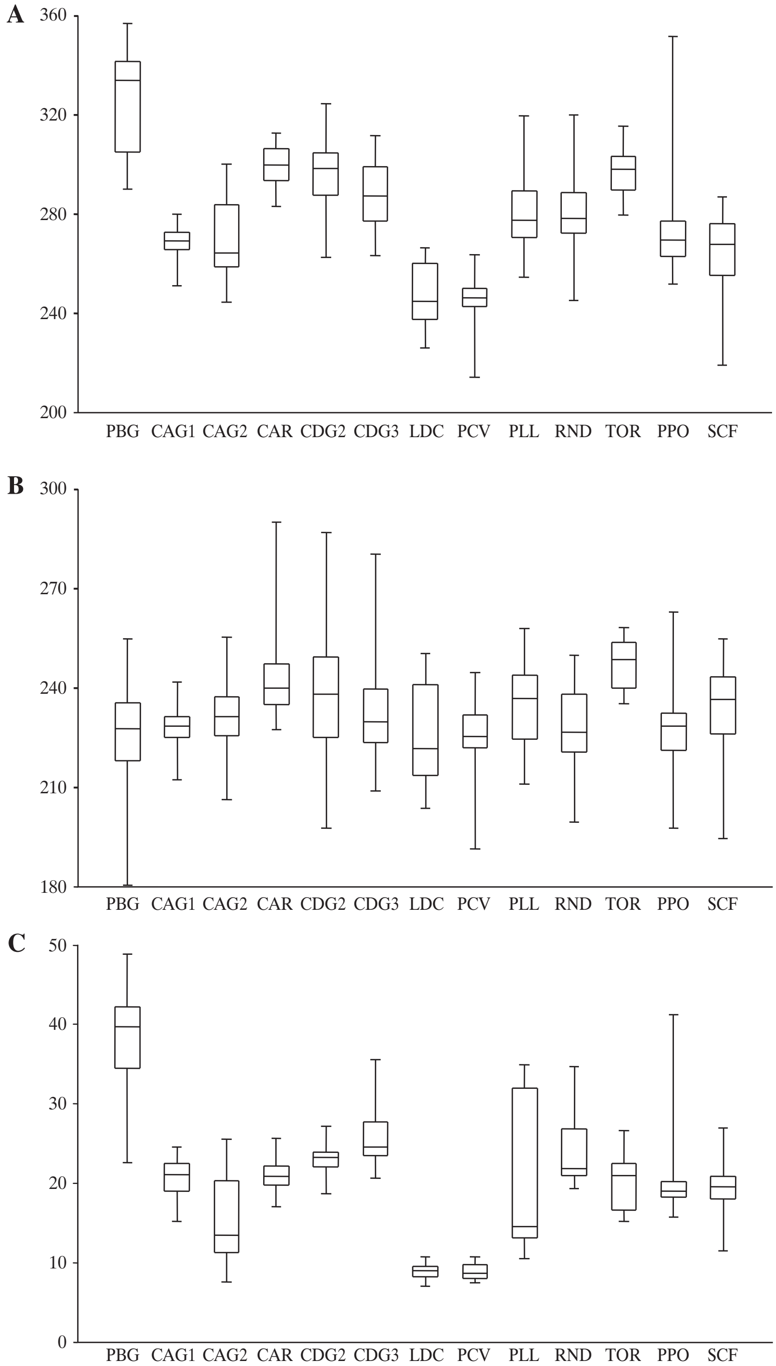

FIG. 15. Box plots showing the median (horizontal bar), interquartile range (box), and full range (whiskers) of the cyst outer diameters (A), inner diameters (B), and height of the ridges (C). All values in µm. Pool codes as in Table 1.

TABLE 4. Summary of cyst measurements (μm) (od: outer diameter; id: inner diameter; hr: height of ridges) and associated descriptive statistics for the 13 populations investigated (between brackets: number of examined cysts for each population)

	PBG (31)			CAG1 (28)			CAG1 (30)			CAR (28)			CDG2 (32)			CDG3 (32)			LDC (20)		
	od	id	hr	od	id	hr	od	id	hr	od	id	hr	od	id	hr	od	id	hr	od	id	hr
min	290.17	180.65	22.63	250.96	212.25	15.27	244.47	206.24	7.63	283.04	227.56	17.10	262.65	197.86	18.75	263.46	208.97	20.68	226.25	203.75	7.04
max	356.77	254.84	48.83	279.99	241.94	24.55	300.00	255.32	25.56	312.81	289.99	25.60	324.36	287.06	27.20	311.69	280.32	35.50	266.50	250.50	10.72
mean	326.20	226.30	37.32	268.71	228.22	20.83	269.53	230.27	15.10	298.90	243.60	20.98	295.97	237.48	23.13	287.45	232.86	25.87	247.70	226.60	8.94
s. e.	3.53	2.92	1.29	1.22	1.25	0.45	2.76	2.21	0.98	1.57	2.78	0.35	2.88	3.46	0.31	2.26	2.42	0.63	2.80	3.33	0.24
variance	387.27	263.49	51.41	42.00	43.55	5.65	227.75	146.86	29.02	69.31	216.55	3.51	265.79	382.34	3.13	162.87	188.08	12.66	156.74	221.21	1.15
s. d.	19.68	16.23	7.17	6.48	6.60	2.38	15.09	12.12	5.39	8.33	14.72	1.87	16.30	19.55	1.77	12.76	13.71	3.56	12.52	14.87	1.07
median	333.83	227.74	39.70	269.35	228.45	21.10	264.52	231.38	13.46	300.00	240.00	20.86	298.53	238.23	23.28	287.42	229.77	24.57	244.92	221.79	9.04
skewness	–0.36	–0.64	–0.67	–0.67	–0.14	–0.34	0.38	–0.16	0.58	–0.16	2.04	0.31	–0.41	0.11	–0.05	–0.03	1.21	0.87	0.12	0.29	–0.19
kurtosis	–1.27	0.74	–0.71	0.58	0.26	–0.60	–1.04	–0.15	–0.96	–1.16	4.03	–0.05	–0.52	–0.12	0.45	–1.05	2.32	–0.05	–1.42	–1.45	–0.95
geom. mean	325.61	225.71	36.56	268.64	228.13	20.69	269.13	229.95	14.22	298.79	243.20	20.90	295.53	236.70	23.06	287.17	232.48	25.65	247.41	226.14	8.87

TABLE 4 (cont.)

	PCV (31)			PLL (31)			RND (30)			TOR (31)			PPO (32)			SCF (35)		
	od	id	hr	od	id	hr	od	id	hr	od	id	hr	od	id	hr	od	id	hr
min	214.29	191.56	7.48	254.68	210.96	10.59	245.19	199.61	19.40	279.78	235.43	15.23	251.88	197.65	15.78	219.04	194.70	11.50
max	263.64	244.81	10.79	319.79	257.87	34.92	320.00	250.00	34.68	315.65	258.26	26.65	351.77	262.94	41.21	287.06	254.87	26.94
mean	246.51	225.79	8.83	281.63	235.15	19.07	283.15	227.77	24.21	296.40	247.32	20.27	273.90	227.20	20.90	266.44	234.96	19.11
s. e.	1.64	1.72	0.17	2.86	2.15	1.62	3.52	2.28	0.86	1.53	1.29	0.60	3.83	2.02	1.02	2.37	2.15	0.51
variance	83.86	92.03	0.91	254.27	142.85	81.14	372.34	155.52	22.11	72.76	51.59	11.17	470.43	130.31	33.35	196.71	161.19	9.24
s. d.	9.16	9.59	0.95	15.95	11.95	9.01	19.30	12.47	4.70	8.53	7.18	3.34	21.69	11.42	5.78	14.03	12.70	3.04
median	246.10	225.32	8.74	277.66	236.81	14.58	278.29	226.79	21.80	298.04	248.48	20.94	269.65	228.53	19.03	267.71	236.51	19.56
skewness	–1.01	–1.03	0.41	0.61	–0.16	0.87	0.60	–0.18	1.06	–0.05	–0.07	–0.01	2.36	0.38	2.48	–0.99	–0.77	–0.28
kurtosis	3.07	3.34	–0.97	–0.38	–0.91	–1.15	–0.50	–0.68	–0.39	–0.75	–1.43	–1.25	5.65	2.02	5.61	1.52	0.92	0.42
geom. mean	246.34	225.58	8.78	281.20	234.86	17.37	282.53	227.43	23.81	296.28	247.22	20.00	273.16	226.93	20.35	266.07	234.61	18.86

DISCUSSION

The present investigation provides evidence for extreme variability in the morphology of the resting eggs of *C. ruffoi*, as previously described in several papers, in particular by Mura & Rossetti (2002). The already known presence of some morphotypes, for example M1 in LDC, M5 in PPL, M2 in SCF, and M3 in CAG1 (see figures in Mura & Rossetti, 2002), is confirmed by the new observations, although many additional patterns are described here. Furthermore, two morphotypes observed by Zarattini *et al*. (2001) (M1 for RND) and by Mura & Rossetti (2002) (M3 for RND and TOR), have not been found in the present study despite the fact that a much larger number of cysts were examined. This means, most likely, that the variability might be much higher than that reported. It is also worthy to note the existence of a surprisingly divergent morphology described for a single cyst from CAG2 (Fig. 14).

The intraspecific variation in anostracan cyst morphology has never been investigated in the same detail as in the case of *C. ruffoi*. Former studies on this subject were unable to draw definite conclusions about the intraspecific specificity of egg morphology. Brendonck & Coomans (1994a, b) noted that the variability recorded in a *Streptocephalus proboscideus* population from Sudan was higher than that found in populations of this species from different geographical regions, but these results were based on a small number (< 10) of cysts. Similar conclusions were drawn by Beladjal & Mertens (2003), also studying *Streptocephalus* species from Algeria and Tunisia. These authors even recorded variation at the individual level, in agreement with the results by Mura & Zarattini (1999) for *C. ruffoi* and by Nagorskaya *et al*. (1998) for *C. josephinae*.

It is still to be ascertained whether parallel intraspecific or individually-based variations are expressed also in the appendages of the adults, i.e. the characters that are commonly used in the taxonomic identification, since studies on this subject are scarce. Petkowski (1991) and subsequently Marinček & Petrov (1995) reported intraspecific differences in adults of *C. brevipalpis* and *C. diaphanus* from the Balkans, interpreted as "different geographical and ecological forms", but unfortunately the investigation did not consider the cyst morphology. On the other hand, preliminary observations on *C. ruffoi* by Zarattini *et al*. (2001) reported interpopulation differences in egg morphology that were unrelated to clear differences in the morphological characters of adult males; however, only two populations were examined in that study.

Miličić and Petrov (2009) analysed the morphological patterns of the eggs in a number of *Branchipus* populations from the Pannonian plain, aiming to define their taxonomical position. The cyst morphologies of different populations overlapped to a certain extent, although the observed differences supported a statistically significant separation of geographic groups previously recorded in the adults (Miličić, 2007). At the moment, the meaning of these findings remains rather obscure. It can be hypothesised that such differences are ascribable to the geographical origin of the populations examined, in particular to the presence of a contact area between the distributional ranges of two *Branchipus* species (*B. schaefferi* and *B. intermedius*), and hence to the existence of "transitional populations".

Such a hypothesis does not seem to hold for the morphological intrapopulation variability recorded for populations of *C. diaphanus carinatus* from the Balkans (Mura *et al*., 2002), which seems to be unrelated to their geographical location. Whatever the explanation, all of the above studies did not consider in detail also intrapopulation variation, both in the eggs and in the adults.

A next step of our research will be to examine also the adults of the populations we investigated, in order to ascertain whether the morphological plasticity observed in eggs is expressed in some characters of the adults as well.

Differently from other studies (e.g. Belk, 1977; Belk *et al*., 1990; Mura, 1991b), our results showed that not only cyst size, but also cyst morphology seem to be unrelated to the biotope characteristics considered in the present study. It cannot be ruled out, however, that other environmental factors may be responsible for these differences, for example the physicochemical composition of water. In any case, it is still rather enigmatic the remarkable morphological heterogeneity observed in apparently similar nearby sites or even within the same population.

A parallel investigation on the genetic diversity of individuals of the same populations considered in the present study is now in progress. Comparing morphological and genetic diversity will hopefully contribute to a better understanding of the spatial patterns observed. Such an approach will also help evaluate our results in a quantitative way, therefore overcoming the limitations of the qualitative approach presented here.

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REFERENCES

Beladjal L, Mertens J, 2003. Interstitial remains for fauna reconstruction of desert pools using fairy shrimps as example (Anostraca). *Journal of Crustacean Biology*, 23: 60-68.

Belk D, 1977. Zoogeography of the Arizona fairy shrimps (Crustacea, Anostraca). *Journal of the Arizona-Nevada Academy of Science*, 12: 70-78.

Belk D, Anderson G, Hsu S-Y, 1990. Additional observations on variation in egg size among populations of *Streptocephalus seali* (Anostraca). *Journal of Crustacean Biology*, 10: 128-133.

Belk D, Mura G, Weeks S, 1998. Untangling confusion between *Eubranchipus vernalis* and *Eubranchipus neglectus* (Branchiopoda: Anostraca). *Journal of Crustacean Biology*, 18: 147-152.

Brendonck L, Coomans A, 1994a. Egg morphology in African Streptocephalidae (Crustacea, Branchiopoda, Anostraca). Part I. South of Zambezi and Kunene rivers and Madagascar. *Archiv für Hydrobiologie*, 99: 313-334.

Brendonck L, Coomans A, 1994b. Egg morphology in African Streptocephalidae (Crustacea, Branchiopoda, Anostraca). Part II. North of Zambezi and Kunene rivers. *Archiv für Hydrobiologie*, 99: 335-356.

Brendonck L, Hamer M, Thiéry A, 1992. Occurrence of tetrahedral eggs in the Streptocephalidae Daday (Branchiopoda, Anostraca) with descriptions of a new subgenus, *Parastreptocephalus*, and a new species, *Streptocephalus* (*Parastreptocephalus*) *zuluensis* Brendonck & Hamer. *Journal of Crustacean Biology*, 12: 282-297.

Cottarelli V, Mura G, 1984. *Chirocephalus ruffoi* n.sp. (Crustacea, Euphyllopoda, Anostraca) di acque astatiche d'alta quota dell'Appennino calabro-lucano. *Bollettino del Museo Civico di Storia Naturale di Verona*, 2: 515-523.

Hamer ML, Appleton CC, 1993. Four new *Streptocephalus* (Crustacea, Branchiopoda, Anostraca) species from South-eastern Africa. *South Africa Museum*, 103: 167-181.

Hammer Ø, Harper DAT, Ryan PD, 2001. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4: 9.

Marinček M, Petrov B, 1995. Contribution to the taxonomy of species of the *diaphanus* group within the genus *Chirocephalus* Prévost, 1803 (Anostraca, Crustacea). *Hydrobiologia*, 298: 233-237.

Miličić D, 2007. Morphological variability and taxonomic status of the genus *Branchipus* Schaeffer, 1766 (Branchiopoda, Crustacea) in Serbia. Ph. D. Thesis, University of Belgrade.

Miličić D, Petrov B, 2009. SEM morphological analysis of fertilized cysts in the genus *Branchipus* (Branchiopoda, Anostraca). Can cyst morphology be used as a reliable taxonomic character? *Archives of Biological Sciences Belgrade*, 61: 563-569.

Mura G, 1986. SEM morphological survey on the egg shell in the Italian anostracans (Crustacea, Branchiopoda). *Hydrobiologia*, 134: 273-286.

Mura G, 1991a. SEM morphology of resting eggs in the species of the genus *Branchinecta* from North America. *Journal of Crustacean Biology*, 11: 432-436.

Mura G, 1991b. Additional remarks on cyst morphometrics in anostracans and its significance. Part I: Egg size. *Crustaceana*, 61: 241-252.

Mura G, 1992a. Pattern of egg shell morphology in thamnocephalids and streptocephalids of the New World (Anostraca). *Crustaceana*, 62: 300-311.

Mura G, 1992b. Additional remarks on cyst morphometrics in anostracans and its significance. Part II: Egg morphology. *Crustaceana*, 63: 225-246.

Mura G, 2001. Morphological diversity of the resting eggs in the anostracan genus *Chirocephalus* (Crustacea, Branchiopoda). *Hydrobiologia*, 450: 173-185.

Mura G, Thiéry A, 1986. Taxonomical significance of scanning electron microscope morphology of the euphyllopods resting eggs from Morocco: part I. Anostraca. *Vie et Milieu*, 36: 125-131.

Mura G, Zarattini P, 1999. Influence of parental rearing conditions on cyst production and hatching of *Chirocephalus ruffoi*, an endemic fairy shrimp from Italy. *Crustaceana*, 72: 449-465.

Mura G, Rossetti G, 2002. On the rediscovery of *Chirocephalus ruffoi* Cottarelli & Mura, 1984 in temporary pools of the Secchia and Panaro valleys (Tuscan-Emilian Apennines, Northern Italy) (Branchiopoda, Anostraca). *Crustaceana*, 75: 969-977.

Mura G, Zarattini P, Petkowski S, 2002. Morphological divergences and similarities among *Chirocephalus diaphanus carinatus* populations (Crustacea, Anostraca) from the Balkan area. *Journal of Crustacean Biology*, 22: 162-172.

Nagorskaya L, Brtek J, Mura G, 1998. Notes on the occurrence of Anostraca in the Republic of Belarus. *Hydrobiologia*, 367: 21-30.

Petkowski ST, 1991. The fairy shrimp *Chirocephalus brevipalpis* (Orghidan, 1953), (Crustacea: Anostraca) in Yugoslavia. *Hydrobiologia*, 212: 261-266.

Rabet N, Thiéry A, 1996. The neotropical genus *Dendrocephalus* (Crustacea, Anostraca, Thamnocephalidae) in Brazil (South America) with a description of two new species. *Journal of Natural History*, 430: 479-503.

Rebecchi L, Leonardi S, Sabatini MA, 1990. Presenza di *Chirocephalus ruffoi* Cottarelli & Mura, 1984 (Crustacea, Anostraca) nell'Appennino tosco-emiliano e descrizione del cariotipo. *Rivista di Idrobiologia*, 29: 587-595.

Thiéry A, 1986. *Linderiella africana* n.sp. premier représentant des Linderiellidae (Branchiopoda, Anostraca) en Afrique du Nord (Maroc). *Crustaceana*, 51: 155-162.

Thiéry A, Gasc C, 1991. Resting eggs of Anostraca, Notostraca and Spinicaudata (Crustacea, Branchiopoda) occurring in France: identification and taxonomical value. *Hydrobiologia*, 212: 245-259.

Thiéry A, Fugate M, 1994. A new American fairy shrimp *Linderiella santarosae* (Crustacea, Anostraca, Linderiellidae) from vernal pools of California, U.S.A. *Proceedings of the Biological Society of Washington*, 107: 641-656.

Thiéry A, Champeau A, 1998. *Linderiella massaliensis*, new species (Anostraca: Linderiellidae), a fairy shrimp from southeastern France, its ecology and distribution. *Journal of Crustacean Biology*, 8: 70-78.

Thiéry A, Brtek J, Gasc C, 1995. Cyst morphology of European branchiopods (Crustacea: Anostraca, Notostraca, Spinicaudata, Laevicaudata). *Bulletin du Musée d'Histoire Naturelle de Paris*, 17: 107-139.

Zarattini P, Ketmaier V, De Matthaeis E, Mura G, 2001. Phenotypic and genotypic variation in the two known populations of *Chirocephalus ruffoi* Cottarelli & Mura, 1984 (Anostraca), endemic to Italy. *Crustaceana*, 74: 377-388.