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A comparative SEM morphological study on the egg shell in some Anostracans (Crustacea: Branchiopoda) from East Azerbaijan Province of Iran

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Abstract: The surface morphology of resting eggs of Anostracan species from East Azerbaijan Province of Iran and that of *Artemia urmiana* was compared using Scanning Electron Microscopy (SEM). Anostracan fauna of East Azerbaijan temporary pools were determined as follows:
Chirocephalus skorikowi (Chirocephalidae), *Branchinecta orientalis* (Branchinectidae), *Streptocephalus torvicornis* (Streptocephalidae), *Branchinella spinosa* (Thamnocephalidae) and *Artemia urmiana* (Artemiidae). However other species may also exist in these pools. Therefore, evaluating the morphology of these cysts by SEM appeared to accentuate that study of cyst ornamentation is a valuable taxonomical factor and more importantly that it can be used to identify the species even when the adults are not present at the time of sampling or in the absence of water.

Keywords: Egg Morphology, SEM, Anostraca (Fairy Shrimps), East Azerbaijan, Iran

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Introduction

Fairy shrimps (Anostraca) are found in vernal pools around Tabriz city as a unique crustacean, which swim upside down. They produce cyst instead of egg that is necessary to cope with fluctuating and unpredictable changes to their habitats (Mura, 2001). This enables them to tolerate alterations in temperature, oxygen level, pH, salinity and turbidity and protect themselves from natural predators.

Egg shell morphology studies on Crustacea "Anostraca" have received little attention, especially on the species present in East Azerbaijan province of Iran. As already noted on most studies associated with the dispersion of anostracan, very frequently more than one species is present in the same temporary pond. Therefore, the number of species detected in each area is not precise and this can be influenced by various factors such as sampling techniques, the frequency of sampling and relative population density (Mura, 1986) and so on. As a result, the study on the distribution of Crustacean Anostraca has been puzzling. Therefore, evaluating the morphology of the cysts by Scanning Electron Microscopy (SEM) appeared to be a valuable taxonomical factor (Thiéry & Charles, 1991) and more importantly that it can be used to identify the species even when the adults are not present at the time of sampling or when the temporary pools are dried out (Mura and Thiéry, 1986). It can also highlight the morphological variations among the cysts of fairy shrimps present in the region.

Consequently, the aim of this study was to examine the cyst morphology of Anostracan species, to provide a clearer understanding of the type of the species present in the region by using SEM as a useful taxonomic tool (Brtek & Thiéry, 1995).

Moreover, the surface pattern of Anostracan cysts and *Artemia urmiana* eggs were also examined by SEM in detail in order to stress any probable differences, because the ornamentation of the cyst wall of Anostracan eggs may play an important role as a physical protection against natural enemies such as copepods, mites, flatworm, etc., which are only abundant in freshwater ponds and are absent in hypersaline Urmia Lake.

Materials and methods

Fairy shrimp and mud samples were collected from the vernal pools in Taymoorlou, Alagheya, Aigher Goli, Ghom Tappeh, Zinal Hajilou, and Khasellou regions, which are all located in East Azerbaijan Province of Iran. The species identification was determined by referring to the relevant earlier reports (Abatzopoulos *et al.*, 1997; Mura & Thiery, 1986; Mura, 1986; Mura, 1992a, Mura and Azari Takami, 2000). All of the species were confirmed by Prof. G. Mura (Department of Animal Biologia, University of Roma)

Cysts of fairy shrimps were collected directly from the ovisac of female specimens or isolated from mud of temporary ponds (natural populations). The removal of cysts directly from brood pouches prevents the effect of pollution by debris and also eliminates the special cleaning procedure. They were quickly preserved in formaldehyde 10% to prevent pollution, knowing that resting eggs coming from dehydrated mud have the same morphology of freshly laid eggs (Mura & Azari Takami, 2000). To study their external feature, cysts were first dehydrated for nearly a day at room temperature and for two hours in dehydration chamber of the electronic microscope, then mounted on standard metal stubs, gold coated (≥10nm) and observed with LEO 440i SEM at an accelerating voltage of 10kv. Furthermore, cysts of *Artemia urmiana* originated from Urmia Lake were also studied by SEM with the same techniques for comparison.

Results

According to the images taken from the species of Anostracans in East Azerbaijan province of Iran, all cysts were spherical or nearly spherical, but they showed distinct differences in diameter and shell textures from one species to another. The geographical position of the regions that these Anostracan cysts were sampled and the type of the species found within each area are summarized in Table1.

Table 1: Geographical distribution of Iranian species of Anostraca

Name of the region	Geographical position	Altitude from the Sea level (m)	Name of Species
Ghom Tappeh	N= 37°, 13′ E= 46°, 2′	13500	*Streptocephalus torvicornis*
Aigher Goli	N= 37°, 46′ E= 46°,35′	2590	*Chirocephalus skorikowi*
Alagheya	N= 37°, 23′ E= 46°,48′	1675	*Branchinecta orientalis*
Taymorlou	N= 37°, 49′ E= 45°,53′	1304	*Branchinecta orientalis*
Zinal Hajilou	N=37°, 58′ E=45°, 56′	1320	*Branchinecta orientalis*
Khasellou	N= 37°, 49′ E= 45°, 50′	1297	*Branchinella spinosa*

As far as our observations showed, the resting eggs of Iranian fairy shrimps on the basis of their morphological pattern can be categorized into five groups. They showed very obvious variation in diameter and shell texture from one species to another. Therefore, SEM studies can remark these variations and it may be a useful tool in taxonomical studies, although it can not be the only procedure.

Figure 1, illustrates the cysts of *Branchinecta orientalis*, sampled from vernal pools of Taymoorlou, Zinal Hajilou and Alagheya. These cysts showed a few number of smaller polygons and have developed a wrinkled appearance. Moreover, irregular polyglonal pattern was evident on its surface and their mean diameters were 440μm, 415μm and 400μm, respectively.

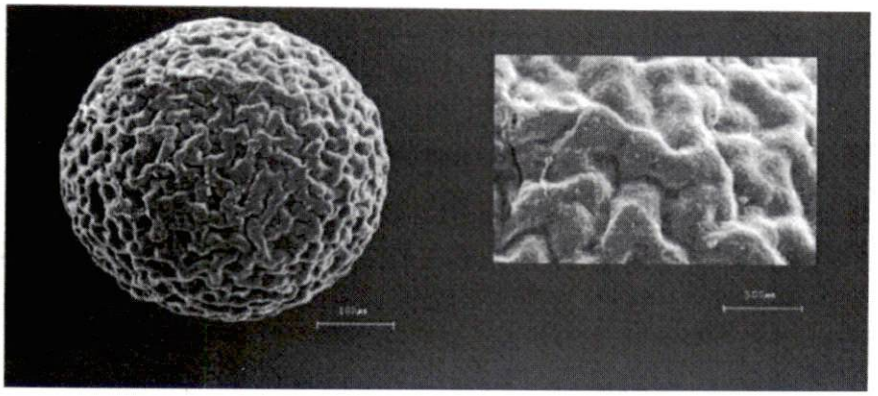

Figure 1: SEM images of *Branchinecta orientalis* from Taymoorlou, Zinal Hajilou and Alagheya regions

Branchinella spinosa's resting eggs, sampled from Khasellou were spherical and showed more developed ribs. Furthermore, there was an irregular polygonal pattern formed by a continuous network of low confluent ridges (Fig. 2). There were no pores evident on *B. spinosa* cyst and the mean diameter of the cysts was 300μm.

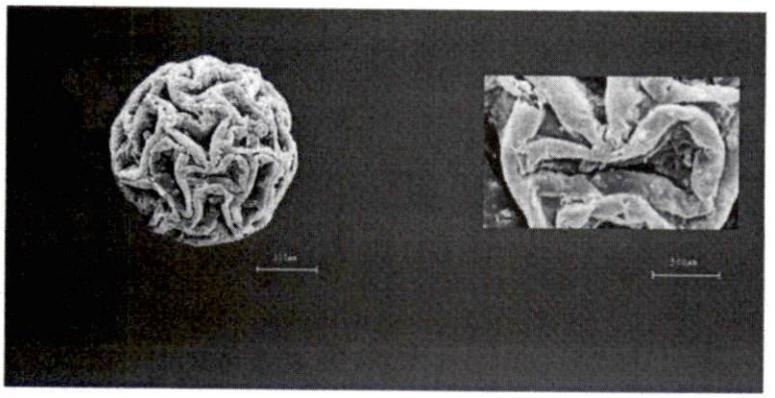

Figure 2: *Branchinella spinosa* from Khasellou district.

The cysts of *Chirocephalus skorikowi,* found in Aigher Goli district, had a less ornamented surface. However, the surface of the egg shell was ornamented by very

thin ridges forming a slightly wrinkled surface (Fig. 3). The mean diameter of these eggs was 450μm.

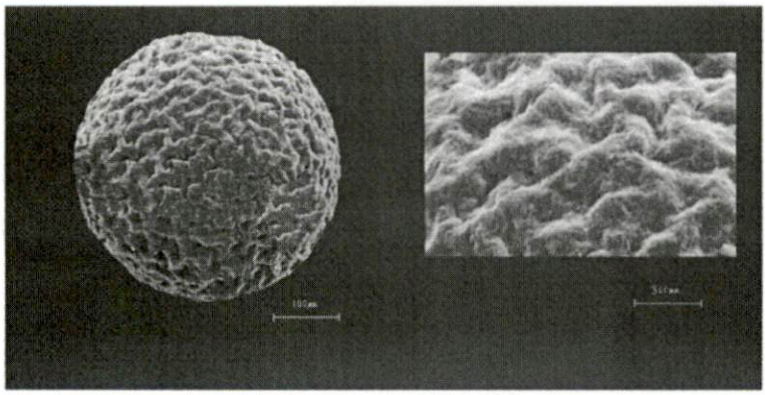

Figure 3: *Chirocephalus skorikowi* cysts from Aigher Goli

Cysts of *Streptocephalus torvicornis* (Fig. 4) were characterized by more regular spherical shape. Their surface was ornamented by a continuous network of ribs, which formed polygonal areas of different extent making the cyst surface deeply wrinkled. In *Streptocephalus torvicornis* eggs, the ridges were more raised, which gave the egg surface a folded appearance. These eggs were sampled from Ghom Tappeh region and they represented a mean diameter of 330μm.

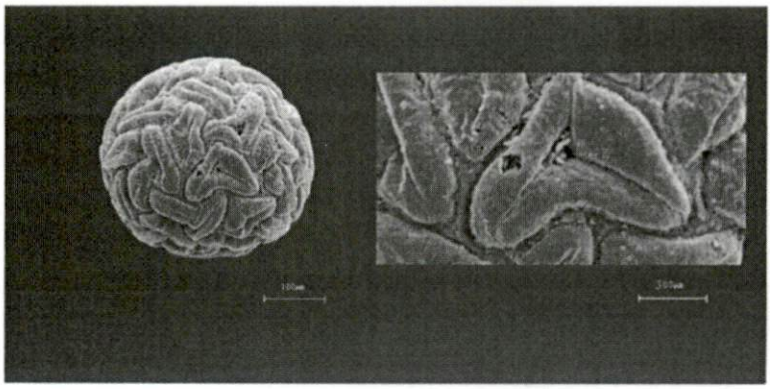

Figure 4: *Streptocephalus torvicornis* Sampled from Ghom Tappeh

Moreover, the egg surfaces of fairy shrimps were also compared with *Artemia urmiana* cysts sampled from Urmia Lake for further analysis. The cysts of *Artemia*

urmiana (Fig.5) have smooth surface with no evident of pores and a mean diameter of 301μm.

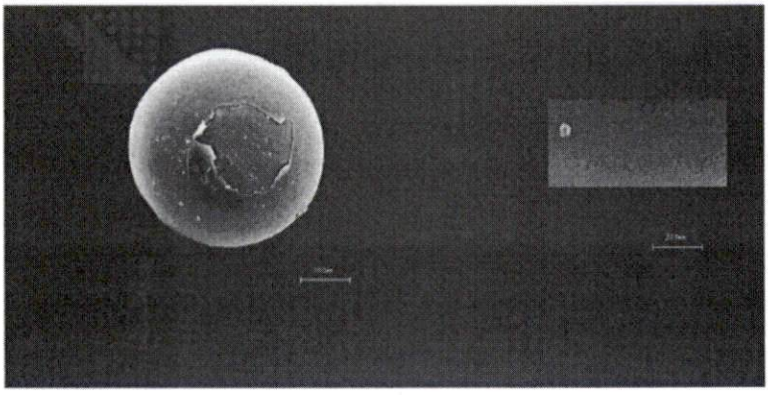

Figure 5: *Artemia urmiana* cysts sampled from Urmia Lake

Egg shell morphology of the Anostraca from East Azerbaijan is shown in Table 2.

Table 2: Egg shell morphology of the Anostraca from East Azerbaijan province (Iran)

Globe shaped	smooth surface	→	*Artemia urmiana*
	ribs (big polygons) →	irrigurlar polygonal pattern (network of ridges) →	*Branchinella spinosa*
	higher ribs (more smaller polygons) →	irrigurlar polygonal pattern (wrinkly look) →	*Branchinecta orientalis*
	more developed ribs →	folded look →	*Streptocephalus torvicornis*
	thin ridges →	slightly wrinkled →	*Chirocephalus skorikowi*

Discussion

Scanning Electron Microscopy (SEM) studies on the external morphology for the resting eggs of some Anostrcans from East Azerbaijan province of Iran provided evidence for consistent interspecific differences and confirmed the previous literature based on the taxonomical importance of this characteristic in species identification, even during dry periods of the pool and the absence of adult specimens (Mura, 1992b; Mura *et al.*, 1989). In agreement with other authors (Gilchrist, 1978; Mura, 1986; Mura & Azari Takami, 2000), there was no difference in the shell structure between the fresh or preserved cysts. However, the shape of the polygonal network and ridges heights and even cysts mean diameters

were not strong enough to give clear variations amongst species (Mura, 1986), and morphological evidence, based on some taxonomical features such as structure of the basal lamina and second antenna in adult males and studying the genetical biodiversity on Iranian species of Anostraca may be necessary.

Fairy shrimp's main strategy to survive in dry periods is dormancy (escape in time) by laying resting eggs. Besides, delayed hatching is also common (Lahr, 1997).

The spongy nature (Hill & Shepard, 1997) and convolution of the cyst wall may have various functions such as physical protection, thermal insulation of the embryo, protection from dense light, salinity and stressful conditions of such temporary vernal pools (De Walsche *et al.*, 1991; Purcell *et al.*, 1999; Dumont *et al.* 2002), which they dwelled.

However, we demonstrated that *Artemia urmian*a, like other *Artemia* spp., that live in low predation environment with salinity of nearly 300mg/L has cyst surface which are almost perfectly smooth and cyst walls that are relatively thin and simple in structure (Mura, 1989). Therefore, it seems that the spongy nature of Anostracan cyst ornamentation may have a protective role in keeping them as safe as possible and provide more protection against stressful conditions in temporary vernal pools.

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