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Subitaneous egg type and focus on ancestral ruins possibility in *Cypris pubera* O.F. Müller, 1776 (Ostracoda, Crustacea)

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Abstract

The most important evolutional gain of some non-marine ostracods are dry- resistant eggs. The "pause-wait" is carried out until suitable conditions are developed during the evolution of dry-resistant eggs. Through the egg-resting period, survival of ostracod individuals depends on a temporary water body. In this paper, we investigated *Cypris pubera* O.F. Müller, 1776, eggs via Scanning Electron Microscope (SEM). We observed two different types of eggs in the life cycle of one specimen. The subitaneous egg type of the *C. pubera* is shown first time here. Subitaneous egg can be only seen in first laying period which explains its role in the growth stage during the *C. pubera* life cycle.

Keywords: Ostracoda, Cyprididae , *C. pubera*, subitaneous egg, life cycle.

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Cypris pubera O.F. Müller, 1776 (Ostracoda, Crustacea)' da subitaneous yumurta tipi ve atasal kalıntı olasılığı

Özet

Bazı tatlısu ostracodlarında en önemli evrimsel kazanım kurumaya dirençli yumurtalardır. Kurumaya direnç kazanmış bu yumurtalar, "dur – bekle" politikasıyla, gelişebilmesi için gerekli olan uygun şartların oluşmasını beklerler. Yumurtanın bu istirahat dönemi sayesinde geçici su kaynaklarında yaşayan bireylerin geçici su kaynaklarındaki soylarının devamlılığı sağlanır. Cyprididae ailesine ait olan *Cypris pubera* O.F. Müller, 1776, türünün de yumurtaları SEM ile incelenmiştir. Çalışmanın sonucunda, bir bireyin hayatında sadece kurumaya dirençli yumurta olmadığı, iki farklı özellikte yumurta olduğu görülmüştür. Bu yumurtalardan hemen gelişime başlayan (subitaneous) tipindeki yumurta *C. Pubera* için ilk kez gösterilmektedir. Bekleme yapmadan gelişen yumurta tipi, kurumaya dirençli yumurtadan gelişen ilk erginin, ilk yumurtalamada bıraktığı yumurta tipidir. Bu yumurta tipinin türün yayılmasında ve varlığının devamındaki rolünü açıklayabilmek için yumurtaların hayat döngüsündeki yerini gösteren bir hayat döngüsü şeması da oluşturulmuştur.

Anahtar Kelimeler: Ostracoda**,** Cyprididae, *C. pubera,* subitaneous yumurta, hayat döngüsü.

Introduction

The non-marine ostracod species, which are dominant in inland waters, can survive despite the desiccation period their aquatic habitat faces. This trait is not seen only in cypridoidean species of Ostracoda but also some anostracan and notostracan species within the crustacean group that can deal with drought (Gilchrist 1978; Fanid et al. 2007; Özuluğ and Suludere 2012). The eggshell morphology and structural traits have been previously investigated and figured in several studies (e.g., Kesling 1951; Smith 1999; Matzke-Karasz 2005; Özuluğ and Suludere 2012). Those eggs consist of two layers of shell. After the egg expelled, outer layer is filled with water (Kesling 1951). The egg cysts of non-marine ostracods are especially important as previous studies have shown the resistance potential of the eggs, against to drought, freezing, and digestive enzymes (Kornicker and Sohn 1971; Sonh and Kornicker 1979; Vandekerhove et al. 2013; Radzikowski 2013). Due to the outer layer (egg cyst), some cypridoi dean ostracods can survive in conditions of dry or freezing periods of water bodies and also in the presence of some digestive enzymes. This egg cyst is the secondary gain against the drought period of inland waters. Several studies have named the eggs differently based on these acquired features, such as "resting egg", "dormant egg" "dry-resistant egg" or "diapausing egg" (e.g., Angell and Hancock 1989; Rossi et al. 1996; Horne and Martens 1998). For some reason these acquired features help cypridoidean ostracods to survive in temporary water bodies and also distribute through inland waters with passive transportation by the help of birds and wind (Mc Kenzie and Hussainy 1968; Horne and Martens 1998). There is a sufficient knowledge on the dry-resistant egg while subitaneous egg type is not yet characterized. The subitaneous eggs had been first mentioned in Angell and Hancock's (1989) study. The study has reported that there are two different types of eggs; one that develops instantly after being laid and the other is a dormant egg. These assumption on the two types of egg, despite studies on ostracods egg (Mc Kenzie and Hussainy 1968; McLay 1978; Kornicker and Sohn 1971; Smith 1999; Spencer and Blaustein 2001; Vandekerkhove 2013), had not been proved or described for subtaneous egg type. At the same time, many studies on the population dynamics and population biology were interpreted according to single type eggs (e.g., Mc Lay 1977; Rossi et al. 1996; Spencer and Blaustein 2001; Rossi et al. 2011). Aim of this study is first, to demonstrate the existence of subitaneous egg type as seen in SEM figures of both resting egg (with egg cyst not develops instantly) and subitaneous egg (without egg cyst develops instantly) of the same specimen. Finally, we emphasize the role of the subitaneous egg type in life cycle of *C. pubera*.

Materials and Methods

For this study, sediment was collected from Lake Büyükçekmece (41.09556°N, 28.52101°E) on May 2011. To obtain the eggs that were expelled after dry period, sediments in the jar were dried at room temperature. The dry material was stored for eight months in a laboratory where the temperature oscillated between 24 °C and 35°C. Distillate water was added to the jar at 25th day during observation of ostracod specimens and a few of them were removed from the culture jar and put into petri dishes. Under the stereomicroscope, two specimens were selected and cultured in a 6-well tissue culture plates (each container in 37 mm diameter and 20 mm deep). Within a day one of specimens have died and observation was further performed with single specimen. Water from culture jar was filtered by planktonic Müller fabric (mesh size: 0,025mm) and added to 6-well culture plate once a week. After 21 days, first offspring was observed and on the same day, seven eggs were collected and fixed in 3% glutaraldehyde. After one egg expelled mature specimen was fixed (50 % ethyl alcohol) and dissected for species identification and *Cypris pubera* was determined.

For SEM studies, all fixed eggs were rinsed twice with phosphate buffer. This was followed by dehydration in a graded series of ethanol (from 70% to 100%). Eggs were then passed through amyl acetate. The samples were dried

at the critical point with CO_2 (Polaron, CPD 7501). Samples were mounted with double sided adhesive tape on SEM stubs, coated with gold using Polaron SC 502 Sputter coater and examined with Jeol JSM 6060 LV SEM at 5-20kV in Gazi University, Faculty of Science, Electron microscopy unit.

Results

Due to the culture conditions, only one specimen, *C. pubera*, could continue to maturity stage and first eggs that expelled from single specimen were investigated using SEM. The eggs were also first eggs that expelled after dry period. SEM studies have revealed two morphologically different type of eggs as with egg- cyst and without which can be easily distinguished. It was known that eggs with egg-cyst are resting eggs. The surface ornamentation of resting eggs is smooth or irregular due to cyst. The egg, without egg- cyst was defined as subitaneous egg. The surface of the eggs that not covered with cyst was ornamented with spines (Fig. 1). In *C. pubera*, surface ornamentation of subitaneous egg is regular at low magnifications. There are many pits on the surface (Fig. 2) and tiny spines between those pits (Fig. 3). The other egg type of *C. pubera*, covered with cyst and attached at a piece of carapace, had a surface pattern of irregular sub-circular flat areas enclosed by low, narrow walls (Fig. 4).

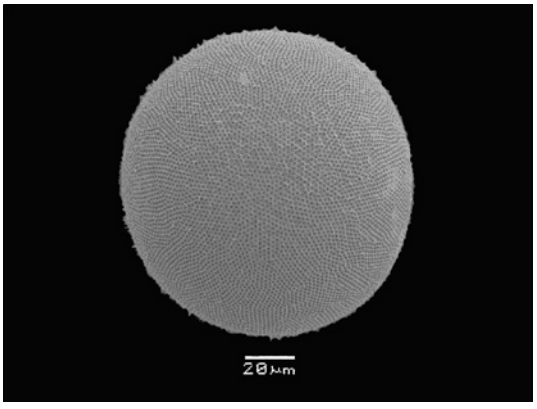

Figure 1. SEM image of subitaneous egg type of *C. pubera.* Whole egg view.

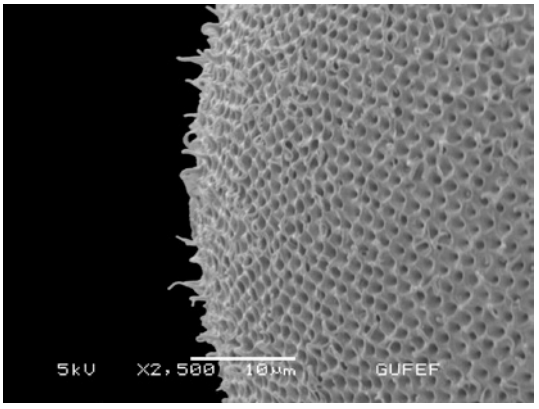

Figure 2. SEM image of subitaneous egg type of *C. pubera*. Detailed view of the egg surface.

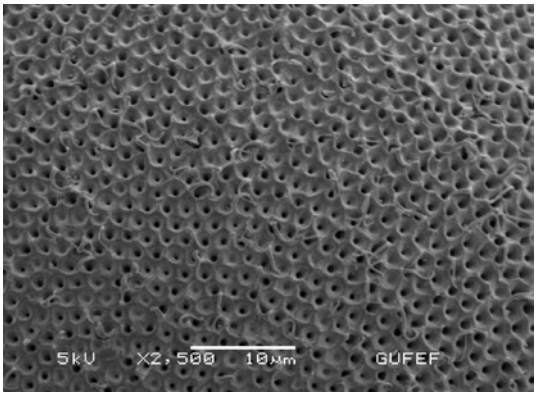

Figure 3. SEM image of subitaneous egg type of *C. pubera*. Detailed view of surface ornamentation; pits and tiny spines between pits can be observded on the egg surface.

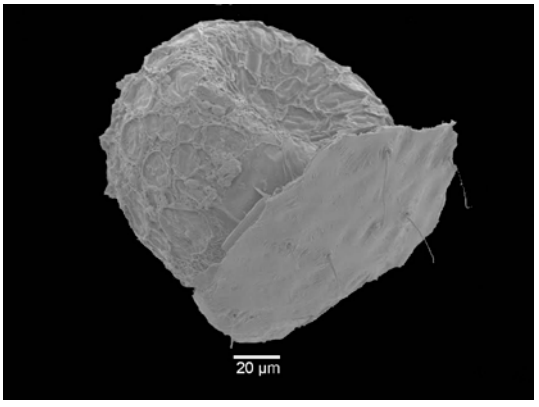

Figure 4. SEM image of *C. pubera*'s resting egg type as attached to a piece of carapace. Surface pattern of resting egg with many sub-circular flat areas enclosed by low, narrow walls can be observed.

Discussion

The egg surface patterns have been shown for many crustacean species and some eggs were used for species identification by the patterns or spines on their surfaces (e.g., Mura 2001; Fanid et al. 2007). In addition, two types of eggs that can be seen seasonally are known in some copepods (Castellani and Lucas 2003). In cypridoiden ostracods, it can be suggested that surface ornamentation exists, and species identification are possible in theory. However, it is not possible to distinguish resting egg or subitaneous egg by surface pattern using conventional light microscopy methods due to its small size.

The subitaneous egg type with surface sculpture or spiny pattern is very rare and the resting egg type is dominant in the life of many non- marine ostracods that combat with desiccation.

Therefore, subitaneous egg type has never been observed in many studies that carried out for different purpose on the eggs (Sohn and Kornicker 1979; Angel and Hancock 1989; Dumont et al. 2002; Özuluğ and Suludere 2012). There is one study shows images from ornamented eggs (Smith 1999), however, there is not any information or description on subitaneous and resting egg. Our study is the first to show both subitaneous and resting egg that expelled from a single specimen. Also, roles of subitaneous egg were discussed.

This rare subitaneous egg type has been found only in the first offspring of the individual, produced by exposing the desiccated egg to water. In our previous studies (Özuluğ and Suludere 2012), experiments have been performed with an abundant population of the individuals hatched from dry eggs. This clarifies why subitaneous egg types were not observed in our earlier studies. The two types of eggs in the life cycle that were expelled by single specimen are important, and brings questions: 1.Why does a species have two types of eggs? It is known the egg cyst is acquired property for living in the temporary freshwater habitats. If the resting egg type is more advantageous than subitaneous egg well, then why is subiteneous egg still produced? Is the subitaneous egg a relic property? 2. What is the role of the subitaneous egg in the life cyle of *C. pubera*? Life cycle of *C. pubera* should be known to understand the benefit of the subitaneous egg. . For this purpose, we have schematized the life cycle based on the existing knowledge on subitaneous and resting eggs (Fig. 5). It has been observed a resting egg that meets the water tarries for a while. Tarry time (T) also contains embryonic development process (T+ E.D). It is known that the embryonic development starts when the egg is laid (unpublished data), and its path will change per egg type. Subitaneous egg develops instantly after being laid but the resting egg has two ways that varies depending on the presence or absence of the water. However, the mechanisms which control the length of tarry time for a resting egg in both wet period and after drought period is not known. We have seen the tarry time is followed by a short Nauplius larval stage and then molting stages from A-8 to A-1. This process was schematized as molting stages (MS). The period from the egg meets water, to adult that can lay eggs is equal to growing time (G).

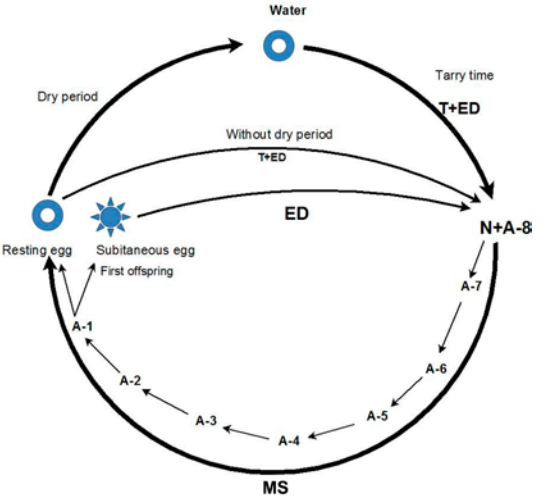

Figure 5. Life cycle of *C. pubera* in family Cyprididae. T: Tarry time, ED: Embryonic development. N: Nauplius. MS: Molting stages, from juvenile (A-8) to adult (A-1) specimen.

Growing time is equal for resting egg, G = "T+ ED" + MS and also for subitaneous egg, G= ED+ MS' (Fig. 5). The important

difference between subitaneous and resting egg was growing time. Growing time of subitaneous eggs is shorter than the resting eggs. Desiccations, which can occur suddenly in aquatic environment will not threat the resting eggs in "T" period, but will cause the individuals in "MS" period to die without laying any eggs and will threat the future of population. In this condition, short growing period by subitaneous egg as ancestral ruins has big importance because of the need for individuals who will be able to produce resting eggs that can maintain their fertility for a short time in wet period. Hereby persistence of population would be ensuring to the utmost short time. At the same time the individuals which have short "G" periods will distribute more widely in inland waters. In other words, duration of the wet period in continental temporary water habitats will limit the *C. pubera* species that are capable of living in these habitats.

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