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Research Article

Studying the details of the life cycle and reproduction in the Tardigrade of Mashhad

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Abstract

Tardigrades are microscopic sized Ecdysozoans. In Iran, in the last year and for the first time, these animals were identified and reported in Mashhad, and it immediately became an important question that what is the extent of tolerance and biological and reproductive details of these animals in Iran. Therefore, in this study, it has been tried to study the mechanism of biological and physiological adaptations, especially to temperature and humidity stresses. Also, the development stages of the living details of these animals should be studied and compared with other studies in other climates. For this purpose, sampling was conducted from valleys and basins in Mashhad city during four seasons of 2014 to 2016. After identification of the species, films were recorded for these animals at least 6 hours per day, and the results of this study have been gathered on the favorable environmental conditions, reproductive details and development of eggs, nutrition, and so on.

Keywords: Protostomata, Arthropoda, Tardigrade, Iran, Khorasan.

Introduction

Tardigrades are animals that found and introduced by Goeze in 1773. These animals never die, but enter into the inactive phase in the unfavorable environmental conditions that remain them alive for many years, and since they have extraordinary capacity to tolerance any change in environmental conditions, attention has been paid to recent conferences for this beast. The important point is to identify the species of these animals and to study their lifestyle in different climates. They range in size is from 0.1 to 1.5 millimeters. This animals are cosmopolitan and found in drought, semi-humid, and aquatic habitats. The extreme tolerance of these animals and the existence of a cryptobiosis period have caused them to be highly resistant to many environmental factors (Bertolani 2001). A lot of studies have been done to identify the species of these animals and describe the apparent traits of these species, but little information is available about their details of life. In this research, we tried to study the life cycle of these animals in the eastern part of Iran for the first time.

Material and methods

The study was conducted in the valleys around Mashhad (Dehbar, Zoshk, Kalateh Ahan, Golestan, Shandiz, Naghandar, and Taragh) between 2014-2014 (Figure 1 and 2). Samples of the habitat: were collected from dried mosses and Watershed, wetlands, mosses floating in water, and depositional debris. Sampling days were selected randomly from the week days. The study time and the sampling range were designed in such a way that the results can be generalized to the statistical population of the study. According to the standards (Molleda *et al.* 2003, 2004), the samples were stacked in a 200-cubic glass prepared for this purpose. Samples were immediately transferred to the Zoology Museum of Mashhad Azad University after collection. The aqueous samples have been deposited after 2 to 5 hours, then 90% of the water has been removed through the siphon. In dry samples, an environment with about 20cc of water and after six hours, their studies were begun. Then about 25 lumens were made from each container. The samples were observed by a microscope (Olympus with digital camera) and a magnification of × 100. A digital camera (100x zoom digital CCD camera) was used to bottle, previously washed and recording photos and videos. Photographing and filming are done daily, at least six hours a day, and sometimes overnight. The biological and reproductive details of the animal were obtained by reviewing 450 films and about 2500 registered photographs. Maintaining and studying samples was carried out in natural conditions. Fixation of samples was carried out using a sample drying method, and in some cases by Hoyer's medium. Temperature stresses were studied in the range of 25 ± 50^{o}c and in the 48-hour period.

Figure 1. Sample collection sites in Mashhad.

Figure 2. The Main habitat of Tardigrades.

Statistical analysis

At each stage of the study, groups including 15 animals were examined. There was a significant difference between control and test conditions by unpaired, two-tailed t-test with a significant level of 0.05 ($P \leq 0.05$).

Results and discussion

Habitat

We have collected these animals from algae in rivers, mosses on rocks and trees, in the mud and bottom of the pond (Figure 2). It should be noted that this creature is found to be inaccurate in dry seasons over dry algae and mosses out of the water, and if they are immersed in water, they will be active and visible within 2 to 5 hours. Their accumulation is in regions where algae and mosses are more abundant. These animals are observed only in areas that are not in the wind, that is, in the gaps and branches that are not windy, and not found in naked areas and exposed to air flow. In flowing waters, they live exclusively in mud and water, but they are found in uncontaminated water on plants, margins of water and even immersion in water. In 1990, Bertolani and his colleagues announced the habitat of this creature as mossy massif. And according to Bohberg (2006), which showed that the propagation of these animals is the displacement of eggs or samples of the carnivores with wind currents, it is expected that, within the regions of the distribution of these animals, they will also be observed in in the dry soils as cryptobiosis.

Cryptobiosis

The characteristic of this stage is stopping all vital acts of the beast that can last forever (Lemloh 2011). Contrary to expectations of observation of these animals in the cold winter and hot summer; however, in all the sampling that took in the coldest and warmest days of the year, the Diapause and cryptobiosis forms were rarely seen naturally, and it seems that these forms are only observed in a very benevolent environmental condition that does not occur in the city's climate. The limited cases in which cryptobiosis form can be found was related to the highly dried mosses in the very dry months of the year such as early September. However, it is possible to enter the beasts into this special form in a laboratory through rapid drying of the environment stages within 2 hours, while the animals are active at temperatures between 25 and $50+^{0}$c. It seems that these animals have the highest resistance to temperature variations and the least sensitivity to environmental humidity. The salinity of the water that changes constantly due to evaporation in the laboratory environment does not have an effect on the activity of the animal, but if any of the minerals (sodium, potassium, etc.) is added to the water so that the salinity of the water reaches the top of 11ppt, animal enters to the cryptobiosis stage within 5 days. It should be noted that the animal can enter cryptobiosis stage from the active state at all stages of life (different stages of development, infancy and maturation). In order to exit from the cryptobiosis stage, the animal can be placed in natural conditions, especially in terms of environmental humidity, and become active within 2 to 5 hours (Figure 3).

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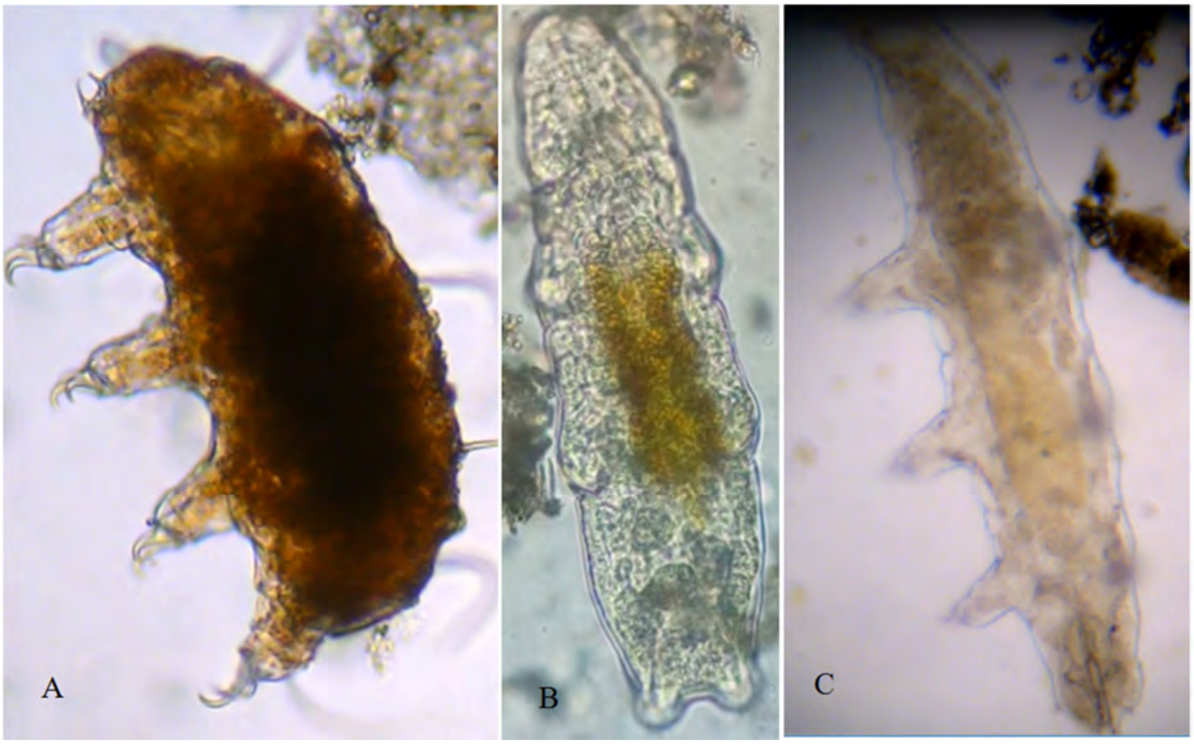


Figure 3. Cryptobiosis of three genera A: Echiniscus and B: Macrobiotus C: Milensium

Moulting

This animal like other Ecdysozoans has moulted several times throughout its life. The number of moulting times depend on the amount of nourishment of these animals. The moulting of the beast is calm and takes between two and seven days. In 1982, Walz announced the moulting period from 5 to 10 days. But in 36 cases, the observation of moulting showed that this process occurred in two days. Probably the reason for this conflict can be that larger samples will have a longer moulting period, because the process of absorbing water and enlarging the body to make a larger skin is longer than those with a smaller average size. In addition to the outer skin of the body, the forks, the lining of the intestinal tract and the intestinal tract also fall (Figure 4.A), and since the cover of the oral and throat sections fall, the animal does not feed until the complete restoration of the area. In general, mosquito samples have less number of moulting than aquatic samples and are smaller in size. The moulting process has the following steps. The first animal eats a large amount of food and increases the size of the body(Figure 4.B), and if it is mature, the eggs are formed inside the body and the size of the animal increases (Figure 4.C). The animal then releases its claws out of the skin, and it seems that the tip of the fork falls on the skin and animal restores the foreskin's branches. After that, the animal collects the front and back segments of the body and separates it from the skin, and at this stage the ends and the initial parts of the digestive system are separated. Then, the animal performs very fast movements, and so it clogs itself to the wall to create a gap, and as eggs leave the gap, the eggs are released inside the skin (Figure 4.D).

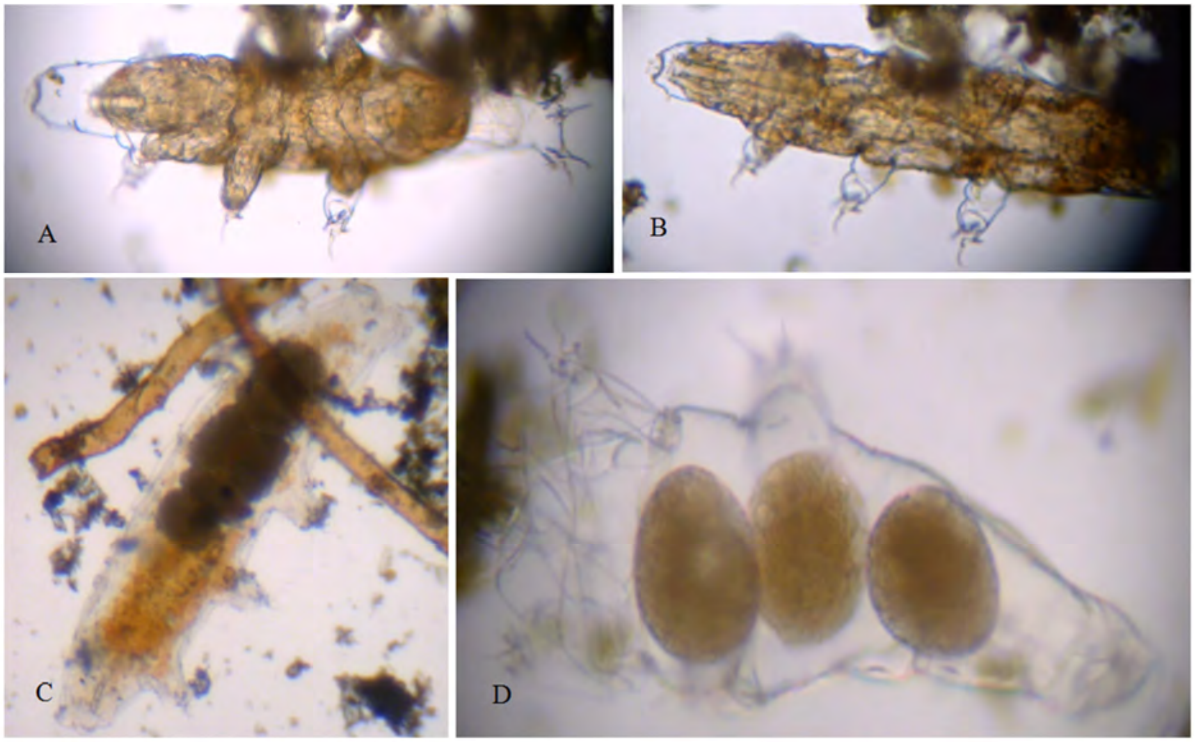


Figure 4. Stages of molting in tardigrada: figures are according to text.

Annual Cyclomorphosis

Annual changes have not been observed in mildew in the studied samples, while Halberg and colleagues in 2009 for *Halbiotus crispae* and Rebecchi along with Bertolani for Bertolanius sp have reported this phenomenon. This dwarf has usually been associated with winter and summer spells, which is usually seen in winter in the form of a beast, and is seen as a sexually active animal in the summer.

Nutrition

The pharynx and pharyngeal muscles are the most important tools for feeding this animal, and the structure of these two parts is different in different species and is one of the main attributes of the key identification of these animals. A group is vegetarians, especially those that live in algae and mosses, have these vegetarian diets (Figure 5.A), and a number of this animals are carnivorous and feed from smaller Lophotrochozoans or smaller Ecdysozoans (Figure 5.B). Similar to what has been reported

before in the samples that feed on plants have very tall forks that easily perforate the cell wall of the bait (Morgan, 1977) (Figure 5.C). But in the carnivorous samples, the forks are short and their muscular part is very large and thick. There are also some powerful appendages in these animals (Figure 5.D).

Reproduction method

Bertolani in 2001, following a report, announced that the mechanism of reproduction of tarigrades varies in different habitats, but his main purpose was the difference between freshwater and saline water habitats, that, since we did not have a saltwater habitat in the area, it wasn't possible to investigate this type of habitat. On the other hand, the results of

Hofmann in 1987 showed that the rate of propagation of these animals depends on the environmental conditions, and our study also confirms this conclusion, especially in a medium with a drying medium, the speed of replication is slowly stopped and ultimately stopped. It can be said that the strategy of this animal in reproduction is R. In this study, the length of the embryonic period varied from five days to eighteen days in different species.

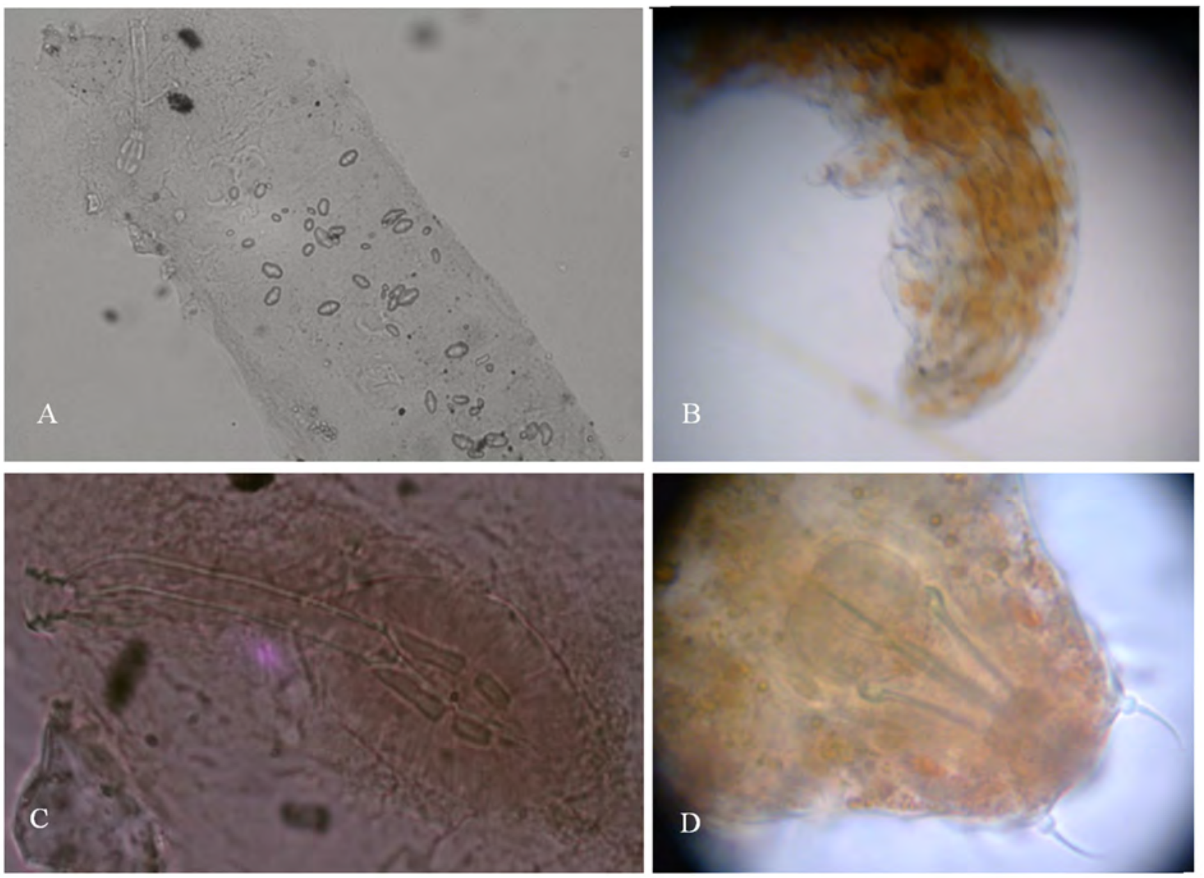


Figure 5. A: A vegetarian tardigrad with some Diatoms in its digestive system. B: A carnivorous tardigrad with smaller animal in its digestive system. C: The pharynx and pharyngeal muscles in a vegetarian tardigrad. D: The pharynx and pharyngeal muscles in a carnivorous tardigrad.

In this research all samples were parthenogenesis, also the hermaphrodite sample was not found either. Probably the unsteadiness of the living environment is the reason for this type of reproduction to ensure species survival and on the other hand, it facilitates the distribution of the animal. As the results of Pilato in 1979 and Kristensen in 1982 and 2005 reveal, probably the distribution of eggs or cryptic samples with wind is the reason for the distribution of these samples. The samples observed in this study did not release their eggs in the water and looked at them in their shells until they were removed from the egg (Fig 7). And, possibly, saline samples will leave the eggs in the water because they do not have a problem with drying water. Also, the development of eggs does not coincide (Fig 5.A). Martin declared in 2010 that the number of eggs varies from a few to more than 30. In this study, the number of eggs varies in one sample depending on the species, age, and amount of food in the environment. In this study, it was found that by changing the five factors of temperature, humidity, salinity and light, the effect of temperature during egg hatching was proved and the temperature is the most important factor in the hatching period at temperatures below 15 ° and above 40 ° Hatching, and at the same time with temperature rise the speed of hatching will increase. These animals tolerate temperatures up to -25 ° C. Altieri and colleagues showed that in bad environmental conditions and when the eggs have a low metabolism, development of the eggs elongates 90 days or more, but if the conditions are appropriate, this process elongates about 30 to 40 days. Our study also showed that, if the environmental conditions are unfavorable, the eggs at each stage enter a non-essential phase and remain in that state until it becomes desirable, and it is not possible to specify a specific day. The eggs that enter the cryptobiosys phase become denser and lighter and can be dispersed by wind to other places (Fig. 6).

Egg development can be prolonged to 90 days . In non-resting (subitaneous) eggs may hatch in as little as 20-40 days. The two-cell and four-cell stages occur quickly(Fig. 8). However, the next divisions of the egg are slow and sometimes they take about 20 to 50 days to reach a multi-cell embryo (Fig. 9). After the the Morula, the egg has a simple fetus within one to three weeks(Fig. 10). After the embryo is complete (Fig. 11), it break the egg shell (Fig. 12). Newborn babies are about a week old in nest shells and then removed the shell.

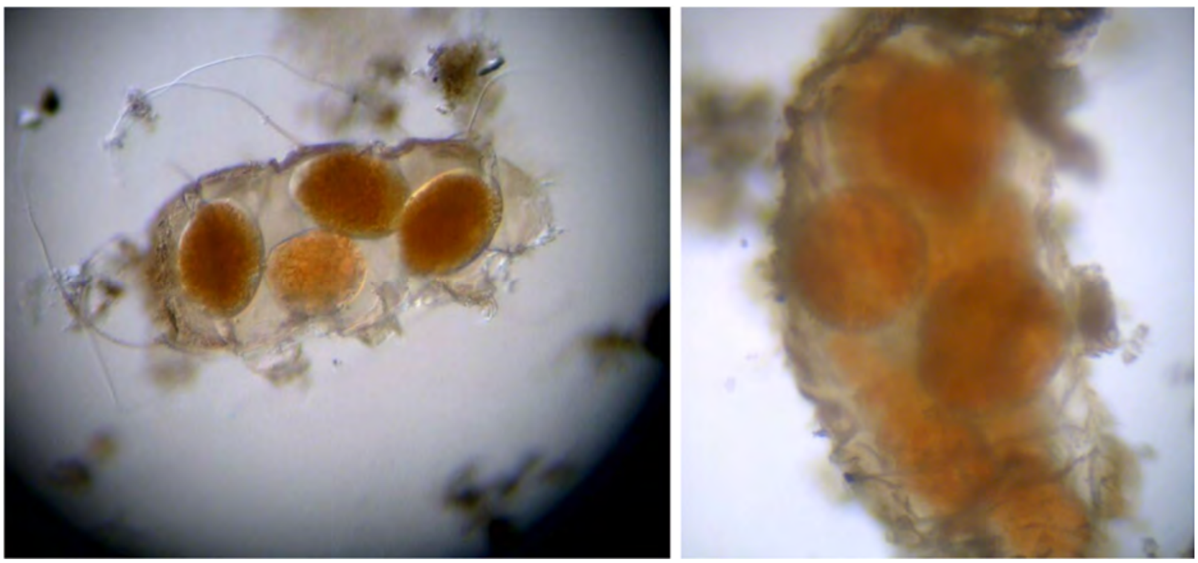
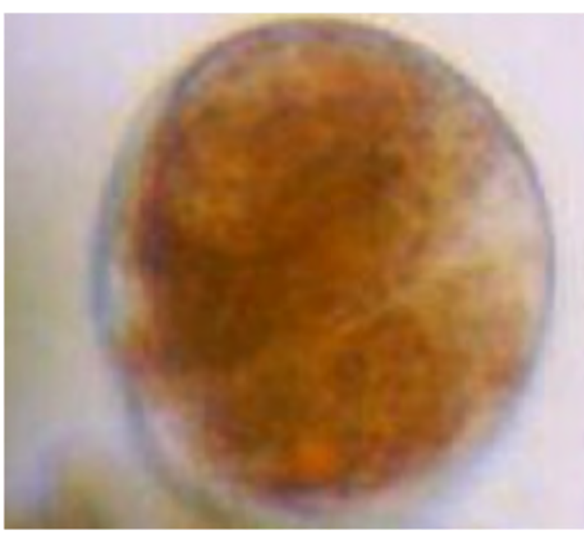


Figure 6: Individual of Tardigrade with four and eight eggs in the shed exuvia



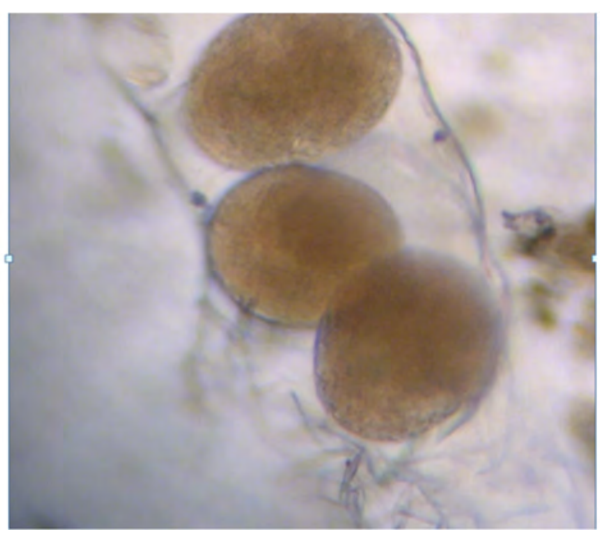


Figure 7: Tardigrada exuvia with eggs (embryos) after first division.

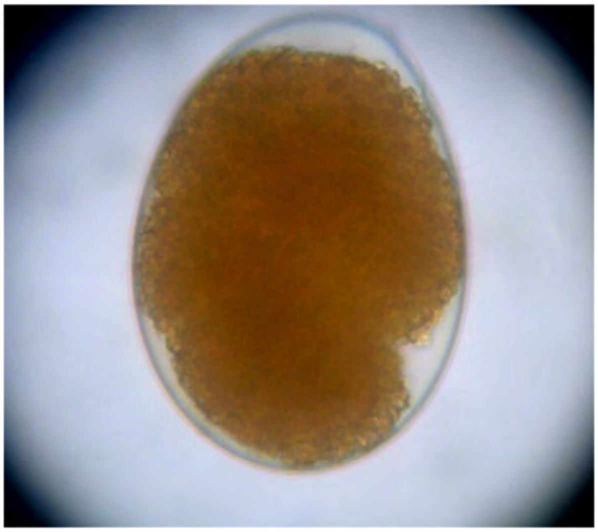


Figure 8: Tardigrada embryo after two divisions.

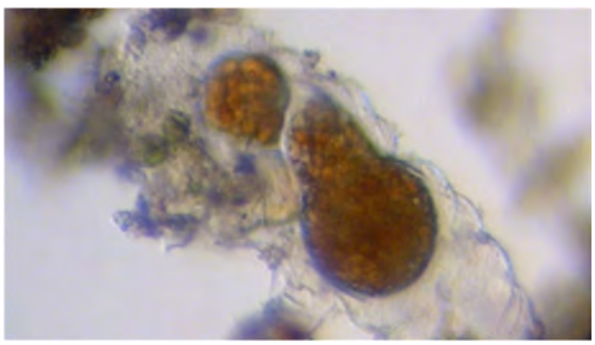


Figure 9: Multicellular tardigrad embryo.

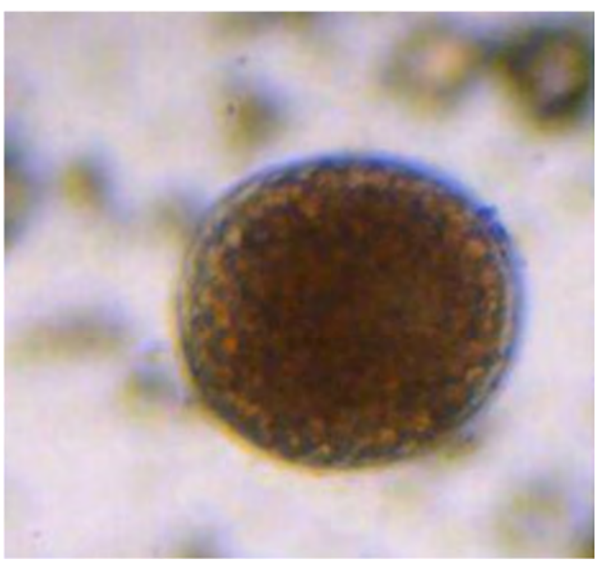


Figure 10: Morula stage in the embryo development of Tardigrade.

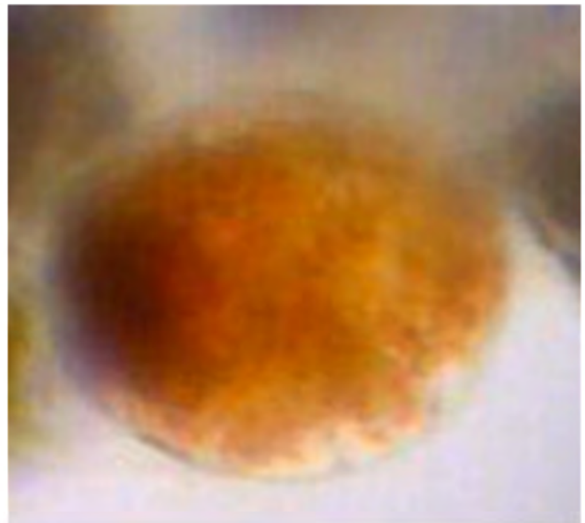


Figure 11: The mature egg of Tardigrade.

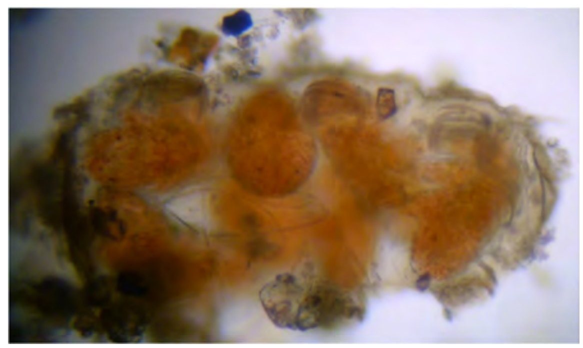


Figure 12: Tardigrade hatching from its eggs.

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