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First record of *Macrobiotus beotiae* Durante & Maucci, 1979 (Tardigrada: Eutardigrada: Macrobiotidae) in Ukraine
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In July 2007 samples of moss *Tortula ruralis* and lichen *Xanthoria calcicola* were collected on the plateau of Mountain Opuk (Opuk Nature Reserve, Crimea, Ukraine). These samples appeared to contain eggs of an uncommon tardigrade species *Macrobiotus beotiae* previously known only from two localities in Greece. A distinguishing character of this species is a hyaline envelope, surrounding an egg, in which the processes of two different types are partially immersed (longer processes are conical while shorter ones are round cylindrical or club-shaped).

Key words: *Tardigrada*, *water bears*, *Macrobiotus beotiae*, *Opuk Nature Reserve*, *Crimea*.

Introduction

Tardigrades, commonly known as water bears or moss piglets, constitute a phylum of microscopic invertebrates, which appears to be a sister group of the arthropods (Nelson, 2000). Hitherto, nearly a thousand of species have been described from bodies of water as well as from thin water films in various terrestrial habitats (Guidetti, Bertolani, 2005). Tardigrades have no apparent economic importance and are studied mainly due to their ability to enter cryptobiosis and thus survive extreme conditions such as low and high temperatures, high levels of X-ray radiation, high pressure, vacuum, some noxious chemicals and even exposure to outer space (Jönsson et al., 2008). However, tardigrades are rather widespread and abundant animals and their role in most ecosystems remains largely unknown.

Two extant classes can be distinguished. The class Heterotardigrada includes mainly the marine and "armoured" terrestrial species while the class Eutardigrada encompasses primarily "unarmoured" freshwater and other terrestrial species. Heterotardigrades are rather diverse, whereas eutardigrades display low morphological variability, and so most of them seem to be nearly identical to an unexperienced eye.

Eutardigrade taxonomy is based primarily on the morphology of the claws, buccal-pharyngeal apparatus, cuticle, eggs and (in some extent) of spermatozoa. Besides, a number of morphometric indices is used to distinguish some close species (Schuster et al., 1980; Pilato, 1982; Nelson, 2000; Guidetti, Bertolani, 2005). Fine details of most internal organs structure are not used in identification since they are scarcely seen on permanent slides and rarely preserved in type specimens. In the last decade sequencing of ribosomal DNA and some other molecular methods were successfully used for identification and differentiation of tardigrade taxa (Blaxter, 2003; Schill, Steinbrück, 2007), though molecular data are not available for the waist majority of species.

Tardigrades of Ukraine are insufficiently studied. Hitherto, only 75 species have been recorded (check-list in Kiosya, 2007), mostly in Subcarpathian region and Eastern Ukraine, while tardigrades of Crimean peninsula remain virtually unexplored in spite of obvious biogeographical peculiarity of this region. Despite their passive dispersal, most tardigrade species seem to have substantial altitudinal and some other ecological preferences and thus can provide valuable zoogeographical information at continental level (Pilato, Binda, 2001). Therefore, we decided to study tardigrade fauna of Crimea.

Located in southern Ukraine, the Crimean peninsula juts into the Black Sea and connects to the mainland by a narrow strip of land at Perekop. Dry steppes cover more than two-thirds of the peninsula, and the Crimean Mountains in the south rise up to 1,500 m before they drop down sharply to the Black Sea. The southern coast, protected by the mountains, has a mild climate. Opuk Nature Reserve situated in the South of the Kerch peninsula lies away from Crimean Mountains, though it also has some highlands and lower mountains. Mountain Opuk composed mainly of reef limestone is the highest of them reaching 185 m. A comparatively small area of mountain Opuk is a mosaic of diverse landscapes characterized by heterogeneity of flora and vegetation. The diversity of ecotops favours to existence here both steppe and mountain elements of flora and fauna, many of which are relic and endemic. Present study revealed there a tardigrade species previously not cited for Ukraine.

Material and methods

In July 2007 V.Inshina examined the plateau of Mountain Opuk and its southern slopes and collected about 115 small samples of lichens and dry mosses (Categories IV and V according to (Ramazzotti, Maucci,

1958), i.e. subject to regular desiccation). The samples were air-dried and placed in labeled paper-bags, so that they could be stored for a long time, as tardigrades entered the state of cryptobiosis.

In a laboratory tardigrades were extracted from samples using a simple technique, developed by Morgan & King (Morgan, King, 1976), with slight modifications. Before being examined each sample was left to soak in small amount of tap water for about 6–24 hours, whereupon an equal volume of 20% alcohol was added in order to narcotize tardigrades and prevent them from holding on a substrate. After 10 minutes in alcohol-tap water medium a sample was intensively agitated and squeezed over a dish; this step was repeated several times, until most tardigrades got to water-alcohol solution. Then a sample itself was removed, whereas the solution was searched for tardigrades under the 25–90 magnification of a stereomicroscope (incident light and dark field were used). Tardigrades and their eggs found were transferred in a drop of distilled water on a slide, air-dried and mounted in Faure's medium. After 2–3 weeks coverslips were sealed with nail polish to prevent excessive drying of the medium.

Tardigrades were identified on permanent slides according to the 3rd edition of "Il Phylum Tardigrada" (Ramazzotti, Maucci, 1983). Measurements were made with a micrometer at the highest magnification ($\times 700$) under a light microscope. Mounted specimens and eggs are deposited in our personal collection at the Department of Zoology and Animal Ecology of Kharkiv National University.

Results and discussion

Holotype and paratypes of *M. beotiae* were collected from moss sample on a rock in Beozia, Greece in 1975 and described in 1979 by M.V.Durante Pasa and W.Maucci (Durante Pasa, Maucci, 1979), who also collected eggs in Edessa (Greece). Since then tardigrades of this species haven't been recorded from any other localities till we have registered it in Crimea (fig. 1).



Fig. 1. Distribution of *M. beotiae*: 1 – Beozia, Greece – Durante Pasa & Maucci, 1975; 2 – Edessa, Greece – Durante Pasa & Maucci, 1975; 3 – Crimea, Ukraine – Kiosya & Inshina, 2007

We have found altogether 8 eggs, which seem to belong to *M. beotiae*, in 2 samples of moss *Tortula ruralis* (Hedw.) Gaertn., Meyer & Scherb on soil and 4 samples of lichen *Xanthoria calcicola* Oxner on limestone from the plateau of Mountain Opuk.

Description: Spherical ornamented eggs are of yellowish color, laid free and are rather large (150–190 μm in diameter including processes and 120–165 μm excluding them). The surface of the egg is surrounded with an uniform hyaline envelope 5–7,5 μm thick, in which the processes of two different types are partially immersed (fig. 2 a, b, c). Larger projections in form of thin spine-like cones (12–18 μm excluding the immersed part) are alternating with much shorter processes (4–6 μm), which are round cylindrical or club-shaped. The processes are spaced irregularly (i.e. not in exact rows) and there are approximately 20 larger ones and 20–25 smaller ones in an optical section (around circumference of an egg). Surface of the processes is smooth, whereas egg shell between processes appears finely granulated, still without any reticulation or “platelets” (fig. 2 d).

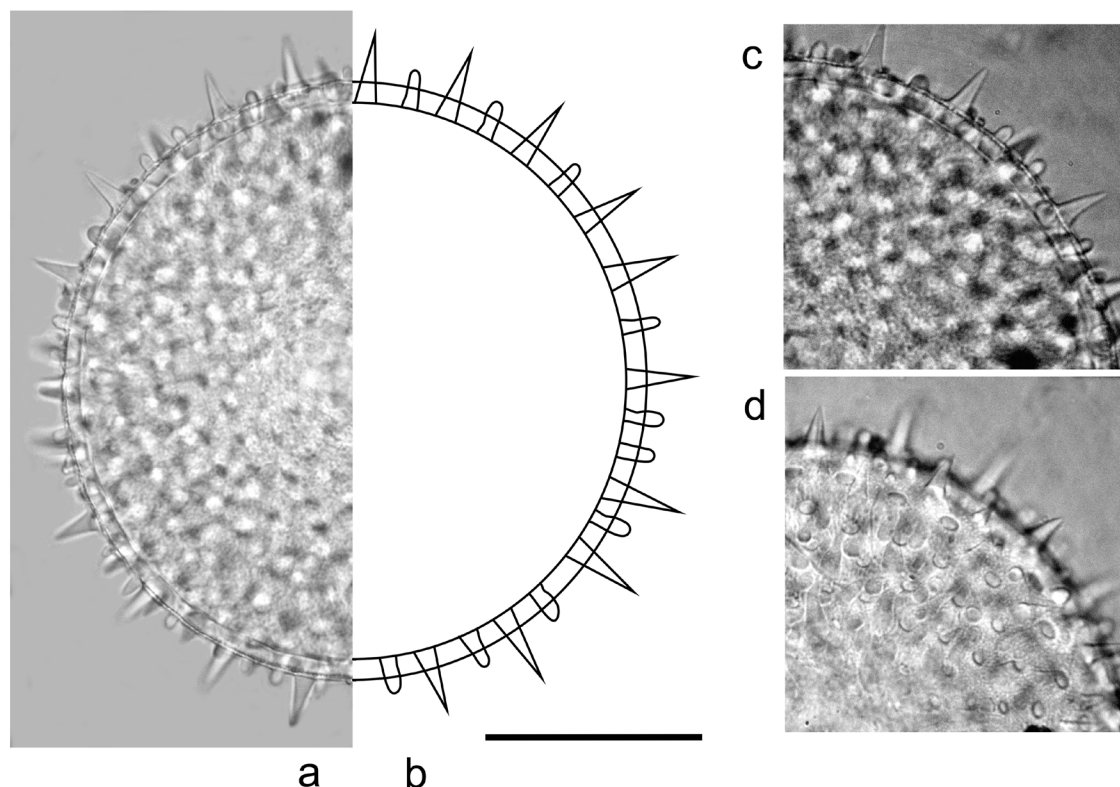


Fig. 2. Egg morphology of *Macrobiotus beotiae*: a, b – general morphology, c – details of the outer parts of the processes, d – details of egg shell sculpture between processes. Scale bar: 50 μm

Associated tardigrade fauna was represented by *Echiniscus testudo* (Doyère, 1840), *Macrobiotus* cfr. *hufelandi* C.A.S.Schultze, 1834, *Macrobiotus* cfr. *occidentalis* Murray, 1910, *Macrobiotus persimilis* Binda & Pilato, 1972, *Macrobiotus* sp. gr. *tenuis*, *Ramazzottius* cfr. *oberhaeuseri* (Doyère, 1840) and *Milnesium tardigradum tardigradum* Doyère, 1840 (both adult / juvenile specimens and eggs). Other associated fauna was represented by various species of bdelloid rotifers, nematodes, mites, ciliates and testate amoebae.

None of eight examined eggs contained a visible embryo and no adult specimens of *Macrobiotus beotiae* have been found along with eggs. Nevertheless we had no doubt ascribing the eggs to *Macrobiotus beotiae*, as all eight studied eggs precisely fit the original diagnosis of Durante Pasa and Maucci, all associated species of tardigrades lay eggs of considerably different morphology, and no other species of tardigrades having similar eggs are known. Besides, the initial record of *M. beotiae* in Edessa was also based solely on eggs. Meanwhile it is hardly possible to judge, whether the absence of adult specimens in studied samples is a consequence of low population density, seasonal dynamics or some other unknown factors. However, further research in the same region is needed.

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Первая находка тихоходки *Macrobiotus beotiae* Durante & Maucci, 1979 (Tardigrada: Eutardigrada: Macrobiotidae) на территории Украины
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В июле 2007 г. на плато горы Опук (Опукский природный заповедник, АР Крым, Украина) были собраны пробы мха *Tortula ruralis* и лишайника *Xanthoria calcicola*, в которых были найдены яйца тихоходки *Macrobiotus beotiae*. Это редко встречающийся вид, ранее известный лишь по находкам в Греции. Отличительной особенностью данного вида является т.н. стекловидная (гиалиновая) оболочка, покрывающая яйцо, в которую частично погружены выросты двух различных типов – длинные конические и короткие округло-цилиндрические или булавообразные.

Ключевые слова: *Tardigrada*, тихоходки, *Macrobiotus beotiae*, Опукский природный заповедник, Крым.

Перша знахідка тихохода *Macrobiotus beotiae* Durante & Maucci, 1979 (Tardigrada: Eutardigrada: Macrobiotidae) на території України
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У червні 2007 р. на плато гори Опук (Опукський природний заповідник) були зібрані зразки моху *Tortula ruralis* та лишайнику *Xanthoria calcicola*, у яких були знайдені яйця тихохода *Macrobiotus beotiae*. Це рідкісний вид, раніше відомий лише за знахідками у Греції. Характерною особливістю цього виду є склоподібна (гіалінова) оболонка, що вкриває яйце; у неї частково занурені відростки двох різних типів – довші конічні та коротші заокруглено-циліндричні або булавоподібні.

Ключові слова: *Tardigrada*, тихоходи, *Macrobiotus beotiae*, Опукський природний заповідник, Крим.

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