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First record of the tardigrade *Echiniscus testudo* (Doyère, 1840) from northeast of Iran (Heterotardigrada: Echiniscidae)

Molavi, F.*, Mokhtari, A., Nayebi Moghaddam, S., Abedian, S.M. J. and Azimi Taraghdari, Z.

Department of Biology, Faculty of Science, Mashhad Branch. Islamic Azad University, Mashhad, Iran

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Tardigrada is a phylum closely allied with the arthropods. They are small (0.05-1.20 mm), hygrophilous micrometazoans, have four pairs of lobe-like legs and are either carnivorous or feed on plant material. Most of the tardigrade species are limno-terrestrial. In two moss and lichen samples collected in Mashhad, one heterotardigrade species was found. *Echiniscus testudo* (Doyère, 1840) belongs to the Echiniscidae group and differs from most of other species in this group mainly by having a different claw configuration and by some morphometric characters. This study is the first report of tardigrades from Iran.

Key words: *Water bears, Middle East, Tardigrada, Biogeography, Diversity.*

INTRODUCTION

Tardigrades are water-dwelling, eight-legged, segmented micro-animals. The length of tardigrades is between 50 µm in the smallest juveniles to 1200 µm in largest adults; average length of mature adults is between 250 – 500 µm. Tardigrades live in diverse marine, freshwater and terrestrial habitats throughout the world from oceanic depths (they were found approximately 4000 m under sea level) to tops of mountains (Ciobanu, 2005). They inhabit the interstices of sediments or surface of submerged plants in freshwaters and seas as well as soil, algae, mosses, lichens, liverworts, and other plants, which are able to accumulate water on their surface (Ciobanu, 2005). They were first discovered by the German zoologist Johann August Ephraim Goeze in 1773. In 1777, an Italian professor of natural history, Lazzaro Spallanzani, named these animals "il Tardigrado" (slow stepper) because its slow crawling movement resembled that of a small tortoise. Their biology has been reviewed by Kinchin (1994), Nelson & Marley (2000) and Nelson (2002). The tardigrades in terrestrial environments are the most well-known. Tardigrades often live alongside rotifers, nematodes and protozoans. Their taxonomy is based on cuticular structures on their body surfaces, claws and buccal apparatus as well as the egg shell structures (in some groups). Geographical distribution of tardigrades is not well known because of insufficient faunistic studies. The current study is the first experimental investigation into the study of tardigrades in NE Iran.

MATERIAL AND METHODS

In April 2015 to September 2016 moss and lichen samples were collected in all countries around the Mashhad (Fig. 1). During a regularly (each week) annual sampling, the tardigrades was observed for the first time in this area were also recorded for environmental monitoring. All individuals and eggs were extracted according to Dastych (1980, 1985) and mounted on microscope slides in

*Corresponding Author: fm_yazdan@yahoo.com

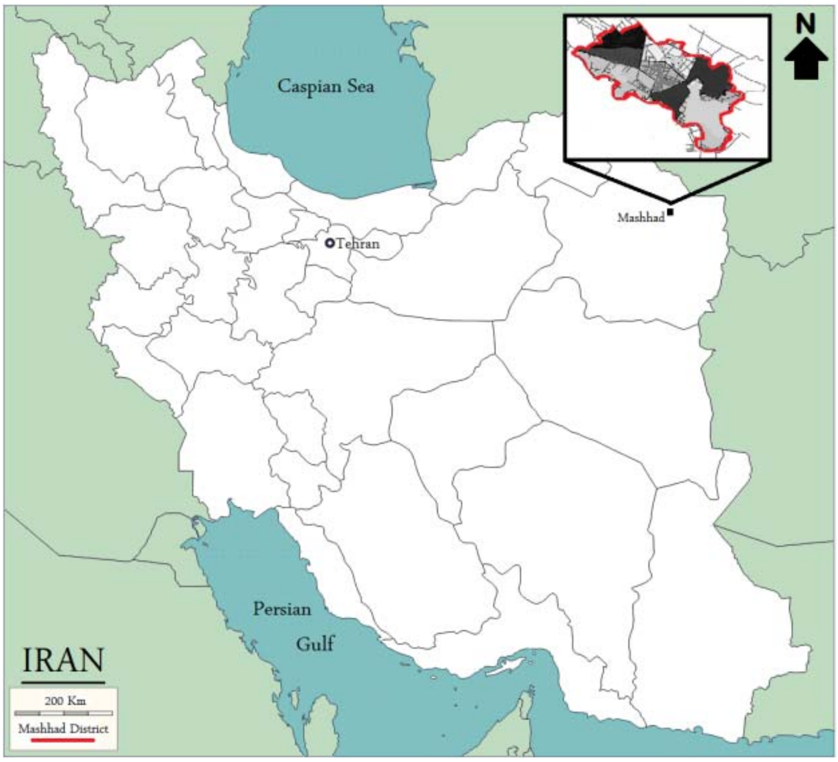

FIGURE 1. Locality of recorded species of Tardigrada in Iran.

Hoyer's medium. 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 recording photos and videos. The specimens were identified according to the common key (Ciobanu, 2005). All measurements were taken using Olympus cell Sens imaging software (Standard Version 1 CS-ST-V1).

RESULTS

In this study, 67 specimens (four in simplex stage), 22 exuvia with eggs, 10 exuvia without eggs and eight free laid eggs were extracted.

Taxonomic account
Phylum Tardigrada Doyère, 1840
Class Heterotardigrada Marcus, 1927 – heterotardigrades
Order Echiniscoidea Marcus, 1927
Family Echiniscidae Thulin, 1928
Genus *Echiniscus* C.A.S. Schultze, 1840
Species *Echiniscus testudo* (Doyère, 1840)

Description of the adults. Body brownish or yellowish (in live specimens) or transparent (after fixation in Hoyer's medium). Eyes present (visible before and after mounting) (Fig. 2). Body length excluding fourth pair of legs, 234 μm. No males were recorded in our samples. This species has six lateral filaments (Fig. 3). Cephalic appendages present, No placoids or one placoid in a pharynx, Cephalic Without cuticular dorsal plates, four peribuccal papillae and four peribuccal lamellae around the mouth opening present Buccal tube slightly funnel-shaped, wider anteriorly (posterior diameter on average 90% of the anterior diameter). Pharyngeal bulb elongated, pear-shaped without

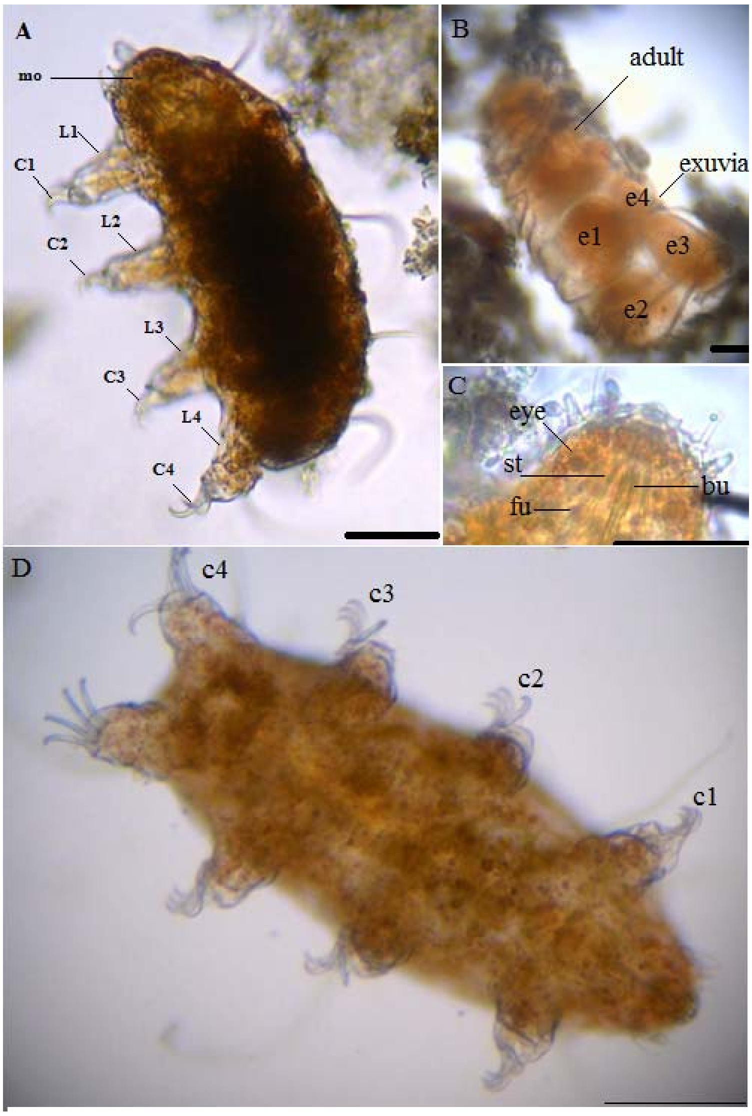

FIGURE 2. General anatomy of *E. testudo*. Scale bars equal 25 µm (A) Differential interference micrograph showing ventrolateral view of the left side of an adult *E. testudo* specimen. The anteroposterior axis includes a head and four trunk segments, each with a pair of legs (left legs are in focus). (B) Light micrograph of an adult laying eggs in its exuvia during molting. (C) DIC micrograph of the ventral head of an adult *E. testudo* specimen. (D) DIC micrograph of the leg claws of the anterior three right legs. Abbreviations: bu, buccal tube; C, claw; e, eggs; fu, furca; L, leg; mo, mouth; st, stylet.

placoids or septulum (Fig. 4). Four cephalic papillae, positioned laterally (Fig. 4). Claws slender, primary claws on all legs simple, unbranched with small accessory points detaching from the branch at its greatest curvature. Secondary claws on all legs with rounded basal thickenings (lunules) (sometimes barely visible). Secondary claws on all legs with three branches (Fig. 5).

Eggs. Oval, smooth and deposited in all samples of this species (Fig. 2).

Comments. A parthenogenetic population of tardigrads is reported in this study for the first time from the similar habitats**.** The density of tardigrads population in studied areas was high but male individuals were rarely observed**.**

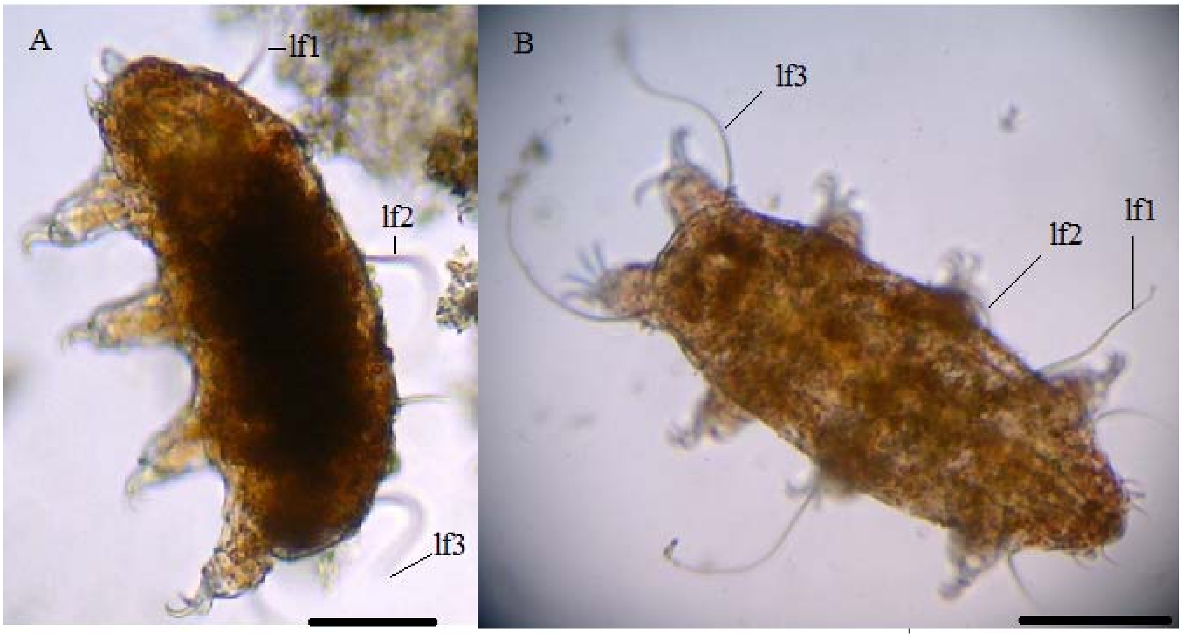

FIGURE 3. General morphology of *E. testudo*. Scale bars equal 25 µm (A) lateral view of lateral filaments of this species. (B) Dorsal view of lateral filaments in *E. testudo*. (lf: lateral filaments).

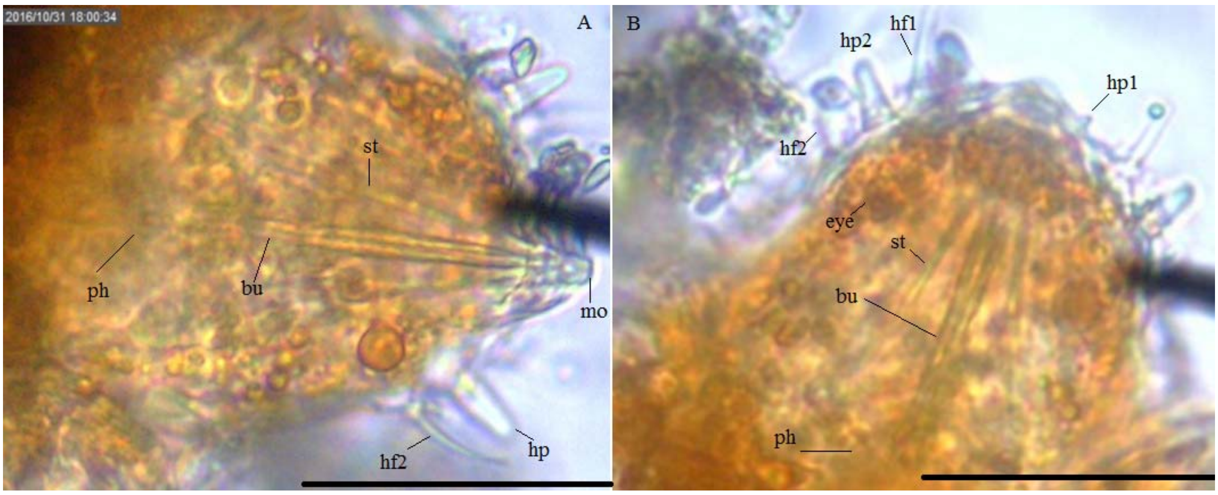

FIGURE 4. Structure of the *E. testudo* head and pharynx. Scale bar equals 25 µm. (A) Ventral -mounted specimens. (B) Dorsal -mounted specimens. The ventral lobe extends on both sides of the buccal tube, but dorsal lods are short. Abbreviations: bu, buccal tube; C, claw; hf, head filaments; hp, head papila ; fu, furca; mo, mouth; st, stylet.

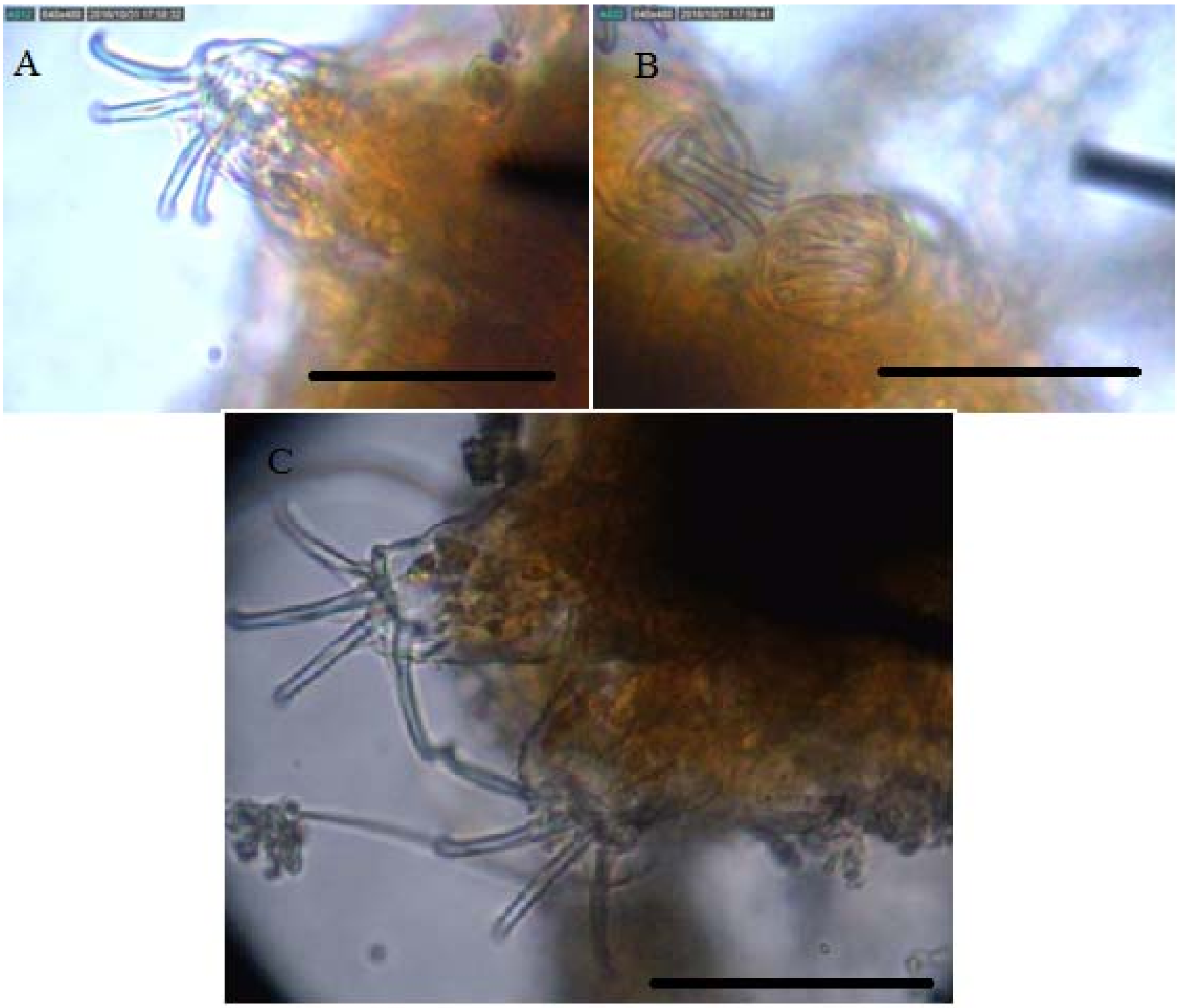

FIGURE 5. General morphology of *E. testudo* claws. Scale bars equal 25 µm (A) First legs (B) Second legs (C) Thirty legs.

DISSCUSSION

In tardigrads, the main intra-phylum division occurs between the classes *Heterotardigrada* Marcus, 1927 and *Eutardigrada* Richters, 1926. The heterotardigrades are a group of species that possess a particular cephalic structure known as cirrus A, they also have a separate gonopore and anus, and often have plated cuticles. Eutardigrade species lack cirrus A and have a cloaca. A third monospecific class, *Mesotardigrada*, was described by Rahm (1937) from a Japanese hot spring. However, both the type specimen and locality are no longer extant and there is some doubt of the validity of the class (Nelson, 2002).

The *Heterotardigrada* are divided into two orders, the marine *Arthrotardigrada* Marcus, 1927 and *Echiniscoidea* Richters, 1926. *Echiniscoidea* comprises four families: *Echiniscoididae* Kristensen & Hallas, 1980; *Carphaniidae* Binda & Kristensen, 1986; *Oreellidae* Puglia, 1959; and the *Echiniscidae* Thulin, 1928. Of these, only *Echiniscoididae* is generally regarded as marine. Species of the other three families occur in limnic or limno-terrestrial environments (DeMilio, 2016).

In the heterotardigrade genus *Echiniscus* has parthenogenesis reproduction and only produce females (Thelytoky) (Kristensen, 1987). Males have recently been found in some *Echiniscus* species from Himalaya, Australia and Antarctica (reviewed in Miller *et al.*, 1999). When continuous parthenogenetic (or asexual) reproduction occurs in a species it results in the absence of gene flow between populations, reduced genetic variability and if parthenogenesis is prolonged it might eventually lead to speciation (Pannebakker *et al*., 2004).

Echiniscus testudo (also known as Turtle Water Bear) (Doyère, 1840) have been recorded from Castlebar (County Mayo, Ireland) by Murray (1911). *Echiniscus testudo* is native to China, Greenland, and Mongolia as well as Europe, the Middle East and North Africa. This species has variation (Ramazzotti & Maucci, 1983). This variation in appendages and in the arrangement of the lateral filaments among *Echiniscus* species are common (Murray, 1911). The difficulties in identifying individuals of such species is further complicated by the lack of supporting genetic data for the delineation of species in these series (Guil & Giribet, 2009).

However, recent investigations indicate that at least the parthenogenetic bdelloid rotifers have undergone substantial speciation (Birky *et al.,* 2005). Parthenogenesis occurs in tardigrades (Bertolani 1994) and many other invertebrate groups, i.e. aphids, crustaceans, nematodes, rotifers, etc. (reviewed in Lushai *et al.,* 2003; Normark *et al.,* 2003). In Echiniscid tardigrades that have a high dispersal potential the ability to reproduce asexually might represent a strong advantage because these species are not limited by the necessity of finding a mate. Much of the speciation (based on phenotypic divergence) within Echiniscus may be a result of the asexual reproduction resulting in the divergence of asexual lineages. Miller *et al.,* (1999) suggested that the *Echiniscus* originated and speciated as sexually reproducing species in Gondwanaland (180 MYA). Though this age of origin might be overestimated many of the Echiniscus species could represent examples of ancient asexuals. The parthenogenetic bdelloid rotifers, darwinulid ostracods and some lineages of oribatid mites are regarded as ancient asexuals (Mark-Welch & Meselson, 2000; Schön & Martens, 2003; Maraun *et al*., 2004). However, this ancient asexual status has recently been questioned for the mites and ostracods (Schaefer *et al.,* 2006; Smith *et al.,* 2006). Tardigrades have just recently been subject to molecular studies, which are mainly phylogenetic (Garey *et al.,* 1996; Regier & Schultz, 2001; Jørgensen & Kristensen, 2004; Guidetti *et al.,* 2005) but also include biodiversity estimation (Blaxter *et al.,* 2004) and the transcription of heat shock proteins (Schill *et al.,* 2004).

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