Short Communication

First Record of *Milnesium* Doyère, 1840 (Tardigrada: Apochela) from the Western Ghats, India.

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Received: July 20, 2022; Revised: September 7, 2022; Accepted: September 17, 2022

Abstract

Tardigrades are the strongest micro-invertebrates on the planet, and their ability to thrive in climatic and pressure extremes is currently gaining attention. The researchers report for the first time the presence of *Milnesium* (Order: Apochela, Class: Eutardigrada) from mosses in the high-altitude of the Nilgiris (2500 msl) in the Western Ghats, as well as observations on its clutch size and egg-laying behaviour. *Milnesium* is a highly tolerant species that has been offered as a candidate for space travel. Until now, just one *Milnesium* species has been documented in India, making the present observation extremely significant. The genus is distinguished by its cuticle sculpture, cuticular characteristics, six peribuccal papillae and two lateral ones, six peribuccal lamellae, and a bucco-pharyngeal bulb devoid of placoids or septum, in addition to its distinctive claws. By providing rotifers and ciliates to the species, the researchers were able to cultivate them successfully in the hay infusion medium. *Milnesium* was observed moulting and laying between one to seven eggs per clutch, which were always found adhered to the exuvia. In this instance, a high-altitude sighting of this species during extreme weather is crucial because the genus is able to withstand harsh conditions such as low temperatures and ionising radiation.

Key words: Tardigrades, *Milnesium*, Eggs, Exuvia, Moulting

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Introduction

Due to their ability to survive in hostile conditions, tardigrade research has recently gained a great deal of scientific attention (Devasurmutt and Arpitha, 2016). They are classified as a different phylum, Tardigrada, with morphological similarities to arthropods and onychophorans, and molecular similarities to nematodes (Richards, 2018). They thrive in a variety of terrestrial and aquatic habitats, including cryptograms (mosses and liverworts), lichens, leaf litter, beaches, sand dunes, and soil, among others (Nelson *et al.,* 2018). They live by slowing down their metabolism, a mechanism known as cryptobiosis, and reviving on a thin layer of water. Tardigrades can survive dehydration and high stress by entering a dormant state known as the tun stage. Various bioprotectants, such as trehalose, LEA proteins, antioxidants, heat shock proteins, tardigrade specific proteins (TDPs), Aquaporin proteins (AQPs), etc., are involved in the stress response (Kamilari *et al.,* 2019; Neves *et al.,* 2020). Due to this ability, the species are appropriate study models for a variety of industries, including agriculture, biotechnology, biomedicine, ecology, and astrobiology (Clegg, 2001; Jonsson *et al.,* 2008).

Milnesium Doyère, 1840, is a genus of the Order Apochela (Class: Eutardigrada) with a great tolerance to harsh environments like ionising radiation (Beltrán-Pardo *et al.,* 2013), desiccation (Schill and Fritz, 2008), and sub-zero temperatures (Hengherr *et al.,*

2010). *Milnesium tardigradum*, a species with a high tolerance, was flown to space for research and has recently attracted notice for demonstrating its enormous capacity to withstand vacuum and solar radiation (Jonsson *et al.,* 2008). *Milnesium* is widely found in mosses and lichens in temperate temperatures and high-altitude locations (Hengherr *et al.,* 2010). *Milnesium* is represented globally by forty-eight species, excluding one subspecies and a species (Rocha *et al.,* 2022). India is home to only one species of *Milnesium tardigrade,* the *Milnesium longiungue* (Tumanov, 2006) discovered from Himachal Pradesh. This paper reports for the first time the presence of *Milnesium* sp. in the high-altitude region of the Nilgiris in the Western Ghats, together with observations of its clutch size and egg-laying habit.

Methodology

The Western Ghats are an ancient mountain range on the west coast of the Indian peninsula. The Nilgiri Hills, located in the southern portion of the Western Ghats, are a very biodiverse region due to their altitude (2500 m asl) and climate conditions. *Milnesium* was identified from mosses growing at high altitudes in the Nilgiris. The genus is characterised by smooth, pseudoporous, or reticulated cuticle sculpturing; cuticular structures (gibbosities, spines, or pseudoplates); six peribuccal papillae and two lateral ones; six peribuccal lamellae; a bucco-pharyngeal bulb devoid of placoids or septum. The secondary branch is distinguished from the long primary branch by the presence of small supplementary points on all claws, which aided in identifying the genus, and by the presence of a spherical thickening at its base.

For this study hay was collected from the market, cut it into pieces, and was washed twice with tap water and distilled water. In a conical flask, hay was cooked after being autoclaved. The hay infusion was stored under sterile conditions for three days. Utilizing hay infusion aliquots, tardigrades and their food sources were cultured. Initially, moss samples were collected in a Petri dish and soaked in distilled water for twelve to twenty four hours. The extra water in the petri dish was drained, and the moss clump was squeezed to a watch glass. Using a micropipette, isolated tardigrades, rotifers, nematodes, and ciliates were put into hay infusion media. After a month, aliquots of tardigrades were examined under an Olympus stereomicroscope. Before being put on Hoyer's medium, the existing tardigrades were fixed in Carnoy's fluid, dehydrated in alcohol, and cleared in lactic acid. The species was identified using the aforementioned identifying characteristics (Moreno-Talamantes *et al.,* 2020) and photographed using an NLCD-307 Lawrence and Mayo microscope. The species of *Milinesium* that were observed living under a microscope in the extract were subcultured in a medium containing an infusion of hay and studied.

Results

Within a month, the medium demonstrated ecological succession through a high density of rotifers (50 per drop) and ciliates on which tardigrades fed. *Milnesium* tardigrades were isolated from the culture and grown individually in scratched plastic petri dishes with ciliates and rotifers as prey. The observed *Milnesium* species displayed notable differences from *M. tardigraum* and modest differences from *M. longiungue,* but the species status cannot be determined. (Figure 1). The researchers watched *Milnesium* moulting and made the observation that it lays between one to seven eggs at a time, with the eggs always seen attached to the exuvia (Figure 2). When separated and cultured in culture plates, the cuticular shed (exuvia) with eggs hatched at a rate of 92%, and the hatched ones were identified as females. Females who had moulted were seen to re-moult after a sixty to seventy day interval.

Figure 1. (a) *Milnesium* observed from the high altitude of the Nilgiris. (b) *Milnesium* ready to moult with eggs

Discussion

The *Milnesium* species found in the Nilgiris are distinct from *Milnesium longiungue*, the sole species found in India. However, further research on the specimen is necessary to determine the species status. Limno-terrestrial tardigrades are sexually monogamous with intermittent hermaphroditism (Altiero and Rebecchi, 2001). Self-fertilization and thelytoky have been observed in species found in mosses (Altiero *et al.,* 2018). When parthenogenesis and self-fertilisation do not occur in the same species, self-fertilisation is reported as an evolutionary mechanism (Bertolani, 2001). Female limno-terrestrial

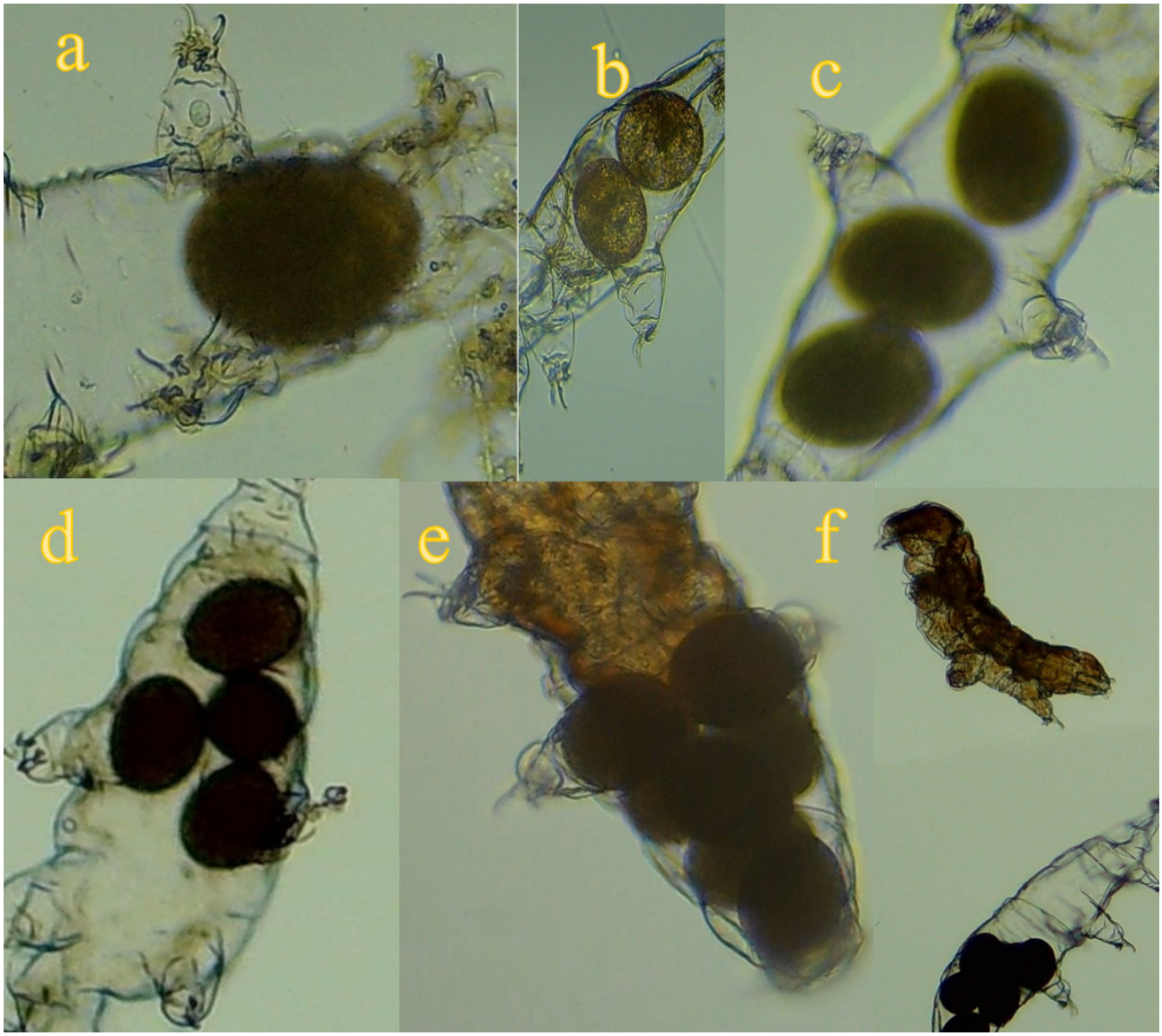

Figure 2. Exuvia of *Milnesium* with eggs (a: Single egg, b: with two eggs, c: with three eggs, d: with four eggs, e: with seven eggs, f: post moulting stage).

tardigrades are said to be iteroparous, which means they can lay eggs freely or in their own exuvia.

Milnesium lives in the moss's upper layer and feeds on ciliates, nematodes, and rotifers (Beasley and Miller, 2007). *Milnesium inceptum* and *Milnesium pentapapillatum* have been shown in the past to reproduce parthenogenetically (Morek *et al.,* 2019-2020). The *Milnesium* species found in the Nilgiris could possibly be parthenogenetic, as only females were observed in the culture. Ontogenetic variation in claw configuration has been reported between instars and adults (Morek *et al.,* 2016). Instar identification was not attainable in this study and requires additional observations under controlled settings. The unique abilities of tardigrades, such as anoxybiosis, cryobiosis, osmobiosis, chemobiosis, and anhydrobiosis (Weronika and Łukasz, 2017), have enabled the development and isolation of biochemicals in industrial and medical applications. At water loss, tardigrades produce a bioprotectant called trehalose, which protects macromolecules such as proteins and nucleic acids from losing their native structure (Kinchin, 2008). Even though tardigrades are extremotolerant, they have a temperature tolerance limit (Neves *et al.*, 2020). Researchers found that a unique protein in tardigrades called the Damage suppressor (Dsup) protein binds to human cells, reducing the amount of DNA damage

caused by radiation up to 40% (Hashimoto *et al.,* 2016). *Milnesium* is a genus that has been extensively used in space research to assess the survival rate. This demonstrates the significance of distinguishing *Milnesium* from climate extremes such as the high altitudes of the Nilgiris.

Acknowledgments

The authors would like to thank the Department of Science and Technology, Government of India, for funding the purchase of instruments through the FIST Scheme (SR/FST/College-2017/11). The first author is grateful to the University Grants Commission, New Delhi, for providing financial assistance under the UGC-NET JRF scheme (476/(CSIRNETJUNE2019).

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