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A NEW SPECIES OF *Milnesium* DOYÈRE, 1840 (TARDIGRADA: APOCHELA: MILNESIIDAE) FROM AN URBAN PARK, SOUTHEASTERN BRAZIL.

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Abstract
Tardigrades are bilaterally symmetrical micrometazoans, generally less than 0.5 mm in size, bearing four pairs of legs with claws. They have the ability to undergo cryptobiosis, which have created an interest in the medical community. Despite this, little is known about their diversity and distribution, mainly in the Neotropical region. In Brazil, most of the taxonomic studies of this phylum were done between 1930 and 1940. Here we provide the first description of a new species belonging to genus *Milnesium* Doyère, 1840 found in mosses on trees from an urban park in Brazil. This tardigrade is characterized by cuticle smooth, short cuticular bars under the claws IV, claw configuration [3-3][3-3] with internal and anterior spurs larger than external and posterior spurs, and dorsal pseudoplates. This is the first of a series of studies dedicated to increase the knowledge of the Brazilian limnoterrestrial tardigrates that has been neglected for years in terms of its taxonomy.

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Introduction

Tardigrades or “water bears” are bilaterally symmetrical animals with a characteristic bucco-pharyngeal apparatus and four pairs of lobopodous legs, usually terminating in claws and/or adhesive disks. In most of the species mature adults have between 0.25–0.5 mm in length. The name “il Tardigrado” (slow-stepper) was introduced by Lazzaro Spallanzani in 1776 to describe the slow, tortoise-like movement of the animal (Nelson *et al*., 2015). Like arthropods and nematodes, tardigrades grow through ecdysis and it has been suggested that they belong to a taxon known as Ecdysozoa that contains all molting animals (Aguinaldo *et al*., 1998). These microinvetebrates are known for the ability to undergo cryptobiosis. In this ametabolic state they are able to tolerate exposure to various chemical and physical extremes (Wełnicz *et al.*, 2011).

Tardigrada specimens can inhabit distincts terrestrial, freshwater, and marine habitats, from the abyss in the deep sea to the highest mountains (Ramazzotti & Maucci, 1983). Despite this widespread distribution, little is know about their diversity and distribution, mainly in Neotropical region. The publication of species description found in this region occurs as abrupt steps, rather than the gradual steady increase showed in other biogeographic regions (Guil & Cabrero-Sanudo, 2007).

In Brazil, most of what we know about the limnoterrestrial tardigrades (that is, that live in mosses, lichens, leaf litter, etc) were done between the decades of 1930 and 1940, when the pioneering studies performed by Rahm (1931, 1932), Marcus (1937, 1939), De Barros (1938, 1939, 1942, 1943), and du Bois-Reymond Marcus (1944) were done. Since this period, only the studies done by Pilato (2000) and Pilato *et al.* (2004) were carried out in Brazil with regard to the limnoterrrestrial tardigrades. These taxonomic studies were based on a limited number of morphological characters and using only the techniques available by that time. Thus, in the present study we provide the first description of a new species belonging to genus *Milnesium* Doyère, 1840 found in Brazil, using modern morphological techniques. We aim to contribute to reduce the knowledge gap about the diversity of a taxon in Brazil that has been neglected for many years.

Methods

Several moss samples were collected by the authors between November, 2017 and May, 2018, from trees growing near to the Department of Animal Biology, Institute of Biology, UNICAMP, Campinas, SP, Brazil (22°49’13.5”S 47°04’09.1”W; 606 m a.s.l). The samples were collected and examined for tardigrades using standard methods (Ramazzotti & Maucci, 1983). At least three different morphotypes were found and one of them was selected for a more detailed study. All live specimens destined to morphometric and morphological analysis were subjected to thermal relaxation according to the protocol by Stec *et al*. (2015) then stored in 70% ethyl alcohol.

Specimens for light microscopy were mounted on microscope slides in a standard quantity of Fluoromount Aqueous Mounting medium. Dried slides were examined under a Zeiss Axio Imager M2 light microscope equipped with Differential Interference Contrast optics (DIC) associated with a camera.

Photomicrographs and measurements were taken using the software ZEN - blue edition. Individuals and their traits were measured only if their orientation was suitable, according to Bartels *et al.* (2011) and Stec *et al.* (2016) recommendations. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs, according to Morek *et al*. (2016). Claw and buccal tube measurements were made according to Tumanov (2006), and additional buccal tube widths and ratios were determined based on Michalczyk *et al*. (2012).

The slides were also digitally documented using an Olympus BX60 epifluorescence microscope equipped with Nomarski DIC condenser and lenses, and filters for detecting UV autofluoresence (excitation filter: 330–385 λ, barrier filter: 420 λ, with a mercury lamp illuminator).

The most preserved specimens were destined for scanning electron microscopy (SEM). They were processed according to the protocol by Mitchell & Miller (2008) and examined under a JEOL JSM 5800LV Scanning Electron Microscope. Photomicrographs were taken using SemAfore 5.21.

Results and Discussion

The morphotype analyzed was identified as a member of the class Eutardigrada that, by having paired cephalic papillae, peribuccal papillae and claws with primary and secondary branches completely separated, belongs to the family Milnesiidae. Comparing the data obtained from our morphological and morphometric analysis with the literature, we were able to describe it as a new species of *Milnesium* Doyère, 1840.

The *Milnesium* sp. nov. has body yellowish to brownish (before and sometimes after preparation). Eyes present in most of fixed specimens. Cuticle smooth without granulation or reticular design, and without pseudopores (Fig. 1 A). The dorsum is covered with pseudoplates (delineated geometric areas of cuticle) (Figs. 2 A and C). Six peribuccal papillae (ventral papilla smallest) and six triangular peribuccal lamellae (of unequal size, 4+2) are present around the mouth opening (Figs. 1 B and 2 B). The internal surface of peribuccal lamellae is longitudinally striped. Two cephalic papillae positioned laterally, smaller in all proportions than the peribuccal papillae. Pharyngeal bulb elongated, pear-shaped, without placoids or septulum. Primary branches on all legs with small but distinct accessory points detaching from the branch at its greatest curvature. Secondary claws on all legs with rounded basal thickenings, sometimes barely visible. Secondary claws on all legs with three branches (claw configuration: [3-3]-[3-3]) (Figs. 1 C and D). Internal and anterior spurs considerably larger and longer than external and posterior spurs. Single, long and transverse cuticular bars under claws I–III present (Fig. 1C). Short cuticular bars on legs IV (Figs. 1 C and D). Eggs: oval, smooth and deposited in exuvia as in all other known *Milnesium* species.

Milnesium sp. nov., with smooth cuticle, the presence of accessory points and the [3-3]-[3-3] claw configuration, is similar to other eight *Milnesium* species. Among these species only *Milnesium shilohae* Meyer, 2015, from Hawaii, exhibit asymmetrical spurs on external and internal and on anterior and posterior branches, as seen in *Milnesium* sp. nov. However, the former species can be distinguished from the latter by the presence of short cuticular bars on legs IV and by having the dorsum covered with pseudoplates.

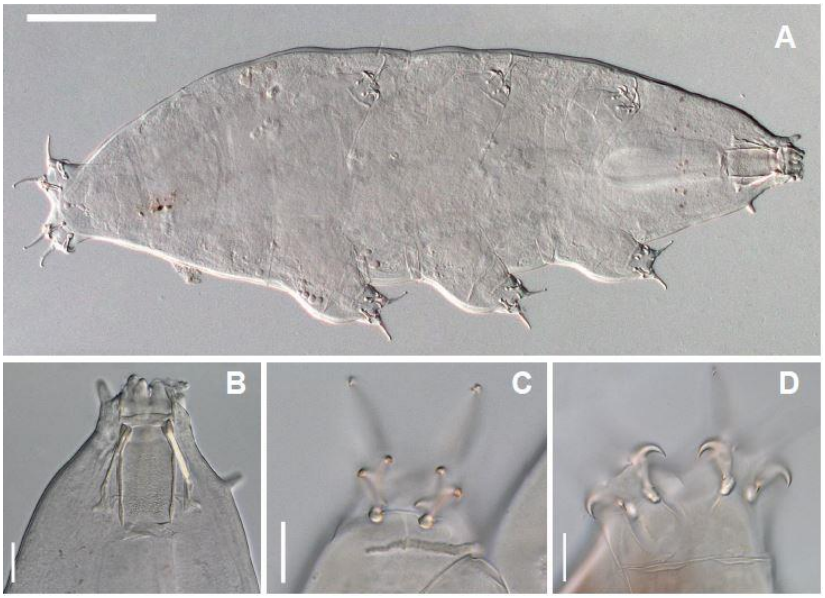

Figure 1. A- Habitus, ventral view, scale bar = 100µm; B- Buccal apparatus, scale bar = 20µm; C- Claws of legs I, scale bar = 20µm; D- Claws of legs IV, scale bar = 10µm.

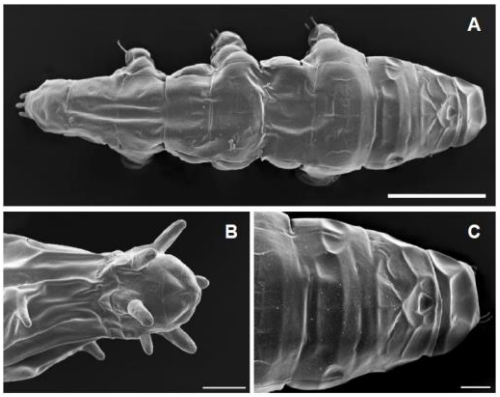

Figure 2. A- Habitus, dorsal view, scale bar = 100µm; B- Buccal apparatus, scale bar = 10µm; C- Dorsal pseudoplates, scale bar = 20µm.

Conclusions

At a first glance, Palaearctic and Nearctic regions, when compared with other biogeographical regions, appears to support most of the known limnoterrestrial tardigrades diversity. However, this pattern seems to be bias, as most of the researches are housed in North American and European institutions and the sampling efforts in these two regions is much more intense than in Afrotropic, Indomalaya, Australasia and Neotropical regions. In this study we could observe that this bias about the tardigrade's diversity can be real because, despite that the knowledge about Brazilian limnoterrestrial tardigrades is incipient and limited, we could sample a new taxon for the science collected in mosses from an urban area and at least two other morphotypes (data not shown).

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