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ORIGINAL ARTICLE

PRELIMINARY DATA ON TARDIGRADA FROM THE ARMENIAN FORESTED AREA

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ABSTRACT

Tardigrada is a phylum that has never been studied in Armenia, along with many other arthropod groups. Our research is about terrestrial tardigrade species. This study is the first paper on tardigrades from Armenia. Six species of tardigrade were identified, belonging to five genera: *Macrobiotus hufelandi* C.A.S. Schultze, 1834; *Paramacrobiotus areolatus* (Murray, 1907); *Paramacrobiotus richtersi* (Murray, 1911); *Ramazzottius cf. oberhaeuseri* (Doyère, 1840); *Milnesium tardigradum* Doyère, 1840; and *Echiniscus testudo* (Doyère, 1840), which are present in lichen and moss from the northern forests of Tavush Province in Armenia. All the mentioned are new to the fauna of Armenia.

Keywords: Armenia, Forest diversity , New records, Tardigrada, Water bears.

INTRODUCTION

Tardigrades inhabit diverse ecosystems, ranging from terrestrial environments such as mosses and lichens to the deepest abysses of the oceans (Nelson *et al*., 2015). Notably, their ability to withstand extreme conditions, including desiccation, high levels of radiation, and even the vacuum of space, has elevated them to the status of extremophiles (Jönsson, 2007; Møbjerg *et al.*, 2011). These remarkable adaptations challenge our understanding of the limits of life on Earth and have led researchers to explore the potential implications for astrobiology, biotechnology, and environmental science (Jönsson *et al.*, 2008).

Early taxonomic studies in the Caucasus region have identified Tardigrada species belonging to genera such as *Echiniscus* C. A. S. Schultze, 1840, and *Milnesium* Doyère, 1840 (Ramazzotti and Maucci, 1983; Dastych, 1988). However, the overall diversity and distribution patterns remain poorly explored. Recent advancements in molecular techniques and imaging technologies offer unprecedented opportunities to unveil the hidden biodiversity

of Tardigrada in this region (Topstad *et al.*, 2021), but the Armenian fauna of Tardigrada remains unexplored.

Armenia, nestled at the crossroads of Western Asia and Eastern Europe, boasts a diverse topography ranging from high mountainous terrains to lowland areas, creating a mosaic of ecosystems. Preliminary studies have hinted at the presence of Tardigrada in Armenian environments, but a comprehensive understanding of their diversity and ecological roles within the region is yet to be established. Only one species belonging to aquatic Tardigrada has been described from Sevan Lake *Pseudobiotus megalonyx* (Thulin, 1928) (Krilov *et al.*, 2010).

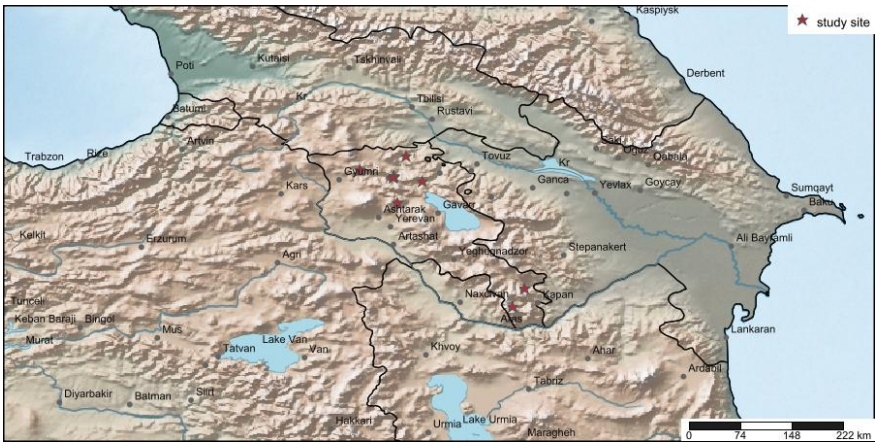
The interdisciplinary nature of Tardigrada research is evident in its relevance to fields such as astrobiology, where the study of extremophiles informs our understanding of potential life beyond Earth (Sloan, 2018). So, this paper seeks to bridge this knowledge gap by offering a comprehensive examination of the Tardigrada fauna in Armenia, shedding light on their taxonomy, distribution, and potential ecological significance.

MATERIALS AND METHODS

Sampling and specimens examined: Nine sites in the mountainous forested area of Armenia were sampled in 2022-2023. Geographic coordinates, temperature during the collection season, and elevation are provided in Table (1) and Map (1). The flora of these forests includes diverse species, mainly *Acer campestre* L., *Celtis caucasica* Willd., *Carpinus orientalis* Mill., *Elaeagnus angustifolia* L., *Fagus orientalis* Lipsky, *Fraxinus excelsior* L., *Quercus macranthera* Fisch. & C. A. Mey. ex Hohen, *Juglans regia* L., *Juniperus foetidissima* Willd., *Pistacia mutica* F &M, *Pinus kochiana* Klotzsch ex K. Koch, *Populus tremula* L., *Salix caprea* L., *Taxus baccata* L., and *Tilia cordata* Mill.

The specimens were examined in the experimental zoology laboratory of the Scientific Center of Hydroecology and Zoology of NAS RA using a Levenhook D95 LCD digital microscope and a Bresser trinocular microscope, and examined for terrestrial tardigrades, using a standard method described by Dastyc (1980).

Identification: The species were identified based on keys mentioned in many references: Bertolani (1971), Dewel and Clark (1973), Dewel and Wallis (1973), Bertolani and Mambrini (1977), Ramazzotti and Maucci (1983), Biserov (1990, 1998), Bertolani and Rebecchi (1993), Biserov and Trumanov (1993), Guidetti and Bertolani (2005), Guidetti *et al*. (2005, 2013, 2019, 2021), Horikawa *et al*. (2006), Bertolani *et al*. (2010, 2011), Wełnicz *et al*. (2011), Kaczmarek *et al*. (2012, 2015), Vicente and Bertolani (2013), Suzuki (2016, 2022), Kaczmarek and Michalczyk (2017), Fontoura *et al.* (2017), Stec *et al*. (2018, 2020), Morek *et al*. (2019), and Kayastha *et al*. (2023a, b). The material is kept in the Scientific Center of Hydroecology and Zoology of NAS RA invertebrate collection as preparations.



Map (1): The locality of recorded species of Tardigrada in Armenia (by Simple mapper).

Table (1): The study area of Tardigrada.

Site No.	Site name	Coordinates	Altitude m.a.s.l	The highest temperature of collected day ˚C
1	Dilijan	40.769095 N, 44.922545 E	1200	+13
2	Karaberd	40.837661 N, 44.553144 E	2400	+18
3	Kapan	41.094901 N, 44.711738 E	922	+24
4	Pambak	40.82788 N, 44.55829 E	1250	+22
5	Vanadzor	40.815524 N, 44.546165 E	1240	+23
6	Zangezur National Park	39.152721 N, 46.089392 E	2050	+19
7	Tatev	39.38611 N, 46.24451 E	1503	+35
8	Trchkan	40.915187 N, 44.114010 E	1860	+4
9	Aghveran	40.4903 N, 44.6001 E	1720	+24

RESULTS AND DISCUSSION

The current study documented six species belonging to five genera. These species of tardigrade will be shown as follows:

Class: Eutardigrada
Order: Parachela
Family: Macrobiotidae
Genus ***Macrobiotus*** C.A.S. Schultze, 1834
Macrobiotus hufelandi C.A.S. Schultze, 1834 (Pl. 1)
Synonym: *Macrobiotus schultzei* Greef, 1866
Material examined: Syunik Prov., Zangezur National Park, 26. vii.2023; Tavush Prov., Dilijan, 09.x.2022.

Distribution: Worldwide (Kaczmarek and Michalczyk, 2019).

Habitat in Armenia: This species has been recorded from soil and moss.

Description of adults: Light-brown body with eye spots. Body length: 510 µm; cuticle with small pores; buccal armature characterized by an anterior and posterior band of well-defined teeth, followed dorsally and ventrally by three distinct transverse crests. Ventral median crest rounded; two pillar-shaped macroplacoids and microplacoid, the first macroplacoid longer than the second. Legs have infrequent granulation located behind the claws on fourth pair, limited and located laterally on the first three legs. Y-shaped claws, with conspicuous accessory points on the main branch; small lunulas with smooth margins present on the first three pairs of legs; larger on the fourth pair.

Comments: There are no certain records for this species in the Caucasus or neighboring Iran, but it has been recorded in Turkey (Berdi *et al.*, 2024). This is the first record for Armenia and the region.

Genus ***Paramacrobiotus*** Guidetti, Schill, Bertolani, Dandekar and Wolf, 2009
Paramacrobiotus areolatus (Murray, 1907) (Pl. 2)
Synonym: *Macrobiotus areolatus* Murray
Material examined: Tavush Prov., Dilijan, 09.x.2022.

Distribution: Worldwide distribution (Kayastha *et al.*, 2023 a, b).
Habitat in Armenia: This species was recorded from forest mosses and other moist habitats, where *P. richtersi* was found too.

Description of the adults. This species has a white body. Body length 450 µm, without microplacoid; a thin cuticular thickening is present in its place, and granulations are situated on I-IV pair of legs. Eyes present, lunules under the claws of IV, and three rows of macroplacoids visible. Body of cuticle smooth, with the number of rows in the oral cavity armature ranging from I to III.

Comments: This species was not recorded in Iran nor in the Caucasus, but it was recorded in Turkey (Berdi and Altındag, 2020), and is the first record for the fauna of Armenia.

Paramacrobiotus richtersi (Murray, 1911) (Pl. 3)
Synonym: *Macrobiotus richtersi* Murray, 1911
Material examined: Tavush Prov., Dilijan, 09.x.2022,
Distribution: The species has a worldwide distribution (Guidetti *et al*., 2019).
Habitat in Armenia: This species is primarily found in humid environments, especially in forest trees.
Description of the adults: body brownish with length 300 µm, microplacoid exists, fine granulations situated on the I-III pair of legs, eyes absent, lunules under claws IV smooth, dorsal cuticle covered with small polygons; pt values of macroplacoid more than 48; number of rows in the oral cavity armature I-III.

Comments: This species was recorded in the neighboring country (Turkey) (Kaczmarek *et al*., 2012), but this is the first record for the fauna of Armenia.

Family: Ramazzottiidae
Genus ***Ramazzottius*** Binda & Pilato, 1987
Ramazzottius cf. oberhaeuseri (Doyère, 1840) (Pl. 4)
Synonym: *Ramazzottius oberhaeuseri* (Doyère, 1840)
Material examined: Lori Prov., Karaberd, 26.iii.2023
Distribution: Cosmopolitan (Stec *et al*., 2018).
Habitat in Armenia: This species was recorded from mosses and lichens in sparse forests north of Armenia.

Description of the adults: Body with red-brown colored and length 490 µm, microplacoid absent, inner claws short and thick. maroon granules are arranged in nine horizontal bands, featuring visible longitudinal rows devoid of pigment. Eyes absent, and hind claws with small and smooth pseudolunules, but more developed under claw IV. Two separated macroplacoids are visible; the body cuticle is smooth; oral cavity armature consists of two posterior bands of teeth.

Comments. This species was recorded in Turkey (Kaczmarek *et al*., 2012) but not in the Caucasus, and is the first record for the fauna of Armenia.

Order: Apochela
Family: Milnesiidae
Genus ***Milnesium*** Doyère, 1840
Milnesium tardigradum Doyère, 1840 (Pl. 4)
Synonyms: *Arcrophanes schlagintweitii* Ehrenberg, 1859
Arctiscon tardigradum Schrank, 1803
Material examined: Lori Prov., Karaberd, 26.iii.2023.
Distribution: Cosmopolitan (Brotto-Guidetti *et al.*, 2024).
Habitat in Armenia: This species was recorded from mosses of forested areas in Armenia.

Description of the adults: Body with brownish transparent, Body length 926 µm, black eyes present, and pharyngeal bulb elongated without placoids. Placoids absent, basal spur on outer claws present with rounded basal thickenings. body cuticle without pores.

Comments: This species was not recorded in neighboring countries and is the first record for the Armenian fauna.

Class: Heterotardigrada
Order: Echiniscoidea
Family: Echiniscidae
Genus ***Echiniscus*** Schultze, 1840
Echiniscus testudo (Doyère, 1840) (Pl. 6)

Synonyms: *Echiniscus bellermanni C*.A.S. Schultze, 1840
Echiniscus biunguis C.A.S. Schultze, 1861
Echiniscus filamentosus Plate, 1888
Echiniscus glaber Bartoš, 1937
Echiniscus inermis Richters, 1902
Echiniscus peruvianus Binda & Pilato, 1994
Emydium testudo Doyère, 1840
Echiniscus trifilis Rahm, 1925

Material examined: Lori Prov., Karaberd, 26.iii.2023.

Distribution: Europe, Africa, Asia, N. America and S. America (Kaczmarek *et al*., 2015, 2016).

Habitat in Armenia: This species was recorded from mosses and lichens in sparse forests north of Armenia.

Description of the adults: Body with orange-colored and dorsal spines present. Body length 442 µm. Microplacoid absent. Eyes present and red, and all internal claws with recurved spurs, and all external claws simple; macroplacoids absent. Body cuticle composed of polygons and light granulation on all legs. Body appendage configurations A, B, C, D, E are visible.

Comments: This species was recorded in Turkey and Iran (Berdi and Altında, 2020; Mokhtari *et al.*, 2018) and wasn't recorded in the Caucasus countries. It is the first record for the fauna of Armenia.

Forest cover, comprising 11.2% of Armenia, represents one of the country's less-studied ecosystems. However, with a growing focus on climate change mitigation, research in these forests is expanding, highlighting all components of their biodiversity. Given the mountainous terrain of Armenia's forested regions, access is challenging, and field conditions are suitable only during a brief period in summer. Consequently, findings on Tardigrada in these areas remain preliminary.

All species included in this study represent the first records for Armenian fauna. Six species *Macrobiotus hufelandi, Paramacrobiotus richtersi, Paramacrobiotus areolatus, Ramazzottius oberhaeuseri*, *Echiniscus testudo*, and *Milnesium tardigradum* were previously unknown from both the Caucasus and the Armenian Highland, likely due to the scarcity of studies in this region and the historical neglect of Tardigrada research here.

Armenia's forest biodiversity, particularly that of Tardigrades, is believed to be quite rich, yet the results from preliminary research are insufficient to clarify their distribution and diversity. Future studies may reveal more about the distribution, traits, and potentially even new species of these fascinating organisms.

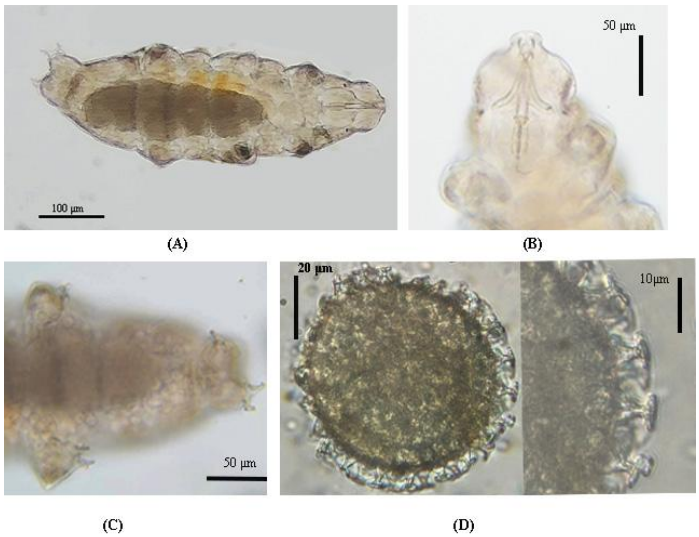


Plate (1): *Macrobiotus hufelandi*; (A) General view, (B) Buccal apparatus, (C) Claws, (D) Egg.

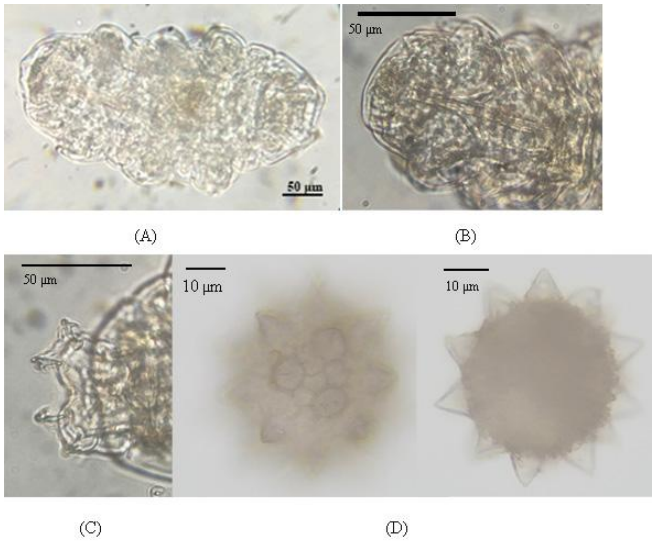


Plate (2): *Paramacrobiotus areolatus*; (A) General view, (B) Buccal apparatus, (C) Claws, (D) Egg.

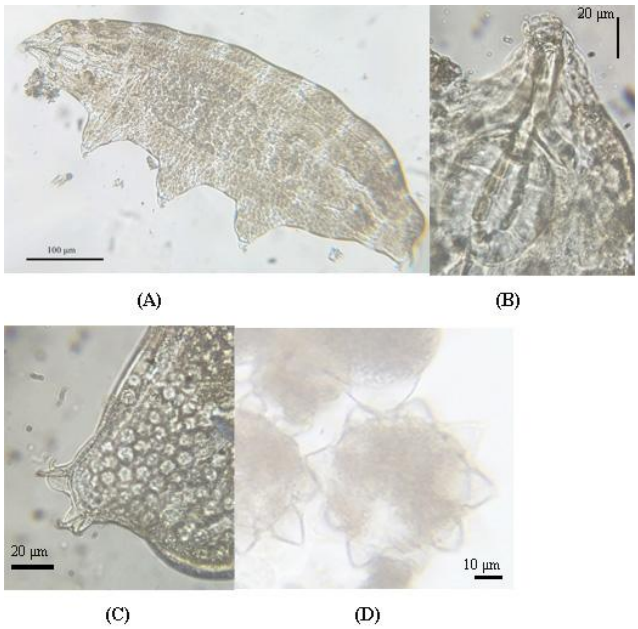


Plate (3): *Paramacrobiotus richtersi*; (A) General view, (B) Buccal apparatus, (C) Claws, (D) Egg.

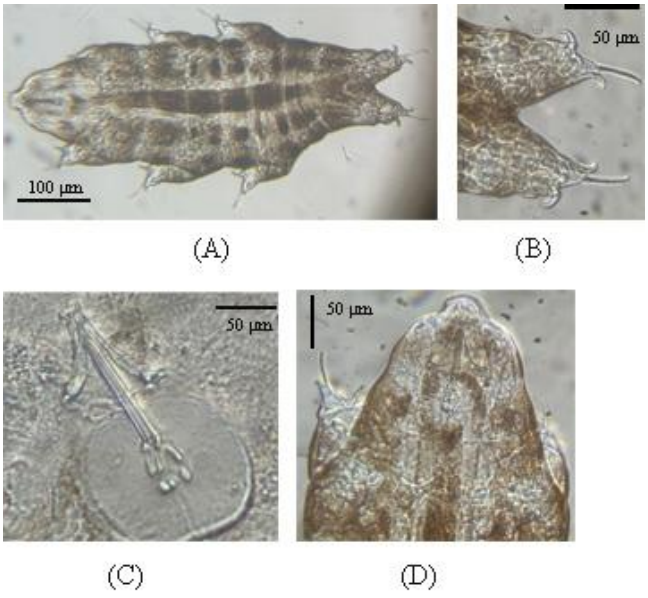


Plate (4): *Ramazzottius cf. oberhaeuseri*; (A) General view, (B) Claws, (C) Buccal apparatus, (D) Buccal apparatus.

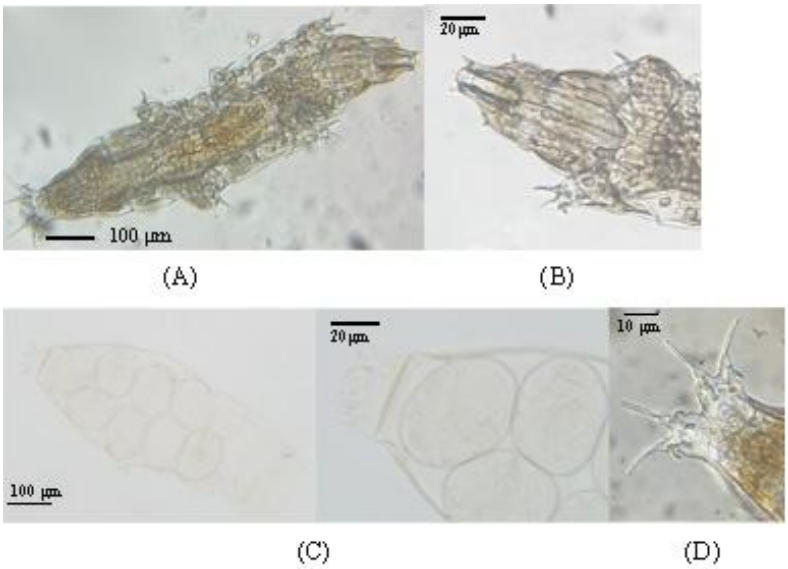


Plate (5): *Milnesium tardigradum*; (A) General view, (B) Buccal apparatus, (C) Eggs, (D) Claws.

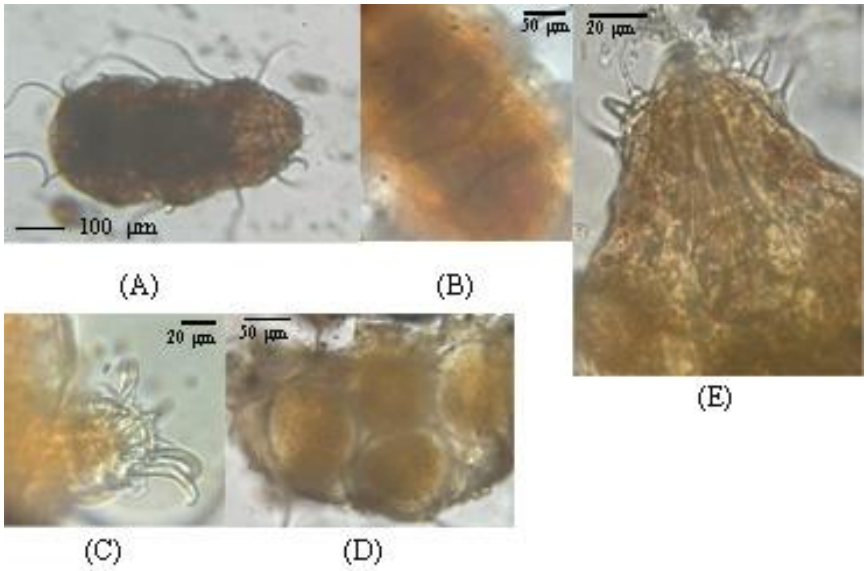


Plate (6): *Echiniscus testudo;* (A) General view, (B) Cuticle, (C) Claws, (D) Buccal apparatus, (E) Eggs.

CONCLUSIONS

This study presents the first comprehensive record of forest-dwelling Tardigrada species in Armenia, documenting six species *Macrobiotus hufelandi*, *Paramacrobiotus richtersi*,

Paramacrobiotus areolatus, *Ramazzottius cf. oberhaeuseri*, *Milnesium tardigradum*, and *Echiniscus testudo* none of which had previously been reported from Armenia, the Caucasus, or the Armenian Highland. These findings significantly expand the known distribution ranges of these taxa and highlight the underexplored nature of Armenia's forest ecosystems.

Given the challenging field conditions and limited seasonal window for research, the data presented here should be regarded as preliminary. Nevertheless, the discovery of these cosmopolitan and widespread taxa in Armenia underscores the potential richness of tardigrade diversity in the region. The mountainous forest habitats of Armenia, though covering a modest portion of the country, appear to harbor a distinct and potentially diverse tardigrade fauna.

Although this study does not include quantitative measures of abundance, diversity indices, or statistical analyses correlating species occurrence with environmental variables, this was a deliberate methodological choice given its baseline, exploratory nature. Our primary aim was to document, for the first time, the terrestrial tardigrade fauna of Armenia through accurate morphological identification and distributional reporting. For regions and taxa with no prior terrestrial records, such faunistic inventories are an essential first step before more complex quantitative analyses can be meaningfully applied. The environmental metadata we provide—including precise coordinates, altitudes, and microhabitat details—form a foundational dataset that will enable future studies to incorporate multi-season sampling, standardized abundance assessments, diversity metrics, and ecological modeling.

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CONFLICT OF INTEREST STATEMENT

"The authors declare that there is no conflict of interest".

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بينات أولية عن بطيئات الخطوTardigrada من المناطق الغابية في أرمينيا

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الخلاصة

تُعد البطيئات الخطو Tardigrada من الشُعب التي لم تُدرس قط في أرمينيا، إلى جانب العديد من مجموعات المفصليات الأخرى. يركّز بحثنا على أنواع البطيئات الخطو البرية. وتُعد هذه الدراسة أول عمل علمي يتناول البطيئات الخطو في أرمينيا. تم تحديد ستة أنواع من هذه الكائنات، تنتمي إلى خمسة أجناس وهي:

Macrobiotus hufelandi (C.A.S. Schultze, 1834)

Paramacrobiotus areolatus (Murray, 1907)

Paramacrobiotus richtersi (Murray, 1911)

Ramazzottius cf. *oberhaeuseri* (Doyère, 1840)

Milnesium tardigradum (Doyère, 1840)

Echiniscus testudo (Doyère, 1840)

تم العثور عليها في الأشنيات والطحالب الموجودة في غابات مقاطعة تافوش الشمالية في أرمينيا. وتُعد جميع هذه الأنواع جديدة على عالم الاحياء الأرمنية.