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Echiniscus ehrenbergi sp. n., a new water bear from the Himalayas (Tardigrada)

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(With 12 figures)

Abstract

Echiniscus ehrenbergi sp. n., a new tardigrade from the Himalayas (Nepal, the Mt. Everest Region) is described and illustrated. The new species resembles *E. testudo* (Doyère, 1840) but differs from the latter mainly by its brown-blackish body, regular dorsal sculpture, the presence of a distinct spur on the internal claws and the presence of males.

Introduction

Tardigrada (or water bears) are among the most widely distributed animals, including a surprisingly wide altitudinal span, from the abyssal plains of the deep see up to the highest peaks in the glacier mountains. The highest altitude recorded for this group, quoted in many zoological handbooks as "6000 m in the Himalayas", goes back to Ehrenberg (1858), who reported three tardigrade species at an altitude of 18- and 20 000 feet, respectively (= 5486 and 6096 m). That material was collected from the Milnum and Ibi Gamin Passes in the "...true Himalayas or the southernmost of three mountain ranges between India and Yarkand" (*op. cit.*, our translation).

Our knowledge of the Himalayan tardigrades, particularly about those from the highest altitudes, is still very poor and limited to a few short contributions, listing a total of 40 nominal taxa (*op. cit.*, Murray 1907, Ramazzotti 1968, Dastych 1975, 1976, 1986). The last two papers deal with water bears found within the upper zone of the Himalayan meadows; Ehrenberg and Ramazzotti (*op. cit.*) examined this fauna in the nival zone. The tardigrades of the neighbouring mountain regions are even less known. Kristensen (1987) reports two species from Kashmir (Ladakh, 4900 m), and Dastych (1973) lists six species from the Karakorum.

When recently processing our bryophyte samples collected in the Mt. Everest Region at 5150 and 5546 m (Khumbu Himal), we encountered many specimens of an unusually dark coloured tardigrade in the genus *Echiniscus*. The species proved to belong to a new taxon and its description is given below. This material represents

the highest verifiable altitudinal record for any tardigrade, aside from the data reported by Ehrenberg (*op. cit.*) with his sites which are now difficult to locate.

The type material is mounted in Faure's medium and deposited in the Zoological Museum Hamburg (ZMH), the Zoological Museum, University of Copenhagen (ZMUC) and in Pilato's collection (Department of Animal Biology, the University of Catania). The measurements given are those of the holotype, unless otherwise indicated. Abbreviations used in Figs 9-12: *B, C, E* - lateral appendages; D_2 - dorsal appendages; *mp* - the third median plate; *s* - claw spur; *sf* - spine fringe; *sep* - IVth leg sensory papilla; *sp* - shoulder plate; *tp* - terminal plate; *ts* - transverse strip on paired plates; *vs* - vertical strip.

Description of the new species

Echiniscus ehrenbergi sp. n.
E. testudo: Dastych, 1976
E. aff. *testudo*: Dastych, 1987
(Figs 1-5, 7-12)

DIAGNOSIS: Median sized, brown-blackish *Echiniscus* with long lateral appendages *B, C* and *E* and short, spine-like dorsal appendages D_2. Third median plate covered with regular granulation. External claws smooth, internal with a distinct spur.

HOLOTYPE: Female, 358 µm long, from lichen sample, April 1980, coll. W. Wróz; the slide No. 8 (N10c) is deposited in ZMH (Reg. No. A32/95).

LOCUS TYPICUS: The Himalayas, NE Nepal, the Mt. Everest Region. The mountainee-ring base Gorak Shep at the moraine of the Khumbu Glacier, *5150* m a.s.l., in lichens and mosses from non-calciferous rocks, April 1980, coll. W. Wróź.

PARATYPES: 195 specimens (48 females, 72 males; 75 individuals with undet. sex, including juveniles) mounted on 11 slides (No. 1-11: Reg. No. A31/95), data as for holotype. One paratype [slide No. 9(N 10b)] is deposited in Pilato's collection, University of Catania, Italy; the remaining paratypes in ZMH. Other localities: as above; front of the Khumbu Glacier, mosses and lichens from stones and rock crevices, about 4600 m, January 1975, coll. A. Heinrich; see Dastych 1976 (5 specimens: two females, two males, one individual with undet. sex; slides No. 12-16, Reg. No. A31/95: housed in ZMH); as above, mosses from S slope of Kola Pathar, above Gorak Shep, 5554 m, 20 November 1986, coll. Å. Jespersen and C. Schou: three females and four males mounted on four slides (RMK Hi-112086.1 to Hi-112086.4) are deposited in ZMUC.

ETYMOLOGY: We dedicate the species to the German 19th Century zoologist, Christian G. Ehrenberg, one of the pioneers of high altitudinal microfaunal studies and the first student of the Himalayan tardigrades.

DESCRIPTION. The body light-brown, its plates brown, dark-brown or brown-blackish in the slide mounted specimens, living animals in incident light brown-blackish with a light-green metalic sheen. The black eye-dots are lacking.

The body is 134-440 µm long (holotype: 358 µm), including two-clawed larvae. Dorsal plates are well developed; ventral plates are absent. Anteriorly the head plate is deeply incised in the middle, usually with a small oval pigmented area in front of the incision (Fig. 2). The neck plate forms a narrow, transversely pigmented area and is located just behind the posterior edge of the head plate (Fig. 2). Sometimes the neck plate is divided into two separate small pieces. The first segmental plate (= scapular or shoulder plate, I) is composed of three pieces, i.e. a larger one, forming the central main part of the shoulder plate and two small pieces located laterally, separated from the main part by a narrow unpigmented strip (Fig. 2). Similar unpigmented strips often occur vertically in the middle of the shoulder plate, and, when the strip reaches the posterior margin of the shoulder plate, it apparently divides the plate into two separate areas. However, this strip often reaches only halfway on the shoulder plate (Figs 2, 9) or it is lacking. Segmental paired plates II and III each have a narrow and weakly pigmented transverse strip, usually deprived of cuticular sculpture. The strips are located in the anterior part of each plate. The terminal plate has two distinct incisions, relatively wide at the bases (Figs 2, 9). In some specimens this plate has weakly marked faceting (Fig. 9). The first and the third intersegmental plates are undivided; the second one has a transverse line in its anterior part, dividing the plate into two parts of different size.

The dorsal plates are covered either with small, flat-sided cuticular knobs or with their derivatives, formed by the fusion of such knobs. In the first type of sculpturing the knobs are closely spaced but still distinctly separated, usually rounded (or polygonal) in shape and with several tiny pore-like structures around their edges (Fig. 10, 11). They occur in the anterior part of the paired segmental plates (II and III), being located just above and under the transverse, unpigmented narrow strip. Moreover, they are usually present in the anterior part of the intersegmental plates I and II and on the whole intersegmental plate III (Figs 10, 11). On the latter plate the knobs are most distinct and the largest, with a diameter up to 2.4 µm (usually 1.8-2.2 µm; in the holotype 1.5-2.2 µm). In the dorsal view this sculpturing is visible as regularly spaced and distinct granulation (Figs 2, 10). The sculpturing greatly resembles the one illustrated by Schuster et al. (1975: Fig. 13) in *E. arctomys* Ehrenberg, 1853, i.e. in a taxon with unclear taxonomic status. On the remaining (posterior) parts of these plates (excluding median III) and on the head, shoulder and terminal plate, the knobs are strongly flattened; their edges are more or less fused and they form an almost uniform cuticular surface. In this second type of sculpturing, the cuticular surface is mainly provided with the remaining tiny pores, arranged in small, more or less regular circles. In the most advanced stage of such fusion, occurring particularly in the largest specimens, knobs are no longer distinguishable and the remaining irregularly and sparsely distributed pores (diameter up to 1µm) are visible as bright-light points on the dark background.

On all dorsal plates, spine fringe (= dorsal leg spines), the bases and anterior parts of legs, the anal, genital and subcephalic areas, densely spaced and very tiny, regularly shaped granules (diameter about 0.3 µm) occur just under the cuticle. The granulation, together with brown pigment, makes up part of the plate-like structures, particularly on the anterior part of the head and neck region and, with thickened cuticle, forms small and weakly marked transverse plates just under the bases of the claws on the ventral part of the legs I- III.

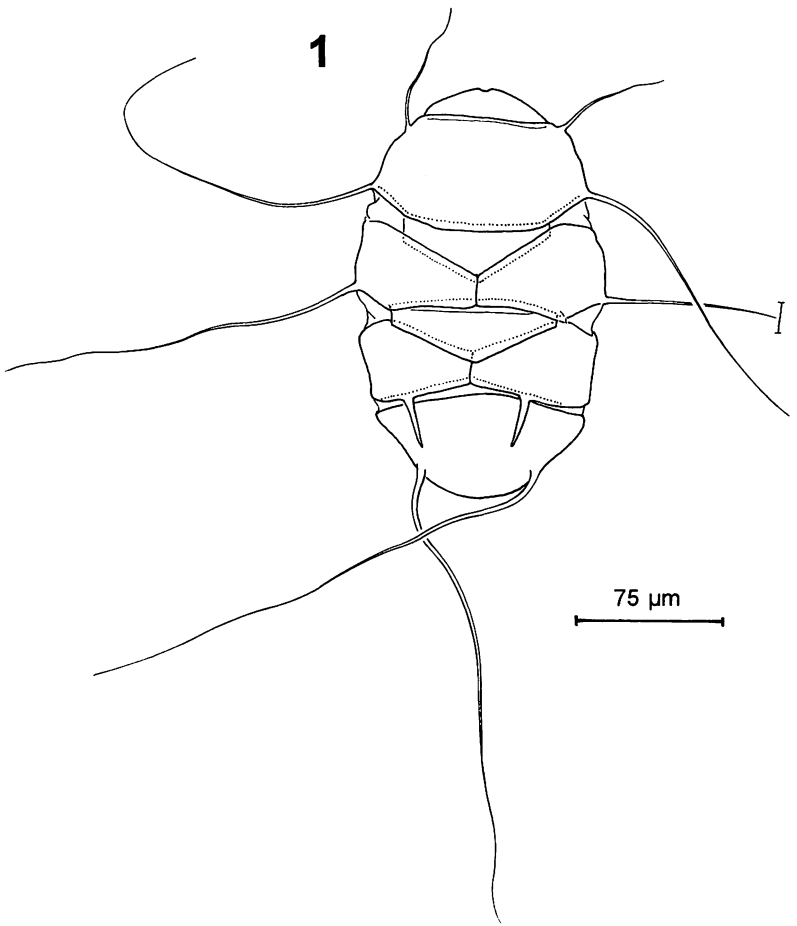


Fig. 1. *Echiniscus ehrenbergi* sp. n.: whole animal in dorsal view, male (paratype).

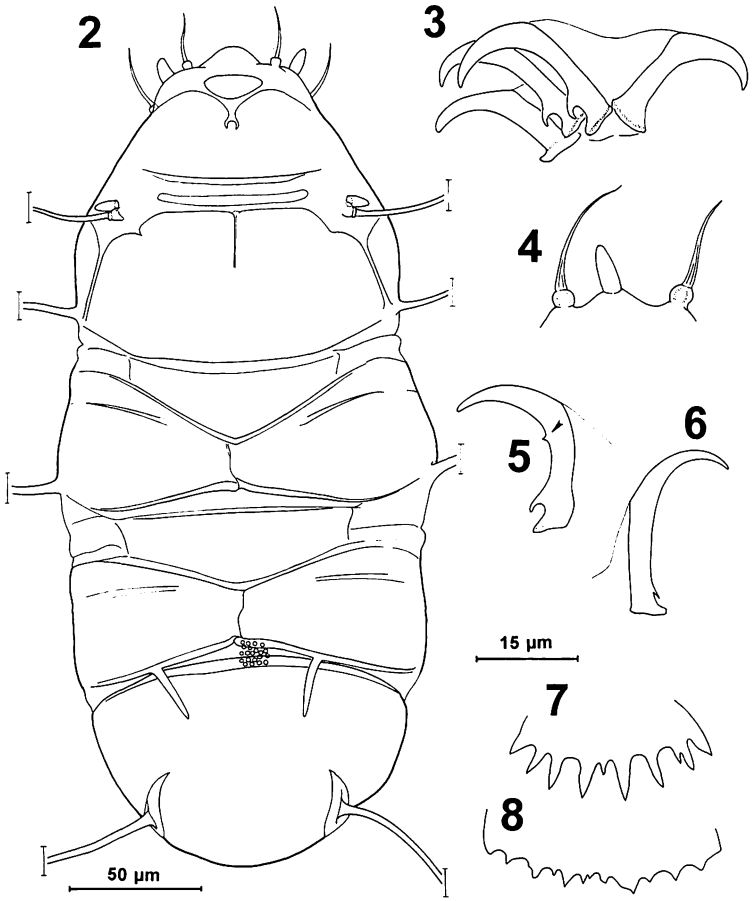
The head segment has external cirri (25 µm) slightly longer than the internal ones (21µm). Between the cirri occurs the secondary clava (= papilla cephalica: 9 µm long). This clava is located almost in the middle of the distance between both cirri, being slightly shifted towards the external cirrus (Fig. 4). Both cirri have distinct cirrophores. The trunk appendages are represented by lateral appendages *A, B, C, E* and dorsal appendages D_2. The lateral appendages, except *A*, very long and strong. The appendages *A* are distinctly shorter and more weakly developed (Fig. 1). Their length formula is as follows: $A < B \leq C \leq E$; in the holotype they are 53, 232, 230 and 287 µm long, respectively. Sometimes the appendages *E* are slightly shorter than *C* or *C* are slightly shorter than *B*. The length of all appendages is subject to small variation. At the base of the appendage *A* occurs a small (8 µm) primary clava (= clava); the appendage itself is located on a distinct cirrophore. The appendages *B* in two-clawed juveniles are lacking. Dorsal appendages D_2 are medium-sized spines (32 and 34 µm, respectively) and their lengths vary between 1/4 - 1/2 (usually 1/3) of the length of the terminal plate (Figs 1, 2, 9, 10). No additional teeth or spines occur either in the incisions on the terminal plate (*E*) or on other plates.

Legs I have a small spine-like sensory organ and legs IV have a small sensory papilla (3.5 and 4.5 µm, respectively). The spine fringe on legs IV is variable in shape (Figs 7, 8) and is usually composed of a dozen or so sharp teeth. Claws are of medium size; the external claws are smooth, the internal claws have a distinct, downwards directed spur (tooth: Figs 3, 5, 12). The spur is located above the claw base, at a height approximately 1/4 of the claw length. In the holotype the claws I are 13 µm long and claws IV are 21 µm long.

The mouth tube has weakly sclerotized (and poorly visible) stylet supports.

Males are usually more slender and slightly smaller than the females; they have relatively larger secondary clavae and a simple round to oval gonopore, as compared to the rosette-like structure surrounding the female genital opening. The male gonopore is composed of a thin and "doubled" cuticular walls surrounding a small area with an anteriorly located tiny semi-lunular slit. The latter is hardly visible or not detectable in slide preparations. However, a somewhat similarly shaped roundish structure is present also in young females, prior to the full formation of the typical rosette-shaped structure present in mature forms. Nevertheless, the bordering wall of a such young female gonopore is less distinct, not doubled and without crescent-like slit. Moreover, the distance between the gonopore and anus is different in both sexes. In females it is distinctly greater ($\bar{x}$ = 27.3 µm; min-max = 19.8-40.7 µm, n = 29), compared to that in males ($\bar{x}$ = 15.7 µm; min-max = 6.6-44.0 µm, n = 55) (measured from external edge in both openings). In less abundant material from Kola Pathar (housed in ZMUC) these distances range between 28-32 µm and 15-17 µm, respectively. The observed sex ratio was distinctly in favour of males, being 1.47 : 1. Mature spermatozoa were observed in the two seminal vesicles. The spermatozoon is of the typical heterotardigrada-type, with two free mitochondria adhering to the flagellum, and is similar to that of *Echiniscoides sigismundi sigismundi* (see Kristensen & Hallas 1980: Fig. 17).

Specimens from the *locus typicus* were found in three of eight lichen samples and in one of two moss samples, co-occuring there with two other tardigrades, *Milnesium tardigradum* Doyère and *Ramazzottius* cfr. *anomalus* (Ramazzotti). In other localities (see Dastych 1976) the new species was found with *Echiniscus blumi* Richters,



Figs 2-8. *Echiniscus ehrenbergi* sp. n.: 2- habitus, dorsal view (male, allotype); 3- claws of IVth pair of legs, ventral view; 4- cephalic sensory organs, ventral view (male); 5- internal claw IV with aberrant tiny tooth (arrowhead); 6- *E. testudo* (Doyère), internal claw IV (specimen from Hamburg); 7 and 8: *E. ehrenbergi* sp. n., dorsal leg spines (Fig. 3 drawn from holotype).

Testechiniscus spitsbergensis (Scourfield) and *Pseudechiniscus suillus* (Ehrenberg), and at Kola Pathar (5554 m) it occurred with *Echiniscus reticulatus* (Murray) and *M. tardigradum* (Doyère).

Comments

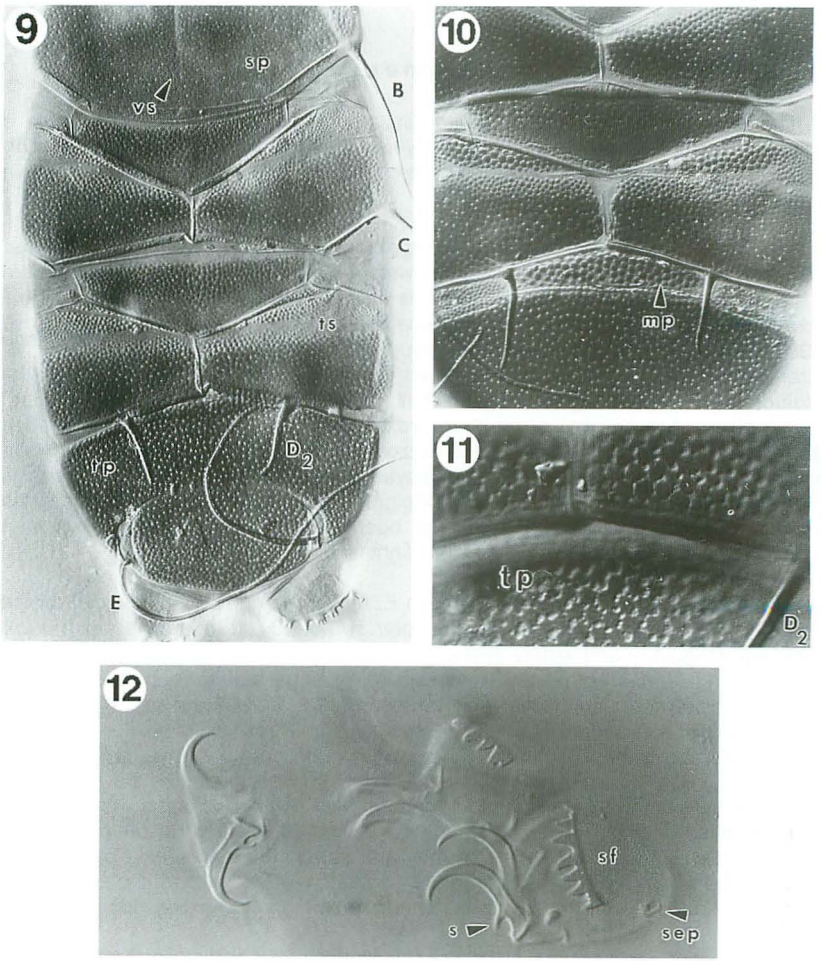
The arrangement of lateral and dorsal appendages in *E. ehrenbergi* sp. n. strikingly resembles that of *E. testudo* (Doyère, 1840). However, the body colour, the sculpturing of the dorsal plates, and the internal claws separate well the new species from the latter taxon.

The brown-blackish body of the new species discerns it not only from yellowish-red or red *E. testudo*, but also from all known species of this tardigrade genus, which is the richest taxon. The only exception is an undescribed black species of the *E. viridis*-group from the Arctic, the presence of which was mentioned by Kristensen (1987). However, since in the *viridis*-group only lateral appendages *A* occur, the latter taxon can be easily differentiated from *E. ehrenbergi* sp. n., which has additionally the appendages *B, C* and *D*. It should be noted that dark body colour is known also in two species of another tardigrade order, vis. *Hypsibius klebelsbergi* Mihelcic, 1959 and *H. janetscheki* Ramazzotti, 1968. Both of these species dwell in cryoconite holes on the Alpine and Himalayan glaciers and probably are conspecific (Dastych 1993). The function of the dark pigmentation and its significance for these high altitudinal tardigrades has still not been clarified. The dark pigment might protect these animals against a high UV - light level.

The sculpturing of the dorsal plates in *E. testudo* is composed of small cavities (pores?) of irregular shape and the diameter of 0.5-1.3 µm (usually about 1.0 µm). They are irregularly and widely spaced on the cuticular surface, particularly on the scapular and terminal plate and resemble small bright points in dorsal view. The roundish or polygonal knobs, as those occurring in *E. ehrenbergi* sp. n., are lacking in the former species. Hence, the whole sculpture in *E. testudo* is similar to the most advanced stage of the second type of sculpturing described in *E. ehrenbergi* sp. n. (see above). On the third intersegmental plate in *E. testudo* the cavities (never knobs) are often more closely spaced and form then some kind of net with irregularly sized meshes. However, the meshes are never larger than the diameter of the cavities on the bordering plates and they are usually even smaller. Conversely, in the new species this plate is covered with distinct, relatively large knobs of regular shape; the knobs are larger than those on the remaining plates (Fig. 10).

The claws of *E. ehrenbergi* sp. n. are relatively smaller and more stumpy than the larger and slender claws of *E. testudo*. In the new species a distinct, well developed spur occurs at some distance from the base of each internal claw (Figs 3, 5, 12). In *E. testudo* the spur is tiny, hardly visible, and shaped as a sharp, thin spine directed downwards. Moreover, the spine is located only just above the claw base (Fig. 6).

Both species are also separated by the shape of the scapular plate (two additional small lateral areas in *E. ehrenbergi* sp. n., undivided plate in *E. testudo*), the shape of the incisions on the terminal plate (narrower at the base in *E. testudo*), the length of the lateral appendages (shorter and more weakly developed in *E. testudo*), and the type of reproduction. *E. ehrenbergi* sp. n. is an gonochoristic



Figs 9-12. *Echiniscus ehrenbergi* sp. n.: 9- female dorsum; 10- fragment of dorsum with well visible median plate III (= IIIm); 11- fragment of dorsum: median plate III concealed under the paired plates II; 12- fragment of the posterior venter of two-clawed larva (all paratypes).

species with an unusually high ratio of males but rather poorly marked secondary sexual dimorphism. In contrast, *E. testudo* probably has the parthenogenetic type of reproduction as indicated by the lack of males in this common, widely distributed and one of the first described tardigrades. On the other hand, the presence of males in *Echiniscus* has been discovered only recently (Dastych 1987) and earlier the whole genus was considered a purely parthenogenetic taxon.

Zusammenfassung

Eine neue Tardigraden-Art, *Echiniscus ehrenbergi* sp. n., wird aus den Moosen und Flechten aus dem Himalaja (Nepal, Mt. Everest Region) beschrieben. Die neue Art, die *E. testudo* (Doyère, 1840) am nächsten steht, unterscheidet sich von ihr durch den schwarzbraunen Körper, regelmäßige dorsale Struktur, größere Nebenhaken an Innenkrallen und das Vorhandensein von Männchen.

Acknowledgements

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