A new discovery of *Novechiniscus armadilloides* (Schuster, 1975) (Tardigrada, Echiniscidae) from Utah, USA with considerations on non-marine Heterotardigrada phylogeny and biogeography

Lorena Rebecchi\textsuperscript{a,*}, Tiziana Altiero\textsuperscript{a}, Jette Eibye-Jacobsen\textsuperscript{b}, Roberto Bertolani\textsuperscript{a}, Reinhardt M. Kristensen\textsuperscript{b}

\textsuperscript{a}Department of Animal Biology, University of Modena and Reggio Emilia, Via Campi 213\,ID, 41100 Modena, Italy
\textsuperscript{b}Zoological Museum, The Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen Ø, Denmark

Received 28 March 2006; accepted 8 November 2006

**Abstract**

The discovery of a new population of the non-marine heterotardigrade *Novechiniscus armadilloides* from Utah, USA, allowed us to reanalyse the species by means of scanning electron microscopy and differential interference contrast microscopy. This analysis confirmed the presence of bar shaped, unpaired segmental plates and of a long filament E in addition to the filament A always present in the class Heterotardigrada. It also provided additional information on characters not explicitly cited in the previous descriptions of this monotypic genus, such as details of the head cirri and clavae, details of the buccal tube and pharyngeal bulb, sculpture of the dorso-lateral and leg plates, details of the claws. The population is bisexual, but no secondary sexual dimorphism was observed. The male and female gonopores were described. New characters such as red eyes and red body colour were used in analysing the phylogeny of the family Echiniscidae. The phylogeny and biogeography of non-marine heterotardigrades provide intriguing questions for further research.

© 2007 Gesellschaft für Biologische Systematik. Published by Elsevier GmbH. All rights reserved.

**Keywords:** Heterotardigrada; Echiniscidae; Phylogeny; Zoogeography; SEM

**Introduction**

The marine Arthrotardigrada, one of the two orders of the class Heterotardigrada, are considered the most ancient order within the phylum Tardigrada (Kristensen 1976, Renaud-Mornant 1982). At the beginning of the 20\textsuperscript{th} century (Thulin 1928, Marcus 1929) and in more recent taxonomic works (Ramazzotti and Maucci 1983), its family Halechiniscidae was considered the stem group of all other tardigrades. However, Renaud-Mornant (1982) stated that *Parastygarctus* (family Stygarctidae) should be considered the tardigrade genus with the greatest number of plesiomorphic characters. One of the latter features are the dorsal cuticular plates, which are present in some marine Arthrotardigrada, but above all, these plates characterize the terrestrial family Echiniscidae (belonging to Echiniscoidea, the other order of Heterotardigrada). Phylogenetic discussions of the so-called ancestral tardigrade have nearly neglected the terrestrial heterotardigrades, represented by the families Oreellidae and
Echiniscidae (Kristensen 1987, Jørgensen 2000). Recently, Nichols et al. (2006) suggested the Oreellidae without plates as the basal heterotardigrade family. In the latter case, the dorsal plates should have developed several times by convergence in heterotardigrades (Jørgensen 2000), i.e. the dorsal plates of Echiniscidae and Renaudarctidae/Stygarctidae would be convergent structures. This again places the focus on the family Echiniscidae, in which the two genera with the most plesiomorphic characters are *Parechiniscus* and *Novechiniscus*. In this paper, we present new information on the genus *Novechiniscus*.

*Parechiniscus armadilloides* Schuster, 1975 was described and illustrated with two scanning electron microscope (SEM) photographs. Schuster (1975) examined a population collected in moss and lichen from Logan Canyon near Logan, Utah (USA), the only site known for that species so far. Kristensen (1987) reconsidered the taxonomic position of *P. armadilloides* in his revision of the Echiniscidae (Echiniscoidea), the most diverse family of semiterrestrial Heterotardigrada, and proposed the erection of the genus *Novechiniscus* for that species. He also redescribed *Novechiniscus armadilloides* (Schuster, 1975) from two paratypes. His description was accurate and detailed, but due to the demanding task of describing or redescribing all the genera of Echiniscidae in the same paper, he represented *N. armadilloides* with one habitus drawing and two cladograms (buccopharyngeal apparatus and claw). However, all observations were made on poorly preserved specimens on old microslides. The discovery of a new population of *N. armadilloides* allows us to report additional information on those characters not specifically cited in the previous descriptions or to modify the characters, and to discuss the phylogeny and biogeography of non-marine heterotardigrades in general. This paper indicates that *Parechiniscus* and *Novechiniscus* belong to two different evolutionary lines in the family Echiniscidae, and that the two genera are not closely related (sister groups).

**Material and methods**

A moss, *Grimmia laevigata* (Bridel) Bridel, growing on basaltic rock was collected (sample code C1934, 4 August 1995) from St. George, Snow Canyon, Utah (USA), about 550 km from the locus typicus in the opposite corner of the State. The moss sample contained large amounts of red soil. The tardigrades living in that moss belonged to two species: the heterotardigrade *Novechiniscus armadilloides* (Schuster, 1975) and the eutardigrade *Milnesium tardigradum* Doyère, 1840.

About 60 specimens of *N. armadilloides* were extracted from the moss and used for this study. Specifically, 34 specimens and three exuviae containing 1 or 2 eggs each were directly mounted in polyvinyl lactophenol. Females and males were found; eight specimens were juveniles with only two claws per leg and without anus or gonopore (‘two-clawed larvae’ according to the terminology used by tardigradologists; see Ramazzotti and Maucci 1983, Bertolani et al. 1984). Slides were examined with a Leitz DM RB microscope equipped with phase contrast and differential interference contrast (DIC) up to the 100 × objective, and connected to a Polaroid DMC Ie Low Light Kit digital camera. Fifteen specimens of *N. armadilloides* were prepared for SEM analysis, following the protocols by Guidetti et al. (2000). In addition, two specimens were treated with NaClO and the buccopharyngeal apparatus

![Fig. 1. *Novechiniscus armadilloides* (in vivo; DIC): (A) Head with a red eye (arrow). (B) Carrot-red colour of body. All scale bars = 20 μm.](image)
was gently torn from the head with a needle, according to Eibye-Jacobsen (2001a). Spermatozoa for SEM analysis were prepared according to Rebecchi and Guidi (1991). SEM photographs were taken either on a Philips SEM XL 40 available at the ‘Centro Interdipartimentale Grandi Strumenti’ of the University of Modena and Reggio Emilia, or on a JEOL JSM-840 at the University of Copenhagen.

Results

The body length of adult *N. armadilloides* from St. George ranges between 308 and 407 mm. Males and females seem to be of the same size; no secondary sexual dimorphism was observed in the clavae. The colour of the body is carrot red (Fig. 1B), due to pigment within both the epithelial cells and the storage cells. Large red eyes, previously undescribed, are present (Fig. 1A). The distinctive taxonomic characters of the species were confirmed in the specimens from this new population. The most evident are the presence of only filament A and a long filament E both in adults (Fig. 2A and B) and in ‘two-clawed larvae’ (Fig. 2C and D). In addition, all segmental plates are unpaired and median plates are bar shaped (Fig. 2A and C).

The mouth opening, buccal cirri, primary and secondary clavae (Figs. 3A, B and 4A) are present on the head. Each internal cirrus is inserted on the top of a dome which is always visible with LM, while sometimes another dome (previously undescribed) is visible at the base of each external cirrus (Fig. 3A). The primary and secondary clavae have a strong cuticular ring (an annulus; Fig. 3A) at the base. The mouth opening is located terminally (Fig. 4A). The buccal tube is rigid and very long (70 μm in an adult 380 μm in length). In *Novechiniscus* it is presumably CaCO$_3$ encrusted all the way down to the pharyngeal bulb, and is clearly not flexible after the insertion of the very thin stylet supports, located far (15 μm) from the pharyngeal bulb (Fig. 4B). The flexible part of the buccal tube is located inside the pharyngeal bulb, where the tube is not encrusted. The cuticle at the anterior end of the buccal tube (stylet sheaths) adheres very strongly to the tip of the stylets. With LM and SEM (Figs. 3B and 4C), two

---

*Fig. 2. Novechiniscus armadilloides (SEM): (A, B) Dorsal and ventral views of adult specimens in toto; in B male gonopore is clearly identifiable (arrow). (C, D) Dorsal and ventral views of a ‘two-clawed larva’; filaments A (arrow) and E (arrowheads) are clearly identifiable. All scale bars = 50 μm.*
Fig. 3. Novechiniscus armadilloides (phase contrast): (A) Head with a dome (arrow) at base of external cirrus and an annulus (arrowhead) at base of secondary clava. (B) Head; note shape of styllet sheaths and a teardrop structure (arrow) in the bulb. (C, D) Latero-caudal curved projections on scapular plate (*) and plate II (arrow) of an adult specimen. (E) Anterior body region of a ‘two-clawed larva’. (F) Coarse irregular sculpture of plate IV and leg plate (arrow). (G) Second and third pairs of legs of an adult specimen, showing spurs (arrow) on claws. (H) Claws on hind pair of legs with spines (arrows). All scale bars = 15 μm.
oval openings were observed in the sheaths. The stylets are very thin and long, but shorter (63 μm) than the buccal tube. The furcae of the stylets are relatively large and seem to lack CaCO₃ (Fig. 4B). The swollen base of the stylet clearly contains CaCO₃. The cuticular formation, like a cuff, located in the middle part of each stylet (Kristensen 1987, Fig. 47c) could not be observed in the NaClO-treated animals. With LM, the stylet supports seem to consist of cuticular material; only with SEM, however, crystals of CaCO₃ (the irregular swellings of the stylet supports seen in Fig. 4B) are observed, although the stylet supports are flexible and bend smoothly. The placoids are the so-called fused placoids, consisting of the normal bar-like structure. Posteriorly, each placoid has a teardrop-shaped cuticular thickening (Fig. 3B); however, this structure is not homologous to the macroplacoid in eutardigrades, which is a solid cuticular structure. With SEM, the teardrop structure of Novechiniscus seems hollow, and a double cuticular lining (Fig. 4E) is discernible. The cuticular lining of the pharyngeal bulb has many fine cuticular fibres that are embedded inside the muscular part of bulb (Fig. 4E).

The head plate has a simple fine sculpture (pillars); only in a few cases was a median pore (or depression) visible. plate II and especially the scapular plate appear slightly raised (Figs. 2A, 3C and D). Both plates show pronounced latero-caudal curved projections (Figs. 2A, 3C and D). On the scapular plates, the two projections are connected by a band that is evident as a distinct line in the anterior part of the plate (Fig. 3D). The band is absent in plate II. In the 'two-clawed larvae', only the projections of the scapular plates are present (Fig. 3E). A double sculpture is confirmed in all segmental plates. With LM, this sculpture appears as coarse irregular elevations and fine granulations. Fig. 5A shows the irregular elevations of the cuticle of the scapular plate with SEM. Double sculpturing is also present in the caudal part of two intersegmental plates (according to Kristensen 1987: m1a and m2a). Coarse irregular sculpturing is most evident in plate IV, even though the elevations are often smaller on that plate than on the

---

**Fig. 4.** Novechiniscus armadilloides (SEM): (A) Buccal cirri (arrows) and secondary clavae (arrowheads). (B) Buccalpharyngeal apparatus; arrow indicates irregular swelling of stylet support. (C) Anterior region of buccal tube and stylets; note one of the two oval openings in the sheaths (arrow). (D) Longitudinal cuticular fibres within bulb. (E) Teardrop structures of pharynx (arrows). Scale bars: A–C = 10 μm; D, E = 5 μm.
others (Fig. 3F). The short incision in plate IV is sometimes visible.

Leg plates are present on the fourth pair of legs (Fig. 3F). They are not visible in the ‘two-clawed larvae’. On each adult leg, internal claws have a spur directed downwards, while external claws have one or two, rarely three, right spines inserted perpendicularly or forwards on the claw (Figs. 3G, H and 5B). These spines are longer on the fourth pair of legs (Figs. 3H and 5B). Both males and females are abundant; the sex ratio is close to 1:1. The female gonopore is a typical rosette (Fig. 5C). In the male, the gonopore is nearer to the anus than in the female, and it is represented by a short tube with a transverse, semilunar fold as the opening (Figs. 2B and 5D). ‘Two-clawed larvae’ do not have any openings, neither anus nor gonopore (Fig. 2D).

In adult males, the oval testis can be voluminous and very rich in spermatozoa (Fig. 6A). The male gamete is about 30 μm in length and made up of a thin head with an elongated cylindrical acrosome followed by a carrot-shaped nuclear region and a tapering tail. An elongated mitochondrial sack, consisting of two tubular parts, originates from the posterior part of the head and extends beyond the main axis of the cell (Fig. 6B and C).

**Discussion**

The respective validity of the genus *Novechiniscus*, of *N. armadilloides* and its specific characters is confirmed. Nevertheless, the new observations of *N. armadilloides* have provided some interesting surprises. Some new characters have emerged with respect to the previous description by Kristensen (1987), in particular the colour of the eyes and the body which could not be
observed in the type material. Moreover, some differences between the two populations may be due to omissions in the previous descriptions (Schuster 1975; Kristensen 1987).

The pharyngeal bulb of *N. armadilloides* with its many fine cuticular fibres (Fig. 4D and E) is unique for an echiniscid, but not for a heterotardigrade. The cuticular lining of the pharyngeal bulb with many fine cuticular fibres was observed for the first time in the marine echiniscoid *Echiniscoides hoepneri* Kristensen & Hallas, 1980 (Kristensen and Hallas 1980); recently those fibres were also detected with TEM in *Echiniscus viridissimus* Petereï, 1956 (Eibye-Jacobsen 2001b).

Kristensen (1987) stated that, superficially, the habitus of *Novechiniscus* looks like an *Echiniscus* with unpaired segmental plates. He believed this to be a case of parallel evolution. However, the eyes are clearly red (as in the *Echiniscus* line), not black as in the *Pseudechiniscus* line and in *Parechiniscus chitonides* Cuénot, 1926. Moreover, a new observation is the carrot-red colour of the cellular pigment of *Novechiniscus*. It is clear that the animal contains carotene. These new characters further remove *Novechiniscus* from *Parechiniscus*, which like *Hyperchiniscus* lacks carotene in its cells. *Novechiniscus* and *Parechiniscus* may still be the most basal genera within the Echiniscidae (see Jørgensen 2000), because they lack the paired dorsal segmental plates. However, both already belong to two separate evolutionary lines. The mineralization of the cuticular elements of the buccal tube can also be used to distinguish the different genera in the family Echiniscidae.

On the other hand, sexual dimorphism of the gonopore of *N. armadilloides* is common to the other Echiniscidae with males and to most Heterotardigrada (Rebecchi et al. 2000). The shape of the male gamete of *N. armadilloides* confirms what has already been described briefly by Rebecchi et al. (2003), showing that the spermatozoon is also closely related to that of the other Echiniscidae and of the marine echiniscoidid species *Echiniscoides sigismundi* (M. Schultze, 1865) (Rebecchi et al. 2003).

If the most basal heterotardigrades are not the marine Arthrotardigrada, as Renaud-Mornant (1982) suggested, but the terrestrial family Oreellidae, as proposed by Nichols et al. (2006), the biogeography and the phylogeny of the terrestrial Echiniscidae should be reconsidered. However, there is no support for the new theory of Nichols et al. (2006) in our new investigation on *Novechiniscus*. Dorsal plates may have developed several times in both Arthrotardigrada and Echiniscoidea (Jørgensen 2000). The dorsal unpaired plates of both *Parechiniscus* and *Novechiniscus* may be plesiomorphic characters inside the family Echiniscidae; however, paired plates have developed in both Arthrotardigrada and Echiniscoidea (including Echiniscooidae, Oreellidae and Echiniscidae) by convergence. That does not make the terrestrial family Oreellidae the basal heterotardigrade family. We therefore still believe in the theory of Renaud-Mornant (1982) that tardigrades were primarily marine and that both families Oreellidae and Echiniscidae have become terrestrial later.

*Novechiniscus* is still a monospecific genus, with the shape of the plates easily distinguishable from those of the other genera of *Echiniscus* with unpaired segmental plates. He believed this to be a case of parallel evolution. However, the eyes are clearly red (as in the *Echiniscus* line), not black as in the *Pseudechiniscus* line and in *Parechiniscus chitonides* Cuénot, 1926. Moreover, a new observation is the carrot-red colour of the cellular pigment of *Novechiniscus*. It is clear that the animal contains carotene. These new characters further remove *Novechiniscus* from *Parechiniscus*, which like *Hyperchiniscus* lacks carotene in its cells. *Novechiniscus* and *Parechiniscus* may still be the most basal genera within the Echiniscidae (see Jørgensen 2000), because they lack the paired dorsal segmental plates. However, both already belong to two separate evolutionary lines. The mineralization of the cuticular elements of the buccal tube can also be used to distinguish the different genera in the family Echiniscidae.

On the other hand, sexual dimorphism of the gonopore of *N. armadilloides* is common to the other Echiniscidae with males and to most Heterotardigrada (Rebecchi et al. 2000). The shape of the male gamete of *N. armadilloides* confirms what has already been described briefly by Rebecchi et al. (2003), showing that the spermatozoon is also closely related to that of the other Echiniscidae and of the marine echiniscoidid species *Echiniscoides sigismundi* (M. Schultze, 1865) (Rebecchi et al. 2003).

If the most basal heterotardigrades are not the marine Arthrotardigrada, as Renaud-Mornant (1982) suggested, but the terrestrial family Oreellidae, as proposed by Nichols et al. (2006), the biogeography and the phylogeny of the terrestrial Echiniscidae should be reconsidered. However, there is no support for the new theory of Nichols et al. (2006) in our new investigation on *Novechiniscus*. Dorsal plates may have developed several times in both Arthrotardigrada and Echiniscoidea (Jørgensen 2000). The dorsal unpaired plates of both *Parechiniscus* and *Novechiniscus* may be plesiomorphic characters inside the family Echiniscidae; however, paired plates have developed in both Arthrotardigrada and Echiniscoidea (including Echiniscooidae, Oreellidae and Echiniscidae) by convergence. That does not make the terrestrial family Oreellidae the basal heterotardigrade family. We therefore still believe in the theory of Renaud-Mornant (1982) that tardigrades were primarily marine and that both families Oreellidae and Echiniscidae have become terrestrial later.

*Novechiniscus* is still a monospecific genus, with the shape of the plates easily distinguishable from those of the other genera of *Echiniscus* with unpaired segmental plates. He believed this to be a case of parallel evolution. However, the eyes are clearly red (as in the *Echiniscus* line), not black as in the *Pseudechiniscus* line and in *Parechiniscus chitonides* Cuénot, 1926. Moreover, a new observation is the carrot-red colour of the cellular pigment of *Novechiniscus*. It is clear that the animal contains carotene. These new characters further remove *Novechiniscus* from *Parechiniscus*, which like *Hyperchiniscus* lacks carotene in its cells. *Novechiniscus* and *Parechiniscus* may still be the most basal genera within the Echiniscidae (see Jørgensen 2000), because they lack the paired dorsal segmental plates. However, both already belong to two separate evolutionary lines. The mineralization of the cuticular elements of the buccal tube can also be used to distinguish the different genera in the family Echiniscidae.

On the other hand, sexual dimorphism of the gonopore of *N. armadilloides* is common to the other Echiniscidae with males and to most Heterotardigrada (Rebecchi et al. 2000). The shape of the male gamete of *N. armadilloides* confirms what has already been described briefly by Rebecchi et al. (2003), showing that the spermatozoon is also closely related to that of the other Echiniscidae and of the marine echiniscoidid species *Echiniscoides sigismundi* (M. Schultze, 1865) (Rebecchi et al. 2003).

If the most basal heterotardigrades are not the marine Arthrotardigrada, as Renaud-Mornant (1982) suggested, but the terrestrial family Oreellidae, as proposed by Nichols et al. (2006), the biogeography and the phylogeny of the terrestrial Echiniscidae should be reconsidered. However, there is no support for the new theory of Nichols et al. (2006) in our new investigation on *Novechiniscus*. Dorsal plates may have developed several times in both Arthrotardigrada and Echiniscoidea (Jørgensen 2000). The dorsal unpaired plates of both *Parechiniscus* and *Novechiniscus* may be plesiomorphic characters inside the family Echiniscidae; however, paired plates have developed in both Arthrotardigrada and Echiniscoidea (including Echiniscooidae, Oreellidae and Echiniscidae) by convergence. That does not make the terrestrial family Oreellidae the basal heterotardigrade family. We therefore still believe in the theory of Renaud-Mornant (1982) that tardigrades were primarily marine and that both families Oreellidae and Echiniscidae have become terrestrial later.
cosmopolitan. Oreella, Antechiniscus and Mopsochiniscus have been found only in Gondwanan areas. Cornechiniscus and Parechiniscus are Palaearctic, while Proechiniscus is Nearctic. Testechiniscus has a bipolar distribution, and Bryochoeerus has been found in Australia, Hawaii, Greenland, Svalbard and Germany, whereas the remaining genera (Bryodelphax, Hypechiniscus, Pseudechiniscus and Echiniscus) can be considered as cosmopolitan. Echiniscus and Pseudechiniscus are the genera of Echiniscidae most rich in species, most of which are parthenogenetic. The species of Echiniscus show a particular geographic distribution: the unisexual species are widespread in the world and exclusive in the Palaearctic region, whereas the bisexual species are frequent or present in some regions of Gondwanan origin, such as Australia, Antarctica and Nepal (Miller et al. 1999) and in some states of the USA. (D.R. Nelson, pers. comm. 2006; Mitchell and Romano 2007). The problem of the relationship between phylogenetic trees and biogeographic distributions of the ‘terrestrial’ heterotardigrades remains unresolved. According to Kristensen (1987) and Jørgensen (2000), the genera Oreella, Parechiniscus, and Novechiniscus are close to the stem group of the ‘terrestrial’ heterotardigrades. However, this does not correspond with their biogeographic regions.

Acknowledgements

Particular thanks are given to Professor Diane Nelson (East Tennessee State University) for useful suggestions and for English revision, and to Dr. Gert Steen Mogensen (Botanical Museum, The Natural History Museum of Denmark, University of Copenhagen) for taxonomic diagnosis of the moss. We also thank the anonymous reviewers for their helpful suggestions. Financial support for the research came from a FAR grant from the University of Modena and Reggio Emilia to L.R., and from the Carlsberg Foundation, Denmark.

References


