A new Eutardigrade (Tardigrada: Milnesiidae) in amber from the Upper Cretaceous (Turonian) of New Jersey

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Abstract

The oldest true tardigrade known thus far, *Milnesium swolenskyi* n.sp., is described on the basis of a single, well-preserved specimen in amber from the Turonian of New Jersey. The new species bears an extremely close similarity to the living, widespread species *Milnesium tardigradum* Doyère. Antiquity of the Tardigrada *sensu lato*, as based on the existence of stem-group tardigrades from the Cambrian, corresponds with our discovery of a modern lineage of eutardigrade that has persisted for at least 90 million years (Ma).

Introduction

Tardigrades are a phylum of approximately 830 described species, traditionally placed in or near the Arthropoda, but sometimes near to the Annelida or Nematoda (Ramazzotti and Maucci, 1983; Kinchin, 1994). They are small (generally 500 µm or less), soft-bodied, rather morphologically basic, segmented animals with clawed legs; a metameric, ventral nerve cord; and they molt. Most species live in water films within the interstices or on the surfaces of bryophytes, lichens, and leaf litter, where they feed on fluid contents of individual cells, on hyphae, algae, or even small metazoans like other tardigrades. They feed using highly modified mouthparts composed of a pair of stylets, a mouth tube, and a muscular pharynx. Approximately 10% of the species are marine intertidal. Perhaps the most famous feature of tardigrades regards several remarkable instances of cryptobiosis (Crowe, 1975; Crowe and Cooper, 1971), and in particular anhydrobiosis. These are states of suspended animation where a highly dormant form of the animal (naturally induced by desiccation) can endure complete desiccation for up to seven years (according to Baumann, 1927), as well as extreme temperatures well above boiling point (150°C) and near absolute zero (Rahm, 1921, 1924, 1925), X-rays (May *et al.*, 1964), and very high pressures (Seki and Toyoshima, 1998).

The phylogenetic position of tardigrades as closely related to the arthropods has recently been confirmed with nucleotide sequences. Garey *et al.* (1996) sequenced the 18S rRNA gene of a species in the *Macrobiotus areolatus* group; and Giribet *et al.* (1996) and Giribet and Ribera (1999) sequenced the 18S rDNA gene of a different *Macrobiotus* species (in the *hufelandi* species-group). Despite the different genes and analyses used for comparing the sequences, results consistently grouped the tardigrades as the sister group to the arthropods, with the notable exception of the study by Giribet and Ribera (1999), which grouped Tardigrada + Nematoda as the sister group to the arthropods. On phylogenetic posi-

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tion alone, tardigrades are clearly an ancient group of animals. Unfortunately, as is expected, the phylum is virtually devoid of a fossil record.

The first fossil tardigrade was reported 35 years ago, based on two specimens preserved in Upper Cretaceous amber from Cedar Lake, Manitoba, Canada (Cooper, 1964). Amber is a matrix renowned for exquisite preservation of minute, soft-bodied organisms, even of internal tissues (Grimaldi, 1996). These specimens are in the Legg collection of Canadian amber fossils housed in the Department of Entomology, Museum of Comparative Zoology, Harvard. One specimen, *Beorn leggi* Cooper, was sufficiently well preserved to diagnose and describe as a new genus and species; the author also classified it in a family of its own. The other, much smaller specimen (presumably a juvenile) is apparently distinct from *Beorn* and considered to be a heterotardigrade. At the end of this paper, we provide a few comments on the possible systematic affinities of *Beorn*. Canadian amber comes from several western deposits, one of which (from Grassy Lake, Alberta) is Campanian (c. 72 Ma) (Pike, 1994).

Dramatic new fossils of animals bearing obvious close relationships to the Tardigrada were recently reported in limestone from the Kuonamka Formation of northern Siberia (mid Cambrian, ca. 550 Ma) (Müller *et al.*, 1995). Soft-bodied animals of Onychophora, Pentastomida, and other phyla are preserved in this and similar Cambrian deposits from Sweden, Australia, and Newfoundland, all of which have the original tissues replaced by phosphates. Scanning electron microscopy of these fossils reveals that, at least externally (since the fossils are hollow), fine structure is preserved with a fidelity better than even those in amber. Specimens from the Kuonamka Formation have exceptionally well preserved integument, peribuccal structures, and claws. Based on the presence of three pairs of legs in these fossils instead of the four pairs seen in all living tardigrades, Müller *et al.* (1995) justifiably interpret them as stem-group tardigrades. Apparently, according to Müller *et al.* (1995), the fourth (posterior-most) pair of legs in modern tardigrades (including the youngest juveniles) would represent a loss of anamorphosis. Clearly, phylogenetic and paleontological evidence indicates antiquity of the Tardigrada, including their involvement in the "Cambrian explosion."

Besides these two reports, and a report of eggs of *Macrobiotus macrocalix* Bertolani and Rebecchi from Quaternary sediments (originally cited as *Macrobiotus hufelandi* Schultze) (Durante and Maucci, 1972), no other fossil tardigrades have been reported until now, even from the vast deposits of Oligocene and Eocene amber of the Baltic region (Larsson, 1978). The specimen reported here is the oldest true tardigrade, and provides another valuable record of the phylum from the Mesozoic.

Methods, Material, Acknowledgments

The piece of amber containing the tardigrade was collected by Mr. Stephen Swolensky, at the Old Crossman's clay pits ("White Oaks" locality) in Sayreville, New Jersey. Stratigraphically, this amber deposit is attributed to the Turonian on the basis of palynology and lithology (reviewed by Grimaldi *et al.*, 2000, this volume). Upon examining the piece, Grimaldi recognized the inclusion as a tardigrade, wherein Mr. Slowensky generously donated the specimen to the AMNH, and for which we are grateful. The specimen was studied by first embedding the amber piece in epoxy under vacuum, and trimming and polishing the preparation to 3 mm in thickness, using the technique described in detail by Nascimbene and Silverstein (2000, this volume). The thin preparation was placed on a glass microscope slide, a drop of glycerine applied to the surface and then a coverslip; it was examined using a Leitz stereoscope (144x) and a Zeiss compound scope (400x) outfitted with transmitted light and reflected fiber optic lights. The preparation was studied at the University of Modena and Reggio Emilia using objectives up to 20x magnification on a Leica DM RB compound microscope with transmitted light, phase contrast, and differential interference contrast (DIC) as well as with the use of a confocal microscope Leica TCS 4D. Drawings were made by the authors with the use of a drawing tube on a Zeiss compound scope. Photomicrographs of

Milnesium tardigradum are presented for detailed comparison to the fossil; they were made using a Leica DM RB microscope and a Philips XL 40 scanning electron microscope, in the Centro Interdipartimentale Grandi Strumenti at the Univ. Modena and Reggio Emilia.

Systematics

Class Eutardigrada
Family Milnesiidae Ramazzotti, 1962
Genus *Milnesium* Doyère, 1840

Milnesium swolenskyi, new species

Diagnosis: Two lateral papillae, six peribuccal papillae and peribuccal lamellae present; buccal tube of apparently moderate size (as in *M. tardigradum*); both claws of the same leg with main branch of similar length, distinguished best from other species in the genus by the short claws (ca. 12 µm long, vs. 26 µm in specimens of *M. tardigradum* having a body length comparable to that of the fossil).

Description: A fully extended specimen with total length of 0.85 mm. Cuticle smooth, not textured. Anterior end with apparently two lateral papillae (but only papilla on one side observed, length 6.5 µm); 6 peribuccal papillae present (observed with confocal microscope), length ca. 8 µm; 6 buccal lamellae present and close to mouth, but shape cannot be resolved. Portion of head between lateral and peribuccal papillae is abruptly narrowed. Details of internal, buccal apparatus (pharynx, stylets,...) not discernable, apart from a silhouette of the buccal tube.

Legs in four pairs, posterior pair is terminal and shortest; length of first 3 leg pairs increasing from first to third (44.8 µm, 49.8 µm, and 55.8 µm, respectively), as in other *Milnesium* (*e.g.*, in a specimen of *M. tardigradum* (fig. 2a), lengths of these legs were 44.8 µm, 54.8 µm, and 59.8 µm). Each leg with two "double" claws, each claw with a main branch ca. 12 µm long (vs. ca. 26 µm in specimens of *M. tardigradum* of same body length as the fossil), separated by a basal claw (only partially discernable); whether this basal claw has a bifid or trifid tip (cf. figs 3b, c for *M. tardigradum*) cannot be discerned.

Type specimen: Holotype, AMNH NJ-796, a complete and extremely well preserved specimen in Turonian amber from Sayreville, New Jersey. Collected by Stephen Swolensky, 1998, deposited in the Dept. of Entomology, AMNH.

Etymology: Patronym, for Mr. Stephen Swolensky, collector and donator of the amber piece that contains this wonderful microscopic inclusion.

Discussion

There is no doubt about attributing this fossil to the Milnesiidae, and to the genus *Milnesium* in particular. Family placement is based on the claw structure and the pair of lateral papillae. *Milnesium* is distinguished from the other genus in the family, *Limmenius* Horning *et al.* 1978, by the presence of six peribuccal papillae (fib. 2b) – the most obvious and definitive taxonomic structures on the fossil. *Limmenius* lacks the peribuccal papillae and has a buccal tube of different structure (unfortunately, this latter feature not observable in the fossil).

There are four species described in *Milnesium*, all of which are moss- or lichen-dwelling: *M. tardigradum*, which is a common, cosmopolitan species; *M. tetralamellatum* Pilato and Binda, 1991 (found once, based on a series of seven specimens from Tanzania); *M. brachyungue* Binda and Pilato, 1990 (found once, based on a series of 10 specimens from Chile); and *M. eurystomum* Maucci, 1988 (found once, based on two specimens from Greenland). Unfortunately, the most reliable diagnostic differences among species of the genus are based on features not readily observable in the fossil, such as the number of buccal lamellae (six in *M. tardigradum* and *M. eurystomum*, four in *tetralamellatum*), and width of the buccal tube (fig. 2d)(wide in *M. eurystomum*; *M. tetralamellatum* larger than in *M. tardigradum*). The main comparative feature for the species that is externally observable is the length of the claws; these are shorter in the fossil than in any of the living species. A buccal tube of average size in the fossil (but details not discerned) agrees most with *M. tardigradum* (cf. figs 3d, e); these two species are extremely similar.

The only other fossil of a true tardigrade is *Beorn leggi*, in Canadian amber. Its systematic placement is somewhat obscure, either because of the original description or due to the fossil itself. According to Pilato (1979), the general shape of *Beorn* is very similar to the living eutardigrades. In our opinion, it is similar specifically to some living hypsibiid genera with two claws and with a relatively long secondary branch on each leg (*Isohypsibius*, *Doryphoribius*, or others with the *Isohypsibius*-type of claws), and possibly to the living macrobiotid *Dactylobiotus*. The original description of *Beorn* with an inner diploclaw having the main branch longer than the outer diploclaw of the same leg may be just a misinterpretation, since in all living eutardigrades these claws are either of equal length or the outer one is longer. Given the remarkable stasis we have observed in a fossil at least 10 Ma older than *Beorn*, it would be important to more carefully compare the type specimen with living taxa. On the basis of our finding, also, it can be predicted that any tardigrades found in the much more abundant, younger ambers from the Baltic region, the Dominican Republic, or Mexico, will also be extremely similar – perhaps indistinguishable – from modern species. A species of tardigrade approximately 90 million years old that appears in all observable features to be modern is consistent with a Cambrian appearance of the stem group of the modern tardigrades.

References

Baumann, H. 1927. Bemerkungen zur Anabiose der Tardigraden. Zool. Anz. 72: 1-4.

Binda, M.G. & G. Pilato 1990. Tardigradi di Terra del Fuoco e Magellanes. I. *Milnesium brachyungue*, nuova specie di tardigrado Milnesiidae. Animalia 17: 105-110.

Cooper, K.W. 1964. The first fossil tardigrade: *Beorn leggi* Cooper, from Cretaceous amber. Psyche 71: 41-48.

Crowe, J.H. & A.F. Cooper, Jr. 1971. Cryptobiosis. Sci. Amer. 225: 30-36.

Crowe, J.H. 1975. The physiology of cryptobiosis in Tardigrada. Mem. Ist. Ital. Idrobiol. 32 suppl.: 37-59.

Durante, M.V. & W. Maucci 1972. Descrizione di *Hypsibius* (*isohypsibius*) *basalovoi* sp-nov. e altre notizie sui tardigradi del Veronese. Mem. Mus. Civ. St. Nat. Verona 20: 275-281.

Garey, J.R., M. Krotec, D.R. Nelson & J. Brooks 1996. Molecular analysis supports a tardigrade-arthropod association. Inv. Biol. 115: 79-88.

Giribet, G. & C. Ribera 1999. The position of arthropods in the animal kingdom: a search for a reliable outgroup for internal arthropod phylogeny. Mol. Phylog. Evol. (in press)

Giribet, G., S. Carranza, J. Baguña, M. Riutort & C. Ribera 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Mol. Biol. Evol. 13: 76-84.

Grimaldi, D. 1996. Amber: Window to the Past. New York: Amer. Mus. Nat. Hist. and H.N. Abrams.

Grimaldi, D., A. Shedrinsky, & T.P. Wampler 2000. A remarkable deposit of fossiliferous amber from the Upper Cretaceous (Turonian) of New Jersey. Backhuys Publishers, Leiden: 1-76 [this volume].

Horning, D.S. Jr., R.O. Schuster & A.A. Grigarick 1978. Tardigrada of New Zealand. New Zealand J. Zool. 5: 185-280.

Kinchin, I.M. 1994. The Biology of Tardigrades. London: Portland Press. 186 pp.

Larsson, S.G. 1978. Baltic amber – A paleobiological study. Entomonograph 1: 192 pp. Denmark: Klampenborg.

Maucci, W. 1988. Tre nuove specie di Eutardigradi della Groenlandia meridionale. Boll. Mus. Civ. St. nat. Verona 15: 279-289.

May, R.M. 1948. La vie des Tardigrades. Gallimard, Paris. 133 pp.

May, R.M., M. Maria & J. Guimard 1964. Action différentielle des rayon X et ultraviolets sur le Tardigrade *Macrobiotus areolatus* à l'état actif et desséché. Bull. Biol. Fr. Bel. 48: 349-367.

Müller, K.J., D. Walossek & A. Zakharov. 1995. "Orsten" type phosphatized soft-integument preservation and a new record from the Middle Cambrian Kuonamka Formation in Siberia. N. Jb. Geol. Paläont. Abh. 197: 101-118.

Nascimbene, P. & H. Silverstein 2000. The preparation of fragile Cretaceous ambers for conservation and study of organismal inclusions. Backhuys Publishers, Leiden: 93-102 [this volume].

Pike, E.M. 1994. Historical changes in insect community structure as indicated by hexapods of Upper Cretaceous Alberta (Grassy Lake) amber. Can. Ent. 126: 695-702.

Pilato, G. & M.G. Binda 1991. *Milnesium tetralamellatum*, new species of Milnesiidae from Africa (Eutardigrada). Trop. Zool. 4: 103-106.

Rahm, G. 1921. Biologische und Physiologische Beiträge zur Kenntnis der Moosfauna. Zeitschr. Allg. Physiol. 20: 1-34.

Rahm, G. 1924. Weitere physiologische Versuche mit niederen Temperaturen. Ein Beitrag zur Lösung des Kaltenproblems. Verhand. Deutsch. Zool. Ges. 29: 106-111.

Rahm, G. 1925. Beiträge zur Kenntnis der Moostierwelt der preußischen Rheinlande. Arch. Naturgesch., Abt. A. 90: 153-214.

Ramazzotti, G. & W. Maucci 1983. Il Phylum Tardigrada. III edizione riveduta e aggiornata. Mem. Ist. Ital. Idrobiol. 41: 1-1012.

Seki, K. & M. Toyoshima 1998. Preserving tardigrades under pressure. Nature 395: 853-854.

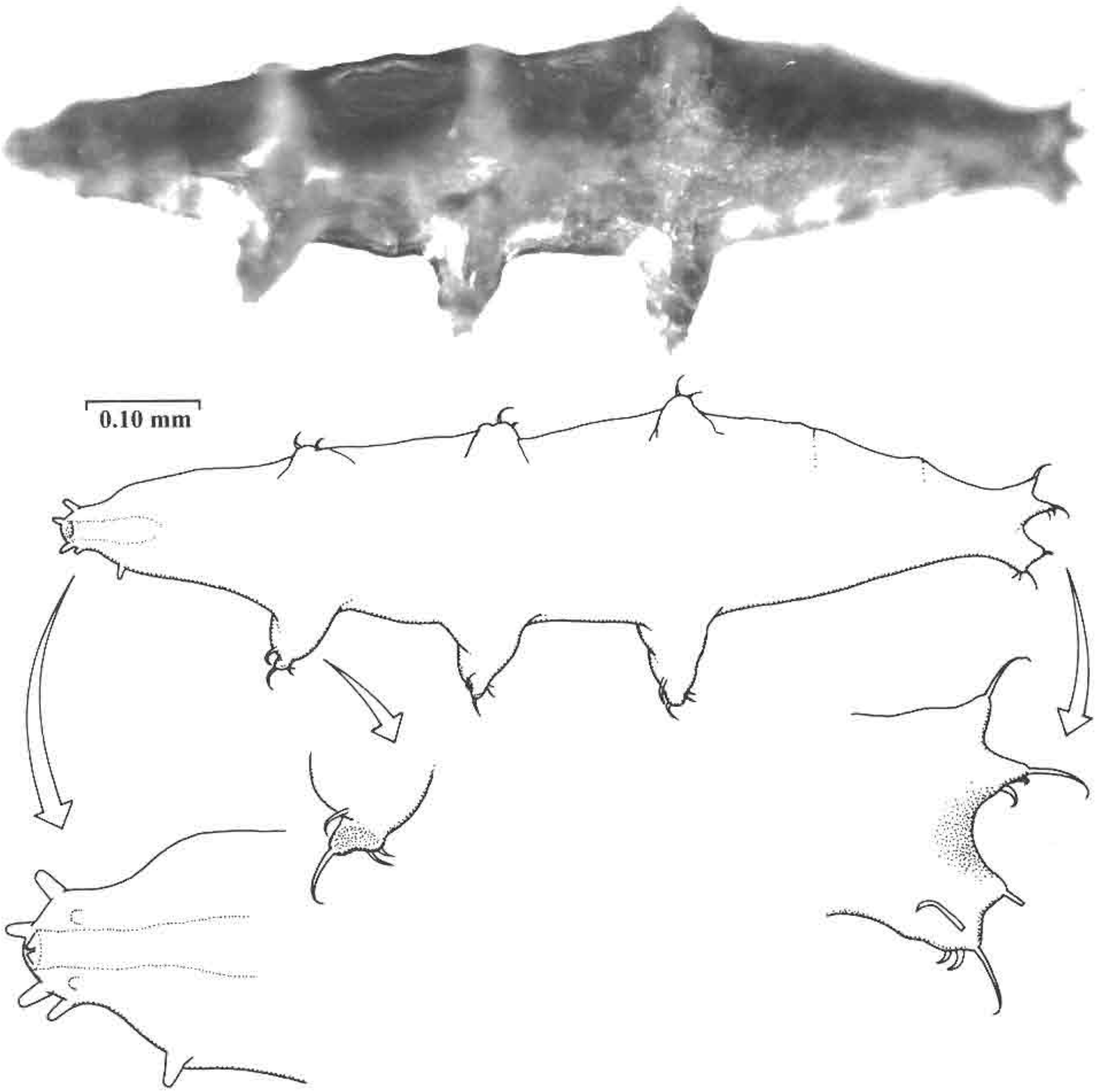

Fig. 1. *Milnesium swolenskyi*, n.sp. above, Photomicrograph (phase contrast); below, Drawing of entire animal, showing details of mouth region and claws.

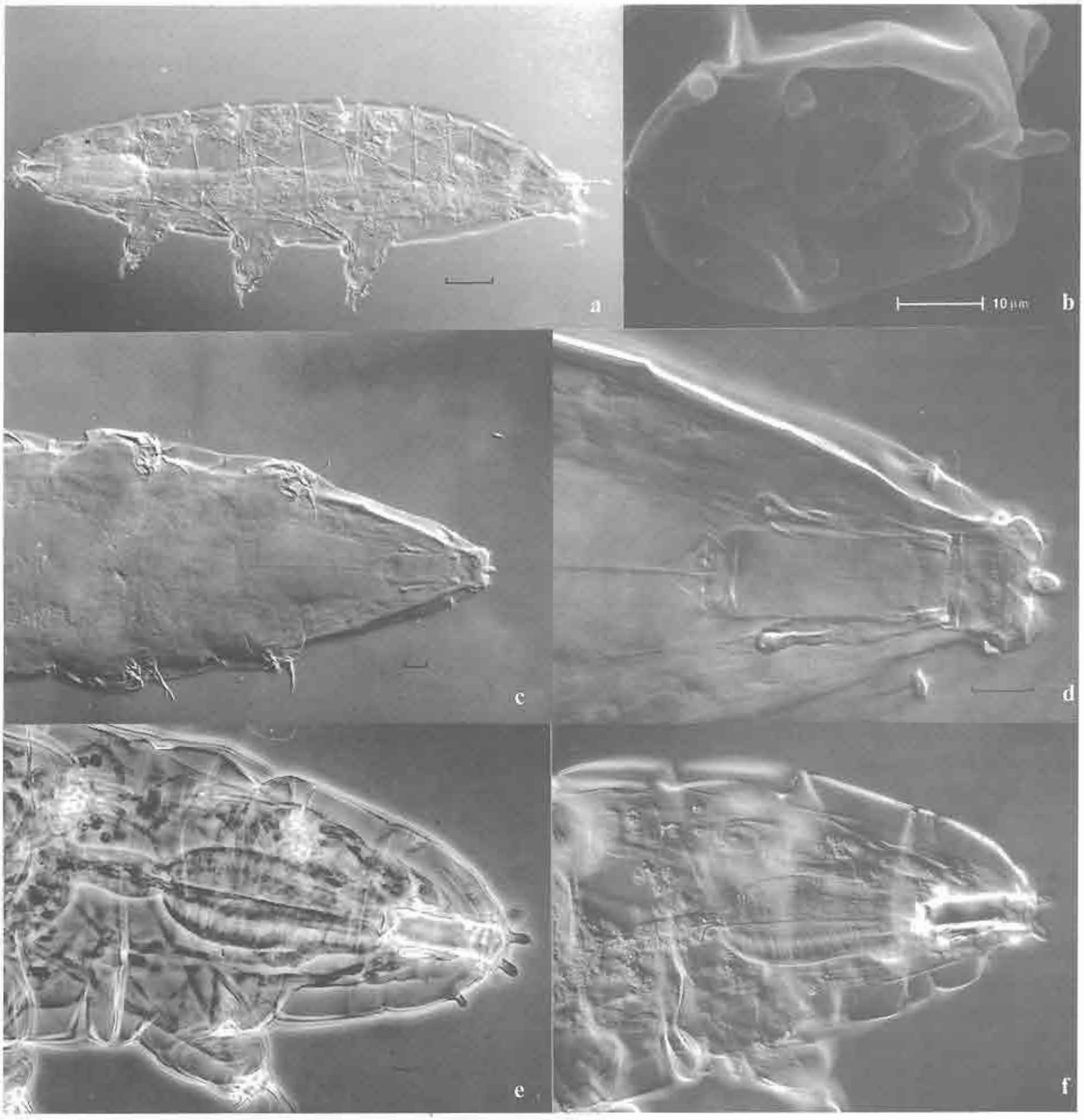

Fig. 2. *Milnesium tardigradum*, a common, widespread living species closely related to the fossil. a. Entire in vivo specimen (from Sweden), lateral view, under Differential Interference Contrast (DIC)[Nomarski optics[. Scale = 50 µm. Digestive tract and muscles are clearly visible. b. Scanning electron micrograph (SEM) of buccal region. c. Anterior half of specimen (from Sardinia), under DIC. d. Head region of same specimen as in c, showing internal details of buccal apparatus (DIC). e. Anterior end of specimen under Phase contrast, showing cephalic papillae and (internally) pharynx. f. Same specimen as in e, under DIC. Scales c-f: 10 µm.

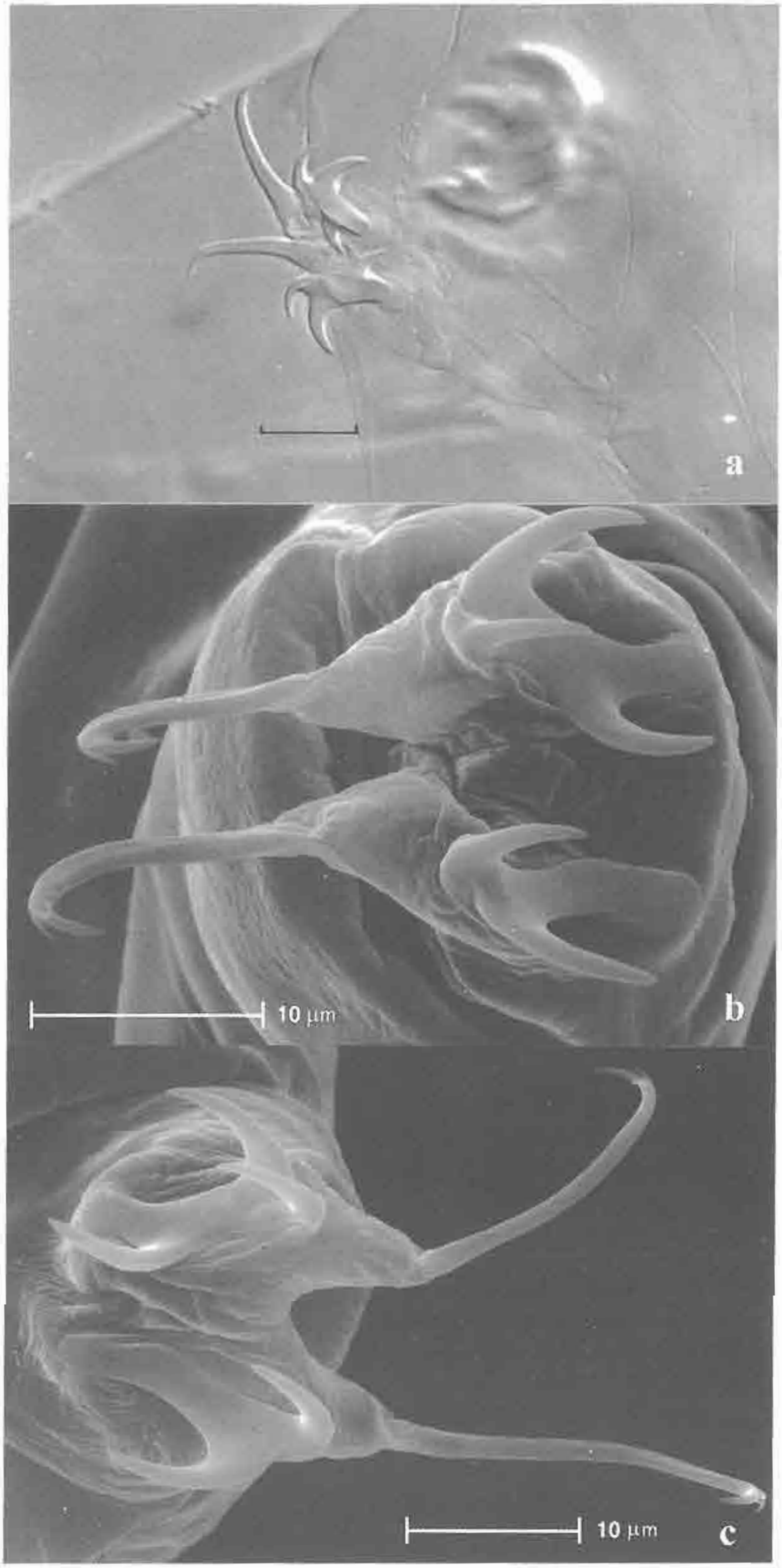

Fig. 3. *Milnesium tardigradum*, details of claws. a. Claws on leg from pair 1. (cf. fig. 3b). Specimen C263 (Sardinia). DIC, scale = 10 µm. b, c: SEMs. b. Claws from leg of pair 1. c. Claws from leg of hind pair (pair 4).