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A New Bisexual Species of *Echiniscus* C.A.S. Schultze, 1840 (Heterotardigrada: Echiniscidae) from Tasmania, Australia

Eine neue bisexuelle Art der Gattung *Echiniscus* C.A.S. Schultze, 1840 (Heterotardigrada: Echiniscidae) aus Tasmanien, Australien

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Summary: The terrestrial tardigrade fauna of Tasmania is insufficiently studied; only 14 species have been recorded to date, which number includes several rare and interesting species and genera restricted to the southern cool temperate regions. The new bisexual species of *Echiniscus* C.A.S. Schultze, 1840 described herein and named *Echiniscus lentiferus* sp. n. is remarkable because of the presence of a prominent round lens-shaped structure on the dorsal mid-line of the terminal plate of both sexes and juveniles. This trait neither has been described in any other species of the genus nor in any other tardigrade; its function is not clear. Morphometric data of males and females of *E. lentiferus* sp. n. suggest sexual dimorphism, i.e. males appear shorter and thinner than females. Clear differences were obtained by comparing the dimensions of dorsal plates. Five plates were significantly wider in females than in males relative to the scapular plate length.

Key words: Tardigrada, Echiniscidae**,** *Echiniscus lentiferus* sp. n, sexual dimorphism, Tasmania, Australia

Zusammenfassung: Die Tardigradenfauna von Tasmanien ist nur wenig untersucht. Bis heute sind nur 14 Arten bekannt, unter denen sich eine Reihe von seltenen und interessanten Arten und Gattungen findet, deren Vorkommen auf die südlichen kühl-temperierten Regionen begrenzt ist. Die hier beschriebene neue bisexuelle Art der Gattung *Echiniscus* C.A.S. Schultze, 1840 mit dem Namen *Echiniscus lentiferus* sp. n. ist bemerkenswert, weil beide Geschlechter eine auffällige linsenförmige Struktur (Beule) in der Mitte der cuticularen Endplatte besitzen. Eine solche Struktur ist bisher weder von *Echiniscus*-Arten noch von irgendeiner anderen Tardigradenart beschrieben worden; ihre Funktion ist unbekannt. Morphometrische Daten für beide Geschlechter von *E. lentiferus* sp. n. scheinen einen Geschlechtsdimorphismus in der Größe zu belegen. Dies wird besonders deutlich, wenn man die Breite bestimmter Cuticulaplatten in Relation zur Schulterplatte bei Männchen und Weibchen vergleicht, die bei Weibchen signifikant größer ist.

Schlüsselwörter: Tardigrada, Echiniscidae, *Echiniscus lentiferus* sp. n., Sexualdimorphismus, Tasmanien, Australien

1. Introduction

Tasmania, with a land mass of 68,401 km^2 (approximately the size of Ireland), lies in the path of the 'roaring forties' wind that encircles the globe in the southern hemisphere. Surrounded by the Indian and Pacific Ocean and separated from mainland Australia by Bass Strait (240 km) it has a cool temperate climate, and in the highlands, e.g. Cradle Mountain, rainfall is about 2700 mm/a. The few species of tardigrade so far cited as occurring in Tasmania include the rare *Antechiniscus moscali* Claxton, 2001, *Limmenius porcellus* Horning, Schuster & Grigarick, 1978, *Mopsechiniscus tasmanicus*

Dastych & Moscal, 1992 and *Milnesioides exsertum* Claxton, 1999. All of these genera have been found only in cool temperate rainforests in the southern hemisphere as the species *Oreella mollis* Murray, 1910 (cited in Tasmania by DASTYCH et al. 1998 and BERTOLANI et al. 1996).

To date, 22 species of the genus *Echiniscus* have been cited for Australia but only one for Tasmania, the bisexual New Zealand species *Echiniscus vinculus* Horning, Schuster & Grigarick, 1978 (cited by CLAXTON 1996, 2001 as *Echiniscus* cf. *vinculus*). CLAXTON (1996) noted that eight of 22 species (36%) of *Echiniscus* found in Australia at that time were bisexual, a large proportion considering the low proportion (16%) of bisexual species amongst the 163 species and subspecies known to date (DEGMA et al. 2017). Sexual dimorphism in *Echiniscus* species involves the shape of the gonopore, body length (males are, on average, shorter, although the size ranges overlap), the lengths of buccal appendages (longer in males), claw lengths (longer in males) and in *Echiniscus curiosus* Claxton, 1996 the presence and lengths of body appendages (longer in males) and males lack spine C^d, which is present in females (CLAXTON 1996).

In this article a new bisexual species of *Echiniscus* is described that has an unusual cuticular structure on the terminal plate. In order to examine sexual dimorphism in this species, measurements of all appendages, lengths and widths of plates and lengths of claws were recorded and their variation was examined using the scapular plate (sp) index introduced by DASTYCH (1999).

2. Materials and methods

The material examined for this article came from the same source – moss and mixed cryptogam samples collected by A.M. MOSCAL in Tasmania and distributed to both authors as dried samples in paper bags complete with extensive field data including names of all cryptogams (see "Material examined").

The methods of preparation and observation for the type material are given in CLAXTON (1996). For them, photomicrographs were made using an Olympus BH-2 phase contrast microscope and a Nikon Coolpix 995 digital camera or ZEISS Photomikroskop III. Measurements were taken with eyepiece micrometer. For SEM examination specimens were transferred from BOUIN'S medium into 70% ethanol, dehydrated, critical-point-dried and carbon-coated; photomicrographs were taken with LEO 1525. The type specimens were mounted in HOYER'S medium with a small amount of iodine. Other specimens were mounted in FAURE'S medium. Most specimens (including the holotype) are mounted with specimens of other species found in the sample.

The scapular plate (sp) index from DASTYCH (1999) was used. This index expresses the percent ratio between the length of a structure and the length of the scapular (= shoulder) plate. The scapular plate length (measured along the dorsal mid-line from the anterior edge of the plate to the posterior edge: see Fig. 7, spl) was used instead of buccal tube length (the latter used in eutardigrades as the pt index of PILATO 1981) because of the relative resistance of the scapular plate length to distortion by mounting methods and because the buccal tube in heterotardigrades is difficult to see under the sclerotized cuticular plates. Body length was measured from the anterior tip to the end of the body not including the fourth pair of legs. The dorsal plates tend to slide over one another naturally and they also do this quite variably in slide mounted specimens. Therefore body length is given to zero decimal places and is not used for comparisons between specimens. When comparing sp indices of selected characters, their means ($\bar{x}$) and standard deviations (SD) are presented. Using the sp index, differences and similarities in body appendages between

males and females of the new species were investigated. Because breakages (unbroken filaments came to a fine sharp point, broken filaments had a blunt end) occurred, the sp value for either the only remaining entire filament or the longest filament at each of the four lateral and three dorsal positions was used. All characters were calculated based on n=10 for females and n=9 for males (Tab. 1), unless otherwise indicated. The means of sp values for females and males were compared using the two-way Student's t-test at 0.05 significance levels, applying Minitab Statistical Software, ver 17.

2.1. Abbreviations and symbols used

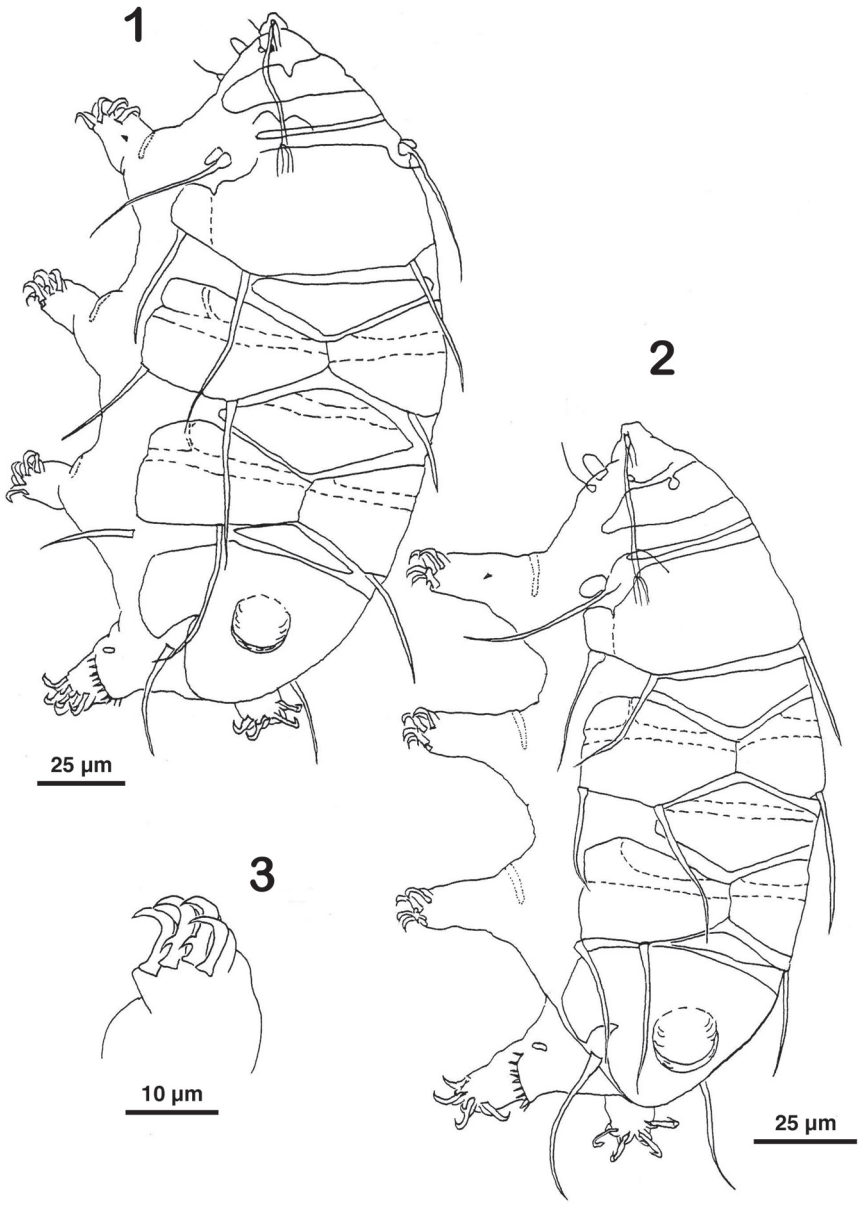
I – first pair of dorsal paired plates [erste paarige Rumpfplatte]; II – second pair of dorsal paired plates [zweite paarige Rumpfplatte]; B – lateral appendage (= filament) [lateraler Anhang B]; B^d, = Bd – dorso-lateral appendage [dorso-lateraler Anhang B^d, = Bd]; C – lateral appendage [lateraler Anhang C]; cA – cirrus (= appendage) A [lateral Cirrus (= Anhang) A]; C^d, = Cd – dorso-lateral appendage [dorso-lateraler Anhang C^d, = Cd]; ce – external cirrus [äußerer Mediancirrus]; ci – internal cirrus [innerer Mediancirrus]; cp – cirrophore [Cirrophorus]; D – lateral appendage [lateraler Anhang D]; dc – dentate collar [Dornfalte]; D^d, = Dd – dorso-lateral appendage [dorso-lateraler Anhang D^d, = Dd]; E – lateral appendage [lateraler Anhang E]; HP – head plate [Kopfplatte]; lp – leg papilla [Beinpapille]; M1– first median plate [erste Schaltplatte]; M2 – second median plate [zweite Schaltplatte]; M3 – third median plate [dritte Schaltplatte]; n – sample size [Stichprobengröße]; NP – neck plate [Nackenplatte]; pc – primary clava [primäre Clava]; s – leg spine [Beindorn]; sc – secondary clava [sekundäre Clava]; SD – standard deviation [Standardabweichung]; spl – length of scapular plate [Länge der Schulterplatte]; SP – scapular (= shoulder) plate [erste Rumpf-(= Schulter-)platte]; TP – terminal plate [vierte Rumpf-(= End-) platte]; $\bar{x}$ – arithmetic mean [arithmetischer Mittelwert].

3. Systematics

Class Heterotardigrada Marcus, 1927
Order Echiniscoididae Kristensen & Hallas, 1980
Family Echiniscidae Thulin, 1928

Echiniscus C.A.S. Schultze, 1840
Type species: *Emydium testudo* Doyère, 1840
Diagnosis (after Kristensen 1987): Red eye spots. Rigid buccal tube, stylet supports absent or not visible. Primary and secondary clavae papillate. Median plates undivided, terminal plate notched. Pseudo-segmental plates IV, and ventral plates absent. Leg plate on fourth pair of legs with dentate collar.

Echiniscus lentiferus sp. n.
(Figs 1-13, Tab. 1)
Type material examined: Australia: TASMANIA: Central Highlands Region, Mt. Kate, Cradle Mountain-Lake St. Claire National Park, 41° 37' E 145° 58' S, 1080 m. a.s.l., 18 Jan 1997, A.M. Moscal, mixed mosses and lichens on skeletal soil hump in stunted *Athrotaxis* sp. stand, 22 specimens (10 females, 9 males, 2 juveniles and one 2-clawed juvenile (called "larva" by some sources). HOLOTYPE (female: slide number 28899-38, KS 41620), ALLOTYPE (male: slide number 28899-24, KS 41621) and PARATYPES (2 females, 2 males and one 2-clawed juvenile, KS 41622-41626). The type materials is deposited in the Australian Museum, Sydney (AM acquisition numbers are KS 411620-411626). Remaining specimens in the collection of S.K. Claxton.
Other material examined: Australia**:** TASMANIA: North West Region, Mount Montgomery, Mt Montgomery State Reserve, 41° 08' E 146° 03' S, 470 m. a.s.l., 20 Oct 1994, A.M. Moscal, moss on rock in low woodland on mountain summit, 2 specimens (female, male). Central Highlands (Southern



Figs 1-3: *Echiniscus lentiferus* sp. n. **1** Latero-dorsal, female. **2** Ditto, male. **3** Leg II. Note the lens-shaped structure on terminal plate.
Abb. 1-3: *Echiniscus lentiferus* sp. n. **1** Latero-dorsal, Weibchen. **2** Ditto, Männchen. **3** Bein II. Man beachte die linsenförmige Struktur auf der Endplatte.

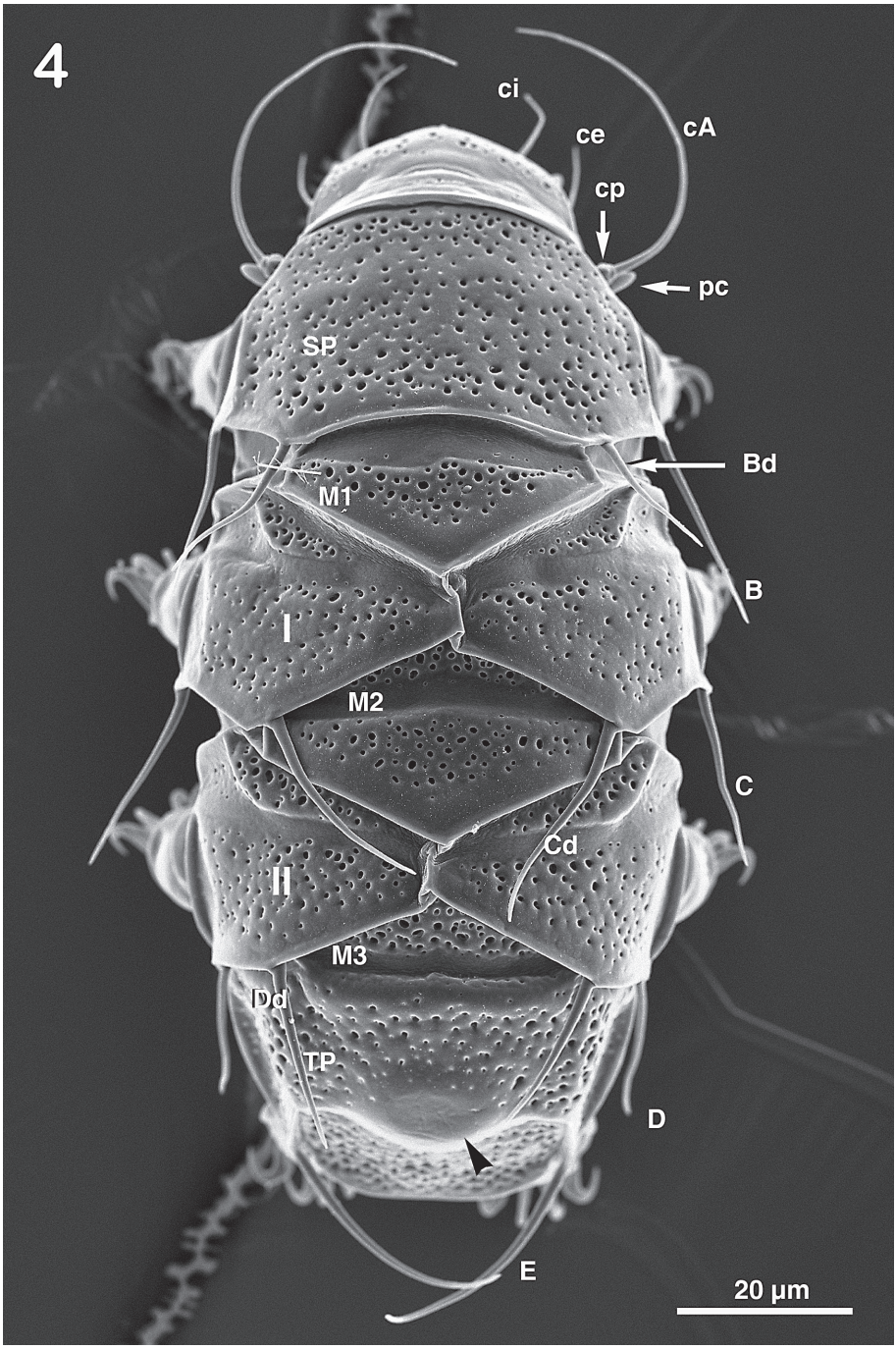


Fig. 4: *Echiniscus lentiferus* sp. n., dorsal view (SEM image). Note the lens-shaped structure on the terminal plate (arrowhead). Abbreviations see page 107.
Abb. 4: *Echiniscus lentiferus* sp. n., Dorsalansicht (REM-Bild). Man beachte die linsenförmige Struktur auf der Endplatte (Pfeilkopf). Abkürzungen s. Seite 107.

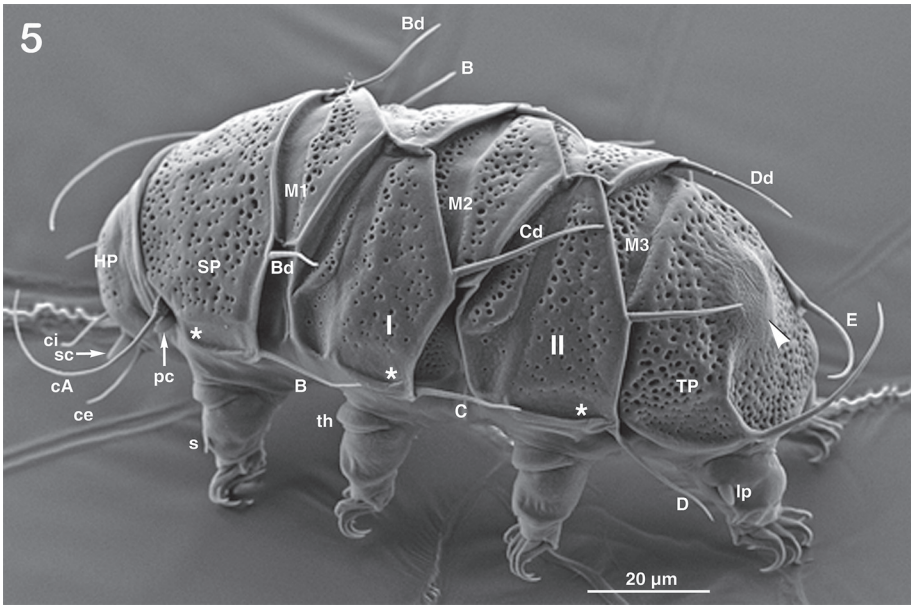
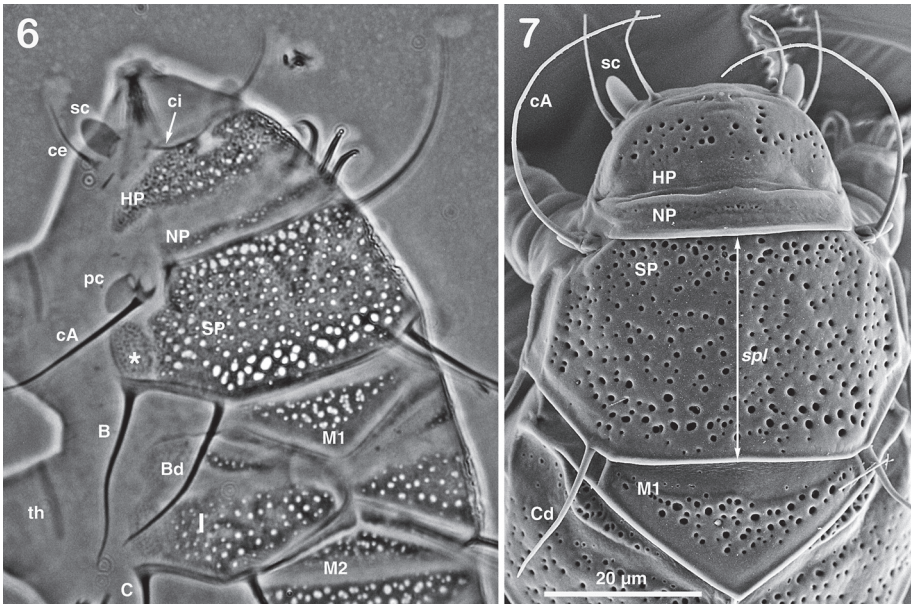
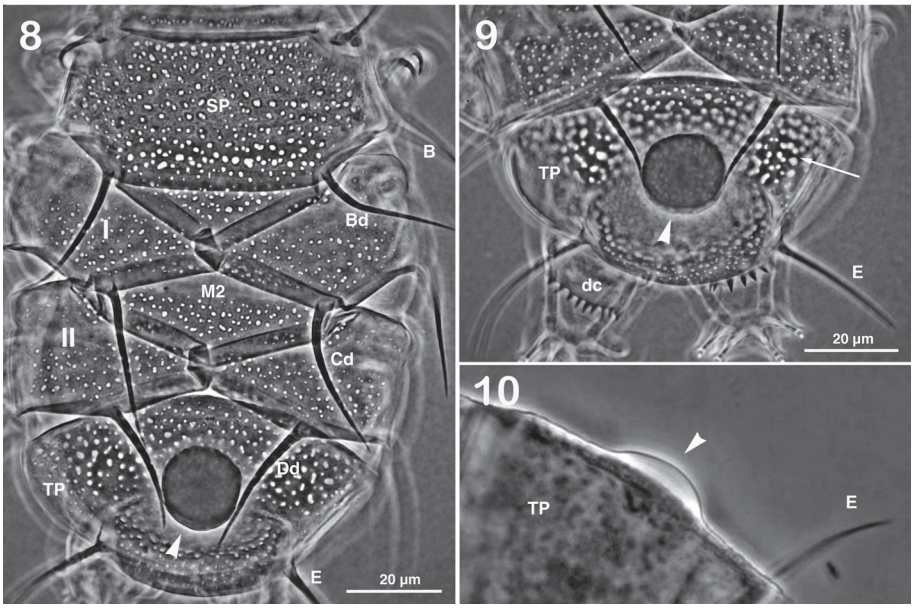


Fig. 5: *Echiniscus lentiferus* sp. n., lateral view (SEM). Lens-shaped structure on the terminal plate (arrowhead). Small lateral platelets (asterisks). Abbreviations see page 107.
Abb. 5: *Echiniscus lentiferus* sp. n., Lateralansicht (REM). Linsenförmige Struktur (Beule) auf der Endplatte (Pfeilkopf). Lateralplättchen (Sterne). Abkürzungen s. Seite 107.



Figs 6-7: *Echiniscus lentiferus* sp. n. **6** Latero-dorsal view (allotype: phase contrast). **7** Dorsal view (SEM). Abbreviations see page 107.
Abb. 6-7: *Echiniscus lentiferus* sp. n. **6** Latero-dorsal (Allotypus: Phasenkontrast:). **7** Dorsal (REM). Abkürzungen s. Seite 107.



Figs 8-10: *Echiniscus lentiferus* sp. n. **8** Habitus, dorsal view. **9** Rear of the body, dorsal view; note the honeycomb pattern (arrow). **10** Terminal plate with lens-shaped structure, view in profile (phase contrast). Abbreviations see page 107.

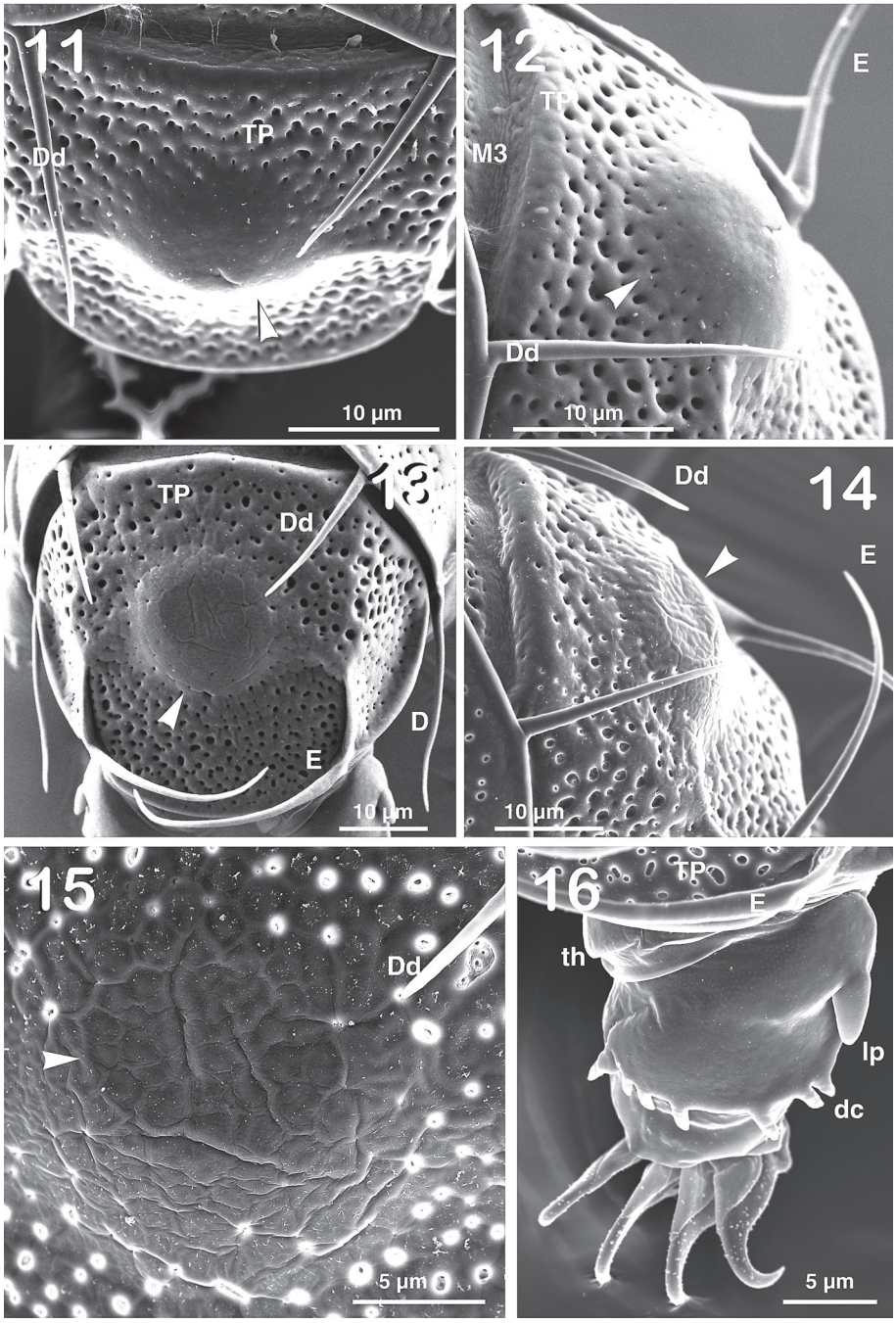
Abb. 8-10: *Echiniscus lentiferus* sp. n. **8** Habitus, dorsal. **9** Hinterer Teil des Körpers, dorsal. Man beachte das wabenförmige Muster (Pfeil). **10** Endplatte mit linsenförmiger Struktur, im Profil. (Phasenkontrast). Abkürzungen s. Seite 107.

Region), Mount Field National Park (146° 38' E 42° 41' S), Lady Barron Creek, 980 m a.s.l., 12 March 1992, A.M. Moscal, mixed liverworts and lichens on stream bank decaying log, 10 specimens (3 males, 4 juveniles; 3 animals in SEM preparation, sex unknown). A slide with two specimens (male, juvenile) from the Lady Barron Creek is deposited in the Zoological Museum, Center of Natural History, University of Hamburg.

Diagnosis: Sculpture double, first, small unevenly sized and spaced black dots, and second, large unevenly sized and spaced white spots overlying the first. Filaments at B, C, D, E, B^d, C^d and D^d. Terminal plate with long, curved notches and a distinct round lens-shaped thickening in the outer cuticular layer in the midline of the plate. Males and females present.

Description: Body cells yellow-orange. Body length 90 (2-clawed juvenile) to 211 mm; females 171-211 mm, males 150-178 mm. Eye spots not discernible (slide preparation). In 192 mm long female-holotype and 162 mm long male-allotype cirrus A 52.8 (44.0) mm long (measured with cirrophores), primary clava 5.7 (7.9) mm long, ovoid. Internal and external buccal cirri 15.8 (15.3) and 18.5 (17.2) mm long, both measured with cirrophores, secondary clava 6.6 (8.3) mm long, ovoid (Figs 1, 2, 4-7).

Mouth antero-ventral on a mouth cone. Buccopharyngeal apparatus often obscured by plate sculpture, consisting of a very narrow, thin-walled buccal tube, very fine, straight stylets and very fine, curved stylet supports inserted with pt value of 81-84% of buccal tube length. Anteriorly, two stylet sheaths with spherical enlargements are visible within buccal crown. In the pharynx (not visible in mounted material thus not measured) three curved rods have pt index value (Pilato 1981) of 27-31% of buccal tube length.



Figs 11-16: *Echiniscus lentiferus* sp. n. (SEM). **11** Terminal plate with lens-like structure, dorsal view. **12** Ditto, lateral view. **13** Ditto, hind view. **14** Ditto, lateral view. **15** Hind view. Note that the surface of the lens does not contain pores. **16** Leg IV, dorso-lateral view; cuticular thickening (th). Abbreviations see page 107.

Sculpture on dorsal plates double (elsewhere cuticle smooth), consisting of minute, unevenly sized and spaced black dots, and white spots or pores variable in size (0.5-2.0 µm) and shape across plates. In SEM only white spots are discernible on the surface of plates, being visible there as small cavities, "pores" (Figs 4, 5, 7, 11-15). Varying the focus of the microscope reveals an internal honeycomb-like pattern of deeper located sculpturing on scapular and terminal plates (Fig. 9, arrow).

Head plate with anterior median notch, with double sculpture with few spots in centre of plate over a "forehead"; on both sides are triangular areas with single sculpture of black dots (Fig. 6). Neck plate narrow with double sculpture and few very small spots (pores) (Figs 6, 7: NP).

Scapular plate large, with double sculpture, with unevenly spaced and sized spots. In most specimens, spots appear in three bands. In the holotype, scapular plate 34.3 µm long – anterior band (about 8 µm wide) with spots 1.0 to 1.3 µm rather close together sometimes coalescing in a dark matrix, middle and widest band (16-18 µm wide) with spots 0.7-1.0 µm about 2 µm apart in a pale matrix in which dark spots are noticeable; posterior band (8 µm wide) with spots 1.3 to 2.0 µm diameter rather close and sometimes coalescing, in a darker matrix than elsewhere on this plate followed posteriorly by a thin unsculptured band. Small lateral plates (platelets: comp. e.g. Dastych 1999) of holotype (allotype) are 13.9 (10.6) µm long and 9.2 (7.0) µm wide with only minute black dots, under phase contrast they appear separated from the main scapular plate by a thin band without sculpture (Fig. 6, asterisk). A surface of the platelet when observed in SEM is covered by hardly discernible pores and the plate is separated from the scapular one by a short longitudinal unsculptured band (Fig. 5, asterisk). The platelets are also formed on both paired plates and are covered by larger pores (Fig. 5, asterisks). Length of scapular plate at mid-dorsal line, 34.3 (29.0) µm, width 87.1 (66.0) µm including lateral plates. Scapular plate is significantly wider, relative to the length of the scapular plate, in females than in males (sp for 10 females is 2.50 ± 0.06, for nine males 2.24 ± 0.06). Both, cirrus A and primary clavae occur in antero-lateral notches on each side of scapular plate. Clavae lie close to and to the sides of cirrus A cirrophores. Trunk appendages on scapular plate are long, strong filaments at positions B, 39 and 33 (both 32) and B^d 52 and 35 (28 and 33) µm; B slightly wider at bases than B^d filaments (Fig. 6).

Paired segmental plates I and II divided transversely by unsculptured bands into anterior third and posterior two thirds. Anterior third subdivided by a smooth line into a lateral area in which sculpture is very faint or missing and a medial area which is strongly sculptured with a dark matrix and large pores (Figs 4-8). Posterior two thirds with small pores, uniform in size (1.0 µm) and 2.6-3.0 µm apart in a pale matrix. Long strong filaments at C, D 26-37 µm long on holotype (25-32 µm long on allotype) and C^d, D^d 36-44 (33-40) µm. Lengths of paired plate I and II from widest points anteriorly and posteriorly are identical 31.7 (26.4) µm; widths from midline to lateral extremity 52.8 (37.0) µm in paired plates I and II (sp is statistically significantly larger in females than in males – for 10 females 1.52 ± 0.04 and for nine males 1.26 ± 0.04.

Median plate 1 triangular, with double sculpture and plain borders (Figs 4-8),

Abb. 11-16: *Echiniscus lentiferus* sp. n. (REM). **11** Endplatte mit linsenförmiger Struktur, dorsal. **12** Ditto, lateral. **13** Ansicht von hinten-oben. **14** Ditto; lateral. **15** Ansicht von hinten. Man beachte, dass die linsenförmige Struktur keine Poren hat. **16** Bein IV, dorso-lateral: Verdickung der Cuticula (th). Abkürzungen s. Seite 107.

15.8 (11.9) μm long at mid-line and 40.9 (31.7) μm wide. This plate is significantly wider in females than in males – for 10 females 1.22 ± 0.02 and in nine males 1.10 ± 0.04. Median plate 2, diamond-shaped, with small anterior sculptured area (Figs 1, 2, 4-6) 7.8 (8.5) μm long, transverse plain band 4.0 (2.0) μm long and posterior sculptured area 17.2 (11.9) μm long (this plate is significantly wider in females than males – in 10 females 0.82 ± 0.02 and in nine males 0.75 ± 0.03); plate width 52.8 (38.3) μm (Figs 4-6). Median plate 3 triangular, with double sculpture, plain posterior border, 9.2 (7.9) μm long and 39.6 (23.8) μm wide.

Terminal plate with two wide incisions curving towards mid-line almost meeting at round to oval, prominent and clear cuticular lense-shaped structure in mid-line (Figs 1, 2, 4, 5, 8-15), 16.5 (16.5) μm wide, present on all specimens 37-45% of the length of the terminal plate. Terminal plate 46.2 μm long in holotype (40.9 μm long in allotype) at mid-dorsal line, 87.1 (66.0) μm wide (measured from mid-point of the structure to lateral extremity of plate). The plate width is statistically significantly greater (relative to the length of the scapular plate) in females than in males – for 10 females sp index is 2.56 ± 0.06 and for nine males 2.17 ± 0.06. Sculpture similar to that on posterior band of scapular plate – large pores in a dark matrix. Long filaments present at E 40 and 48 μm long on holotype (44 and 44 μm long on allotype).

Ventral surface without sculpture.

Legs long, first three pairs about 26 μm long on allotype, two cuticular thickenings occur antero-laterally on legs I-III (Figs 1, 2, 5, 6, 16, th). The most proximal is about 1.3 μm wide and occurs where the greatest flexion of the leg occurs. The distal thickening is wider, about 2.6 μm and shorter, on first leg it is anterior to the spine.

Small spine on first pair of legs 1.7 (1.7) μm long and small papilla 4.0 (4.0) μm long on fourth pair. Fourth pair of legs with plate with single sculpture of fine internal black dots, its surface smooth, i.e. lacking pores (Figs 5, 16). Dentate collar with 9-12 long, slender teeth, uneven in length 1.0-4.0 μm (Figs 1, 2, 9; 16, dc). A small crescent-shaped cuticular thickening occurs just above the papilla.

Claws rather short and robust, internal claws with long, strongly curved and relatively thin spurs which arise 3.0 μm from claw base on internal claw of fourth leg – 9.2 μm long. The placement of the spurs of "merokensis"-type, i.e. they are located below or well below the middle of the claw length (comp. e.g. THULIN 1911: Fig. 4b). Claws on first pair of legs shortest, internal claws 9.2 (7.5) μm and external 8.6 (7.3) μm long, second and third pairs of claws equal in length and similar or equal to lengths of first pair of claws, internal 9.2 (7.9) μm and external 8.6 (7.5) μm and slightly shorter than those on fourth - internal claws 10.6 (9.2) μm, external 9.9 (8.6) μm in 192 (163) μm specimens. The lengths of the claws on males and females are the same relative to the lengths of the scapular plates e.g. fourth internal claws have sp values of 30.8 (31.9).

No eggs found.

Etymology: L. *lentiferus,* lens-bearing, after the strange lens-shaped structure on the terminal plate.

Remarks: The 2-clawed juvenile, 89 μm long, has cirrus A 20 μm long, primary clava 3.0 μm long. Internal and external buccal cirri 5.3 and 7.4 μm long, secondary clava 4.0 μm long. Sculpture double on all plates, white spots, large, up to 2.0 μm diameter, especially on the trailing edge (4.0 μm long band) of the scapular plate 17.8 μm long (Fig. 12). Trunk appendages B 13 and 17 μm long, B^d 16 μm on one side, absent or broken off on the other, C^d 19 and 20 μm long, D^d both 16 μm long. Lateral filaments C 17 and 15 μm and D 15 and 11 μm long and E 17 and 21 μm. The lens on terminal plate is 13.2 μm in diameter (56% of the terminal plate length). Both claws on each

leg carrying spurs, claws on first leg 4.6 µm, second and third legs claws 5.0 µm long, on fourth legs 5.5 µm.

Differential diagnosis: *E. lentiferus* sp. n. differs from all other species of *Echiniscus* described so far, by having a clear lens-shaped structure on the terminal plate. The presence of appendages at B^d separates the new species from all but six of 162 species described. It differs from four of these six in having different sculpture on the dorsal plates. *E. apuanus* M. Bertolani, 1946, *E. militaris* Murray, 1911, *E. hexacanthus* Maucci, 1973 and *E. weisseri* Maucci, 1978 have polygonal sculpture, very different from sculpture of *E. lentiferus* sp. n. *E. africanus* Murray, 1907 differs from the new species by having six thin spines on the caudal margin of the scapular plate (three on each side of the median line), the new species has only one filament (B^d) on each side. *E. arcangelii* Maucci, 1973-74 differs from the new species by having six small triangular teeth on the anterior margin of the scapular plate where the new species has none. *E. lentiferus* sp. n. is similar to another new hitherto undescribed species from south-eastern Australia (Claxton in prep.) in having the lens-shaped structure on the terminal plate, the general dimensions of the body and the lengths of the cephalic appendages. However, it differs from this species by having a different plate sculpture and filaments at all positions and particularly at B and B^d.

Distribution: This species has so far only been found in Tasmania and not on mainland Australia.

The Cradle Mountain sample consisted of a mixture of several mosses – *Polytrichum juniperinum* Hedw., *Dicranoloma billiarieri* (Brid.) Par., *Campylopus clavatus* (R. Br.) Wils., *Rhacomitrium crispulum* (Hook. F. & Wils) Dix. var. *tasmanicum, Rhacocarpus purpurescens* (Brid.) Par. and *Hypnum chrysogaster* C. Muell., liverworts – *Jamesoniella colorata* (Lehm.) Spruce ex Schiffn., *Lepidozia ulothrix* (Schwaegr.) Lindenb. and *Frullania rostrata* (J.D. Hook & Tayl.) J.D. Hook & Tayl. ex Gott. et al. – and lichens, *Cladia aggregata* (Sw.) Nyl., 1870, *Alectoria nigricans* (Ach.) Nyl., Lich. Scand. 71. 1861 and *Pannaria* sp. on a skeletal soil hump under an old growth stand of *Arthrotaxis selaginoides* D. Don 1838 over low *Phyllocladus aspleniifolius* (Labill.) Hook. f., *Leptospermum scoparium* J.P. Forst. & G. Forst. 1776, *L. lanigerum* (Sol ex Aiton) Sm. and *Richea scoparia* Hook. f.

At Mount Montgomery the new species was found in a sample of *Hedwigidium integrifolium* P. Beauv. on conglomerate rock in a low wood-heathland of *Eucalyptus amygdalina* Labill. over *Banksia marginata* Cav. and *Exocarpos cupressiformis* Labill. but not in fifteen other cryptogam samples from the site.

At Lady Barron Creek *E. lentiferus* sp. n. was found in a mixed sample of liverworts (*Heteroscyphus conjugatus* (Mitt.) J.J. Engel & R.M. Schuster, *H. cymbaliferus* (Hook. f. & Taylor) J.J. Engel & R.M. Schuster) and lichens (*Sphaerophorus melanocarpus* (Sw.) DC., *Cladia aggregata* (Sw.) Nyl.) collected from a decaying log at dry stream bed in alpine-subalpine woodland on dolerite background. The following tardigrade species were found in the only sample examined from the type locality – *Limmenius porcellus* Horning et al., 1978, *Echiniscus vinculus* Horning et al., 1978, *Calohypsibius ornatus* (Richters, 1900), *Diphascon langhovdense* (Sudzuki, 1964), *Macrobiotus* sp. n. 1, Gen. n. sp. n. (Macrobiotidae?), *Minibiotus* sp. n., *Minilentus* sp. n.

At Mount Montgomery the new species was found with *Pseudechiniscus* sp. n., *Oreella mollis* Murray 1910, *Diphascon higginsi* Binda 1971, *Hebesuncus* cf. *mollispinus* Pilato, McInnes & Lisi, 2012, *Macrobiotus* sp. n. 1, *Mesobiotus* sp. n., *Paramacrobiotus* sp., *Minibiotus asteris* Claxton, 1998, *Milnesium* sp.

At Lady Barron Creek *E. lentiferus* sp. n. co-occurred with *Mopsechiniscus tasmanicus* Dastych & Moscal, 1992, *Minibiotus* cf. *kappelensis* Claxton, 1998, Gen. n. sp. n. (Macrobiotidae?), *Diphascon langhovdense*

(Sudzuki, 1964), *D.* cf. *higginsi* Binda, 1971 and *Limmenius porcellus* Horning et al., 1978.

4. Discussion

4.1. Buccopharyngeal apparatus

Usually, the buccopharyngeal apparatus is not mentioned in descriptions of new *Echiniscus* species, as in many specimens the apparatus is obscured by the sculpture of the cuticle and by the fact that some parts, e.g. the stylet furcae, become dissolved. This may result in a loss of valuable information on inter- and intraspecific variation. In *Echiniscus lentiferus* sp. n. fine, long curved stylet supports were visible in some specimens and similar structures have been noted for other *Echiniscus* species, e.g. *E. viridissimus* (DEWEL & EBYE-JACOBSEN 2006) and *E. blumi* (GUIDETTI et al. 2013). The latter authors also noted (l.c., Fig. 3b) the presence of two spherical enlargements located within the buccal crown. These are also present in the new species.

4.2. Sexual dimorphism

As in other bisexual species of *Echiniscus,* males of the new species are distinguished from females by the structure of the gonopore, a simple round pore in males and a rosette in females. In reports on bisexual *Echiniscus* species the female : male ratio varies considerably, e.g. no females and five males were found in *E. crebraclava* (SUN, LI & FENG 2014), in *E. jenningsi* the ratio was 63 : 2 (DASTYCH 1987) and in *E. ehrenbergi* 48 : 72 (DASTYCH & KRISTENSEN 1995). Often it is close to 1 : 1, e.g., 395 : 296 in *E. jenningsi* (MILLER & HEATWOLE 1995) and 23 : 19 in *E. jamesi* as well as 34 : 36 in *E. rodnae* (CLAXTON 1996). The two very different ratios for *E. jenningsi* may indicate that tardigrades are more or less randomly distributed. Therefore, one can state only that males are common or uncommon in a given sample. In *E. lentiferus* sp. n. the ratio appears to be close to 1 : 1, i.e. males were common in the sample containing the type specimens.

In *Echiniscus lentiferus* sp. n. the body length appears to differ between sexes although in ten females and nine males of the type population the body length ranges overlap: females measured 171 to 211 μm and males 150 to 178 μm in length. This corresponds with other *Echiniscus* species, e.g. in Australia *E. curiosus, E. jamesi, E. rodnae, E. duboisi* Richters, 1902 and *E. vinculus* (CLAXTON 1996), in the Himalayas *E. ehrenbergi* (DASTYCH & KRISTENSEN 1995) and in China *E. clevelandi* (BEASLEY 1999).

To study possible differences between sexes, measurements of the widths of several cuticular plates were compared. In *E. lentiferus* sp. n. widths of five dorsal plates, the scapular, paired plates I and II, median plate 1 and the terminal plate are statistically significantly wider in females than in males relative to the scapular plate length. Females also have a statistically significantly longer median plate 2 than males relative to the scapular plate length. In one of the two juveniles, paired plates I and II were wide, with sp values 1.46 and 1.50 respectively, i.e. within the range of females, although sp values for the other plates were within the range of males. In the second juvenile sp values for the paired plates were both 1.31, i.e. within the range for males (as were the other dorsal plates). In order to state categorically that sp values of dorsal plates are useful in assigning juveniles to a sex (in population studies for instance) a much larger sample of juveniles would be needed.

In *E. lentiferus* sp. n. lengths of buccal appendages (relative to the scapular plate length) are not significantly different in males and females. In *E. clevelandi* the primary clavae were longer in males than in females but the secondary clavae were not significantly different in length (BEASLEY 1999). In *E. ehrenbergi* only the secondary

clavae were longer in males than in females whilst the primary clavae were not significantly different in length (Dastych & Kristensen 1995). Cirrus A was often damaged, and it was not clear whether this happened in the processing of material or naturally. A t-test determined that cirrus A is similar in length, relative to the scapular plate length, in males and females.

Differences in claw lengths between males and females have been reported for *E. curiosus, E. jamesi* and *E. rodnae* and males had longer claws than females of the same length. In *E. lentiferus* sp. n., sp values indicate that both sexes have the same claw lengths relative to the scapular plate length (Tab. 1).

Sexual dimorphism regarding the lengths of and/or the presence of body appendages has been described in two species, *E. curiosus* and *E. rodnae* (Claxton 1996). Both show a reduced set of appendages in 2-clawed juveniles and a large variation in appendage complement in other juveniles. Males of both species had statistically significantly longer body appendages than females. In *E. curiosus* females had spines at position C^d which were completely lacking in males. These means of sp values for females and males in *E. lentiferus* sp. n. were compared

Tab. 1. Summary of measurements and sp values of characters for a sample of ten females and nine males of *Echiniscus lentiferus* sp. n. (HT – holotype, AT – allotype, pl – plate, lgth – length, wth – width). Significant values in bold letters. Other abbreviations see page 107.

Tab. 1. Zusammenfassung der Messungen und sp-Werten verschiedener Merkmale von zehn Weibchen und neun Männchen von *Echiniscus lentiferus* sp. n. (HT – Holotypus, AT – Allotypus, pl – Platte, lgth – Länge, wth – Breite). Signifikanzen fett. Andere Abkürzungen s. S. 107.

	Females n=10				Males n=9						*Females n=10*				*Males n=9*					
	Min µm	Max µm	Mean µm	SD µm	Min µm	Max µm	Mean µm	SD µm	HT µm	AT µm	*Min sp*	*Max sp*	*Mean sp*	*SD sp*	*Min sp*	*Max sp*	*Mean sp*	*SD sp*	*HT sp*	AT sp
Body length	171	211	189	11	150	178	162	10	192	163	*5*	*5.93*	*5.46*	*0.32*	*5.17*	*5.76*	*5.5*	*0.16*	*5.6*	5.62
Scapular length	29.7	38.3	34.6	2.3	27.7	33	29.5	1.8	34.3	29										
Scapular width	73.9	92.4	86.3	5.6	63.4	71.3	66.2	2.6	87.1	66	***2.41***	***2.62***	***2.5***	***0.06***	***2.13***	***2.29***	***2.24***	***0.06***	*2.54*	*2.28*
internal cirrus	13.9	18.5	15.9	1.5	13.2	15.8	14.6	1.2	15.8	15.3	*0.42*	*0.52*	*0.46*	*0.03*	*0.46*	*0.53*	*0.49*	*0.03*	*0.46*	*0.53*
Secondary clava	5.7	7.5	6.6	0.5	6.3	8.6	7.3	0.8	6.6	8.3	*0.18*	*0.2*	*0.19*	*0.01*	*0.22*	*0.29*	*0.25*	*0.03*	*0.19*	*0.29*
external cirrus	15.8	20	18.1	1.5	14.5	17.8	16.7	1.1	18.5	17.2	*0.47*	*0.56*	*0.52*	*0.03*	*0.5*	*0.6*	*0.56*	*0.04*	*0.54*	*0.59*
Primary clava	5.3	6.2	5.7	0.3	5	7.9	6.5	0.8	5.7	7.9	*0.15*	*0.18*	*0.17*	*0.01*	*0.18*	*0.27*	*0.22*	*0.03*	*0.17*	*0.27*
Cirrus A	43.6	52.8	48.3	3	31.7	51.5	38.6	6	52.8	44	*1.14*	*1.56*	*1.4*	*0.14*	*1*	*1.56*	*1.31*	*0.18*	*1.54*	*1.52*
B	20	53	36	9.6	20	40	29.7	6.5	39	32	*0.56*	*1.38*	*1.02*	*0.23*	*0.68*	*1.29*	*1*	*0.21*	*1.14*	*1.09*
C	22	40	32	6	22	53	33	9.1	37	32	*0.63*	*1.12*	*0.91*	*0.15*	*0.81*	*1.6*	*1.11*	*0.25*	*1.08*	*1.09*
D	28	42	35	4.8	22	59	33.4	8.4	36	32	*0.78*	*1.22*	*1.01*	*0.13*	*0.81*	*1.86*	*1.14*	*0.31*	*1.04*	*1.09*
E	29	55	42	7.5	29	55	43.4	8.7	48	44	*0.93*	*1.56*	*1.21*	*0.18*	*1.05*	*1.83*	*1.47*	*0.26*	*1.38*	*1.5*
Bd	17	52	40	10.7	26	53	38	9.4	52	33	*0.48*	*1.5*	*1.16*	*0.29*	*0.95*	*1.91*	*1.29*	*0.32*	*1.5*	*1.14*
Cd	24	61	40	10.7	25	53	36.3	10.8	44	40	*0.72*	*1.71*	*1.16*	*0.28*	*0.79*	*1.91*	*1.24*	*0.4*	*1.27*	*1.37*
Dd	24	45	34	7.5	21	45	33.4	8.5	37	33	*0.67*	*1.26*	*0.99*	*0.19*	*0.76*	*1.57*	*1.13*	*0.28*	*1.08*	*1.14*
Paired pl I lgth	26.4	35.6	31.6	2.7	25.1	27.7	26	0.9	31.7	26.4	*0.88*	*0.94*	*0.91*	*0.02*	*0.83*	*0.91*	*0.88*	*0.03*	*0.92*	*0.91*
Paired pl I wth	44.9	58.1	52.6	3.9	34.3	40.9	37.1	2	52.8	37	***1.45***	***1.58***	***1.52***	***0.04***	***1.18***	***1.34***	***1.26***	***0.04***	*1.54*	*1.28*
Paired pl II lgth	27.7	33	31	2	23.8	27.7	25.5	1.3	31.7	26.4	*0.86*	*0.93*	*0.9*	*0.03*	*0.82*	*0.91*	*0.87*	*0.04*	*0.92*	*0.91*
Paired pl II wth	44.9	58.1	52.6	4.2	34.3	40.9	37.1	2	52.8	37	***1.45***	***1.57***	***1.52***	***0.03***	***1.18***	***1.34***	***1.26***	***0.04***	*1.54*	*1.28*
Median pl 1 lgth	14.5	19.8	16.7	1.5	11.9	14.5	12.8	1.3	15.8	11.9	*0.44*	*0.52*	*0.48*	*0.03*	*0.36*	*0.5*	*0.43*	*0.04*	*0.46*	*0.41*
Median pl 1 wth	35.6	46.2	42.1	2.9	29.7	34.3	32.3	1.2	40.9	31.7	***1.17***	***1.25***	***1.22***	***0.02***	***1.02***	***1.14***	***1.1***	***0.04***	*1.19*	*1.09*
Median pl 2 lgth	23.8	31.7	28.5	2.3	19.8	23.8	22.1	1.2	29	22.4	***0.78***	***0.86***	***0.82***	***0.02***	***0.7***	***0.77***	***0.75***	***0.03***	*0.85*	*0.77*
Median pl 2 wth	46	59.4	53.5	3.4	37	52.8	41.5	5	52.8	38.3	*1.5*	*1.58*	*1.55*	*0.02*	*1.32*	*1.6*	*1.4*	*0.09*	*1.54*	*1.32*
Median pl 3 lgth	7.9	11.9	9.6	1.2	5.9	8.6	7.6	1	9.2	7.9	*0.24*	*0.31*	*0.28*	*0.02*	*0.21*	*0.27*	*0.26*	*0.03*	*0.27*	*0.27*
Median pl 3 wth	34.3	43.6	39.7	2.5	23.8	38.3	27.4	5.3	39.6	23.8	*1.11*	*1.18*	*1.15*	*0.02*	*0.82*	*1.16*	*0.92*	*0.12*	*1.15*	*0.82*
Terminal pl lgth	39.6	55.4	48.6	4.5	37	44.9	39.9	2.5	46.2	40.9	*1.33*	*1.45*	*1.4*	*0.04*	*1.29*	*1.41*	*1.35*	*0.03*	*1.35*	*1.41*
Terminal pl wth	73.9	100.3	88.5	7.4	60.3	68.6	64.1	2.8	87.1	63.4	***2.46***	***2.62***	***2.56***	***0.06***	***2.08***	***2.28***	***2.17***	***0.06***	*2.54*	*2.19*
Lens length	16.5	21.1	17.9	1.5	14.6	17.2	16.1	0.8	17.2	15.8	*0.46*	*0.57*	*0.52*	*0.03*	*0.5*	*0.59*	*0.55*	*0.04*	*0.5*	*0.54*
Lens width	16.5	20.5	17.8	1.3	15.2	17.2	16.3	0.8	16.5	16.5	*0.46*	*0.58*	*0.51*	*0.04*	*0.48*	*0.59*	*0.55*	*0.04*	*0.48*	*0.57*
Spine leg 1	1.3	2.6	1.7	0.4	1	2	1.5	0.4	1.7	1.7	*0.04*	*0.07*	*0.05*	*0.01*	*0.04*	*0.06*	*0.05*	*0.01*	*0.05*	*0.06*
Papilla leg IV	3.2	4.6	3.8	0.6	3	4	3.5	0.5	4	4	*0.09*	*0.13*	*0.11*	*0.01*	*0.09*	*0.14*	*0.12*	*0.02*	*0.12*	*0.14*
Claw I internal	7.9	10.6	9.2	0.7	7.3	8.6	8	0.4	9.2	7.5	*0.25*	*0.28*	*0.27*	*0.01*	*0.25*	*0.29*	*0.27*	*0.01*	*0.27*	*0.26*
Claw I external	7	9.6	8.5	0.7	6.6	7.9	7.3	0.4	8.6	7.3	*0.24*	*0.26*	*0.25*	*0.01*	*0.23*	*0.25*	*0.25*	*0.01*	*0.25*	*0.25*
Claws II-III int	8.3	11.1	9.7	0.7	7.9	9.2	8.4	0.5	9.2	7.5	*0.27*	*0.29*	*0.28*	*0.01*	*0.27*	*0.3*	*0.28*	*0.01*	*0.27*	*0.27*
Claws II-III ext	7.5	9.9	8.9	0.6	7	8.6	7.6	0.5	8.6	7.3	*0.25*	*0.27*	*0.26*	*0.01*	*0.24*	*0.27*	*0.26*	*0.01*	*0.25*	*0.26*
Claw IV int	9.2	11.9	10.9	0.8	8.6	10	9.3	0.5	10.6	9.2	*0.31*	*0.32*	*0.31*	*0.01*	*0.3*	*0.32*	*0.31*	*0.01*	*0.31*	*0.32*
Claw IV ext	8.6	11.1	10.1	0.8	8.2	9.2	8.6	0.4	9.9	8.6	*0.28*	*0.31*	*0.29*	*0.01*	*0.28*	*0.3*	*0.29*	*0.01*	*0.29*	*0.3*
Teeth	6	12	10	1.7	9	12	10	1	10	10										

using a two-way t-test. Females and males were found to have very similar filament lengths, relative to the length of the scapular plate, at all filament positions.

4.3. The terminal plate lens-like thickening

The round clear lens-shaped structure in the terminal plate of this species is unique amongst tardigrades and its function is, at present, unclear. It is present in all specimens examined including the 2-clawed juvenile. It seems to be made up smooth and transparent cuticle – there are no spots or black dots within or over the top of the structure itself (Figs 8-10, 11-15). This lens has a diameter about half the length of the scapular plate in females and in males and 37-45% of the terminal plate length. Small folds on its surface and somehow flattened apex represent SEM preparation artifacts (e.g. Figs 5, 14, 15).

4.4. Variability of appendages

Intraspecific variability in number and length of body appendages has been noted previously in *Echiniscus* species, e.g. in *E. blumi* (GUIL 2008), in the bisexual *E. curiosus* and *E. rodnae* (CLAXTON 1996). In *E. lentiferus* sp. n. symmetry of filaments on individuals appears to be rather striking and was examined in some detail. Equal pairs of filaments (defined as up to 5 μm difference) occurred in females, i.e. in 33 to 100% of pairs; in males 50 to 100% of pairs were very close in length. Over 71% of pairs of filaments in females and over 60% in males were within 10 μm of each other.

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