

OBSERVATIONS OF IRISH *PAECHINISCUS* CUÉNOT, 1926 (HETEROTARDIGRADA: ECHINISCIDAE) CONVEY NEW INFORMATION (AND QUESTIONS) ABOUT THE GENUS

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ABSTRACT

Parechiniscus Cuénot, 1926 is a monotypic genus of tardigrades that includes among its characteristic features the uncommon trait of unpaired dorsal cuticular plates at the position of each of the first three leg pairs. Our morphological analyses of Irish *Parechiniscus* specimens, which included scanning electron microscopy, led to a new interpretation of configuration and details of the dorsal cuticular plate morphology in this genus. We also provide comments on *Parechiniscus* morphometry, sensory organs and sexual system. New emendations to the genus diagnosis based on our findings are proposed. Additionally, we record *Echiniscus spiniger* Richters, 1904 and *Testechiniscus spitsbergensis spitsbergensis* (Scourfield, 1897) from Ireland for the first time.

INTRODUCTION

Tardigrades (phylum Tardigrada Doyère, 1840) are microscopic animals (adults typically 100µm–1,000µm) that periodically moult chitinous cuticles (Greven *et al.* 2016). The tardigrade cuticle has taxonomically important characteristics (Czerneková and Vinopal 2021) that in many heterotardigrades include the configuration and morphology of dorsal and ventral cuticular plates. Cuénot (1926) described *Parechiniscus chitonides* based on tardigrade specimens, collected from several French locations, that exhibited unique characteristics including bar-shaped, unpaired dorsal cuticular plates that are weakly developed at the body anterior. Considering the unusual condition of the plates of the specimens as a possible orthogenetic representation between unplated marine species and the plated terrestrial taxa, the new genus *Parechiniscus* Cuénot, 1926, was erected for the specimens. This preceded the definition and division of the main classes of Tardigrada, Heterotardigrada Marcus, 1927 and Eutardigrada Richters, 1926. Additional morphological details, including observant representations of the dorsal plates of *P. chitonides*, were given by Thulin (1928), Cuénot (1932) and Marcus (1936), but these were limited by the early stage of tardigrade taxonomy in which they were produced.

A second *Parechiniscus* species, *P. unispinosus* da Cunha, 1947, was described from Portugal. However, this taxon was later regarded as synonymous with *Hypechiniscus gladiator* (Murray, 1905) (Ramazzotti and Maucci 1983; Fontoura *et al.* 2009; Gąsiorek *et al.* 2021a). Another taxon, also with bar-shaped unpaired plates, was later collected from Utah, U.S.A. and was originally attributed to *Parechiniscus* by Schuster (1975). This species was subsequently designated *Novechiniscus armadilloides* (Schuster, 1975), the type of a new genus by Kristensen (1987) in his important revision of the Echiniscidae. Thus, Kristensen (1987) regarded *Parechiniscus* as monotypic and revised the genus with emendations based upon specimens identified as '*P. chitonides*' from the Thulin collection and fresh specimens collected from Sweden. Kristensen (1987) determined that *Parechiniscus* and *Novechiniscus* Kristensen, 1987 were not closely related despite the superficial resemblance in the form of some dorsal plates. This was later supported by the detailed morphological analyses of *Novechiniscus* involving SEM by Rebecchi *et al.* (2008) and phylogenetic inferences by Jørgensen (2000) and Gąsiorek *et al.* (2018a). The uniqueness of the *P. chitonides* morphology was emphasised through these works along with its distinct phylogenetic position within Echiniscidae, also inferred via molecular analyses (e.g. Jørgensen *et al.* 2011; Guil *et al.* 2013).

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Parechiniscus was only recently reported from Ireland for the first time (DeMilio *et al.* 2022). A sample of moss containing specimens identified as *Parechiniscus* cf. *chitonides* was collected at Glensleade, County Clare, Republic of Ireland as part of the All-Ireland Tardigrade Survey (AITS). Studies of these specimens conducted in light microscopy (LM) and scanning electron microscopy (SEM) presented the opportunity to contribute new information about *Parechiniscus* morphology. As part of the present study, additional moss material of Tumanov (2005) was revisited, which was also found to contain *P. cf. chitonides* as well as two other heterotardigrade taxa, *Echiniscus spiniger* Richters, 1904 and *Testechiniscus spitsbergensis spitsbergensis* (Scourfield, 1897). Both of these species represent new records for Ireland (Republic of Ireland and Northern Ireland).

MATERIALS AND METHODS

Specimens from a population of *Parechiniscus* cf. *chitonides* were collected at Glensleade, County Clare, Republic of Ireland in moss on limestone pavement as reported by DeMilio *et al.* (2022). Mosses were collected from the substrate surface and allowed to dry in paper envelopes for storage. Following a 24-hour rehydration period in distilled water, moss material was agitated vigorously in a glass jar. The contents were passed through a set of sieves of 1.22mm and 36µm diameter. The retained material was inspected under a stereomicroscope at magnifications of at least 40× to manually remove tardigrade specimens. Retrieved specimens were either mounted on microscope slides in Heinz's polyvinyl alcohol (PVA) or Hoyer's medium or prepared for scanning electron microscopy (SEM). Specimens intended for SEM analysis were prepared according to the protocol described by Perez-Pech *et al.* (2020) or Tumanov (2020). Slide mounted specimens were examined with differential interference contrast (DIC) and phase contrast (PhC) up to the highest available magnification (100× oil objective and 2× magnification changer) on an Olympus BX51 or Leica DM2500 microscope. An AMScope T720 with PhC kit was also used to view some specimens. Micrographs were made with a digital camera and AMScope capture software (Version 3.7) or using a Nikon DS-Fi3 digital camera with NIS software. Figures were designed using Corel Draw Graphics Suite X6.

Additional material that had been collected as part of the study by Tumanov (2005) from the vicinity of Bellharbour, Co. Clare, Republic of Ireland was further studied. Taxa that were not previously reported were identified to species level.

Morphometric data were obtained as described by DeMilio *et al.* (2022). Statistical analyses were

performed with JASP (Version 0.12.2). The methodology of Bartels *et al.* (2011) was followed to check for evidence of allometric growth in morphological analyses.

RESULTS

A total of 27 *P. cf. chitonides* individuals and a single exuvium containing 2 eggs were extracted from moss collected at Glensleade. Twenty-three individuals were mounted on slides (10 females, 6 males, 5 of undeterminable gender, and 2 four-clawed juveniles) and four individuals were prepared for SEM.

The revisited material of Tumanov (2005) contained previously unreported specimens of *P. cf. chitonides* from the vicinity of Bellharbour from moss on rock (rock type unspecified). A total of 37 *P. cf. chitonides* individuals were extracted. Twenty-seven individuals were mounted on slides and ten individuals were prepared for SEM. Representative specimens of Irish *P. cf. chitonides* are presented in Figures 1–9.

DESCRIPTION OF IRISH *PARACHINISUCUS* CF. *CHITONIDES*

Body morphology and morphometry

Cuticle, translucent to light brown before and after mounting in PVA or Hoyer's media, with distinctive sculpture elements (Figs 1, 2) (described below). Body length range 122.9–202.3µm (Glensleade population). Additional measurements of selected structures are given in Table 1 and Table 2. None of the measured characters showed evidence of allometric growth (i.e. when log transformed, the slope (b) of the regression line of the measured trait values relative to body length was not significantly different from a slope of 1 as shown by t-tests ($p < 0.05$)), and therefore trait measurements were determined to be suitable for use in a morphometric ratio (*pcap*, see below). The slope (b) of the regression line of each measure trait relative to body length with log-log transformed data is given in Table 1. Although only isometric growth was indicated, the Y-intercept (a^*) of Thorpe's transformed traits are also given in Table 1 for potential comparisons with future studies as recommended by Bartels *et al.* (2011).

Cephalic structures

Black, round eyes, not composed of granules, persist after mounting in PVA. Cephalic structures observed in LM (Fig. 3) and SEM (Fig. 4). Cephalic appendages include small internal and external cirri (Fig. 3B), both without true cirrophores, with slightly swollen bases. External cirri positioned upon an elevated lobe (Fig. 4A). Secondary clava

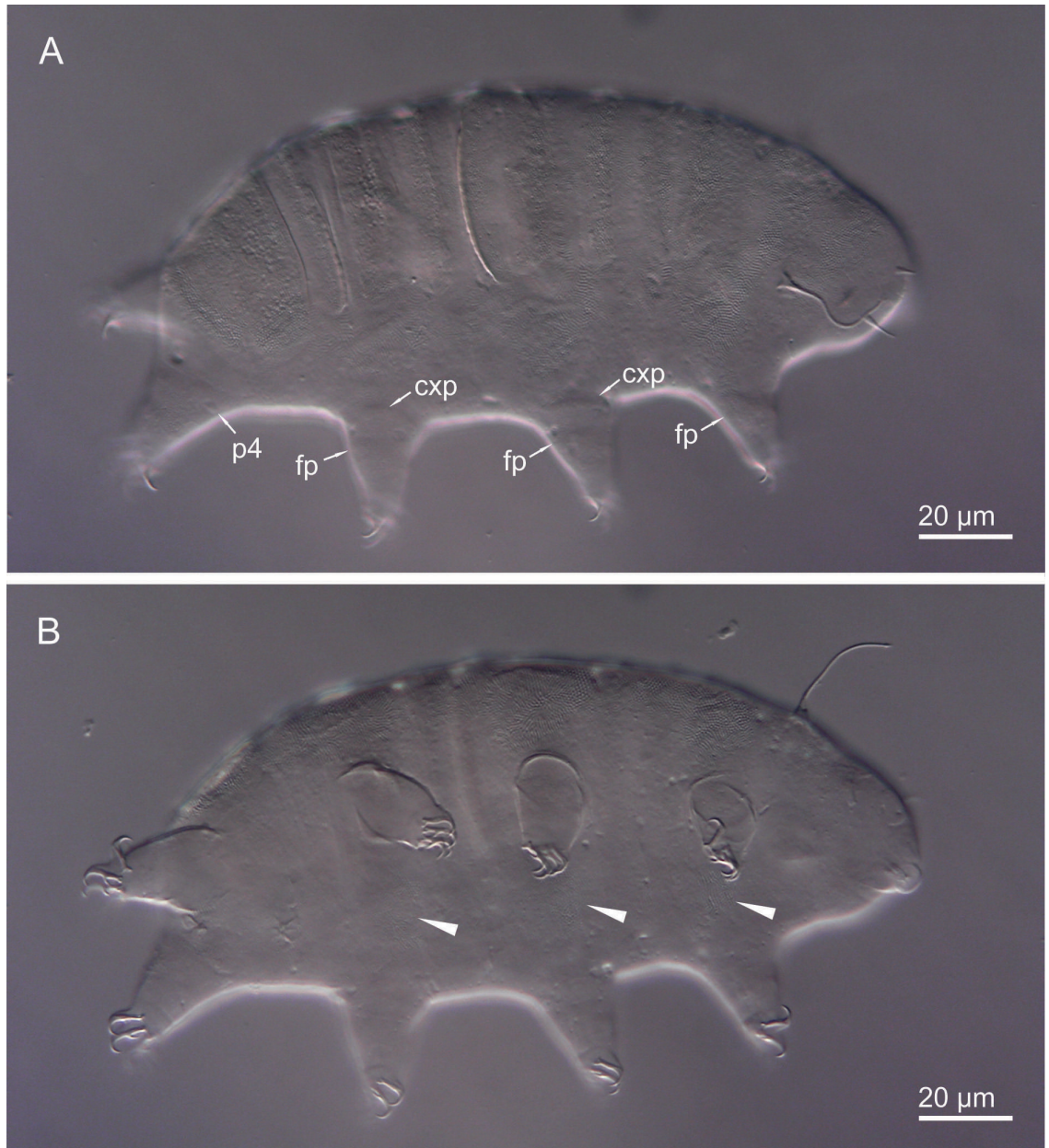


Fig. 1—Irish *Parechiniscus* cf. *chitonides* habitus (DIC). **A** female, dorsal; **B** female, ventral. **cxp**= coxal plate, **fp**= femoral plate, **p4**= leg sense organ IV, filled white arrowhead indicates evident area of large intracuticular pillars.

with narrow base and expanded, laminar paddle shaped distal part (Figs 3; 4A,C). Paired planate, rounded tertiary clavae (Figs 3; 4A,C) present rostral to secondary clavae. Primary clava in the form of an elongated papilla with a blunt apex (Fig. 3C), situated at the cirrophore base of cirrus A (Fig. 3A), a long flexible filament. Other dorsal and lateral sensory appendages absent. Ten peribuccal papulae present, eight fused in pairs and two singles (Fig. 4B). Single papulae positioned along median sagittal plane. Internal buccal-pharyngeal structures not well observed.

Dorsal cuticular plates and sculpture elements

Dorsal plates unpaired, distinctly sculptured. Dorsal plates sometimes poorly defined, especially towards the cephalic end (Figs 1, 2). Nine dorsal plates (Figs 5, 6) include: cephalic plate (cep), neck plate (np), three single transverse plates (t), three median plates (m), and caudal plate (cap). Lateral supplementary plates (lsp) also present.

Dorsal sculpture elements (Figs 3C; 4C,D; 7A,B) include: visible intracuticular pillars (some finer and diffuse) that appear as small, raised circular

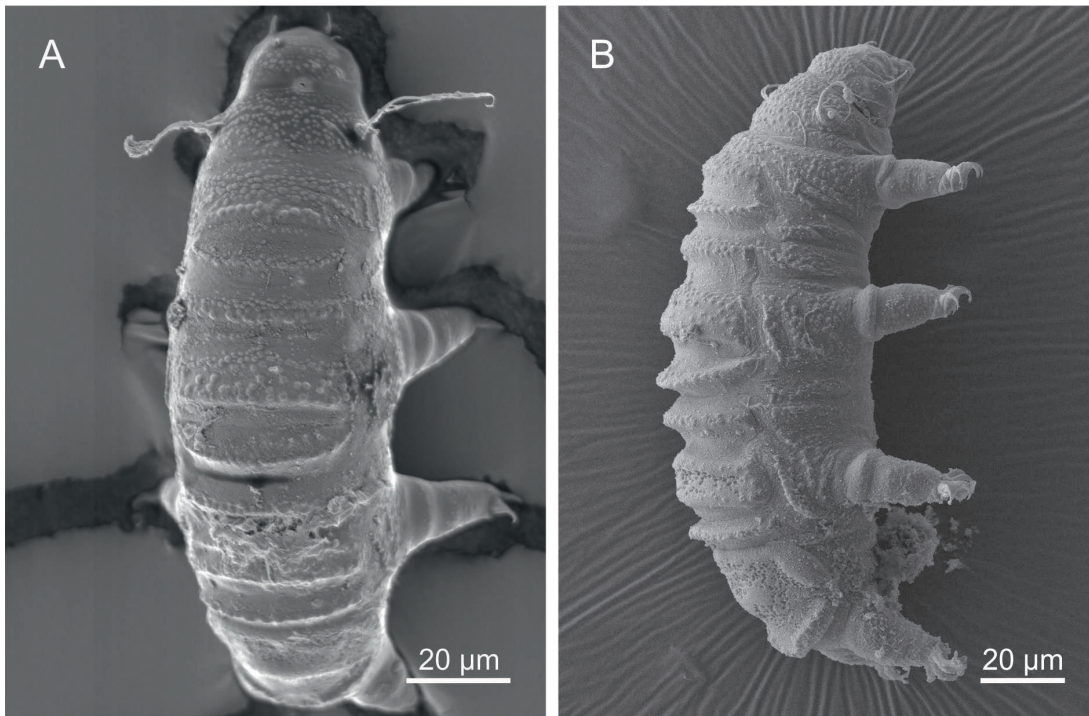


Fig. 2—Irish *Parechiniscus* cf. *chitonides* habitus (SEM). A dorsal; B lateral.

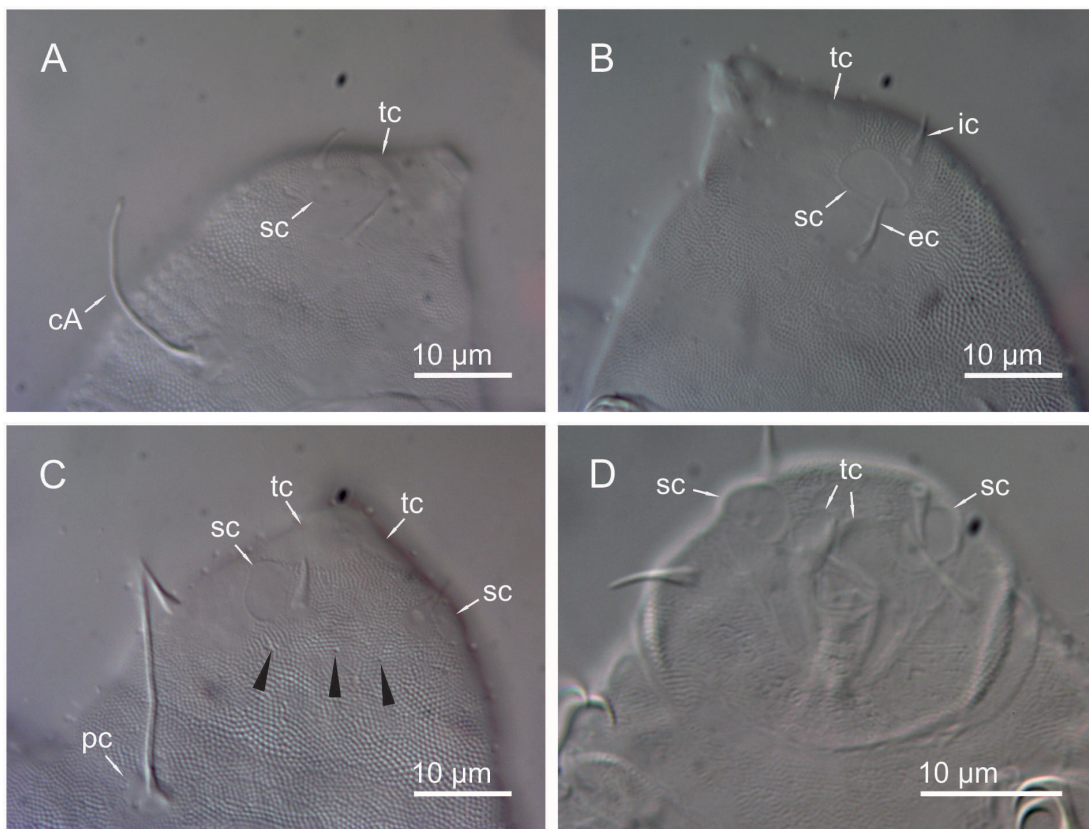


Fig. 3—*Parechiniscus* cf. *chitonides* cephalic structures from various perspectives (DIC). A lateral, B lateral; C dorsal; D ventral. cA= cirrus A, ex= external cirrus, ic= internal cirrus, pc= primary clava, sc= secondary clava, tc= tertiary clava, filled black arrowhead indicates cuticular pore.

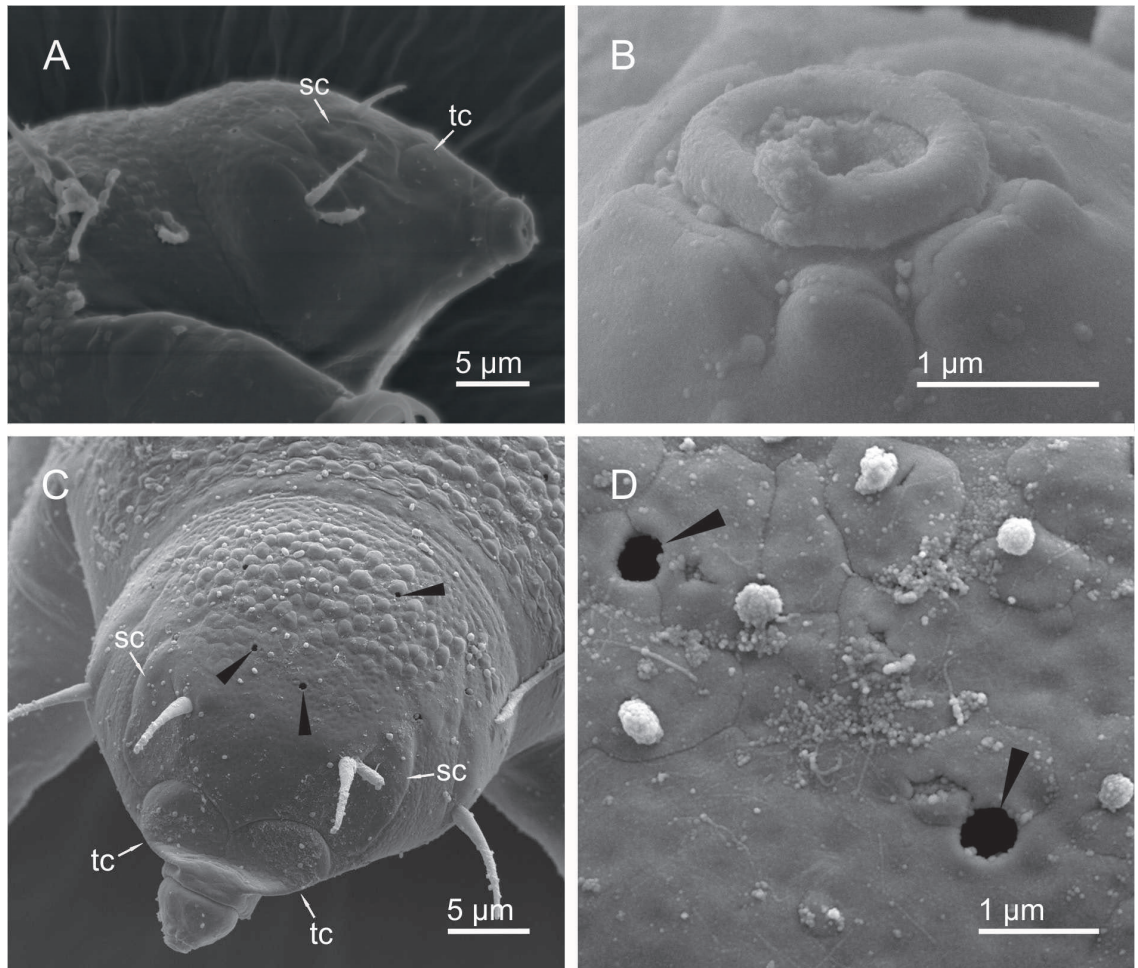


Fig. 4—*Parechiniscus* cf. *chitonides* cephalic structures (SEM). A head, lateral; B mouth ring with peribuccal papulae; C head, dorsal; D cephalic plate pores. sc= secondary clava, tc= tertiary clava, filled black arrowhead indicates cuticular pore.

points in DIC or regular, round dark dots in PhC; pores that appear as small pits between pillars in DIC or as minute, light points in PhC; depressions that appear as rounded or irregular, singular or adjoining indentations in DIC or as light areas creating a mottled pattern in PhC; and epicuticular elements (EEs) that appear as evident, rounded bumps in DIC or evident, dark bulky circular or subcircular areas in PhC.

Cephalic plate poorly defined, undivided (Fig. 1A). In LM cep appears sculptured with visible intracuticular pillars on its entirety and diffuse fine pillars surrounding all margins, scattered pores, and large, round, wart-like EEs of varying sizes (Figs 3A,C; 5A). In LM, EEs appear not as well defined on cep as on other dorsal plates. In SEM three sculpture element types well visible on cep including surface indications of pillars, large pores along cep anterior- anterolateral margin (Figs 4C,D) with additional pores at the plate interior, and wart-like EEs equally as well

developed as on other dorsal plates (Fig. 2B). Depressions absent from cep. Cep terminates caudally at a shallow crevice delimiting the cephalic region (Figs 2, 4C). Lateral cephalic plate absent but corresponding area clearly sculptured with visible intracuticular pillars and surrounded by surface devoid of visible pillars (Figs 3A,B).

Neck plate broad, without divisions, sometimes with poorly defined margins (Figs 1A; 3A,C; 5A). Cirrus A and primary clavae insertion positions encompassed by np (Figs 2A; 5; 6A,B). Np sculptured with visible intracuticular pillars, scarce pores, and wart-like EEs on its entirety (Figs 2; 3A,C; 5; 6A,B). Depressions absent. In LM, border between cep and np may be difficult to discern more evident in SEM. Np terminates caudally at a crevice in which EEs are absent or very sparse (Figs 2; 5B; 6A,B).

Transverse plate 1 (t1), unpaired, bar-shaped with lateral broadenings, positioned at level of legs I, evidently narrower than np. T1 sculptured with visible intracuticular pillars, scarce pores, and large,

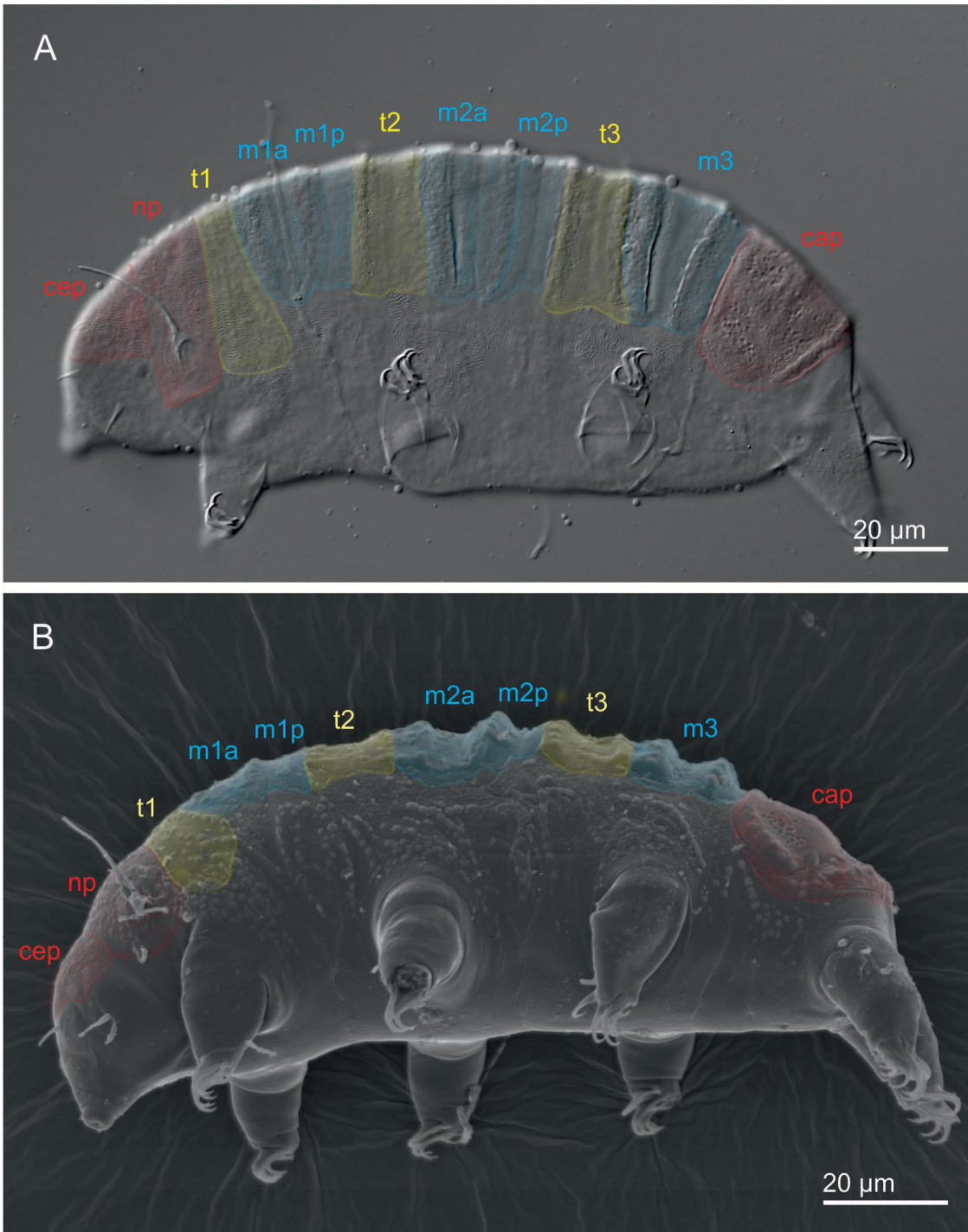


Fig. 5—Colourisation demonstrating *Parechiniscus* dorsal cuticular plate configuration. A lateral (DIC); B lateral (SEM). cep= cephalic plate, np= neck plate, t= transverse plate, m= median plate (a= anterior part, p= posterior part), cap= caudal plate.

densely distributed wart-like EEs on entire plate (Figs 1A; 2; 5; 6A,B). Depressions absent and median transverse groove absent from t1. Transverse plate 2 (t2) positioned slightly anteriorly to level of legs II, formed as an anterior and posterior latitudinal ridge separated by a broad median transverse groove that

is interrupted at its median by a longitudinal line of EEs upon a ridge (sometimes incomplete) (Figs 2; 5; 6A,C). Epicuticular elements may be present within median transverse groove. Larger, more densely distributed EEs occur on both anterior and posterior t2 ridges than within median groove. Visible

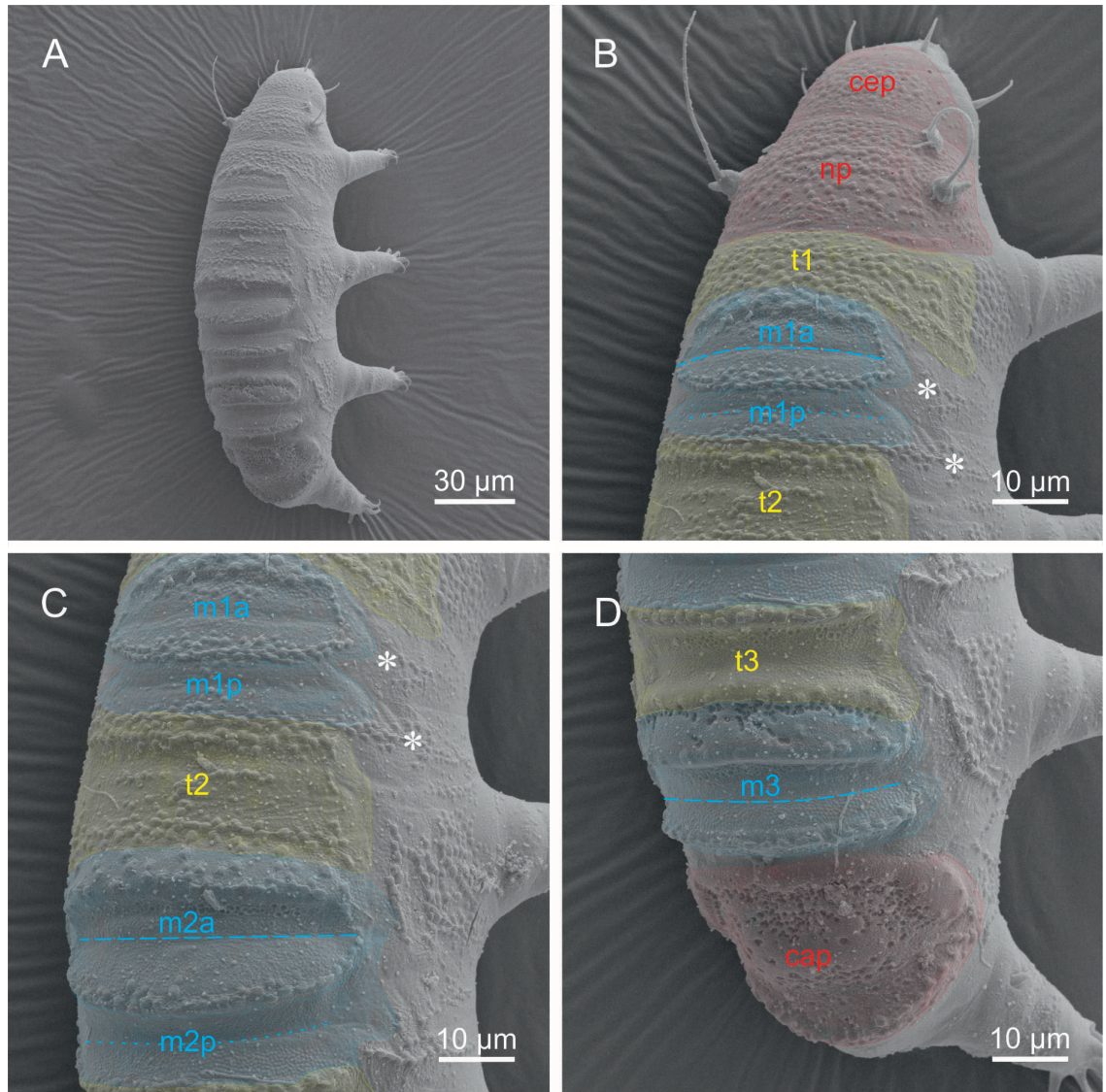


Fig. 6—Colourisation demonstrating *Parechiniscus* dorsal cuticular plate configuration (SEM). **A** uncoloured overview, whole animal; **B** anterior body region; **C** middle body region; **D** posterior body region. cep= cephalic plate, np= neck plate, t= transverse plate, m= median plate (a= anterior part, p= posterior part), cap= caudal plate, long-dashed line indicates median transverse furrow, short-dashed line indicates fold, asterisk indicate lateral supplementary plate.

intracuticular pillars present, more evident on ridges than within median groove. Pores scattered on t2 anterior and posterior ridges. Depressions not observed on t2. T3 positioned slightly anterior to level of legs III, similar in morphology to t2 (Figs 5; 6A,D) but with conspicuous depressions present at the base of its anterior ridge (Figs 2B; 6D). Posterior ridge of t3 with EEs more poorly developed than EEs on posterior ridge of t2 (Figs 2B; 6C,D).

Median plate 1 positioned between t1 and t2. M2 positioned between t2 and t3. M3 positioned between t3 and caudal plate. M1 and m2 share a similar morphology. M1 and m2 each divided into a larger anterior (a) part and smaller inconspicuous

posterior (p) part (Figs 5; 6B,C). M1a and m2a lenticular in form and each composed of heavily sculptured anterior and posterior ridges separated by a deep median transverse furrow (Figs 2; 5; 6B,C). Dense visible intracuticular pillars, cuticular pores and largest wart-like EEs occur on m1a and m2a ridges, especially developed on anterior ridges (Figs 1; 2; 5B; 6A–C). Small depressions present at m1a anterior ridge and m2a bases, anterior to the median transverse furrows (Fig. 6C). Depressions not observed on m1a or m2a posterior ridges. M1a and m2a both divided from smaller, narrower, m1p and m2p at a crest (Figs 2; 5). M1p and m2p with intracuticular pillars, mostly smooth but sparse pores

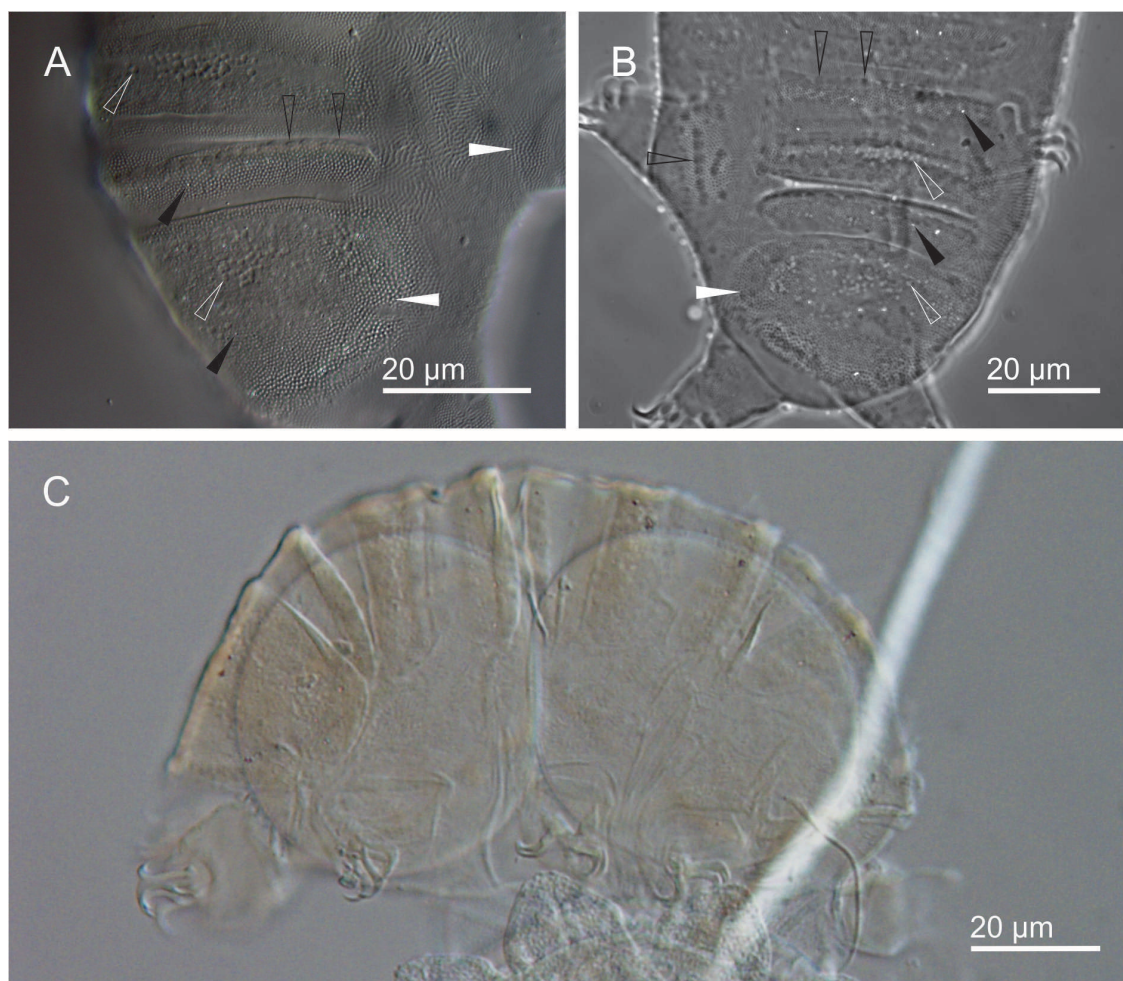


Fig. 7—*Parechiniscus* cuticle. A cuticular sculpture elements (DIC); B cuticular sculpture elements (PhC); C exuvium containing two eggs (DIC). white filled arrowhead indicates intracuticular pillar, white hollow arrowhead indicates depression, black filled arrowhead indicates cuticular pore, black hollow arrowhead indicates epicuticular element (EE).

and small EEs may be present (Figs 6B,C). Small, difficult to observe depressions present on m1p and m2p anterior regions. M1p and m2p each with a median fold (Figs 6B,C). M1p and m2p posterior margins occur at a shared crest upon t2 and t3 anterior ridges respectively (Figs 2; 5). M3 undivided (i.e. without a posterior portion), with a similar morphology to m1a and m2a (Figs 1A; 2B; 5; 6A,D), but with conspicuous and densely distributed depressions present along m3a anterior margin (Figs 1A; 2B; 5A; 6A,D; 7A,B) and sparsely within its transverse median furrow (Fig. 6D).

Caudal plate ovoid. Cap anterior and posterior margins formed as ridges heavily sculptured with large wart-like EEs, pores and visible intracuticular pillars. All three types of sculpture elements present in cap interior along with numerous large, evident, depressions producing an unlevel surface (Figs 1A; 2B; 5; 6A,D; 7A,B). At dorsal plate lateral margins fine cuticular pillars

are visible in diffuse areas extending along the lateral body sides (Fig. 7A).

Wart-like EEs, similar to those of dorsal plates present in a network of lines along lateral body sides. Lateral EE lines particularly developed and dense in arcs around areas of insertion of legs I–III to body (Figs 1A; 2B; 5; 6; 7B). Lateral supplementary plates (lsp) present, weakly demarcated and difficult to observe (LM (Figs 1A; 5A) and SEM (Figs 2; 5B), but with clusters of large wart-like EEs (Figs 6A–C). Lsps positioned at either lateral side of m1a and m1p.

Leg morphology and leg sense organs

Legs with weakly demarcated coxal and femoral plates (Fig. 1A). Coxal and femoral plates sculptured with wart-like EEs (Figs 2B, 6B,D, 8C). Pores observed on femoral plates (Figs 8A, B). Tarsus well differentiated and sculptured on ventral side with

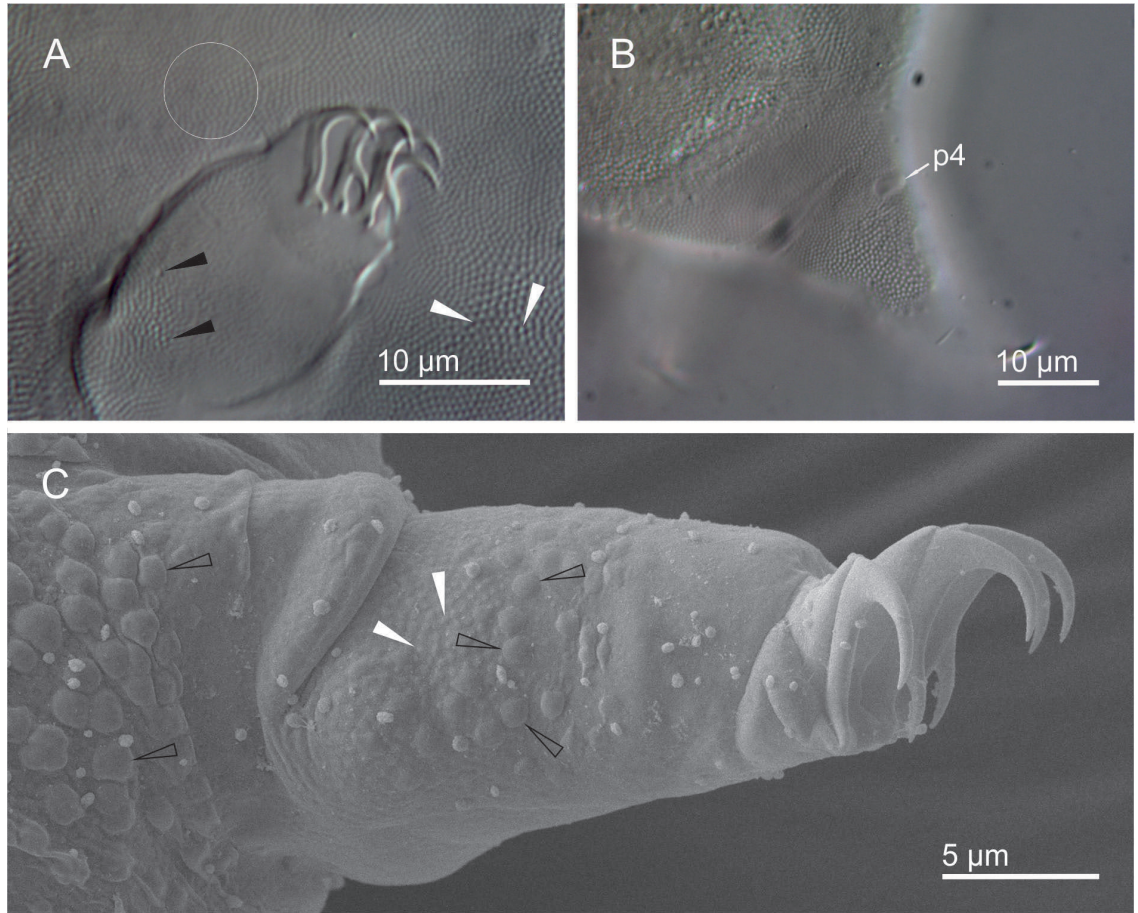


Fig. 8—*Parechiniscus* cf. *chitonides* leg structures. A leg II and claws (DIC); B leg IV (DIC); C leg I and claws (SEM). p4= leg sense organ IV, white filled arrowhead indicates intracuticular pillar, solid line circle indicates area of visible fine pillars, black filled arrowhead indicates cuticular pore, black hollow arrowhead indicates epicuticular element (EE).

intracuticular pillars (Fig. 9B). Dense intracuticular pillars visible elsewhere on leg surfaces (Fig. 8). Leg sense organs I–III absent (Fig. 8C). Large, rounded cone-shaped, sense organ evident on leg IV (p4) positioned between coxal and femoral plates on external dorsal leg surface (Figs 1A; 8B). Claws well developed (Figs 8A,C). External claws smooth. Internal claws with robust downward curving spur positioned distantly from claw base, near to but more proximally than the midpoint of the main branch.

Ventral structures

Ventral body surface almost entirely covered with dense and clearly visible intracuticular pillars in LM (Figs 3D; 8A; 9A,B). Visible pillars appear larger than surrounding fine pillars at several positions on ventral body surface (subcephalic area, between each leg pair, area surrounding gonopore and anus, and particularly evident in line with each leg pair I–III (Fig. 1B)). Large, wart-like EEs present in a band composed of several rows (hardly visible in LM,

well-visible in SEM (Figs 9C,D) located between the female and male gonopores and anus.

Female gonopore (Figs 9A,C) of six-petaled rosette shape, large, unsculptured, but surrounded on all sides by visible dense intracuticular pillars. Male gonopore visible as a pore within a round shallow, unsculptured depression in LM surrounded by dense intracuticular pillars (Fig. 9B), smaller in diameter than female gonopore (μm and *pcap*, Table 1). In SEM the central thickened structure upon the male gonopore aperture is visible (Fig. 9D). Anal system (Fig. 9 and presented in detail in DeMilio *et al.* 2022) with two rectangular lateral lobes and a fusiform terminal lobe, all lobes well differentiated and clearly observable in LM.

Reproduction and developmental stages

Sexual dimorphism: female longer body length than male (Table 1), mean female gonopore to anus distance longer than male (DeMilio *et al.* 2022).

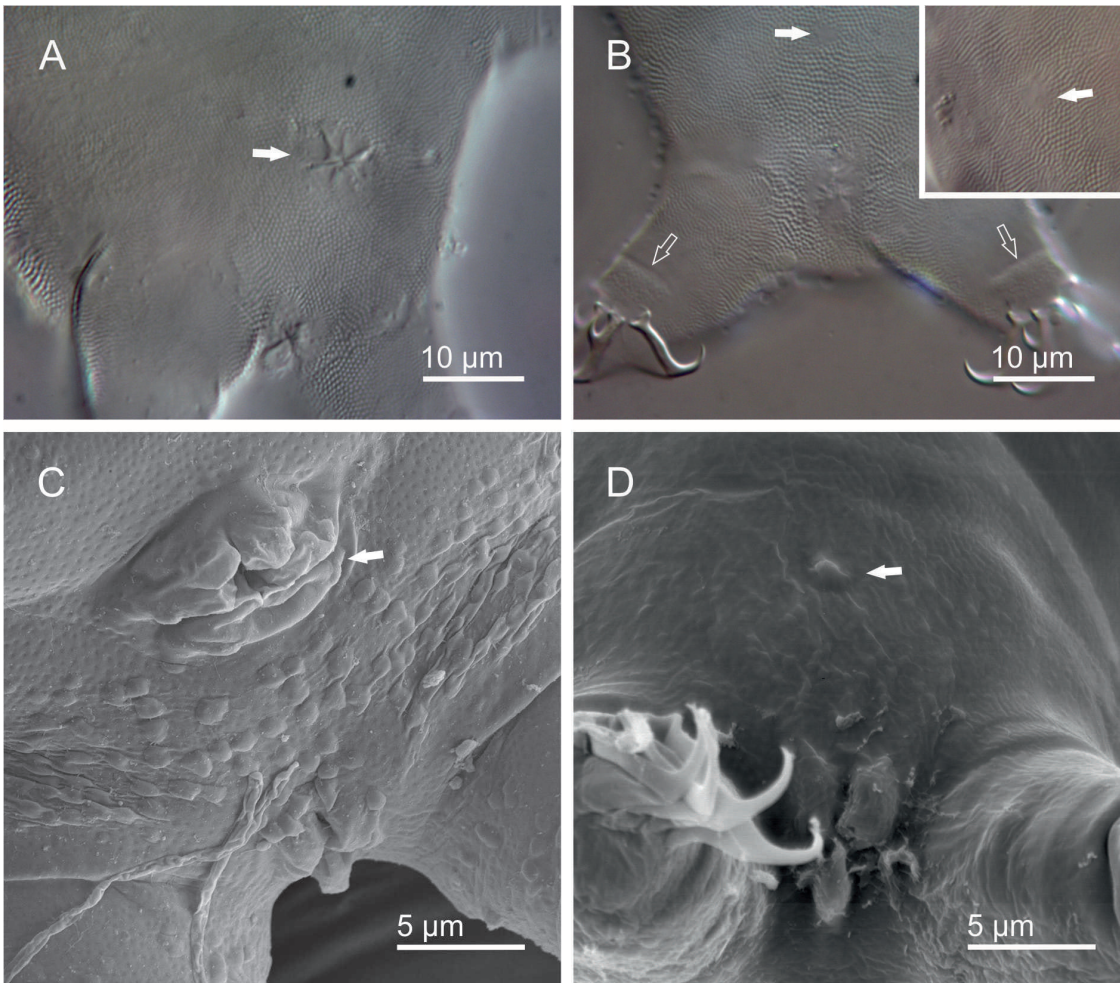


Fig. 9—*Parechiniscus* cf. *chitonides* ventral structures. A female gonopore and anus (DIC); B male gonopore and anus, insert with focus on male gonopore (DIC); C female gonopore and anus (SEM); D male gonopore and anus (SEM). filled arrow indicates gonopore, hollow arrow indicates sculptured tarsus.

Ontogeny: two smooth eggs (48.5µm and 51.2µm) observed within an exuvium (Fig. 7C). No two-clawed *Parechiniscus* larvae collected. Two four-clawed juveniles collected (measurements in Table 2) with anal system developed as in adults.

NEW IRISH SPECIES RECORDS

The moss material of Tumanov (2005), also collected from County Clare, contained previously unreported tardigrade taxa. In addition to a second population of *P. cf. chitonides* from the vicinity of Bellharbour, specimens of *Echiniscus spiniger* Richters, 1904 and *Testechiniscus spitsbergensis spitsbergensis* (Scourfield, 1897) were obtained (Fig. 10). These taxa represent new species records and the first report of the genus *Testechiniscus* Kristensen, 1987 from Ireland (Republic of Ireland and Northern Ireland).

Echiniscus spiniger

Six specimens (adult females) were extracted from a moss sample collected at the same location as the Bellharbour sample containing *P. cf. chitonides*. *E. spiniger* belongs to the 'spinulosus group' and undoubtedly requires redescription based on material from the type location (Miller *et al.* 2010; Gašiorek and Degma 2018; Gašiorek and Michalczyk 2020; see also Pilato *et al.* 2005). The Irish specimens conform to previous descriptions from other locations in several distinguishing characters. The Irish *E. spiniger* (Fig. 10A) is similar to that of Maucci (1986) in the dorsal cuticular plate sculpture element morphology (Fig. 10C) and distribution (e.g. paired plates with a band of poorly developed sculpture elements at the anterior margin) and dorsal and lateral appendage morphology (e.g. laterally and dorsally appendages in positions B–D are spinous with the dorsal spines at C^d longer than those

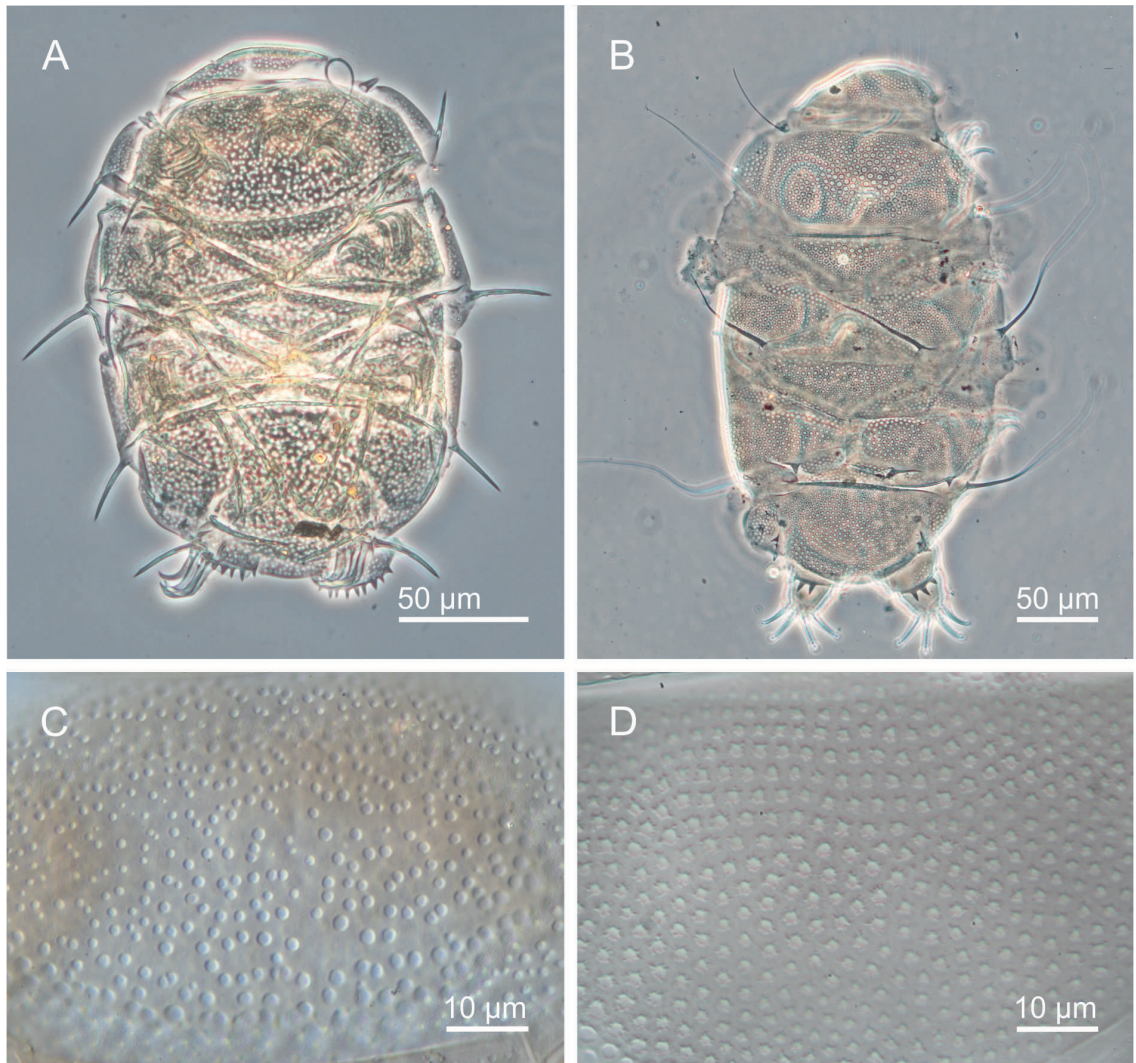


Fig. 10—Species representing new records for Ireland. **A** *Echiniscus spiniger* Richters, 1904 *habitus* (PhC); **B** *Testechiniscus spitsbergensis spitsbergensis* (Scourfield, 1897) *habitus* (PhC); **C** *E. spiniger* scapular plate cuticular sculpture elements (DIC); **D** *T. s. spitsbergensis* scapular plate cuticular sculpture elements (DIC).

at D^d ($C = 33.0\mu\text{m}$, $C^d = 58.3\mu\text{m}$, $D = 25.9\mu\text{m}$, $D^d = 43.4\mu\text{m}$, as measured on the best-preserved specimen). See also Pilato *et al.* (2008), Gąsiorek and Degma (2018) and Gąsiorek and Michalczyk (2020) for further details on the comparative morphology of the species.

Testechiniscus spitsbergensis spitsbergensis

A single specimen (adult female) was extracted from the same sample with *P. cf. chitonides*. The morphology of this specimen (Fig. 10B) conforms to the modern redescription of the nominative subspecies by Gąsiorek *et al.* (2018b), representing a morph with the genus typical cuticular sculpture (Fig. 10D) and the D^d appendages in the form of a short spine and at least two small spines at the caudal

plate posterior margin. Currently, reliable records of this subspecies indicate a distribution confined to the Western Palearctic only (but see discussions in Dastych 1973; Gąsiorek *et al.* 2018b).

DISCUSSION

IRISH *PARACHINISCUS* CF. *CHITONIDES*

Observations in LM and SEM of Irish *Parachiniscus* cf. *chitonides* specimens yielded new information about the dorsal plate configuration, cuticular sculpture elements, leg plates, sensory organs (cephalic and leg sense organs) and reproductive structures.

Table 1—Morphometric values for selected characters of adult Irish *Parechiniscus* cf. *chitonides* specimens (Glenslade population). cap= caudal plate, l= length, w= width, h= height, \varnothing = diameter; \bar{x} = mean, SD= standard deviation, b= slope, a^* =Y-intercept. *see DeMilio *et al.* (2022) for descriptive statistics by sex.

Character	Females			Males			\bar{x}			SD	b	a*	
	n	μm	<i>pcap</i>	n	μm	<i>pcap</i>	μm	<i>pcap</i>	μm				<i>pcap</i>
body length	9	139.3–202.3	479.2–650.5	6	126.1–171.5	475.3–684.7	15	160.8	561.1	18.7	59.0	-	-
cap	9	28.4–32.7	-	6	24.8–28.3	-	15	28.7	-	2.3	-	0.35	28.56
internal cirrus	8	4.2–6.0	14.5–19.6	5	4.5–5.6	17.0–21.6	13	5.1	18.0	0.5	1.9	0.62	5.34
external cirrus	9	6.9–8.6	23.6–28.5	5	6.1–9.2	23.3–37.2	14	7.6	26.6	0.8	3.4	0.63	7.71
cirrus A	9	27.1–32.6	87.2–105.5	6	25.8–31.0	97.9–124.8	15	29.0	101.4	2.0	8.2	0.24	29.51
cirrus A <i>pbl</i>	9	13.4–20.6%	-	6	17.2%–20.6%	-	6	18.2%	-	1.9	-	-	-
primary clava	7	3.2–5.0	11.0–15.4	6	3.5–4.7	12.4–18.8	13	4.1	14.3	0.6	1.9	0.46	4.13
secondary clava l	6	5.6–8.5	19.3–28.0	3	6.3–7.4	22.5–26.2	9	7.1	24.3	1.0	2.7	0.73	7.16
secondary clava w	6	4.1–5.0	13.7–16.3	3	4.5–5.2	16.0–18.9	9	4.6	15.9	4.1	13.7	0.13	4.81
external claw I h	9	6.6–7.7	21.7–24.9	6	6.3–7.2	23.5–29.1	15	6.9	24.2	0.3	1.7	0.18	6.90
internal claw I h	9	7.3–8.2	23.2–27.1	6	6.9–7.3	25.0–29.6	15	7.5	26.1	0.4	1.5	0.28	7.44
spur I l	9	1.5–2.1	5.0–7.2	6	1.4–1.8	5.5–6.5	15	1.7	5.9	0.2	0.5	0.34	1.70
spur I insertion point	9	1.8–2.4	5.9–8.3	6	1.7–2.3	7.0–8.1	15	2.1	7.4	0.2	0.7	0.19	2.09
external claw IV h	7	7.7–8.9	25.6–29.4	3	8.0–8.5	28.2–34.3	10	8.3	28.4	0.3	2.5	0.10	8.28
internal claw IV h	6	8.5–9.9	27.3–31.4	3	8.9–9.1	31.5–36.8	9	9.1	31.2	0.4	2.6	0.08	9.06
spur IV l	6	1.8–2.6	6.0–8.3	3	1.9–2.4	6.6–9.8	9	2.2	7.5	0.3	1.2	0.74	2.16
spur IV insertion point	6	2.9–3.6	9.4–11.5	3	3.1–3.4	10.9–12.4	9	3.1	10.7	0.2	1.1	0.04	3.19
sense organ leg IV	7	2.6–3.4	9.2–10.9	6	2.3–3.3	8.7–13.0	13	2.9	10.4	0.3	1.2	-0.12	2.88
gonopore \varnothing^+	7	8.8–10.6	26.9–35.5	6	2.2–3.5	7.7–12.6	-	-	-	-	-	-	-
gonopore to anus ⁺	8	15.3–22.6	51.3–79.1	6	13.7–17.1	51.9–67.7	-	-	-	-	-	-	-
gonopore to anus <i>pbl</i> ⁺	8	10.1%–13.4%	-	6	9.3–11.6%	-	-	-	-	-	-	-	-

Table 2—Mean morphometric values for selected characters of four-clawed juvenile Irish *Parechiniscus* cf. *chitonides* specimens (Glenslade population). cap=caudal plate, l=length, w=width, h=height, \bar{x} =mean, SD=standard deviation.

Character	Juveniles				
	n	\bar{x}		SD	
		μm	<i>pcap</i>	μm	<i>pcap</i>
body length	2	128.9	608.2.1	8.4	116.6
cap	2	21.5	-	2.8	-
internal cirrus	2	3.6	16.8	0.3	0.8
external cirrus	2	7.6	26.6	0.8	3.4
cirrus A	2	21.7	102.5	2.3	23.6
cirrus A <i>pbl</i>	2		16.8%		0.7
primary clava	2	2.6	12.4	0.1	2.0
secondary clava l	0	-	-	-	-
secondary clava w	0	-	-	-	-
external claw I h	2	5.2	24.5	0.3	4.3
internal claw I h	2	6.2	29.1	0.6	6.9
spur I l	2	1.1	5.3	0.1	1.2
spur I insertion point	2	1.6	7.5	0.1	1.6
external claw IV h	2	6.4	29.9	0.1	3.3
internal claw IV h	2	7.0	32.6	0.1	4.0
spur IV l	2	1.3	6.2	0.1	1.7
spur IV insertion point	2	1.9	8.8	0.3	0.3
sense organ leg IV	2	1.9	8.5	0.2	0.3

Characterisation of the dorsal cuticular plates

The conformation and the morphology of the dorsal cuticular plates of *Parechiniscus* is unique among Echiniscidae. We therefore proposed a dorsal plate terminology slightly modified from that in general use for other echiniscids. Our LM and SEM observations found that nine dorsal cuticular plates are present: cephalic plate (cep), neck plate (np), three single transverse plates (t), three median plates (m) and caudal plate (cap). Weakly developed lateral supplementary plates (lsp) are also present.

Cuénot (1926) did not specify the number of plates present in the original description of *P. chitonides*. Thulin (1928) gave a detailed account of the dorsal morphology, which he regarded as a system of plates, crests and furrows with associated musculature. He was however, limited to what could be seen in LM, and so could not discern the anterior most weakly developed plates, or the precise divisions between more posterior plates, as were observed in our analyses. In his revision, Kristensen (1987) determined the presence of 8–9 dorsal plates and illustrated their general form (Fig. 36 in Kristensen 1987) differently to Cuénot (1926) and Thulin (1928). Although the number of dorsal plates we identified on the Irish specimens overlaps with Kristensen's (1987) count, we concluded a different plate configuration to each of these earlier authors. The flexibility of the ridge-shaped dorsal plates when

subjected to coverslip pressure for slide mounting in LM and the use of SEM in the present study likely account for these differences.

Cuénot (1926), Thulin (1928) and Kristensen (1987) all commented on the poor definition of the anteriormost plates. The cep and np were not recognised by Thulin (1928) nor Kristensen (1987) due to the weak sclerotisation of these plates, although their borders are recognisable with modern LM and in SEM. The *Parechiniscus* np is much broader than in other echiniscid genera in which it typically occurs in the form of a narrow bar, sometimes indiscernible from the adjacent plates in LM (e.g. compare to np of *Hypechiniscus* Thulin, 1928 (Kristensen 1987; Gąsiorek *et al.* 2021a) *Pseudechiniscus* Thulin, 1911 (Kristensen 1987; Tumanov 2020) and *Testechiniscus* (Gąsiorek *et al.* 2018b)).

Another difference in dorsal plate morphology is that *Parechiniscus* deviates from the condition of most echiniscid genera, which typically have a scapular plate (scp) and paired plates (sometimes considered as 'segmental plates' (Kristensen 1987)) located at the level of each of the first three leg pairs. In most other echiniscid genera the scp is typically positioned dorsal to legs I and extends at its anterior margin to the posterior of the primary clava and cirrus A. The scp can often be well defined and strongly sclerotised (e.g. in the genera *Bryodelphax* Thulin, 1911 (DeMilio *et al.* 2022), *Echiniscus* Schultze, 1840

(Kristensen 1987; Gąsiorek *et al.* 2017) and *Mopsechiniscus* (Guidetti *et al.* 2014)). In other genera it is common for the scp to be poorly sclerotised and demarcated (e.g. *Hypechiniscus* (Kristensen 1987; Gąsiorek *et al.* 2021a) and *Pseudechiniscus* (Kristensen 1987; Tumanov 2020)). The scp has had importance in the use of its measurement as the basis for the *psc* value, the standard per cent ratio between the length of a structure and the scapular plate length (Dastych 1999; Fontoura and Morais 2011) widely used in echiniscid comparative morphometrics.

In *Parechiniscus*, our observations led to a new interpretation of the dorsal plates that correspond to the scp and paired plates in most other echiniscid genera. These plates are positioned slightly anterior to each the first three leg pairs, are all singular and have a general shape of a transversally oriented bar, therefore we refer to them as transverse plates (t1–t3). The interplate borders between the transverse plates and the adjacent plates are sometimes poorly discernible in LM. We first attempted to utilise t1 in a morphometric ratio in a similar way to the scp of other genera but found that in LM the margins of t1 were often not sufficiently defined as to be able to obtain a reliable measurement. Therefore, we used the strongly sclerotised caudal plate (cap) measurement (μm) to calculate a morphometric index, *pcap*, which we define as the per cent ratio between the length of a structure and the caudal plate length. Although only a small sample size was available, we did not find evidence of allometric growth in *Parechiniscus* caudal plate compared to body length calculations. We suggest the use of the *pcap* ratio in place of the *psc* ratio for *Parechiniscus* to reduce body size effect in morphometric comparisons of structures showing isometric growth (Bartels *et al.* 2011). If future morphometric analyses involving larger sample sizes find evidence of allometry in *Parechiniscus* structures, Thorpe's normalisation techniques may be used as discussed in the context of tardigrades by Bartels *et al.* (2011) to eliminate body size effects in comparisons between populations.

Thulin (1928) distinguished, what he termed annular plates ('annularplatten') at the approximate positions of t2 and t3. Kristensen (1987) figured segmental plates II and III at similar locations. The precise interplate borders and individual plate morphologies as interpreted by these earlier authors are not entirely clear from their illustrations. In our attempt to characterise the *Parechiniscus* dorsal plates, we found that SEM was critical to understanding the transverse plate morphology, as the actual forms of these plates may be considered counterintuitive to how they can appear in LM. Only in SEM can t2 and t3 be clearly seen to have structure composed of a double ridge separated by a median transverse groove, homologous in appearance to the paired plates of other echiniscid genera.

Similarly, SEM enabled us to observe the form of all three median plates in greater detail than was possible for previous authors. Only Kristensen (1987) recognised the general divided condition of median plates 1 and 2 in LM, but SEM enabled us to more precisely distinguish and characterise the crests and furrows associated with the median plates in *Parechiniscus*. We only found a single previously published SEM image of an Italian *Parechiniscus* (Fig. 111 in Maucci 1986), but we were not able to make detailed comparisons with this image due to its low resolution. Similarly, we lacked material from the type population for comparative studies to determine the range of diversity of dorsal cuticular sculpture elements in *Parechiniscus*. However, based upon our observations and our inferences from previous reports (e.g. Cuénot 1926; Marcus 1936; Arcidiacono 1964; Kristensen 1987) large, wart-like EEs may be a characteristic component of the *Parechiniscus* dorsal cuticular sculpture.

Sense organs

The presence of tertiary clavae has not been previously reported for *Parechiniscus*. The occurrence of tertiary clavae within each of the two heterotardigrade orders, Echiniscoidea Richters, 1926 and Arthrotardigrada, Marcus 1927, is uncommon. In Echiniscoidea tertiary clavae have been previously reported only in the echiniscid genus *Cornechiniscus* Maucci and Ramazzotti, 1981 (Kristensen 1987), and according to Gąsiorek *et al.* (2019b) to occur 'occasionally' in *Nebularmis* Gąsiorek and Michalczyk, 2019 in Gąsiorek *et al.* 2019a. The tertiary clavae reported in these other genera differ in form and/or position to those that we observed on *Parechiniscus*. Kristensen (1987) showed small dome-shaped tertiary clavae located near the base of the internal cirri in specimens identified as *Cornechiniscus* cf. *holmeni* (Petersen, 1951). The tertiary clavae shown on *N. reticulatus* (Murray, 1905) by Gąsiorek *et al.* (2019b) are papillate and positioned at the level of, but caudal to, the internal cirri. In comparison, on our *Parechiniscus* specimens we observed considerably large planate, rounded tertiary clavae that were positioned more rostrally in relation to the internal cirri and other cephalic sense organs than in the other genera.

While it does not meet modern taxonomic standards, in our opinion, the original description of *Pseudechiniscus megacephalus* Mihelčič, 1951 (*nomen dubium* according to Dastych 2015 and Gąsiorek *et al.* 2021b), might suggest the presence of tertiary clavae (see Dastych 1993 on the status of the Mihelčič collection). Although the identification of the described cephalic structures as potential tertiary clavae was not stated by Grobys *et al.* (2019), we agree with those authors that the presence of such sensory structures should place the taxon in another genus. The thorough re-examination of

Novechiniscus by Rebecchi *et al.* (2008) involving SEM imaging provided new information on the cephalic sense organs of that taxon. In addition to the dome-like structure upon which Kristensen (1987) reported the internal cirrus to sit, Rebecchi *et al.* (2008) suggested the presence of an additional dome at the base of the external cirrus. We interpret the published *Novechiniscus* micrographs (Fig. 3A (LM) and Fig. 4A (SEM) in Rebecchi *et al.* 2008) to suggest that the dome structure reported as constituting the base of the internal cirrus may actually be a potential tertiary clava positioned adjacent to and arching around the internal cirrus.

In Arthrotardigrada, tertiary clavae were reported in the original descriptions of *Renaudartus* Kristensen and Higgins, 1984, *Paradoxipus* Kristensen and Higgins, 1989, *Neoarctus* de Zio Grimaldi, D'Addabbo Gallo and Morone De Lucia, 1992, and in *Angursa* Pollock, 1979 by later authors (Noda 1985; Bussau 1992; Fujimoto and Hansen 2019). Tertiary clavae fused to secondary clavae have also been suggested to occur in *Coronarctus* Renaud-Mornant, 1974 (Hansen *et al.* 2012) and in *Nodarctus* Fujimoto and Yamasaki, 2017. The tertiary clavae are lamellar in form in all arthrotardigrade genera in which they are known to occur, except for *Neoarctus* in which they are papillate. The extent of variation in the morphology of tertiary clavae and their position in relation to other cephalic structures in both Arthrotardigrada and Echiniscoidea may not be fully known, as suggested by the new recognition of these structures in *Parechiniscus*.

The original description of *Parechiniscus chitonides* stated the presence of a sense organ at the base of leg I in the form of a very small, difficult to observe spine (Cuénot 1926). In the original drawing it appeared coxally positioned but in a later illustration, the leg I sense organ is shown in the femoral region (Cuénot 1932). Kristensen (1987) also reported a small, pointed sense organ on leg I of his Swedish specimens but in his illustration (Fig 36. in Kristensen 1987) suggests a femoral position different to that indicated by Cuénot (1926). We observed some Irish *Parechiniscus* specimens with a well-developed EE in the femoral region of leg I that could potentially be mistaken for a sense organ in LM. However, our SEM analyses confirmed that the Irish *Parechiniscus* lack a leg I sense organ. The absence of this structure, specifically stated to be present in the original description of the taxon, required us to use open nomenclature in the identification of the Irish specimens. *P. chitonides* apparently lacking a leg I sense organ were also reported from Sicily (Arcidiacono 1964). In other echiniscid genera, the presence/absence and morphology of leg sense organs is commonly utilised as a species level character (e.g. *Barbaria* Michalczyk, Gąsiorek, Morek and Stec, 2019 in Gąsiorek *et al.* 2019a

(Gąsiorek *et al.* 2022); *Bryodelphax* (DeMilio *et al.* 2022); *Claxtonia* Gąsiorek and Michalczyk, 2019 in Gąsiorek *et al.* 2019a (Degma *et al.* 2021); *Pseudochiniscus* (Tumanov 2020) and *Testechiniscus* (Gąsiorek *et al.* 2018b)). The reported variability of the leg I sense organ and any value of this character in discriminating potential species of *Parechiniscus* warrants further investigation.

Sexual system

Both females and males were extracted from the Irish *P. chitonides* population. Our sample had a sex ratio approximately 2:1, suggesting that males were not uncommon among the Irish *Parechiniscus*. This is in contrast to the samples of Kristensen (1987) who reported that he collected several hundred *P. chitonides* specimens from moss on rocks in Sweden but stated that males were not found. This stark difference in the sex composition of these populations could be the result of many factors including the unknown influence of environmental variables, chance of sampling, or a representation of an actual difference in sexual system of two distinct taxa yet to be delineated. The uncertainty of the cause of discrepancy in the occurrence of sexes between two minimally sampled populations should prohibit premature conclusions about the sexual systems or reproductive mode(s) of tardigrade species.

NEW RECORDS FOR IRELAND

The two echiniscid species found during the present reinvestigation of Tumanov's (2005) material, *E. spiniger* and *T. spitsbergensis spitsbergensis* increase the number of records of Irish heterotardigrades to 22 species in 9 genera (DeMilio *et al.* 2016; DeMilio and Lawton 2020; DeMilio *et al.* 2022). However, the current numbers of recorded tardigrade taxa should not be considered as the final indicators of the extent of the phylum's biodiversity in Ireland. Historically, faunistic surveys involving tardigrades have been rare and highly localised (DeMilio *et al.* 2016), but the number of Irish taxa will certainly increase as further results from other AITS samples are published.

CONCLUSION

Parechiniscus chitonides was described at a relatively early stage of tardigrade taxonomy at a time when modern imaging techniques and molecular analyses did not exist. Cuénot (1926) did include in his original description a few uncommonly specific details on the morphology and distribution of some sculpture elements on the dorsal and lateral body

sides and a clearly stated presence and position of a sense organ on leg I. Despite this and the later attempts to better characterise the species, many traits that are now recognised as important for separating heterotardigrade species and genera have remained unreported for *Parechiniscus* (e.g. morphology of the leg plates, ventral cuticle sculpture pattern, and anal system). The missing information about these and other taxonomically important characters from other populations and a lack of modern re-description of type specimens make it difficult to determine if records of '*Parechiniscus chitonides*' from a broad swath of Europe and North Africa (McInnes 1994; McInnes *et al.* 2017 and references within) all truly represent a single taxon, or if unrecognised hidden species diversity is present.

A new redescription involving the type material (if it still exists) from one of the original French locations or a designated neotype will provide a baseline for the comparison of specimens from other populations including the Irish population described herein. The integrative re-description of *Pseudechiniscus suillus* (Ehrenberg, 1853) by Grobys *et al.* (2020) together with integrative analyses by Cesari *et al.* (2020) and the comprehensive review of morphological characters by Tumanov (2020) catalysed the delimitation of several new species morphologically similar to *Pseudechiniscus suillus* that were previously impossible to separate (e.g. Roszkowska *et al.* 2020; Kayastha *et al.* 2020). Such research focus on *Parechiniscus chitonides* might produce a similar outcome. For now, our observations on the Irish *Parechiniscus* specimens provide new data on several characters. We therefore suggest that this new information justifies that the genus diagnosis is further emended from Cuénot (1926) and Kristensen (1987) as follows:

PARECHINISCUS CUÉNOT, 1926 EMENDED
DIAGNOSIS

Small, black eyes. Cephalic structures include three pairs of clavae. Tertiary clavae planar in form. Rigid buccal tube. Nine unpaired dorsal plates present (cephalic plate, neck plate, three single transverse plates, three median plates and caudal plate).

Composition: *Parechiniscus chitonides* Cuénot, 1926

Furthermore, the heterotardigrades *Echiniscus spiniger* and *Testechiniscus spitsbergensis spitsbergensis* are added to the checklist of species known from Ireland.

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