

# Analysis of non-morphometric morphological characters used in the taxonomy of the genus *Pseudechiniscus* (Tardigrada: Echiniscidae)

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*Pseudechiniscus*, the second-largest genus of the family Echiniscidae (Tardigrada: Heterotardigrada: Echiniscoidea), is notoriously difficult for taxonomic studies. In this study, I performed a morphological analysis of a new species from Croatia, based on a light microscopic and scanning electron microscopic examination of 45 specimens from the same sample. Furthermore, I have summarized all available data on *Pseudechiniscus* species, including their original descriptions, and have analysed the following complexes of morphological characters: (1) arrangement and morphology of dorsal cuticular plates, (2) ventral sculpture, (3) morphology of cephalic, trunk and leg sensory organs and (4) claw morphology. The applicability of these characters in the taxonomy and their distribution in the genus are discussed. Some of the characters traditionally used for species delimitation were shown to be unsuitable and others in need of a thorough reinvestigation. The meaning of the old term ‘faceted’, commonly used but often misapplied, has been clarified, based on the initial definition. Several characters of the claw structure were suggested as potentially useful for species delimitation. The taxonomic status of several old forms and species was discussed.

**KEYWORDS:** Echiniscoidea – Heterotardigrada – morphology – scanning electron microscopy – taxonomy.

## INTRODUCTION

*Pseudechiniscus* is the second-largest genus in the family Echiniscidae (Tardigrada: Heterotardigrada: Echiniscoidea). It was separated from the genus *Echiniscus* by Thulin (1911) for having a different configuration of the dorsal cuticular plates. Compared to *Echiniscus* and related taxa, members of the genus *Pseudechiniscus* have an additional pseudosegmental plate positioned between the third median plate and the caudal (terminal) plate. Several groups of species were later excluded from the genus *Pseudechiniscus* and assigned to new genera, mostly on the basis of morphological characters. Du Bois-Reymond Marcus (1944) erected the genus *Mopsechiniscus* characterized by a reduced set of cephalic sensory organs (both internal and external cirri absent). Maucci & Ramazzotti (1981) separated the genus *Cornechiniscus*, the members of which have horn-shaped cirri A, and Kristensen (1987) isolated the genera *Antechiniscus*, which has a

different configuration of dorsal cuticular plates, and *Proechiniscus*, which has a different configuration of the dorsal cuticular plates and a different structure of the buccopharyngeal apparatus. Subsequently, Schulte & Miller (2011) erected the genus *Multipseudechiniscus* on the basis of a different configuration of the dorsal cuticular plates and a different structure of the buccopharyngeal apparatus (see also: Miller *et al.*, 2012). Most recently, the genus *Acantechiniscus* has been erected based on a combined morphological and molecular analysis (Vecchi *et al.*, 2016).

The genus *Pseudechiniscus* currently comprises 36 species from the former *Pseudechiniscus suillus/conifer* group of species. Taxonomic studies of this genus are complicated by the presence of numerous poorly or inadequately described species. Eighteen species (half of the total number) were described before 1980, mostly without the use of high-resolution optics and scanning electron microscopy (SEM). Some descriptions were based on juvenile specimens, e.g. that of *P. jiroveci* (Bartoš, 1963). Thus, redescrptions using modern techniques are much desired. However, this is often

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difficult because of the absence of the type material, and as a result, most of these 18 species have not yet been redescribed.

Another problem is that *Pseudechiniscus* species are often present in samples in low numbers, which impedes a parallel investigation of the morphology of a given population with different methods and securing material for genetic analyses. Given the low number of available type or neotype DNA sequences, qualitative morphological characters are still the basis of the species-level taxonomy within the genus. At the same time, traditional and geometric morphometrics have recently been successfully used to discriminate cryptic (or pseudocryptic) species in *Pseudechiniscus* (Fontoura & Morais, 2011).

The terminology used in the descriptions of the echiniscid cuticular plates is rather outdated, and some terms have lost connection with their initial meanings. Moreover, many of the *Pseudechiniscus* species bear weakly sclerotized cuticular plates, and their borders and configuration are poorly distinguishable in light microscopy.

During examination of a moss sample from Croatia, I found a new species of tardigrade representing the genus *Pseudechiniscus*, possibly closely related to *Pseudechiniscus ramazzottii* Maucci, 1952 [in this paper I refer to it as *Pseudechiniscus* sp. (Croatia)]. More than 40 specimens of the new species were present in the sample, making it possible to conduct a parallel study of its morphology with Contrast Light Microscopy (CLM) and SEM. The following complexes of characters were analysed: (1) arrangement and sculpturing of the dorsal cuticular plates, (2) ventral cuticular sculpture, (3) morphology of cephalic, body and leg sensory organs and (4) claw morphology. In addition to the morphological analysis of the new species, in this study also I aim to summarize the data available on the genus *Pseudechiniscus*, to evaluate the applicability of morphological characters currently used in its taxonomy, to analyse character distribution within the genus, to identify problems arising from an uncritical use of ambiguous morphological terms and to propose new characters potentially useful for taxonomic studies.

## MATERIAL AND METHODS

Forty-five specimens of *Pseudechiniscus* sp. (Croatia) were found in a single moss sample collected by Oksana Orlova in Park Šuma Golubinjak (Golubinjak Forest Park), Primorje-Gorski Kotar County, Croatia (45.35216°N; 14.76557°E; c. 750 m a.s.l.) on 10 September 2005.

Microscope slides from the collection of Walter Maucci (Museum of Natural History of Verona,

Italy) were used for comparison. Type specimens of *Pseudechiniscus bartkei bartkei* Węglarska, 1962, *P. facettalis* Petersen, 1951, *P. insolitus* Maucci, 1991, *P. ramazzottii* Maucci, 1952 and specimens attributed by Maucci to *P. facettalis*, *P. pseudoconifer* Ramazzotti, 1943, *P. novaezeelandiae* (Richters, 1908) and *P. suillus* (Ehrenberg, 1853) were examined.

I also investigated the type material of *P. jubatus* Biserov, 1990 and *P. nataliae* Biserov & Maucci in Biserov, 1986 (collection of V. Biserov, Museum of Natural History of Verona, Italy); specimens of *P. santomensis* Fontoura *et al.*, 2010 (collection of R. Bertolani, University of Modena and Reggio Emilia, Italy) and specimens attributed to *P. jiroveci* Bartoš, 1963 by Kaczmarek & Michalczyk (2006) (Mongolian population, collection of Ł. Kaczmarek, Adam Mickiewicz University in Poznań, Poland) and by G. Pilato (South African population, Binda and Pilato's collection, Museum of Department of Biological, Geological and Environmental Sciences of the University of Catania, Italy and collection of R. Bertolani, University of Modena and Reggio Emilia, Italy); specimens attributed by G. Iharos to *P. ramazzottii* f. *facettalis* Iharos, 1964 from Chile (Soil Zoological Collections, Hungarian Natural History Museum) and specimens of several undescribed *Pseudechiniscus* species from my own collection of European (Russia, Valaam Archipelago) and South American (Argentina) populations. A microphotograph of *P. spinirectus* Pilato *et al.*, 2001 was kindly provided by Giovanni Pilato (University of Catania, Italy).

Original descriptions of all *Pseudechiniscus* species and forms were analysed in order to define the set of the morphological characters used in the taxonomy of this genus (Ehrenberg, 1853; Richters, 1904a, b, 1908; Murray, 1907, 1910; Bartoš, 1934, 1963; Iharos, 1936, 1969; Mihelčič, 1938, 1951, 1955; de Barros, 1939; Ramazzotti, 1943; Petersen, 1951; Franceschi, 1952; Maucci, 1952, 1991; Węglarska, 1962; Dastyh, 1980, 1987; Biserov, 1986, 1990; Kendall-Fite & Nelson, 1996; Abe *et al.*, 1998; Pilato *et al.*, 2001; Yang, 2002; Li *et al.*, 2005, 2007; Pilato & Lisi, 2006; Li, 2007; Wang, 2009; Fontoura *et al.*, 2010; Vecchi *et al.*, 2016; Xue *et al.*, 2017; Wang *et al.*, 2018). Additional information from recent partial redescriptions was used for some 'old' species [Pilato *et al.* (2005) for *P. novaezeelandiae* and Pilato & Lisi (2006) for *P. juanita* de Barros, 1939], but only if the new findings originated from the locality close to the *locus typicus* and if the morphology of specimens from the new material corresponded to the original description. Notes on *P. jiroveci* (Binda, 1984), *P. cf. quadrilobatus* Iharos, 1969 (Pilato *et al.*, 2004) and *P. cf. papillosus* Li *et al.*, 2005 (Beasley & Miller, 2012) were excluded from the analysis, because the new material could not be reliably attributed to the original species.

Specimens of *Pseudechiniscus* sp. (Croatia) were extracted from rehydrated samples using a standard technique of washing them through two sieves (Tumanov, 2018). The content of the fine sieve and the material that passed through both sieves were examined under LOMO MBC-10 Stereo Microscope. The tardigrades found were fixed with acetic acid and mounted on slides in Hoyer's medium.

Permanent slides were examined under a Leica DM2500 microscope equipped with phase contrast (PCM) and differential interference contrast (DIC). Photographs were made using Nikon DS-Fi3 digital camera.

For SEM, specimens were prefixed with acetic acid, fixed with formaldehyde, dehydrated in an ascending ethyl alcohol series (10%, 20%, 30%, 50%, 70%, 96%) and acetone, critical-point dried in CO<sub>2</sub>, mounted on stubs and sputter-coated with gold. Tescan MIRA3 LMU SEM was used for observations (Centre for Molecular and Cell Technologies, St. Petersburg State University).

## RESULTS

### ARRANGEMENT AND SCULPTURING OF DORSAL CUTICULAR PLATES

The degree of the development of the dorsal plates varied considerably within the genus from thin poorly demarcated plates to well-sclerified plates with raised crest-like margins.

#### *Cephalic plate (cp)*

The first dorsal plate covering the anteriormost part of the body is called the cephalic plate (Fig. 2A: cp). Its pattern, usually referred to as W-shaped, is often mentioned in the descriptions of *Pseudechiniscus* species. The presence or absence of this pattern was used to discriminate some species by Maucci (1986) in his key to the genus *Pseudechiniscus*. In fact, a W-shaped pattern is characteristic of all studied specimens of *Pseudechiniscus*. It can usually be clearly seen in PCM as a transverse line with a reduced cuticular sculpture separating two anterior areas with larger dots (Fig. 1A–E: arrow a). In SEM this structure is usually visible as an elevated crest-like folding (Fig. 1F: arrow a), but the degree of its elevation could vary considerably within the same species. An additional poorly developed, straight, transverse crest separating the anteriormost part of the cephalic plate is found on SEM images (Fig. 1F: arrow d). It was also discernible in CLM, but only in well-compressed specimens (Fig. 1B: arrow d).

Two parallel lines often go backwards from the back corners of the W-shaped sculpture (Fig. 1A–E: arrow b). The degree of their development varies within the

population. In SEM photographs they are often visible as grooves, in contrast to the lines of the W-shaped sculpture, usually visible as crests (Fig. 1G: arrow b).

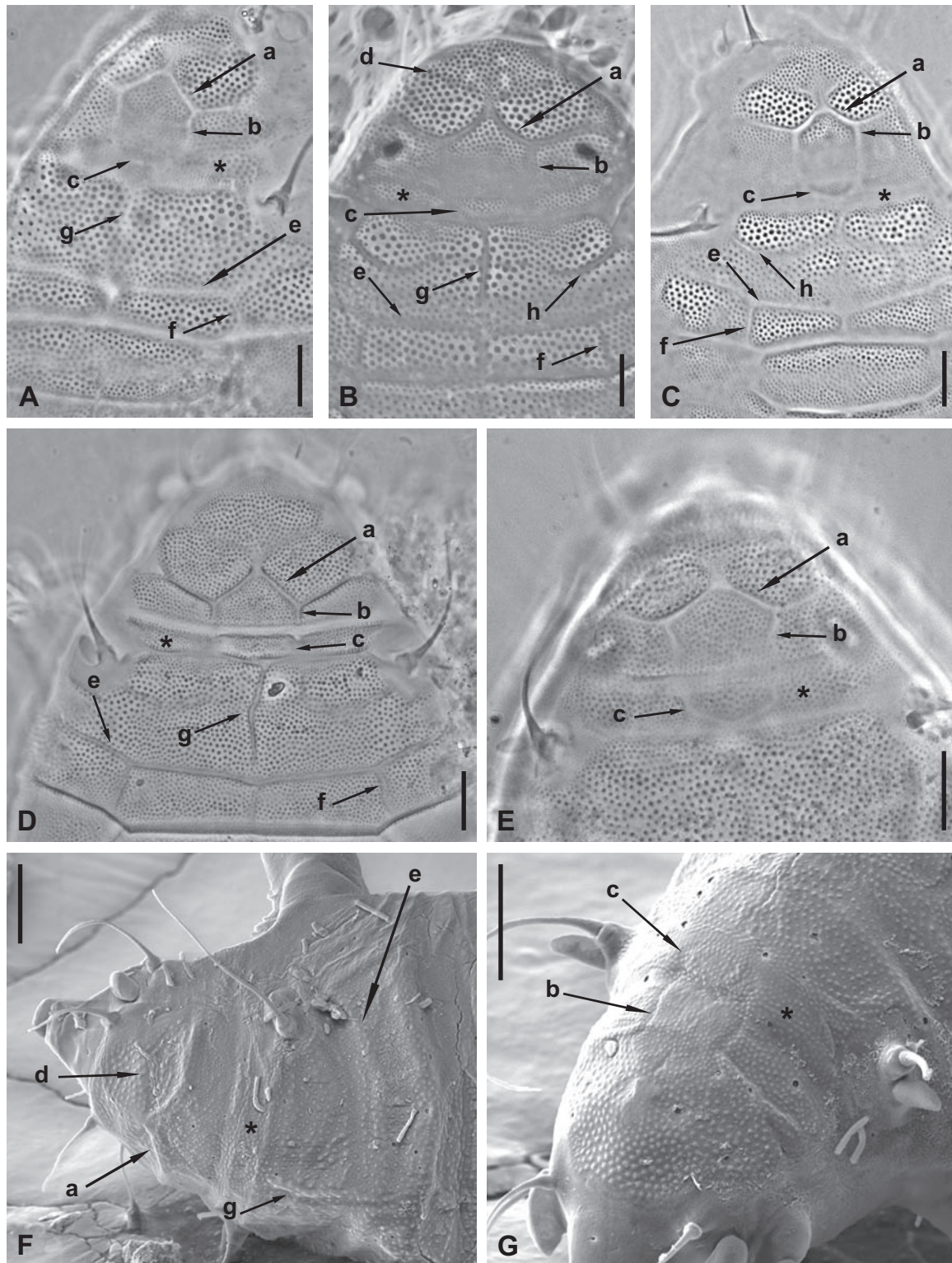
An ambiguous term 'faceted', often used in descriptions of the cephalic plate in *Pseudechiniscus*, seems merely to indicate the presence of the W-shaped sculpture (see section 'Caudal (terminal) plate' of the Results for a detailed discussion on this term).

#### *Neck plate (np)*

The neck plate is a narrow, transverse plate between cephalic and scapular plates (Fig. 1A–G: asterisk). It is often weakly sclerified and poorly demarcated, especially from the cephalic plate (Fig. 1C). The neck plate can be hidden under the anterior margin of the scapular plate in unstretched specimens. The plate usually bears two lines, which are continuations of the paired lines of the cephalic plate connecting caudally and forming a U-shaped figure on the neck plate (Fig. 1A–E: arrow c). This U-shape is a good marker of the neck plate, even if its borders are indiscernible. Lateral incisions may also provide information about the position of the border between the cephalic and the neck plate (Fig. 2A: arrowhead). Similar to the paired lines of the cephalic plate, the lines of the U-shaped figure can be seen as grooves in SEM images (Fig. 1G: arrows b and c). The U-shaped structure often forms a well-visible closed figure together with the W-shaped sculpture and the paired lines of the cephalic plate (Fig. 1A–E, G).

#### *Scapular plate (scp)*

This is a large plate covering the anterior part of the body (Fig. 2A: scp). The line between the bases of cirri A marks its frontal margin. The anterior zone of the reduced sculpture, noted in some older descriptions (e.g. Maucci, 1952), seems to be the unrecognized neck plate. In most species, the scapular plate is divided by a transverse ridge into the anterior and the posterior parts (Fig. 1A–D, F: arrow e). The latter is typically divided into four parts by three short longitudinal ridges (Fig. 1A–D: arrow f). Rarely, the medial longitudinal ridge continues anteriorly, dividing the anterior part of the scapular plate into two sections (e.g. in *P. spinerectus* and *P. pseudoconifer*) (Fig. 1A, B, D, F: arrow g). The degree of development of the ridge may vary within a population from well-developed to indiscernible (this might also depend on the coverslip pressure). When poorly developed, this system of ridges can form a T-shaped pattern (*P. occultus* Dastych, 1980). In some species (*P. nataliae*, *P. pseudoconifer* and in some specimens attributed by Maucci to *P. suillus*), additional oblique ridges go back from the cirri A bases (Fig. 1B, C: arrow h). In some specimens with a



**Figure 1.** Anterior part of the body dorsal surface of different *Pseudechiniscus* species. A, *P. jiroveci* (Mongolia). B, *P. pseudoconifer*. C, *P. nataliae* (holotype). D, *P. suillus*. E–G, *Pseudechiniscus* sp. (Croatia). a, W-shaped sculpture; b, lines moving backwards from the back corners of the W-shaped sculpture; c, U-shaped figure of the neck plate; d, anterior transverse

well-developed relief, these ridges extend to the middle of the plate, forming an indistinct second W-shaped pattern (Fig. 1B). A developed W-shaped sculpture of the scapular plate is also known in *P. alberti* Dastych, 1987.

#### Paired segmental plates (s1, s2)

There are two pairs of segmental plates in the middle part of the dorsal body side (Fig. 2A: s1, s2). The right and the left plates in each pair are usually distinctly divided with a longitudinal crest. The shape of these plates is variable, typically hexagonal or pentagonal, with the contact zone of the paired plates being the shortest side. The anterior margin of each plate usually protrudes slightly, the protrusion having the shape of an obtuse angle or, rarely, being slightly convex or straight. Acute triangular processes of the anterior margins of segmental plates of both pairs are known in *P. transsylvanicus* Iharos, 1936. Additional short longitudinal ridges of the segmental plates 1 and 2 are described in *P. spinirectus* (Pilato *et al.*, 2001).

#### Median plates (m1, m2, m3)

Unpaired plates are located between the scapular plate and segmental plates 1 (m1), between segmental plates 1 and 2 (m2) and between segmental plates 2 and the pseudosegmental plate (m3) (Fig. 2A: m1, m2, m3). Median plates can be undivided or divided by a transverse crest or fold into two parts, the anterior and the posterior one. In species with poorly sclerified dorsal plates, the borders between the parts are difficult to distinguish, especially when additional cuticular folds are present.

At least two types of division could be distinguished for the first median plate (m1). In the case of the primary division, the anterior part (Fig. 2: m1a) of the plate is wide and more or less hexagonal. The posterior part (Fig. 2A: m1p), caudally attached to the anterior part, is usually narrower and has an angled or a convex caudal margin. In the case of the secondary division, a reduced posterior part is poorly visible, because it is weakly demarcated (especially its lateral margins) and often hidden in the folding of the dorsal cuticle (Fig. 2B, D). The anterior part of m1 may be divided by a newly formed transverse fold, and this additional division complicates the pattern (Fig. 2C, E: arrow a). Sometimes, if the folding of the dorsal cuticle does not cross the anterior part of m1, but goes through

the reduced posterior plate, m1 seems undivided in contracted specimens, yet its posterior part is visible in stretched specimens [e.g. see the description of *P. xiai* in Wang *et al.* (2018: figs 1, 3)].

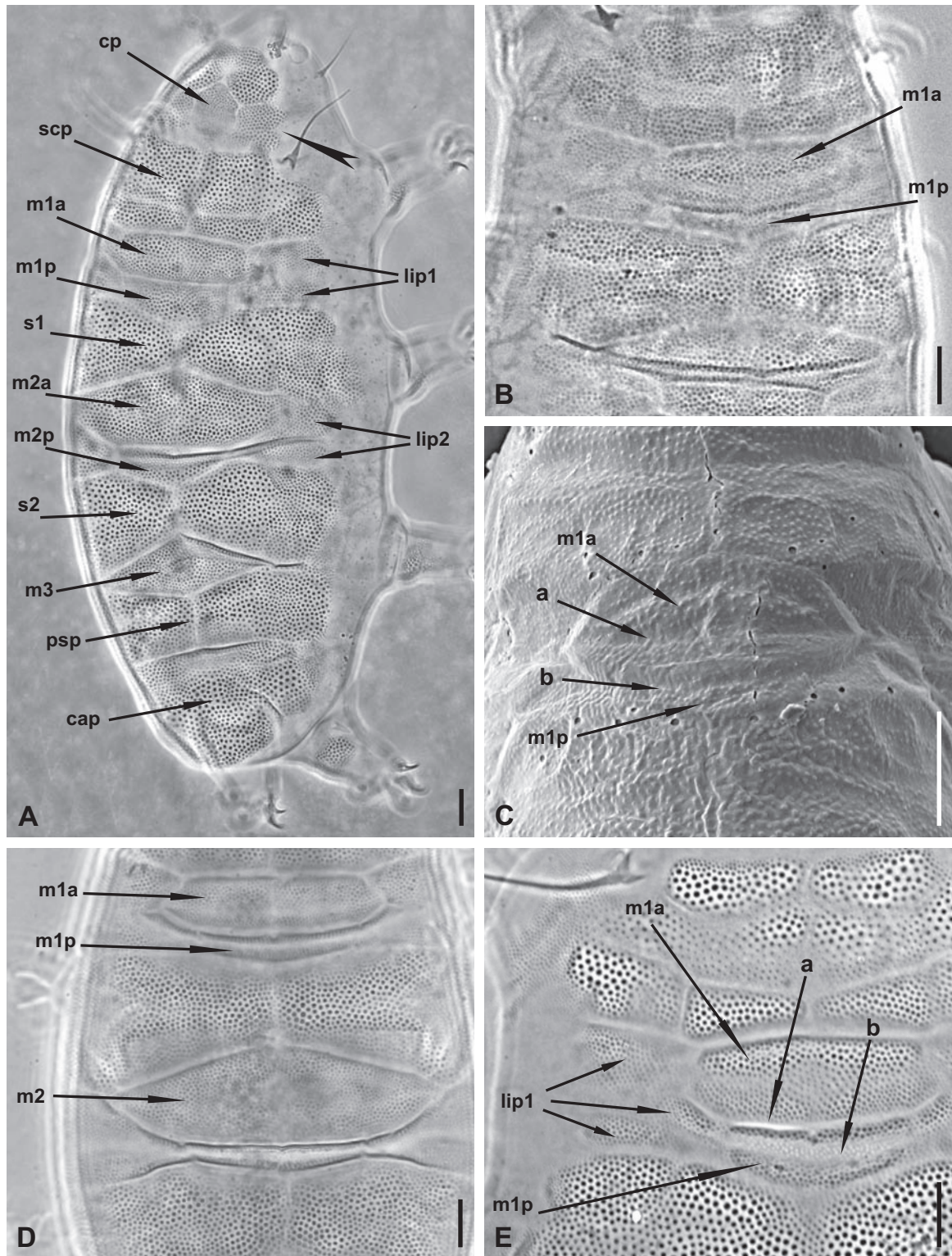
In some other species, the anterior part of m1 may be reduced, forming a narrow band anterior to the well-developed triangular posterior part, as in *P. titianae* (Vecchi *et al.*, 2016), or almost indistinguishable, as in *P. bartkei* [although Węglarska (1962) noted that a thin and weakly sclerified anterior part could be visible in compressed specimens]. The latter configuration corresponds to the undivided state of m1 plate (Fig. 3A: m1).

In *P. dicrani* Mihelčič, 1938, m1 is described as divided into three parts. The description of this species is brief and incomplete, and it has not been reported since the original publication. In Mihelčič's drawing (Mihelčič, 1938: 95, fig. 1) of this species, the second part of m1, which is wide and hexagonal, and its third part, which is narrower and subtriangular, correspond to the typical anterior and posterior parts of the m1 plate. The anteriormost part, a transverse band tightly attached to the scapular plate, may be the misidentified posterior part of the scapular plate, divided by the cuticular crest. This configuration is typical for *Pseudechiniscus* species (see also: Maucci, 1973–74).

The second median plate (m2) is usually similar in its configuration to m1. It usually consists of two parts: a larger anterior part and a smaller posterior part (Fig. 2A: m2a, m2p). Albeit rarely, it has been described as undivided, but this impression might have arisen from the two modifications of its initial double structure. In one case, a strongly sclerified m2 loses the structure separating it into the anterior and the posterior part (as in *P. gullii* Pilato & Lisi, 2006, *P. bartkei* and possibly in *P. yunnanensis* Wang, 2009) (Fig. 3A: m2), while in the other case the posterior part of m2 is reduced (as it may also happen with m1), leaving only an undivided anterior part (Fig. 2D: m2). In the latter case, a thin posterior part may be concealed within the cuticular folding.

The third median plate (m3), rhomboid or triangular (Fig. 2A: m3), is usually described as undivided, but a slightly developed folding goes transversely through it in some species. The folding may be invisible in CLM, but visible in SEM (Fig. 3B: arrow a). In two species [*P. bartkei* and *P. conifer* (Richters, 1904)] this fold is well-developed dividing m3 into two parts (Fig. 3C: arrows a). In three species (*P. bidenticulatus* Bartoš,

crest of the cephalic plate; e, transverse ridge of the scapular plate; f, lateral longitudinal ridges of the scapular plate; g, median longitudinal ridge of the scapular plate; h, oblique ridges of the scapular plate; \*, neck plate. A–E, PCM; F, G, SEM. Scale bar: 10 µm. Species names '*jiroveci*' and '*suillus*' are given in quotes because the identification of the specimens is questionable.



**Figure 2.** Dorsal cuticular plates of different *Pseudechiniscus* species. A, *P. jiroveci* (Mongolia), habitus. B, C, *Pseudechiniscus* sp. (Croatia), first median plate. D, *Pseudechiniscus* sp. (Russia), first and second median plates. E, *P. nataliae* (holotype), first median plate and lateral intersegmental plates. cp, cephalic plate; cap, caudal plate; lip1, first lateral intersegmental plates; lip2, second lateral intersegmental plates; m1a, anterior part of the first median plate; m1p, posterior part of the

1963, *P. jiroveci* and *P. scortecii* Franceschi, 1952) m3 has been reported to be missing, but because of the poor quality of these descriptions it is unclear whether it is the real condition or an erroneous observation.

#### *Lateral intersegmental plates (lip)*

These are small plates located in pairs (anterior and posterior plates) laterally to m1 and m2, between the scapular plate and segmental plate 1 (lip1) and between segmental plates 1 and 2 (lip2) (Figs 2A, E, 3D). These plates are usually present even in species with a poorly sclerified cuticle, but are often not mentioned in older descriptions, possibly because of the limitations of the optics. The degree of the development of these plates may vary within a pair, e.g. in *P. bartkei* the anterior plates in lip1 and lip2 groups have better-developed cuticular sculpture than the posterior ones (Fig. 3A).

Rarely, in some species (e.g. *P. nataliae*), an additional third lateral intersegmental plate is visible between the anterior and posterior lip1 plates, attached to the posterolateral margin of the anterior part of m1 (Fig. 2E; see also: Wang *et al.*, 2018: fig. 4a).

#### *Pseudosegmental plate (psp)*

This is a large transverse plate, situated between m3 and the caudal plates (Figs 2A, 3E, F: psp). The plate is often divided into two parts by a median longitudinal crest, but the degree of its development varies much within a population and the crest may be completely absent in some specimens (Kristensen, 1987; personal observations). Three pairs of lateral tooth-like appendages are described in *P. marinae* Bartoš, 1934 (see Discussion for a review of the taxonomic status of this species).

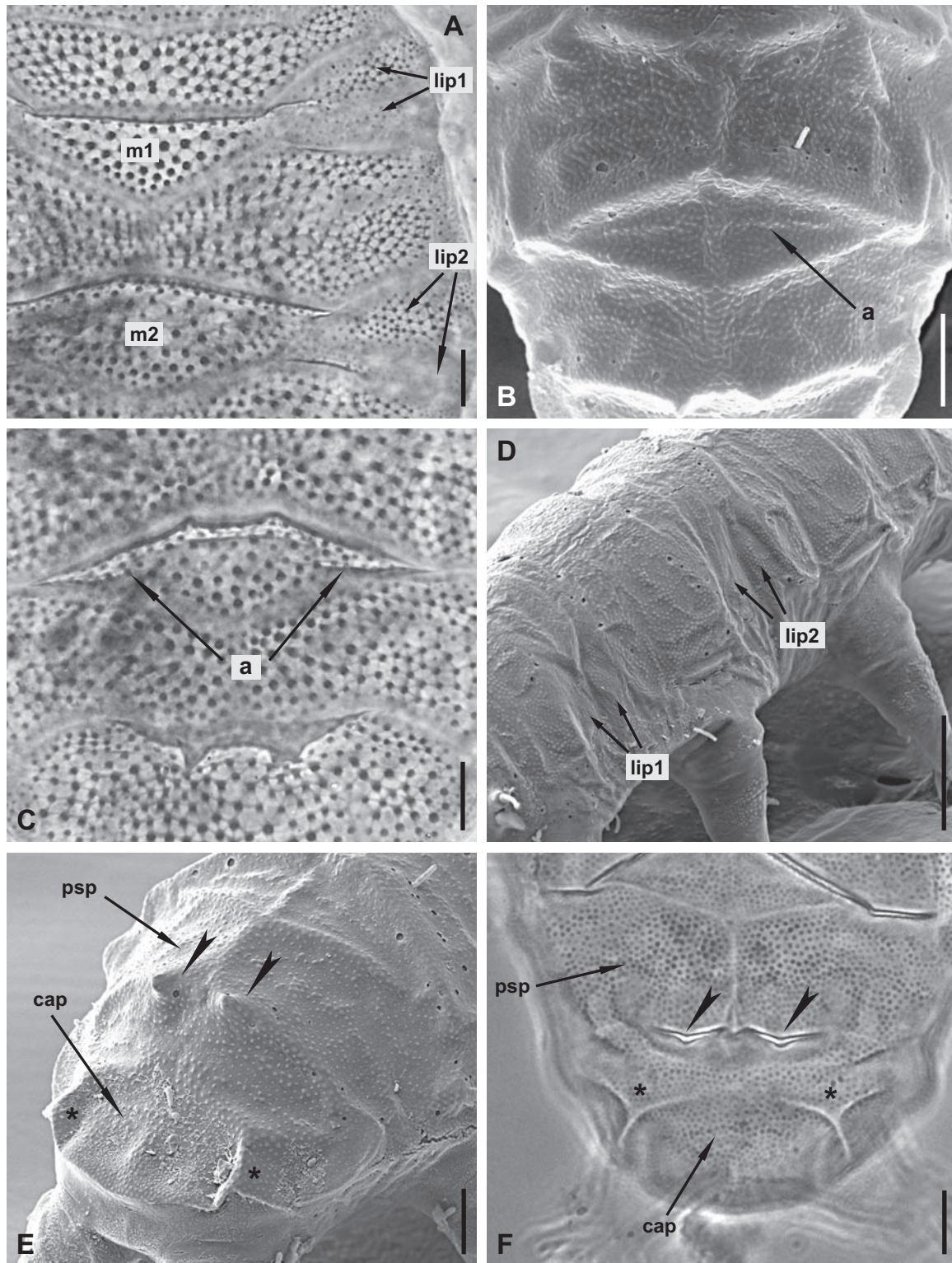
#### *Caudal (terminal) plate (cap)*

This large posteriormost plate is located on the caudal body end (Figs 2A, 3E, F: cap). Numerous terms have been coined for the description of its structure and shape in various *Pseudechiniscus* species. The most controversial term is 'faceted'. It seems it was used by Murray (1910) for the first time, who wrote in the description of the caudal plate in the genus *Echiniscus*: 'Often the middle and lateral flaps thus produced are

bent downwards at an angle to the small median portion of the plate, which is then said to be faceted' (Murray, 1910: 86). It is clear from this explanation that Murray understood 'faceted' as having several intersecting planes. In the early 20<sup>th</sup> century, this term was used in a similar sense in several fundamental studies by various authors (Thulin, 1911; Marcus, 1929, 1936). However, after that it has mostly been accepted as self-explanatory. The term '*sfaccettatura*' is widely used in the authoritative monographs of the 20<sup>th</sup> century (Ramazzotti, 1962, 1972; Ramazzotti & Maucci, 1983), but without an explanation. As a result, it has evidently been misunderstood by several authors. For example, Biserov (1986) in his description of *P. nataliae* (in co-authorship with Maucci) stated: '*Terminal plate with a large, evenly distributed granulation, with right and left strips, devoid of sculpture (the so-called sfaccettatura of the Italian authors)*' [in Russian, translated by D. Tumanov]. In some languages (e.g. in Russian) this term could be associated with a flat surface with a meshwork pattern.

Within the genus *Pseudechiniscus*, the term 'faceted' is usually applied to the morphology of the caudal plate. In CLM, this plate appears to consist of several flat surfaces, but the structure of the caudal plate of *Pseudechiniscus* sp. (Croatia), as seen in SEM, was different from Marcus's definition (Fig. 3E). A relatively flat surface of the caudal plate forms two raised folds, starting anteriorly from the caudal margin of the plate and forming a Y-shaped bifurcation. It is also visible in CLM, if an observed specimen is not flattened (Fig. 3F). In CLM, these ridges are often visible as regions devoid of sculpture when the microscope is focused on the plate surface. Careful CLM and SEM observations show that the surface of these ridges is covered with the sculpture of the same type as the rest of the plate. In specimens strongly flattened during the slide preparation, these ridges could be deformed, giving the appearance of incisions, or notches, often mentioned in the descriptions of *Pseudechiniscus* species. This kind of caudal plate structure was observed in all investigated species of the genus *Pseudechiniscus*, but the degree of the development of the ridges could vary much and the Y-shaped bifurcation was not always visible, even in the specimens attributed to the same species, so the taxonomic value of this character needs verification.

first median plate; m2a, anterior part of the second median plate; m2p, posterior part of the second median plate; m3, third median plate; psp, pseudosegmental plate; s1, first paired segmental plates; s2, second paired segmental plates; scp, scapular plate; a, additional folding dividing the anterior part of the m1; b, border between the anterior and the posterior part of m1; arrowhead, incision between the cephalic and the neck plate. A, B, D, E, PCM; C, SEM. Scale bar: 10 µm. Species name '*jiroveci*' is given in quotes because the identification of the specimen is questionable.



**Figure 3.** Dorsal cuticular plates of different *Pseudechiniscus* species. A, *P. bartkei* (paratype), first and second median plates and lateral intersegmental plates. B, *Pseudechiniscus* sp. (Croatia), third median plate. C, *P. bartkei* (paratype), third median plate. D, *Pseudechiniscus* sp. (Croatia), lateral intersegmental plates. E, F, *Pseudechiniscus* sp. (Croatia), pseudosegmental



### Additional lateral segmental plates (*lsp*)

*Pseudechiniscus alberti* bears a set of additional plates lateral to *sc*, *s1*, *s2* and *psp*. These additional plates are separated from the main plates by developed sutures. Additional lateral plates connected with *sc* and *s1* and *s2* are mentioned in the description of *P. asper* Abe *et al.*, 1998, while plates connected only with *s1* and *s2* are mentioned in the description of *P. beasleyi* Li *et al.*, 2007. However, in the latter two species, these plates are poorly described and illustrated, thus it is impossible to establish their relationship with the additional plates of *P. alberti*.

### Relief of the dorsal plates

In most cases, the surface of the dorsal plates is more or less flat, with the exception of ridges dividing the plates longitudinally or transversely (see above). Rarely, indistinct transverse grooves are detectable on the paired segmental plates [*P. spinerectus*, *P. pseudoconifer* and *Pseudechiniscus* sp. (Croatia)] (Fig. 4A: arrow a) and on pseudosegmental plate (*P. xiai*, see Wang *et al.*, 2018: fig. 4c). In CLM, these grooves usually appear as bands of modified or reduced sculpture, which probably results from the sculpture of the groove being out of focus. SEM investigation of *Pseudechiniscus* sp. (Croatia) also revealed a system of poorly developed ridges on *s1* and *s2* (Fig. 4A: arrow b). In some species (*P. facettalis*, *P. jubatus* and *P. xiai*) these ridges are well developed, forming a network pattern over all the dorsal plates (Fig. 4B).

### Sculpture of the dorsal plates

In CLM, the sculpture of the dorsal plates of *Pseudechiniscus* species is represented by dark dots of different size and shape (from common rounded ones to rare slightly polygonal ones) (Figs 1–4). These dots are made up by intracuticular pillars protruding through the epicuticle and forming round granules on the surface. In some species of the genus *Pseudechiniscus*, these pillars are long and the picture visible in CLM is, in consequence, different, depending on whether the image is focused on the surface tubercles or on the pillars. It is often difficult to distinguish the intracuticular pillars from the surface tubercles in CLM, but it can be done based on the differences in shape (rounded surface granules *vs.* slightly polygonal intracuticular pillars). The size of the visible elements of the sculpture may vary within the cuticular plate. In SEM, larger granules are usually located on the ridges

of the plate relief (Fig. 4C, D). ‘Elongated dots’ of the cuticular sculpture mentioned in some descriptions [e.g. that of *P. jiroveci* in Bartoš (1963)] are an artefact, resulting from a strong compression of the specimen, in which case the pillars are viewed slightly laterally (Fig. 3A). In some species, the size of the surface granules varies along the body length of the animal, with larger granules being usually located caudally (Fig. 4E).

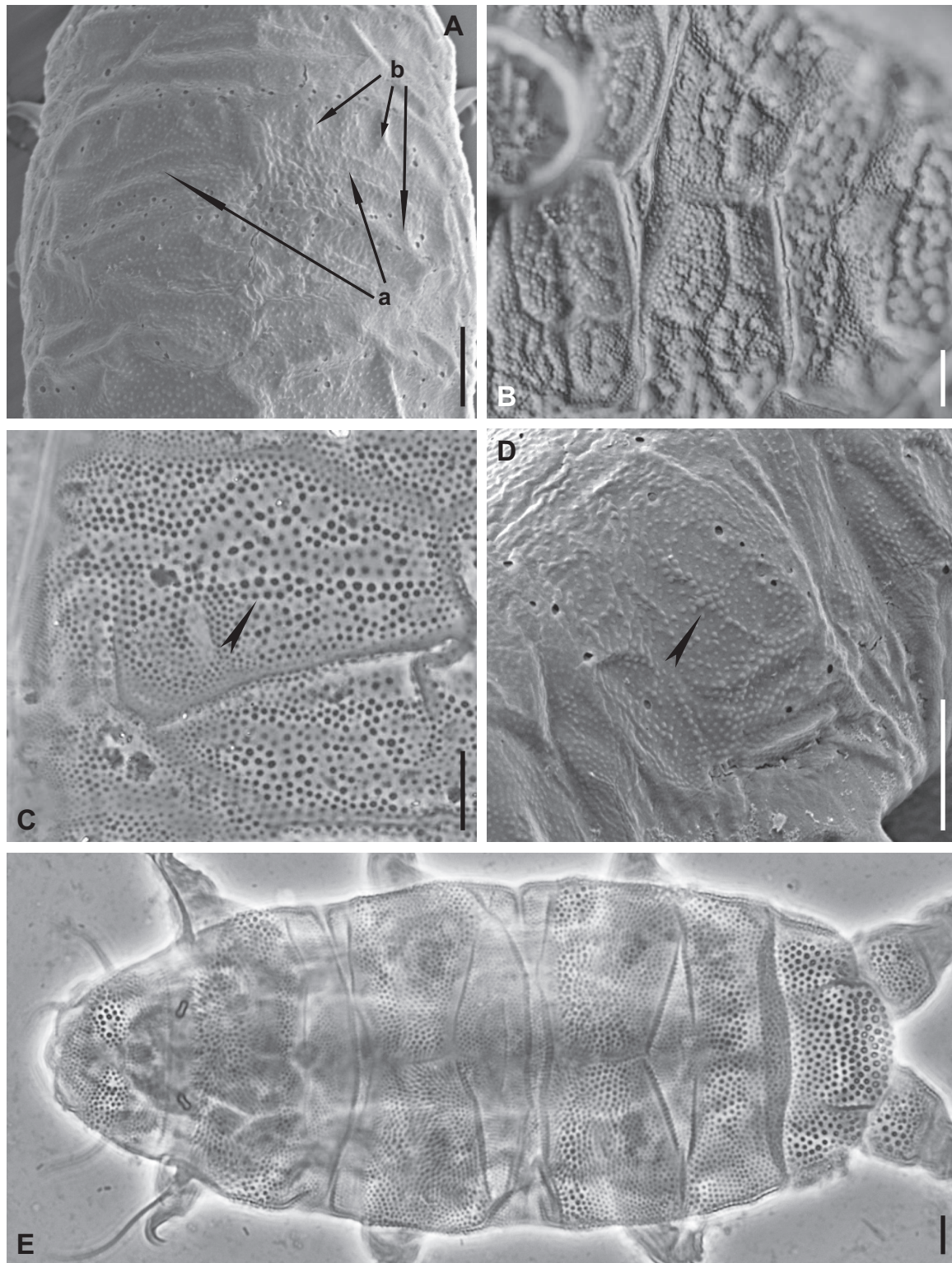
The number of granules of the sculpture on a given square of the dorsal plate was sometimes used as the taxonomic character within the genus *Pseudechiniscus* (Biserov, 1986; Maucci, 1986; Fontoura *et al.*, 2010), but the usefulness of this trait is limited, because of the small number of the species investigated and difficulties in standardization of the region of the measurements and its dimensions. The presence of thin striae connecting the intracuticular pillars in the internal structure of the cuticular plates has been recently recognized as taxonomically significant (Pilato *et al.*, 2001; Pilato & Lisi, 2006; Fontoura *et al.*, 2010; Vecchi *et al.*, 2016). The distribution of these structures in *Pseudechiniscus* spp. is unclear, because it was probably omitted in early species descriptions. Some species have well-visible striae (e.g. *P. bartkei*, *P. gullii*, *P. spinerectus* and likely *P. quadrilobatus*; see Pilato *et al.*, 2004); Fig. 3A, C), whereas in others they are poorly visible and detectable only in high-quality PCM at a maximum magnification and when the image is focused on the intracuticular part of the plate structure, rather than on the surface tubercles (Fig. 5A, C).

Small pores in the outer layer of the cuticle are also visible in SEM (Nelson, 1975; Schuster *et al.*, 1975; Figs 1G, 2C, 3D, E, 4D). Grigarick *et al.* (1983) used this character to differentiate *P. facettalis* (as *P. suillus facettalis*) from *P. novaezeelandiae* and Biserov (1986) used it to differentiate *P. nataliae* from *P. suillus*. However, this character cannot be accepted as a good taxonomical trait until its distribution in the genus has been evaluated and the possibility of artefact excluded.

### STRUCTURE OF THE VENTRAL CUTICLE

The ventral cuticle in *Pseudechiniscus* has no distinct cuticular plates. Distinct surface tubercles are absent, and the dot-like pattern visible in CLM is formed solely by the intracuticular pillars. Areas of large dots, often visible ventrally between the legs I–III, are not detectable in SEM and seem to be groups of the pillars

and caudal plates. *cap*, Caudal plate; *lip1*, first lateral intersegmental plates; *lip2*, second lateral intersegmental plates; *psp*, pseudosegmental plate; *a*, transversal crest of the third median plate; arrowheads, dorsal processes of the pseudosegmental plate; \*, foldings of the caudal plate. A, C, F, PCM; B, D, E, SEM. Scale bar: 10 µm.



**Figure 4.** Dorsal cuticular plates of different *Pseudechiniscus* species. A, *Pseudechiniscus* sp. (Croatia), relief of the first segmental plates. B, *P. facettalis* (paratype), relief of the second segmental, pseudosegmental and caudal plates. C, *P. jubatus* (holotype), sculpture of the first segmental plate. D, *Pseudechiniscus* sp. (Croatia), sculpture of the first segmental plate.

with an increased thickness but of normal height. However, the reticular pattern between the pairs of legs is formed by elongated pillars and appears as a system of surface ridges (Fig. 5C–F). This structure is known since Maucci described ‘*una finissima granulazione (difficilmente visibile) disposta a liste irregolari formanti una specie di reticolo*’ [‘a very fine granulation (hardly visible) arranged in irregular strips forming a kind of network’] in *P. pseudoconifer* (Maucci, 1973–74: 123). The presence or absence of the ventral network has been considered as a valuable character for distinguishing similar species. Some doubts, mainly related to the understudied polymorphism in *P. suillus* species complex, were expressed (Dastych, 1984). In older descriptions of *Pseudechiniscus* species, this character was often not mentioned, which could lead to errors. For example, Maucci (1986) used the presence of the ‘ventral labyrinth’ in *P. pseudoconifer* as a valid character discriminating it from *P. facettalis*, but a reinvestigation of Petersen’s type material of the latter species has shown that a well-developed ventral network is present in the latter species.

The pattern of the ventral network is sometimes used in taxonomic descriptions for discrimination of species (Pilato *et al.*, 2001), but its variability is poorly investigated. My investigation revealed an evident sexual dimorphism of the ventral network in *Pseudechiniscus* sp. (Croatia). Network meshes are smaller and mostly isodiametric in females, and larger and often elongated in males (Fig. 5C–F). This means that the shape and proportions of the ventral network meshes should be used cautiously in species delineation until this trait has been studied in a larger number of species.

The ventral side of the head region usually bears an indistinctly bordered zone of enlarged granules. In SEM, the surface of the cuticle in this area has developed tubercles similar to the sculpture of the dorsal plates. A well-demarcated subcephalic plate has been described in *Pseudechiniscus asper* (Abe *et al.*, 1998).

#### CEPHALIC APPENDAGES

Species of the genus *Pseudechiniscus* have a typical set of cephalic sensory appendages, consisting of two pairs of short cirri (internal and external buccal cirri), a pair of papilliform organs (cephalic papillae or secondary clavae) and, typically, have developed thin setiform cirri A with small, elongated, primary clavae (with the exception of the doubtful species *P. clavatus* Mihelčič, 1955, in which the primary clavae were described as

large and rounded). Light microscopy observations of dorsoventrally oriented specimens show two types of the cephalic papillae within the genus *Pseudechiniscus*: elongated papillae protruding anteriorly, e.g. in *Pseudechiniscus* sp. (Argentina) (Fig. 6A) and spherical, hemispherical or mushroom-like papillae, e.g. in *Pseudechiniscus* sp. (Croatia) (Fig. 6B). However, SEM investigation of *Pseudechiniscus* sp. (Croatia) shows that cephalic papillae, seemingly subspherical in CLM, were in fact elongated with an acuminate apex, but attached laterally to the body and directed vertically (Fig. 6C). The real shape could sometimes even be seen in CLM if the specimen was laterally oriented (Fig. 6D).

Three species (*P. alberti*, *P. jubatus* and *P. occultus*) have bifurcated tips of the cirrus A and of the buccal cirri. This character, also known in *Acantechiniscus*, *Bryodelphax* and *Echiniscus* (Dastych, 1987), seems to be species-specific but its exact distribution in *Pseudechiniscus* is unknown.

#### TRUNK APPENDAGES

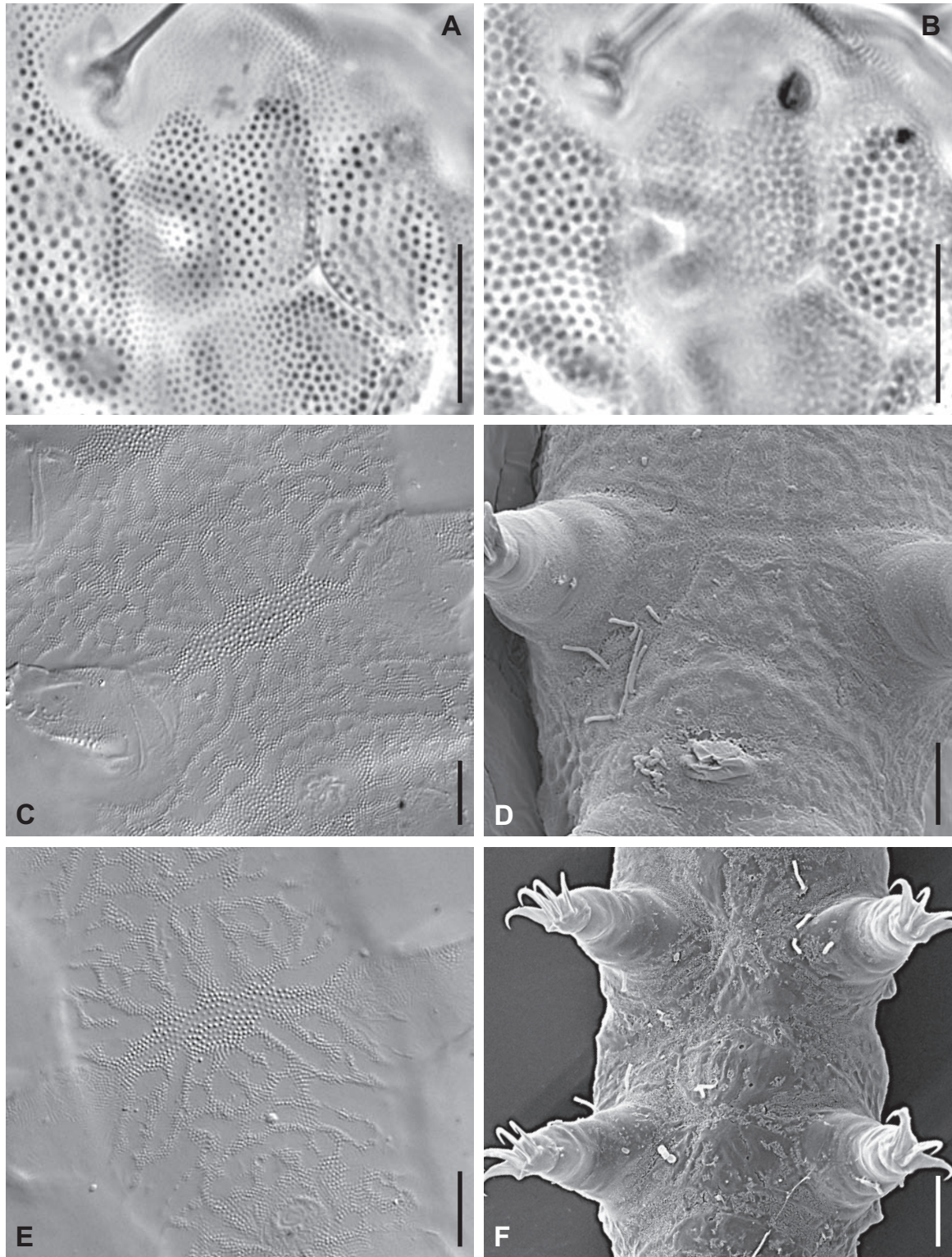
Lateral appendages, except cirri A, are poorly developed. Two species [*P. bispinosus* (Murray, 1907) and *P. transsylvanicus*] have relatively long setiform appendages C, and one species [*P. pulcher* (Murray, 1910)] has long setiform appendages E (see below for the discussion of its taxonomic position). Both *P. bispinosus* and *P. transsylvanicus* were described almost a hundred years ago and have never been reinvestigated.

Spiniform appendages E were described only in *P. alberti*, *P. insolitus* and *P. occultus*. In these three species, spines E seem to be derivatives of the caudal plate crests (Fig. 6E).

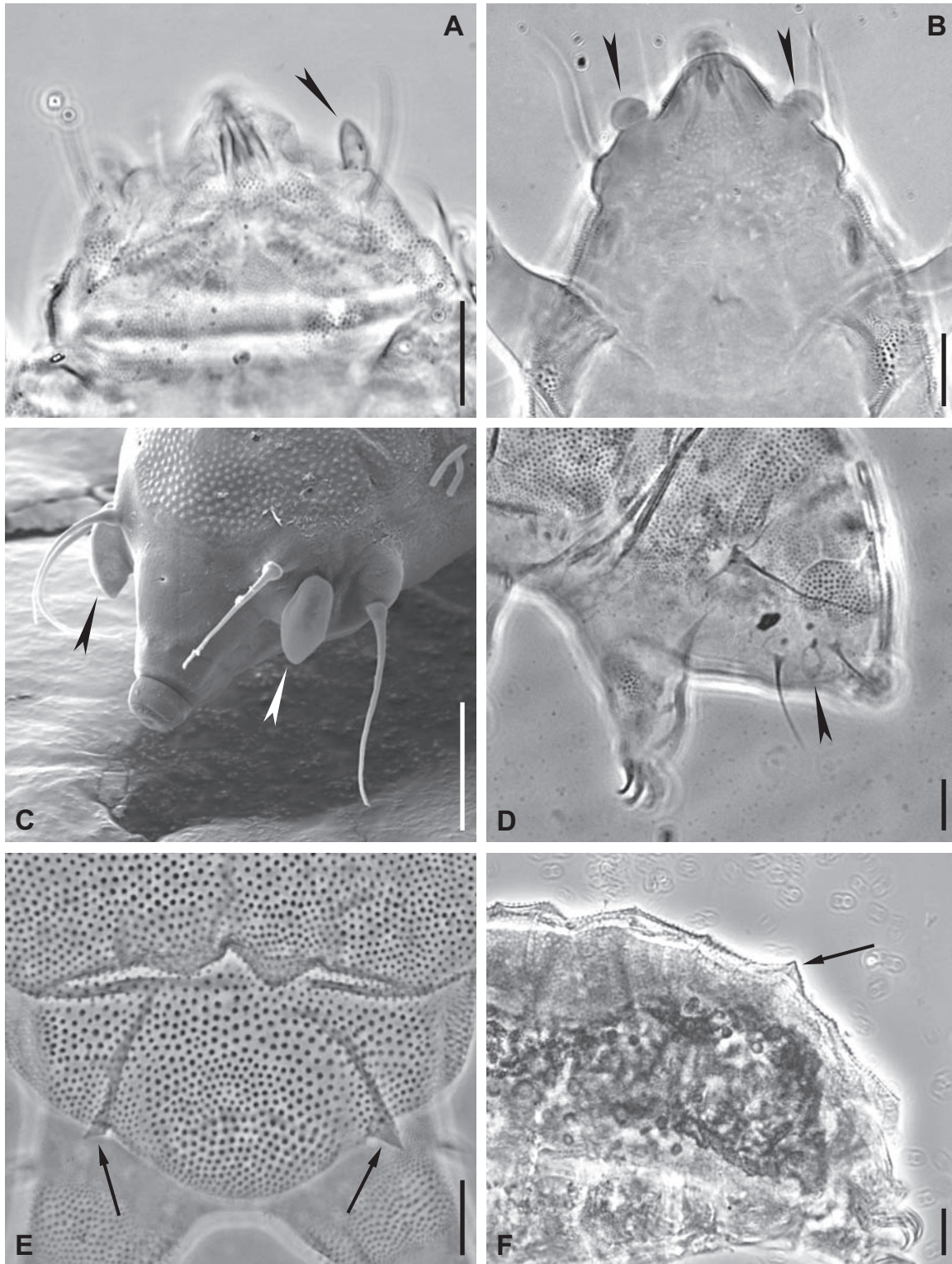
Some *Pseudechiniscus* species have small papilliform lateral appendages, mostly in position D (rarely in B and C). They form the so-called *conifer*-group of species named after *P. conifer*. The lateral papillae are usually difficult to see, and the degree of their development varies much within the population (Ramazzotti, 1943; Biserov, 1986). In some species (*P. alberti*, *P. insolitus*, *P. occultus* and a poorly described *P. shilinensis* Yang, 2002), these lateral appendages look like short spines.

Paired dorsal appendages in *Pseudechiniscus* are most often present on the posterior margin of the pseudosegmental plate, usually as short lobes, spines or lobes with distal spines. In permanent slides, these spines usually seem to be directed backwards, but in living specimens, they are directed upwards (Pilato

E, *Pseudechiniscus* sp. (Russia), dorsal sculpture. a, transversal grooves of the first segmental plate; b, additional ridges of the first segmental plate; arrowheads, enlarged tubercles of the cuticular sculpture on the ridges of the dorsal plates. A, D, SEM; B, DIC; C, E, PCM. Scale bar: 10 µm.



**Figure 5.** Dorsal and ventral cuticular sculpture of different *Pseudechiniscus* species. A, B, *P. 'jiroveci'* (Mongolia), dorsal cuticular sculpture of the same specimen with the microscope focused on the cuticle surface (A) and on the intracuticular structures (B). C, D, *Pseudechiniscus* sp. (Croatia), ventral sculpture of a female. E, F, *Pseudechiniscus* sp. (Croatia), ventral sculpture of a male. A, B, PCM; C, E, DIC; D, F, SEM. Scale bar: 10  $\mu$ m. Species name '*jiroveci*' is given in quotes because the identification of the specimen is questionable.



**Figure 6.** Cephalic and body appendages of different *Pseudechiniscus* species. A, *Pseudechiniscus* sp. (Argentina), cephalic papilla. B, *Pseudechiniscus* sp. (Croatia), cephalic papillae. C, D, *Pseudechiniscus* sp. (Croatia), cephalic sensory organs. E, *P. insolitus* (paratype), caudal plate. F, *Pseudechiniscus* sp. (Chile), caudal part of the body, laterally. Arrowheads, cephalic papillae; arrows, spines of the caudal (E) and pseudosegmental (F) plates. A, B, D–F, PCM; C, SEM. Scale bar: 10  $\mu$ m.

*et al.*, 2001), which is confirmed by SEM (Fig. 3E) and by CLM investigation of laterally mounted specimens (Fig. 6F).

An unusual feature of the genus *Pseudechiniscus* is a high variability of appendages on the posterior margin of the pseudosegmental plate in some species. In *P. ramazzottii*, *P. santomensis* and *Pseudechiniscus* sp. (Croatia), this plate can bear paired short spines, paired lobes or an unpaired median lobe. In *P. bartkei*, the plate bears paired spines or unpaired median lobe (if *P. bartkei* f. *unilobata* is not a separate species; see Discussion). Paired appendages of the pseudosegmental plate can be absent or present in *P. insolitus* (as spines) and *P. pseudoconifer* (as short lobes).

Apart from the pseudosegmental plate, paired dorsal appendages can be present on the scapular plate (*P. insolitus* and *P. occultus*), segmental plates 1 and 2 (*P. alberti* and *P. insolitus*) and even on median plate 2 (*P. insolitus*). In one species (*P. insolitus*), a variable set of dorsolateral spiniform appendages is present on the scapular plate, and on the paired segmental plates 1 and 2.

A unique feature of the genus *Pseudechiniscus* is the development of unpaired median lobes on the caudal margins of dorsal plates. These lobes could be developed on the scapular plate (*P. alberti*, *P. jubatus* and *P. quadrilobatus*), paired segmental plates 1 and 2 (*P. jubatus*, *P. quadrilobatus* and *P. yunnanensis*), median plates 1 and 2 (*P. alberti*) and pseudosegmental plates (*P. gullii*, *P. jubatus*, *P. pilato* Li, 2007 and *P. quadrilobatus*).

#### LEGS AND LEG SENSORY STRUCTURES

Legs in *Pseudechiniscus* bear a zone of well-developed sculpture consisting of surface tubercles (Fig. 7A, F, G). There is no evidence that these areas have distinct borders and form a true pedal plate in any species. A dentate collar on legs IV is usually absent, except for *P. alberti*, *P. cf. papillosus* (Beasley & Miller, 2012) and a poorly described *P. dicrani*, but even in these species it is strongly reduced and consists of one or two teeth.

Sensory spines on legs I were described in three species (*P. alberti*, *P. marinae* and *P. scortecii*), but SEM investigation revealed the presence of a small papilla (not visible in CLM) on the legs of the first pair in *Pseudechiniscus* sp. (Croatia) (Fig. 7A). A small papilla is also detectable in CLM on legs I in a Mongolian population of, presumably, *P. jiroveci* (Fig. 7B).

Legs IV usually bear a sensory papilla on their outer sides. For 19 species (nearly a half of all known species), there is no information on the state of this character. Usually, the papilla on the hindlegs is poorly described and often no measurements are given. It is often supposed that only one species, *P. ramazzottii*,

has an elongate spiniform hindleg papilla (e.g. Maucci, 1986; Kendall-Fite & Nelson, 1996; Pilato *et al.*, 2001). However, a reinvestigation of the type material of this species revealed that its appearance could be very variable in CLM. The SEM investigation of the similar new species from Croatia, which shows the same pattern of variability of the leg IV papilla in CLM (Fig. 7C), showed that this structure has a more complex morphology than a simple spine or papilla. Specifically, it is elongated, slightly curved and often has a thinner base, which gives it a banana-like appearance (Fig. 7D). The same shape could be seen in some suitably oriented *P. ramazzottii* specimens (Fig. 7E).

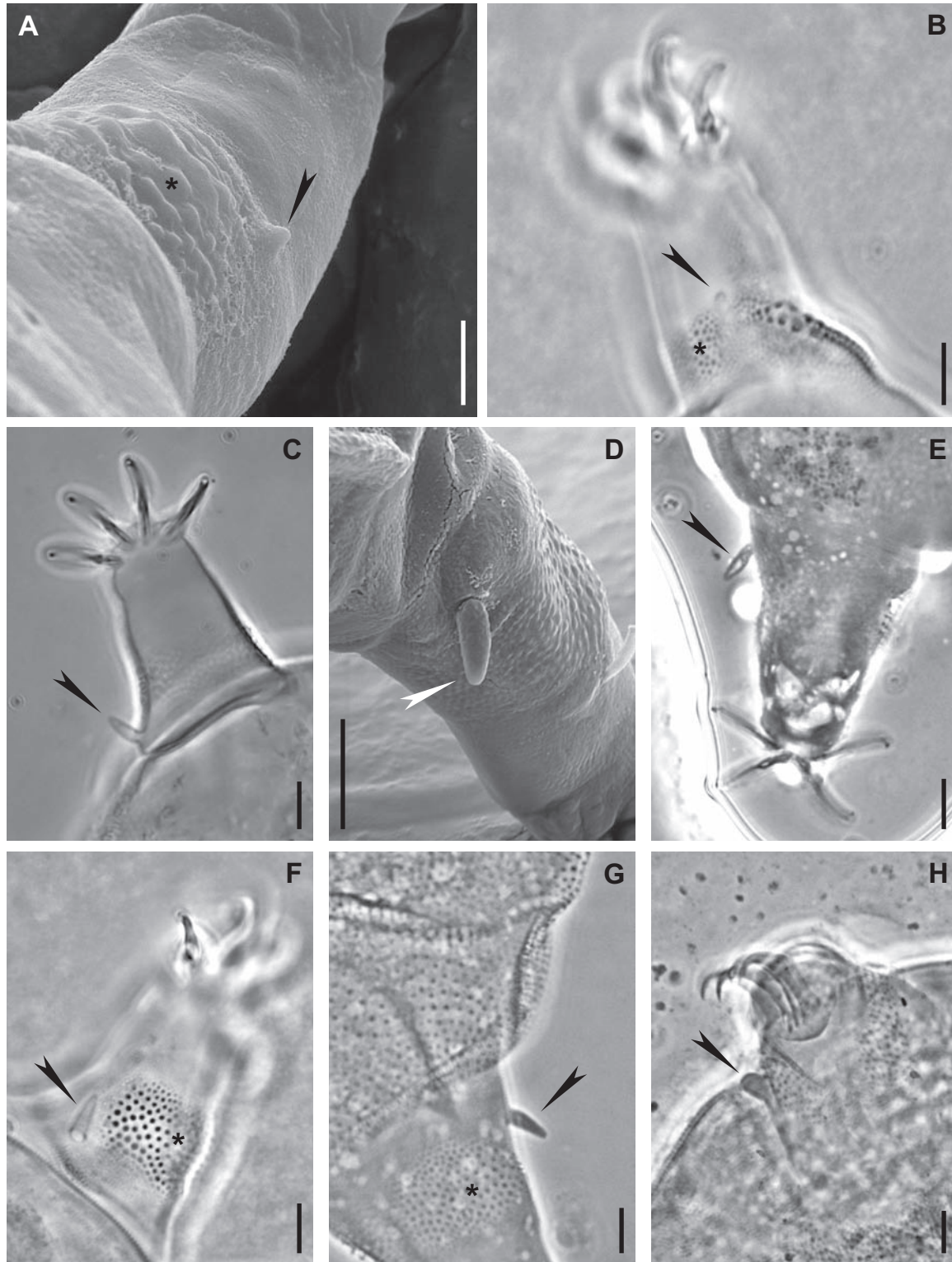
An investigation of the available material showed that elongated and curved papillae on the hindlegs are widely distributed within the genus *Pseudechiniscus* (Fig. 7F, G). Their appearance on permanent slides depends strongly on their orientation, so that several specimens should be analysed to ascertain their shape. Besides the species with elongate and acuminate papillae of legs IV, there are also some species with distinctly short, blunt papillae (Fig. 7H).

Iharos, in his drawings of newly described species and forms of the genus *Pseudechiniscus* (Iharos, 1963: fig. 2, 1964: fig. 2, 1969: fig. 1), showed distinct papillae on the inner surface of legs IV. However, this structure was mentioned in the text only for *P. ramazzottii* forma *facettalis* (Iharos, 1964) and *P. novaezeelandiae* forma *laterospinosa* (or *laterospina* – both names are used in the publication) (Iharos, 1963). In fact, there are no papillae on the inner surface of legs IV in Echiniscidae, but the retraction of the legs during the slide preparation could result in the formation of cuticular folds similar to papillae when observed in CLM, especially considering the quality of the optics at that time. Therefore, these structures should be considered as artefacts.

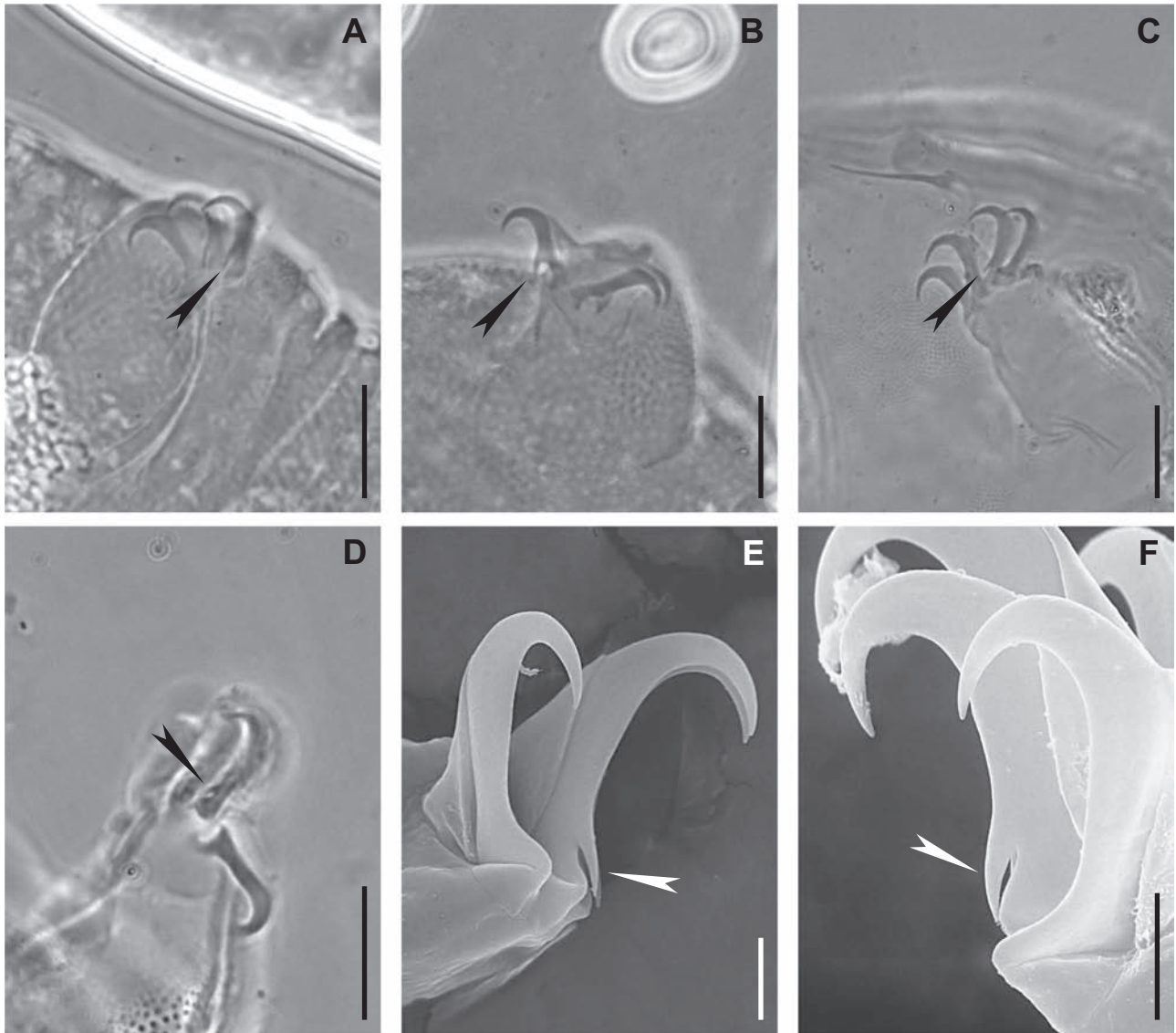
Biserov (1990) described wide tubercles near the claw bases on legs I–III of *P. jubatus*. These structures are similar to the ‘cushion-like structures’ described for *Mopsechiniscus franciscaae* Guidetti *et al.*, 2014 (Guidetti *et al.*, 2014) and *Acantechiniscus victor* (Ehrenberg, 1853) (Vecchi *et al.*, 2016).

#### CLAW MORPHOLOGY

In most cases, only the presence or absence of the basal spurs on internal and external claws was mentioned in the descriptions of *Pseudechiniscus* species. The length of the claws is sometimes provided, but often only for the hindlegs. The investigation of *Pseudechiniscus* sp. (Croatia) and *P. ramazzottii* revealed that both species, which are morphologically similar, have basal spurs on the inner claws of all legs. However, they could be easily distinguished by the characteristic spurs of the hindlegs in *P. ramazzottii*, which differ from the spurs



**Figure 7.** Leg sensory appendages of different *Pseudechiniscus* species. A, *Pseudechiniscus* sp. (Croatia), papilla of leg I. B, *P. 'jiroveci'* (Mongolia), papilla of leg I. C, D, *Pseudechiniscus* sp. (Croatia), papilla of leg IV. E, *P. ramazzottii* (paratype), papilla of leg IV. F, *P. 'jiroveci'* (Mongolia), papilla of leg IV. G, *P. insolitus* (paratype), papilla of leg IV. H, *P. barthei* (paratype), papilla of leg IV. Arrowheads, leg papillae; asterisks, areas of the dot-like sculpture on the legs. A, D, SEM; B, C, E, F–H PCM. Scale bars: A, 2  $\mu$ m; B–H, 5  $\mu$ m. Species name '*jiroveci*' is given in quotes because the identification of the specimen is questionable.



**Figure 8.** Claws of different *Pseudechiniscus* species. A, *P. ramazzottii* (paratype), claws of leg II. B, *P. ramazzottii* (paratype), claws of leg IV. C, E, *Pseudechiniscus* sp. (Croatia), claws of leg I–II. D, F, *Pseudechiniscus* sp. (Croatia), claws of leg IV. Arrowheads, basal spurs of the claws. A–D, PCM; E, F SEM. Scale bars: A–D, 10 µm; E, F, 2 µm.

on legs I–III in that they outbranch from the claw at a greater distance from its base and are more divergent from the claw stem (Fig. 8A, B). In *Pseudechiniscus* sp. (Croatia) there is no such difference between claws I–III and the hind claws (Fig. 8C–F).

## DISCUSSION

### NOTES ON SOME SPECIES

#### *Pseudechiniscus pulcher* Murray (1910)

This species was described by Murray (1910) from Australia. It is the only *Pseudechiniscus* species

with long setiform appendages *E.* Claxton (2004) investigated newly found specimens attributed to this species and suggested that it should be transferred to the genus *Antechiniscus* (publication in preparation, S. Claxton, *pers. comm.*).

#### *Pseudechiniscus novaezeelandiae* (Richters, 1908) *s.l.*

The problematic status of this species is recognized (Fontoura *et al.*, 2010; Kaczmarek *et al.*, 2015). Currently it includes several forms with an unclear status described from different zoogeographic regions and attributed to *P. novaezeelandiae s.l.* by different authors (Ramazzotti & Maucci, 1983; Degma *et al.*, 2009–18).



*Pseudechiniscus novaezeelandiae* s.s. was described by Richters (1908) from the North Island, New Zealand. The description is short, but clear, and is accompanied by a photograph of a specimen *in toto* and a drawing of the pseudosegmental plate (Richters, 1908). To note, Richters did not mention any lateral papillae in the species description. On the contrary, he stated that ‘Außer den üblichen Borsten neben der Sinnespapille an der Schnauze und dem Borstenpaar vor II keine Anhänge’ [‘Apart from the usual bristles next to the sensory papilla on the snout and the bristle pair before II no attachments’] (Richters, 1908: 205). It seems unlikely that Richters could have missed these structures considering that several years earlier he described lateral papillae in *P. conifer* (Richters, 1904a, b).

Two years later, Murray (1910) attributed to this species specimens found in New Zealand, Australia and Hawaii. He tried to emend Richters’ diagnosis, but his additions were mainly based on the Australian material and he presented drawings of the Australian and Hawaiian specimens only. Murray (1910) noted lateral papillae in *P. novaezeelandiae*, but commented that these structures were not seen in the New Zealand material. He also reported a spine on legs I, which had not been mentioned in Richters’ description. The dorsal plate pattern in his drawings is different from that described by Richters (1908). At that time, a high variability of species with a world-wide distribution was a dominant concept in the tardigrade taxonomy, while nowadays such differences are considered as species-specific (Faurby *et al.*, 2011; Guidetti *et al.*, 2016, 2019; Stec *et al.*, 2018). Later, Richters (1911) reported a single specimen of *P. novae-zeelandiae* (*sic!*) from South America (Colombia), but did not provide any detailed description or drawing noting only ‘Von Murray auf Hawaii beobachtet’ [‘Seen by Murray on Hawaii’] (Richters, 1911: 276). We may assume that this specimen was similar to Murray’s material from Hawaii but Murray’s specimens, given their morphological distinctiveness, would currently be attributed to a different species than *P. novaezeelandiae* (see above).

Marcus also adhered to the idea of a great intraspecific variability in tardigrades in his influential monographs (Marcus, 1929, 1936). His diagnosis for *P. novaezeelandiae* was based on Murray’s description and drawings, and so the presence of lateral papillae was accepted as typical of the species. Marcus (1936) synonymized *P. marinae* Bartoš, 1934, a species described from a very distant zoogeographic region (Europe, Moravia), with *P. novaezeelandiae*, accepting it as *P. novaezeelandiae* forma *marinae*. According to the description of Bartoš, *P. marinae* is characterized by the presence of lateral papillae and a spine on legs I, and an extremely developed relief on the caudal plate

and lateral teeth of the pseudosegmental plate, which is a unique feature within *Pseudechiniscus*.

Marcus’s monograph was the basis of the tardigrade taxonomy for a long time. His diagnoses were accepted mostly uncritically and so Murray’s description of the Australian specimens was treated as representing the species described by Richters from New Zealand. The situation became more complicated when Iharos (1963) published the descriptions of two new forms of *P. novaezeelandiae*, forma *aspinosa* and forma *laterospina*, from Argentina. Both these new forms have no lateral papillae and no spines, only blunt lobes on the pseudosegmental plate. Iharos included Murray’s drawing of *P. novaezeelandiae* designating it as ‘forma *typica*’. He also presented a drawing of a form, named in the legend as ‘f. *dorsospinosa* Richt.’ (Iharos, 1963; Fig. 2B), without giving any diagnosis or description. This name probably refers to the form described by Richters. The source of the depicted specimen is unclear, but in comments to table 1 (Iharos, 1963: 295), Iharos stated that *P. novaezeelandiae* was represented in his material by three forms and that the typical form was absent. So, it may be assumed that specimens attributed to *P. f. dorsospinosa* were also found in the material from Argentina except the two new forms described in the paper. The drawing by Iharos is different from the description of Richters: there are no spines, but lobes are present on the pseudosegmental plate, and the shape of the third median plate is different and could not belong to the species described by Richters.

Horning *et al.* (1978) reported specimens of *P. novaezeelandiae* from New Zealand that corresponded perfectly to the description of Richters. A reinvestigation of this material (Pilato *et al.*, 2005) revealed striations between the dots of the cuticular sculpture, but other characters, such as the absence of lateral papillae or spines on legs I, matched the original description.

Jørgensen *et al.* (2011) sequenced *COI*, 18S rRNA and 28S rRNA markers from specimens from Chile and attributed these specimens to *P. novaezeelandiae* (*sic!*). However, the presence of this species in South America is doubtful (see above) and most published findings of *P. novaezeelandiae* in this region are poorly documented (Heinis, 1914; du Bois-Reymond Marcus, 1944; Séméria, 1993; Garitano-Zavala, 1995; Jerez Jaimes & Narváez Parra, 2001; Nickel *et al.*, 2001). The only record of a South American *Pseudechiniscus* similar to the original description is found in the publication of Grigarick *et al.* (1983) and derives from Venezuela. The authors compared their material with New Zealand specimens and reported that, in general, they were similar, but also noted the presence of the basal spurs on the inner claws, which are absent in

*P. novaezeelandiae* (Horning *et al.*, 1978; Pilato *et al.*, 2005). It is likely that they compared their Venezuelan material with New Zealand specimens representing a yet undescribed species of the genus *Pseudechiniscus*. This assumption is supported by the presence of the specimen from New Zealand exhibiting basal spurs on the claws and attributed to *P. novaezeelandiae* in the collection of Maucci (slide 6859). This means that the sequences published by Jørgensen *et al.* (2011) cannot be attributed to *P. novaezeelandiae* until this material is reinvestigated and its identity to the original description is confirmed. Specimens of *Pseudechiniscus* found in Australia by Sandra Claxton and attributed to *P. novaezeelandiae* also have some differences compared to the original description (S. Claxton, pers. comm.).

Summarizing, only New Zealand specimens conforming to the original description by Richters (1908) with corrections by Pilato *et al.* (2005) should be considered as nominal *P. novaezeelandiae*. The presence of this species in other regions should be confirmed by new findings or a reinvestigation of the material in collections. Specimens described by Murray (1910) from Australia and Hawaii should be considered as belonging to a new, yet undescribed species. The taxa described as *P. novaezeelandiae* forma *aspinosa* Iharos, 1963, *P. novaezeelandiae* forma *laterospina* Iharos, 1963 and *P. novaezeelandiae* forma *dorsospinosa* Iharos, 1963 are, in my opinion, yet undescribed species of the genus *Pseudechiniscus* and should be considered as *nomina dubia* until the investigation of the type material of these forms is performed. *Pseudechiniscus novaezeelandiae* forma *marinae* Bartoš, 1934 should be re-evaluated as a bona species *Pseudechiniscus marinae* Bartoš, 1934.

#### *Pseudechiniscus jiroveci* Bartoš, 1963

This species was described by Bartoš (1963) from China based on five specimens at the larval stage (a two-clawed larva = first instar). Later, specimens attributed to this species were reported from Africa (Binda, 1984; Pilato *et al.*, 1991), Hawaii (Binda & Pilato, 1994), Mongolia (Kaczmarek & Michalczyk, 2006) and China (Kaczmarek & Beasley, 2002; Beasley *et al.*, 2006). The only character on which the attribution of these specimens to *P. jiroveci* was based, was a clearly divided posterior part of the scapular plate. However, this character is common in the genus *Pseudechiniscus*, and in the absence of other characters, cannot be used for the species determination. Unfortunately, postembryonic development of the taxonomically significant traits is completely unknown in *Pseudechiniscus*, and recent studies on other tardigrade groups show clearly that ontogenetic variability may lead to significant errors

in species identification (Morek *et al.*, 2016), thus the attribution of any adult specimens to the species described by Bartoš should be considered as doubtful. An investigation of the specimens from Mongolia (Kaczmarek collection) and Africa (Pilato and Binda collection) suggests that they represent two different undescribed species. Taking into account that the original description of *P. jiroveci* is incomplete and the type material is lost (*P. Gąsiorek*, pers. comm.), I suggest designating *P. jiroveci* as *nomen dubium* in order to avoid erroneous identification of new taxa.

#### *Pseudechiniscus bartkei* Węglarska, 1962

This species was described by Węglarska (1962) from Vietnam. The presence of a trilobed median plate 1 is sometimes considered to be a distinctive character for this species (e.g. in Kendall-Fite & Nelson, 1996). This seems to be the result of an erroneous attribution of Węglarska's drawings in the authoritative 20<sup>th</sup>-century monographs on tardigrade taxonomy (Ramazzotti, 1972; Ramazzotti & Maucci, 1983). In fact, a trilobed first median plate is characteristic of *P. bartkei* forma *unilobata* described in the same publication. This form may actually belong to another, undescribed species because of its differences from the nominative form of *P. bartkei* (a single median process of the pseudosegmental plate instead of two, and longer cirrus A).

#### *Pseudechiniscus suillus* (Ehrenberg, 1853)

This species is the type species of the genus and at the same time the most problematic taxon in *Pseudechiniscus*. Its original description is short and incomplete, and the original drawing by Ehrenberg (1854) lacks taxonomically important details. As a result, none of the existing records of *P. suillus* can be considered valid. Until this species is redescribed, based on new material from the *locus typicus*, no specimens can be attributed to *P. suillus* with certainty.

## CONCLUSION

Tardigrades are traditionally considered as a group with a relatively small set of morphological characters. However, a system of highly diversified cuticular plates, and a progressive development of the body appendages in the family Echiniscidae, provide the possibility to perform a morphological analysis of the diversity within this group. Even though molecular methods are crucial in resolving phylogenetic relations (e.g. Guidetti *et al.*, 2005, 2016; Sands *et al.*, 2008; Bertolani *et al.*, 2014; Dabert *et al.*, 2014; Cesari *et al.*, 2016; Vecchi *et al.*, 2016; Zawierucha *et al.*, 2018; Gąsiorek *et al.*, 2019a, b), morphological analysis remains a tool for the study

of diversity (e.g. Guidetti *et al.*, 2000; Jørgensen, 2000; Miller *et al.*, 2012; Gąsiorek *et al.*, 2018).

The analysis of the morphological traits used in the taxonomy of the genus *Pseudechiniscus* reveals a gap between the old and the modern species descriptions. In older descriptions, the number of features used for species discrimination was limited, and some of them were not clearly defined or illustrated; some descriptions were even based on immature specimens [see, for example, descriptions of *P. suillus* (Ehrenberg, 1853), *P. clavatus* (Mihelčič, 1955), *P. megacephalus* (Mihelčič, 1951) and *P. jiroveci* (Bartoš, 1963)]. Recent descriptions are usually more detailed, but in the absence of detailed redescrptions, comparison with older ones is often difficult, if not impossible. Moreover, in the case of many species, redescription requires the collection of fresh material because their type material was lost or has never been preserved. It is highly desirable that new species descriptions and redescrptions, based on the new material, have genetical data included.

Some of the characters, traditionally used in the taxonomy of the genus *Pseudechiniscus*, are ambiguous and should be clarified to avoid misidentifications. The term ‘faceted’ should be avoided or clearly defined when describing the structure of the cuticular plates, because it often does not match the original meaning. An analysis of the suitability of the term ‘faceting’ within different genera of the family Echiniscidae is outside the scope of this study. In my opinion, a true faceting is present only in some Echiniscidae species (see, for example: Schuster & Grigarick, 1971: fig. 3, SEM photo of *Diploechiniscus horningi*, or Michalczyk & Kaczmarek, 2006: figs. 4, 10, SEM photos of *Barbaria bigranulata*; both species were recently transferred from *Echiniscus* to new genera, see Gąsiorek *et al.*, 2019a), whereas in other echiniscid genera, the caudal plate is not faceted in Murray’s sense. It is interesting that even in the diagnosis of the genus *Pseudechiniscus*, Thulin (1911) stated: ‘Die Endplatte hat immer Kleeblattkerben aber keine Fazettierung’ [‘The end plate always has cloverleaf notches but no faceting’] (Thulin, 1911: 18). Thus, considerable differences in the structure of the caudal plate between the genera *Echiniscus* and *Pseudechiniscus* were revealed at the moment of the establishment of the genus *Pseudechiniscus*, but a wide, uncritical use of the term ‘faceted’ has rendered it almost useless.

The presence or absence of the anterior bifurcation of the ridges of the caudal plate and its configuration seem to be a constant character at the species level, but this hypothesis needs to be confirmed on more abundant material, including the comparisons of CLM and SEM images. It is not enough simply to record a divided or an undivided state of the median plates 1 and 2, because several modifications of their initial double

configuration may result in their appearance as paired or single. Their structure should be described based on the observations of several specimens, because their appearance depends on the degree of the compression of the specimen and its orientation on the slide.

The shape of the hindleg papilla should also be carefully investigated, using as many specimens as possible in order to reveal its true shape. The presence or absence of the lateral intersegmental plates and their number should be indicated, as well as the presence or absence and the pattern of the ventral reticular sculpture. In the latter case, a possible sexual dimorphism should be taken into account. The degree of the basal spur development on the claws of all legs should be documented, because the differentiation of the claws of the hind pair of legs seems to be a good species-delimiting character.

Striation connecting the dots of the cuticular sculpture seems to be a constant character within a given species, but its distribution within the genus *Pseudechiniscus* should be clarified. It may well be visible at the CLM level (e.g. in *P. bartkei*). At the same time, we cannot rule out the possibility that a well-developed striation may be manifested externally, in which case it would be visible in SEM. This is the case of *Acantechiniscus victor*, where striation can be seen as ridges on the cuticular plate surface (Vecchi *et al.*, 2016). On the other hand, striation may be weak and easy to overlook if the microscope is focused on the cuticular tubercles rather than the inner structure of the epicuticle.

The morphology of the cephalic papilla should be investigated carefully for all existing *Pseudechiniscus* species to confirm the hypothesis that there are two types of the organization of the head sensory structures: with protruding papillae and with papillae attached to the head surface along their lateral side (recognized as hemispherical or dome-shaped in CLM). Interestingly, a recent molecular investigation of the genus *Pseudechiniscus* has shown that it could be divided into two evolutionary lineages: the *P. novaezeelandiae* lineage and the ‘*P. suillus*–*facetialis* group’ lineage, with the structure of the cephalic papillae being an evident morphologically discriminating character (Cesari *et al.*, 2020). The protruding form of the cephalic papilla seems to correlate with the presence of the striation of the cuticular sculpture, but this hypothesis should be tested using SEM investigation of other *Pseudechiniscus* species.

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