

***Echiniscus pardalis* n. sp., a new species of Tardigrada (Heterotardigrada, Echiniscidae, *arctomys* group) from the Parco Naturale delle Alpi Marittime (NW Italy)**

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ABSTRACT

A new species of Tardigrada Doyère, 1840, *Echiniscus pardalis* n. sp., is described from two moss samples collected in the Parco Naturale delle Alpi Marittime (NW Italy). It belongs to the *Echiniscus arctomys* species-group, but differs from other 49 known members of the group mainly by the irregularly and distantly scattered deep pores on the plates and by a unique subsurface cuticular pattern on the plates, resembling that of a leopard's fur. The new species is most similar to eight species from the *arctomys* group: *E. barbarae* Kaczmarek & Michalczyk, 2002, *E. crebraclava* Sun, Li & Feng, 2014, *E. dearmatus* Bartoš, 1935, *E. mosaicus* Grigarick, Schuster & Nelson, 1983, *E. nigripustulus* Horning, Schuster & Grigarick, 1978, *E. nobilis* Mihelčič, 1967, *E. tardus* Mihelčič, 1951 and *E. vinculus* Horning, Schuster & Grigarick, 1978. The differences between the new species and these eight species are thoroughly discussed. They mainly concern the presence/absence and relative size of shallow dimples surrounding individual tubercles, the relative length of cirrus A, the presence/absence of a spine on the first pair of legs, the presence/absence of a spur on the internal/external claws and the number of teeth on the collar of the hind legs. This paper is the first result published on Tardigrada collected during the European Distributed Institute of Taxonomy's in All Taxa Biodiversity Inventories+Monitoring programme.

KEY WORDS
Tardigrada,
Heterotardigrada,
Italy,
Maritime Alps,
Echiniscus arctomys
group,
new species.

RÉSUMÉ

Echiniscus pardalis n. sp., une nouvelle espèce de Tardigrada (Heterotardigrada, Echiniscidae, groupe *arctomys*) du Parco Naturale delle Alpi Marittime (NW de l'Italie).

Une nouvelle espèce de Tardigrada Doyère, 1840, *Echiniscus pardalis* n. sp., est décrite à partir de deux échantillons de mousse récoltés dans le Parco Naturale delle Alpi Marittime (NW de l'Italie). Elle appartient au groupe d'espèces *Echiniscus arctomys*, mais elle diffère des 49 autres représentants connus de ce groupe par la disposition irrégulière et très espacée des pores profonds sur les plaques, et la présence, sous la surface des plaques, d'un patron cuticulaire original, qui ressemble à la fourrure d'un léopard. La

MOTS CLÉS
Tardigrada,
Heterotardigrada,
Italie,
Alpes-Maritimes,
Echiniscus arctomys group,
espèce nouvelle.

nouvelle espèce est proche de huit espèces du groupe *arctomys* : *E. barbarae* Kaczmarek & Michalczyk, 2002, *E. crebraclava* Sun, Li & Feng, 2014, *E. dearmatus* Bartoš, 1935, *E. mosaicus* Grigarick, Schuster & Nelson, 1983, *E. nigripustulus* Horning, Schuster & Grigarick, 1978, *E. nobilis* Mihelčič, 1967, *E. tardus* Mihelčič, 1951 et *E. vinculus* Horning, Schuster & Grigarick, 1978. Les différences entre la nouvelle espèce et ces huit autres espèces sont discutées en détail. Elles sont basées essentiellement sur les éléments suivants : présence/absence et taille relative des fossettes peu profondes entourant les tubercules isolés, longueur relative des cirres *A*, présence/absence d'épine sur la première paire de pattes, présence/absence d'un éperon sur les griffes internes/externes et nombre de dents sur la collerette des pattes postérieures. Cet article est la première publication sur les tardigrades réalisée par European Distributed Institute of Taxonomy dans le cadre des inventaires ATBI+M (inventaire biologique généralisé de la biodiversité et monitoring).

INTRODUCTION

The phylum Tardigrada Doyère, 1840 currently consists of about 1170 known species (Degma *et al.* 2014), of which about 980 are terrestrial or freshwater. The non-marine fauna of Italian tardigrades is relatively well known, with 226 species (valid according to Degma *et al.* 2014) being reported up to July 2014 (Pilato & Catanzaro 1989; Binda & Rebecchi 1992; Pilato & Rebecchi 1992; Bertolani & Rebecchi 1993, 1996; McInnes 1994; Bertolani *et al.* 1994a, b, 1996, 2011; Binda *et al.* 1995; Pilato & Binda 1997/98; Pilato *et al.* 2000, 2005a, 2014; Guidetti & Bertolani 2001; Pilato & Bertolani 2005; Marley *et al.* 2008; Pilato 2009; Lisi *et al.* 2014).

The European Distributed Institute of Taxonomy (EDIT) was a consortium of leading European, North American and Russian institutions devoted to the management of biodiversity data, formed in 2004. In the aim of the program "Applying Taxonomy to Conservation" (Workpackage 7), EDIT organized the "All Taxa Biodiversity Inventories+Monitoring" (ATBI+M) as an intensive community effort to identify and record all living species that exist within a given area. In 2007, the first European ATBI+M pilot site was established in the protected areas Parc national du Mercantour (SE France) and adjacent Parco Naturale delle Alpi Marittime (NW Italy), both in the Maritime Alps Mts.

In this publication, a new *Echiniscus* Schultze, 1840 species of the *arctomys* group, found in moss cushions within the Parco Naturale delle Alpi Marittime, Italy, is described.

MATERIAL AND METHODS

More than 300 moss samples from the ATBI+M site were collected and processed in 2007. Two of them contained specimens of a new *Echiniscus* species.

Each moss sample was placed into a labelled small paper bag immediately after its collection and was then slowly air-dried. Their geographical coordinates were measured using GPS-equipment Garmin GPSmap 60CSx.

In the laboratory, each dried sample was rehydrated by adding spring water (Volvic®, Danone Waters, Wiesbaden,

Germany) and extracted specimens were directly mounted in polyvinyl lactophenol medium (Chroma, Münster, Germany) without any previous fixation. The body colour or presence of eye spots were not observed before or promptly after slides preparation. Coverslips were ringed with nail varnish.

The slide-mounted material was examined, measured and photographed using light microscopes: an Olympus BX51 light microscope with phase contrast (PhC; lenses 40× and 100× oil immersion) and Nomarski differential interference contrast (DIC; 100× oil immersion lens) equipped with CCD ColorView III FW digital camera (operated with AnalySIS Five software); and a Leica DM2500 with DIC (40× lens) equipped with a Leica DFC 290 HD camera (operated with LAS 3.5.0 software). Photomicrographs were finally adjusted using Adobe Photoshop CS3 Extended V.10.0 software.

Body lengths were measured in a straight line from the anterior extremity to the end of the body, excluding hind legs, or, when the body was bent, along the curved line of its median axis. Reduced body length (LUT in Lattes & Gallelli 1972) was measured along the median antero-caudal straight line between the anterior margins of the scapular and terminal plates. Measurements of all structures were taken using a 100× lens with DIC, so that they would not be affected by the halo effect of PhC. All cirri were measured without cirriphori as lengths of arcs. Scapular plate length was measured along the median antero-caudal line. Claw lengths were measured from the centre of the base to the top level of apical curvature along the basal part of the claw as the length of a straight line segment. Claw spur lengths were measured as the length of a line segment between the distal point of their connection with the claw from the claw base to the top of the spur. The position of a spur on a claw was measured as the distance from its distal connection point to the base of the claw. Each character was measured only when its position was suitable (i.e. focus changes during measuring were not significant). Morphometric indices *pb* and *psc* were calculated for structures according to Fontoura & Morais (2011).

The new species was compared with all known *Echiniscus* species described up to July 2014, using original and amended descriptions and those of Ramazzotti & Maucci (1983) as translated by Beasley (1995).

SYSTEMATICS

Family Echiniscidae Thulin, 1928

Genus *Echiniscus* Schultze, 1840*Echiniscus pardalis* n. sp.

(Figs 1-4; Tables 1-3)

TYPE LOCALITY. — North-western Italy, Maritime Alps, Parco Naturale delle Alpi Marittime, EDIT ATBI+M focal site 2a – Vallone di Brocan valley, about 1100 m SE of the hydroelectric dam of Lago del Chiotas, GPS position 44°09'44.9"N 07°20'36.9"E-07°20'37.7"E, 2063-2064 m asl.

TYPE MATERIAL. — **Holotype:** from moss sample (*Polytrichum piliferum* Hedw.) on a rock, GPS position 44°09'44.9"N 07°20'37.7"E, position accuracy ± 27 m, 2063 m asl., collected 05.VIII.2007, leg. R. Schill (slide with the label "# 71c, 9449 377"). **Paratypes:** 6 specimens from same sample as the holotype (3 paratypes on slide "# 71a, 9449 377" and 3 paratypes on slide "# 71b, 9449 377") and 5 specimens from another moss sample from a rock, GPS position 44°09'44.9"N 07°20'36.9"E, position accuracy ± 27 m, 2064 m asl, 05.VIII.2007, leg. R. Schill (3 paratypes on slide "# 70a, 9449 369" and 2 paratypes on slide "# 70c, 9449 369").

TYPE DEPOSITORIES. — The type material is deposited in the collection of Roberto Bertolani (Department of Life Sciences, University of Modena and Reggio Emilia, Italy – holotype and 5 paratypes on slides "70a" and "70c") and in the collection of Maria Grazia Binda & Giovanni Pilato (Department of Animal Biology "Marcello La Greca", University of Catania, Italy – 6 paratypes on slides "71a" and "71b").

ETYMOLOGY. — The species name refers to the similarity of the subsurface dorsal plates pattern (when observed using 100 $\times$ PhC) to a leopard's spots.

DIAGNOSIS

Echiniscus species with lateral cirri in position A, shorter than half of body length. Other appendages absent with the exceptions of cephalic cirri, clava and sensorial papilla on hind legs. All dorso-lateral plates undivided and without facetation. Each lateral side of median plates 1 and 2 with joined lateral platelet visible in small specimens. Median plate 3 and ventral plates absent. Surface of cuticular plates (with the exception of the neck plate, leg plates and lateral platelets) with flat polygonal tubercles arranged in the manner of tiling. Narrow space between individual tubercles with a crown of shallow dimples. Unequal sparse deep pores irregularly distributed on plates. Under the cuticle surface, the perimeter of each tubercle is reinforced by a rosette of cuticular pillars, visible as dark spots (in PhC) and forming a pattern resembling that of a leopard (sometimes also their interior with several pillars, especially under larger tubercles). Spine or papilla absent on the first three pairs of legs. Papilla and dentate collar present on hind legs. All internal claws with well developed protruding spur.

DESCRIPTION OF THE HOLOTYPE

Adult female, orientated ventro-laterally. Body of prepared holotype indistinctly reddish and without visible eye spots, 258.1 μ m long (Fig. 1).

Internal cephalic cirrus 11.9 μ m long (length of cirrophorus 1.9 μ m) and external cephalic cirrus 17.2 μ m long (cirrophorus 1.8 μ m long). Broad cephalic papilla with broadly rounded apex 7.7 μ m long, with basal diameter 3.6 μ m and maximal width 5.4 μ m half-way along its length. Lateral cirrus A in the shape of a filament, 76.6 μ m long (29.7% of body length; its cirrophorus 3.5 μ m long). Clava with rounded apex, 6.8 μ m long, with basal diameter 2.2 μ m and maximal width 2.9 μ m in its basal third (Fig. 2A, B). Apart from cirrus A, no other trunk appendages present (Fig. 1).

Dividing line between cephalic plate and neck plate weakly visible in the holotype. Scapular plate 40.4 μ m long and without any ridges (Fig. 2C). Segmental paired plates II and III shortest along saggital line. Posterior margin of segmental plate II forming a distinct angle ($c. 150^\circ$) in its lateral third on both sides, while the posterior margin of segmental plate III is moderately arched (Fig. 2D, E). Terminal plate with two postero-lateral incisions, 12.4 μ m long, without facetation and with indistinct depression in central part of plate (Fig. 2F-H). Cephalic, neck, scapular, median 1, median 2 and terminal plates undivided. Four narrow lateral platelets apparent, each connected with lateral margin of median plate 1 or median plate 2, but these have faint edges in the holotype (Fig. 1). Median plate 3 (Figs 1; 2F-H) and ventral plates absent. Anterior and posterior margins of dorso-lateral plates well marked (with the exception of neck plate and lateral platelets), but lateral margins marked only by their sculpture. Similarly, edges of leg plates indistinct in the holotype, especially for the first three pairs of legs (Fig. 1).

Sculpture of surface of plates (with some exceptions described below) consists of simple, rather flat, polygonal (mostly hexagonal) tubercles up to 1.9 μ m in diameter (up to 1.3 μ m on cephalic plate, both median plates 1 and 2 and both segmental plates, up to 1.6 μ m on scapular plate, largest on caudal plate), densely spaced in a manner like tiling. This sculpture has the appearance of a system of dark patches when using a 40 $\times$ PhC lens. Similar, but less distinct, sculpture in narrow areas between median plate 1 and segmental paired plate II, between median plate 2 and segmental paired plate III and in area corresponding to median plate 3 (Fig. 1). Only tiny granulation (0.2-0.4 μ m in diameter), instead of tubercles, covers a narrow transverse band on the neck plate just before the scapular plate, the posterior margin and posterior corners of the sculptured field on the cephalic plate, the whole of the lateral platelets (where the granulation is only faint) and the leg plates on outer surface of legs. Anterior notch in the shape of an hourglass on the cephalic plate; rest of neck plate, narrow posterior margins of both median plates 1 and 2 and of segmental paired plate II and wider anterior margins of both segmental paired plates II and III unsculptured (Figs 1B; 2A, C-H; 4C).

Deeper focusing an oil immersion 100 $\times$ lens on a space separating surface tubercles reveals a crown of round, not perfectly focusable in PhC (apparently just weakly sloped), shallow dimples (0.2-0.5 μ m in diameter) that form a more or less distinct hexagonal pattern around each tubercle (Fig. 2G, H). Apart from above mentioned surface sculpture (large tubercles

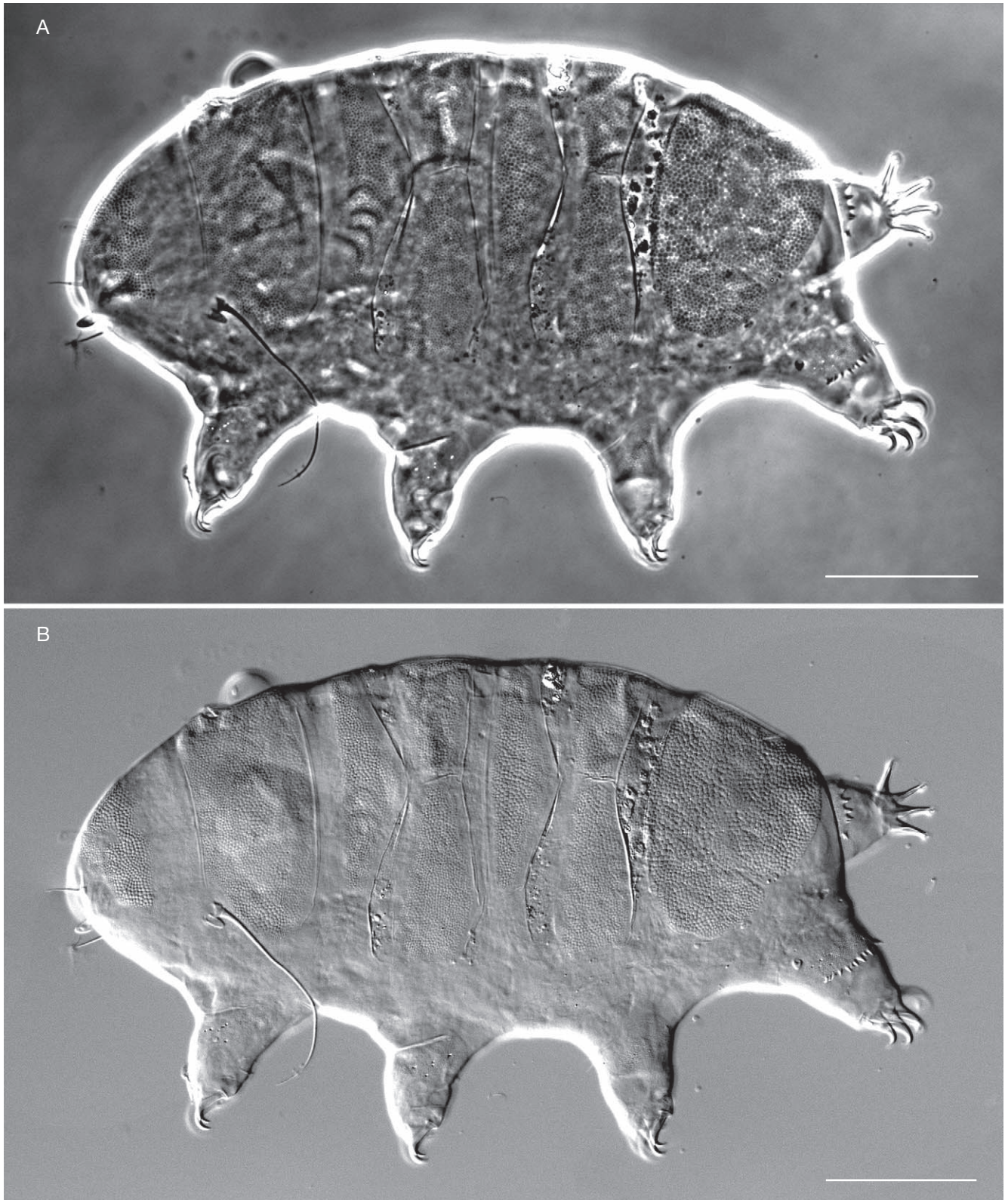


FIG. 1. — *Echiniscus pardalis* n. sp., habitus of holotype: **A**, PhC; **B**, DIC. Scale bars: 50 µm.

and tiny granulation), there are very irregularly and widely distributed deep pores of different sizes (having an appearance of round or elongated white spots in PhC), up to 1.3 µm, on

the plates with the exception of the leg plates, on which there is a small group of about 5-7 pores on each plate (Figs 1; 4C). When a 100× PhC oil immersion lens is focused under the

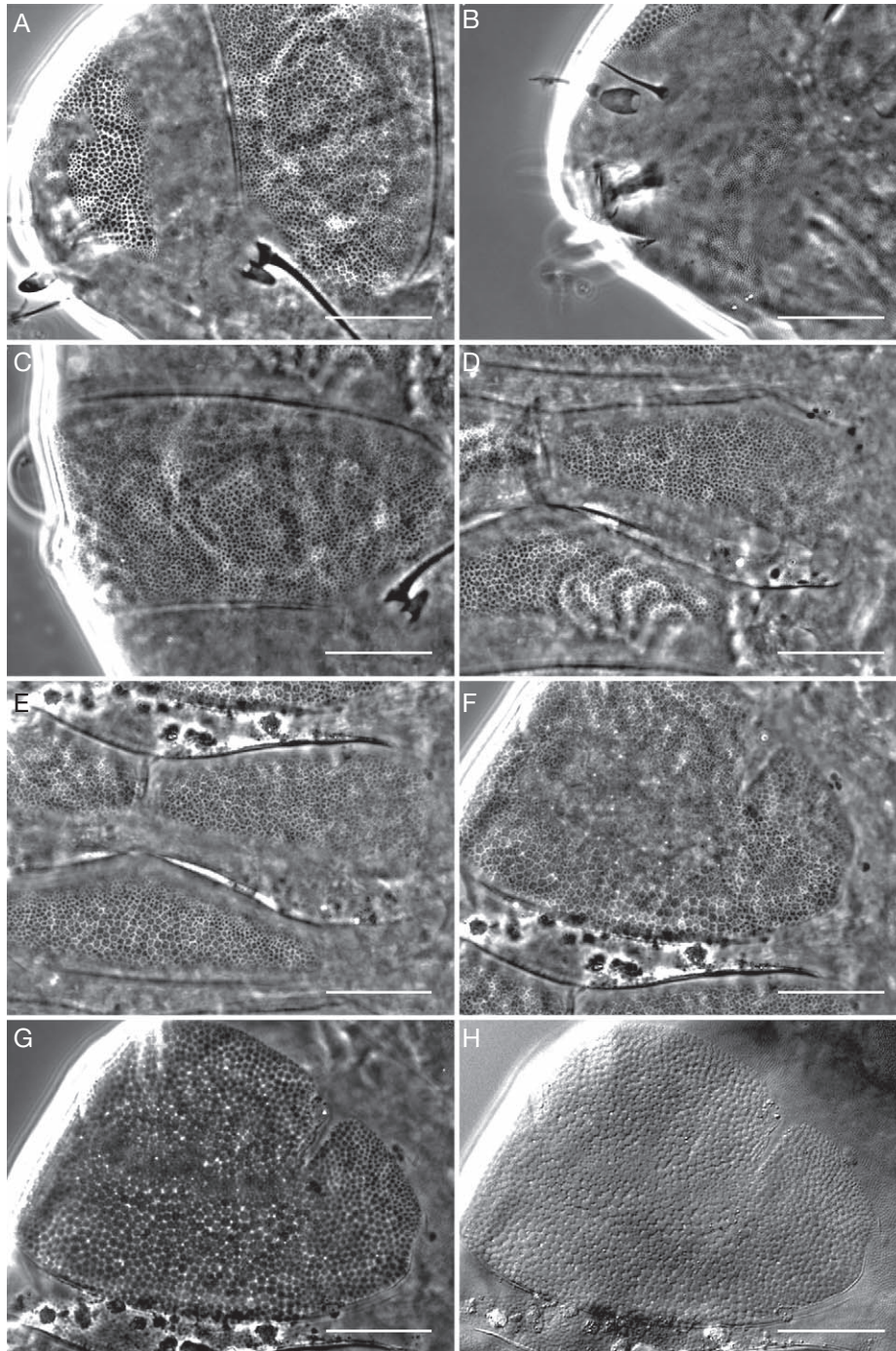


FIG. 2. — *Echiniscus pardalis* n. sp., holotype: **A**, cephalic papilla, clava and surface sculpture of cephalic plate (dorso-lateral view), PhC; **B**, cephalic cirri, cephalic papilla and cuticular pillars in ventral cuticle (ventro-lateral view), PhC; **C**, scapular plate, PhC; **D**, median plate 1 and segmental plate II (note its angular posterior margin), PhC; **E**, median plate 2 and segmental plate III, PhC; **F**, area corresponding to median plate 3 and caudal plate, PhC; **G, H**, shallow dimples around tubercles on caudal plate; **G**, PhC; **H**, DIC. **C-F**, focused on subsurface structures and posterior body part orientated towards upper side of pictures; **G-H**, focused on surface of plate. Scale bars: 20 µm.

surface of the plates, the above-mentioned shallow dimples, as well as deeper pores, disappear and the subsurface structure of each tubercle appears in the form of a rosette of several more or less round and well-focusable dark spots under its perimeter. Some other spots can also be present in a space delimited by the peripheral spots, especially under larger tubercles (about 0.2 µm

in diameter, about 1-10 spots per tubercle, depending on its size) (Fig. 3). These spots are apparently cuticular pillars that reinforce the tubercles internally. Rest of cuticle, including that of ventral body side, has an appearance of tiny, dense and regularly distributed dots not protruding above the cuticle surface (thus formed by cuticular pillars; 0.1-0.2 µm in diameter) (Fig. 2B).

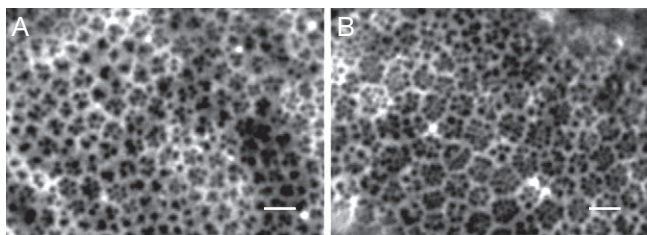


FIG. 3. — *Echiniscus pardalis* n. sp., details of subsurface structure of plates, holotype, PhC: A, scapular plate; B, caudal plate. Scale bars: 2 µm.

Leg claws well developed, straight in their basal portion, bent in their distal third and thereafter in a fluent arc to their apex (Fig. 4A, B). External claws slightly shorter than internal ones, claws on the hind legs longest (external and internal claws on the first pair of legs 11.6 and 12.4 µm long, respectively, those of fourth pair of legs 13.7 and 15.6 µm long, respectively). Internal claws of all legs with a well-developed, rather protruding spur orientated downwards (4.1 µm long on first legs, 4.8 µm long on hind legs, angle between claw and spur $c.$ 45°), situated at about $\frac{2}{3}$ of claw height (spurs of first and fourth pairs of legs respectively 4.4 µm and 6.8 µm from base, representing 36% and 44% of claw height respectively) (Fig. 4A, B). All external claws without spurs. First three pairs of legs without spine or papilla. Broadly drop-shaped papilla with rounded apex (3.5 µm long, with maximal width 3.0 µm) and a dentate collar with 8 and 12 tall triangular sharp teeth of unequal height (1.2–3.5 µm; each tooth always higher than broad) and unequally spaced (however, the space between adjoining teeth is always shorter than the width of their base) present on fourth pair of legs (Fig. 4C).

Eggs unknown.

VARIABILITY

Paratypes similar to the holotype, except the edges of their neck plates and lateral platelets, which are much more distinct in paratypes than in the holotype (Fig. 4D, E).

The variability of specimens manifests itself primarily in the arrangement and density of deep pores (perforations) on plates, which is diverse without any common pattern (e.g., pores in Fig. 4E).

In both the paratypes orientated in a lateral position (slides “70a” and “71b”) lateral continuations of both median plates 1 and 2 are well visible in the form of lateral platelets (one on each side of each median plate 1 and 2) although they fluently join with the rest of cuticle in their lower end, without a distinct margin. Anterior margin of first pair of these lateral platelets is angular (Fig. 4E). Similarly, leg plates appear to be well delimited in these laterally orientated paratypes. However, it should be noted that all paratypes have more or less crumpled cuticle around the plates and for this reason it is difficult to decide whether a clearer delimitation of leg plates, as well as lateral platelets, is natural in small specimens (all paratypes are distinctly smaller than the holotype with body length 113.7–183.3 µm) or if it is instead a consequence of deformations.

Measurements and morphometric indices of the holotype and all paratypes are given in Tables 1–3.

DISCUSSION

The genus *Echiniscus* Schultze, 1840, includes 156 species and six subspecies as of July 2014 (Degma *et al.* 2014). Among these species, only those of the *E. viridis* group and the *E. arctomys* group *sensu lato* (i.e. as defined by Ramazzotti & Maucci 1983) lack lateral, dorso-lateral or dorsal trunk appendices other than cirri in position A. It is worth noting that *E. mauccii* Ramazzotti, 1956, which has two pairs of lateral hemispherical or slightly conical projections, was not included in either of these two groups (Ramazzotti & Maucci 1983: 335).

Currently, the *Echiniscus viridis* group consists of six species, including *E. rufoviridis* du Bois-Reymond Marcus, 1944 and *E. viridissimus* Péterfi, 1956, both of which have an unclear affiliation with the group. The cuticle of members of the *viridis* group is characterised by plate ornamentation comprised of dark tubercles, fine dots and light spots, as well as by the green colour, although this is not restricted to species of this group (Pilato *et al.* 2007, 2008; Fontoura *et al.* 2011).

The *Echiniscus arctomys* group *sensu lato* is composed of 49 known species: 27 species included in Ramazzotti & Maucci (1983: 335; another species in their list, *E. robertsi* Schuster & Grigarick, 1965, cannot belong to the group because it has lateral appendages in two positions) and 22 other species, viz. *E. aliquantillus* Grigarick, Schuster & Nelson, 1983, *E. barbarae* Kaczmarek & Michalczyk, 2002, *E. bisculptus* Maucci, 1983, *E. charrua* Claps & Rossi, 1997, *E. cirinoi* Binda & Pilato, 1993, *E. corrugicaudatus* McInnes, 2009, *E. crebraclava* Sun, Li & Feng, 2014, *E. ganczareki* Michalczyk & Kaczmarek, 2007, *E. jenningsi* Dastych, 1984, *E. latifasciatus* Dudichev & Biserov, 2000, *E. madonnae* Michalczyk & Kaczmarek, 2006, *E. malpighii* Biserov, 1994, *E. marginoporus* Grigarick, Schuster & Nelson, 1983, *E. mosaicus* Grigarick, Schuster & Nelson, 1983, *E. ollantaytamboensis* Nickel, Miller & Marley, 2001, *E. palmai* Dastych, 1997, *E. pseudelegans* Séméria, 1994, *E. pseudowendti* Dastych, 1984, *E. quitensis* Pilato, 2007, *E. rackae* Dastych, 1986, *E. tamus* Mehlen, 1969 and *E. walteri* Pilato & Lisi, 2003 (Mehlen 1969; Maucci 1983; Grigarick *et al.* 1983; Dastych 1984, 1986, 1997; Binda & Pilato 1993; Biserov 1994; Séméria 1994; Claps & Rossi 1997; Dudichev & Biserov 2000; Nickel *et al.* 2001; Kaczmarek & Michalczyk 2002; Pilato & Lisi 2003; Michalczyk & Kaczmarek 2006, 2007; Pilato 2007; McInnes 2009; Sun *et al.* 2014). Cuticular ornamentation in the *arctomys* group *sensu lato* is very diverse and some individual species groups were separated from the group after publication of Ramazzotti & Maucci’s (1983) monograph: the *E. bigranulatus* group, characterized by “double sculpturing” of cuticular plates; the *E. reticulatus* group, with a reticulate cuticular pattern; and the *E. tessellatus* group, with tessellating patterns (McInnes 2009). In addition, McInnes (2009: Table 3) defined the *E. arctomys* group *sensu stricto* as a residual group of 21 species after separation



FIG. 4. — *Echiniscus pardalis* n. sp.: **A**, claws of first pair of legs, holotype, PhC; **B**, claws of hind legs, holotype, PhC; **C**, papilla and dentate collar on hind legs, holotype, DIC; **D**, cephalic plate and neck plate (note clear delimitation of neck plate), paratype on slide "71a", DIC; **E**, lateral view (note distinct neck plate, angular posterior margin of segmental plate II and both lateral platelets with angular anterior margin of the first one; white spots are deep punctures in the cuticle), paratype on slide "71b", PhC. Scale bars: A-D, 20 μ m; E, 40 μ m.

of the *bigranulatus*, *reticulatus* and *tesselatus* groups from the *arctomys* group *sensu lato*, but it is necessary to point out that *reticulatus* and *tesselatus* groups have not been clearly defined and their composition remains uncertain. As she stressed, the redefined *arctomys* group requires major revision because species with quite different plate ornamentation are attributed to this group. We note that at least *E. cirinoi* Binda & Pilato, 1993 should be transferred to the *reticulatus* group, since it has the same cuticular sculpture as *E. reticulatus* Murray, 1905 (see Binda & Pilato 1993). The *arctomys* group *s.s.*, together with the *bigranulatus* group with its seven known species (Michalczyk & Kaczmarek 2007; McInnes 2009) account for

28 species of the former *arctomys* group *s.l.*, thus the remaining 21 species are not clearly attributed to either of the leftover species complexes (*reticulatus* and *tesselatus* groups). Because of these ambiguities we do not consider the *reticulatus*, *tesselatus* and *arctomys s.s.* species groups well defined, hence we compared *E. pardalis* n. sp. separately with the adequately defined *E. viridis* and *E. bigranulatus* species groups, as well as with the remaining 42 species of the *E. arctomys* group *sensu lato*.

Only three species of the *Echiniscus viridis* group (*E. clavispinosus* Fontoura, Pilato & Lisi, 2011, *E. perviridis* Ramazzotti, 1959 and *E. viridianus* Pilato, Fontoura & Lisi, 2007) have the surface of plates covered with tubercles having mutual

TABLE 1. — Measurements of selected morphological structures of type specimens of *Echiniscus pardalis* n. sp. Abbreviations: **N**, number of specimens/structures measured; **Min/Max**, smallest and largest values for the structure; **SD**, standard deviation; *, 10 collars in 5 specimens (holotype and 4 paratypes).

Character	N	Min	Max	Mean	SD	Holotype
Body length (µm)	12	113.7	258.1	159.85	36.77	258.1
Reduced body length (µm)	10	71.9	154.9	102.05	26.14	154.9
Internal cephalic cirrus length (µm)	8	8.2	12.8	9.86	1.75	11.9
Cephalic papilla length (µm)	9	5.5	7.7	6.63	0.66	7.7
External cephalic cirrus length (µm)	9	10.9	17.2	13.82	1.87	17.2
Clava length (µm)	11	4.2	7.6	6.10	0.99	6.8
Cirrus A length (µm)	2	47.0	76.6	61.78	20.92	76.6
Scapular plate length (µm)	10	28.3	40.9	34.09	4.98	40.4
Terminal plate incision length (µm)	3	12.4	13.8	12.99	0.73	12.4
Internal claw of leg I length (µm)	10	10.1	13.1	11.79	1.11	12.4
External claw of leg I length (µm)	11	9.5	12.8	10.78	1.02	11.6
Internal claw of leg IV length (µm)	9	11.3	17.4	14.21	1.98	15.6
External claw of leg IV length (µm)	8	9.8	15.4	13.09	1.86	13.7
Spur on internal claw I length (µm)	12	2.6	4.5	3.78	0.56	4.1
Spur on internal claw IV length (µm)	10	3.9	6.6	4.95	0.82	4.8
Papilla on leg IV length (µm)	8	2.6	4.5	3.55	0.58	3.5
Number of teeth on leg IV collar	*10	8.0	12.0	9.60	1.26	8+12
Cirrus A/Body length ratio (%)	2	29.7	36.1	32.86	4.52	29.7
Cirri int./Cirri ext. lengths ratio (%)	7	58.9	86.2	70.90	8.98	69.2
Cirri int./Cirri A lengths ratio (%)	2	15.5	17.9	16.74	1.67	15.5
Cirri ext./Cirri A lengths ratio (%)	2	22.4	25.7	24.13	2.27	22.4
Scapular plate/Body lengths ratio (%)	6	15.7	23.3	21.20	2.87	15.7
Spur I/Claw I lengths ratio (%)	10	23.9	39.0	31.59	4.65	33.1
Spur IV/Claw IV lengths ratio (%)	9	30.8	38.8	35.56	2.50	30.8

TABLE 2. — Values of morphometric index *pb* of *Echiniscus pardalis* n. sp. type specimens as the percent ratio between the measurement of a given structure and the reduced body length. Abbreviations: **N**, number of indices calculated; **Min/Max**, smallest and largest values; **SD**, standard deviation.

Character (%)	N	Min	Max	Mean	SD	Holotype
Internal cephalic cirrus length	6	7.7	12.5	9.93	1.86	7.7
Cephalic papilla length	9	4.9	8.4	6.63	1.31	5.0
External cephalic cirrus length	7	9.8	16.9	13.15	2.77	11.1
Clava length	9	4.4	8.8	6.12	1.68	4.4
Cirrus A length	2	49.4	65.3	57.37	11.22	49.4
Internal claw of leg I length	8	8.0	15.0	12.35	2.90	8.0
External claw of leg I length	9	7.5	14.4	10.97	2.67	7.5
Internal claw of leg IV length	8	10.1	20.1	14.21	3.78	10.1
External claw of leg IV length	7	8.8	17.6	13.12	3.56	8.8
Spur on internal claw I length	10	2.6	5.2	3.81	0.80	2.6
Spur on internal claw IV length	8	3.1	7.6	5.05	1.50	3.1
Papilla on leg IV length	7	2.3	5.2	3.43	0.90	2.3

TABLE 3. — Values of morphometric index *psc* of *Echiniscus pardalis* n. sp. type specimens as the percent ratio between the measurement of a given structure and the scapular plate length. Abbreviations: **N**, number of indices calculated; **Min/Max**, smallest and largest values; **SD**, standard deviation.

Character (%)	N	Min	Max	Mean	SD	Holotype
Reduced body length	9	236.6	383.4	295.25	41.39	383.4
Internal cephalic cirrus length	7	24.9	35.8	29.59	3.49	29.5
Cephalic papilla length	8	15.2	23.0	18.90	2.52	19.1
External cephalic cirrus length	8	31.0	52.2	40.22	6.33	42.6
Clava length	9	13.8	23.8	17.34	3.58	16.8
Cirrus A length	2	154.5	189.6	171.96	24.64	189.6
Internal claw of leg I length	8	28.3	43.4	35.12	5.50	30.7
External claw of leg I length	9	24.6	42.6	31.90	5.82	28.7
Internal claw of leg IV length	7	33.6	54.3	39.49	7.16	38.6
External claw of leg IV length	6	30.4	51.1	36.56	7.68	33.9
Spur on internal claw I length	10	8.7	14.8	11.36	1.95	10.2
Spur on internal claw IV length	8	11.9	18.1	13.83	2.30	11.9
Papilla on leg IV length	6	8.7	9.9	9.30	0.54	8.7

distances shorter than their diameter, as in *E. pardalis* n. sp. (Ramazzotti 1959; Pilato *et al.* 2007; Fontoura *et al.* 2011). However, the new species differs from them mainly by the absence of numerous small light spots covering tubercles and in the presence of sparsely distributed deep pores (Ramazzotti 1959; Pilato *et al.* 2007; Fontoura *et al.* 2011).

Species of the *Echiniscus bigranulatus* group have typical “double granulation” on most of the dorsal plates. Actually, the simultaneous presence of surface pseudopores and/or pores together with subsurface cuticular pillars is the reason for this characteristic appearance in a light microscope. Regularly and densely distributed pillars are visible as dark dots and randomly distributed pseudopores and/or pores as light spots when observed at different depths of focus (Michalczyk & Kaczmarek 2006). *Echiniscus pardalis* n. sp. strongly differs from all known species of the *bigranulatus* group in having tubercles as a main structural element of the surface of plates (plates completely without tubercles, at most having granulation on very restricted areas in the *bigranulatus* group); in the quite differently arranged shallow surface dimples (pseudopores in the terminology of Michalczyk & Kaczmarek 2006) and pores; in having subsurface cuticular pillars in the plates (pseudopores are arranged in circles around tubercles in the new species, whereas they are not so distributed in the *bigranulatus* group, the pores are very sparse in the new species, whereas they are more regular and significantly more numerous in the *bigranulatus* group, and the cuticular pillars on the plates are arranged under tubercles only, as opposed to being densely and regularly distributed in species of the *bigranulatus* group) (Dastyh 1984; Michalczyk & Kaczmarek 2006, 2007).

Echiniscus pardalis n. sp. is rather similar to eight species from the rest of *arctomys* group *sensu lato* that have 1) surface of plates mainly covered with tubercles that are more or less densely arranged; 2) plates without groups of true granules, as for example in *Echiniscus reticulatus* Murray, 1905 (see Binda & Pilato 1993); 3) scapular and caudal plates without any subdivision, found in *E. tessellatus* Murray, 1910 and similar species (see Murray 1910); and 4) caudal plate without any ridges, such as in *E. corrugicaudatus* (see McInnes 2009); *E. barbara*e, *E. crebraclava*, *E. dearmatus* Bartoš, 1935, *E. mosaicus*, *E. nigripustulus* Horning, Schuster & Grigarick, 1978, *E. nobilis* Mihelčič, 1967, *E. tardus* Mihelčič, 1951 and *E. vinculus* Horning, Schuster & Grigarick, 1978 (Bartoš 1935; Mihelčič 1951, 1967; Horning *et al.* 1978; Grigarick *et al.* 1983; Kaczmarek & Michalczyk 2002; Pilato *et al.* 2005b; Sun *et al.* 2014). The new species strongly differs from these eight species in having scattered deep pores on the plates and by the subsurface ornamentation pattern of the plates arranged like a leopard's spots. Moreover, *E. pardalis* n. sp. differs from these species in the following characters:

- from *E. barbara*e (known only from Cuba) by having pseudopores around tubercles (pores or pseudopores absent in *E. barbara*e), in lacking a spine on the first pair of legs (present in *E. barbara*e) and by the relatively longer cirrus *A* (longer than 20% of body length in the new species, but shorter than 20% of body length in *E. barbara*e) (Kaczmarek & Michalczyk 2002);

- from *E. crebraclava* (known only from China) by the absence of a spine on the first pair of legs (present in *E. crebraclava*) and by the more numerous teeth on the hind legs collar (8–12 teeth in the new species, *versus* 7 in *E. crebraclava*) (Sun *et al.* 2014);

- from *E. dearmatus* (described from Slovakia and later found in Canada and Argentina, see McInnes 1994) by the absence of a spine on the first pair of legs (present in *E. dearmatus*), the absence of spurs on the external claws (present on both internal and external claws of *E. dearmatus*) and by the shape of the plate ornamentation (according to Guil *et al.* 2013: 732, *E. dearmatus* belongs to the *Echiniscus blumi-canadensis* complex, with which it shares a similar granulation; for the shape of that granulation see Guil *et al.* 2013: fig. 1B) (Bartoš 1935; Guil *et al.* 2013);

- from *E. mosaicus* (known only from Venezuela) by the absence of a spine on the first pair of legs (present in *E. mosaicus*), a relatively longer cirrus *A* (longer than 20% of body length in the new species and shorter than 20% of body length in *E. mosaicus*) and by the relatively smaller diameter of dimples surrounding tubercles (their diameter is evidently smaller than that of tubercles in the new species, whereas it is nearly same as that of the tubercles in *E. mosaicus*, in which the dimples have a diameter of 1–2.8 µm, while the tubercles have a diameter 2–3 µm on the scapular plate) (Grigarick *et al.* 1983);

- from *E. nigripustulus* (known only from New Zealand) by the relatively smaller diameter of dimples surrounding tubercles (diameter of dimples evidently smaller than that of tubercles in the new species, whereas the diameters are nearly the same in *E. nigripustulus*; compare Fig. 2G with Pilato *et al.* 2005b: fig. 9A) and by the absence of any sensory organ on the first pair of legs (first pair of legs with a small papilla in *E. nigripustulus*) (Horning *et al.* 1978; Pilato *et al.* 2005b);

- from *E. nobilis* (known only from Slovenia and Italy) by having cirrus *A* shorter than 50% of the body length (cirrus *A* is longer than 50% of the body length in *E. nobilis*), in having spurs on internal claws only (*E. nobilis* has spurs on external claws only) and by having incisions on the terminal plate (absent in *E. nobilis*) (Mihelčič 1967); the description of cuticular sculpture of *E. nobilis* is somewhat confusing and we are not sure if the large surface elements forming the appearance of tiling (Mihelčič 1967: fig. 2C) are tubercles or depressions – unfortunately the type material of this species has been lost according to Dastyh (1993);

- from *E. tardus* (only known from Austria; see Mihelčič 1952) by having pseudopores around tubercles (pores or pseudopores are not present in *E. tardus*), in the different shape of the claws (stout and straight in the new species, *versus* slim and sickle-shaped in *E. tardus*), by the absence of small cuticular projections in the place of eye spots (present in *E. tardus*) and by the different shape of the cephalic cirri (straight in the new species, whereas the cephalic cirri almost form an arch over the cephalic papilla in *E. tardus*) (Mihelčič 1951);

- from *E. vinculus* (known only from New Zealand) by not having smooth areas on scapular and terminal plates (compare Figure 2C, G with Pilato *et al.* 2005b: fig. 1A, B) and by the generally less numerous teeth on hind legs collar (8–12 teeth

in the new species and 11-15 in *E. vinculus*) (Horning *et al.* 1978; Pilato *et al.* 2005b).

In any case, the presence of only lateral body appendages in position *A* together with the unique leopard's coat-like cuticular pattern under the surface of the plates allows *Echiniscus pardalis* n. sp. to be easily distinguished from all other known species of the genus *Echiniscus*.

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