Echiniscidae in the Mascarenes: the wonders of Mauritius

Yevgen Kiosya¹, Katarzyna Vončina², Piotr Gąsiorek²

1 School of Biology, V. N. Karazin Kharkiv National University, Svobody Sq. 4, 61022 Kharkiv, Ukraine
2 Department of Invertebrate Evolution, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland

http://zoobank.org/22050C34-40A5-4B7A-9969-222AE927D6AA

Corresponding author: Piotr Gąsiorek (piotr.lukas.gasiorek@gmail.com)

Abstract

Many regions of the world remain unexplored in terms of the tardigrade diversity, and the islands of the Indian Ocean are no exception. In this work, we report four species of the family Echiniscidae representing three genera from Mauritius, the second largest island in the Mascarene Archipelago. Two species belong in the genus Echiniscus: Echiniscus perarmatus Murray, 1907, a pantropical species, and one new species: Echiniscus insularis sp. nov., one of the smallest members of the spinulosus group and the entire genus, being particularly interesting due to the presence of males and supernumerary teeth-like spicules along the margins of the dorsal plates. The new species most closely resembles Echiniscus tropicalis Binda & Pilato, 1995, for which we present extensive multipopulation data and greatly extend its distribution eastwards towards islands of Southeast Asia. Pseudechiniscus (Meridioniscus) mascarenensis sp. nov. is a typical member of the subgenus with elongated (dactyloid) cephalic papillae and the pseudosegmental plate IV in reduced posterior projections in males. Finally, a Bryodelphax specimen is also recorded. The assemblage of both presumably endemic and widely distributed tardigrade species in Mauritius fits the recent emerging biogeographic patterns for this group of micrometazoans.

Key Words

Biodiversity, distribution, Heterotardigrada, insular fauna, morphology, sculpturing

Introduction

Tardigrades, as many micrometazoan taxa, remain mostly ignored in biodiversity surveys, although molecular techniques indicate the presence of multiple lineages and high potential for cryptic speciation (Blaxter et al. 2003, Cesari et al. 2020). Recent estimates augment the increasing evidence for the existence of numerous species complexes (Faurby et al. 2012; Jørgensen et al. 2018; Guidetti et al. 2019; Morek and Michalczyk 2020), however, rather mediocre species richness of this phylum emerges when compared to other animal groups characterised by greater species abundance by an order of magnitude (Bartels et al. 2016). Many regions of the world have never been sampled in a search for tardigrades, although the collection of these animals is very easy and not costly (Degma 2018). Archipelagos in the Western Indian Ocean are known as centres of insular endemism and local biodiversity hotspots (Goodman and Benstead 2005; Cheke and Hume 2008), but the scarcity of faunistic tardigrade studies precludes a more in-depth look into the evolutionary history of the phylum in this area. Tardigrades were particularly intensively sampled in the Seychelles (Biserov 1994; Binda and Pilato 1995, Pilato et al. 2002, 2004, 2006, 2009a, 2009b) and Madagascar (see Gąsiorek and Vončina 2019; Kaczmarek et al. 2020 for summary). Single papers were devoted to either limno-terrestrial or marine tardigrades of Mauritius (Grimaldi De Zio et al. 1987), Maldives (De Zio Grimaldi et al. 1999), and Réunion (Séméria 2003). Other archipelagos, like the Comoros or Socotra, have not been explored for these animals. Among the smaller islands of the Western Indian Ocean, the fauna of Mauritius has received the
greatest attention and appeals for conservation effort of the best-studied insects, especially beetles (Motala et al. 2007).

The purpose of this contribution is to provide the first integrative data for the Mauritian members of the armoured tardigrades from the family Echiniscidae (Heterotardigrada). They include detailed DNA barcoding and morphological information for two species new to science, extracted from two moss samples. The new species represent the genera *Echiniscus* and *Pseudechiniscus*, the most speciose echiniscid taxa. Novel morphological characters are depicted for the *Echiniscus spinulosus* complex based on the smallest and dioecious member of this extraordinarily species-rich, by tardigrade standards, group. We also elaborate on *Echiniscus tropicalis*, the cognate taxon of the new species. Finally, the records of species with wide tropical or even pantropical distribution support the supposition that very broad geographic ranges may be typical for tropical tardigrade taxa (Gąsiorek et al. 2019). This is in accordance with data for oribatid mites inhabiting the Madagascan region, a significant fraction of which comprises pantropical species (Niedbała 2017).

Materials and methods

Sample collection and processing

Tardigrades were extracted from two moss samples (MU.001–2) collected from Sophie Nature Walk in the vicinity of Mare aux Vacoas (ca. 20°22′S, 57°29′E, 580 m asl; Mauritius, Mascarene Archipelago, Western Indian Ocean; O. Garmish leg. on 7th September 2019). Samples were rehydrated in Petri dishes, and then processed according to standard protocols (Dastych 2010; Stec et al. 2015). The animals were used in three analyses: (I) qualitative and quantitative morphology investigated with phase contrast microscopy (PCM); (II) high-resolution imaging with scanning electron microscopy (SEM); (III) DNA sequencing. Additionally, populations of *Echiniscus tropicalis* were obtained and underwent an identical procedure (Table 1).

Table 1. List of the populations of *Echiniscus tropicalis* examined in this study. Types of analyses: (LCM) imaging and morphometry in PCM, (SEM) imaging in SEM, (DNA) DNA sequencing. Number in each analysis indicates how many specimens were utilised in a given method (a – adults, j – juveniles, l – larvae).

| Sample code | Coordinates altitude | Locality | Sample type | Collector | Analyses |
|-------------|----------------------|----------|-------------|-----------|----------|
| ID.032      | 8°16′35″S, 115°29′29″E, 521 m asl | Indonesia, Bali, Karangasem Regency | moss from tree bark | Łukasz Michalczyn | LCM 23a SEM 20a DNA 10a |
| ID.071      | ca. 2°10′N, 97°26′E, 0–20 m asl | Indonesia, coastline of Sumatra, Palambak Island | moss from tree bark | Łukasz Skoczylas | a 9a |
| ID.858      | 0°39′47″N, 127°24′11″E, 1717 m asl | Indonesia, the Moluccas, Tidore, Gunung Kiematubu | moss and lichen from rock | Piotr Gąsiorek | 2a |
| ID.939      | 1°15′33″N, 124°55′57″E, 696 m asl | Indonesia, Celebes, Sulawesi Utara, shores of Danau Tondano | moss and lichen from tree bark | Piotr Gąsiorek and Łukasz Krzywański | 36a + 16j + 101 |
| ID.951      | 1°10′02″N, 124°49′22″E, 743 m asl | Indonesia, Celebes, Sulawesi Utara, Rano Lewo | moss and lichen from palm tree | Piotr Gąsiorek and Łukasz Krzywański | 1a |
| MY.068      | 5°58′54″N, 116°04′42″E, 30 m asl | Malaysia, Borneo, Sabah, Kota Kinabalu, Bukit Bendera Street | moss from concrete wall | Piotr Gąsiorek | 1a + 1j |
| SG.001      | 1°21′39″N, 103°53′24″E, 12 m asl | Singapore | moss from tree bark | Tan Pal Chun | 9a |

Microscopy, imaging and morphometrics

Permanent microscope slides were made using Hoyer’s medium and examined under Olympus BX53 phase contrast microscope (PCM) equipped with a digital camera Olympus DP74. In order to obtain ideally dorso-ventrally or dorso-laterally positioned and flattened specimens, specimens were first completely air-dried, then mounted in a minuscule drop of medium which did not fill the entire space between the slide and cover slip, and, eventually, the missing portion of medium was added at the edges of the cover slip to refill the empty space after 30 minutes. Specimens were prepared for SEM according to the protocol by Stec et al. (2015). All figures were assembled in Corel Photo-Paint X7. All measurements are given in micrometres (μm) and were performed under PCM. Structures were measured only when not broken, deformed or twisted, and their orientations were suitable. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. The sp ratio, the ratio of the length of a given structure to the length of the scapular plate, was expressed as a percentage (Dastych 1999). Morphometric data were handled using the Echiniscoidea ver. 1.3 template available from the Tardigrada Register, www.tardigrada.net (Michalczyn and Kaczmarek 2013). Raw data are presented as Suppl. materials 1–5. Scientific drawing of the ventral sculpturing pattern of the new *Pseudechiniscus* species was made in Microsoft PowerPoint using microphotographs and direct observations of specimens in PCM.

Genotyping, genetic comparisons and phylogenetics

DNA was extracted from individual animals following the Chelex 100 resin (Bio-Rad) extraction method (Casquet et al. 2012; Stec et al. 2015). Each specimen was observed in a drop of distilled water on a temporary slide under a 400× magnification prior to investigation. Hologenophores (Pleijel et al. 2008) were obtained for *E. insularis* sp. nov., *E. perarmatus* and *P. mascarensensis* sp. nov. Five
DNA fragments were sequenced: four nuclear and one mitochondrial in the case of *E. insularis* sp. nov., four for *E. perarmatus* (excluding ITS-2, all will be presented in another contribution) and three for *P. mascarenensis* sp. nov. (excluding ITS-2 and COI). Both ITS-2 and COI are highly variable markers and are often difficult to amplify, as in these cases. All fragments were amplified and sequenced according to the protocols described in Stec et al. (2015). Primers and PCR programmes are presented in Table 2.

ITS-1 and ITS-2 sequences were used to reconstruct a concatenated Maximum Likelihood (ML) phylogeny for *E. insularis* sp. nov.; GenBank accession numbers for the sequences retrieved from GenBank are presented in the Suppl. material 6. Alignments were 741 bp (ITS-1) and 546 bp (ITS-2) long. SequenceMatrix was used for concatenation (Vaidya et al. 2011). ModelFinder (Kalyaanamoorthy et al. 2017) was used to choose the best-fit models: TIM3+F+G4 (ITS-1 partition) and TPM2+F+G4 (ITS-2 partition), chosen according to the Bayesian information criterion. W-IQ-TREE was used for ML reconstruction (Nguyen et al. 2015; Trifinopoulos et al. 2016). One thousand ultrafast bootstrap (UFBoot) replicates were applied to provide support values for branches (Hoang et al. 2018). Trees were rooted on *Diploechiniscus olphonae* (Richerts, 1903). The final consensus trees were visualised with FigTree ver. 1.4.3 (available at: http://tree.bio.ed.ac.uk/software/figtree/).

Results

Systematic account

**Phylum:** Tardigrada Doyère, 1840

**Class:** Heterotardigrada Marcus, 1927

**Order:** Echiniscoida Richters, 1926

**Family:** Echiniscidae Thulin, 1928

**Genus:** *Echiniscus* C.A.S. Schultze, 1840

*Echiniscus insularis* sp. nov. Gasiorek, Vončina & Kiosya

http://zoobank.org/1C027A99-2712-4825-A41A-3479E3768C3A

Figures 1–8, Tables 3–5

**Locus typicus and type material.** ca. 20°22’S, 57°29’E, 580 m asl; Sophie Nature Walk, vicinity of Mare aux Va- coas (Plaines Wilhems, Mauritius, Mascarene Archipelago, Western Indian Ocean); mosses from tree trunks. Holotype (mature female on slide MU.002.04), allotype (mature male on slide MU.002.02), seven paratypic females, fourteen paratypic males, and five juveniles (slides MU.001.01–3, MU.002.01–6). One hologenophore on slide MU.001.24, and three hologenophores the slide MU.002.07. All deposited in the Department of Invertebrate Evolution.

**Etymology.** From Latin *insula* = island. The name refers to *locus typicus*. Adjective in the nominative singular.

**Description.** Mature females (i.e. from the third instar onwards; measurements in Table 3). Body small and plump (Figs 1, 3, 6A), yellow to orange, with minute red eyes absent after mounting. Ordinary primary and secondary (cephalic papillae) clavae of the *Echiniscus*-type; peribuccal cirri with well-developed cirrophores. Cirrus *A* very short (<25% of the body length), with cirrophore. Body appendage configuration *A-(B)-(C)-^D^-E, with the majority of appendages developed as spicules, slightly longer spines can occur only in the positions *C*^1^, *D*^2^, and *E* (Figs 1, 3B, 6A). Asymmetries frequent, but only rarely are more appendages absent (Fig. 3A). Additionally, supernumerary spicules occur along the margins of all dorsal plates and sometimes on their surface (Fig. 6A), particularly frequent (up to five) along the caudal incisions (Fig. 6D, E). Spines and spicules are always smooth and simple, not ramified. Dorsal plates strongly sclerotised and well-demarcated from each other, with the *spinulosus* type sculpturing, i.e. only pores are present (Figs 1, 3, 6A). Pores are densely arranged and may be of various size: from medium (Figs 1B, C, 3B, 6A) to large (Fig. 1A), even merging into groups of two/three pores (Fig. 3A). Dark endocuticular rings usually absent (Figs 1, 3B, 6A), or present, but only in the largest pores (Fig. 3A). Only in one female are pores absent and irregular dark epicuticular swellings are

Table 2. Primers and references for specific protocols for amplification of the four DNA fragments sequenced in the study.

| DNA fragment | Primer name | Primer direction | Primer sequence (5’-3’) | Primer source | PCR programme* |
|--------------|-------------|------------------|-------------------------|---------------|----------------|
| 18S rRNA     | 18S_Tar_F1  | forward          | AGCCGAAAACCCGGAATGCTC  | Stec et al. (2017) | Zeller (2010) |
|              | 18S_Tar_R2  | reverse          | CTGATCGCCTTCGAACCTCTAACTTTCG | Gasiorek et al. (2017) | Mironov et al. (2012) |
|              | 28S_Eutar_F | forward          | ACCCGCTGAACCTTTAAGCATAT | Mironov et al. (2012) | Welchiz et al. (2011) |
|              | 28SR0990   | reverse          | CTTGTCGCTACTACCGATTGG  | Gasiorek et al. (2019) | Mironov et al. (2012) |
| ITS-1        | ITS1_Echi_F | forward          | CCGTCGCTACTACCGATTGG  | Gasiorek et al. (2019) | Mironov et al. (2012) |
|              | ITS1_Echi_R | reverse          | GTTCGAAAACCCGGAATGCTC  | Gasiorek et al. (2019) | Mironov et al. (2012) |
| ITS-2        | ITS-3      | forward          | GCAFCGAGAAGAAGACCGACG | Gasiorek et al. (2019) | Mironov et al. (2012) |
|              | ITS-4      | reverse          | TCCTCCGCTTTATGATATGC  | Gasiorek et al. (2019) | Mironov et al. (2012) |
| COI          | bcdF01     | forward          | CATTTTCHACTAAYCATAARGATATTGG | Dabert et al. (2008) | Mironov et al. (2012) |
|              | bcdR04     | reverse          | TATAACACGTCCGGATGNNCAAAAAA | Dabert et al. (2008) | Mironov et al. (2012) |

* – All PCR programmes are also provided in Stec et al. (2015).
developed, most visible on the scapular plate (Fig. 4A). The cephalic plate consists of two halves, with an anterior chalice-like incision (Figs 1A, 3A). The cervical (neck) plate is in the form of a narrow grey belt, weakly delineated anterior to the scapular plate (Figs 1A, 3). The scapular plate non-faceted, with the usual lateral sutures delineating small rectangular portions (Figs 1, 3). Three median plates: m1, m3 unipartite, and m2 bipartite (Figs 3A, 6A); sculpture well-developed in all portions of the median plates with a narrow stripe, and m2 bipartite (Figs 3A, 6A); sculpture delineating small rectangular portions (Figs 1, 3). Three incisions (Figs 1, 3) and may be facetted (Figs 3B, 6A).

Ventral cuticle smooth. Sexpartite gonopore located ventrally (neck) plate is in the form of a narrow grey belt, weakly delineated anterior to the scapular plate (Figs 1A, 3). The cephalic plate consists of two halves, with an anterior chalice-like incision (Figs 1A, 3A). The cervical (neck) plate is in the form of a narrow grey belt, weakly delineated anterior to the scapular plate (Figs 1A, 3). The scapular plate non-faceted, with the usual lateral sutures delineating small rectangular portions (Figs 1, 3). Three median plates: m1, m3 unipartite, and m2 bipartite (Figs 3A, 6A); sculpture well-developed in all portions of the median plates with an incision (Fig. 5). Gonopore absent.

Mature males (i.e. from the third instar onwards; measurements in Table 4). Body slender (Fig. 2). Only one male has rudimentary developed pores (Fig. 4B). Males have often comparatively better developed supernumerary spicules than females (Fig. 2B). Clavae males have often comparatively better developed supernumerary spicules than females (Fig. 2B). Clavae enlarged, more prominent than in females (Figs 2, 6B). Subcephalic region with a pair of weakly developed oval swellings (probably rudimentary subcephalic plates). Gonopore circular, with a U-shaped slit; semicircular bulge resembling a genital plate with a cracked surface present anterior to the gonopore (Fig. 6C).

Juveniles (i.e. from the second instar onwards; measurements in Table 5). No morphometric gap between adults and juveniles (likely a result of general miniaturisation of the species). Qualitatively similar to adults (Fig. 5). Gonopore absent.

Larvae. Unknown.

Eggs. One egg per exuviae was found in few examined exuviae.

Table 3. Measurements [in µm] of selected morphological structures of mature females of Echiniscus insularis sp. nov. mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                  | N  | µm   | sp  | µm   | sp  | µm   | sp  | µm   | sp  | Holotype |
|----------------------------|----|------|-----|------|-----|------|-----|------|-----|----------|
| Body length                | 7  | 122  | 169 | 466  | –   | 591  | 150 | 374  | 16  | 41       | 136  | 592 |
| Scapular plate length      | 7  | 25.7 | 35.4| –    | 29.1| –    | 3.2 | –    | 25.7| –       |
| Head appendages lengths    |    |      |     |      |     |      |     |      |     | –        |
| Cirrus internus            | 7  | 8.5  | 13.2| 32.4 | –   | 40.9 | 10.8| 37.1 | 1.7 | 3.4      | 9.0  | 35.0|
| Cephalic papilla           | 7  | 5.0  | 7.0 | 16.9 | –   | 22.2 | 5.9 | 20.3 | 0.6 | 1.8      | 5.7  | 22.2|
| Cirrus externus            | 7  | 10.9 | 15.9| 40.7 | –   | 32.2 | 13.5| 46.3 | 1.6 | 4.2      | 12.9 | 50.2|
| Clava                      | 7  | 3.6  | 5.5 | 11.9 | –   | 17.5 | 4.4 | 15.2 | 0.6 | 1.9      | 4.5  | 17.5|
| Cirrus A                   | 7  | 24.2 | 37.0| 86.1 | –   | 106.2| 28.3| 96.9 | 4.1 | 7.0      | 27.3 | 106.2|
| Cirrus A/Body length ratio | 7  | 17%  | 22% | –    | 19% | –    | 2%  | –    | 20% | –       |

Claw 1 heights

- Branch: 7 7.0 – 10.3 26.2 – 30.7 8.3 28.5 1.0 1.8 7.9 30.7
- Spur: 4 1.7 – 2.1 5.8 – 8.2 1.8 6.6 0.2 1.1 2.1 8.2
- Spur/branch length ratio: 4 20% – 27% – 23% – 3% – 27% –

Claw 2 heights

- Branch: 7 7.2 – 9.4 24.5 – 30.8 7.9 27.2 0.8 2.5 7.7 30.0
- Spur: 5 1.3 – 2.5 4.4 – 7.1 1.7 5.5 0.5 1.1 7
- Spur/branch length ratio: 5 18% – 27% – 21% – 3% – 7 –

Claw 3 heights

- Branch: 7 6.9 – 9.9 25.5 – 32.7 8.1 27.8 1.0 2.9 8.4 32.7
- Spur: 5 1.3 – 2.2 4.4 – 6.2 1.7 5.5 0.4 0.7 7
- Spur/branch length ratio: 5 16% – 24% – 20% – 3% – 7 –

Claw 4 heights

- Branch: 7 8.2 – 10.8 28.8 – 35.8 9.2 31.7 0.8 2.1 9.2 35.8
- Spur: 3 2.0 – 2.7 6.8 – 8.7 2.4 7.7 0.4 0.9 7
- Spur/branch length ratio: 3 21% – 27% – 24% – 3% – 7 –
Figure 1. Habitus of females of *Echiniscus insularis* sp. nov. (PCM): **A** dorsal view (cA – cirrus A, ce – cirrus externus, ci – cirrus internus, cl – (primary) clava, cp – cephalic papilla), **B** dorsolateral view, **C** lateral view. Note irregularly distributed spicules along margins of the dorsal plates. Scale bars in μm.
Figure 2. Habitus of males of *Echiniscus insularis* sp. nov. (PCM): A dorsal view, B dorsolateral view, C lateral view. Note differences between the density of supernumerary spicules at margins of the dorsal plates. Scale bars in μm.
Figure 3. Habitus of females of *Echiniscus insularis* sp. nov.: A specimen with appendages (arrowheads) greatly reduced in number and aberrantly large, merging pores (PCM, dorsolateral view), B typical female with ordinary set of appendages (SEM, dorsal view). Scale bars in μm.

**DNA sequences and phylogenetic position.** Two haplotypes in all markers were found, corresponding with the populations MU.001 and MU.002: 18S rRNA (MW180887, MW180888), 28S rRNA (MW180879, MW180880), ITS-1 (MW180910, MW180911), ITS-2 (MW180898, MW180899), and in COI (MW178242, MW178243). *p*-distance in COI between the two populations is 4.9%. *Echiniscus insularis* sp. nov. belongs
Figure 4. Habitus of *Echiniscus insularis* sp. nov. – individuals with aberrantly developed dorsal sculpturing (PCM): A female, dorsal view, B male, dorsolateral view. Note differences in the development of appendages. Scale bars in μm.

in the *spinulosus* complex, being a sister species to the clade composed of *E. manuelae* da Cunha & do Nascimento Ribeiro, 1962 + *E. tristis* Gašíorek & Kristensen, 2018 (Fig. 8).

**Remarks.** The species is easily recognisable because of the additional supernumerary dorsal spicules along margins of all plates and sometimes on the plates, making it an unusual member of the *spinulosus* group and of the entire genus. Besides, it is one of the smallest representatives of *Echiniscus* with the average adult body length at ca. 150 μm, whereas adults of *Echiniscus* spp. usually reach 200–250 μm at least. There is one species
### Table 4. Measurements [in µm] of selected morphological structures of mature males (one hologenophore included) of *Echiniscus insularis* sp. nov. mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                      | N     | Range | Mean | SD  | Allotype |
|--------------------------------|-------|-------|------|-----|----------|
|                                | µm    | sp    | µm   | sp  | µm      |
| Body length                    | 15    | 113   | 167  | 596 | 145      | 548 |
| Scapular plate length          | 15    | 22.2  | 28.8 | –   | 26.4     | –   |
| Head appendages lengths        |       |       |      |     |          |     |
| Cirrus internus                | 14    | 5.6   | 13.8 | 23.0 | 27.8     |
| Cephalic papilla               | 15    | 4.5   | 7.2  | 18.5 | 27.8     |
| Cirrus externus                | 15    | 7.9   | 18.0 | 32.5 | 13.7     |
| Clava                          | 15    | 3.2   | 6.1  | 14.4 | 23.1     |
| Cirrus A                       | 15    | 17.2  | 29.9 | 77.5 | 24.1     |
| Cirrus A/Body length ratio     | 15    | 15%   | 20%  | 17%  | 1%       |
| Body appendages lengths        |       |       |      |     |          |     |
| Spine B                        | 6     | 1.6   | 2.9  | 6.0  | 11.1     |
| Spine C                        | 15    | 2.2   | 5.0  | 8.3  | 17.4     |
| Spine C*                       | 5     | 2.4   | 8.0  | 10.8 | 32.9     |
| Spine D                        | 15    | 1.9   | 3.5  | 6.8  | 13.5     |
| Spine D*                       | 14    | 3.2   | 13.2 | 13.1 | 10.1     |
| Spine E                        | 13    | 3.9   | 7.7  | 14.8 | 28.2     |
| Supernumerary spicules         | 32    | 0.6   | 1.0  | 2.1  | 11.5     |
| Spine on leg I length          | 15    | 1.2   | 2.4  | 4.9  | 9.2      |
| Papilla on leg IV length       | 15    | 2.3   | 4.0  | 9.5  | 13.9     |
| Number of teeth on the collar  | 15    | 7     | 11   | –    | 9.2      |
| Claw 1 heights                 |       |       |      |     |          |     |
| Branch                         | 13    | 5.7   | 9.7  | 23.5 | 17.4     |
| Spur                           | 9     | 1.5   | 2.3  | 6.2  | 8.7      |
| Spur/branch length ratio       | 9     | 20%   | 26%  | –    | 22%      |
| Claw 2 heights                 |       |       |      |     |          |     |
| Branch                         | 14    | 5.7   | 9.2  | 23.5 | 34.2     |
| Spur                           | 10    | 1.3   | 2.4  | 5.2  | 9.4      |
| Spur/branch length ratio       | 10    | 17%   | 29%  | –    | 21%      |
| Claw 3 heights                 |       |       |      |     |          |     |
| Branch                         | 15    | 5.0   | 9.3  | 20.6 | 33.1     |
| Spur                           | 7     | 1.2   | 2.0  | 4.9  | 7.8      |
| Spur/branch length ratio       | 7     | 17%   | 25%  | –    | 22%      |
| Claw 4 heights                 |       |       |      |     |          |     |
| Branch                         | 15    | 6.4   | 11.2 | 26.3 | 42.4     |
| Spur                           | 8     | 1.5   | 2.6  | 6.2  | 10.2     |
| Spur/branch length ratio       | 8     | 22%   | 28%  | –    | 25%      |

**Figure 5.** Habitus of juvenile of *Echiniscus insularis* sp. nov. with fully developed appendages (PCM, dorsolateral view). Scale bar in µm.
Figure 6. Morphological details of *Echiniscus insularis* sp. nov.: A female in lateral view (SEM), B male cephalic appendages (SEM), C male gonopore (SEM), D, E appendages along the caudal incision (PCM). Scale bars in μm.
Table 5. Measurements [in µm] of selected morphological structures of juveniles of *Echiniscus insularis* sp. nov. mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                        | N  | µm  | Range | Mean | SD  |
|----------------------------------|----|-----|-------|------|-----|
| Body length                      | 5  | 100 | 140   | 422  | 625 |
| Scapular plate length            | 5  | 18.4| 29.4  | 24.2 | 4.3 |
| Head appendages lengths          |    |     |       |      |     |
| *Cirrus internus*                | 5  | 5.3 | 9.5   | 22.4 | 34.7|
| *Cephalic papilla*               | 5  | 3.5 | 5.6   | 16.8 | 21.5|
| *Cirrus externus*                | 5  | 6.6 | 13.0  | 35.9 | 50.6|
| *Clava*                          | 5  | 3.0 | 4.7   | 13.5 | 18.5|
| *Cirrus A*                       | 5  | 15.3| 27.3  | 83.2 | 101.3|
| *Cirrus A/Body length ratio*     | 5  | 13% | 19%   | 4%   | 2%  |
| Body appendages lengths          |    |     |       |      |     |
| *Spine B*                        | 1  | 2.2 | 9.3   | 9.3  | 2.2 |
| *Spine C*                        | 5  | 2.2 | 12.0  | 18.9 | 3.7 |
| *Spine C′*                       | 4  | 2.1 | 7.1   | 35.4 | 5.6 |
| *Spine D*                        | 5  | 1.3 | 7.1   | 15.6 | 2.7 |
| *Spine D′*                       | 5  | 6.7 | 36.4  | 54.9 | 10.6|
| *Spine E*                        | 4  | 4.1 | 22.1  | 30.8 | 5.8 |
| Supernumerary spicules           | 18 | 1.1 | 3.0   | 12.7 | –   |
| Spine on leg I length            | 4  | 1.1 | 2.6   | 8.8  | 1.8 |
| Papilla on leg IV length         | 5  | 2.1 | 2.9   | 12.2 | 2.6 |
| Number of teeth on the collar    | 5  | 6   | 10    | 7.8  | 1.5 |
| Claw 1 heights                   |    |     |       |      |     |
| Branch                           | 5  | 5.6 | 8.1   | 32.1 | 7.0 |
| Spur                             | 3  | 1.0 | 4.5   | 7.1  | 1.5 |
| Spur/branch length ratio         | 3  | 17% | 26%   | 22%  | 5%  |
| Claw 2 heights                   |    |     |       |      |     |
| Branch                           | 5  | 4.9 | 7.7   | 25.2 | 28.7|
| Spur                             | 3  | 1.0 | 1.7   | 5.4  | 5.8 |
| Spur/branch length ratio         | 3  | 20% | 23%   | 22%  | 1%  |
| Claw 3 heights                   |    |     |       |      |     |
| Branch                           | 5  | 5.3 | 7.3   | 24.8 | 28.8|
| Spur                             | 3  | 1.0 | 1.6   | 5.4  | 5.9 |
| Spur/branch length ratio         | 3  | 19% | 24%   | 21%  | 2%  |
| Claw 4 heights                   |    |     |       |      |     |
| Branch                           | 4  | 6.3 | 8.6   | 27.6 | 34.2|
| Spur                             | 1  | 2.0 | 2.0   | 6.8  | 6.8 |
| Spur/branch length ratio         | 1  | 25% | 25%   | 25%  | 7%  |

Figure 7. Claws of *Echiniscus insularis* sp. nov.: A claws I (PCM, empty arrowhead indicates the asymmetric lack of internal spur), B claws II (SEM), C claws III (SEM), D claws IV with dentate collar (SEM). Scale bars in µm.
resembling specimens of *E. insularis* sp. nov. with a lower number of spicules – *E. tropicalis* Binda & Pilato, 1995 described from the Seychelles. For the purpose of the comparison *E. insularis* sp. nov. – *E. tropicalis*, we present updated description of the latter species below.

Due to the fact that *E. manuelae* and *E. tristis* currently emerge as species closest phylogenetically to *E. insularis* sp. nov., we compare them with the new species accordingly:

- *E. manuelae* has larger and more sparsely distributed pores in dorsal plates (see fig. 3 in da Cunha & do Nascimento Ribeiro (1962) and fig. 5 in Gasiorek and Kristensen (2018)), and appendages $C^v + D^d$ are long and serrated (smooth and short in *E. insularis* sp. nov.);
- *E. tristis* is a larger species (adult females $\geq 180 \mu m$ in *E. tristis* vs $< 170 \mu m$ in *E. insularis* sp. nov.) and has larger claw spurs that are more divergent from branches than in *E. insularis* sp. nov.

**Echiniscus tropicalis** Binda & Pilato, 1995

Figures 8–11, Tables 6–8

**Material.** Together 402 adult females, 17 juveniles and 10 larvae mounted on slides.

**Description.** **Mature females** (i.e. from the third instar onwards; measurements in Table 6). Body small and plump (Figs 9A, 11A), yellow to orange, with minute red eyes absent after mounting. Ordinary primary and secondary (cephalic papillae) clavae of the *Echiniscus*-type; peri-buccal cirri with well-developed ciriophores. Cirrus $A$ very short ($<25\%$ of the body length), with ciriophore. Body appendage configuration $A-B-C-C^v-D-D^d-E$, with all appendages developed as spines or spicules, which are smooth or only sometimes spines $E$ are serrated (Figs 9A, 10, 11A). Asymmetries frequent, especially in the lateral positions.

Dorsal plates strongly sclerotised and well-demarcated from each other, with the *spinulosus* type sculpturing, i.e. only pores are present (Figs 9A, 10, 11). Pores are densely arranged and rather of uniform size. Dark endocuticular rings absent (Figs 10, 11B, C). The cephalic plate consists of two halves, with an anterior chalice-like incision. The cervical (neck) plate is in the form of a narrow grey belt, weakly delineated anterior to the scapular plate (Fig. 10). The scapular plate non-facetted, with the usual lateral sutures delineating small rectangular portions (Figs 9A, 10). Three median plates: $m_1$, $m_3$ unipartite, the latter reduced to a narrow stripe; $m_2$ bipartite (Figs 9A, 10, 11). Two pairs of large segmental plates, their narrower anterior portions with two thin belts devoid of sculpture (Fig. 10). The caudal (terminal) plate with evident incisions (Figs 9A, 10) and may be facetted (Fig. 11A). Ventral cuticle smooth or with densely arranged endocuticular pillars. Sexpartite gonopore located anteriorly of legs IV and a trilobed anus between legs IV. Pedal plates and pulvini present (Fig. 9A). Spine I thin and minute (Fig. 9A). Dentate collar IV composed of numerous acute teeth (Figs 9A, 11A). Papilla on leg IV present (Fig. 9A). Claws IV slightly higher than claws I–III. External claws on all legs smooth. Internal claws with heteromorphic spurs positioned at ca. 1/4–1/3 of the claw height.

**Mature males.** Absent.

**Juveniles** (i.e. from the second instar onwards; measurements in Table 7). No morphometric gap or qualitative differences between adult and juvenile females found. Gonopore absent.

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**Figure 8.** Phylogenetic position of *Echiniscus insularis* sp. nov. on the Maximum Likelihood consensus phylogenetic tree; the *E. spinulosus* complex is marked in green and *Diploechiniscus oihonnae* was used as an outgroup. ML bootstrap values are presented above the branches.
Figure 9. Habitus of *Echiniscus tropicalis* in dorsolateral view (PCM): A adult female (black arrowheads indicate pulvini, whereas white arrowheads – pedal plates), B larva. Scale bars in μm.

Figure 10. Dorsal plate sculpturing of *Echiniscus tropicalis* in close-up (PCM). Scale bar in μm.
Larvae (i.e. the first instar; measurements in Table 8). Clear morphometric gap between juveniles and larvae exists (compare Tables 7, 8). Body appendage configuration $A-C^4-D^4-E$ (Fig. 9B). Anterior portions of paired segmental plates weakly sclerotised. Gonopore and anus absent.
Eggs. One egg per exuviae was found in few examined exuviae.

DNA sequences and phylogenetic position. Two haplotypes in all markers were found, corresponding with the populations ID.032 and ID.939: 18S rRNA (MW327546, MW327547), 28S rRNA (MW327542, MW327543), ITS-2 (MW327549, MW327550), with the exception of ITS-1, characterised by one haplotype (MW327551, MW327552). The sister species of *E. tropicalis* within the *spinulosus* complex is *E. siticulosus* (Fig. 8).

Phenotypic differential diagnosis. *Echiniscus tropicalis* was originally described based on two adult females (Binda and Pilato 1995). We compared the newly found Southeast Asian specimens with the microphotographs of the holotype that confirmed our suspicions after reading the description, i.e. the lack of sound morphological discrepancies between the type material from the Seychelles and abundant material from the Malay Archipelago and the Malay Peninsula. The only difference is the serration of spines *E* that may be well-developed in Asian populations (Fig. 10), whereas this trait was not reported by Binda and Pilato (1995). The original description mentions “primary and secondary points” in the paratype = a potential ramification. As there is a considerable intrapopulation variability regarding this trait, the Seychellois and Asian populations should be ascertained as conspecific unless DNA data from the Seychelles reject this hypothesis.

There is a plethora of differences between adult females of *E. insularis* sp. nov. and *E. tropicalis* after the description of the latter was supplemented with new data:

- the presence of supernumerary spicules along the margins of dorsal plates and in the caudal incisions (present in *E. insularis* sp. nov. vs absent in *E. tropicalis*);
- the presence of pedal plates (absent in *E. insularis* sp. nov. vs present in *E. tropicalis*);
- the relative length of cirrus *A* (86.1–106.2 in *E. insularis* sp. nov. vs 41.1–79.3 in *E. tropicalis*);
- the absolute lengths of lateral spines *B–D* (B 2.5–3.2 μm, C 2.0–5.2 μm, D 2.5–4.0 μm in *E. insularis*);
- the presence of supernumerary spicules along the margins of dorsal plates and in the caudal incisions (present in *E. insularis* sp. nov. vs absent in *E. tropicalis*);
- the presence of pedal plates (absent in *E. insularis* sp. nov. vs present in *E. tropicalis*);
- the relative length of cirrus *A* (86.1–106.2 in *E. insularis* sp. nov. vs 41.1–79.3 in *E. tropicalis*);
- the absolute lengths of lateral spines *B–D* (B 2.5–3.2 μm, C 2.0–5.2 μm, D 2.5–4.0 μm in *E. insularis*).

### Table 6. Measurements [in μm] of selected morphological structures of mature females of *Echiniscus tropicalis* (pooled data from the populations ID.032, ID.939 and SG.001) mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                        | N     | Range | Mean | SD  |
|----------------------------------|-------|-------|------|-----|
|                                 | µm    | µm    | µm   | µm  |
| Body length                      | 26    | 137   | 223  | 384 |
| Scapular plate length            | 26    | 33.6  | 45.2 | –   |
| Head appendages lengths          |       |       |      |     |
| Cirrus *A*                       | 25    | 19.9  | 33.8 | 41.1 |
| Cirrus *B*                       | 25    | 17.9  | 33.8 | 41.1 |
| Cirrus *C*                       | 25    | 11.2  | 18.2 | 27.5 |
| Cirrus *D*                       | 25    | 5.7   | 13.8 | 13.5 |
| Cirrus *D*                       | 25    | 4.3   | 14.9 | 9.9  |
| Spine *A*                        | 26    | 4.3   | 12.7 | 9.9  |
| Spine *B*                        | 26    | 6.5   | 14.7 | 14.9 |
| Spine *C*                        | 26    | 3.0   | 10.8 | 7.7  |
| Spine *D*                        | 22    | 5.7   | 13.8 | 13.5 |
| Spine *D*                        | 25    | 4.3   | 14.9 | 9.9  |
| Spine *E*                        | 26    | 7.1   | 15.8 | 16.3 |
| Spur *i*                         | 25    | 9.6   | 12.4 | 24.7 |
| Spur *ii*                        | 25    | 1.5   | 2.6  | 4.0  |
| Spur *iii*                       | 25    | 15%   | 21%  | –   |
| Spur/branch length ratio         | 23    | 15%   | 21%  | –   |
| Claw 1 heights                   |       |       |      |     |
| Branch 1                         | 25    | 9.6   | 12.4 | 24.7 |
| Spur 1                           | 23    | 1.5   | 2.6  | 4.0  |
| Spur/branch length ratio         | 23    | 15%   | 21%  | –   |
| Claw 2 heights                   |       |       |      |     |
| Branch 2                         | 26    | 9.2   | 12.0 | 22.9 |
| Spur 2                           | 25    | 1.6   | 2.7  | 3.9  |
| Spur/branch length ratio         | 25    | 15%   | 23%  | –   |
| Claw 3 heights                   |       |       |      |     |
| Branch 3                         | 25    | 8.7   | 12.3 | 23.2 |
| Spur 3                           | 23    | 1.5   | 2.7  | 3.8  |
| Spur/branch length ratio         | 23    | 15%   | 23%  | –   |
| Claw 4 heights                   |       |       |      |     |
| Branch 4                         | 26    | 10.7  | 14.8 | 25.9 |
| Spur 4                           | 18    | 2.0   | 3.1  | 5.3  |
| Spur/branch length ratio         | 18    | 18%   | 24%  | –   |

Eggs. One egg per exuviae was found in few examined exuviae.

DNA sequences and phylogenetic position. Two haplotypes in all markers were found, corresponding with the populations ID.032 and ID.939: 18S rRNA (MW327546, MW327547), 28S rRNA (MW327542, MW327543), ITS-2 (MW327549, MW327550), with the exception of ITS-1, characterised by one haplotype (MW327551, MW327552). The sister species of *E. tropicalis* within the *spinulosus* complex is *E. siticulosus* (Fig. 8).

Phenotypic differential diagnosis. *Echiniscus tropicalis* was originally described based on two adult females (Binda and Pilato 1995). We compared the newly found Southeast Asian specimens with the microphotographs of the holotype that confirmed our suspicions after reading the description, i.e. the lack of sound morphological discrepancies between the type material from the Seychelles and abundant material from the Malay Archipelago and the Malay Peninsula. The only difference is the serration of spines *E* that may be well-developed in Asian populations (Fig. 10), whereas this trait was not reported by Binda and Pilato (1995). The original description mentions “primary and secondary points” in the paratype = a potential ramification. As there is a considerable intrapopulation variability regarding this trait, the Seychellois and Asian populations should be ascertained as conspecific unless DNA data from the Seychelles reject this hypothesis.

There is a plethora of differences between adult females of *E. insularis* sp. nov. and *E. tropicalis* after the description of the latter was supplemented with new data:

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- the presence of pedal plates (absent in *E. insularis* sp. nov. vs present in *E. tropicalis*);
- the relative length of cirrus *A* (86.1–106.2 in *E. insularis* sp. nov. vs 41.1–79.3 in *E. tropicalis*);
- the absolute lengths of lateral spines *B–D* (B 2.5–3.2 μm, C 2.0–5.2 μm, D 2.5–4.0 μm in *E. insularis*).
Table 7. Measurements [in µm] of selected morphological structures of juveniles of *Echiniscus tropicalis* (population ID.939) mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                         | N  | µm   | Range | Mean | SD  |
|-----------------------------------|----|------|-------|------|-----|
| Body length                       | 10 | 143  | – 169 | 444  | –   |
| Scapular plate length             | 10 | 29.9 | – 35.4| –    | 3.2 |
| Head appendages lengths           |    |      |       |      |     |
| *Cirrus interus*                  | 9  | 7.6  | – 9.6 | 22.4 | –   |
| Cephalic papilla                  | 10 | 4.6  | – 5.5 | 13.3 | –   |
| *Cirrus externus*                 | 10 | 10.2 | – 13.7| 34.1 | –   |
| Clava                             | 10 | 3.6  | – 4.5 | 11.2 | –   |
| *Cirrus A*                        | 9  | 22.2 | – 25.6| 63.3 | –   |
| *Cirrus A* / Body length ratio    | 9  | 13%  | – 16% | –    | –   |
| Spine B                           | 9  | 3.5  | – 7.4 | 10.4 | –   |
| Spine C                           | 10 | 6.5  | – 10.5| 19.9 | –   |
| Spine C*                          | 10 | 4.6  | – 7.9 | 14.1 | –   |
| Spine D                           | 7  | 2.1  | – 8.8 | 6.4  | –   |
| Spine D*                          | 10 | 10.1 | – 14.3| 28.9 | –   |
| Spine E                           | 10 | 7.8  | – 13.0| 23.9 | –   |
| Spine on leg I length             | 10 | 1.8  | – 2.6 | 3.3  | –   |
| Papilla on leg IV length          | 10 | 2.4  | – 3.0 | 6.8  | –   |
| Number of teeth on the collar     | 10 | 7    | – 12  | –    | 10.3|
| Claw 1 heights                    |    |      |       |      |     |
| Branch                            | 10 | 7.3  | – 9.3 | 23.4 | –   |
| Spur                              | 9  | 1.3  | – 1.7 | 5.7  | –   |
| Spur/branch length ratio          | 9  | 16%  | – 22% | –    | 18% |
| Claw 2 heights                    |    |      |       |      |     |
| Branch                            | 10 | 7.2  | – 8.6 | 22.0 | –   |
| Spur                              | 7  | 1.3  | – 1.9 | 5.2  | –   |
| Spur/branch length ratio          | 7  | 17%  | – 22% | –    | 19% |
| Claw 3 heights                    |    |      |       |      |     |
| Branch                            | 10 | 7.7  | – 8.7 | 22.0 | –   |
| Spur                              | 7  | 1.4  | – 1.7 | 4.0  | –   |
| Spur/branch length ratio          | 7  | 17%  | – 21% | –    | 19% |
| Claw 4 heights                    |    |      |       |      |     |
| Branch                            | 10 | 8.5  | – 10.9| 26.3 | –   |
| Spur                              | 6  | 1.7  | – 2.4 | 5.8  | –   |
| Spur/branch length ratio          | 6  | 17%  | – 24% | –    | 21% |

Table 8. Measurements [in µm] of selected morphological structures of larvae of *Echiniscus tropicalis* (population ID.939) mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                         | N  | µm   | Range | Mean | SD  |
|-----------------------------------|----|------|-------|------|-----|
| Body length                       | 3  | 110  | – 124 | 559  | –   |
| Scapular plate length             | 3  | 19.4 | – 22.0| –    | 21.0|
| Head appendages lengths           |    |      |       |      |     |
| *Cirrus interus*                  | 3  | 4.2  | – 4.6 | 20.9 | –   |
| Cephalic papilla                  | 3  | 3.7  | – 4.1 | 18.5 | –   |
| *Cirrus externus*                 | 2  | 6.0  | – 6.4 | 29.6 | –   |
| Clava                             | 3  | 2.6  | – 3.1 | 13.4 | –   |
| *Cirrus A*                        | 3  | 13.3 | – 15.5| 60.9 | –   |
| *Cirrus A* / Body length ratio    | 3  | 11%  | – 13% | –    | 12% |
| Spine C                           | 3  | 0.9  | – 3.2 | 4.1  | –   |
| Spine C*                          | 3  | 3.2  | – 6.0 | 16.5 | –   |
| Spine E                           | 3  | 3.9  | – 5.5 | 20.1 | –   |
| Spine on leg I length             | 3  | 1.2  | – 1.6 | 6.2  | –   |
| Papilla on leg IV length          | 3  | 1.7  | – 2.0 | 8.8  | –   |
| Number of teeth on the collar     | 3  | 6    | – 7   | –    | 6.7 |
| Claw 1 heights                    |    |      |       |      |     |
| Branch                            | 3  | 5.3  | – 5.8 | 26.4 | –   |
| Spur                              | 3  | 1.1  | – 1.4 | 5.7  | –   |
| Spur/branch length ratio          | 3  | 21%  | – 25% | –    | 23% |
| Claw 2 heights                    |    |      |       |      |     |
| Branch                            | 3  | 5.1  | – 5.5 | 24.5 | –   |
| Spur                              | 3  | 1.0  | – 1.1 | 4.6  | –   |
| Spur/branch length ratio          | 3  | 18%  | – 22% | –    | 26% |
| Claw 3 heights                    |    |      |       |      |     |
| Branch                            | 3  | 5.1  | – 5.7 | 24.5 | –   |
| Spur                              | 3  | 1.2  | – 1.3 | 5.5  | –   |
| Spur/branch length ratio          | 3  | 22%  | – 24% | –    | 23% |
| Claw 4 heights                    |    |      |       |      |     |
| Branch                            | 3  | 6.0  | – 6.1 | 27.7 | –   |
| Spur                              | 2  | 1.2  | – 1.6 | 5.5  | –   |
| Spur/branch length ratio          | 2  | 20%  | – 27% | –    | 23% |

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Echiniscus perarmatus Murray, 1907

Material. Single adult female and a juvenile used for DNA sequencing (juvenile retrieved as a hologenophore on slide MU.001.23), larva on slide MU.001.01.

Remarks. This pantropical species (McInnes 1994) will likely appear to be one of the most common members of Echiniscidae in tropical climates, providing that the conspecificity of populations originating from different continents is demonstrated (data in preparation).

Genus: Pseudechiniscus Thulin, 1911
Subgenus: Meridioniscus Gąsiorek et al., 2021

Pseudechiniscus mascarenensis sp. nov. Kiosya, Vončina & Gąsiorek
http://zoobank.org/E5E6328D-D431-4BEA-88CB-0C24F6172B65
Figures 12–17, Tables 9–12

Pseudechiniscus sp. 5 in Gąsiorek et al. (2021)

Locus typicus and type material. ca. 20°22’S, 57°29’E, 580 m asl; Sophie Nature Walk, vicinity of Mare aux Vacoas (Plaines Wilhems, Mauritius, Mascarene Archipelago, Western Indian Ocean); mosses from tree trunks. Holotype (mature female on slide MU.001.04), allotype (mature male on slide MU.001.05), sixty paratypic females, seven paratypic males, ten juveniles, and six larvae (slides MU.001.01–21). Single hologenophore on slide MU.001.22. All deposited in the Department of Invertebrate Evolution.

Etymology. The name indicates the Mascarenes, terra typica of the new species. Adjective in the nominative singular.

Description. Mature females (i.e. from the third instar onwards; measurements in Table 9). Body yellow to orange, with minute, round black eyes absent after mounting (Fig. 12A). Elongated (dactyloid) cephalic papillae (secondary clavae) and elongated (primary) clavae (Figs 12A, 13, 15, 16); peribuccal cirri with poorly developed cirrophores. Cirrus A short, with cirrophore.

Dorsal plates are both poorly sclerotised and demarcated from each other, with the Pseudechiniscus-type sculpturing, i.e. endocuticular pillars protruding through the epicuticle and visible as dark dots in PCM (Fig. 12A). Striae present, but not visible in SEM (Figs 15A, 16). Epicuticular ornamentation absent. The cephalic plate pentapartite, with the anterior bi-halved portion and three posterior portions, roughly equal in size. The cervical (neck) plate absent. The scapular plate with sutures, separating wide anterior portion and four rectangular posterior portions. Three median plates: m1 and m3 unipartite, the latter indistinctly merged with the anterior margin of the pseudosegmental plate IV* (Fig. 12A), clearly delimited in SEM (Fig. 15A); m2 bipartite and

Table 9. Measurements [in µm] of selected morphological structures of mature females of Pseudechiniscus mascarenensis sp. nov. mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                        | N | µm   | RANGE | sd   | µm | RANGE | sp | µm | RANGE | sp  | µm | RANGE | sp | µm | RANGE | sp | µm | RANGE | sp |
|----------------------------------|---|------|-------|------|----|-------|----|----|-------|----|----|-------|----|----|-------|----|----|-------|----|
| Body length                      | 10| 151  | 177   | 627  | 843| 163   | 712| 9  | 64    | 167| 732|       |    |     |       |    |     |
| Scapular plate length            | 10| 21.0 | 24.8  | 521  | 23.0| 1.4   | 22.8|    |       |    |     |       |    |     |       |    |     |
| Head appendages lengths          |   |      |       |      |    |       |    |    |       |    |     |       |    |     |       |    |     |
| Cirrus internus                  | 10| 5.4  | 7.6   | 23.1 | 32.9| 6.7   | 29.2| 0.6| 2.9   | 6.8| 29.8|       |    |     |       |    |     |
| Cephalic papilla                 | 10| 4.3  | 5.7   | 17.3 | 25.2| 5.0   | 21.8| 0.5| 2.3   | 4.4| 19.3|       |    |     |       |    |     |
| Cirrus externus                  | 10| 7.7  | 13.0  | 33.2 | 36.5| 10.8  | 47.3| 1.6| 7.7   | 11.5| 50.4|       |    |     |       |    |     |
| Clava                           | 10| 3.4  | 5.2   | 13.9 | 21.2| 4.2   | 18.2| 0.5| 2.3   | 4.0| 17.5|       |    |     |       |    |     |
| Cirrus A                        | 9 | 13.8 | 23.4  | 38.1 | 93.5| 18.9  | 82.9| 3.0| 14.6  | 20.5| 89.9|       |    |     |       |    |     |
| Cirrus A/Body length ratio       | 9 | 9.9  | 13%   | 13%  | 12% | 2%    | 12% |    |       |    |     |       |    |     |       |    |     |
| Papilla on leg IV length         | 10| 1.5  | 2.4   | 7.0  | 11.0| 2.0   | 8.9 | 0.3| 1.2   | 2.1| 9.2 |       |    |     |       |    |     |
| Claw 1 heights                   |   |      |       |      |    |       |    |    |       |    |     |       |    |     |       |    |     |
| Branch                          | 10| 6.9  | 8.6   | 29.7 | 39.5| 7.8   | 34.1| 0.6| 3.0   | 8.0| 35.1|       |    |     |       |    |     |
| Spur                            | 9| 1.3  | 2.1   | 5.7  | 8.6 | 1.6   | 7.2 | 0.3| 1.0   | 1.3| 5.7 |       |    |     |       |    |     |
| Spur/branch length ratio         | 9| 16%  | 25%   | 100% | 21% | 3%    | 16% |    |       |    |     |       |    |     |       |    |     |
| Claw 2 heights                   |   |      |       |      |    |       |    |    |       |    |     |       |    |     |       |    |     |
| Branch                          | 10| 6.8  | 8.7   | 29.1 | 38.6| 7.8   | 33.9| 0.6| 2.9   | 7.8| 34.2|       |    |     |       |    |     |
| Spur                            | 9| 1.2  | 1.8   | 5.2  | 7.6 | 1.5   | 6.4 | 0.2| 0.8   | 1.4| 6.1 |       |    |     |       |    |     |
| Spur/branch length ratio         | 9| 17%  | 25%   | 100% | 19% | 3%    | 18% |    |       |    |     |       |    |     |       |    |     |
| Claw 3 heights                   |   |      |       |      |    |       |    |    |       |    |     |       |    |     |       |    |     |
| Branch                          | 10| 6.5  | 8.4   | 28.9 | 40.0| 7.6   | 33.3| 0.7| 3.2   | 7.6| 33.3|       |    |     |       |    |     |
| Spur                            | 8| 1.2  | 1.7   | 5.6  | 7.2 | 1.5   | 6.4 | 0.2| 0.5   | 1.4| 6.1 |       |    |     |       |    |     |
| Spur/branch length ratio         | 8| 18%  | 24%   | 100% | 20% | 2%    | 18% |    |       |    |     |       |    |     |       |    |     |
| Claw 4 heights                   |   |      |       |      |    |       |    |    |       |    |     |       |    |     |       |    |     |
| Branch                          | 10| 7.5  | 9.1   | 30.2 | 42.4| 8.3   | 36.2| 0.6| 3.4   | 8.0| 35.1|       |    |     |       |    |     |
| Spur                            | 6| 1.3  | 2.2   | 6.0  | 9.0 | 1.8   | 7.6 | 0.4| 1.3   | 1.8| 7.9 |       |    |     |       |    |     |
| Spur/branch length ratio         | 6| 17%  | 25%   | 100% | 21% | 3%    | 23% |    |       |    |     |       |    |     |       |    |     |

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Figure 12. Habitus of *Pseudechiniscus mascarenensis* sp. nov. (PCM): A female, dorsolateral view, B, C males, dorsal view. Scale bars in μm.
large. Four pairs of lateral intersegmental platelets flanking the boarders of m1–2 (Figs 12A, 16). Two pairs of large segmental plates. The pseudosegmental plate IV’ undivided by a median longitudinal suture; the posterior margin of the plate sinusoid and smooth. The caudal (terminal) plate with short, very poorly marked incisions (Figs 15A, 16).

Ventral cuticle with a pronounced species-specific pattern reaching the lateroventral sides of the body (Figs 12A, 13, 14, 15B), being a typical reticulum composed of belts of pillars. The pattern is relatively stable and well developed in the majority of individuals. The subcephalic zone with a wide belt of pillars. No epicuticular thickenings. Sexpartite gonopore located anterior to legs IV and a trilobed anus between legs IV.

Pedal plates and dentate collar IV absent; instead large patches of pillars are present centrally on each leg (Fig. 12A). Pulvini faint. Papilla on leg I absent (Figs 12A, 16). Two pairs of large segmental plates. The pseudosegmental plate IV’ (undivided in P. mascarenensis, referred to the smallest and the largest structure among all measured specimens; SD – standard deviation).

**Table 10. Measurements [in µm] of selected morphological structures of mature males of *Pseudechiniscus mascarenensis* sp. nov. mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).**

| Character                          | N   | RANGE  | MEAN   | SD     | Allotype |
|------------------------------------|-----|--------|--------|--------|----------|
|                                    | µm | 4.6    | sp     | 4.1    | 3        |
| Body length                         | 6   | 118    | –      | 146    | 605      |
| Scapular plate length               | 6   | 17.6   | –      | 22.4   | –        |
| Head appendages lengths             |     |        |        |        |          |
| Cirrus internus                     | 6   | 5.1    | –      | 7.9    | 23.9     |
| Cephalic papilla                    | 6   | 3.5    | –      | 4.9    | 19.9     |
| Cirrus externus                     | 5   | 8.0    | –      | 10.0   | 37.2     |
| Claw A                              | 6   | 3.5    | –      | 4.6    | 18.3     |
| Cirrus A                            | 5   | 13.8   | –      | 18.8   | 73.2     |
| Papilla on leg IV length ratio      | 6   | 1.7    | –      | 2.4    | 7.6      |
| Claw 1 heights                      |     |        |        |        |          |
| Branch                              | 6   | 6.7    | –      | 8.3    | 39.9     |
| Spur                                | 3   | 1.0    | –      | 1.5    | 4.7      |
| Spur/branch length ratio            | 3   | 14%    | –      | 20%    | –        |
| Claw 2 heights                      |     |        |        |        |          |
| Branch                              | 6   | 6.2    | –      | 7.5    | 29.1     |
| Spur                                | 5   | 1.1    | –      | 1.5    | 5.2      |
| Spur/branch length ratio            | 5   | 15%    | –      | 21%    | –        |
| Claw 3 heights                      |     |        |        |        |          |
| Branch                              | 6   | 6.3    | –      | 7.2    | 29.6     |
| Spur                                | 3   | 1.2    | –      | 1.5    | 5.4      |
| Spur/branch length ratio            | 3   | 18%    | –      | 21%    | –        |
| Claw 4 heights                      |     |        |        |        |          |
| Branch                              | 6   | 6.6    | –      | 8.1    | 31.3     |
| Spur                                | 2   | 1.5    | –      | 1.5    | 6.7      |
| Spur/branch length ratio            | 2   | 19%    | –      | 21%    | –        |

**Larvae** (i.e. the first instar; measurements in Table 12). Morphometric gap exists between juveniles and larvae. Gonopore and anus absent.

**Eggs.** One egg per exuviae was found in few examined exuviae.

**DNA sequences and phylogenetic position.** Single haplotypes in 18S rRNA (MW031972), 28S rRNA (MW032061), and ITS-1 (MW032151) were found. *Pseudechiniscus mascarenensis* sp. nov. has no close relatives according to the phylogeny presented in Gąsiorek et al. (2020) (see fig. 2 therein), constituting a separate evolutionary lineage within the subgenus *Meridioniscus*.

**Phenotypic differential diagnosis.** The species must be compared to other members of *Meridioniscus* with no projections on the pseudosegmental plate IV’ or with rudimentarily developed projections. *Pseudechiniscus mascarenensis* sp. nov. is differentiated from:

- *P. angelusalas* Roszkowska et al., 2020, described from Madagascar, by the body length (151–177 µm in females of *P. mascarenensis* sp. nov. vs 113–143 µm in females of *P. angelusalas*), the cirrus A/ body length ratio (9–13% in females of *P. mascarenensis* sp. nov. vs 19–22% in females of *P. angelusalas*), and by the division of the pseudosegmental plate IV’ (undivided in *P. mascarenensis* sp. nov. vs with median longitudinal suture in *P. angelusalas*);
- *P. dastychi* Roszkowska et al., 2020, described from the Argentine Islands (maritime Antarctic), by the presence of males (present in *P. mascarenensis* sp. nov. vs absent in *P. dastychi*), and by the division of the pseudosegmental plate IV’ (undivided in
Figure 13. Ventral sculpturing pattern of female of *Pseudechiniscus mascarenensis* sp. nov. (PCM). Scale bar in μm.

Table 11. Measurements [in μm] of selected morphological structures of juveniles of *Pseudechiniscus mascarenensis* sp. nov. mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character                     | N   | Range        | Mean | SD        |
|-------------------------------|-----|--------------|------|-----------|
|                              |     | μm           | sp   | μm        | μm | sp |
| Body length                   | 5   | 115 – 129    | 63.5 | 72.6 – 124| 67.2| 5  | 33 |
| Scapular plate length         | 5   | 17.5 – 19.3  | –    | 18.4 – 0.7| –  | 0.7 |
| Head appendages lengths       |     |              |      |           |    |     |
| Cirrus internus               | 5   | 5.2 – 6.9    | 27.8 | 38.1 – 5.9| 32.3| 0.7| 4.4 |
| Cephalic papilla              | 5   | 3.4 – 3.9    | 17.6 | 21.2 – 3.5| 19.3| 0.2| 1.4 |
| Cirrus externus               | 5   | 6.9 – 7.8    | 38.1 | 42.3 – 7.5| 40.7| 0.4| 1.6 |
| Clava                         | 5   | 2.8 – 3.9    | 15.0 | 21.2 – 3.3| 18.0| 0.5| 2.7 |
| Cirrus A                      | 5   | 12.7 – 16.4  | 65.8 | 89.1 – 14.3| 77.7| 1.9| 10.0|
| Cirrus A/Body length ratio    | 5   | 10% – 13%    | –    | 12% – 2%  | –   |     |
| Papilla on leg IV length      | 4   | 1.5 – 2.0    | 8.0  | 10.9 – 1.8| 9.5 | 0.3| 1.4 |
| Claw 1 heights                |     |              |      |           |    |     |
| Branch                        | 5   | 6.1 – 6.4    | 33.2 | 34.9 – 6.3| 34.0| 0.1| 0.8 |
| Spur                          | 2   | 1.2 – 1.3    | 6.2  | 7.4 – 1.3 | 6.8 | 0.1| 0.9 |
| Spur/branch length ratio      | 2   | 19% – 21%    | –    | 20% – 2% | –   |     |
| Claw 2 heights                |     |              |      |           |    |     |
| Branch                        | 5   | 5.2 – 5.8    | 28.0 | 31.3 – 5.6| 30.3| 0.3| 1.5 |
| Spur                          | 3   | 1.3 – 1.4    | 6.7  | 7.6 – 1.3 | 7.1 | 0.1| 0.5 |
| Spur/branch length ratio      | 3   | 22% – 24%    | –    | 24% – 1% | –   |     |
| Claw 3 heights                |     |              |      |           |    |     |
| Branch                        | 5   | 5.6 – 6.4    | 29.5 | 35.4 – 6.0| 32.5| 0.3| 2.6 |
| Spur                          | 3   | 1.1 – 1.3    | 5.7  | 7.1 – 1.2 | 6.5 | 0.1| 0.7 |
| Spur/branch length ratio      | 3   | 19% – 21%    | –    | 20% – 1% | –   |     |
| Claw 4 heights                |     |              |      |           |    |     |
| Branch                        | 5   | 6.4 – 6.9    | 33.2 | 37.7 – 6.6| 36.0| 0.2| 1.9 |
| Spur                          | 2   | 1.1 – 1.2    | 6.0  | 6.6 – 1.2 | 6.3 | 0.1| 0.5 |
| Spur/branch length ratio      | 2   | 16% – 18%    | –    | 17% – 2% | –   |     |

*P. mascarenensis* sp. nov. vs with median longitudinal suture in *P. dastychi*;

- *P. indistinctus* Roszkowska et al., 2020, described from Norway, by the division of the pseudosegmental plate IV (undivided in *P. mascarenensis* sp. nov. vs with median longitudinal suture in *P. indistinctus*), and by the presence of males (present in *P. mascarenensis* sp. nov. vs absent in *P. indistinctus*);

- *P. santomensis* Fontoura et al., 2010, considered endemic to the island São Tomé (Gulf of Guinea), by the presence of males (present in *P. mascarenensis* sp. nov. vs absent in *P. santomensis*), and the morphology of the posterior margin of pseudosegmental plate IV in females (smooth in *P. mascarenensis* sp. nov. vs with two projections in *P. santomensis*, as in males of *P. mascarenensis* sp. nov.).
Figure 14. Schematic ventral sculpturing pattern of female of *Pseudechiniscus mascarenensis* sp. nov.
overall, these two species are most similar within the genus.

Moreover, only the ventral sculpturing pattern of *P. santomensis* resembles that of *P. mascarenensis* sp. nov.; the remaining species have a very different ventral arrangement of pillars. *Pseudechiniscus juanitae* de Barros, 1939 should be treated as unidentifiable due to the lack of knowledge on its morphology (Grobys et al. 2020), although one attempt was made to characterise this species based on individuals from Central America (Pilato and Lisi 2006; Tumanov 2020), whereas its *locus typicus* lies in Brazil. Consequently, it is not included within the differential diagnosis.

**Discussion**

The fauna of Mauritius has previously been illustrative for an isolated oceanic island, consisting of a small number of mostly endemic species (Cheke and Hume 2008; Kehlmaier et al. 2019). However, many native species have been extirpated and replaced by allochthonous, often invasive taxa, a process which has been documented for numerous islands (Drake et al. 2002). Although the interactions between tardigrades inhabiting a given microhabitat are poorly understood (Meyer et al. 2020), in some cases it is easy to pinpoint with high level of certainty that a species is not native to a region. For example, *Echiniscus testudo* (Doyère, 1840) was report-
ed from the Seychelles (Pilato et al. 2002), and it is highly probable that it was brought there by the Europeans during the colonialism era. The impact of such successfully colonising tardigrade species on local communities is unknown.

Our contribution provides first faunistic data on limno-terrestrial tardigrades for Mauritius, and reveals one species (E. perarmatus) probably widely distributed in the tropics (McInnes 1994). This concurs with pantropical records of another echiniscid, E. lineatus (Gąsiorek et al. 2019), and the wide geographic range of E. tropicalis (Fig. 18). The remaining two species are potentially endemic to the island, and the morphological similarity of E. insularis sp. nov. and E. tropicalis (Binda and Pilato 1995) would suggest that autochthonous tardigrades inhabiting Mauritius and Seychelles likely share a common origin. However, E. tropicalis is not closely related to E. insularis sp. nov. since its immediate kin is E. siticulosus (Fig. 8), an Australian endemic. Niedbała (2017) showed that oribatid mites of the Madagascan region are mostly endemic, but, at the same time, its fauna is more similar to the Afrotropical than to

Figure 16. Habitus of females of Pseudechiniscus mascarenensis sp. nov. (SEM) in lateral view. Scale bars in μm.
Figure 17. Claws of *Pseudechiniscus mascarenensis* sp. nov.: A claws I (PCM), B claws II (SEM), C claws III (PCM), D claws IV with papilla (SEM). Scale bars in μm.

Table 12. Measurements [in μm] of selected morphological structures of larvae of *Pseudechiniscus mascarenensis* sp. nov. mounted in Hoyer’s medium (N – number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

| Character | N  | Range   | Mean   | SD  |
|-----------|----|---------|--------|-----|
|           |    | μm      | sp μm  | μm  |
| Body length | 5  | 89 – 105 | 603 – 699 | 99  |
| Scapular plate length | 5  | 14.3 – 17.4 | 30.6 – 37.3 | 1.4  |
| Head appendages lengths | | | | |
| Cirrus internus | 4  | 3.7 – 5.2 | 25.9 – 28.7 | 0.6  |
| Cephalic papilla | 5  | 2.8 – 3.6 | 17.2 – 21.1 | 0.4  |
| Cirrus externus | 5  | 5.2 – 6.6 | 31.9 – 37.3 | 0.6  |
| Clava | 5  | 2.5 – 3.4 | 14.4 – 17.6 | 0.4  |
| Circus d | 4  | 10.3 – 14.2 | 59.8 – 75.8 | 1.8  |
| Cirrus d/Body length ratio | 4  | 10% – 14% | – – 12% | – 2%  |
| Papilla on leg IV length | 4  | 11% – 15% | 6.6 – 8.3 | 0.2  |
| Claw 1 heights | | | | |
| Branch | 5  | 5.0 – 5.9 | 30.7 – 35.0 | 0.4  |
| Spur | 4  | 1.0 – 1.6 | 6.0 – 8.2 | 0.3  |
| Spur/branch length ratio | 4  | 20% – 30% | – – 23% | – 4%  |
| Claw 2 heights | | | | |
| Branch | 5  | 4.6 – 5.1 | 28.7 – 35.0 | 0.2  |
| Spur | 5  | 0.9 – 1.5 | 6.1 – 10.5 | 0.2  |
| Spur/branch length ratio | 5  | 18% – 30% | – – 23% | – 5%  |
| Claw 3 heights | | | | |
| Branch | 5  | 4.7 – 5.3 | 29.5 – 36.4 | 0.3  |
| Spur | 2  | 0.9 – 1.0 | 6.2 – 6.8 | 0.1  |
| Spur/branch length ratio | 2  | 19% – 21% | – – 20% | – 2%  |
| Claw 4 heights | | | | |
| Branch | 5  | 5.4 – 6.2 | 32.5 – 43.4 | 0.4  |
| Spur | 1  | 1.3 – 1.3 | 8.8 – 8.8 | 0.3  |
| Spur/branch length ratio | 1  | 24% – 24% | – – 24% | – 3%  |
the Oriental realm. Given that the wind dispersal is an important movement mechanism for both oribatids and tardigrades (Lehmitz et al. 2011; Nelson et al. 2018; Gąsiorek et al. 2019), it is probable that the closest relatives of endemic Mauritian echiniscids should also be sought in Africa.

The supernumerary dorsal appendages of *E. insularis* sp. nov. are a morphological peculiarity, atypical for *Echiniscus*. Other species exhibiting appendages along the plate margins are very rare: *E. africanus*, *E. baloghi*, and *E. semifoveolatus* (Murray 1907; Iharos 1973; Ito 1993; Gąsiorek and Vončina 2019). These appendages, usually in the form of spicules, should be regarded as morphological convergence, appearing at the same time in the distantly related genus *Acanthechiniscus* (Vecchi et al. 2016). However, in the new Mauritian species, these spicules grow out of the surface of the dorsal plates, which is not present in any other echiniscids. Together with the small body size as for an *Echiniscus*, the presence of these spicules make *E. insularis* sp. nov. easily distinguishable and characteristic taxon. Contrarily, *P. mascarenensis* sp. nov. is a typical representative of *Pseudechiniscus*, more precisely of the subgenus *Meridioniscus*, which has been poorly represented in the molecular dataset under the term “*novaezeelandiae* group” (Cesari et al. 2020), until recently when *Pseudechiniscus* was divided into subgenera thanks to augmenting the phylogenetic inference for this genus based on a large molecular dataset (Gąsiorek et al. 2021). In summary, this limited sampling in the centre of Mauritius reflects the restricted knowledge about insular tardigrade faunas not only in the region of Indian Ocean, but throughout the world.

**Acknowledgements**

We are most grateful to Olena Garmish, Ekaterina Vasilenko, Łukasz Michalczyk, Łukasz Krzywański, Łukasz Skoczylas, and Tan Pal Chun for providing us with the samples. We deeply appreciate help of Oscar Lisi who kindly shared photos of the holotype of *E. tropicalis* with us for comparisons. The Deputy Editor-in-Chief Andreas Schmidt-Rhaesa, Diane Nelson and an anonymous reviewer helped in improving the manuscript, and are gratefully acknowledged. The study was performed in the framework of Preludium (2019/33/N/NZ8/02777 to PG supervised by LM) and Sonata Bis (2016/22/E/NZ8/00417 to LM) grants funded by the National Science Centre. Sampling in Asia was supported by the Polish Ministry of Science and Higher Education (DI2015 014945 to PG). PG is a recipient of the ‘Etiuda’ (2020/36/T/NZ8/00360, funded by the National Science Centre) and ‘Start’ stipends (START 28.2020, funded by the Foundation for Polish Science). Łukasz Michalczyk is acknowledged for advice and constant support.

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**Figure 18.** Distribution of species discussed in the present study. Map downloaded from [www.freeworldmaps.net](http://www.freeworldmaps.net).
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**Supplementary material 1**

*Echiniscus insularis*, MU.001+MU.002
Authors: Piotr Gąsiorek
Data type: Raw morphometric data
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Link: https://doi.org/10.3897/evolsyst.5.59997.suppl1

**Supplementary material 2**

*Echiniscus tropicalis*, ID.032
Authors: Piotr Gąsiorek
Data type: Raw morphometric data
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Link: https://doi.org/10.3897/evolsyst.5.59997.suppl2

**Supplementary material 3**

*Echiniscus tropicalis*, ID.939
Authors: Piotr Gąsiorek
Data type: Raw morphometric data
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Link: https://doi.org/10.3897/evolsyst.5.59997.suppl3

**Supplementary material 4**

*Echiniscus tropicalis*, SG.001
Authors: Piotr Gąsiorek
Data type: Raw morphometric data
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Link: https://doi.org/10.3897/evolsyst.5.59997.suppl4

**Supplementary material 5**

*Pseudechiniscus mascarenensis*, MU.001
Authors: Piotr Gąsiorek
Data type: Raw morphometric data
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Link: https://doi.org/10.3897/evolsyst.5.59997.suppl5

**Supplementary material 6**

GenBank accession numbers
Authors: Yevgen Kiosya, Katarzyna Vončina, Piotr Gąsiorek
Data type: GenBank accession numbers
Explanation note: GenBank accession numbers for the sequences used in the present study.
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