

# Phylogeography and morphological evolution of *Pseudechiniscus* (Heterotardigrada: Echiniscidae)

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**Supplementary Material 2.** Morphological and biogeographic characteristics of species used in analyses. Taxonomic notes on affinities of some taxa are provided below the table (ps IV' = pseudosegmental plate IV').

| Species                              | Sample code | Cephalic papilla | Striae                     | Posterior margin<br>of the ps IV'                              | Ventral<br>ornamentation<br>pattern                         | Claw spurs       | Males in<br>population | Region       |
|--------------------------------------|-------------|------------------|----------------------------|----------------------------------------------------------------|-------------------------------------------------------------|------------------|------------------------|--------------|
| <i>Pseudechiniscus (M.)</i> sp.<br>1 | ID.411      | elongated        | present, mostly<br>reduced | with two evident<br>teeth                                      | complicated, with<br>additional epicuticular<br>thickenings | absent           | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>2 | MY.063      | elongated        | present                    | smooth                                                         | complicated                                                 | present, reduced | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>3 | MY.588      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | ID.057      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | ID.518      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | ID.689      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | present                | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | ID.691      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | ID.693      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | ID.846      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | present                | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | ID.887      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | not found              | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>4 | MY.776      | elongated        | present                    | smooth                                                         | complicated                                                 | present          | present                | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp.<br>5 | MU.001      | elongated        | present                    | smooth or with<br>two poorly<br>elevated teeth                 | complicated                                                 | present          | present                | Afrotropical |
| <i>Pseudechiniscus (M.)</i> sp.<br>6 | ID.842      | elongated        | present, reduced           | usually with two<br>poorly elevated<br>teeth, rarely<br>smooth | complicated                                                 | present          | not found              | Oriental     |

| Species                            | Sample code | Cephalic papilla    | Striae                                                  | Posterior margin of the ps IV'                            | Ventral ornamentation pattern | Claw spurs       | Males in population | Region       |
|------------------------------------|-------------|---------------------|---------------------------------------------------------|-----------------------------------------------------------|-------------------------------|------------------|---------------------|--------------|
| <i>Pseudechiniscus (M.)</i> sp. 6  | ID.954      | elongated           | present, reduced                                        | usually with two poorly elevated teeth, rarely smooth     | complicated                   | present          | not found           | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp. 7  | MY.026      | elongated           | present, reduced                                        | smooth                                                    | complicated                   | present          | present             | Oriental     |
| <i>Pseudechiniscus (M.)</i> sp. 8  | MY.090      | elongated           | present, reduced                                        | smooth                                                    | complicated                   | present          | present             | Oriental     |
| <i>Pseudechiniscus (P.)</i> sp. 9  | AT.050      | pseudohemispherical | absent                                                  | smooth                                                    | reduced                       | present          | present             | Palaearctic  |
| <i>Pseudechiniscus (P.)</i> sp. 9  | ES.188      | pseudohemispherical | absent                                                  | smooth                                                    | reduced                       | present          | present             | Palaearctic  |
| <i>Pseudechiniscus (P.)</i> sp. 9  | ES.202      | pseudohemispherical | absent                                                  | smooth                                                    | reduced                       | present          | present             | Palaearctic  |
| <i>Pseudechiniscus (P.)</i> sp. 9  | GB.023      | pseudohemispherical | extremely reduced, discernible only in the caudal plate | smooth, secondarily thickened or with a longitudinal lobe | reduced                       | present          | present             | Palaearctic  |
| <i>Pseudechiniscus (P.)</i> sp. 9  | GB.035      | pseudohemispherical | absent                                                  | smooth                                                    | reduced                       | present          | present             | Palaearctic  |
| <i>Pseudechiniscus (P.)</i> sp. 9  | TN.018      | pseudohemispherical | absent                                                  | smooth or secondarily thickened                           | reduced                       | present          | present             | Palaearctic  |
| <i>Pseudechiniscus (P.)</i> sp. 10 | ZA.366      | pseudohemispherical | absent                                                  | with single longitudinal lobe                             | complicated                   | present          | present             | Afrotropical |
| <i>Pseudechiniscus (P.)</i> sp. 11 | ZA.246      | pseudohemispherical | absent                                                  | smooth                                                    | complicated                   | present          | not found           | Afrotropical |
| <i>Pseudechiniscus (P.)</i> sp. 12 | MG.005      | pseudohemispherical | absent                                                  | smooth                                                    | complicated                   | present, reduced | present             | Afrotropical |
| <i>Pseudechiniscus (P.)</i> sp. 13 | US.036      | pseudohemispherical | absent                                                  | smooth                                                    | complicated                   | present          | present             | Nearctic     |
| <i>Pseudechiniscus (P.)</i> sp. 14 | ME.008      | pseudohemispherical | absent                                                  | smooth                                                    | complicated                   | present          | present             | Palaearctic  |

| Species                                            | Sample code | Cephalic papilla    | Striae                                                  | Posterior margin of the ps IV'  | Ventral ornamentation pattern | Claw spurs | Males in population | Region       |
|----------------------------------------------------|-------------|---------------------|---------------------------------------------------------|---------------------------------|-------------------------------|------------|---------------------|--------------|
| <i>Pseudechiniscus (P.)</i> sp. 15                 | PL.189      | pseudohemispherical | absent                                                  | smooth                          | complicated                   | present    | present             | Palaearctic  |
| <i>Pseudechiniscus (P.)</i> sp. 16                 | AR.439      | pseudohemispherical | absent                                                  | smooth                          | complicated                   | present    | not found           | Neotropical  |
| <i>Pseudechiniscus (P.)</i> sp. 16                 | BR.016      | pseudohemispherical | extremely reduced, discernible only in the caudal plate | smooth                          | complicated                   | present    | present             | Neotropical  |
| <i>Pseudechiniscus (P.)</i> sp. 17                 | US.037      | pseudohemispherical | absent                                                  | with two evident teeth          | complicated                   | present    | not found           | Nearctic     |
| <i>Pseudechiniscus (P.)</i> sp. 18                 | IT.120      | pseudohemispherical | absent                                                  | smooth                          | complicated                   | present    | present             | Palaearctic  |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ID.368      | elongated           | present                                                 | smooth                          | complicated                   | present    | not found           | Oriental     |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ID.417      | elongated           | present                                                 | smooth                          | complicated                   | present    | not found           | Oriental     |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ID.483      | elongated           | present                                                 | smooth                          | complicated                   | present    | not found           | Oriental     |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ID.485      | elongated           | present                                                 | smooth                          | complicated                   | present    | not found           | Oriental     |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ID.526      | elongated           | present                                                 | smooth                          | complicated                   | present    | not found           | Oriental     |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | VN.026      | elongated           | present                                                 | smooth or secondarily thickened | complicated                   | present    | not found           | Oriental     |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ZA.177      | elongated           | present                                                 | smooth or secondarily thickened | complicated                   | present    | present             | Afrotropical |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ZA.178      | elongated           | present                                                 | smooth or secondarily thickened | complicated                   | present    | present             | Afrotropical |
| <i>Pseudechiniscus (M.)</i> cf. <i>angelusalas</i> | ZA.256      | elongated           | present                                                 | smooth or secondarily thickened | complicated                   | present    | present             | Afrotropical |

| Species                                    | Sample code | Cephalic papilla    | Striae  | Posterior margin<br>of the ps IV'     | Ventral<br>ornamentation<br>pattern | Claw spurs       | Males in<br>population | Region       |
|--------------------------------------------|-------------|---------------------|---------|---------------------------------------|-------------------------------------|------------------|------------------------|--------------|
| <i>Pseudechiniscus (P.) asper</i>          | JP.012      | pseudohemispherical | absent  | with two evident<br>teeth             | complicated                         | present, reduced | present                | Palaeartic   |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ID.464      | pseudohemispherical | absent  | smooth                                | complicated                         | present          | not found              | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ID.466      | pseudohemispherical | absent  | smooth                                | complicated                         | present          | not found              | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ID.467      | pseudohemispherical | absent  | smooth                                | complicated                         | present          | present                | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ID.507      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | present                | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ID.546      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | not found              | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ID.547      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | present                | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ID.548      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | not found              | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | MM.010      | pseudohemispherical | absent  | smooth                                | complicated                         | present          | present                | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | VN.042      | pseudohemispherical | absent  | smooth or<br>secondarily<br>thickened | complicated                         | present          | not found              | Oriental     |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ZA.157      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | not found              | Afrotropical |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ZA.183      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | not found              | Afrotropical |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ZA.190      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | not found              | Afrotropical |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ZA.202      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | not found              | Afrotropical |
| <i>Pseudechiniscus (P.) cf. ehrenbergi</i> | ZA.246      | pseudohemispherical | absent  | smooth                                | reduced                             | present          | not found              | Afrotropical |
| <i>Pseudechiniscus (M.) quadrilobatus</i>  | ID.407      | elongated, swollen  | present | with large lobe                       | absent                              | absent           | not found              | Oriental     |

| Species                                   | Sample code | Cephalic papilla    | Striae  | Posterior margin of the ps IV' | Ventral ornamentation pattern                   | Claw spurs | Males in population | Region      |
|-------------------------------------------|-------------|---------------------|---------|--------------------------------|-------------------------------------------------|------------|---------------------|-------------|
| <i>Pseudechiniscus (M.) quadrilobatus</i> | ID.474      | elongated, swollen  | present | with large lobe                | absent                                          | absent     | not found           | Oriental    |
| <i>Pseudechiniscus (M.) cf. saltensis</i> | AR.251      | elongated           | present | with two evident teeth         | complicated                                     | present    | present             | Neotropical |
| <i>Pseudechiniscus (M.) cf. saltensis</i> | AR.266      | elongated           | present | with two evident teeth         | complicated                                     | present    | present             | Neotropical |
| <i>Pseudechiniscus (P.)shintai</i>        | JP.012      | pseudohemispherical | absent  | smooth                         | complicated                                     | present    | present             | Palaeartic  |
| <i>Pseudechiniscus (P.)suillus</i>        | GB.008      | pseudohemispherical | absent  | secondarily thickened          | reduced, uniform pillars covering entire venter | present    | present             | Palaeartic  |
| <i>Pseudechiniscus (P.)suillus</i>        | GB.028      | pseudohemispherical | absent  | secondarily thickened          | reduced, uniform pillars covering entire venter | present    | present             | Palaeartic  |
| <i>Pseudechiniscus (P.)suillus</i>        | NO.002      | pseudohemispherical | absent  | smooth                         | complicated                                     | present    | present             | Palaeartic  |
| <i>Pseudechiniscus (P.)suillus</i>        | NO.190      | pseudohemispherical | absent  | smooth                         | complicated                                     | present    | present             | Palaeartic  |

1. After the examination of the type material of *P. (M.) quadrilobatus*<sup>1</sup>, *P. gullii*<sup>2</sup> and the description of *P. pilatoi*<sup>3</sup>, combined with obtaining new populations from Celebes (the Malay Archipelago), there are no morphological arguments that would substantiate keeping these three species as separate entities. Specifically: (i) the sculpturing is homogeneous in all three species, consisting of widely spaced endocuticular pillars joined by evident *striae*; (ii) the posterior margin of the scapular plate exhibits considerable intra-population variability that exceeds inter-specific variation (the posterior margin may form a lobe, a dimple, or be completely smooth); and (iii), claws have an identical morphology, being very delicate and short, spurless, which is unusual for Echiniscidae, and for *Pseudechiniscus* in particular (= a homoplasious autapomorphy). To conclude, in our opinion, *P. gullii* and *P. pilatoi* are younger synonyms of *P. (M.) quadrilobatus* that is characterised by a wide tropical distribution in the Indopacific area.

2. *Pseudechiniscus (M.)* sp. 5 bears much resemblance to *Pseudechiniscus (M.) santomensis*<sup>4</sup> from the Gulf of Guinea. However, males are unknown for the latter, and the posterior margin of pseudosegmental plate IV' is smooth in females of *Pseudechiniscus (M.)* sp. 5, whereas the plate is equipped with two teeth in *Pseudechiniscus (M.) santomensis*.

3. *Pseudechiniscus (M.)* sp. 6 is similar to *Pseudechiniscus (M.) bidenticulatus*<sup>5</sup> described from Java, however the *Pseudechiniscus (M.)* sp. 6 has evident spurs on internal claws of whereas the claws in *Pseudechiniscus (M.) bidenticulatus* are spurless, thus species are not conspecific.

4. *Pseudechiniscus (P.)* sp. 17 is very characteristic because of lateral bumps in the positions B–E, which, together with the place of origin (Roan Mountain, Tennessee, USA), suggest it is conspecific with an undescribed *Pseudechiniscus* species mentioned in the description of *Pseudechiniscus (P.) brevimontanus*<sup>6</sup>.

5. Results of COI comparisons (see the phylogeny below):

– *Pseudechiniscus* (*P.*) cf. *ehrenbergi* likely corresponds with *Pseudechiniscus* (*P.*) *ehrenbergi* s.s. (the morphological disparities embrace e.g. different ventral sculpturing patterns)<sup>7</sup>;

– *Pseudechiniscus* (*P.*) sp. 18 likely corresponds with *Pseudechiniscus* aff. *suillus* (Ca1)<sup>8</sup>, especially that both taxa were found in Italy.

6. All remaining unnamed species were identified as new.

## References

<sup>1</sup>Iharos, G. Einige Angaben zur Tardigradenfauna Vietnams. *Opusc. Zool.* **9**, 273–277 (1969).

<sup>2</sup>Pilato, G. & Lisi, O. Notes on some tardigrades from southern Mexico with description of three new species. *Zootaxa* **1236**, 53–68 (2006).

<sup>3</sup>Li, X. Tardigrades from the Tsinling Mountains, central China with descriptions of two new species of Echiniscidae (Tardigrada). *J. Nat. Hist.* **41**, 2719–2739 (2007).

<sup>4</sup>Fontoura, P., Pilato, G. & Lisi, O. First record of Tardigrada from São Tomé (Gulf of Guinea, Western Equatorial Africa) and description of *Pseudechiniscus santomensis* sp. nov. (Heterotardigrada: Echiniscidae). *Zootaxa* **2564**, 31–42 (2010).

<sup>5</sup>Bartoš, E. Die Tardigraden der Chinesischen und Javanischen Moosproben. *Acta Soc. Zool. Bohem.* **27**, 108–114 (1963).

<sup>6</sup>Kendall-Fite, K. & Nelson, D.R. Two new species of tardigrades from Short Mountain, Tennessee, U.S.A. *Zool. J. Linn. Soc.* **116**, 205–214 (1996).

<sup>7</sup>Roszkowska, M. *et al.* Integrative description of five *Pseudechiniscus* species (Heterotardigrada: Echiniscidae: the *suillus-facettalis* complex). *Zootaxa* **4763**, 451–484 (2020).

<sup>8</sup>Cesari, M. *et al.* An integrated study of the biodiversity within the *Pseudechiniscus suillus-facettalis* group (Heterotardigrada: Echiniscidae). *Zool. J. Linn. Soc.* **188**, 717–732 (2020).

