



First record of Tardigrada from São Tomé (Gulf of Guinea, Western Equatorial Africa) and description of *Pseudechiniscus santomensis* sp. nov. (Heterotardigrada: Echiniscidae)

PAULO FONTOURA¹, GIOVANNI PILATO² & OSCAR LISI²

¹Eco-Ethology Research Unit (FCT-331/94) and Department of Biology, Faculty of Sciences, University of Porto. R. Campo Alegre s/n, FC4, 4169-007 Porto, Portugal. E-mail: pfontoura@fc.up.pt

²Dipartimento di Biologia Animale “Marcello La Greca”, Università di Catania Via Androne 81, 95124 Catania, Italy. E-mail: pilato@unict.it

Abstract

The first six species of tardigrades from São Tomé Island (Democratic Republic of São Tomé and Príncipe) were recorded: the heterotardigrade *Pseudechiniscus santomensis* sp. nov., and five eutardigrades: *Macrobiotus* sp., *Paramacrobiotus gerlachae* (Pilato, Binda & Lisi, 2004), *Minibiotus intermedius* (Plate, 1889), *Doryphoribius* sp., *Diphascon* (*Diphascon*) sp.. *Pseudechiniscus santomensis* sp. nov. belongs to the group of species that lack all trunk cirri except filament A; have a pseudosegmental plate with triangular projections on the posterior margin, and dorsal plates ornamented by small dots joined by very delicate striae. The ventral surface of the body has small dots, without striae, forming patches and a reticular design. The new species differs from *Pseudechiniscus spinerectus* Pilato, Binda, Napolitano & Moncada, 2001, the most similar species, in lacking longitudinal folds on scapular and paired plates; the pseudosegmental plate undivided and with shorter triangular, backward oriented projections that are never erect; only one intersegmental platelet lateral to the median plates 1 and 2; slightly shorter buccal cirri, filament A and papilla of the hind legs; and in having slightly different the ornamentation of the ventral body surface.

Key words: Tardigrada, African meiofauna, *Pseudechiniscus*, *Macrobiotus*, *Paramacrobiotus*, *Minibiotus*, *Doryphoribius*, *Diphascon*

Introduction

The importance of faunistic studies has been renewed at the beginning of the 21st century as a consequence of the current biodiversity crisis. Actually, despite all the efforts done by conservation biologists, the number of species extinctions resulting from climate changes and habitats loss is still constantly increasing worldwide, justifying the urgent need of species inventories with special emphasis on neglected regions (Dubois, 2003). The study of the geographic distribution of the limno-terrestrial tardigrades is not an exception to that paradigm. In the last three decades the number of known tardigrade species has almost doubled, reaching c. 1000 species, but this knowledge is still centred mostly in the Palearctic and Nearctic regions. By comparison, some other geographic regions are barely explored. This is the case for Africa with one of the lowest rates of tardigrade paper publications in the last few years, probably because tardigradologists do not live there (Guill & Cabrero-Sañudo, 2007; McInnes & Pugh, 2007). In Africa only about 200 species are known from 24 countries (Jørgensen, 2001; Pilato & Binda, 2001; McInnes & Pugh, 2007; Garey *et al.* 2008; Kaczmarek *et al.* 2008). In reality, the knowledge of the African tardigrade fauna is scattered and sparse with most records located along the coastline and in the vicinity of touristic destinations (Jørgensen, 2001). As an exception we may record the Seychelles, where 34 species have been recorded, 19 of which are currently endemic for that archipelago (Biserov, 1994; Binda & Pilato 1995a; b; Pilato *et al.* 2002, 2004, 2006; Pilato & Lisi 2009a, b). It is evident that other African regions and enclaves require further investigation. This is the

case of the Guinea Gulf, an area of the Western Equatorial Africa that is traditionally shared by nine countries: Benin; Cameroon; Equatorial Guinea; Gabon; Ghana; Ivory Coast; Nigeria; São Tomé and Príncipe, and Togo. It is a region characterised by a very particular geomorphology, having a tropical wet climate with high rainfall, which is propitious to the development of important areas of tropical rainforest with the associated specific fauna and flora. Despite this peculiarity there were only three very old studies on tardigrades carried out in four countries of this geographical region: Ivory Coast (Marcus, 1936); Bioko Island (former Fernando Póo) in Equatorial Guinea (Rodríguez-Roda, 1948) and Cameroon and Gabon (Iharos, 1969b). In this paper, the first record of tardigrades in São Tomé and Príncipe, we include the description of a species new to science, *Pseudechiniscus santomensis*.

Material and methods

Tardigrades were extracted from dried moss samples collected from rocks in Cascata de São Nicolau (0°16' 38"N; 6°38' 46"E), São Tomé Island, Democratic Republic of São Tomé and Príncipe, in March 22, 2008, by N. G. Oliveira (Parque Biológico de Gaia, Portugal). The specimens were mounted on slides in Hoyer's medium with a small amount of potassium iodide solution (Horning *et al.*, 1978). Measurements, given in micrometers (μm), and photomicrographs were made using a Zeiss Axioskop Phase Contrast Microscope (100x oil immersion) equipped with digital camera and using Axiovision 4.7 Imaging System Software. Structures were measured only if they were undamaged and their orientation was suitable. Body length was measured from the anterior margin to the end of the body, excluding the hind legs. Measurements of the bucco-pharyngeal apparatus, claws, and the assessment of the *pt* index, were obtained according to Pilato (1981). For the species of the subgenus *Diphascion*, the *ptd* indexes were obtained according to Pilato & Binda (1997/98). The percent ratio between the body length or the scapular plate length and another structure, were computed according Lattes & Gallelli (1972) for specimens of the genus *Pseudechiniscus*.

For comparison, we examined: the holotype and paratypes of *Pseudechiniscus spinerectus* Pilato, Binda, Napolitano & Moncada, 2001, *Pseudechiniscus gullii* Pilato & Lisi 2006, *Paramacrobotus gerlachae* (Pilato, Binda & Lisi, 2004), *Doryphoribius maranguensis* Binda & Pilato, 1995b, *Diphascion* (*Diphascion*) *claxtonae* Pilato & Binda, 1998 and *Diphascion* (*Diphascion*) *faialense* Fontoura & Pilato, 2007, paratypes of *Pseudechiniscus bartkei* Węglarska, 1962 and specimens of *Peudechiniscus quadrilobatus* Iharos, 1969c, *Pseudechiniscus suillus* (Ehrenberg, 1853), *Pseudechiniscus novaezeelandiae* (Richters, 1908a) and *Minibiotus intermedius* (Plate, 1889). Holotypes and paratypes were supplied from the collection of Binda & Pilato (Museum of the Department of Animal Biology "Marcello La Greca", University of Catania, Italy), whilst comparisons with other species were only based on descriptions in the literature.

Taxonomic accounts

Pseudechiniscus santomensis sp. nov.

(Figs. 1A–D; 2A)

Material examined: 32 specimens, one of them is a two clawed larva.

Type locality: Cascata de São Nicolau (0°16' 38"N; 6°38' 46"E), São Tomé Island, Democratic Republic of São Tomé and Príncipe.

Type repository: The holotype (slide No. 5401) and one paratype (slide No. 5402) are deposited in the collection of Binda & Pilato, (Museum of the Department of Animal Biology "Marcello La Greca", University of Catania, Italy). The other paratypes including the larva (slides CII59 - CII89) are deposited in the collection of P. Fontoura at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Specific diagnosis: *Pseudechiniscus* with tiny projections on the posterior margin of the undivided pseudosegmental plate. Those projections, generally triangular in shape, are sometimes reduced into two small teeth or assume the shape of a wide protruding flap poorly developed with a central indentation; median

plate 1 with a transverse fold; median plate 2 divided; median plate 3 undivided; terminal plate not faceted but with two indentations. One intersegmental platelet present on each side of the median plates 1 and 2. Ornamentation of the dorsal plates comprised of dots joined by very delicate striae. Ventral dots unjoined by striae and forming a reticular patched design. Cephalic appendages and very short filaments A, present; all other lateral, dorsal filaments, or projections, absent. Internal claws with a thin and straight spur oriented towards the claw base. No dorsal leg spines. Short papilla on hind legs present; dentate collar, absent.

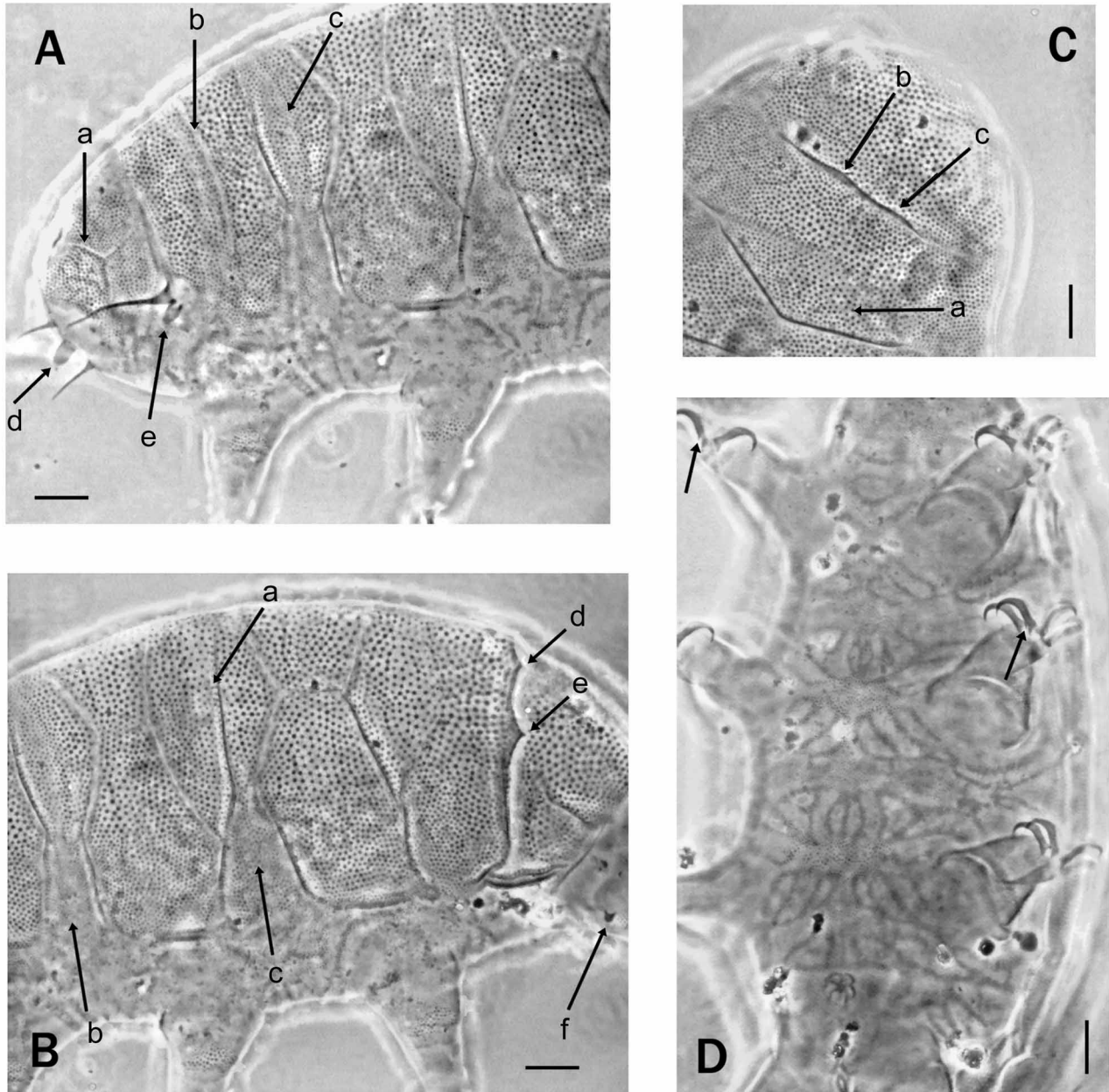


FIGURE 1. *Pseudechinscus santomensis* sp. nov., A, Anterior portion of the body of the holotype; the W-shaped fold of the head plate (arrow a), the transverse fold dividing the scapular plate into an anterior and a posterior portion (arrow b), the transverse fold dividing the median plate 1 into an anterior and a posterior portion (arrow c), the buccal cirri, the cephalic papilla (arrow d), the cirrus A and the adjacent clava (arrow e) are visible. B, posterior portion of the body of the holotype; the transverse fold dividing the median plate 2 into an anterior and a posterior portion (arrow a), the intersegmental platelets lateral to the median plates 1 and 2 (arrows b, c), the triangular marginal projections of the undivided pseudosegmental plate (arrows d, e), the papilla on the hind legs (arrow f) are visible. C, posterior portion of the body of a paratype; the undivided median plate 3 (arrow a) and the pseudosegmental plate with reduced marginal projections (arrows b, c) are visible. D, cuticular ornamentation of the ventral body surface of a paratype. The arrows indicate spurs of the internal claws. Scale bars = 10 µm.

Description of the holotype: Female; body length 168 μm ; colour reddish. Eye spots not observed either in slide mounted specimens or in live specimens. Head plate with a W-shaped fold (Fig. 1A, arrow a). Scapular plate divided into an anterior and a posterior portion by a transverse fold (Fig. 1A, arrow b); lateral longitudinal folds absent. Median plate 1 divided by a transverse fold into an anterior and posterior portion with clearly different sized dots (Fig. 1A, arrow c); median plate 2 also clearly divided but dots similar in size in anterior and posterior portion (Fig. 1B, arrow a); median plate 3 undivided (Fig. 1C, arrow a). An intersegmental platelet on each side of median plates 1 and 2 (Fig. 1B, arrows b, c). Median and lateral longitudinal folds absent in all the median plates and in the pseudosegmental plate. Two small triangular projections, backward oriented, extending about 4.2 μm from the posterior margin of the pseudosegmental plate (Fig. 1B, arrows d, e). Terminal plate unafaceted with two indentations.

TABLE 1. Measurements (in μm) of the largest (holotype) and the smallest specimens, and mean values (mean; standard deviation; number of specimens (n); minimum and maximum) of *P. santomensis* **sp. nov.** (% bo = percentage of the given structure to the body length; % sc = percentage of the given structure to the scapular plate length).

Character	Holotype (largest)			Smallest			Mean values and range		
	μm	%bo	%sc	μm	%bo	%sc	μm	%bo	%sc
Body length	168	-	-	119	-	-	143.6 \pm 15.2(21) 119–168	-	-
Scapular plate length	26.2	15.6	-	-	-	-	23.0 \pm 2.2(15) 18.1–26.2	15.9 \pm 1.6(13) 13.8–18.6	-
Internal buccal cirrus	9.4	5.6	35.9	6.1	5.1	-	7.8 \pm 0.9(22) 6.1–9.8	5.4 \pm 0.1(21) 4.6–6.7	35.1 \pm 3.8(14) 28.9–44.7
External buccal cirrus	12.8	7.6	48.9	7.2	6.1	-	11.1 \pm 1.6(22) 7.2–14.4	7.8 \pm 1.0(21) 5.7–9.6	50.0 \pm 5.9(14) 39.9–55.7
Cephalic papilla	4.8	2.9	18.3	3.7	3.1	-	4.3 \pm 0.45(22) 3.5–5.4	3.0 \pm 0.3(20) 2.4–3.6	19.0 \pm 1.6(14) 15.6–22.7
Clava	4.8	2.9	18.3	3.3	2.8	-	4.1 \pm 0.6(22) 2.7–5.2	2.8 \pm 0.4(20) 2.3–3.5	18.2 \pm 3.5(15) 12.3–24.3
Cirrus A	18.7	11.1	71.4	10.5	8.8	-	17.4 \pm 2.3(21) 10.5–21.8	12.0 \pm 1.4(19) 8.8–14.2	77.9 \pm 7.7(14) 67.6–96.8
Pseudosegmental plate projections	4.2	2.5	16.0	1.0	0.8	-	3.6 \pm 1.2(20) 1.4–6.3	2.5 \pm 0.8(18) 1.1–4.1	16.8 \pm 6.7(15) 6.1–29.4
Internal claws I	9.0	5.4	34.4	-	-	-	8.0 \pm 0.7(14) 6.8–9.0	5.3 \pm 0.2(13) 4.9–5.6	34.4 \pm 1.9(9) 30.8–37.9
External claws I	8.0	4.8	30.5	5.5	4.6	-	7.1 \pm 0.8(15) 5.5–8.2	5.0 \pm 0.6(15) 3.8–6.3	32.3 \pm 5.5(9) 23.7–43.1
Internal claws II	8.4	5.0	32.1	-	-	-	7.8 \pm 0.4(13) 7.2–8.4	5.3 \pm 0.3(12) 5.0–6.2	34.0 \pm 3.4(11) 30.4–42.5
External claws II	8.0	4.8	30.5	-	-	-	7.2 \pm 0.7(14) 5.4–8.1	4.9 \pm 0.4(13) 4.3–5.9	32.5 \pm 3.8(10) 27.3–40.3
Internal claws III	-	-	-	-	-	-	7.4 \pm 0.5(13) 6.3–8.0	5.1 \pm 0.3(11) 4.7–5.6	32.5 \pm 2.7(9) 28.5–37.0
External claws III	8.0	4.8	30.5	-	-	-	7.0 \pm 0.6(15) 6.1–7.9	4.7 \pm 0.4(13) 4.2–5.6	31.4 \pm 3.5(11) 25.7–38.7
Internal claws IV	9.2	5.5	35.1	6.7	5.6	-	8.0 \pm 0.7(11) 6.8–9.0	5.6 \pm 0.4(10) 4.7–6.0	37.6 \pm 3.8(7) 34.6–45.3
External claws IV	8.5	5.1	32.4	6.0	5.0	-	7.4 \pm 0.7(13) 6.7–9.2	5.2 \pm 0.4(11) 5.1–6.6	34.1 \pm 3.7(8) 29.8–41.4
Papilla on legs IV	2.2	1.3	8.4	1.8	1.5	-	2.1 \pm 0.2(22) 1.6–2.3	1.4 \pm 0.2(20) 1.2–1.8	9.1 \pm 0.7(15) 7.9–10.5

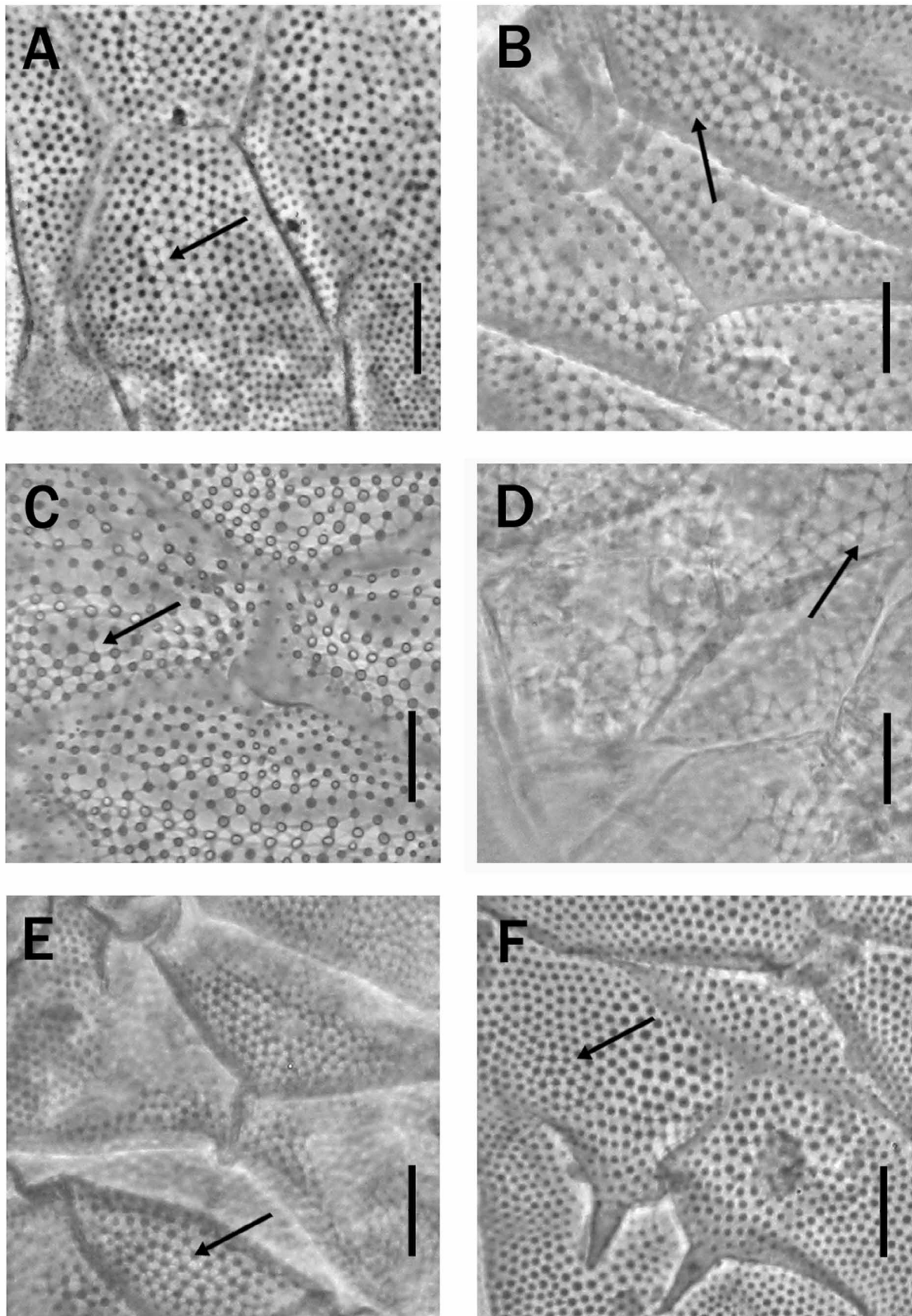


FIGURE 2. Cuticular plate ornamentation of: A, *Pseudechiniscus santomensis* **sp. nov.** (holotype); B, *P. bartkei*; C, *P. gullii*; D, *P. quadrilobatus*; E, *P. spinerectus* and F, *P. novaezeelandiae novaezeelandiae*. Arrows indicate delicate striae connecting the dots. Scale bars = 10 μ m.

All the dorsal plates have a dense and regular ornamentation comprised of dots joined by very delicate striae, which are difficult to see (Fig. 2A, arrow). Dots are slightly larger on the terminal and pseudosegmental plates (with respectively *c.* 50 and 56 dots per 100 μm^2), dorsal portion of paired plates and posterior portion of the scapular plate. Smallest dots are present on the neck plate, anterior portion of median plate 1, lateral portions of paired plates and on the intersegmental lateral platelets. Leg plates also sculptured but striae joining the dots are not visible (Fig. 1A).

Ventral ornamentation comprised of a very fine granulation (without striae joining the dots) forming transverse patches and a reticular design (Fig. 1D). A wide patch is present in the head region, two elliptical meshes follow; after the first pair of legs alternate transverse patches of dots and transverse bands of mesh are present; in some bands all the meshes are elongate in shape, in other bands two large roughly triangular meshes are also present.

Length of external and internal buccal cirri 12.8 μm and 9.4 μm respectively; cephalic papilla 4.8 μm long (Fig. 1A, arrow d).

Cirri A very short, 18.7 μm long; adjacent clava 4.8 μm long (Fig. 1A, arrow e). Apart from the triangular projections of the pseudosegmental plate, all other lateral or dorsal cirri, or any other kind of projection are absent.

No spines on legs I. Hind legs without dentate collar but with a small papilla 2.2 μm long (Fig. 1B, arrow f). Length of the external and internal claws of the legs I, 9.0 μm and 8.0 μm respectively; on the legs II and III, external 8.0 μm and internal 8.4 μm ; external and internal claws of legs IV, 8.5 μm and 9.2 μm long respectively. A very thin and straight spur oriented towards the claw base is present on internal claws (Fig. 1D, arrows).

Eggs unknown.

Remarks: The quantitative and qualitative characters of the paratypes are similar to those of the holotype (Tab. 1), but a degree of variability concerning the shape of the posterior margin of the pseudosegmental plate must be stressed. Most specimens (64 %) have two triangular projections, as in the holotype (Fig. 1B), which can be reduced to two very small teeth; other specimens (32%) have the posterior margin slightly projected forming only one wide protruding flap, sometimes with a very soft median indentation assuming the shape of two rounded lobes (Fig. 1C), Only one paratype (4%) has the posterior margin of the pseudosegmental plate without any kind of projection.

Etymology: The name *santomensis* refers to the *locus typicus*: São Tomé Island; *santomensis* = inhabiting São Tomé.

Differential diagnosis: According to Kristensen (1987), two main groups of species can be distinguished within the genus *Pseudechiniscus* Thulin, 1911: the ‘*victor* group’ composed by species with trunk cirri and the ‘*suillus/conifer* group’ with species characterised by the absence of trunk cirri. *Pseudechiniscus santomensis* **sp. nov.** clearly belongs to the latter.

Within the ‘*suillus/conifer* group’, six species have the following five important characters in common with *P. santomensis* **sp. nov.**: absence of lateral papillae, presence of projections at the posterior margin of the pseudosegmental plate, scapular plate with a transverse fold, terminal plate not faceted, and cuticular ornamentation comprised of dots joined by striae. Those taxa are: *P. bartkei* Węglarska, 1962; *P. quadrilobatus* Iharos, 1969c; *P. spinerectus* Pilato, Binda, Napolitano & Moncada, 2001; *P. gullii* Pilato & Lisi 2006, *P. pilato* Li, 2007 and *P. yunnanensis* Wang, 2009. In addition, we must stress a problem regarding *P. novaezeelandiae* (Richters, 1908a). The nominal subspecies of this species has lateral papillae, but in the subspecies *aspinosus*, described by Iharos (1963), these are lacking. In the description of *P. novaezeelandiae*, and all the subspecies, striae joining the cuticular dots were not described but we noted that they are present, even if very faint, in a specimen from New Zealand (see Pilato *et al.* 2005). Unfortunately we were not able to examine the subspecies *aspinosus* and therefore we may only hypothesize that it has cuticular ornamentation similar to that of the nominal subspecies. If the hypothesis is true, *P. novaezeelandiae aspinosus* would also have the five characters indicated above, and therefore we have included a comparison of *P. santomensis* **sp. nov.** to this subspecies. *Pseudechiniscus santomensis* **sp. nov.** differs from *P. bartkei*, *P. quadrilobatus*, *P. gullii*, *P. pilato* and *P. yunnanensis* in having the median plate 2 divided; more dense cuticular granulation,

and less visible striae joining the dots (Fig. 2). In addition, it does not have the raised margins of the cuticular plates, which are present in *P. gullii*, *P. quadrilobatus* and *P. pilato*; the median plate 1 is divided by a transverse fold, unlike *P. bartkei*, *P. quadrilobatus*, *P. pilato* and *P. yunnanensis*; spurs are present on the internal claws, absent in *P. gullii*, *P. quadrilobatus*, *P. pilato* and *P. yunnanensis*; and the new species has a different ventral ornamentation to that seen on *P. bartkei*, *P. gullii*, *P. quadrilobatus* and *P. yunnanensis* (in the description of *P. pilato* no information was supplied regarding the ventral cuticular ornamentation). *Pseudechiniscus santomensis* can also be distinguished from *P. bartkei* in having the pseudosegmental plate undivided and only one intersegmental platelet lateral to the median plates 1 and 2; from *P. gullii* as the pseudosegmental plate is undivided and with two marginal lobes instead of only one, and the absence of a dip between scapular plate and median plate 1; from *P. quadrilobatus* as the pseudosegmental plate has two marginal projections instead of only one; from *P. pilato* as the pseudosegmental plate is not divided and has two marginal projections instead of only one, plus the terminal plate has two indentations (*n.b.* the description of *P. pilato* does not note presence or absence of intersegmental lateral platelets, which may also be a distinguishing character); and from *P. yunnanensis* as the terminal plate has two indentations and lateral platelets are present.

P. spinerectus is the species most similar to *Pseudechiniscus santonensis* **sp. nov.**, but the new species differs in having the pseudosegmental plate undivided; the marginal projections of this plate less developed and never erected; lateral longitudinal folds absent in the scapular and paired plates; only one intersegmental platelet lateral to the median plates 1 and 2; striae joining the plate dots slightly less visible (Fig. 2A and E); dots on the legs not joined by striae, and some differences in the detail of the ventral cuticular ornamentation. In addition, *P. santomensis* **sp. nov.** differs from *P. spinerectus* in having shorter buccal cirri (external and internal cirri 12.8 μm and 9.4 μm respectively (holotype) and 14.5 μm and 12.1 μm long respectively (*P. spinerectus* specimen 135 μm long)); in having shorter cirrus A (<20 μm long in *P. santomensis* **sp. nov.** and >32 μm in *P. spinerectus*); shorter papilla of hind legs (*c.* 2.2 μm in the new species and *c.* 3.0 in *P. spinerectus*).

The new species differs from *P. navaezeelandiae aspinosus* in having the pseudosegmental plate undivided; intersegmental lateral platelets more clearly defined; striae joining the cuticular dots more visible (if *ssp aspinosus* follows *P. navaezeelandiae* form of striae); completely different ventral cuticular ornamentation and internal claws with spurs.

It is interesting to note that some authors (*e.g.* Dastych, 1984; Kathman & Dastych, 1990; McInnes, 1995; Miller *et al.* 2001) ascribed to specimens of *Pseudechiniscus suillus* the ventral cuticle with small dots forming a reticular design and, with the exception of Miller *et al.* (2001), also a dorsal sculpture comprised of dots joined by very delicate striae. However, they also stated that this kind of sculpture seems to be characteristic of localised populations. This kind of cuticle sculpture is not usually observed in populations of *P. suillus*, and is not mentioned in descriptions made by taxonomists of high reputation (*e.g.* Dastych, 1984; Maucci, 1986) with light microscope (phase contrast) or scanning electron microscope (Schuster *et al.*, 1975). According to McInnes (1995) with whom we agree, those specimens do not belong to *P. suillus* but to different species. For this reason we think superfluous to compare *P. santomensis* **sp. nov.** with populations belonging to undetermined species.

***Macrobotus* sp.**

Material examined: Two specimens (slides CII-90 and CII-91), deposited in the collection of P. Fontoura at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Remarks: The specimens belong to the '*hufelandi* group'. However, the absence of eggs prevents a positive specific diagnosis.

***Paramacrobotus gerlachae* (Pilato, Binda & Lisi, 2004)**

Material examined: 29 specimens (5 in simplex stage) and eight eggs (slides CIII1-to CIII36), deposited in the collection of P. Fontoura at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Remarks: The characteristics of the specimens perfectly fit the original description by Pilato *et al.* (2004) and additional information on cap-like terminal portion of the egg processes by Michalczyk *et al.* (2006). Up to now *Paramacrobotus gerlachae* was reported only from the Seychelles.

***Minibiotus intermedius* (Plate, 1889)**

Material examined: 34 specimens, 9 in simplex stage, and 5 eggs (slides CIII37 to CIII74), deposited in the collection of P. Fontoura at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Remarks: The characteristics of the specimens and eggs fit the amended description of the species made by Claxton (1998). *Minibiotus intermedius* is a very common species widely distributed all over the world, Africa included.

***Doryphoribius* sp.**

Material examined: One specimen (slide CII92), deposited in the collection of P. Fontoura at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Description: Cuticle with transverse undulations and an indistinct sculpture, which is restricted to the posterior portion of the body, and composed of small and irregularly distributed tubercles. The buccal tube, with ventral strengthening bar, 35.3 μm long and 3.7 μm wide ($pt = 10.5$) externally; stylet supports inserted at 73.0 % of the buccal tube length; pharyngeal bulb with apophyses, two rod-shaped macroplacoids and no microplacoid; placoids row 13.7 μm long ($pt = 38.8$); first macroplacoid 9.2 μm long ($pt = 26.1$), the second 4.6 μm ($pt = 13.0$).

Claws with short basal portion; main branches with accessory points; external claws with enlarged bases.

Unfortunately the specimen was partially damaged and other characters are not visible; therefore a confident correct specific diagnosis is not possible.

***Diphascon* (*Diphascon*) sp.**

Material examined: One specimen (slide CII93), deposited in the collection of P. Fontoura at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Description: The examined specimen clearly belongs to the 'pingue group' characterised by having smooth cuticle; pharyngeal bulb with apophyses, three macroplacoids, microplacoid and septulum; lunules and other cuticular thickenings on the legs absent; claw bases without indentations. According to several authors (*e.g.* Pilato & Binda, 1997/98; 1998; 1999; Fontoura & Pilato, 2007) the species belonging to this group are very difficult to separate from one another, differing in some quantitative characters and in only a few cases in qualitative characters.

The only specimen collected in São Tomé, 173 μm long, is not perfectly oriented to measure all the structures necessary to ensure a correct specific diagnosis. As a consequence the measurements indicated here have to be considered approximate. Buccal tube 13.8 μm long and 1.6 μm wide externally ($ptd = 11.6$), its length is about 35.3 % of the total length of the bucco-pharyngeal tube; stylet supports inserted on the buccal tube at about 71.0 % of its length; pharyngeal bulb with apophyses, three rod-shaped macroplacoids, microplacoid and septulum; entire row of placoids (microplacoid and septulum included) 10.0 μm long ($ptd = 72.5$); line of the macroplacoids 8.2 μm long ($ptd = 59.4$); first, second and third macroplacoid 2.7 μm long

(*ptd* = 19.2), 2.4 μm long (*ptd* = 17.7) and 3.6 μm long (*ptd* = 25.7) respectively; microplacoid 0.7 μm long (*ptd* = 5.1); septulum 1.4 μm long (*ptd* = 10.1).

The claws, of ‘*Hypsibius* type’, are well developed and with accessory points on the main branches; external and internal claws of the second and third pairs of legs 7.3 μm long (*ptd* = 52.9) and 5.2 μm long (*ptd* = 37.7) respectively; anterior and posterior claws of the hind legs 5.5 μm long (*ptd* = 39.9) and 7.8 μm long (*ptd* = 56.5) respectively.

For some characters the specimen collected in São Tomé is similar to *Diphascon* (*D.*) *claxtonae* Pilato & Binda, 1998 and *Diphascon* (*D.*) *faialense* Fontoura & Pilato, 2007 but some metric characters are clearly different (the buccal tube is wider; the stylet supports are inserted in a more posterior position and the *ptd* values of the claws length are higher). Due to the poor orientation of the specimen, we prefer not to attribute it to any known species of the ‘*pingue* group’, nor do we think it opportune to describe a new species.

TABLE 2. Limno-terrestrial tardigrade species recorded in the Gulf of Guinea (Western Equatorial Africa).

Species	Gulf of Guinea				
	Cameroon	Equatorial Guinea	Gabon	Ivory Coast	São Tomé & Príncipe
HETEROTARDIGRADA					
<i>Echiniscus bigranulatus</i> Richters, 1908b	+				
<i>Echiniscus pooensis</i> Rodriguez-Roda, 1948		+			
<i>Echiniscus wendti</i> Richters, 1903	+				
<i>Pseudechiniscus suillus</i> (Ehrenberg, 1853)	+				
<i>Pseudechiniscus santomensis</i> sp. nov.					+
EUTARDIGRADA					
<i>Macrobotus hufelandi</i> C.A.S. Schultze, 1834	+	+			
<i>Macrobotus montanus</i> Murray, 1910				+	
<i>Macrobotus topali</i> Iharos, 1969a	+				
<i>Macrobotus</i> sp.					+
<i>Paramacrobotus gerlachae</i> (Pilato, Binda & Lisi, 2004)					+
<i>Paramacrobotus richtersi</i> (Murray, 1911)	+	+	+		
<i>Minibiotus intermedius</i> (Plate, 1889)	+				+
<i>Murrayon pullari</i> (Murray, 1907)				+	
<i>Doryphoribius flavus</i> (Iharos, 1966)	+				
<i>Doryphoribius</i> sp.					+
<i>Hypsibius convergens</i> (Urbanowicz, 1925)	+	+			
<i>Hypsibius dujardini</i> (Doyère, 1840)	+				
<i>Hypsibius maculatus</i> Iharos, 1969b	+				
<i>Isohypsibius cameruni</i> (Iharos, 1969b)	+				
<i>Isohypsibius prosostomus</i> Thulin, 1928	+				
<i>Isohypsibius schaudinni</i> (Richters, 1909)			+		
<i>Isohypsibius tetradactyloides</i> (Richters, 1907)				+	
<i>Diphascon</i> (<i>Adropion</i>) <i>scoticum</i> Murray, 1905	+				
<i>Diphascon</i> (<i>Adropion</i>) <i>scoticum</i> ssp <i>ommatophorum</i> (Thulin, 1911)		+			
<i>Diphascon</i> (<i>Diphascon</i>) sp.					+
<i>Fractonotus caelatus</i> (Marcus, 1928)	+				

Conclusion

The results of this study increases the records of limno-terrestrial Tardigrada in Guinea Gulf (Western Equatorial Africa) to 22 species (plus 3 undetermined) and 1 subspecies from 11 genera (see Tab. 2). Two are endemic (*Pseudechiniscus santomensis* **sp. nov.** and *Echiniscus pooensis* Rodriguez-Roda 1948). However, some records, particularly the early papers, need to be reconfirmed in the light of modern taxonomy. Actually, the potential of ‘misidentifications’ and the need for revision are very high as these early specific diagnoses occurred prior to deep changes in the taxonomy of tardigrades (*e.g.* Pilato, 1969; Schuster *et al.*, 1980).

This survey of a limited habitat indicates that the tardigrade fauna of the Guinea Gulf (Western Equatorial Africa) is certainly much more diverse than may at first be apparent. Such work therefore justifies an intensification of the research effort, which would allow a deeper knowledge of the African fauna and potentially resolve of some problems of tardigrade biogeography.

Acknowledgements

We are very grateful to Nuno Gomes Oliveira, the Director of the Parque Biológico de Gaia (Portugal), for collecting the moss samples from São Tomé. We also want to thank the anonymous referees and especially Sandra McInnes for improving our English and for their valuable critical remarks and comments. This research was supported by the Fundação para a Ciência e Tecnologia (Eco-Ethology Research Unit - FCT-331/94) and also by the University of Catania (Fondo Ricerca d’Ateneo).

References

- Binda, M.G. & Pilato, G. (1995a) Remarks on tardigrades from the Seychelles, with a description of two new species. *Tropical Zoology*, 8, 1–6.
- Binda, M.G. & Pilato, G. (1995b) Some notes on African tardigrades with the description of two new species. *Tropical Zoology*, 8, 367–372.
- Biserov, V. (1994) Some tardigrades from the Seychelles with descriptions of three new species. *Tropical Zoology*, 7, 181–189.
- Claxton, S.K. (1998) A revision of the genus *Minibiotus* (Tardigrada: Macrobiotidae) with descriptions of eleven new species from Australia. *Records of the Australian Museum*, 50, 125–160.
- Dastych, H. (1984) The Tardigrada from Antarctic with descriptions of new species. *Acta Zoologica Cracoviensia*, 27, 377–436.
- Doyère, L.M. (1840) Mémoire sur les Tardigrades. I. *Annales des Sciences Naturelles, Paris, Série 2*, 14, 269–362.
- Dubois, A. (2003) The relationships between taxonomy and conservation biology in the century of extinctions. *Comptes Rendus Biologies*, 326, 9–21.
- Ehrenberg, C.G. (1853) Diagnoses novarum formarum. *Monatsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin*, Berlin, 8, 526–533.
- Fontoura, P. & Pilato, G. (2007) *Diphascon (Diphascon) faialense* sp. nov. a new species of Tardigrada (Eutardigrada, Hypsibiidae) from the Azores and a key to the species of the *D. pingue* group. *Zootaxa*, 1589, 47–55.
- Garey, J.R., McInnes, S.J. & Nichols, P.B. (2008) Global diversity of tardigrades (Tardigrada) in freshwater. *Hydrobiologia*, 595, 101–106.
- Guill, N. & Cabrero-Sañudo, F.J. (2007) Analysis of the species description process for a little known invertebrate group: the limnoterrestrial tardigrades (Bilateria, Tardigrada). *Biodiversity and Conservation*, 16, 1063–1086.
- Horning, D.S., Schuster, R.O. & Grigarick, A.A. (1978) Tardigrada of New Zealand. *New Zealand Journal of Zoology*, 5, 185–280.
- Iharos, G. (1963) The zoological results of Gy. Topal’s collectings in South Argentina. *Annales Historico-Naturales Musei Nationalis Hungarici, Pars Zoologica*, 55, 293–299.
- Iharos, G. (1966) Neue Tardigraden-arten aus Ungarn (Neuere Beiträge zur Kenntnis der Tardigraden-fauna Ungarns, IV). *Acta Zoologica Academia Scientiarum Hungaricae*, 12(1–2), 111–122.
- Iharos, G. (1969a) Beiträge zur Kenntnis der Tardigraden Indiens. *Opuscula Zoologica Budapest*, 9(1), 107–113.
- Iharos, G. (1969b) Tardigraden aus Mittlewestafrika. *Opuscula Zoologica Budapest*, 9(1), 115–120.
- Iharos, G. (1969c) Einige Angaben zur Tardigraden-Fauna Vietnams. *Opuscula Zoologica Budapest*, 9(2), 273–277.

- Jørgensen, A. (2001) Graphical presentation of the African tardigrade fauna using GIS with the description of *Isohypsibius malawiensis* sp. n. (Eutardigrada: Hypsibiidae) from Lake Malawi. *Zoologischer Anzeiger*, 240, 441–449.
- Kaczmarek, Ł., Michalczyk, Ł. & Eggermont, H. (2008) *Dactylobiotus luci*, a new freshwater tardigrade (Eutardigrada: Macrobiotidae) from the Ruwenzori Mountains, Uganda. *African Zoology*, 43(2), 150–155.
- Kathman, R.D. & Dastych, H. (1990) Some Echiniscidae (Tardigrada: Heterotardigrada) from Vancouver Island, British Columbia, Canada. *Canadian Journal of Zoology*, 68, 699–706.
- Kristensen, R.M. (1987) Generic revision of the Echiniscidae (Heterotardigrada), with a discussion of the origin of the family. In: Bertolani R. (Ed.), *Biology of Tardigrades. Selected Symposia and Monographs, U.Z.I.*, 1. Mucchi Editore, Modena Italy, 261–335.
- Lattes, A. & Gallelli, F.T. (1972) Variabilità intraspecifica di *Echiniscus (E.) quadrispinosus* Richters e differenziazione di questa specie da *Echiniscus (E.) merokensis* Richters. *Bollettino dei Musei e degli Istituti Biologici dell'Università di Genova*, 40, 137–152.
- Li, X. (2007) Tardigrades from the Tsinling Mountains, central China with descriptions of two new species of Echiniscidae (Tardigrada). *Journal of Natural History*, 41(41–44), 2719–2739.
- Marcus, E. (1928) Bärthierchen (Tardigrada). In: Dahl F., *Die Tierwelt Deutschlands und der angrenzenden Meeresteile*, Jena, 12(IV), 1–230.
- Marcus, E. (1936) Tardigrada. In: *Das Tierreich*, 66, 1–340. Ed. Walter de Gruyter, Berlin und Leipzig.
- Maucci, W. (1986) Tardigrada. In: *Fauna d'Italia*, 34, 1–338. Calderini, Bologna.
- McInnes, S.J. (1995) Tardigrades from Signy Island, South Orkney Islands, with particular reference to freshwater species. *Journal of Natural History*, 29, 1419–1445.
- McInnes, S.J. & Pugh, P.J.A. (2007) An attempt to revisit the global biogeography of limno-terrestrial Tardigrada. *Journal of Limnology*, 66, Suppl. 1, 90–96.
- Michalczyk, Ł., Kaczmarek, Ł. & Węglarska, B. (2006) *Macrobiotus sklodowskiae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae, richtersi group) from Cyprus. *Zootaxa*, 1371, 45–56.
- Miller, W.R., Horning, D.S. & Heatwole, H.F. (2001) Tardigrades of the Australian Antarctic: Macquarie Island, sub-Antarctica. *Zoologischer Anzeiger*, 240, 475–491.
- Murray, J. (1905) The Tardigrada of the Scottish lochs. *Transactions of the Royal Society of Edinburgh, Part III*, 41, 677–698.
- Murray, J. (1907) Scottish tardigrades collected by the lake survey. *Transactions of the Royal Society of Edinburgh, Part III*, 45, 641–668.
- Murray, J. (1910) Tardigrada. *Reports of the Scientific Investigations of the British Antarctic Expedition, 1907–1909*, London, Vol.1, Biology (Part V), 81–185.
- Murray, J. (1911) Arctiscoidea. *Proceedings of the Royal Irish Academy*, Dublin and London, 31(37), 1–16.
- Pilato, G. (1969) Evoluzione e nuova sistemazione degli Eutardigradi. *Bollettino di Zoologia*, 36(3), 827–845.
- Pilato, G. (1981) Analisi di nuovi caratteri nello studio degli Eutardigradi. *Animalia*, 8, 51–57.
- Pilato, G. & Binda, M.G. (1997/98) A comparison of *Diphascon (D.) alpinum* Murray, 1906, *D. (D.) chilense* Plate, 1989 and *D. (D.) pingue* Marcus, 1936 (Tardigrada), and description of a new species. *Zoologischer Anzeiger*, 236, 181–185.
- Pilato, G. & Binda, M.G. (1998) Two new species of *Diphascon* (Eutardigrada) from New South Wales, Australia. *New Zealand Journal of Zoology*, 25, 171–174.
- Pilato, G. & Binda, M.G. (1999) Three new species of *Diphascon* of the *pingue* group (Eutardigrada, Hypsibiidae) from Antarctica. *Polar Biology*, 21, 335–342.
- Pilato, G. & Binda, M.G. (2001) Biogeography and limno-terrestrial tardigrades: Are they truly incompatible binomials? *Zoologischer Anzeiger*, 240, 511–516.
- Pilato, G. & Lisi, O. (2006) Notes on some tardigrades from southern Mexico with description of three new species. *Zootaxa*, 1236, 53–68.
- Pilato, G. & Lisi, O. (2009a) Description of three new species of Tardigrada from the Seychelles. *Zootaxa*, 2005, 24–34.
- Pilato, G. & Lisi, O. (2009b) Tardigrades of the Seychelles Islands, with the description of three new species. *Zootaxa*, 2124, 1–20.
- Pilato, G., Binda, M.G. & Lisi, O. (2002) Notes on the tardigrades of the Seychelles with the description of two new species. *Bollettino delle sedute dell'Accademia Gioenia di Scienze Naturali, Catania*, 35(361), 503–517.
- Pilato G., Binda, M.G. & Lisi, O. (2004) Notes on tardigrades of the Seychelles with description of three new species. *Italian Journal of Zoology*, 71, 171–178.
- Pilato, G., Binda, M.G. & Lisi, O. (2005) Remarks on some Echiniscidae (Heterotardigrada) from New Zealand with the description of two new species. *Zootaxa*, 1027, 27.45.
- Pilato, G., Binda, M.G. & Lisi, O. (2006) Three new species of eutardigrades from the Seychelles. *New Zealand Journal of Zoology*, 33, 39–48.

- Pilato, G., Binda, M.G., Napolitano, A. & Moncada, E. (2001) Notes on South American tardigrades with the description of two new species: *Pseudechiniscus spinerectus* and *Macrobiotus danielae*. *Tropical Zoology*, 14, 223–231.
- Plate, L.H. (1889) Beiträge zur Naturgeschichte der Tardigraden. *Zoologische Jahrbücher*, 3, 487–550.
- Richters, F. (1903) Nordische Tardigraden. *Zoologischer Anzeiger*, 28, 5, 168–172.
- Richters, F. (1907) Die Fauna der Moosrasen des Gausberg und einiger Südlicher Inseln. *Deutsch Südpolar Expedition, Zoologie*, 9(4), 259–302.
- Richters, F. (1908a) Beitrag zur Kenntnis der Moosfauna Australiens und der Inseln des Pacifischen Oceans. *Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere*, Jena, 26, 196–213.
- Richters, F. (1908b) Moosbewohner. *Wissenschaftliche Ergebnisse der Schwedischen Südpolar Expedition (1901–1903)*, 5(2), 1–16.
- Richters, F. (1909) Tardigraden-studien. *Bericht über die Senckenberische Naturforschende Gessellschaft in Frankfurt am Main*, 2, 28–48.
- Rodríguez-Roda, J. (1948) Algunos tardígrados de Fernando Póo. *Publicaciones del Instituto de Biología Aplicada*, 4, 149–159.
- Schultze, C.A.S. (1834) *Macrobiotus hufelandi* animal e crustaceorum classe novum reviviscendi post diurnam asphyxiam et ariditatem potens, etc. 8, C. Curths, Berlin.
- Schuster, R.O., Grigarick, A.A. & Toftner, E.C. (1975) Ultrastructure of tardigrade cuticle. *Memorie dell'Istituto Italiano di Idrobiologia*, 32, Suppl., 337–375.
- Schuster, R.O., Nelson, D.R., Grigarick, A.A. & Christenberry, D. (1980) Systematic criteria of the Eutardigrada. *Transactions of the American Microscopical Society*, 99, 284–303.
- Thulin, G. (1911) Beitrag zur Kenntnis der Tardigradenfauna Schwedens. *Arkiv för Zoologi Stockholm*, 7, 1–60.
- Thulin, G. (1928) Über die Phylogenie und das System der Tardigraden. *Hereditas*, 11, 207–266.
- Urbanowicz, C. (1925) Sur la variabilité de *Macrobiotus oberhaeuseri*. *Bulletin de Biologie*, 59, 1, 124–142.
- Wang, L. (2009) Tardigrades from Yunnan-Guizhou Plateau (China) with description of two new species in the genera *Mixibius* (Eutardigrada: Hysibiidae) and *Pseudechiniscus* (Heterotardigrada: Echiniscidae). *Journal of Natural History*, 43(41), 2553–2570.
- Węglarska, B. (1962) Die Tardigraden Vietnams. *Acta Societatis Zoologicae Bohemoslovenicae*, XXVI, 4, 300–307.