



Tardigrada from Santo Antão Island (Archipelago of Cape Verde, West Africa) with the description of a new species

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Abstract

Three tardigrade species from Santo Antão Island, Archipelago of Cape Verde are firstly reported from Republic of Cape Verde: *Echiniscus scabrospinosus* Fontoura, 1982, *Echiniscus clavispinosus* **sp. nov.** and *Milnesium tardigradum* Doyère, 1840. *E. clavispinosus* **sp. nov.** belongs to the ‘*viridis* group’ of species characterized by the green colour and plate ornamentation comprised of tubercles, fine dots and light spots, by lacking dorsal and lateral trunk appendages (cirrus A excluded) and well developed claws. *E. clavispinosus* **sp. nov.** differs from all the known species of the ‘*viridis* group’ in having the area between the paired plate III and the terminal plate unsculptured, in details of the cuticular ornamentation, pointed clavae instead of papillate apices, and stronger spur on internal claws. A dichotomous key to the species of the *Echiniscus viridis* group and a list of Macaronesian species are also given.

Key words: Macaronesia, Tardigrada, first records, *Echiniscus clavispinosus* **sp. nov.**, *Echiniscus viridis* group, dichotomous key

Introduction

Cape Verde, an archipelago of ten islands and a few islets, is located in the Atlantic Ocean in the coast of West Africa, in the so called Macaronesia ecoregion consisting of five volcanic archipelagos (Azores, Madeira, Savage, Canaries and Cape Verde) with a common biogeography, but also each having a number of endemic species according to current knowledge. Unlike the other archipelagos of Macaronesia, which have a climate that ranges from tropical to subtropical, Cape Verde (and also the eastern Canaries) is part of the ‘Sahelian arid belt’, having a semi-desert climate, receiving only about 260 mm of annual rainfall.

Information about the tardigrade fauna of Macaronesia is scarce and very scattered. Recently, three of the nine Azorean islands were surveyed with 17 species recorded, five of which were endemic (Fontoura, 1985; Fontoura *et al.*, 2008^{a, b}); 13 species were recorded in the Canaries (Heinis, 1908; Guil & Guidetti, 2005). An earlier study was carried out on tardigrades from Madeira Island (Cunha & do Nascimento-Ribeiro, 1962) reporting 27 species (two of which were endemic). The tardigrade fauna is completely unknown for the small and largely uninhabited Savage islands and the archipelago of Cape Verde. The entire African continent is barely explored with only *c.* 200 known tardigrade species from 25 countries (Jørgensen, 2001; Pilato & Binda, 2001; Pilato *et al.*, 2003; Kaczmarek & Michalczyk, 2004; Kaczmarek *et al.*, 2006; 2008; McInnes & Pugh, 2007; Garey *et al.*, 2008; Fontoura *et al.*, 2010). This scenario justifies the urgent need for species inventories on this previously ignored region, aiming to contribute to a deeper knowledge of the African fauna and to understand the peculiarities of the tardigrade biogeography.

In this paper, the first record of tardigrades in Cape Verde, three species are reported, one of which, *Echiniscus clavispinosus* **sp. nov.**, is new to science.

Material and methods

Tardigrades were extracted from three dried lichen and moss samples collected in Xôxô, Ribeira da Torre, Municipality of Ribeira Grande (17° 08' 13"N; 25° 04' 01"W; 340 meters above sea level), Corda - Cova, Municipality of Paúl (17° 06' 13"N; 25° 03' 40"W; 1260 m a.s.l.) and Fajã Domingas Benta, Ribeira da Torre, Municipality of Ribeira Grande (17° 09'N; 25° 04'W; 200 m a.s.l.), Santo Antão Island, Archipelago of Cape Verde (Republic of Cape Verde), on February 2010. The specimens were mounted 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 under x100 oil immersion, using a Zeiss Phase Contrast Microscope (PCM) and a Zeiss Differential Interference Contrast Microscope (DIC), both equipped with digital cameras and using Axiovision 4.7.1 Imaging System Software. Structures were measured only if undamaged and the orientation was suitable. If different values were obtained when measuring symmetric structures, the larger value was chosen. Body length was measured from the anterior margin to the end of the body, excluding the hind legs.

For comparison, we examined the holotype of *Echiniscus scabrospinosus* Fontoura, 1982 (Serra do Buçaco, Portugal; from the collection of Binda & Pilato, Museum of the Department of Animal Biology "Marcello La Greca", University of Catania, Italy); the holotype and a number of paratypes of *Echiniscus viridianus* Pilato, Fontoura & Lisi, 2007 from Auburn, Alabama, U.S.A (collected by D. Christenberry); specimens (examined together with the paratypes) from New Mexico, Dona Ana, U.S.A. (collected by C. W. Beasley; Binda & Pilato collection), and the Azores (collected in Ribeira Fria, Lages do Pico, by P. Fontoura; from the collection of P. Fontoura, Department of Biology, Faculty of Sciences, University of Porto, Portugal). One paratype of *Echiniscus perviridis* Ramazzotti, 1959 from Pallanza (Novara), Italy (Ramazzotti collection, Museo Civico di Storia Naturale di Verona, Italy) and two specimens from the Azores, (collected at Lages do Pico, Pico Island, by P. Fontoura), and from China, Anhui, (collected by C. W. Beasley and W. R. Miller; both from P. Fontoura collection). Two specimens of *Echiniscus viridis* Murray, 1910 (Hawaiian Islands, collected by A. A. Grigarick; R. O. Schuster collection, the Bohart Museum, U.S.A.); one specimen of *Echiniscus rufoviridis* du Bois-Reymond Marcus, 1944 (Argentina, collected by J. Pelluffo and M. C. Moly de Peluffo; Binda & Pilato collection), and specimens of *Echiniscus viridissimus* Péterfi, 1956 from Alabama, U.S.A, (collected by D. Christenberry; Binda & Pilato collection), and from Guizhou, China, (collected by C. W. Beasley and W. R. Miller; P. Fontoura collection).

Results

Echiniscus clavispinosus sp. nov.

(Figs. 1, 2; Table 1)

Locus typicus. Xôxô, Ribeira da Torre, Municipality of Ribeira Grande, Santo Antão Island, Archipelago of Cape Verde, in a lichen sample growing on rocks.

Material examined. Thirteen specimens, of which four were two-clawed larvae.

Type repository. The holotype (slide N. 5432) and one two-clawed larva (slide N. 5433) 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 (slides CIV88 - CIV96) are deposited in the collection of P. Fontoura at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Specific diagnosis. Colour green; eye spots absent; dorsal plates well marked. Median plate 3 absent; cuticular ornamentation consisting of slightly raised dark tubercle and fine dots. At a higher focal position very dense, fine dots and small light spots appear. Distance between the dark tubercles shorter than the diameter of the tubercles. Anterior portion of median plates 1 and 2 and area between paired plate III and terminal plate unsculptured. Buccal cirri and cirrus A short, elongated clavae with pointed apices; no other lateral or dorsal appendages present. Spine present on the first pair of legs; papilla and dentate collar on the hind legs; claws well developed; internal claws with well developed spur.

Description of the holotype. Body length 220 μm ; plate colour green in specimens observed with transmitted light, after mounting on a slide, but reddish brown when observed with reflected light; eye spots not observed in either living nor slide mounted specimens. A narrow finely dotted transverse band was present anterior to the cephalic plate (Fig. 1A, D); this plate with an anterior notch; a true neck plate was absent but there was a thickened

and faint ornamented transverse area between the cephalic plate and the scapular plate (Fig. 1A, D); the scapular plate, 43.6 μm long in the medium line, was subdivided into a wide central portion and two small lateral, almost triangular, portions (Fig. 1A, D). Median plate 2 with unsculptured anterior portion (Fig. 1B); median plate 3 absent and the area between paired plate III and terminal plate unsculptured (Fig. 1B, C); terminal plate unsculptured and with two indentations (Fig. 1B). Plate sculpture of the adults comprised of slightly raised dark tubercles and very fine, dense dots (Fig. 1 A–D); at a higher focal position small light spots appear (Fig. 1D). Distance between the dark tubercles shorter than the diameter of the tubercles; largest tubercles (diameter *c.* 2.3 μm) are present in the central portion of the scapular plate. Cephalic plate sculptured with only fine dots (Fig. 1D). In the anterior portion of the paired plates dark tubercles (Fig. 1B, C) were present (and a transverse area with almost invisible dots). Leg plates sculptured with only fine dots. Ventral body surface had an extremely fine granulation more visible in the cephalic region. Small spine (2.4 μm long) was present on the first pair of legs (Fig. 2A); small papilla 3.3 μm long and dentate collar with about 10 unequal, sharp teeth (the longest *c.* 2.2 μm long) were present in the fourth pair of legs (Fig. 2C).

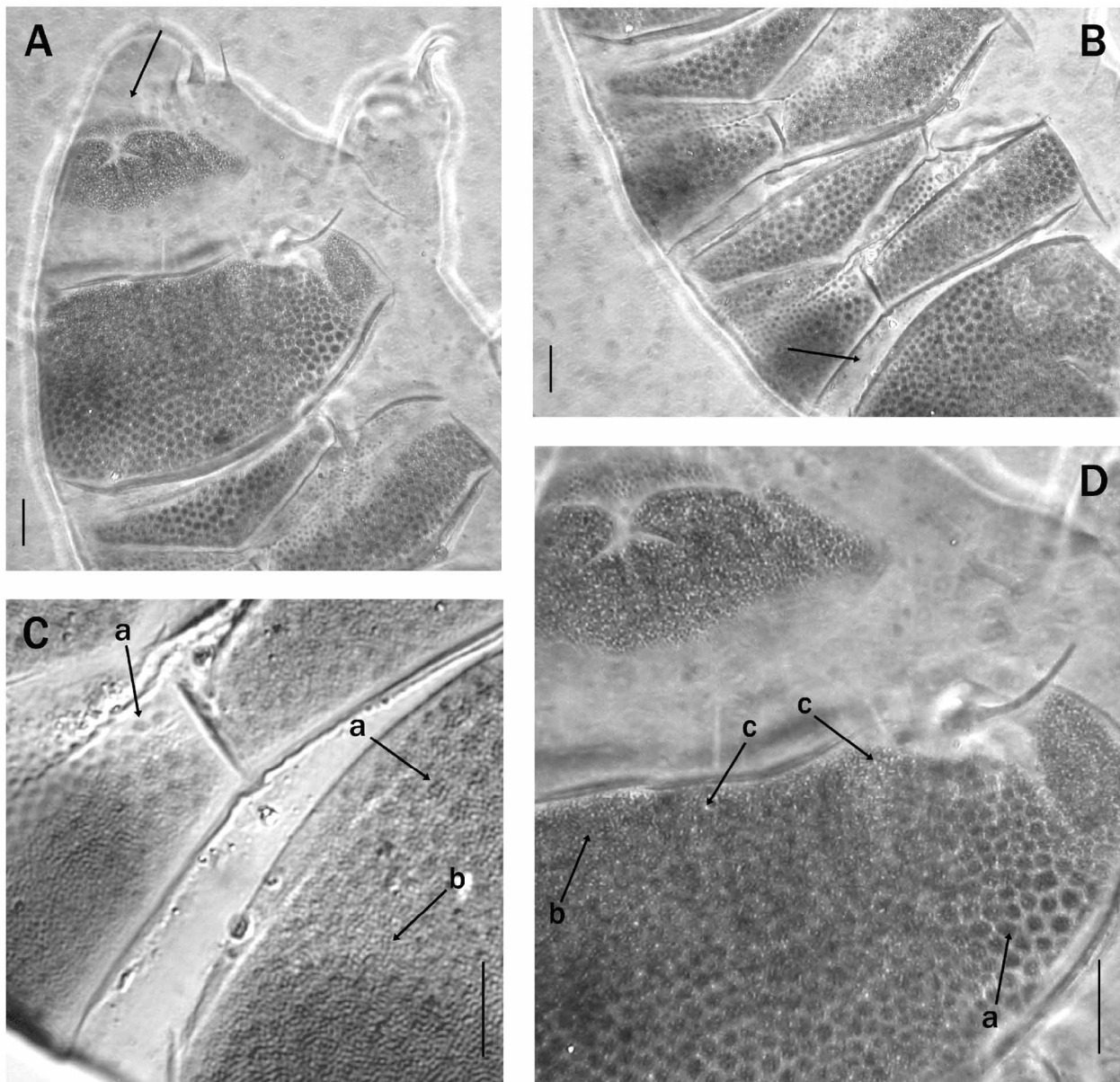


FIGURE 1. *Echiniscus clavispinosus* sp. nov. A, anterior portion of the body. The arrow indicates the sculptured band anterior to the cephalic plate. B, posterior portion of the body. The arrow indicates that median plate 3 is absent and the corresponding area lacks sculpture. C, details of the plate sculpture; arrows a: indicate dark tubercles; arrow b: indicates small dots. D, anterior portion of the body; it is evident that cephalic plate lacks dark tubercles. Arrow a: indicates a dark tubercle; arrow b: small dots; arrows c: indicate small light spots. Scale bars = 10 μm . A, B, D: PCM images; C: DIC image.

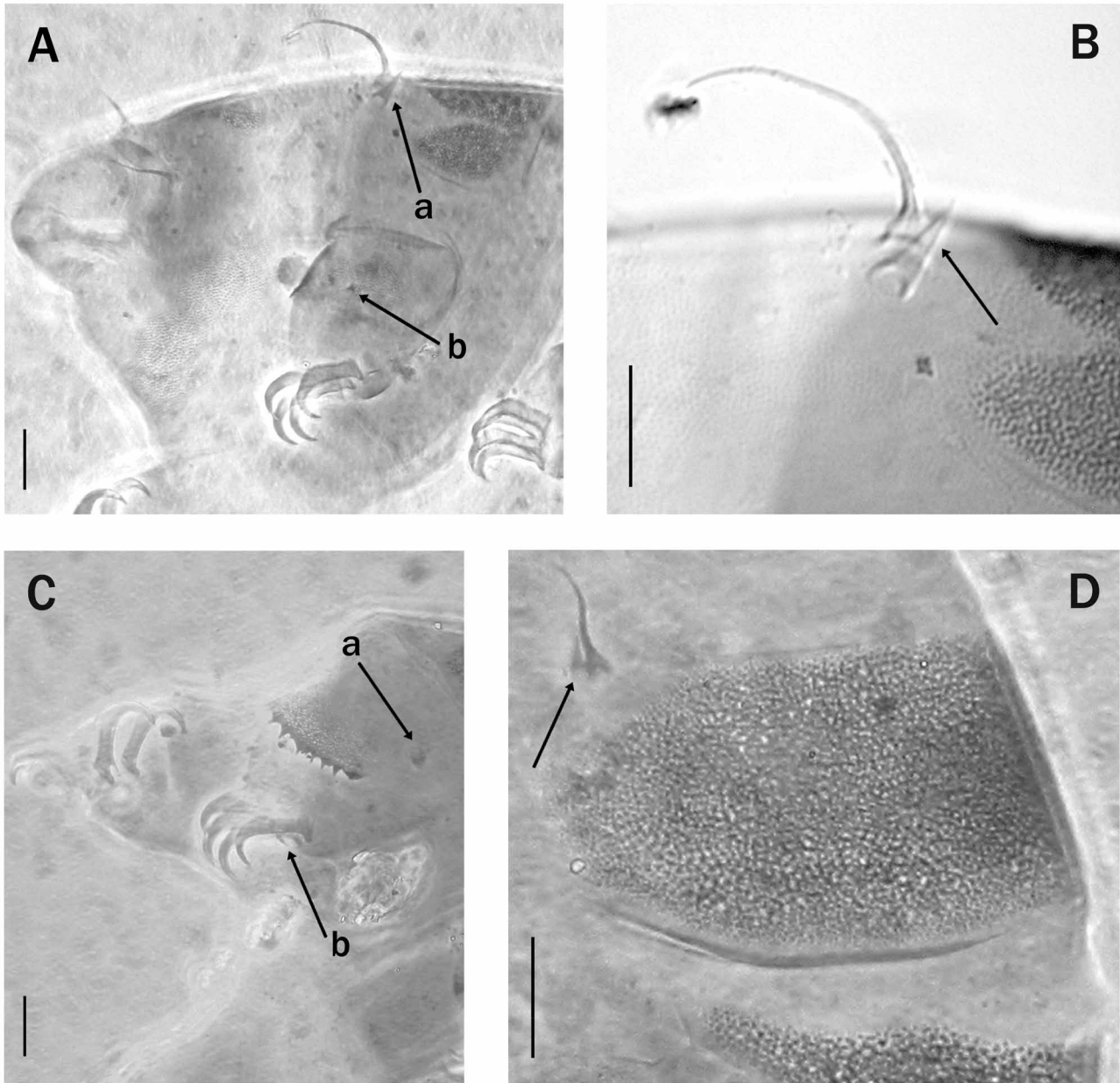


FIGURE 2. *Echiniscus clavispinosus* sp. nov. A, anterior portion of the body; arrow a: indicates the pointed clava; arrow b: indicates a small spine on the first pair of legs. B, anterior portion of the body; arrow indicates the pointed clava. C, Posterior portion of the body; arrow a: indicates the papilla on the hind legs; arrow b: indicates the spur of one of the internal claws. D, anterior portion of a two-clawed larva; it is evident that the plate ornamentation lacks dark tubercles. Arrow indicates the pointed clava. Scale bars = 10 μ m. A, C, D: PCM images; B: DIC image.

Internal buccal cirri 9.6 μ m long; external cephalic cirri slightly longer (11.9 μ m); cephalic papillae 6.8 μ m long (Fig. 2A); clavae, 7.2 μ m long, had a distinctive sharp-tipped, conical shape rather than the rounded apices (Fig. 2B). Cirri A, a short filament 26.0 μ m long (Fig. 2A, B); no other trunk appendages present.

Claws well developed (Fig. 2A, C); internal and external claws on the first pair of legs 13.3 and 11.8 μ m long respectively; they were slightly longer on the fourth pair of legs (14.3 μ m and 13.3 μ m respectively). Internal claws with a well developed spur directed downwards (Fig. 2C).

Eggs unknown.

Variability. The paratypes (body length 133–241 μ m) were similar to the holotype in both qualitative and metric characters (Table 1). However, we would like to emphasise the curiosity found in two of the paratypes. One had a deeply forked buccal cirrus, and the other a deeply forked cirrusi A. The cause of this anomaly was not found.

We consider it important to stress the differences between the two-clawed larvae (body lengths c. 142–144 μ m)

and the adults. In the larvae the cuticular ornamentation lacked the dark tubercles and was instead formed by small, dense dots, sometimes joined together, and by sparse light spots (Fig. 2D); these light spots, more evident in the posterior margins of the scapular, median and paired plates, were larger than those observed on the adults. We were sure that the two-clawed larvae belong to *Echiniscus clavispinosus* **sp. nov.** due to the distinctive (pointed) clavae shape and because they have the smooth unornamented anterior portion of the median plate 2 and the unornamented area between the paired plates III and the terminal plate. The differences of the cuticular ornamentation seemed to be in relation to the developmental stage and not body length because the four two-clawed larvae were slightly larger (142–144 μm long) than a four-clawed paratype (133 μm) which had typical adult cuticular ornamentation.

TABLE 1. Measurements of the holotype, the smallest and the largest specimens of *Echiniscus clavispinosus* **sp. nov.** (% *bo* = percentage of body length; % *sc* = percentage of scapular plate length).

Characters	Smallest specimen			Holotype			Largest specimen		
	μm	% <i>bo</i>	% <i>sc</i>	μm	% <i>bo</i>	% <i>sc</i>	μm	% <i>bo</i>	% <i>sc</i>
Body length	133	-	-	220	-	-	241	-	-
Scapular plate length	26.6	20.0	-	43.6	19.8	-	48.8	20.2	-
Internal buccal cirrus	7.1	5.3	26.7	9.6	4.4	22.0	12.8	5.3	26.2
External buccal cirrus	8.6	6.5	32.3	11.9	5.4	27.3	15.8	6.6	32.4
Cephalic papilla	4.5	3.4	16.9	6.8	3.1	15.6	7.3	3.0	15.0
Clava	4.8	3.6	18.1	7.2	3.3	16.5	8.1	3.4	16.6
Cirrus A	14.5	10.9	54.5	26.0	11.8	59.6	32.4	13.4	66.4
Spine on leg I	?	-	-	2.4	1.1	5.5	3.4	1.4	7.0
Internal claw leg I	9.2	6.9	34.6	13.3	6.1	30.5	15.4	6.4	31.6
External claw leg I	8.1	6.1	30.5	11.8	5.4	27.1	14.4	6.0	29.5
Internal claw leg IV	9.3	7.0	35.0	14.3	6.5	32.8	17.6	7.3	36.1
External claw leg IV	8.1	6.1	30.5	13.3	6.1	30.5	15.5	6.4	31.8
Papilla leg IV	?	-	-	3.3	1.5	7.6	?	-	-

Etymology. The name *clavispinosus* refers to the pointed apex shape of the clava.

Differential diagnosis. *Echiniscus clavispinosus* **sp. nov.** belongs to the *viridis* group, a small group of species within the genus *Echiniscus* characterised by the green colour, by plate ornamentation comprised of dark tubercles, fine dots and light spots, by the absence of dorsal and lateral trunk appendages (cirrus A excluded), and well developed claws. Traditionally, on the basis of the colour, five species were attributed to this group: *E. viridis*, *E. rufoviridis*, *E. viridissimus*, *E. perviridis* and *E. viridianus*. However, two of these species, *E. rufoviridis* and *E. viridissimus*, have distinctly different cuticular ornamentation (Fig. 3) (Pilato *et al.*, 2007; 2008^a) and should probably be excluded from the group. Nevertheless, to avoid misunderstandings and to make the species diagnosis easier, we incorporated all the green coloured *Echiniscus* species lacking dorsal and lateral trunk appendages (including *E. viridissimus* and *E. rufoviridis*) into the key below.

Echiniscus clavispinosus **sp. nov.** differed from the other three true ‘*viridis* group’ species by the unsculptured area between paired plate III and terminal plate; pointed clavae, instead of with rounded apices; and a stronger spur on internal claws. Differences among these species regarding quantitative characters are illustrated in Table 2. It is important to stress that the values relative to the external buccal cirrus, clava and spine on the first pair of legs of *E. viridis* and the values relative to the leg IV claws of *E. viridianus* are different from those indicated by Pilato *et al.* (2008^a; tables 1 and 2) in which they, for a misunderstanding, are wrong.

The new species differed from *E. viridis* in the absence of dark tubercles on the cephalic and neck plates; the smooth anterior portion of the median plate 2; the distance between the dark tubercles much shorter than the diameter of the tubercles (Fig. 3, A and B); more visible fine dots and light spots; and slightly longer cirrus A (Table 2).

Echiniscus clavispinosus **sp. nov.** also differed from *E. perviridis* in lacking tubercles in the cephalic and neck plates, the smooth anterior portion of median plate 2; finer and denser dots and light spots on the plates (Fig. 3, A

and D), less visible green area bordering the trunk plates; dentate collar with smaller teeth; and much shorter cirrus A and shorter claws (Table 2).

The new species differed from *E. viridianus*, the most similar species, by the absence of dark tubercles on the cephalic and neck plates; less visible and more dense fine dots and light spots on the plates (Fig. 3, A and C); smooth anterior portion of the median plate 2; less visible green area bordering the trunk plates.

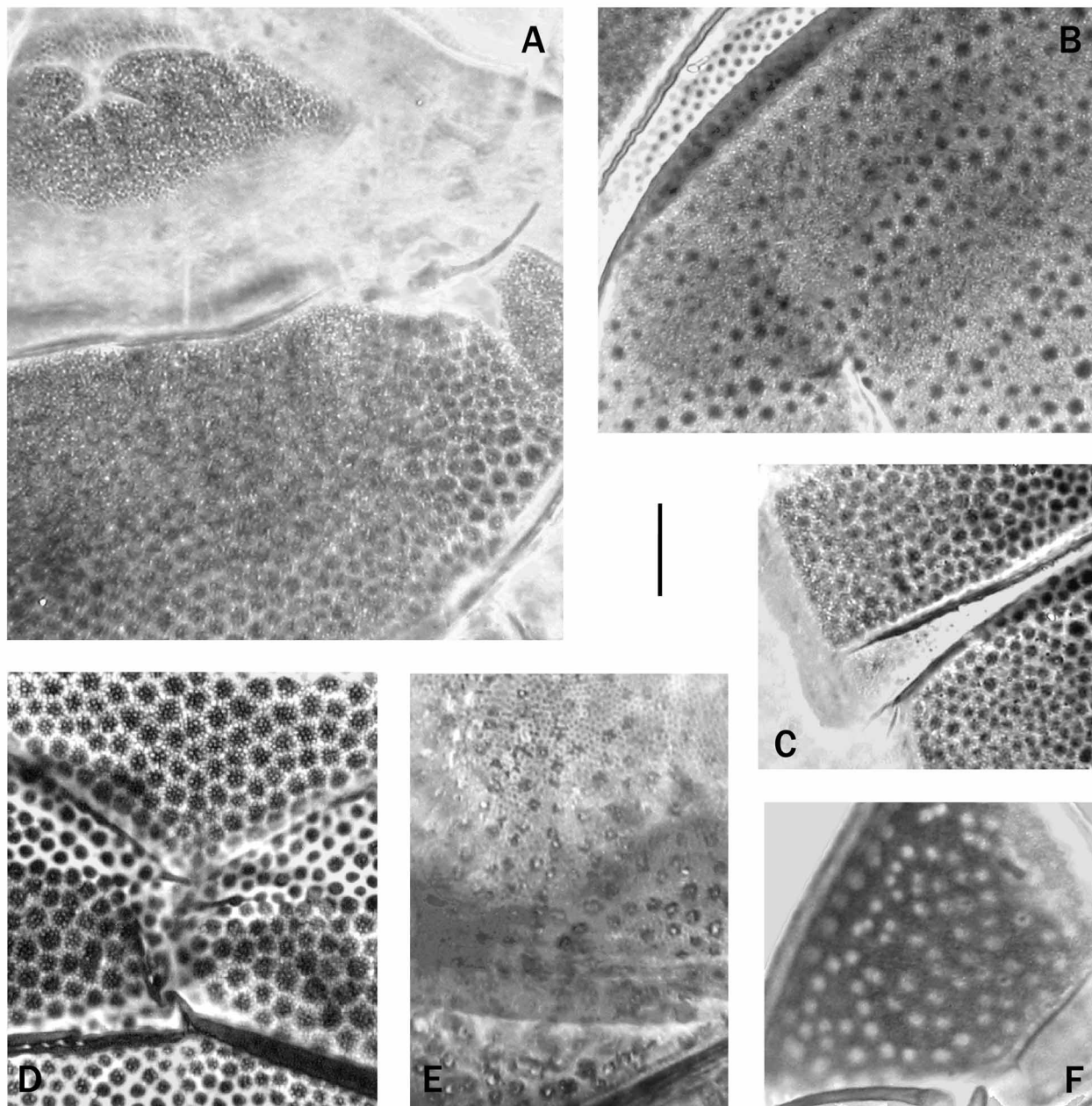


FIGURE 3. Details of the cuticular ornamentation (PCM images) in: A, *Echiniscus clavispinosus* **sp. nov.**; B, *Echiniscus viridis*; C, *Echiniscus viridianus*; D, *Echiniscus perviridis*; E, *Echiniscus rufoviridis* and, F, *Echiniscus viridissimus*. The differences between the first three species on one hand and *Echiniscus rufoviridis* and *Echiniscus viridissimus* on the other hand are very evident. Scale bar: 10 μ m.

TABLE 2. Measurements of single specimens of *Echiniscus clavispinosus* **sp. nov.**, *E. viridis*, *E. perviridis* and *E. viridianus* (% *bo* = percentage of body length; % *sc* = percentage of scapular plate length).

Characters	<i>E. clavispinosus</i> sp. nov.			<i>E. viridis</i> *			<i>E. perviridis</i>			<i>E. viridianus</i> *		
	µm	% bo	% sc	µm	% bo	% sc	µm	% bo	% sc	µm	% bo	% sc
Body length	241	-	-	190			290			231		
Scapular plate length	48.8	20.2	-	41.8	22.0		56.5	19.5		43.1	18.7	
Internal buccal cirrus	12.8	5.3	26.2	9.0	4.7	21.5	-	-	-	13.9	6.0	32.3
External buccal cirrus	15.8	6.6	32.4	10.3	5.4	24.6	-	-	-	14.7	6.4	34.1
Cephalic papilla	7.3	3.0	15.0	6.4	3.4	15.3	6.0	2.1	10.6	5.8	2.5	13.5
Clava	8.1	3.4	16.6	6.0	3.2	14.4	-	-	-	5.5	2.4	12.8
Cirrus A	32.4	13.4	66.4	18.2	9.6	43.5	114.0	39.3	201.8	29.0	12.6	67.3
Spine on leg I	3.4	1.4	7.0	3.3	1.7	7.9	-	-	-	2.6	1.1	6.0
Internal claw leg I	15.4	6.4	31.6	12.6	6.6	30.1	-	-	-	15.1	6.5	35.0
External claw leg I	14.4	6.0	29.5	11.9	6.3	28.5	21.4	7.4	37.9	14.5	6.3	33.6
Internal claw leg IV	17.6	7.3	36.1	15.5	8.2	37.1	30.0	10.3	53.1	16.9	7.3	39.2
External claw leg IV	15.5	6.4	31.8	14.7	7.7	35.2	28.5	9.8	50.4	15.2	6.6	35.3
Papilla leg IV	-	-	-	3.0	1.6	7.2	-	-	-	4.8	2.1	11.1

* As above mentioned in the text, the %*bo* and %*sc* values relative to the external buccal cirrus, clava and spine on the first pair of legs of *E. viridis* and the values regarding the leg IV claws of *E. viridianus* differ from those indicated by Pilato *et al.* (2008^a, tables 1 and 2), which were incorrect.

Key to adult specimens of the species of the *Echiniscus viridis* group

1. Whole body green; median plate 3 absent; the corresponding area may be ornamented or smooth; terminal plate unfaceted; internal claws with a spur 2
- Cephalic portion not green; median plate 3 present; terminal plate faceted; cuticle without dark tubercles, claws without spurs *E. rufoviridis*
2. Plate ornamentation with extremely fine and dense dots and large light patches, but without dark tubercles. Cirrus A long about a third of the body length *E. viridissimus*
- Plate ornamentation with dark tubercles, very fine and dense dots and very fine light spots 3
3. Area between paired plates III and terminal plate smooth; pointed clavae *E. clavispinosus* **sp. nov.**
- Area between paired plates III and terminal plate sculptured; clavae with rounded apices 4
4. Distance between the dark tubercles equal to or greater than the diameter of the tubercles *E. viridis*
- Distance between the dark tubercles less than the diameter of the tubercles 5
5. Cirrus A shorter than the scapular plate *E. viridianus*
- Cirrus A clearly longer than the scapular plate *E. perviridis*

***Echiniscus scabrospinosus* Fontoura, 1982**

Material examined. One specimen (female; body length 196 µm) collected from a moss sample in Corda - Cova, Municipality of Paúl, Santo Antão Island, Archipelago of Cape Verde.

Distribution. Until now the species had been reported from Portugal, *locus typicus* (Fontoura, 1982; Pilato *et al.*, 2008^b), Tanzania (Binda & Pilato, 1995) and the Azores (Fontoura *et al.*, 2008^a). Specimens attributed to this species were also reported from China, Yunnan Province (Beasley & Cleveland, 1996) and Fujian Province (Yang, 2008). However, the examples from Yunnan Province were re-examined and identified as *E. lineatus* Pilato, Fontoura, Lisi & Beasley, 2008 (see Pilato *et al.*, 2008^b). It is possible the examples from Fujian Province have been miss-identified and could be *E. lineatus* or a new species, but this hypothesis needs to be confirmed.

Milnesium tardigradum Doyère, 1840

Material examined. Nine specimens collected from the same sample as *Echiniscus clavispinosus* **sp. nov.**

Distribution. The species is considered cosmopolitan (McInnes, 1994).

Conclusion

This is the first record of tardigrade fauna from Cape Verde, and raises the known species for the Macaronesia ecoregion to 43 (Table 3). Despite the extremely limited exploration of this region for tardigrade fauna, a relatively high number of species (eight) have been described as new to science and are considered endemic to this ecoregion. This confirms the uniqueness of this area and supports the need for more extensive and deeper surveys. The intensification of the research effort is also justified by the need to reconfirm species recorded in the older literature for Canary and Madeira, where miss-identifications certainly have occurred. For example, a record (da Cunha & do Nascimento-Ribeiro, 1962) for *Macrobotus echinogenitus* Richters, 1904 indicated specimens with three macroplacoids, whilst in the true description of this species there are two macroplacoids. A.X. da Cunha & F. do Nascimento-Ribeiro (1962) also reported *Macrobotus schultzei* Greeff, 1866 from Madeira, which is now considered a synonym of *Paramacrobotus richtersi* (Murray, 1911). The Macaronesia ecoregion is an important biogeographic zone which, with further study, would enhance our understanding of tardigrade biogeography, and particularly could prove pivotal to our understanding of the biogeography of the *Echiniscus viridis* group.

TABLE 3. Limno-terrestrial tardigrade species recorded in the Macaronesia. Endemic species are indicated in bold.

Species	Macaronesia			
	Canary	Madeira	Azores	Cape Verde
HETEROTARDIGRADA				
<i>Bryodelphax atlantis</i> Fontoura, Pilato & Lisi, 2008 ^a			+	
<i>Bryodelphax parvulus</i> Thulin, 1928		+		
<i>Echiniscus arctomys</i> Ehrenberg, 1853	+	+		
<i>Echiniscus azoricus</i> Fontoura, Pilato & Lisi, 2008 ^a			+	
<i>Echiniscus canadensis</i> Murray, 1910	+		+	
<i>Echiniscus canedoi</i> Cunha & do Nascimento Ribeiro, 1962		+		
<i>Echiniscus clavispinosus</i> sp. nov.				+
<i>Echiniscus manuelae</i> Cunha & do Nascimento Ribeiro, 1962		+		
<i>Echiniscus mediantus</i> Marcus, 1930	+			
<i>Echiniscus merokensis</i> Richters, 1904		+	+	
<i>Echiniscus perviridis</i> Ramazzotti, 1959			+	
<i>Echiniscus quadrispinosus</i> Richters, 1902	+	+		
<i>Echiniscus quadrispinosus brachyspinosus</i> Bartoš, 1934			+	
<i>Echiniscus quadrispinosus cribrosus</i> Murray, 1907 ^a			+	
<i>Echiniscus scabrospinosus</i> Fontoura, 1982			+	+
<i>Echiniscus spinulosus</i> (Doyère, 1840)		+		
<i>Echiniscus testudo</i> (Doyère, 1840)		+		
<i>Echiniscus trisetosus</i> Cuénot, 1932	+			
<i>Echiniscus viridianus</i> Pilato, Fontoura & Lisi 2007			+	
<i>Pseudechiniscus suillus</i> (Ehrenberg, 1853)		+	+	

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