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ORIGINAL ARTICLE

A trans-isthmus survey of marine tardigrades from Costa Rica (Central America) with descriptions of seven new species

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ABSTRACT
The Central American Isthmus has had profound effects on the evolution and distribution of larger marine organisms, but the impacts on meiobenthic animals have received little attention. Tardigrades are microscopic metazoan lobopodians that are ubiquitous in benthic communities worldwide, but little is known about marine tardigrades in Central America. Only two marine tardigrades have been identified to species level in all of Central America, and these came from a single sample of barnacles from El Salvador collected in 1953. Additionally, multiple haplotypes of *Echiniscoides* were reported from Costa Rica and Panama, but species names were not assigned. Here we report an extensive survey of both intertidal and subtidal marine tardigrades from both the Caribbean and Pacific coasts of Costa Rica. We found 701 individuals in 65 samples. There was a total of 19 taxa, including seven species with a sufficient quality and quantity of specimens to describe here as new to science. The new species are *Archechiniscus murilloi* sp. nov., *Batillipes homocercus* sp. nov., *Batillipes ichthyocercus* sp. nov., *Echiniscoides costaricensis* sp. nov., *Echiniscoides ritavargasae* sp. nov., *Styraconyx vargasi* sp. nov. and *Tanarctus breedyae* sp. nov. Ten taxa were found only in the Caribbean, six were found only in the Pacific, and three were found on both coasts. We discuss the three species with trans-isthmus distributions and note two additional candidate geminate species pairs that warrant further investigation.

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Introduction

The Central American Isthmus has had a profound and well-studied effect on the biogeography of marine animals. For terrestrial life the Isthmus led to the well-known ‘Great American Exchange’ (Stehli and Webb 1985; for terrestrial tardigrades see Kaczmarek and Roszkowska 2016, Kaczmarek et al. 2016), while for marine organisms it created the ‘Great American Schism’. Lessios (2008) coined this term and reviewed the considerable literature on trans-isthmus distributions of marine macroinvertebrates and fish. Trans-isthmus distributions of marine meiobenthic animals, however, have received relatively little attention until very recently.

Meiofaunal interstitial communities contain vast biodiversity including representatives from over 20 phyla (Giere 2009). They play an important role in marine food webs (Danovaro et al. 2007) and contribute to valuable ecosystem services (Schratzberger and Ingels 2017). It was once thought that most meiofauna have very broad or cosmopolitan distributions and therefore lack biogeography, even though most of these microscopic animals are benthic, have low fecundity and lack planktonic larval stages, all of which should limit dispersal and restrict geographic ranges. Giere (2009) called this the ‘meiofauna paradox’. Many recent studies across multiple phyla (e.g. see Derycke et al. 2010 for nematodes; Cornils and Held 2014 for copepods; Leasi and Norenburg 2014 for nemerteans; Garraffoni and Balsamo 2017 for gastrotrichs; Faurby et al. 2012 and Santos et al. 2019 for marine tardigrades) have disentangled cosmopolitan cryptic species clusters and revealed that, in fact, many meiofauna species do have limited ranges sometimes resulting in interesting geospatial

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patterning (e.g. see Bartels et al. 2020). Trans-isthmus of Central America distributions have been reported for very few groups of meiofauna, and only one study previously examined trans-isthmus marine tardigrades (Faurby et al. 2012). We consider these studies in the discussion section.

Only 241 species and subspecies of marine tardigrades (Phylum Tardigrada) are currently known (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Degma et al. 2009–2020; Bartels et al. 2015a), but global diversity could easily approach 1000 species (Bartels et al. 2016), since many areas of the world have not been adequately surveyed (Kaczmarek et al. 2015; Bartels et al. 2015a). Marine tardigrades have been reported from Costa Rica only once before, and only twice in all of Central America.

The only marine tardigrades identified to species level in Central America were two species recorded from a single sample of intertidal barnacles in El Salvador (Schulz 1953), i.e. *Archechiniscus marci* Schulz 1953 and *Echiniscoides sigismundi* s.s. (M. Schultze 1865). *Archechiniscus marci* was a new species and the type species for the genus. At the time of that study *Echiniscoides* was considered monospecific and thought to be cosmopolitan. Since then, eight additional *Echiniscoides* species have been described, eight subspecies have been identified within *Ecn. sigismundi*, and two new genera (*Isoechiniscoides* Møbjerg, Kristensen and Jørgensen, 2016 and *Neoechiniscoides* Møbjerg, Kristensen and Jørgensen, 2016) have been erected (Møbjerg et al. 2016, 2019). Additionally, numerous haplotypes of *Echiniscoides* were discovered indicating cryptic species, but species names were not assigned (Faurby et al. 2011, 2012; Faurby and Barber 2015); these genetic analyses of *Echiniscoides* include the only additional records of marine tardigrades from Central America.

Here we report findings from a survey of intertidal and subtidal habitats on the Caribbean and Pacific coasts of Costa Rica (Central America) and describe seven species new to science. We discuss putative geminate species that could be valuable for understanding the role of the formation of the Isthmus of Central America in allopatric speciation of these model meiobenthic animals.

Materials and methods

Description of the area

The Pacific and Caribbean coasts of Costa Rica are quite distinct. Ecological characterization of these coastal areas of Costa Rica can be found in Cortés (2016a, 2016b). The Caribbean coast, in general, has much less rocky substrate than the Pacific, but has relatively extensive coral reefs and seagrass beds, and two mangrove forests. Tides are small (maximum 50 cm) so rocky intertidal zones are narrow (Cortés 2016a). The Pacific coast of Costa Rica is much larger and with more habitats than the Caribbean coast. The northern section of the coast is exposed to seasonal upwelling with water temperatures dropping to as low as 14.7° C (versus 27–28°C during non-upwelling conditions), tides are around 3 m in amplitude, and intertidal rocky areas are extensive with a varied lithology (Cortés 2016b).

Collections

Collections were made in February 2018. Intertidal sand samples were collected by shovel or with a soil corer which included sand from the surface down to a maximum of about 0.5 m. Barnacles and other intertidal substrates were collected by hand. Shallow subtidal samples were collected while snorkelling or wading and scooping sand into a wide-mouthed bottle. Deeper subtidal samples were not possible in the Caribbean due to rough sea conditions. Deeper subtidal samples on the Pacific coast were collected using a Petite Ponar grab sampler. Soft sediment samples consisted of approximately one litre of sand. A map of the collecting sites is provided in Figure 1. Collections in the Pacific occurred during a cold upwelling period.

To extract tardigrades, the samples were treated with the freshwater shock method (Kristensen and Higgins 1984a). Sand was placed in a two-litre Erlenmeyer flask with fresh water for one minute, agitated and quickly decanted onto a 40 µm sieve. This was repeated three times. For hard substrates such as barnacles, the specimens were treated as above but after the first freshwater bath the substrate was crushed followed by the last two baths. After preservation in 4% buffered formaldehyde solution, tardigrades were sorted with an Olympus SZX-12 stereomicroscope. Specimens were transferred to microslides using Irwin loops, mounted in either glycerine or Fluoromount-G^{TM} and cover slips were sealed with nail polish.

Additional collections from barnacles were made at La Islita (10°57.872′N, 085°41.743′W) in the Golfo de Santa Elena in August 2018. Animals were collected and extracted as above, but preserved in 95% EtOH for SEM and DNA analysis.

Identifications were based on Fontoura et al. (2017) and original species descriptions in the literature.

Generic abbreviations throughout this manuscript follow recommendations of Perry et al. (2019).

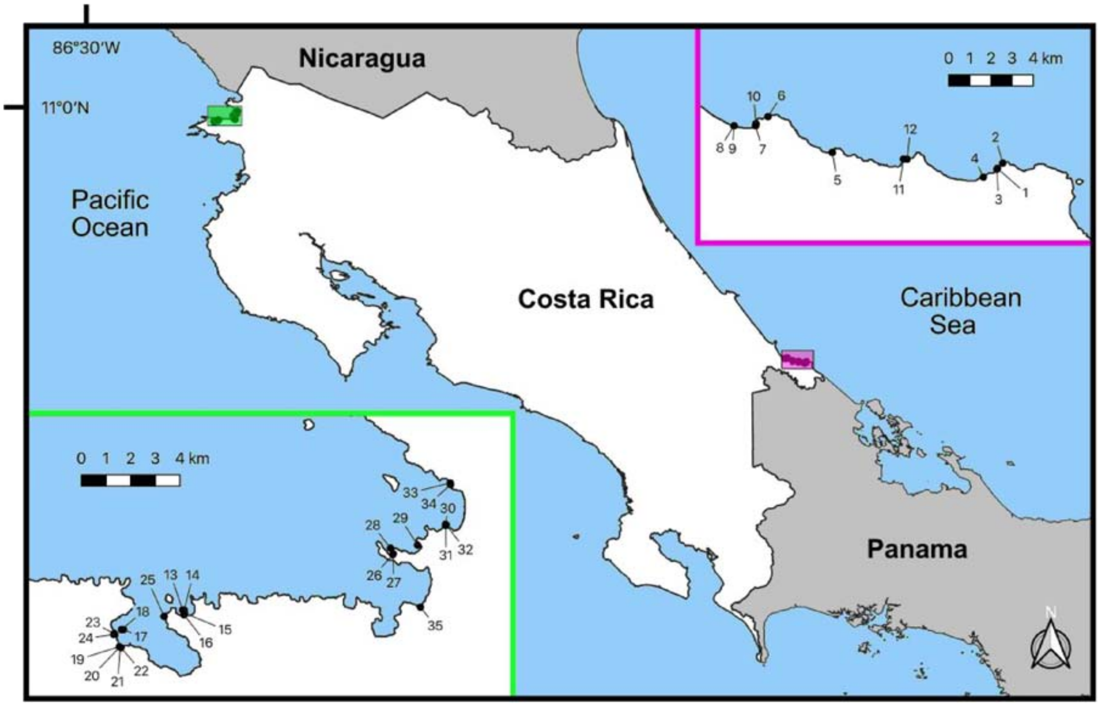

Figure 1. Map of collecting sites. Numbers correspond to site numbers in Table I. White and grey = land masses, blue = ocean. Green inset shows collecting sites in the Pacific, magenta inset shows collecting sites in the Caribbean.

DNA extraction and mitochondrial COI barcode gene amplification

Specimens were rinsed three times in sterile MQ H_2O and each specimen was placed separately in a 1.5 ml Eppendorf microcentrifuge tube in 20 µl of sterile MQ H_2O. Genomic DNA isolation was performed according to the Chelex®100 resin (Bio-Rad) extraction method (Casquet et al. 2012) modified to obtain tardigrade exoskeletons (see Kaczmarek et al. 2019). Tardigrade exoskeletons were fixed on microscope slides in Hoyer's medium.

The *COI* gene fragment was amplified using universal primers: HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) and LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′ (Folmer et al. 1994). The PCR reactions were performed in a BiometraT Professional thermocycler. The polymerase chain reactions were carried out in 20 µl volume containing 0.8 × JumpStart Taq ReadyMix (1 U of JumpStart Taq DNA polymerase, 4 mM Tris-HCl (pH 8.3), 20 mM KCl, 0.6 mM $MgCl_2$, 0.08 mM of dNTP; Sigma-Aldrich, Germany), 0.4 µM of proper forward and reverse primers and ~1 ng of DNA. The PCR protocol was as follows: initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 45°C for 1 min, and 72°C for 1 min and ending with 72°C for 5 min. The PCR products were treated with exonuclease I (20 U/µl, Thermo Scientific) and alkaline phosphatase FastAP (1 U/µl, Thermo Scientific) according to the manufacturer's guidelines and sequenced directly in both directions by Genomed (Warsaw, Poland).

Comparative molecular analysis and phylogenetic relationships

The COI sequences were checked for quality, trimmed to the same length, and unambiguously aligned without inserting gaps in BioEdit v. 7.2.5 (Hall 1999). The COI haplotypes were retrieved using DNASP v.5.10.01 software (Librado and Rozas 2009). To check against pseudogenes, the nucleotide sequences of the *COI* gene fragments were translated into amino acid sequences using the EMBOSS-TRANSEQ application (Rice et al. 2000; Goujon et al. 2010). The translation was carried out with the invertebrate mitochondrial codon table and the −3 reading frame. The BLAST searches (Basic Local Alignment Search Tool; Altschul et al. (1990) were performed to verify the identity and homology of the obtained *COI* gene fragments with sequences deposited in the NCBI database. All obtained COI sequences have been deposited in GenBank under accession numbers: MW046189–MW046193 (see also Table SII, SIII).

To confirm the phenotypic description of the new species, the phylogenetic relationships were estimated between the five COI sequences obtained in our study and all 394 available in GenBank COI sequences of *Echiniscoides*, *Isoechiniscoides* and *Neoechiniscoides* (see Figure A1 and Table SIII for the list of the GenBank accession numbers). Finally, we decided to use 52 COI sequences that represented population/species from each generated subclade/clade. We also applied the closest available outgroup to *Echiniscoides* (Jørgensen et al. 2010), i.e. *Echiniscus tristis* Gąsiorek and Kristensen 2018 (GenBank: MN239904.1 – Bartylak et al. 2019), *Echiniscus blumi* Richters 1903 (GenBank: EU046198.1 – Guil and Giribet 2009) and *Echiniscus testudo* (Doyère 1840) (GenBank: EF620381.1 – Jørgensen et al. 2007) (for details see Table SII). The default settings of MAFFT v 7 (Katoh et al. 2002; Katoh and Toh 2008) were applied to align the COI sequences. The obtained alignment was edited manually in BioEdit. The most appropriate model of sequence evolution was estimated by jModelTest 2 (Darriba et al. 2012) with the assumptions of both the Bayesian Inference Criterion (BIC) and Akaike Information Criterion (AIC) (Posada and Buckley 2004). The GTR + G + I evolution model (General Time Reversible model with gamma distribution and proportion of invariable unchanging sites) was chosen. A phylogenetic tree was computed by applying the Maximum Likelihood (ML) analysis and calculated using MEGA X (Kumar et al. 2018) under the general settings of the selected model with 1000 rapid bootstrap replicates. Bayesian inference (BI) was conducted using MrBayes 3 (Ronquist and Huelsenbeck 2003). Sampling the Markov chains was carried out in every 1000 generations. Random starting trees were used and each of four Metropolis-coupled Marcov chain Monte Carlos ((MC)3) were launched for 40×10^6 generations. Final phylogenetic trees were edited in FigTree v. 1.4.2 software (http://tree.bio.ed.ac.uk/software/figtree).

Calculation for the uncorrected p-distances among our obtained COI sequences and selected representative sequences from clades/subclades of the generated phylogenetic tree was performed using MEGA X. Uncorrected pairwise distances are provided as supplementary materials (Table SII).

Microscopy and imaging

Animals were examined, measured (all measurements in micrometers – μm) and photographed under 1000× oil immersion with a Zeiss Phase Contrast (PhC) microscope and a Zeiss Axioimager 2 Differential Interference Contrast Microscope (DIC), both equipped with digital cameras and Zen Imaging Software (Zeiss) or an Olympus BX-60 PhC/DIC microscope and a Jenoptiks Gryphax camera using iSolutions biometric analysis software. When symmetrical structures were measured, the larger value was recorded if different values were obtained. We report all measurements to the nearest tenth of a micron and estimate that the level of uncertainty in these measurements is ±0.2 microns. All light microscopy, measurements and species descriptions were conducted by PB and PF.

Thirty specimens of *Ecn. ritavargasae* sp. nov. from the La Islita, Golfo de Santa Elena, August 2018 collections were prepared for scanning electron microscope (SEM) analysis according to the protocol from Roszkowska et al. (2018) and examined under high vacuum in a Hitachi S3000N SEM.

All figures were assembled in Affinity Photo 1.8.3. When relevant structures were at different focal planes, multiple images were combined using focus merging with Affinity Photo. Original photos used in focus merged images are deposited in MorphoBank (http://morphobank.org/permalink/?P3907).

Substrate analysis

Granulometric analysis of sediment samples was carried out with a Retsch AS200 sieve shaker and GRADISTAT v. 8 software.

Results

Collecting site characteristics, including the qualitative results of the granulometric analysis, are given in Table I. Quantitative results of the granulometric analysis are available in Table SI.

From a total of 65 samples, 28 contained tardigrades. We found 701 tardigrades and identified 19 taxa including seven species with sufficient type material to describe here as new to science. At least one additional new species in the terrestrial genus *Milnesium* was collected, but additional material is needed to describe the species. The distribution of species at the various collecting sites is shown in Table II with a summary of habitat associations. Ten species were found only in the Caribbean, six species were found only in the Pacific, and three species were found on both coasts (for more details see discussion section).

Table I. Collecting site locations, characteristics and collecting dates.

Site #	Coast	Location	Lat (N) deg min sec	Lon (W) deg min sec	Sample #	Substrate	Depth	Coll Date
1	Caribbean	Manzanillo, loc. 1	09 38 13.0	082 39 07.1	1	*Turbinaria* sp. brown algae on rocks	intertidal	20-Feb-18
2	Caribbean	Manzanillo, loc. 2	09 38 21.1	082 38 58.6	2	turf algae on rocks	intertidal	20-Feb-18
2	Caribbean	Manzanillo, loc. 2	09 38 21.1	082 38 58.6	3-4	calcareous slightly gravelly sand or gravelly sand	intertidal	20-Feb-18
3	Caribbean	Manzanillo, loc. 3	09 38 11.6	082 39 07.9	5-6	calcareous slightly gravelly sand	intertidal	20-Feb-18
4	Caribbean	Manzanillo, loc. 4	09 37 59.3	082 39 28.7	7-10	Calcareous sand	intertidal	20-Feb-18
4	Caribbean	Manzanillo, loc. 4	09 37 59.3	082 39 28.7	28-31	calcareous slightly gravelly sand or sand	0.5–2 m bsl	21-Feb-18
5	Caribbean	Punta Cocles	09 38 37.7	082 43 23.4	11	*Enteromorpha* sp. on rocks	intertidal	21-Feb-18
5	Caribbean	Punta Cocles	09 38 37.7	082 43 23.4	12	*Dictyota* sp. on rocks	intertidal	21-Feb-18
6	Caribbean	Puerto Viejo-Playa Negra, loc. 1	09 39 33.4	082 45 03.1	13	*Padina* sp. on rocks	intertidal	21-Feb-18
7	Caribbean	Puerto Viejo-Playa Negra, loc. 2	09 39 19.0	082 45 22.5	14	*Chthamalus proteus* barnacles on barge	intertidal	21-Feb-18
8	Caribbean	Puerto Viejo-Playa Negra, loc. 3	09 39 19.0	082 45 56.0	15-17	sand, black magnetite	intertidal	21-Feb-18
9	Caribbean	Puerto Viejo-Playa Negra, loc. 4	09 39 19.0	082 45 55.9	18-20	sand, black magnetite	1 m bsl	21-Feb-18
10	Caribbean	Puerto Viejo-River Mouth	09 39 22.2	082 45 21.9	21-22	sand	intertidal	21-Feb-18
10	Caribbean	Puerto Viejo-River Mouth	09 39 22.2	082 45 21.9	23-24	sand or slightly gravelly sand	0.5 m bsl	21-Feb-18
11	Caribbean	Punta Uva, loc. 1	09 38 27.4	082 41 32.7	25	unidentified green algae on rocks	intertidal	21-Feb-18
12	Caribbean	Punta Uva, loc. 2	09 38 27.2	082 41 27.2	26	calcareous sand	1 m bsl	21-Feb-18
12	Caribbean	Punta Uva, loc. 2	09 38 27.2	082 41 27.2	27	calcareous sand	intertidal	21-Feb-18
13	Pacific	Ensenada Mata Palito, loc. 1	10 56 01.9	085 47 34.5	32	gravelly sand	8 m bsl	23-Feb-18
14	Pacific	Ensenada Mata Palito, loc. 2	10 56 01.9	085 47 32.4	33	gravelly sand	6 m bsl	23-Feb-18
15	Pacific	Ensenada Mata Palito, loc. 3	10 55 57.8	085 47 32.1	34	slightly gravelly sand	4 m bsl	23-Feb-18
16	Pacific	Ensenada Mata Palito, loc. 4	10 55 55.5	085 47 32.7	35	slightly gravelly muddy sand	3 m bsl	23-Feb-18
17	Pacific	Ensenada Mata Palito, loc. 5	10 55 35.8	085 48 53.5	36	sandy gravel	13 m bsl	23-Feb-18
18	Pacific	Ensenada Mata Palito, loc. 6	10 55 35.8	085 48 56.4	37	sandy gravel	7 m bsl	23-Feb-18
19	Pacific	Playa Santa Elena, loc. 1	10 55 12.9	085 48 59.0	38-39	sandy gravel or gravelly sand	intertidal	23-Feb-18
20	Pacific	Playa Santa Elena, loc. 2	10 55 12.4	085 48 58.4	40	sandy gravel	intertidal	23-Feb-18
21	Pacific	Playa Santa Elena, loc. 3	10 55 12.0	085 48 57.7	41	sandy gravel	intertidal	23-Feb-18
22	Pacific	Playa Santa Elena, loc. 4	10 55 11.6	085 48 57.0	42	sandy gravel	intertidal	23-Feb-18
23	Pacific	Playa Santa Elena, loc. 5	10 55 30.3	085 49 06.0	43	*Chthamalus panamensis* & *Amphibalanus peruvianus* barnacles on rocks	intertidal	23-Feb-18
23	Pacific	Playa Santa Elena, loc. 5	10 55 30.3	085 49 06.0	44	*Chthamalus panamensis* & *Balanus* sp. barnacles on rocks	intertidal	23-Feb-18
24	Pacific	Playa Santa Elena, loc. 6	10 55 29.2	085 49 06.5	45	*Balanus inexpectatus* barnacles on rocks	intertidal	23-Feb-18
25	Pacific	Punta Pochote	10 55 53.3	085 47 59.3	46	unidentified limpets	intertidal	23-Feb-18
25	Pacific	Punta Pochote	10 55 53.3	085 47 59.3	47	sandy gravel	0.5 m bsl	23-Feb-18
26	Pacific	Playa Jicote, loc. 1	10 57 15.8	085 42 53.9	48	slightly gravelly sand	5 m bsl	24-Feb-18
27	Pacific	Playa Jicote, loc. 2	10 57 17.2	085 42 53.3	49	slightly gravelly sand	6 m bsl	24-Feb-18
28	Pacific	Playa Jicote, loc. 3	10 57 24.6	085 42 56.8	50	slightly gravelly sand	13 m bsl	24-Feb-18
29	Pacific	Playa Corona	10 57 28.6	085 42 20.7	51	slightly gravelly sand	8 m bsl	24-Feb-18
30	Pacific	Islote de Junquillal, loc. 1	10 57 56.3	085 41 43.2	52	gravelly sand	8 m bsl	24-Feb-18
31	Pacific	Islote de Junquillal, loc. 2	10 57 54.8	085 41 42.9	53	sandygravel	9 m bsl	24-Feb-18
32	Pacific	Islote de Junquillal, loc. 3	10 57 55.6	085 41 42.5	54	gravelly sand	9 m bsl	24-Feb-18
33	Pacific	Playa Toyosa, loc. 1	10 58 51.6	085 41 36.9	55	gravelly sand	intertidal	24-Feb-18
33	Pacific	Playa Toyosa, loc. 1	10 58 51.6	085 41 36.9	56	gravelly sand	intertidal	24-Feb-18
33	Pacific	Playa Toyosa, loc. 1	10 58 51.6	085 41 36.9	57	gravelly sand	intertidal	24-Feb-18
33	Pacific	Playa Toyosa, loc. 1	10 58 51.6	085 41 36.9	58-59	gravelly sand	intertidal	24-Feb-18
34	Pacific	Playa Toyosa, loc. 2	10 58 49.4	085 41 36.4	60	unidentified barnacles on rocks	intertidal	24-Feb-18
34	Pacific	Playa Toyosa, loc. 2	10 58 49.4	085 41 36.4	61-62	*Phragmatopoma attenuata* sabellarid tubes on rocks	intertidal	24-Feb-18
34	Pacific	Playa Toyosa, loc. 2	10 58 49.4	085 41 36.4	63	*Tetraclita stalactifera* and *Chthamalus panamensis* barnacles on rocks	intertidal	24-Feb-18
35	Pacific	Playa Cajiniquil	10 56 05.8	085 42 16.9	64	slightly gravelly sand	intertidal	25-Feb-18
35	Pacific	Playa Cajiniquil	10 56 05.8	085 42 16.9	65	*Chthamalus panamensis* barnacles on rocks	intertidal	25-Feb-18

Note: Under 'Substrate,' sediments are named by their textural group except for Sites 8 & 9, for which mineralogical information is added. For further granulometric data see Supplementary Table SI. Depth is metres below estimated average low tide.

Table II. List of species, abundance values, and habitat associations. Note that no algae were collected in the Pacific, no sabellarids were sampled in the Caribbean, and only one population of barnacles was found in a single sample in the Caribbean, which yielded no tardigrades.

Location		Caribbean				Pacific			
Species	Sample #s	Algae	Barnacles	Beach Sand	Subtidal Sand	Sabellarids	Barnacles & Limpets	Beach Sand	Subtidal Sand
Anisonyches eleutherensis Bartels et al. 2018	15			1					
Archechiniscus bahamensis Bartels et al. 2018	12	1							
Archechiniscus murilloi sp. nov. Bartels and Fontoura	46, 62					93	14		
Batillipes homocercus sp. nov. Bartels and Fontoura	62					35			
Batillipes ichthyocercus sp. nov. Bartels and Fontoura	7, 23, 24, 30, 31			4	5				
Echiniscoides costaricensis sp. nov. Bartels and Fontoura	15			11					
Echiniscoides ritavargasae sp. nov. Bartels, Fontoura, Mioduchowska and Kaczmarek	43, 44, 45, 46, 56, 59, 60, 62,63					1	151	3	
Halechiniscus remanei Schulz, 1955	5, 6, 39, 40, 41, 56, 57			12				125	
Mesostygarctus intermedius Renaud-Mornant, 1979	61, 62					8			
Milnesium sp.	64			2					
Orzeliscus sp.	7, 9, 28, 29, 30, 31			2	20				
Parastygarctus sterreri Renaud-Mornant, 1970	4			1					
Raiarctus colurus Renaud-Mornant, 1981	6, 40			15				5	
Stygarctus gourbaultae Renaud-Mornant, 1981	6, 39, 40, 41			131				34	
Styraconyx craticulus (Pollock, 1983)	15			5					
Styraconyx vargasi sp. nov. Bartels and Fontoura	61, 62					18			
Tanarctus breedyae sp. nov. Bartels and Fontoura	40							2	
Tanarctus sp.	6			1					
Wingstrandarctus sp.	4			1					
Totals		1	0	186	25	155	165	169	0

Taxonomic accounts

Taxonomic accounts

Phylum: Tardigrada Doyère, 1840

Class: Heterotardigrada Marcus, 1927

Order: Arthrotardigrada Marcus, 1927

Family: Anisonychidae Møbjerg, Jørgensen and Kristensen, 2019

Genus: *Anisonyches* Pollock 1975 (amended by Bartels et al. 2018 and Møbjerg et al. 2019 and transferred from Echiniscoididae by Møbjerg et al. 2019)

Anisonyches eleutherensis Bartels, Fontoura and Nelson, 2018

Site 8 (Tables I and II, Figure 1)

Material examined: One adult of undetermined sex. Specimen deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Morphological comments: Although some important traits were not observed in the specimen examined (such as eyes and the buccopharyngeal apparatus), the presence of ovoid primary clavae, legs I–III without sensory organs, internal claws longer than external claws (*mauritanius*-type), all claws with long curved distal points and accessory points support the assignment of the specimen to *Ani. eleutherensis*.

Habitat characteristics and distribution: This species was collected from intertidal beach sand at Playa Negra in Puerto Viejo on the Caribbean coast. The fine black sand from this beach is made up exclusively of magnetite and is a unique sediment type on the Caribbean coast. Until now the species was known only from the type locality in the Bahamas (Bartels et al. 2018).

Family: Archechiniscidae Binda, 1978

Genus: *Archechiniscus* Schulz, 1953

Archechiniscus bahamensis Bartels, Fontoura and Nelson, 2018

Site 5 (Tables I and II, Figure 1)

Material examined: One specimen, adult female. Specimen deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Morphological comments: Specimen exactly matches the description in Bartels et al. (2018), including the unique cuticular platelets surrounding the gonopore.

Habitat characteristics and distribution: The species was found on the Caribbean coast at Punta Cocles in *Dictyota* algae growing on intertidal rocks. It was the only tardigrade found in that sample. The species is known from Costa Rica, the Bahamas and Jamaica (Bartels et al. 2018) and the Caribbean coast of Mexico (Pérez-Pech et al. 2020).

Archechiniscus murilloi sp. nov. Bartels and Fontoura
Sites 25 and 34 (Tables I and II, Figure 1)
Figures 2 and 3, Table III

Material examined and type repository: Holotype, adult female (slide CRI-62-1, note that ID numbers refer to sample and slide number), allotype, adult male (slide CRII-62-45), and 29 paratypes: 12 females (slides CRI-46-3, CRI-46-6, CRI-46-14, CRI-62-13, CRI-62-15, CRI-62-17, CRII-62-23, CRII-62-29, CRII-62-40, CRII-62-46, CRII-62-75, CRII-62-100); six males (slides CRI-62-5, CRI-62-21, CRII-62-28, CRII-62-41, CRII-62-58, CRI-62-84); three specimens of undetermined sex (slides CRI-46-8, CRII-62-54, CRII-62-71); three second instar larvae (slides CRI-46-2, CRI-46-12, CRI-62-8); and five first instar larvae (slides CRI-46-4, CRI-62-22, CRII-62-32, CRII-62-42, CRI-62-48), are deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal. Forty-three specimens (including 1 female, 4 males, 35 specimens of undetermined sex and 4 first instar larvae) deposited at the Department of Biology, Warren Wilson College, Asheville, North Carolina, USA. One specimen deposited at the Museo de Zoología, Universidad de Costa Rica.

Type locality: Golfo de Santa Elena at Playa Toyosa, Guanacaste, Costa Rica (Pacific Ocean) (10°58′ 49.4″N, 085°41′36.4″W) on an intertidal rocky beach in the tubes of the sabellarid tube worm *Phragmatopoma attenuata* Hartman, 1944. Additional material was collected from other localities (Tables I and II).

Etymology: This species is named in honour of Dr Manuel M. Murillo, the father of marine science in Costa Rica. Dr Murillo is an exemplar of commitment to the Universidad de Costa Rica and the country and an inspiring mentor to several generations of marine scientists.

Specific diagnosis: *Archechiniscus* with large dark brown eyes and finely punctated cuticle with metameric folds. Lenticular secondary clavae located between the cephalic cirri, but nearer the external cirrus. Internal and external cirri with the distal tip composed of multiple (2–5) leaves, sometimes seen in a fan-like arrangement, but also appearing hollow and tapered like a candle flame. Median cirrus vestigial. Short cirri *A* with enlarged basal portion and flagellum with a blunt tip or with the tip composed of two leaves. Cirri *E* short. Legs with a pair of cuticular bars. Peduncles present on external claws and at the base of internal digits. All claws can be entirely retracted inside claw sheaths. Short spine on legs I and spherical papilla on legs IV. Long arced seminal receptacles with openings located laterally near the female gonopore. Six cell rosette-like female gonopore, circular male gonopore with thick crescent-shape fold. The anus is a slit surrounded by a fan of folds.

Description of the holotype (measurements and statistics in Table III): Female with a cylindrical body (Figures 2 and 3a). Conical cephalic region with large oblong dark brown eyes (about 3.3 µm × 4.0 µm). Rounded caudal region. Cuticle finely punctated (dorsally about 15 pillars/10 µm length) with metameric cuticular folds.

The cephalic appendages are a short external and internal cirri with an enlarged crenate proximal portion and a distal tip made up of multiple leaves compressed such that the tips appear hollow and tapered like a candle flame (Figure 3c,d); lenticular secondary clavae located between external and internal cephalic cirri, but nearer the external cirrus with central annular refracting organelle (Figure 3c); vestigial median cephalic cirrus visible only as a short protuberance (Figure 2); club-shaped primary clavae with a refracting annular van der Land's organ at its base and a distal pore; and short cirrus *A* inserted at the same level as primary clava (Figure 3d). Cirrus *A* with enlarged proximal portion and a distal tip composed of two leaves.

Cirrus *E* (poorly visible) was observed in several paratypes and consists of a short annulated basal portion and a flagellum.

The protruded sub-terminal mouth is followed by a long buccal tube ending in an oval pharyngeal bulb (15.8 µm × 13.3 µm) containing placoids (Figure 3b). Stylets with stylet sheaths. The stylets were not visible in the holotype, but as confirmed in some paratypes, they are terminated by T-shaped furcae with rounded apices (Figure 2). Stylet supports not observed and seem to be absent.

Stubby, non-telescopic legs typical of the genus, having two external sessile robust claws and two internal digits ending with crescent-shaped claws

Table III. Measurements (in μm) of selected morphological structures of the holotype, allotype and paratypes of *Archechiniscus murilloi* sp. nov. (sd = standard deviation; range is the minimum and maximum values; n = number of measurements; * Absent in one specimen).

Characters	Holotype female	Adult females mean±sd (range); n	Allotype male	Adult males mean±sd (range); n	2nd stage larvae mean±sd (range); n	1st stage larvae mean±sd (range); n
Body length	196	188.8 ± 28.4(148–232);13	159	156.0 ± 21.6(122–193);7	125 ± 17.1(111–144);3	89.4 ± 13.6(67–104);5
Body width	68.7	69.4 ± 10.2(51.1–83.1);10	56.3	55.8 ± 4.5(48.3–61.9);6	48.9 ± 2.4(46.9–51.5);3	31.8 ± 6.4(25.0–37.6);4
External cirrus	4.9	4.6 ± 0.5(3.9–5.3);7	?	3.0 ± 0.6(2.7–3.7);3	3.8 ± 0.7(3.0–4.4);3	2.9 ± 0.4(2.6–3.5);5
Internal cirrus	4.8	4.6 ± 0.3(4.2–5.0);8	4.7	4.4 ± 0.8(3.4–5.4);6	3.9 ± 0.6(3.5–4.3);2	3.2 ± 0.1(3.1–3.4);5
Median cirrus	1.8	1.6 ± 0.3(1.0–1.9);8	?	1.5 ± 0.1(1.4–1.6);3	1.6 ± 0.1(1.5–1.6);2	1.4 ± 0.3(1.1–1.7);4
Primary clava	4.9	4.5 ± 0.4(3.9–5.0);12	4.9	4.5 ± 0.5(3.5–5.2);7	3.9 ± 0.0(3.9–3.9);2	3.8 ± 0.4(3.2–4.2);5
Secondary clava (diameter)	6.3	5.6 ± 0.8(4.4–6.9);13	4.6	5.1 ± 0.6(4.5–5.9);7	4.2 ± 0.5(3.7–4.6);3	3.7 ± 0.8(2.9–4.7);4
Cirrus *A*	7.5	7.8 ± 1.5(5.7–10.5);7	6.1	6.8 ± 0.6(6.1–7.4);6	4.8 ± 0.1(4.7–4.8);2	6.3 ± 1.3(5.3–7.8);3
Buccal tube length	31.6	32.2 ± 2.4(29.9–36.8);6	28.9	30.5 ± 3.0(26.3–34.7);6	26.4 ± 4.3(23.3–29.4);2	26.6 ± 1.0(19.3–21.7);4
Stylet sheath length	22.6	20.2 ± 3.4(16.6–25.3);7	?	18.0 ± 2.8(16.6–25.3);2	16.3 ± 1.9(14.9–17.6);2	12.9 ± 1.1(12.9–14.1);3
Stylets	37.2	35.0 ± 2.8(30.9–37.9);5	?	35.5 ± 0.8(34.6–36.2);3	27.4 ± 1.6(26.3–28.5);2	21.7 ± 0.6(21.1–22.4);4
Placoids	9.7	9.3 ± 1.0(7.9–10.5);6	6.9	8.2 ± 1.3(6.5–9.6);6	5.8 ± 0.6(5.3–6.2);2	5.0 ± 0.2(4.7–5.2);4
Pharyngeal bulb (greatest diameter)	15.8	16.6 ± 1.7(15.1–20.3);7	14.1	14.8 ± 1.8(14.0–18.3);6	11.9 ± 0.6(11.5–12.3);2	10.3 ± 0.7(9.5–10.9);3
Papilla leg I	2.5	2.2 ± 0.3(1.7–2.7);7	?	2.1 ± 0.6(1.5–2.6);3	?	1.6 ± 0.0(1.6–1.6);2
External claws I	5.6	5.4 ± 0.5(4.5–5.9);13	4.7	4.6 ± 1.1(2.8–6.3);7	3.5 ± 0.1(3.4–3.6);2	-
Internal claws I	5.1	4.5 ± 0.5(3.6–5.4);13	3.7	4.1 ± 0.8(2.8–4.9);7	3.3 ± 0.2(3.1–3.4);2	3.8 ± 0.3(3.5–4.2);4
External claws II/III	5.8	5.7 ± 0.6(4.9–6.7);12	4.7	4.7 ± 1.0(3.4–5.7);7	3.5 ± 0.6(3.4–3.5);3	-
Internal claws II/III	4.6	4.6 ± 0.4(4.1–5.0);11	3.9	4.2 ± 0.6(3.4–5.1);7	3.0 ± 0.3(2.7–3.3);3	3.7 ± 0.2(3.5–4.1);5
External claw IV	6.9	5.9 ± 0.6(5.2–6.9);12	4.6	4.7 ± 0.9(3.3–5.6);7	3.4 ± 0.2(3.2–3.5);2	-
Internal claw IV	5.3	5.0 ± 0.4(4.2–5.7);11	4.6	4.5 ± 0.7(3.2–5.1);7	3.0 ± 0.2(2.8–3.1);3	3.9 ± 0.3(3.6–4.3);5
Peduncles on external claws IV	3.5	4.1 ± 0.5(3.5–5.1);10	3.7	3.4 ± 0.5(2.6–3.9);6	2.5; 1	-
Basal spur (claws IV)	2.4	2.5 ± 0.2(2.1–2.8);11	2.2	2.0 ± 0.3(1.7–2.3);4	*	-
Leg IV (with claw)	12.6	13.0 ± 0.7(12.2–14.5);10	11.1	9.4 ± 2.6(7.1–12.0);4	8.0; 1	5.2;1
Leg IV papilla	3.8	3.5 ± 0.3(3.1–3.8);9	2.9	2.9 ± 0.4(2.4–3.3);5	2.7 ± 0.5(2.3–3.2);3	2.4 ± 0.3(2.1–2.7);4
Cirrus *E*	?	10.5 ± 2.0(8.0–14.9);9	8.6	8.7 ± 1.0(6.8–9.9);7	7.4 ± 1.5(6.2–9.1);3	6.7 ± 0.5(6.1–7.3);5
Gonopore (diameter)	5.5	4.7 ± 0.8(3.6–5.6);6	3.6	2.8 ± 0.7(1.9–3.6);5	-	-
Gonopore-anus distance	18.6	12.1 ± 3.2(8.8–18.6);8	5.1	5.3 ± 0.5(4.7–5.8);6	-	-
Seminal receptacle length	39.4	30.8 ± 5.3(25.2–39.4);5	-	-	-	-

(Figure 3a, e-g). Two cuticular bars present on legs under external claws. On legs I–III the cuticular bars are separate, roughly triangular in shape, with irregular edges (Figure 3e). On leg IV the bars are composed of two rectangular sections that are connected by a thinner band (Figure 3g). External claws with short spurs directed downwards and a conspicuous peduncle at the base; internal claws with conspicuous accessory points. Short granule-shaped peduncles present at the base of internal digits (Figure 3e). Claws can be entirely retracted inside claw sheaths.

Sensory organs on legs I and IV. Short blunt papilla with enlarged base, having an embedded internal needle-like structure on leg I (Figure 3d), and spherical papilla on leg IV with a refracting annular van der Land's organ at the base. Anterior bulbous papilla on legs I–III absent.

The female gonopore (Figure 3f) consists of a six-cell rosette. Seminal receptacles (Figure 3f) are long arced tubes, having openings 5.4 μm in diameter, located ventro-laterally at the level of the gonopore, from which they are 25.5 μm distant. In their terminal portion, seminal receptacles are very thin and almost in contact with the sagittal line, about 24 μm anterior to the gonopore. The anal fissure straight and surrounded by a fan of folds.

Remarks: Variability in the morphology of cephalic cirri was observed. The distal tip of external and internal cirri often appears hollow and tapered like a candle flame, but may also appear to be composed of multiple (2–5) leaves that are sometimes seen spread apart in a fan-like array. Cirrus *A* can have a blunt tip or it can be composed of, at least, two leaves. Cuticular bars at the base of claws are also somewhat variable in shape. The claw IV cuticular bars are usually fused with a small band, but sometimes they are separate. The males appear to be smaller than females. The male gonopore (Figure 3g) is circular and, in comparison with females, located much nearer to the anus. All other characteristics are very similar to females. First instar larvae lack gonopore, anus and external claws. Second instar larvae lack a gonopore. Otherwise, larvae have all other adult characteristics.

Differential diagnosis: Only five species have been previously assigned to the genus *Archechiniscus*: *Archechiniscus marci* (for a clarification of the status of this

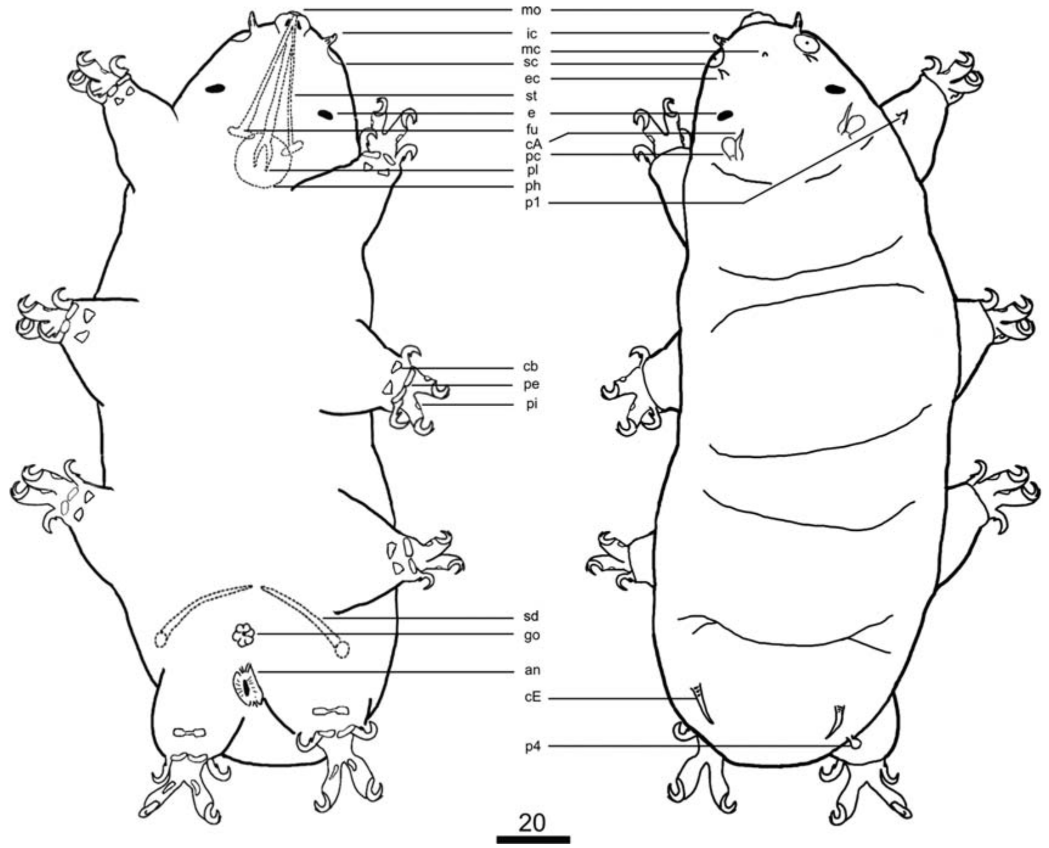

Figure 2. Ventral and dorsal aspects of *Archechiniscus murilloi* sp. nov. an = anus, cA = cirrus *A*, cb = cuticular bars, cE = cirrus *E*, e = eye, ec = external cirrus, fu = furca, go = gonopore, ic = internal cirrus, mc = median cirrus, mo = mouth, pc = primary clava, pe = peduncle of external claw, ph = pharynx, pi = peduncle of internal digit, pl = placoids, p1 = sensory papilla on leg I, p4 = sensory papilla on leg IV, sc = secondary clavae, sd = seminal duct, st = stylet. Scale bar in μm.

species see Bartels et al. 2018); *Archechiniscus minutus* Grimaldi de Zio and D'Addabbo Gallo, 1987; *Archechiniscus symbalanus* Chang and Rho, 1998; *Archechiniscus biscaynei* Miller, Clark and Miller, 2012; and *Archechiniscus bahamensis*.

The new species differ from all those species by the presence of peduncles at the base of internal claws and thick cuticular bars on legs (the cuticular bars are present in *Arc. bahamensis* though less prominent, in other species this trait has not been described) and because:

- *Arc. marci* has sharp-tipped external and internal cephalic cirri (tips composed of 2–5 leaves sometimes seen in a fan-like arrangement, but also appearing hollow and tapered like a candle flame in *Arc. murilloi* sp. nov.), has serrated stylets with rounded stylet furcae (T-shaped in the new species), has secondary clavae that are small papillae under external cirri, accessory points on internal claws and peduncles on external claws were not described in *Arc. marci.*
- *Arc. minutus* lacks a median cirrus, lacks a sensory spine on legs I, has longer internal and external cirri (which range between 4.2–5.0 μm and 3.9–5.3 μm, respectively, in females of the new species, while in the holotype of *Arc. minutus*, a female with a body 140 μm long, they are both 5.5 μm), has much shorter digits (ranging in females of the new species from 12.2–14.5 μm, compared with 5.5 μm in the *Arc. minutus* holotype) and its eyes are not always visible, whereas they are very conspicuous in *Arc. murilloi* sp. nov.
- *Arc. symbalanus* has S-shaped seminal receptacle ducts (arced in the new species), has sharp-tipped external and internal cephalic cirri (tips composed of 2–5 leaves, sometimes seen in a fan-like arrangement, but also appearing hollow and tapered like a candle flame in the new species), has short but evident median cirrus (vestigial in the new species), has rounded stylet furcae, has cirri *E* without annulated proximal portion, lacks cuticular metameric folds and females lack claw sheaths.

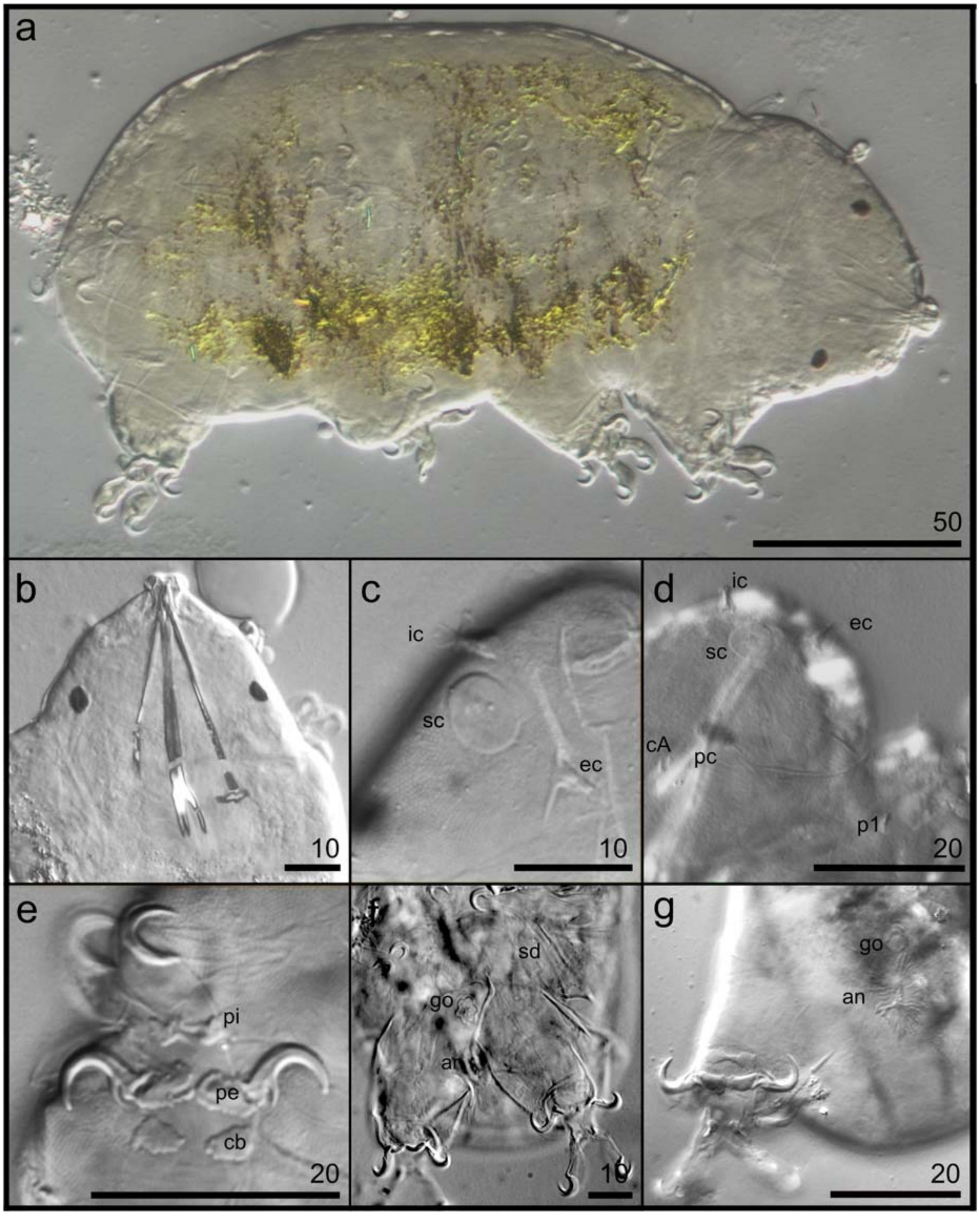

Figure 3. *Archechiniscus murilloi* sp. nov. **a.** Habitus, female paratype (CR-62-30) in dorso-lateral view. DIC, focus merged. **b**. Head showing mouth, buccal apparatus and eyes (CR-62-59). DIC. **c**. Detail of cephalic appendages (CR-62-57). Note fan-like appearance of tip of internal cirrus and bifurcated appearance of tip of external cirrus. DIC. **d.** Detail of anterior appendages on laterally arranged specimen (CR-62-65). Note flame-like appearance of tips of internal and external cirri. DIC. **e**. Claw I (CR-62-57). DIC, focus merged. **f**. Posterior ventral aspect of female (CRI-62-1). PhC. **g**. Posterior ventral aspect of male (CRI-46-6). DIC. In **f** and **g** note peduncles and cuticular bars of claw IV. an = anus, cA = cirrus *A*, cb = cuticular bars, ec = external cirrus, go = gonopore, ic = internal cirrus, p1-4 = leg sensory organs, pc = primary clava, pe = external peduncles, pi = internal peduncles, sc = secondary clava, sd = seminal duct. Scale bars in μm.

- *Arc. biscaynei* has bulbous papillae on the front of legs I, II and III, lacks eyes, lacks sensory spine on leg I and lacks cuticular metameric folds.
- *Arc. bahamensis* has short seminal receptacles with openings located in a depression protected by cuticular platelets, has longer somatic cirri *A* and *E* ($A > 10$ and $E > 20$ µm in *Arc. bahamensis*, opposite in the new species), has bulbous papillae on the front of legs I, II and III.

Habitat characteristics and distribution: *Archechiniscus murilloi* sp. nov. was collected on sabellarid tubes and limpets, which supports the argument for the possibility of different niche specializations within this genus advanced by Bartels et al. (2018). *Archechiniscus minutus* and *Arc. bahamensis* (with the exception of the single specimen reported in this study collected in *Dictyota* algae) were collected in coralline sand (Grimaldi de Zio and D'Addabbo Gallo 1987, 2001; D'Addabbo Gallo et al. 1989; Gallo et al. 2007; Bartels et al. 2018) and calcareous sand with shell and coral fragments (Pérez-Pech et al. 2020) and the holotypes of *Arc. marci*, *Arc. symbalanus* and *Arc. biscaynei* on barnacles (Schulz 1953; Chang and Rho 1998; Miller et al. 2012). *Archechiniscus murilloi* sp. nov. was found only on the Pacific coast.

Family: Batillipedidae Ramazzotti, 1962 (amended by Gallo D'Addabbo et al. 2005)

Genus: *Batillipes* Richters, 1909

***Batillipes homocercus* sp. nov. Bartels and Fontoura**

Sites 33 and 34 (Tables I and II, Figure 1)

Figures 4–5, Table IV

Material examined and type repository: Holotype, adult female (slide CRI-62-25). Allotype, adult male (slide CRI-62-47) and 27 other paratypes: 10 females (slides CRI-62-9, CRI-62-18, CRI-62-35, CRII-62-60, CRII-62-63, CRII-62-64, CRII-62-66, CRII62-88, CRII-62-91, CRI-62-78); seven males (slides CRI-62-20, CRI-62-34, CRI-62-43, CRI-62-44, CRII-62-74, CRII-62-77, CRII-62-81); nine specimens of undetermined sex (slides CRI-62-24, CRI-62-37, CRII-62-53, CRII-62-67, CRII-62-73, CRII-62-83, CRI-62-95, CRI-62-102, CRI-62-105) and one four-clawed larva (slide CRI-62-33). The holotype, allotype and 23 paratypes are deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal; four paratype adults of undetermined sex (slides CR-62-7, CR-62-95, CR-62-102, CR-62-105) are deposited at the Department of Biology, Warren Wilson College, Asheville, USA. One specimen (CR-62-78) is deposited at the Museo de Zoología, Universidad de Costa Rica.

Type locality: Golfo de Santa Elena, Playa Toyosa, Guanacaste, Costa Rica (Pacific Ocean) (10°58′49.4″N, 085° 41′36.4″W) from an intertidal rocky beach on *P. attenuata* sabellarid tubes.

Etymology: The specific name *homocercus* (from the Greek *homós* = equal +*kérkos* = tail) refers to the two equal sharp appendages that constitute the caudal apparatus.

Specific diagnosis: *Batillipes* with tubular primary clavae. Well-developed dome-shaped secondary clavae, appearing as conspicuous ventral platelet-like circular structures. Internal cirri and median cirrus with cirrophores and swollen distal tips. External cirri with swollen rounded distal tips turned inwards. Sensory spines on all legs. Sensory spines on legs I–III with enlarged base (cirrophore) and swollen distal tips. On leg I the swollen tip is separated from the basal portion by an optically dense dot. The sensory organ on leg IV is divided into a cirrophore, a basal portion with a van der Land's organ and a distal swollen tip with an optically dense dot at base. On the fourth legs, middle toes 3 and 4 of different lengths, with toe 3 shorter than toe 4 (toe arrangement pattern B1 according to Santos et al. 2018a). Triangular lateral body projections between legs III–IV, each with an elongated and thick blunt tip turned backwards. Other lateral body projections absent. The caudal apparatus consists of two separated sharp spikes inserted dorsally. Dorsal cuticle uniformly punctated. Rosette-like female gonopore and circular male gonopore with a cuticular crescent-shaped fold.

Description of the holotype (measurements and statistics in Table IV): Female with head continuous with the trunk without a visible neck constriction (Figures 4 and 5a–c). Eye spots not visible in mounted specimens. Internal cirri bearing cirrophores inserted dorsally (Figure 5b). Thick horn-shaped external cirri inserted ventrally near the common pedestal bearing primary clavae and cirri *A*, ending in a swollen rounded tip turned inwards (Figure 5b,c). Long median cirrus with a cirrophore (Figures 4 and 5b). All cephalic cirri with an optically dense dot (van der Land's organs) near their blunt tips. Cirri *A* are located dorsally in relation to the unconstricted primary clavae (Figure 5a,b). The primary clava (Figure 5b,c), with a van der Land's organ at the base, is tubular (13.1 µm long and 1.5 µm wide). In the frontal edge of the head, dome-shaped secondary clavae present. Ventrally, the secondary clavae appear as conspicuous platelet-like circular structures located at the base of external cephalic cirri (Figure 4 and 5c). Indentations in the frontal edge of the head absent.

Ventral mouth opening in a slightly protruded buccal cone. Buccal tube 19.5 µm long and 2.5 µm

Table IV. Measurements (in µm) of selected morphological structures of the holotype, allotype and paratypes of *Batillipes homocercus* sp. nov.

	Females		Males	
Characters	Holotype	mean ± sd (range); n	Allotype	mean ± sd (range); n
Body length	137.8	142.8 ± 19.1 (105.2–170.6);10	130.0	136.8 ± 19.9 (103.3–159.1);8
Body width	54.6	53.6 ± 9.4 (35.8–66.2);9	44.1	47.9 ± 7.8 (37.9–57.4);8
Head width	43.3	44.2 ± 4.1 (37.8–48.8);8	40.6	43.1 ± 3.6 (39.1–49.2);7
Caudal apparatus	13.0	11.6 ± 1.7 (7.2–13.0);10	11.6	11.5 ± 1.4 (8.5–13.2);8
Median cirrus	22.6	21.8 ± 3.2 (16.0–25.2);7	18.0	21.3 ± 3.8 (17.6–28.9);8
Internal cirri	21.2	21.9 ± 2.2 (18.2–24.7);10	22.3	21.7 ± 2.2 (17.5–24.4);8
External cirri	14.1	13.9 ± 1.3 (11.2–15.4);10	13.2	13.4 ± 0.8 (11.7–14.4);8
Cirri *A*	26.3	29.3 ± 2.7 (23.7–32.5);10	23.5	28.6 ± 4.1 (23.2–34.7);6
Primary clava	13.1	13.9 ± 1.5 (10.7–16.0);10	12.9	12.9 ± 1.4 (10.3–15.3);8
Secondary clava (diameter)	5.5	6.4 ± 1.1 (5.2–8.0);8	6.0	6.6 ± 0.9 (5.6–8.3);6
Cirri *E*	17.2	15.8 ± 1.1 (14.3–17.2);5	11.6	14.3 ± 4.0 (10.5–17.0);4
Leg I spine	7.5	7.9 ± 1.0 (6.8–9.5);7	7.2	7.1 ± 1.1 (4.7–8.6);8
Leg II spine	10.8	10.6 ± 1.2 (9.0–12.5);6	11.3	11.3 ± 1.2 (9.8–12.9);6
Leg III spine	12.1	13.0 ± 2.1 (9.3–16.9);8	12.7	12.3 ± 0.9 (10.6–13.6);8
Leg IV sensory organ	15.3	13.5 ± 2.2 (10.3–16.6);7	11.4	13.1 ± 2.3 (9.5–17.5);7
Body projection 4	12.2	12.2;1	?	11.2;1
Legs I–III: Toe 1	8.6	9.4 ± 0.7 (8.6–10.5);6	8.2	9.2 ± 0.6 (8.2–9.9);5
Toe 2	6.0	6.0 ± 0.7 (5.3–7.1);6	4.2	5.3 ± 0.6 (4.2–6.0);5
Toe 3	14.1	13.8 ± 1.2 (11.6–15.4);6	9.5	12.8 ± 2.0 (9.5–15.6);5
Toe 4	9.3	9.1 ± 0.7 (8.0–10.0);6	7.8	8.8 ± 0.8 (7.8–9.9);5
Toe 5	15.8	15.8 ± 0.9 (14.6–17.0);6	14.3	15.0 ± 0.9 (14.0–16.1);5
Toe 6	9.4	9.8 ± 1.1 (8.6–11.3);6	8.6	9.4 ± 0.6 (8.6–10.1);5
Leg IV: Toe 1	11.5	11.2 ± 1.2 (9.5–12.8);10	12.0	11.4 ± 0.9 (9.2–12.2);7
Toe 2	16.3	15.7 ± 1.6 (13.6–18.0);10	?	15.1 ± 1.7 (12.5–17.2);6
Toe 3	6.6	7.1 ± 0.9 (6.2–9.0);10	6.9	7.3 ± 0.6 (6.1–8.1);7
Toe 4	11.5	11.2 ± 1.4 (9.2–13.0);10	10.5	10.4 ± 1.4 (9.7–12.3);7
Toe 5	20.2	19.1 ± 2.5 (16.6–21.6);9	19.9	18.8 ± 2.2 (13.6–20.3);7
Toe 6	11.6	11.8 ± 1.0 (10.2–12.9);9	11.3	11.1 ± 1.3 (8.9–13.4);7
Gonopore-anus distance	11.3	10.7 ± 2.1 (7.9–12.9);4	2.7	2.8 ± 0.4 (2.1–3.4);6
Punctation/10 µm	10.7	11.4 ± 1.8 (9.5–14.8);7	12.0	12.0 ± 1.0 (10.6–13.3);8

Note: Body lengths exclude caudal spikes; body width was measured between legs III–IV; head width was measured between bases of primary clavae; toe lengths include discs (sd = standard deviation; range is the minimum and maximum values; n = number of measurements).

wide. Stylets (20.5 µm long) with strong stylet supports. Spherical pharyngeal bulb, about 18.5 µm in diameter, with placoids (~9.7 µm long).

Ventro-lateral body projections between legs III–IV (body projection 4) are the only body projections present. Each body projection 4 is well-developed, has a triangular base and a long thick blunt tip turned backwards (Figures 4 and 5e,f).

The caudal apparatus (Figures 4 and 5a–d) composed of two sharp spikes inserted on separated swollen bases.

Sensory spines with slightly enlarged bases are present on all legs (Figure 5a,b,d,f,g). Leg sensory spines increase in length from legs I to legs IV. Sensory organs with swollen tips on all legs. Sensory organ on leg I with a distal swollen tip with an optically dense dot at base (Figure 5a). Sensory organ on leg IV is divided into a cirrophore (6.6 µm) and a basal portion (7.2 µm), with a van der Land's organ at the base, separated from a distal swollen tip (1.5 µm) by an optically dense dot (Figure 5d).

Blunt tipped cirri *E* with small cirrophores.

Telescopic legs with toes having the distal stalk enlarged distally, small circular/ovoid suction discs (disc diameter of toes on legs IV about 4.3 µm) with thin braces. On the first three pairs of legs, toe 2 is the shortest (considering toe 1 to be the most cephalically situated), toes 3 and 5 are the longest and toes 1, 4 and 6 are medium-sized. On feet of the fourth pair of legs, toes are arranged according to the B1 pattern (Santos et al. 2018a): [(Toe 3 < Toe 4) ≤ Toe 1 & Toe 6] (Figure 5a,b,d).

Dorsal cuticle (Figure 5f) without perceptible folds punctated with about 10.7 pillars/10 µm (each pillar about 1.4 µm high) in the latero-caudal part of the body and about 12.4 pillars/10 µm near the sagittal line. Ventral cuticle, with folds and finely punctated (Figure 5c,e).

The rosette-shaped gonopore (about 4.5 µm in diameter), has two very small posterior smooth platelets and is separated from the anus by a very thin groove (Figure 5e).

Remarks: Sexual dimorphism is not evident (see Table IV). While there is a slight difference in the diameter of the secondary clavae between males and females (Table IV), the ranges overlap extensively (5.2–8.0 in females, 5.6–8.3 in males). Otherwise, males are very similar to females in both qualitative and quantitative characters, except for their circular gonopore (2.8 µm in diameter in the allotype) with a cuticular crescent-shaped fold and located nearer the anus.

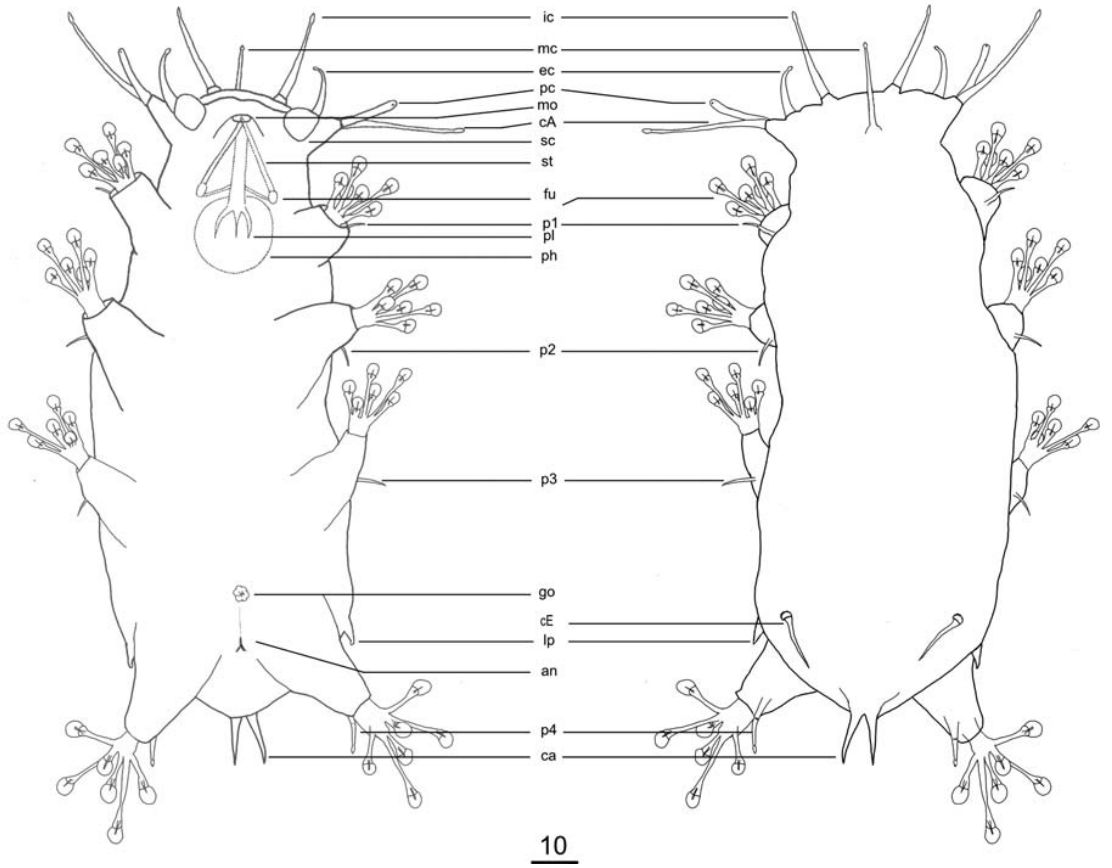

Figure 4. Ventral and dorsal aspects of *Batillipes homocercus* sp. nov. an = anus, lp = lateral body projection IV, ca = caudal appendage, cA = cirri *A*, cE = cirrus *E*, ec = external cirri, fu = furca, go = gonopore, ic = internal cirri, mc = median cirrus, mo = mouth, pc = primary clavae, ph = pharynx, pl = placoids, p1–p4 = sensory organs on leg I–IV, st = stylet. Scale bar in µm.

Among adults, variability in the morphology of the caudal apparatus was not observed.

The only four-toed larva collected (74.1 µm long and 23.7 µm wide between the third and fourth pair of legs and 30.4 µm between primary clavae), despite being very deformed by the slide mounting process, is similar to adults. Cephalic appendages display the same morphology (median cirrus is 16.7 µm; external and internal cirri are 6.0 µm and 14.9 µm, respectively; cirri *A* are 20.2 µm, and the primary clavae are 9.3 µm long and 0.9 µm wide). Secondary clavae were not observed. Sensory organs were visible on legs II (9.3 µm) and IV (10.0 µm long) only. Somatic cirri *E* is 9.6 µm long. The caudal apparatus consists of two small points (about 2.5 µm long). Body projections between legs III–IV, probably folded under the body, were not visible.

Differential diagnosis: Like *Bat. homocercus* sp. nov., four other *Batillipes* species display bifurcated caudal apparatuses: *Batillipes dicrocercus* Pollock 1970; *Batillipes noerrevangi* Kristensen, 1978; *Batillipes potiguarensis* Santos, da Rocha, Gomes Jr. and Fontoura, 2017; and *Batillipes tridentatus* Pollock, 1971. Ontogenetic variability of the caudal apparatus in *Batillipes* species has been reported by many tardigradologists (Grimaldi de Zio and D`Addabbo Gallo 1975; McKirdy 1975; Kristensen 1978; Morone de Lucia et al. 1988; Gallo D'Addabbo et al. 2000; Kristensen and Mackness 2000; Santos et al. 2018a, 2018b, 2019), and although a key character for some species (e.g. *Batillipes bullacaudatus* McGinty and Higgins 1968), the caudal apparatus should be used with caution for taxonomic purposes and associated with other stable taxonomic characters (McKirdy 1975; Gallo D'Addabbo et al. 2000). Toe length arrangement pattern is a stable character for *Batillipes* (Pollock 1970; Kristensen and Mackness 2000; Santos et al. 2017, 2018a), and *Bat. homocercus* sp. nov. belongs to group B1 (Santos et al. 2018a) [(Toe 3 < Toe 4) ≤ Toe 1 & Toe 6 (toes numbered from cephalic to caudal)], while *Bat. dicrocercus*, *Bat. noerrevangi*, *Bat. potiguarensis* and *Bat. tridentatus* are all members of group A [(Toe 3 = Toe 4) ≤ Toe 1 & Toe 6)].

Excluding the new species, only seven from the 36 described species of the genus *Batillipes* have a B1 toe

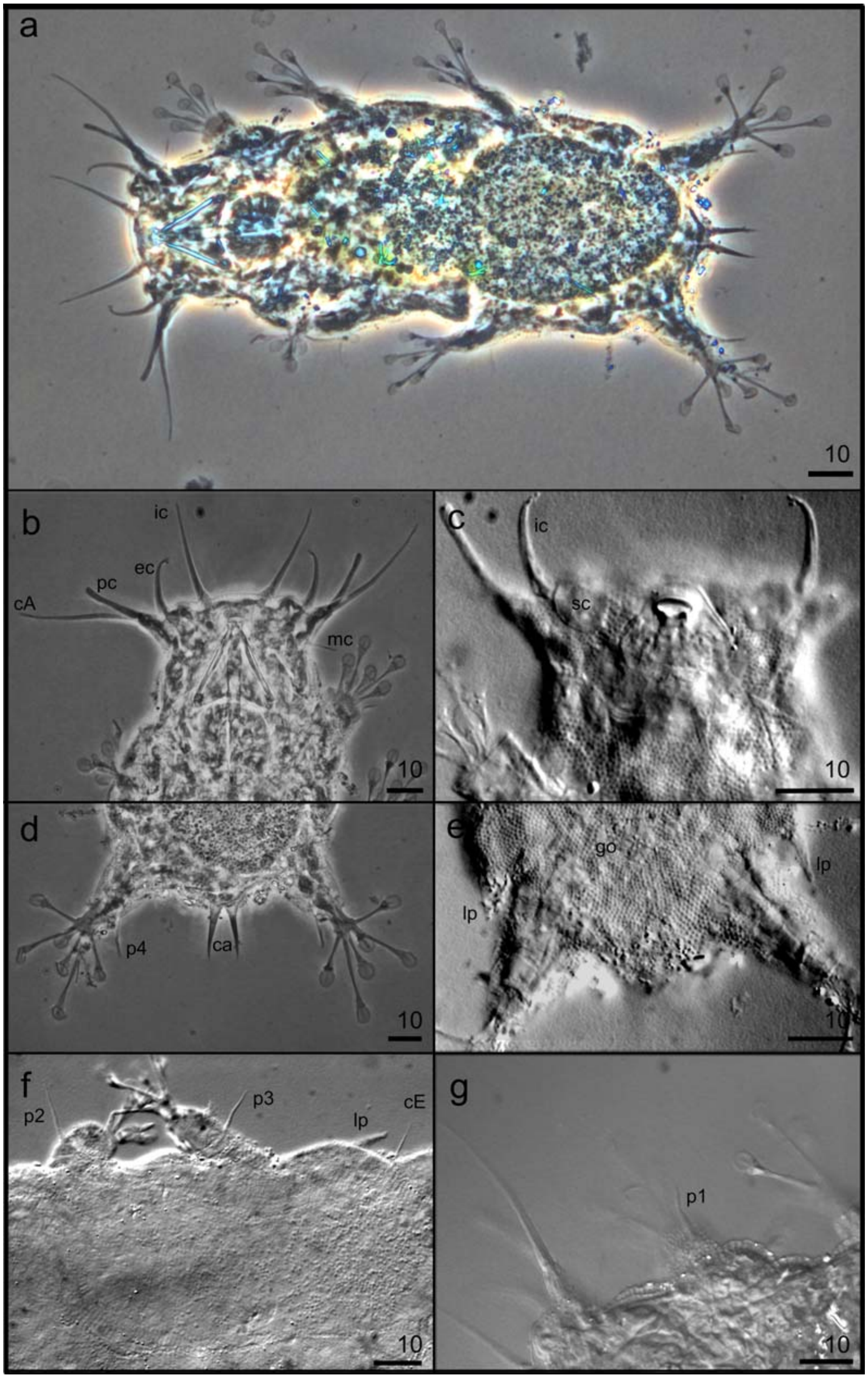

Figure 5. *Batillipes homocercus* sp. nov. **a**. Habitus, female holotype, dorsoventral view. PhC, focus merged. **b**. Head of holotype showing buccal apparatus and cephalic appendages. Note that only tip of median cirrus is visible. PhC, focus merged. **c**. Detail of ventral surface of head of holotype. DIC. **d**. Posterior section of holotype. PhC, focus merged. **e**. Posterior ventral section of female holotype. DIC. **f**. Right lateral area of paratype (CRII-62-63) showing leg II (to left of image) and leg III. DIC. **g**. Right anterio-lateral detail of holotype. DIC, focus merged. ca = caudal appendage, cA = cirrus *A*, cE = cirrus *E*, ec = external cirrus, go = gonopore, ic = internal cirrus, lp = lateral projection, pc = primary clava, mc = median cirrus, p1-3 = leg sensory organs, sc = secondary clava. Scale bars in µm.

Table V. Measurements (in µm) of selected morphological structures of the holotype, allotype and paratypes of *Batillipes ichthyocercus* sp. nov. Body lengths exclude caudal spikes; body width was measured between legs III–IV; head width was measured between bases of primary clavae; toe lengths include discs.

Characters	Holotype Male CRI-24.1	Allotype Female CRI-30.4	Paratype Male CRII-30.6	Paratype Male CRII-7.5	Paratype Female CRI-7.2	Paratype Female CRI-7.3	Paratype Female CRII-31.7	Paratype ? CRII-7.4
Body length	152	129.6	131.3	124.9	137.3	167	144.1	95.3
Body width	59.8	56.7	?	46.6	?	?	?	32.2
Head width	50.8	50.0	?	?	?	?	?	?
Caudal apparatus	12.0	14.2	15.9	16.5	8.8	?	12.3	10.0
Median cirrus	?	19.7	18.2	?	18.7	?	15.8	?
Internal cirri	21.5	19.0	18.3	16.8	19.3	?	?	?
External cirri	16.5	12.0	15.3	12.1	?	?	?	8.5
Cirri *A*	28.9	24.4	21.2	22.3	17.2	?	23.0	21.3
Primary clava	14.2	12.8	11.3	12.4	13.0	9.2	12.5	11.0
Secondary clava (diameter)	5.1	4.4	4.7	?	?	?	?	?
Cirri *E*	?	12.7	19.8	?	?	?	?	?
Leg I spine	9.7	8.7	9.5	7.1	7.0	8.0	8.1	7.3
Leg II spine	9.6	9.1	?	?	?	?	?	7.6
Leg III spine	9.7	?	?	?	?	?	?	?
Leg IV sensory organ	18.7	10.8	10.8	13.2	11.9	10.9	?	7.9
Body projection 4	13.0	11.7	18.2	?	?	?	14.7	7.2
Legs I–III: Toe 1	10.0	8.8	9.3	9.0	?	11.4	?	?
Toe 2	5.2	6.1	5.6	4.8	?	5.2	?	?
Toe 3	14.2	14.0	12.5	12.5	?	16.8	?	?
Toe 4	9.2	9.8	8.3	9.1	?	9.2	?	?
Toe 5	16.6	15.7	16.8	14.5	?	18.0	?	?
Toe 6	11.2	11.0	10.4	9.4	?	11.6	?	?
Leg IV: Toe 1	17.0	16.9	11.1	10.5	?	16.4	?	12.0
Toe 2	22.8	18.3	13.9	?	?	?	?	14.4
Toe 3	9.0	8.7	7.0	9.3	?	8.3	?	7.5
Toe 4	13.4	13.1	10.5	12.0	?	?	?	11.7
Toe 5	21.4	24.5	19.4	17.7	?	24.0	?	21.3
Toe 6	13.9	13.7	14.0	13.0	?	18.0	?	12.2
Gonopore–anus distance	3.8	6.3	4.5	?	?	?	?	?
Punctation/10 µm	10.0	9.9	9.5	?	?	?	9.0	?

arrangement pattern (Santos et al. 2018a). These species are *Batillipes africanus* Morone de Lucia, D'Addabbo Gallo and Grimaldi de Zio, 1988; *Batillipes brasiliensis* Santos, da Rocha, Gomes Jr. and Fontoura, 2017; *Batillipes dandarae* Santos, da Rocha, Gomes Jr. and Fontoura, 2017; *Batillipes friaufi* Riggin, 1962; *Batillipes lesteri* Kristensen and Mackness, 2000; *Batillipes littoralis* Renaud-Debyser, 1959b; and *Batillipes similis* Schulz, 1955. *Batillipes homocercus* sp. nov. can be clearly distinguished from these in having well-developed dome-shaped secondary clavae appearing as conspicuous ventral platelet-like circular structures, and also because:

- *Bat. africanus* lacks the lateral body projection between legs III–IV and has a single pointed caudal apparatus.
- *Bat. brasiliensis* has a body projection between legs III–IV that terminates in five digit-shaped expansions, has well-developed body projections between legs I–II and II–III and has a single ala-like caudal apparatus.
- *Bat. dandarae* lacks the lateral body projection between legs III–IV and lacks a caudal apparatus with two sharp spikes.
- *Bat. friaufi* lacks the lateral body projection between legs III–IV and its caudal apparatus is not bifurcated.
- *Bat. lesteri* has a body projection between legs III–IV terminating in a two-pointed expansion, has well-developed body projections between legs I–II and II–III and has a single ala-like caudal apparatus.
- *Bat. littoralis* has well-developed body projections between legs I–II and II–III, has a tooth on legs IV and has a three-pointed caudal apparatus.
- *Bat. similis* has a body projection between legs III–IV that is conical versus blunt in the new species, has well-developed body projections between legs I–II and II–III, has much longer sensory organs on legs IV (shorter than the hind legs in the new species, longer than the hind legs including toes in *Bat. similis*), has drum-stick like primary clavae with terminal swellings vs tubular in new species, has external cirri ending in rounded tip and has a single conical caudal apparatus.

Habitat characteristic and distribution: All specimens were found on the Pacific coast associated with intertidal sabellarid tubes.

Batillipes ichthyocercus sp. nov. Bartels and Fontoura

Sites 4 and 10 (Tables I and II, Figure 1)

Figures 6–7, Table V

Material examined and type repository: Holotype: adult, male (slide CRI-24-1) collected at the river mouth at Puerto Viejo (Caribbean Sea) in subtidal sand. Allotype: adult, female (slide CRI-30-4) collected in intertidal sand at Manzanillo (Caribbean Sea). Seven paratypes: two females (CRI-7-2, CRI-7-3), one male (CRII-7-5) and one specimen of undetermined sex, probably a second instar larvae (CRII-7-4) collected in intertidal sand at Manzanillo; one female (CRII-31-7) and one male (CRII-30-6) both collected in subtidal sand at Manzanillo; and a specimen of undetermined sex (CRI-23-1, a poor preparation) collected in subtidal sand in the river mouth at Puerto Viejo. The type material (holotype and paratypes) is deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal, except for CRII-31-7 deposited at the Museo de Zoología, Universidad de Costa Rica.

Type locality: Puerto Viejo, Costa Rica (Caribbean Sea) (9°39′22.2″N, 082°45′21.9″W) from shallow subtidal sand. Additional material collected from Manzanillo (Tables I and II).

Etymology: The specific name, *ichthyocercus*, from the Greek *ikhthys* = *fish* + kérkos = tail, refers to the bifurcated caudal cuticular expansion that looks like a fish tail.

Specific diagnosis: *Batillipes* with distinct head, separated from the body by a neck constriction followed by short lateral processes. Primary clavae is tubular with the tip slightly swollen. Dome-shaped secondary clavae present. Cephalic cirri with cirrophores and swollen distal tips. Internal cirri horn-shaped. Sensory spines on all legs. Sensory spine on leg I with a swollen distal tip separated from the basal portion by a van der Land's organ. The sensory organ on leg IV divided into a cirrophore, a basal portion and a distal swollen tip. The basal portion and the distal tip with van der Land's organ at base.

Fourth leg toe pattern group B1 (Santos et al. 2018a) [(Toe 3 < Toe 4) ≤ Toe 1 & Toe 6]. In addition to the short lateral body projection 1, separating the head from the body, only sharp lateral body projections between legs III–IV are present. The caudal apparatus consists of a thin, transparent structure bifurcated into two arced points that merge before the posterior edge of the body, giving the appearance of a lateral view of a fish tail. Dorsal cuticle uniformly punctated. Rosette-like female gonopore and circular male gonopore.

Description of the holotype (measurements and statistics in Table V): Male with trapezoidal head separated from the body by a neck constriction followed by a very small (protruding ~3.8 μm) lateral processes (body projection 1) (Figures 6 and 7a,b). Eye spots not visible in mounted specimens. Internal cirri inserted dorsally, bearing cirrophores (Figure 7a,b). External cirri thick and horn-shaped with short cirrophores inserted more ventrally near the common pedestal bearing cirri *A* and primary clavae (Figure 7a,b). The median cirrus (Figures 6 and 7b) with a cirrophore not measurable in the holotype. Cirri *A* are located dorsally in relation to the unconstricted primary clavae (Figure 6). The primary clavae, with a van der Land's organ at the base, is tubular, displaying a short broader basal portion, the middle portion slightly thinner (1.9 μm wide), terminal portion ending into a swollen tip (Figure 7a,b). In the frontal edge of the head, dome-shaped secondary clavae are present. All cephalic cirri with conspicuous swollen tips (Figure 7a,b). Indentations in the frontal edge of the head not visible.

Ventral mouth opening in a slightly protruded circular structure. Buccal tube 21.3 μm long and 4.1 μm wide. Stylets, with strong stylet supports, ending in a swollen robust sharp point (Figures 6 and 7a,b). Ovoid pharyngeal bulb, ~21.5 μm in diameter, with placoids (~9.2 μm long).

Except for body projections 1 (Figure 7b) and body projections 4 (the ventro-lateral body projections between legs III–IV, Figure 7a-d), no other lateral body projections are present. Body projections 4 (~13.0 μm long and 9.6 μm wide at base), although thin and transparent, well-developed, conical, with sharp tips.

The caudal apparatus (Figures 6 and 7a,e) consists of a thin, transparent structure bifurcated into two arced points that merge before the posterior edge of the body, 10.6 μm wide at base and protruding ~12.0 μm, covered by detritus.

Sensory spines present on all legs, with the same length on legs I–III and longer on legs IV. First and fourth leg sensory organs with swollen tips. The sensory organ on leg IV divided into a cirrophore (8.5 μm long), a basal portion (9.6 μm long) and a distal swollen tip (0.6 μm). The basal portion and the distal tip with, respectively, a van der Land's organ and an optically dense dot at base (Figures 6 and 7a-c).

Blunt tipped cirri *E* with small cirrophores present (not visible in the holotype, but observed in some paratypes) (Figure 6).

Telescopic legs with toes having small circular/ovoid suction discs (discs of toes 2 and 5 slightly

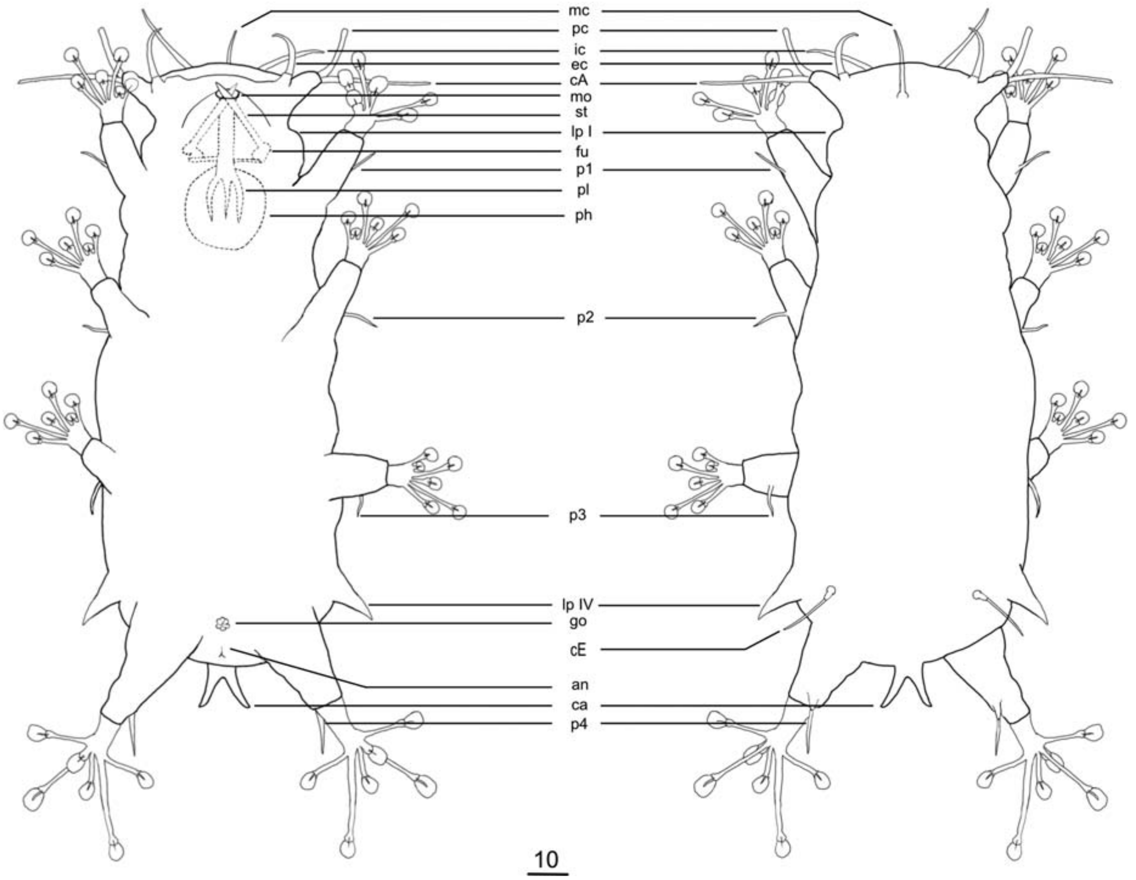

Figure 6. Ventral and dorsal aspects of *Batillipes ichthyocercus* sp. nov. an = anus, ca = caudal appendage, cA = cirri *A*, cE = cirrus *E*, ec = external cirri, fu = furca, go = gonopore, ic = internal cirri, lp I and lp IV = lateral processes, mc = median cirrus, mo = mouth, pc = primary clavae, ph = pharynx, pl = placoids, p1–p4 = sensory organs on leg I–IV, st = stylet. Scale bar in µm.

smaller than discs of the other toes; diameter 4.2 µm measured on toe I of leg IV) with thin braces. On the first three pairs of legs, toe 2 is the shortest, toes 3 and 5 are the longest and toes 1, 4 and 6 are medium-sized. On feet of the fourth pair of legs, the toes are arranged according to the B1 pattern (Santos et al. 2018a): the shortest middle toes 3 and 4 are of different lengths with toe 3 shorter than toe 4; toes 2 and 5 are the longest, and toes 1 and 6 of intermediate size (Figure 7a,c).

Dorsal cuticle (Figure 7d) uniformly punctated, ~10 pillars/10 µm (each pillar ~1.2 µm high); transverse folds are imperceptible. Ventral cuticle (Figure 7e) finely punctated. Body with a considerable amount of adherent detritus, mainly near the caudal projection and the lateral body projection 4 (Figure 7a,e). Circular gonopore with a cuticular crescent-shaped fold (Figure 7e).

Remarks: Females similar to males in both qualitative and all measureable quantitative characters, except for their small (2.5 µm long, 2.3 µm wide in the allotype) rosette-shaped gonopore, located further away from the anus. Four-toed larvae not found. In some paratypes the precise shape and size of lateral body projections and caudal apparatuses difficult to define due to the amount of adherent detritus.

Differential diagnosis: No other congener shares the fish-shaped caudal apparatus. Excluding *Bat. ichthyocercus* sp. nov., only eight described species of the genus *Batillipes* have the group B1 toe pattern (Santos et al. 2018a): *Bat. africanus*, *Bat. brasiliensis*, *Bat. dandarae*, *Bat. friaufi*, *Bat. homocercus* sp. nov. (described above), *Bat. lesteri*, *Bat. littoralis* Renaud-Debyser, 1959b, and *Bat. similis*. *Batillipes ichthyocercus* sp. nov. can be distinguished clearly from these because:

- *Bat. africanus* lacks lateral body projections and has a single pointed caudal apparatus.
- *Bat. brasiliensis* has well-developed body projections between legs I–II and II–III, body projections

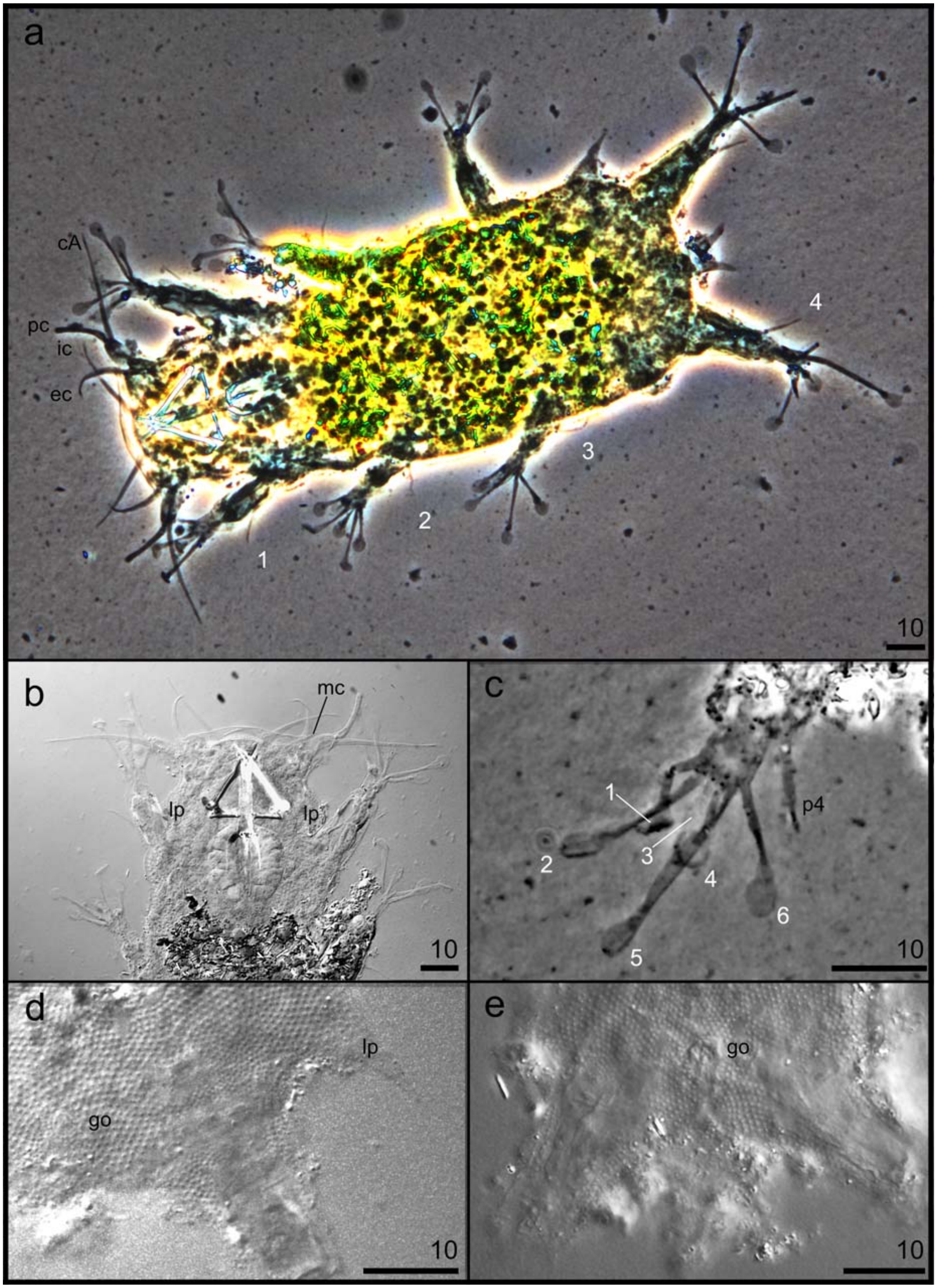

Figure 7. *Batillipes ichthyocercus* sp. nov. **a**. Habitus, male holotype, dorsoventral view. Numbers 1–4 are sensory organs on legs I, II, III and IV (p3 is curved back toward body edge). Also evident are the lateral processes between legs III and IV and the caudal appendage. PhC, focus merged. **b**. Head detail of holotype showing buccal apparatus, lateral processes of neck and tip of median cirrus. Sensory organs of legs I and II also visible. PhC, focus merged. **c**. Claw IV of female paratype (CR-30-4). Numbers are toe numbers from anterior to posterior. Toe 3 barely visible. PhC. **d**. Posterior ventral section of holotype. DIC. **e**. Posterior ventral section of female paratype (CR-30-4). DIC. Caudal apparatus partially visible. an = anus, ca = caudal appendage, cA = cirrus *A*, cE = cirrus *E*, ec = external cirrus, go = gonopore, ic = internal cirrus, lp = lateral projection, mc = median cirrus, p4 = leg IV sensory organ, pc = primary clava, sc = secondary clava. Scale bars in µm.

between legs III–IV terminate in five digit-shaped expansions and has a single ala-like caudal apparatus.

- *Bat. dandarae* lacks lateral body projections and lacks caudal apparatus with two sharp spikes.
- *Bat. friaufi* lacks lateral body projections and the caudal apparatus is not bifurcated.
- *Bat. homocercus* sp. nov., although most similar to the new species (see discussion section on geminate species), lacks lateral body projection 1, has a blunt-tipped lateral body projection 4 versus sharp-tipped in *Bat. ichthyocercus* sp. nov., has well-developed secondary clavae appearing as ventral cuticular platelets and the caudal apparatus has two sharp spikes that appear thickened and rigid as opposed to the thin, transparent caudal apparatus of *Bat. ichthyocercus* sp. nov.
- *Bat. lesteri* has external cephalic cirri that are sharp rather than blunt-tipped, the body projection between legs III–IV terminates in a two-pointed expansion, has well-developed body projections between legs I–II and II–III and has a single ala-like caudal apparatus (often covered with detritus and may sometimes appear bifurcated).
- *Bat. littoralis* has well-developed body projections between legs I–II and II–III, has a tooth on legs IV and has a three-pointed caudal apparatus.
- *Bat.similis* has well-developed body projections between legs I–II and II–III and has a single conical caudal apparatus.

Habitat characteristics and distribution: This species was found at Manzanillo and Puerto Viejo on the Caribbean coast. Samples were from intertidal sand and very near shore shallow subtidal sand.

Family: Halechiniscidae Thulin, 1928 (amended by Fujimoto et al. 2016)

Subfamily: Florarctinae Renaud-Mornant, 1982 (amended by Kristensen 1984)

Genus: *Wingstrandarctus* Kristensen, 1984

***Wingstrandarctus* sp.**

Site 2 (Tables I and II, Figure 1)

Material examined: One adult female. Specimen deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Morphological comments: The specimen exhibits six alae (frontal, two lateral on each side – antero-lateral and postero-lateral, and caudal ala). Alae without caesti. Caudal ala very large with four lobes. Cuticle appears smooth in LM but faint granulation is observable at body contours, crescent-shaped claws, external claws larger with calcar and avicularia. Specimen was in poor condition and it was not possible to assign it to a known species or describe it as a new one.

Habitat characteristics and distribution: This specimen was found in intertidal sand at Manzanillo on the Caribbean coast.

Subfamily: Halechiniscinae Thulin, 1928 (amended by Grimaldi de Zio et al. 1990)

Genus: *Halechiniscus* Richters, 1908 (amended by Grimaldi de Zio et al. 1990)

Halechiniscus remanei remanei Schulz, 1955

Sites 3, 19, 20, 21 and 33 (Tables I and II, Figure 1)

Material examined: One hundred and thirty-seven specimens were found including juveniles and adults. Specimens deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal, the Department of Biology at Warren Wilson College, Asheville, North Carolina, USA, and the Museo de Zoología, Universidad de Costa Rica.

Morphological comments: Specimens match the original description of *Hal. remanei remanei* by Schulz (1955). We were also able to observe circumoral secondary clavae, which have not been previously reported, but the exact contours are difficult to discern in our specimens.

Habitat characteristics and distribution: This species was the dominant species in intertidal beach sand on the Pacific coast and occurred in lower abundance on the Caribbean coast. There are questions concerning subspecies designations, but it appears to be broadly distributed worldwide in both intertidal and subtidal habitats (Bartels et al. 2015a; Kaczmarek et al. 2015).

Subfamily: Orzeliscinae Schulz, 1963 (amended by Gross et al. 2014)

Genus: *Orzeliscus* du Bois-Raymond Marcus, 1952

***Orzeliscus* sp.**

Site 4 (Tables I and II, Figure 1)

Material examined: Thirteen specimens (four females, one male, five specimens of undetermined sex, and three first instar larvae). These specimens are deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal. Additional specimens are at the Department of Biology at Warren Wilson College, Asheville, North Carolina, USA, and the Museo de Zoología, Universidad de Costa Rica.

Morphological comments: The distinction between the three species in this genus is dubious, making the assignment of species difficult (Kaczmarek et al.

2015). Specimens from Costa Rica have sensory papillae on hind legs, therefore they can be differentiated from *Orzeliscus septentrionalis* Schulz, 1953, considered as species *inquirenda* by Guidetti and Bertolani (2005) but recognized as *bona* species by Lee et al. (2017). They also have tubular primary clavae (similar to *Orzeliscus asiaticus* Lee, Rho and Chang, 2017); weak projections in the neck region (similar to *Orzeliscus belopus* du Bois-Reymond Marcus, 1952); body projections between legs III and IV present (similar to *Orz. asiaticus* and *Orz. belopus*). However, Costa Rican specimens differ from *Orz. asiaticus* and *Orz. belopus*: (1) in the shape of sensory organs on legs IV (elongate and tubular in both *Orz. belopus* and *Orz. asiaticus*; much shorter and more roundish in specimens from Costa Rica; (2) in the density of cuticular pillars (about 9–10 per 10 µm in specimens from Costa Rica; about 6–7 in *Orz. asiaticus* and *Orz. belopus*). The Costa Rican specimens seem to be smaller (the largest female is 158 µm, about 200 µm for body length in *Orz. asiaticus* and *Orz. belopus*), but the sample size is too small and additional measurements are needed to confirm this character. Therefore, we are not able to assign specimens from Costa Rica to any described species, nor can we describe it as new species.

Habitat characteristics and distribution: *Orzeliscus* sp. was only found at Manzanillo on the Caribbean coast. Two individuals were found in intertidal beach sand and the others in subtidal sand. *Orzeliscus asiaticus* is known only from Korea and Japan (Lee et al. 2017), while *Orz. belopus* is broadly distributed worldwide in both intertidal and subtidal habitats, but due to identification problems its distribution should be considered tentative (Kaczmarek et al. 2015; Bartels et al. 2015a).

Family: Stygarctidae Schulz, 1951 (amended by Hansen et al. 2012)

Subfamily: Stygarctinae Schulz, 1951

Genus: *Mesostygarctus* Renaud-Mornant, 1979 (amended by Hansen et al. 2012)

Mesostygarctus intermedius Renaud-Mornant, 1979

Site 34 (Tables I and II, Figure 1)

Material examined: Seven specimens (five females, one male and a second instar larva). Specimens deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal and one specimen at the Museo de Zoología, Universidad de Costa Rica.

Morphological comments: All specimens exactly match the original description (Renaud-Mornant 1979), including additional information provided by Hansen et al. (2012).

Genus: *Parastygarctus* Renaud-Debyser, 1965 (amended by Hansen et al. 2012)

Parastygarctus sterreri Renaud-Mornant, 1970

Site 2 (Tables I and II, Figure 1)

Material examined: One adult male. Specimen deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Morphological comments: The entire body was green, and the specimen perfectly matches the description of the species by Renaud-Mornant (1970).

Habitat characteristics and distribution: The specimen was found in intertidal beach sand collected at Manzanillo on the Caribbean coast. This species has a very wide distribution worldwide from both intertidal and subtidal habitats (Bartels et al. 2015a; Kaczmarek et al. 2015).

Genus: *Stygarctus* Schulz 1951

Stygarctus gourbaultae Renaud-Mornant, 1981

Sites 3, 19, 20 and 21 (Tables I and II, Figure 1)

Material examined: One hundred and sixty-five specimens were found and examined, including adult males and females and two-clawed larvae. Specimens deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal; the Department of Biology at Warren Wilson College, Asheville, North Carolina, USA; and the Museo de Zoología, Universidad de Costa Rica.

Morphological comments: Our specimens match all characters of *Stg. gourbaultae*, according to the original description (Renaud-Mornant 1981b). However, we have observed variability in the number of microspines on body plate 2. In some specimens this number was four, as in the original description, but five in others.

Habitat characteristics and distribution: This species was found in intertidal beach sand on both Caribbean and Pacific coasts. It was the dominant intertidal tardigrade in the Caribbean and occurred in lower numbers in the Pacific. Previously, *Stg. gourbaultae* had only been recorded from Florida and the Caribbean Sea (Kaczmarek et al. 2015; Bartels et al. 2015a).

Family: Styraconyxidae Kristensen and Renaud-Mornant, 1983 (amended by Fujimoto et al. 2016)

Genus: *Raiarctus* Renaud-Mornant, 1981

Raiarctus colurus Renaud-Mornant, 1981

Sites 3 and 20 (Tables I and II, Figure 1)

Material examined: Twenty specimens (six females, one first instar larva and 13 specimens of undetermined sex) deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal and one specimen at the Museo de Zoología, Universidad de Costa Rica.

Morphological comments: Five species are currently assigned to the genus *Raiarctus*, i.e. *Raiarctus aureolatus* Renaud-Mornant, 1981; *Raiarctus colurus* Renaud-Mornant, 1981; *Raiarctus variabilis* D'Addabbo Gallo, Grimaldi de Zio and Morone De Lucia, 1986; *Raiarctus jesperi* Jørgensen, Boesgaard, Mobjerg and Kristensen, 2014; and *Raiarctus katrinae* Jørgensen, Boesgaard, Møbjerg and Kristensen, 2014. High variability in some relevant taxonomic characters was observed, making the identification of *Raiarctus* specimens very difficult. The two species described by Renaud-Mornant (1981b) had been considered variable forms of *Rai. variabilis*, then later their taxonomic status as valid species was confirmed (D'Addabbo Gallo et al. 1986). As many characters were not described in the original descriptions, these species are in need of a re-description based on modern taxonomic standards. More recently, Jørgensen et al. (2014) described *Rai. jesperi* and *Rai. katrinae*. These species exhibit unique characters, allowing a clear distinction from all other described species. In having cone-shaped primary clavae with an inverted distal part, a simple weakly-developed frontal ala and a caudal lobe clearly perceptible in the postero-lateral ala, specimens from Costa Rica can be clearly distinguished from *Rai. aureolatus*, *Rai. katrinae* and *Rai. variabilis*. Costa Rican specimens have a much lower number of pillars supporting the alae than *Rai. jesperi* (less than 150 in specimens from Costa Rica vs more than 210 in *Rai. jesperi*) and lack the distal swelling on leg sensory organs typical of *Rai. jesperi*. We noted a few differences between our specimens and *Rai. colurus* (sensory organs on legs IV less elongated, and cuticular droplet in the buccal tube not observed in Costa Rica specimens), but these can be due to the compression of animals and fading caused by the slide mounting process, or they may have been neglected in the original description (division of cephalic cirri in Costa Rican specimens not described in *Rai. colurus*). Otherwise, specimens from Costa Rica are similar to *Rai. colurus* for all the other characters, thus we assigned these specimens to *Rai. colurus* which has previously been found in the Caribbean Sea (Renaud-Mornant and Gourbault 1984).

Habitat characteristics and distribution: This species was found on both the Pacific and Caribbean coasts in intertidal beach sand. This is a rare species (Bartels et al. 2015a; Kaczmarek et al. 2015) previously found in both intertidal and subtidal sands in the Atlantic Ocean, Mediterranean Sea and Caribbean Sea. This is the first report of this species in the Pacific.

Genus: *Styraconyx* Thulin, 1942 (amended by Kristensen and Higgins 1984b)

Styraconyx craticulus **(**Pollock, 1983**)**

Site 8 (Tables I and II, Figure 1)

Material examined and type repository: Five specimens (two adult females, one two-toed larva and two adults of undetermined sex). Specimens deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal and the Museo de Zoología, Universidad de Costa Rica.

Morphological comments: The adult specimens have four peduncles on each foot and well-developed sense organs with elongated primary clavae. The dorsal cuticle has a grid-like pattern as in *Sty. craticulus* and *Sty. craticuliformis*. The primary clava, cirrus *A*, cephalic cirri, and cirrus *E* all match the description of *Sty. craticulus* by Pollock (1983) and additional remarks by Kristensen and Higgins (1984b).

Habitat characteristics and distribution: All specimens were collected from intertidal beach sand at Playa Negra in Puerto Viejo on the Caribbean coast. This species has been described from numerous substrates in both intertidal and subtidal habitats in the Bahamas, the Dominican Republic, Guadeloupe Islands, Fuerteventura (Canary Islands), Haiti, the U.S. Virgin Islands, the Mediterranean Sea, Coral Sea, Tasmanian Sea and the Red Sea (Bartels et al. 2015a; Kaczmarek et al. 2015, 2018a).

Styraconyx vargasi **sp. nov. Bartels and Fontoura**

Site 34 (Tables I and II, Figure 1)

Figures 8–9, Table VI

Material examined and type repository: Holotype, adult female (slide CRII-61-10), allotype, adult male (slide CRII-61-8), and 14 paratypes: seven females (slides CRII-61-1, CRII-61-4, CRII-61-6, CRII-62-10, CRII-62-68, CRIII-62-97, CRIII-62-99); two males (slides CRII-61-12, CRIII-62-107); two first instar larvae (slides CRII-61-5, CRII-62-51), and three specimens of undetermined sex (slides CRIII-62-98, CRIII-62-103, CRIII-62-104) are deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal. Additionally, two specimens (CR-61-11, CR-62-101) deposited at the Department of Biology, Warren Wilson College, Asheville, North Carolina, USA, and one specimen (CRII-61-4) deposited at the Museo de Zoología, Universidad de Costa Rica.

Type locality: Golfo de Santa Elena, Playa Toyosa, Guanacaste, Costa Rica (Pacific Ocean) (10°58′49.4′′N, 085°

Table VI. Measurements (in µm) of selected morphological structures of the holotype, allotype and paratypes of *Styraconyx vargasi* sp. nov.

Characters	Holotype female	Adult females mean ± sd (range); n	Allotype male	Adult males mean ± sd (range); n	Undetermined sex mean ± sd (range); n	1^{st} stage larvae range; n
Body length	134	137.6 ± 15.2(113–162);8	102	105.0 ± 12.4(91–121);3	112.7 ± 15.6(97–134);3	82–94;2
Body width	60.5	58.7 ± 6.2(49.1–65.8);6	40.7	38.8 ± 8.2(28.0–47.8);3	51.8 ± 6.5(44.5–60.3);3	26.3–35.5;2
External cirrus	11.2	11.4 ± 1.3(9.4–12.6);5	9.0	8.6 ± 2.1(5.8–10.9);3	9.1 ± 0.6(8.3–9.8);3	6.5;1
Internal cirrus	8.6	9.0 ± 1.3(7.6–11.1);7	7.8	7.5 ± 2.3(4.5–10.1);3	7.4 ± 0.5(7.0–8.2);3	4.3;1
Median cirrus	?	(6.4–8.1);2	?	4.1;1	?	?
Primary clava	9.5	8.7 ± 0.4(8.2–9.5);6	7.3	(7.3–7.9);2	8.2 ± 0.5(7.6–8.9);3	5.9;1
Secondary clava (diameter)	9.0	9.0 ± 0.0(7.8–9.8);4	7.2	(6.9–7.2);2	6.5;1	?
Cirrus *A*	20.0	18.7 ± 1.3(17.0–20.6);6	16.7	(16.7–19.5);2	(17.3–18.9);2	?
Primary clava/Cirrus *A* ratio	0.48	0.47 ± 0.03(0.42–0.50);6	0.44	(0.41–0.44);2	(0.47–0.47);2	?
Leg I papilla	6.4	6.7 ± 1.0(5.2–7.9);5	5.3	4.9 ± 0.9(3.7–5.8);3	5.1;1	4.2;1
Leg II spine	?	7.0 ± 1.8(5.2–8.8);3	6.4	(6.4–9.0);2	(8.6–9.0);2	?
Leg III spine	13.3	9.5 ± 1.8(7.7–13.3);6	9.9	(5.3–9.9);2	10.0;1	6.1-7.2;2
Leg IV papilla	9.5	8.3 ± 0.7(7.6–9.5);7	7.9	(7.9–7.9);2	(6.2–8.0);2	6.8;1
Cirrus *E*	19.3	20.5 ± 1.3(18.8–22.5);6	18.1	(17.7–18.1);2	(18.3–20.5);2	14.5;1
Internal digit leg I*	7.0	7.0 ± 0.3(6.6–7.4);3	7.0	7.4 ± 1.5(5.8–9.4);3	5.7;1	5.5;1
External digit leg I*	6.0	5.7 ± 0.3(5.5–6.0);4	6.1	5.9 ± 1.2(4.4–7.3);3	4.6;1	-
Internal digit leg IV*	11.0	11.1 ± 0.7(10.1–11.9);5	9.9	8.9 ± 1.3(7.1–9.9);3	(7.2–8.3);2	7.2;1
External digit leg VI*	7.6	7.9 ± 0.8(6.9–8.9);5	6.9	(6.1–6.4);2	(6.3–6.8);2	-
Gonopore (diameter)	5.6	5.0 ± 1.2(3.5–6.6);8	2.4	2.3 ± 0.1(2.2–2.4);3	-	-
Gonopore-anus distance	?	(8.0–8.7);2	3.0	2.3 ± 0.5(2.0–3.0);3	-	-

Note: sd = standard deviation; range is the minimum and maximum values; n = number of measurements; *digit lengths include claw lengths.

41′36.4″W) from intertidal rocks on *P. attenuata* sabellarid tubes.

Etymology: The species epithet honours Dr José A. Vargas for his dedication to the advancement of marine science in Costa Rica.

Specific diagnosis: *Styraconyx* with subterminal mouth cone and brownish/reddish eyespots. Bipartite internal, external and median cirri. Elongate primary clavae and bipartite lateral cirri *A* situated on a common pedestal and enveloped by a common membrane extending almost to claval tip. Lenticular secondary clavae. Undivided cirri *E* on short cirrophore. Peduncles present on all digits: external peduncles thin with two small lateral expansions in the proximal portion as typical for the genus, but the internal lateral projection reduced to a slight protuberance; internal peduncles rod-shaped. Three-pointed claws, with thin basal hook directed downwards and thin accessory spine. Sensory organs present on all legs, bipartite on legs I; spines on legs II–III and club-shaped papilla with terminal spine on leg IV. Cuticle punctated but otherwise smooth. Seminal receptacles and ducts unknown.

Description of the holotype (measurements and statistics in Table VI): Female (Figures 8 and 9a) with brownish/reddish eye spots clearly visible (even after slide mounting). The dorsal cuticle coarsely punctated, consisting of small pillars (~15 pillars/10 µm). The punctation also extends to the ventral cuticle and proximal parts of the legs. The dorsal cuticle smooth, without any ridges, folds or grid-like patterns. Subterminal protruded mouth opening, formed of dome-shaped cuticular annular fold without buccal papillae. Buccal apparatus consists of buccal tube (15.8 µm long and 1.9 µm wide) without apparent apophyses; paired thick stylets (19.0 µm long) with strong furcae; stylet supports with distal swelling and pharyngeal bulb (diameter 15.2 µm) with three placoids (8.7 µm long) (Figure 9b). Complete set of cephalic sense organs present (Figure 9a,b,d). Elongate primary clavae and lateral cirri *A*; both arise from common dorso-lateral pedestal and are enveloped by a very thin transparent membranous sheath extending almost to the clavae tip. Primary clavae/lateral cirri *A* ratio is 0.48. A van der Land's organ is present in the base of the primary clavae. Lateral cirri *A* divided into a scapus, followed by a tubular flagellum, terminated by a blunt tip. Secondary clavae lenticular, located between internal and external cirri. Dorso-frontal internal cephalic cirri comprised of stout scapus (6.0 µm long) followed by short tubular flagellum (~2.6 µm long). Ventrally located external cephalic cirri (11.2 µm long: scapus 8.3 µm and tubular flagellum 2.9 µm long). Dorsal median cirrus only partially visible in the holotype (in a paratype with a body 141 µm long, the median cirrus, not extending beyond front edge of the head, is 6.4 µm long, also constituted by a broad scapus 4.0 µm and tubular flagellum 2.4 µm) (Figure 9b-d). Internal, external and median cirri have blunt tips and lack visible cirrophores (Figure 9a,b,d). Sensory organs present on all legs (Figure 9a-d). Leg I (6.5 µm long) with differentiation

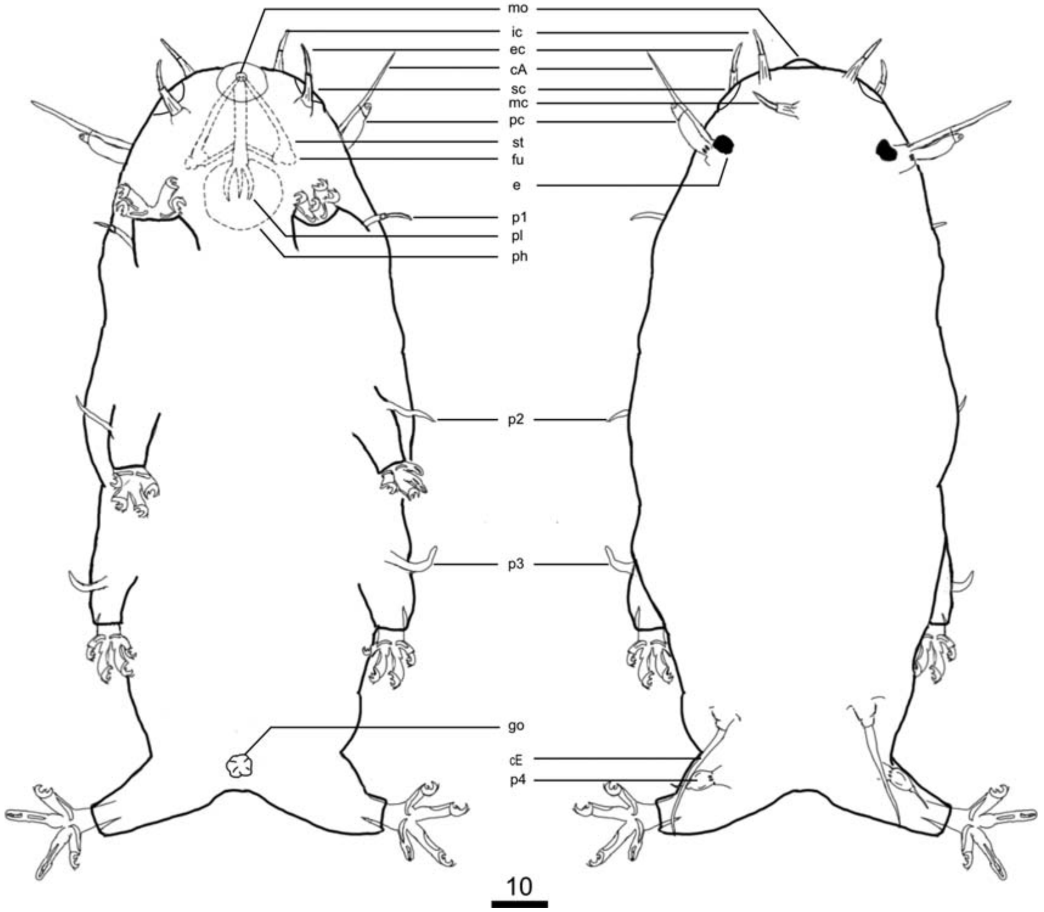

Figure 8. Ventral and dorsal aspects of *Sytraconyx vargasi* sp. nov. cA = cirri *A*, cE = cirrus *E*, e = eye, ec = external cirri, fu = furca, go = gonopore, ic = internal cirri, mc = median cirrus, mo = mouth, pc = primary clavae, ph = pharynx, pl = placoids, p1–p4 = sensory papilla on legs I–IV, sc = secondary clavae, st = stylet. Scale bar in µm.

of scapus (3.7 µm) and tubular flagellum (2.8 µm); on legs II and III unsegmented spines present. Leg IV sensory organ with smooth symmetrical papilla (4.6 µm) and terminal spine (4.9 µm) arising on short cirrophore with basal van der Land's organ. A diffractive structure present between papilla and terminal spine. Undivided cirrus *E* (Figure 9a,c,d) with smooth proximal portion (without accordion-like appearance) arises from a short cirrophore. Legs consist of coxa, femur, tibia and tarsus. Four digits on each leg. First three pairs of legs, digits of similar length. Leg IV digits longer (Figure 9a,e,f). Heart-shaped proximal pads present on internal digits (Figure 9e,f). Peduncles present on both internal and external digits. The external hook-shaped peduncles (5.6 µm long) attached to the base of the claws and with two small basal lateral processes in the proximal portion (1.6 µm distant from base), which have a very small hollowed area. The external peduncles slightly curved at base, very thin and straight, and with internal lateral process reduced to a slight protuberance (Figure 9g). The thin internal rod-shaped peduncles (4.1 µm long) extend from the proximal pads to the claw base. Claws attached to the peduncle at almost a right angle. Claw sheaths were not visible in the holotype and paratypes, and this character needs to be confirmed. Claws on internal and external digits of similar size (~2.9 µm and 3.3 µm long on legs I and IV, respectively). Each claw with three points, comprising thin dorsal spine-like accessory hook, clearly shorter than primary claw hook, and downward-directed secondary hook, slightly shorter than primary hook (Figure 9e,f).

The female gonopore consists of a six-cell rosette. Seminal receptacles and ducts are not observable in studied specimens. The anus (not very visible in the holotype) is a short undulating slit (anal platelets were not visible).

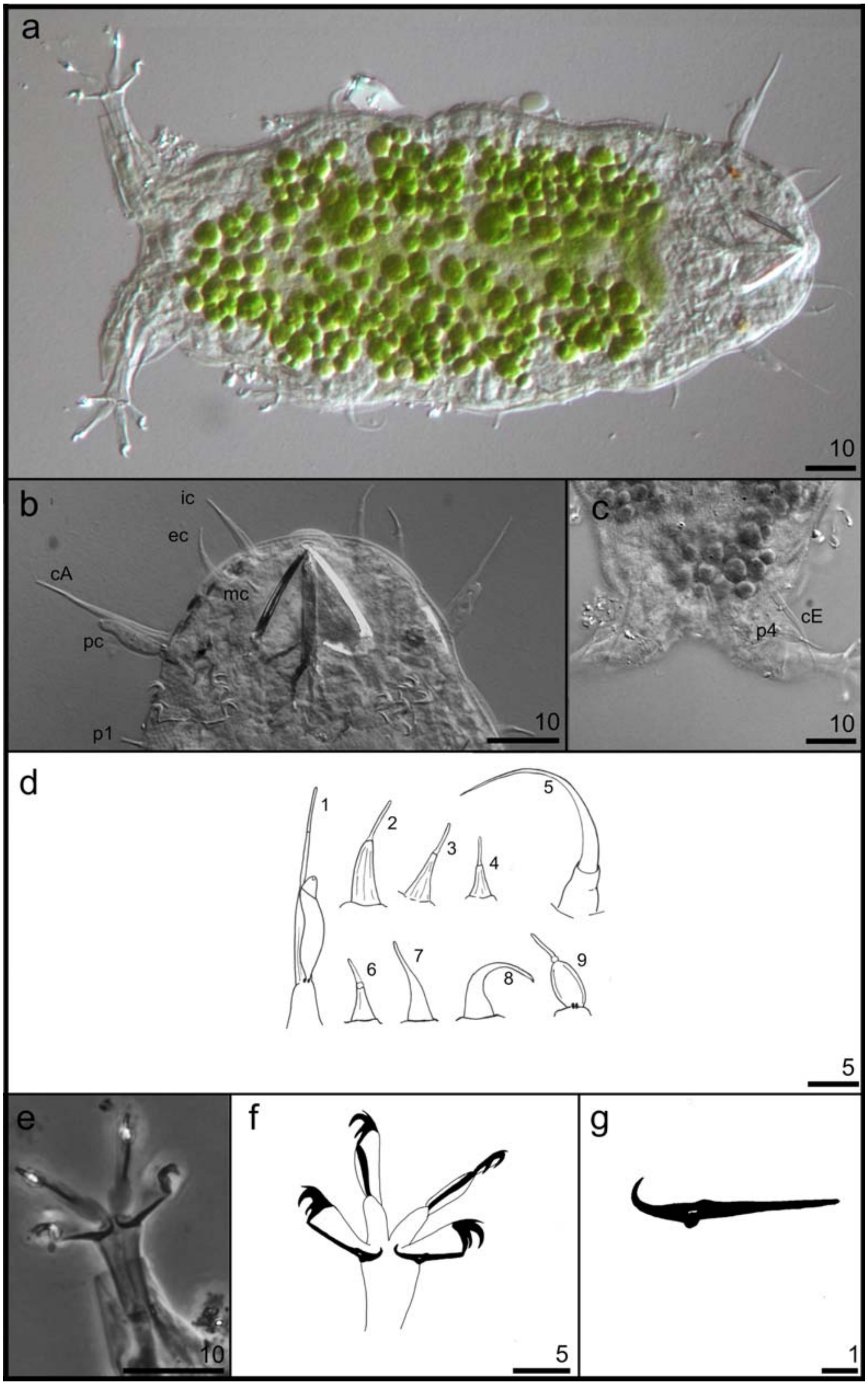

Figure 9. *Styraconyx vargasi* sp. nov. **a**. Female holotype, habitus, dorsoventral view. DIC, focus merged. **b**. Cephalic appendages and buccal apparatus of holotype. DIC, focus merged. **c**. Dorsal posterior surface of holotype. DIC. **d**. Drawing of sensory appendages. 1 = cirrus *A* and primary clava, 2 = external cirrus, 3 = internal cirrus, 4 = median cirrus, 5 = cirrus *E*, 6–9 = sensory organs of legs I, II, III, and IV, respectively. **e**. Foot IV of holotype, PhC, focus merged. **f**. Drawing of foot highlighting key structures. **g**. Drawing showing detail of outer peduncle. cA = cirrus *A*, cE = cirrus *E*, ec = external cirrus, ic = internal cirrus, mc = median cirrus, p1–4 = leg sensory organs, pc = primary clava. Scale bars in µm.

Remarks: The males appear to be smaller than females. The male gonopore is an oval opening, in comparison with females, located almost in contact with the anus (distance between gonopore and anus in the allotype is 1.5 µm). All other characteristics are very similar to females, including peduncles on digits. In the majority of the examined specimens, the division of lateral cirri *A* into scapus and flagellum is very difficult to perceive. First instar larvae, which lack the gonopore, anus and external digits, have all other characteristics as in the adults.

Differential diagnosis: Fourteen species were assigned to the genus *Styraconyx* until now (excluding *Styraconyx testudo* D'Addabbo Gallo, Grimaldi de Zio and Morone De Lucia, 1984, which, according to Jørgensen et al. (2014), should be transferred to the genus *Tholoarctus*). These species can be divided into two major groups according to the number of peduncles on each foot: nine species, constituting the *Sty. sargassi* group, have peduncles on all four digits (we include in this group *Styraconyx robertoi* Pérez-Pech, de Jésus-Navaratte, DeMilio, Anguas-Escalante and Hansen, 2020, which has females with peduncles on all digits and males with peduncles on external digits only), and another five species in the *Sty. hallasi* group with peduncles on external digits only (Kristensen and Higgins 1984b; D'Addabbo Gallo et al. 1989; Chang and Rho 1998; Bartels et al. 2015a; Pérez-Pech et al. 2020; Fujimoto et al. 2020). By having peduncles on all digits, *Sty. vargasi* sp. nov. belongs to the *sargassi* group. Only four other species of the *sargassi* group have well-developed sense organs and elongated primary clavae (club or sausage-shaped and not spine-like) and a smooth cuticle without a grid-like pattern as in the new species. They are *Styraconyx sargassi* Thulin, 1942; *Styraconyx sardiniae* D'Addabbo Gallo, Morone De Lucia and Grimaldi de Zio, 1989; *Styraconyx turbinarium* Bartels, Fontoura and Nelson, 2015; and *Styraconyx tyrrhenus* D'Addabbo Gallo, Morone De Lucia and Grimaldi, 1989. The new species differs from these four by having conspicuous brownish/reddish eyes (clearly visible after slide mounting) and different peduncles on external digits (peduncles with two well-developed lateral processes in the four species; thin and straight peduncles with the internal lateral process reduced to a slight protuberance in the new species). Additionally, the new species can be distinguished from these because:

- *Sty. sargassi* has primary clavae that, although enveloped by a membrane, do not share a common cirrophore with cirrus *A*, and has claws with accessory and secondary points similar in size to the primary point.
- *Sty. sardiniae* has primary clavae and cirri *A* located on a common pedestal but the entire clava is free without a surrounding membrane, with an annulated proximal portion of cirri *E.*
- *Sty. turbinarium* has claws with accessory and secondary points similar in size to the primary point.
- *Sty. tyrrhenus* has shorter primary clavae (primary clavae/cirri *A* ratio about *0.5* in the new species vs *0.3* in *Sty. tyrrhenus*) and undifferentiated cirrus *A*.

Habitat characteristics and distribution: This species was found on sabellarid tubes on intertidal rocks on the Pacific coast.

Family: Tanarctidae Renaud-Mornant, 1980 (elevated to the family level and amended by Fujimoto et al. 2016)

Genus: Tanarctus Renaud-Debyser, 1959a

***Tanarctus breedyae* sp. nov. Bartels and Fontoura**

Site 20 (Tables I and II, Figure 1)

Figures 10–12, Table VII

Material examined and type repository: Holotype, adult female (slide CRII-40-20), and one female paratype (slide CRIII-40-3) are deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Type locality: Golfo de Santa Elena, Playa Santa Elena, Guanacaste, Costa Rica (Pacific Ocean) (10°55′12.4′′N, 085°48′58.4′′W) from intertidal sand.

Etymology: The specific epithet honours Dr Odalisca Breedy, colleague and friend, for her important contributions to the taxonomy of marine organisms, especially octocorals.

Specific diagnosis: Small tanarctid with long smooth primary clavae and flat secondary clavae. Cephalic cirri with enlarged cirrophores and, except on the internal cirri, with a division between scapus and flagellum. Short median cirrus present. Sensory organs on legs I divided. Inarticulate sensory organs on legs II–III and long unbranched appendages on legs IV. Telescopic legs with coxa, femur, lance-like tibia and conical tarsus, as typical of the genus. Internal digits longer than external digits. External digits with cuticular folds at the base. All digits with teardrop-shaped cuticular thickenings at claw bases. Internal claws with minute dorsal spur, external claws simple. All claws with strong calcars. Six cell rosette-like female gonopore. Seminal receptacles are two round vesicles situated laterally with seminal ducts divided into a collection of tiny tubules that form a swollen area near the gonopore and whose tips come together to form a pore, opening just beside the gonopore.

Table VII. Measurements (in µm) of selected morphological structures of the holotype and paratype of *Tanarctus breedyae* sp. nov.

Characters	Holotype Female CRII-40-20	Paratype Female CRIII-40-3
Body length	83	92
Body width	42.1	44.0
Pharyngeal bulb (greatest diameter)	10.6	9.8
Median cirrus	6.1	8.2
Internal cirri	33.4	25.5
External cirri	21.1	18.7
Cirri *A*	11.7	?
Primary clava	109.6	87.4*
Primary Clava /body length ratio	1.32	-
Cirri *E*	30.0	32.8
Leg I spine	9.9	?
Leg II spine	17.1	?
Leg III spine	17.9	?
Leg IV sensory organ	104.3	?
Leg I:		
External digit	8.6	8.7
Internal digit	12.3	12.8
Leg IV:		
External digit	8.7	8.4
Internal digit	14.9	13.6
Gonopore	4.8	5.2
Gonopore–anus distance	7.9	?
N° amoebocytes	12	?

Note: Body width was measured at the level of legs III; digit lengths include claws; asterisk indicates broken structures, not totally measured.

Description of the holotype (measurements and statistics in Table VII): Adult female with a dorso-ventrally flattened body, 83 µm long and 42.1 µm wide (between legs III and IV) (Figures 10 and 11a). Eye spots absent. The dorsal cuticle, with adherent material, is smooth but with wavy folds (Figure 11c) over body segments I, II and III forming a cowl-like structure. The adherent material on the dorsal cuticle surface appears to be associated with these folds, and it is predominantly a diverse assortment of benthic diatoms including boat-shaped, circular, rectangular, and elongate pennate forms. The dorsal cuticle also has sparse epicuticular pillars, ventral cuticle smooth with some folds also. Head, not particularly distinct from the body, with an undefined number of amoebocytes, highly variable in size, around the mouth cone (the smallest ones can be observed randomly distributed at several focus levels) (Figure 11d). Cirri *A* and primary clavae (Figure 11a,b) are inserted on common short but wide cirrophores located on either side of the head. Cirrus *A*, dorso-posterior relative to primary clavae, divided into a swollen proximal portion (a scapus ~8.2 µm long) and, after a constriction, a short distal flagellum (3.5 µm). Primary clavae, without enlarged proximal portion, are very long and smooth and each has a van der Land's organ at base. Flat secondary clavae surrounding the mouth cone. The borders of the secondary clavae difficult to discern, but three internally directed swellings visible. Paired internal and external cephalic cirri (Figure 11a,b,d) with enlarged cirrophores. Each internal cirrus, with indistinct divisions between scapus and flagellum, inserted dorsally and their basal portions connected by a cuticular sheet. Each external cephalic cirrus with scapus (16.1 µm) and tubular flagellum (5 µm long) with enlarged blunt tip. The ventrally located external cirri positioned externally relative to the internal cirri. Short unpaired median cirrus divided into scapus and flagellum (3.3 µm and 2.8 µm, respectively) (Figure 11c). All cephalic cirri with an optically dense dot near the tips.

Mouth opening in a mouth cone protruding ventrally. Buccal apparatus consists of a narrow buccal tube (diameter about 0.7 µm), stylets (15.3 µm long) with bifurcated furcae and stylet supports, and round pharyngeal bulb with hook-shaped placoids about 4.5 µm long (Figure 11d).

Paired cirri *E*, located dorsally between legs III and IV, with accordion-like proximal portion (Figure 11e).

Telescopic legs with indistinct coxa and femur, lance-like tibia and conical tarsus with four digits, as typical of the genus. On all legs, a short sharp cuticular thickening (~1.4 µm) visible ventrally, in the terminal portion of the tibia (Figure 11b) that could be a site for muscle attachment or a cuticular spur. Sensory organs present on all legs, increasing in length from legs I to IV (Figure 11a-c,e). On leg I, sensory organs are divided into a proximal portion (5.5 µm) and a distal portion (4.4 µm) with an optically dense dot near the tip. On legs II and III the sensory organs are inarticulate tapering spines. Leg IV sensory organs are smooth, undivided appendages, arising from a cirrophore (5.3 µm) located dorsally at the base of the hind legs (Figures 10 and 11a,e). A refracting van der Land's organ is present at the base of each leg IV sensory organ. Internal digits, inserted in a common pretarsus, are longer than external digits, which have a cuticular fold at their bases. All digits terminate in crescent-shaped claws (Figure 11g–j) with strong primary points, separated from the basal portion by a slight depression, and with strong blunt calcars. Claws on internal digits with a minute dorsal spur located posteriorly at the base of the primary point. Claws of external digits simple. All digits with tear-drop-shaped cuticular thickenings at claw bases.

The female gonopore (Figures 10, 11f, 12) consists of a six-cell rosette. The seminal receptacles (Figures 10, 11f, 12) are two round vesicles situated laterally with seminal ducts divided into a collection of tiny tubules forming an enlarged swelling near the gonopore. The tips of the tubules come together to form

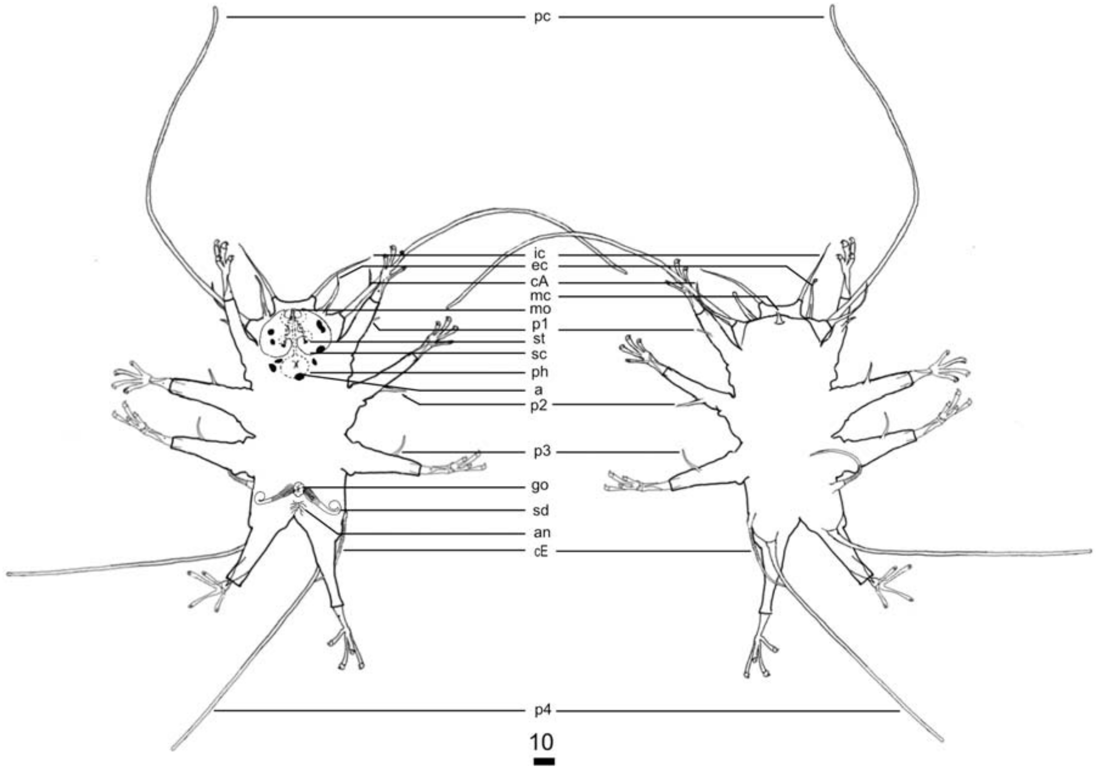

Figure 10. Ventral and dorsal aspects of *Tanarctus breedyae* sp. nov. a = amoebocyte, an = anus, cA = cirri *A*, cE = cirri *E*, ec = external cirrus, go = gonopore, ic = internal cirri, mc = median cirrus, mo = mouth, pc = primary clavae, ph = pharynx, p1–p4 = sensory organs on leg I–IV, sc = secondary clava, sd = seminal duct, st = stylet. Scale bar in µm.

a pore, opening just beside the gonopore. The anus, under a small transverse fold, is a wave-like slit formed by three cuticular folds (like an inverted Y) (Figures 11f, 12).

Remarks: The epicuticular diatoms on the holotype dorsum are reminiscent of the coccoliths reported on *Tanarctus bubulubus* Jørgensen and Kristensen, 2001. Jørgensen and Kristensen (2001) speculated that the coccoliths may serve as a form of mechanical camouflage or protection against abrasion from sediments in a high energy habitat. We wonder if the cuticular folding in the new species is an adaptation to facilitate retention of diatoms. It also would be intriguing to determine if there is any selectivity for diatoms, or if they are accumulated as a random component of the benthic sediment.

Differential diagnosis: Fourteen species are assigned to the genus *Tanarctus*, of these, six species share with *Tan. breedyae* sp. nov. the presence of simple, smooth sensory organs on legs IV: *Tanarctus diplocerus* Fujimoto, Miyazaki and Suzuki, 2013; *Tanarctus gracilis* Renaud-Mornant, 1980; *Tanarctus heterodactylus* Renaud-Mornant, 1980; *Tanarctus minotauricus* Renaud-Mornant, 1984; *Tanarctus ramazzotti* Renaud-Mornant, 1975; and *Tanarctus tauricus* Renaud-Debyser, 1959a. The new species can be distinguished from these by its unique claw morphology with strong primary points separated from the basal portion by a slight depression, by its cuticular teardrop-shaped thickenings at claw bases, by its unique seminal ducts and because:

- *Tan. diplocerus* has internal and external cephalic cirri perfectly aligned at the same distance from the midpoint of the head.
- *Tan. gracilis* has longer sensory organs on leg IV (sensory organ on legs IV/body length = 2.75 vs 1.26 in the new species) with a swollen proximal portion.
- *Tan. heterodactylus* has cuticular thickenings at claw bases, but they are longitudinal and not teardrop-shaped, and has external digits reduced without claws.
- *Tan. minotauricus* has well-developed club-shaped secondary clavae.
- *Tan. ramazzotti* has well-developed club-shaped secondary clavae.
- *Tan. tauricus* has well-developed club-shaped secondary clavae, lacks a median cirrus and has less well-developed primary points on claws.

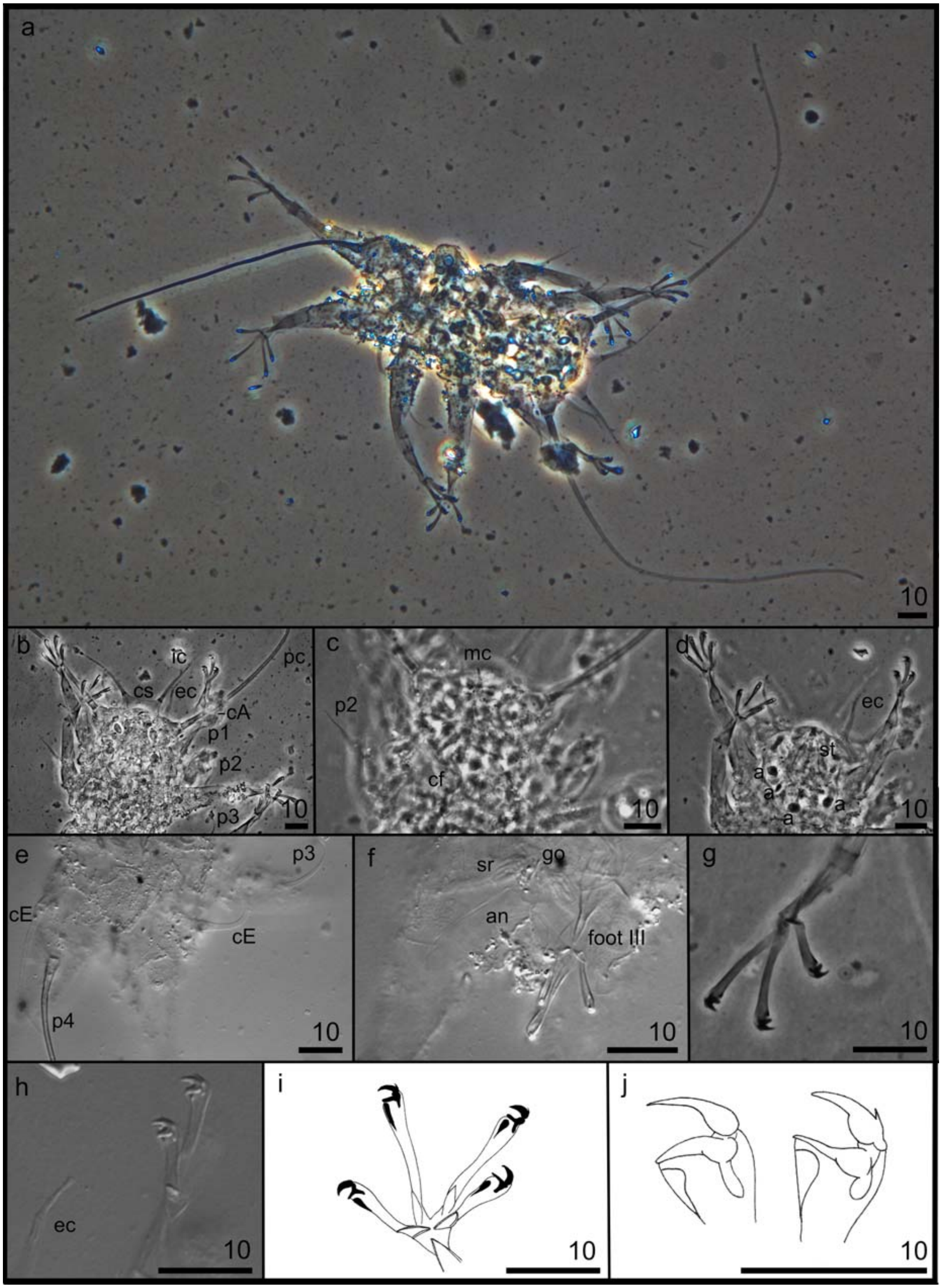

Figure 11. *Tanarctus breedyae* sp. nov. All images from female holotype. **a**. Habitus, dorsoventral view. Right sensory organ for leg IV is missing. PhC, focus merged. **b**. Sensory appendages. PhC, focus merged. **c**. Dorsal anterior aspect. PhC. **d**. Ventral anterior aspect. PhC, focus merged. **e**. Dorsal posterior aspect. DIC, focus merged. **f**. Ventral posterior aspect. Note that foot III is folded under body. DIC, focus merged. **g**. Foot IV with digits in lateral orientation. PhC, focus merged. **h**. Foot I. DIC, focus merged. **i**. Drawing showing detailed foot anatomy. **j**. Drawing detailing claw structure. Claw on left is from outer digit, claw on right is from inner digit. a = amoebocyte, an = anus, cA = cirrus *A*, cE = cirrus *E*, cf = cuticular fold, cs = cuticular sheet, ec = external cirrus, go = gonopore, mc = median cirrus, p1–p4 = leg sensory organs, st = stylet, sr = seminal receptacle. Scale bars in µm.

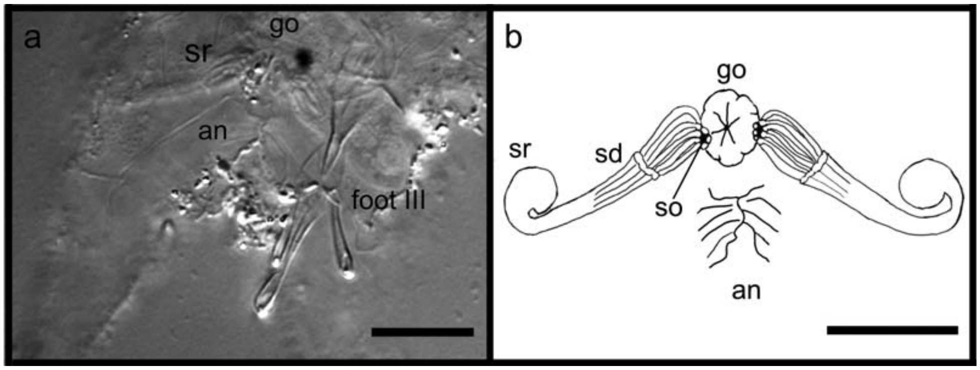

Figure 12. *Tanarctus breedyae* sp. nov. female holotype reproductive structures. **a.** Posterior ventrum. Note that foot III is folded under body, legs IV project toward bottom of image. DIC, focus merged. **b.** Drawing of reproductive structures. an = anus, go = gonopore, sd = seminal duct, so = seminal receptacle opening, sr = seminal receptacle. Scale bars = 10 µm.

Habitat characteristics and distribution: This species was rare, found only in one sample of intertidal beach sand on the Pacific coast.

***Tanarctus* sp.**

Site 3 (Tables I and II, Figure 1)

Material examined: One first instar larva (slide CRII-6-27). Specimen deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Morphological comments: The two-digit larva (61 µm long, 26.1 µm wide) has a complete set of cephalic cirri (paired external and internal cephalic cirri, 12.0 and 19.2 µm, respectively, unpaired median cirrus, 4.7 µm). Cirri *A* (10.8 µm) and long smooth primary clavae (66.8 µm) inserted on common pedestals. Indistinct secondary clavae. Spine-like sensory organs on legs I–III (6.7, 8.1 and 10.5 µm, respectively) and long, possibly unbranched appendage on leg IV. One sensory organ on leg IV is missing and the other is broken before termination (the remaining portion measures 53.8 µm long). Cirri *E* 20.1 µm long. The two digits (about 7.3 µm) have a minute cuticular thickening at claw bases. Crescent-shaped claws as typical of the genus. Despite being collected on the opposite coast, the specimen exhibits some characters similar to *Tan. breedyae* sp. nov., such as the smooth sensory organs on legs IV and the presence of cuticular thickenings on digits at claw bases. Unfortunately, the morphology of cephalic cirri and sensory organs on legs I–III cannot be clearly interpreted, and the fact that the distal portion of the sensory organ on leg IV is unknown prevents identification to species level.

Habitat characteristics and distribution: The single specimen was collected in intertidal beach sand at Manzanillo on the Caribbean coast.

Order: Echiniscoidea Richters 1926

Family: Echiniscoididae Kristensen and Hallas, 1980

Subfamily: Echiniscoidinae Kristensen and Hallas, 1980

Genus: *Echiniscoides* Plate, 1888

***Echiniscoides costaricensis* sp. nov. Bartels and Fontoura**

Sites 8 (Tables I and II, Figure 1)

Figures 13–14, Table VIII

Material examined and type repository: Holotype, adult female (slide CRII-15-16). Allotype, adult male (slide CRII-15-14) and six other paratypes: three females (slides CRI-15-5, CRI-15-7, CRII-15-11); two males (slides CRI-15-2, CRI-15-4); and one first instar larvae (slide CRII-15-17). Specimen CRII-15-11 is deposited at the Museo de Zoología, Universidad de Costa Rica, and the remainder of the type material is deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Type locality: Puerto Viejo, Playa Negra (Caribbean Sea), Costa Rica (9°39′19.0″N, 082°45′56.0″W) in intertidal sand.

Etymology: The specific epithet, *costaricensis*, refers to the type locality, Costa Rica.

Specific diagnosis: Small-sized *Echiniscoides* species with dorsal cuticle strongly sculptured with irregular polygonal and circular tubercles, and dark-brown eyespots. Ventral cuticle smooth. Five to seven anisonych claws on each leg, 7,7,7,6 being the most common adult claw configuration. Buccal tube is moderately long (<44 µm) within the genus. Short (<12 µm) lateral cirri *A* and *E*. Papillary primary clavae with a pore at the top of a very short terminal bead and lens-shaped secondary clavae. Internal and external cirri with swollen bases and short tufted distal tips

Table VIII. Measurements (in μm) of selected morphological structures of the holotype, allotype and paratypes of *Echiniscoides costaricensis* sp. nov.

Characters	Holotype Female CRII-15.16	Allotype Male CRII-15.14	Paratype Female CRI-15.5	Paratype Female CRI-15.7	Paratype Female CRII-15.11	Paratype Male CRI-15.2	Paratype Male CRI-15.4	Paratype 1st instar juvenile CRII-15.17
Body length	173	121	132	150	203	172	129	102
Body width	69.9	54.0	53.4	64.0	97.8	85.5	?	47.7
Buccal tube length	42.4	30.3	30.4	37.8	44.3	?	?	?
Placoid length	12.3	10.6	?	7.5	10.1	?	?	?
Internal cirri	?	5.3	?	?	5.5	4.8	4.0	?
External cirri	?	4.2	?	4.4	2.8	?	?	?
Cirri *A*	8.3	8.4	7.2	9.1	11.5	11.1	8.1	?
Primary clava	3.8	3.6	3.9	4.1	3.9	3.5	?	?
Secondary clava*	8.8	5.5	?	?	8.4	6.7	6.6	?
Cirri *E*	10.4	8.4	6.7	10.6	12.0	8.4	?	?
Leg I sensory org.*	2.3	2.5	?	?	2.4	?	?	?
Leg II sensory org.*	2.5	?	?	?	2.7	?	?	?
Leg III spine	6.6	6.9	6.8	6.6	7.1	6.4	6.2	5.3
Leg IV papilla	?	2.9	?	?	4.2	2.6	?	?
Legs I–III:								
Internal claw	10.3	6.9	5.6	?	10.5	9.1	5.8	?
External claw	7.3	4.5	4.2	?	7.6	6.4	4.3	?
Leg IV:								
Internal claw	11.3	7.7	?	10.2	12.2	10.1	9.1	?
External claw	8.8	5.1	?	7.2	8.7	7.5	?	?
Gonopore*	6.6	3.5	5.5	5.5	5.7	4.2	3.7	?
Gonopore–anus distance	19.3	9.4	?	21.0	19.4	11.7	10.0	?
Claw formula: right	7-7-7-6	7-7-7-0	?-?-?-?	7-7-?-6	6-6-7-5		6-6-6-5	?-?-?-?
left	7-7-7-6	7-7-7-6	7-7-7-?	7-7-7-6	6-7-6-6		6-6-6-5	5-5-5-5

Note: Body width was measured between legs III–IV. * indicates diameter.

with an undistinguished number of projections. Sensory organs on all legs: dome-shaped papillae on legs I–II; spine-like with enlarged bases and thin distal portions on legs III; papillae with a short terminal bead on legs IV. Six cell rosette-like female gonopore. Male gonopore simple oval shape.

Description of the holotype (measurements and statistics in Table VIII): Female with cylindrical body (Figures 13 and 14a). Dorsal cuticle strongly sculptured with irregular polygonal and circular tubercles (~1.5–2.0 μm in diameter) (Figure 14b). Dorsal sculpture also covers head and legs. A few small flat discs arranged symmetrically, interpreted as muscle attachment points, are visible dorsally (Figure 14b). Ventral cuticle smooth. Dark-brownish eyespots. Internal and external cephalic cirri with conical bases and very short tufted distal tips with an undistinguished number of projections (not measured in the holotype due to their orientation on the slide (Figure 14e). Median cephalic cirrus absent. Lateral cirri *A* and *E* short, both with annulated bases and pointed flagella (Figure 13). Papillary primary clavae (Figure 14d) with a pore at the top of a very short terminal bead and a van der Land's organ at base. Lens-shaped secondary clavae (Figure 14e). Subterminal mouth without mouth plates (Figure 14e). Rigid buccal tube, relatively long (buccal tube length/body length ratio = *0.25*) and wide (2.1μm) (Figure 14c). Long stylets ending with large furcae at the anterior edge of the pharyngeal bulb with stylet sheaths (stylet sheaths ~25.5 μm long); stylet supports not visible. Pharyngeal bulb with placoids (~12.3 μm long) with calcium carbonate incrustation at their bases.

Legs with sensory organs (Figure 13): dome-shaped papillae on legs I and II; spine-like with enlarged bases and distal thin and blunt portions on legs III (6.6 μm long; basal portion and thin distal portion ~3.3 μm each); papillae (not measured in the holotype) with a very short terminal bead on legs IV (Figure 14f), similar to primary clavae.

Seven claws on legs I–III and six claws on legs IV (Figure 13). Claws, as typical of the genus, are anisonych (smaller/longer claw ratio is *0.66* on legs I–III and *0.78* on legs IV) (Figure 14a,e,g).

The female gonopore is a six-lobed rosette (Figure 13). As typical of the genus, the anus is trilobed, with two lateral lobes and a short posterior lobe, lacking additional lobes (wings) (Figure 14g).

Remarks: Males are similar to females in qualitative characters, except for their gonopore that is a simple oval and located nearer the anus. The low number of specimens collected does not allow a comparison of quantitative characters between sexes. In the majority of the examined specimens, dome-shaped papillae on legs I and II are difficult to observe. In adults, the number of claws on legs I–III is variable from 6 and 7, and from 5 to 6 on legs IV. The most common configuration is 7,7,7,6. In the only first instar juvenile collected the claw configuration is 5,5,5,5.

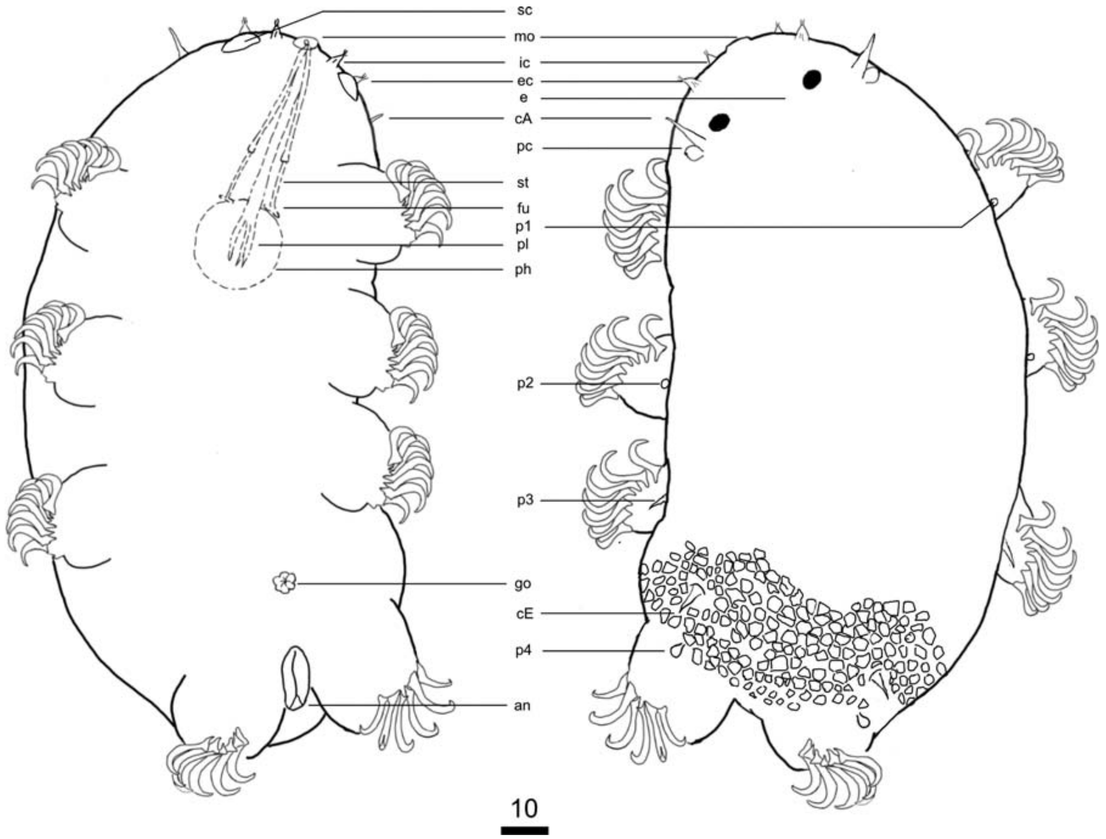

Figure 13. Ventral and dorsal aspects of *Echiniscoides costaricensis* sp. nov. an = anus, cA = cirri *A*, cE = cirrus *E*, e = eye, ec = external cirri, f = furca, go = gonopore, ic = internal cirri, mo = mouth, pc = primary clavae, ph = pharynx, pl = placoids, p1–p4 = sensory organs of leg I–IV, sc = secondary clava, st = stylet. Dorsal cuticular pattern shown only for terminal segment. Scale bar in µm.

Differential diagnosis: Six species have been described in the genus *Echiniscoides*. One of these species, *Ecn. sigismundi* (M. Schultze 1865), is comprised of eight subspecies that probably should be elevated to species level (Faurby et al. 2011, 2012; Faurby and Barber 2015; Møbjerg et al. 2019). Two species, *Echiniscoides bruni* D'Addabbo Gallo, Grimaldi de Zio, Morone De Lucia and Troccoli, 1992 and *Echiniscoides rugostellatus* Perry, Rawson, Ameral, Miller, JD Miller, 2018 and three subspecies of *Echiniscoides sigismundi* (*Echiniscoides s. hispaniensis* Kristensen and Hallas, 1980; *Echiniscoides s. galliensis* Kristensen and Hallas, 1980; *Echiniscoides s. polynesiensis* Renaud-Mornant 1976), have a strongly sculptured dorsal cuticle as in the new *Ecn. costaricensis* sp. nov. The new species can be distinguished from these five taxa because:

- *Ecn. bruni* has a 10,10,10,10 adult claw formula and has unique long spine-like sensory organs on leg I.
- *Ecn. rugostellatus* has a 9,9,9,8 adult claw formula, has a unique dorsal cuticular pattern with many large cuticular discs (muscle attachment points) compared with the new species in which the discs are fewer, smaller, and differently patterned (see Figure 14b here and Figure 1 in Perry et al. 2018), has a shorter buccal tube (mean values of the ratio between the length of the buccal tube and body length is *0.17* vs *0.24* in the new species) and has a longer tip of the external cephalic cirri (one third of the total cirri length vs one fourth of the total in the new species).
- *Ecn. s. galliensis* has a 10,9,10,9 adult claw formula, has cuticular tubercles each containing a small circle of discontinuous lines that are not present in the new species, has reduced highly curved claws and has cephalic cirri with conical rather than tufted tips.
- *Ecn. s. hispaniensis*, although most similar to the new species in size and often with the same claw formula, has tubercles peculiarly arranged like roofing tiles, in adults the number of claws can reach nine, has claws that are more isonych and less curved, has a shorter buccal tube (buccal tube length/body length ratio is ∼*0.20* in the holotype

of *Ecn. s. hispaniensis*,which is a female 157 µm long, vs *0.25* in a female of the new species that is 150 µm long).

- *Ecn. s. polynesiensis* has a 9,9,9,8 adult claw formula, has cuticular tubercles each with an elevation in the centre that is not present in the new species, has central claws bent at a right angle, has cephalic cirri with conical rather than tufted tips and has sensory organs on legs III shorter than sensory organs on legs IV (opposite in the new species).

Echiniscoides s. groenlandicus Kristensen and Hallas 1980 recorded from the North Atlantic in subarctic regions (Kristensen and Hallas 1980; Hallas and Kristensen 1982; Biserov 1999) and from the eastern Pacific on the west coast of North America (Miller et al. 2014), has a cuticular sculpture characterized by a fine granulation. However, some populations also can exhibit cuticular tubercles (Kristensen and Hallas 1980), justifying a comparison with the new species. Differences between these species include body size (specimens of *Ecn. costaricensis* sp. nov. are smaller than 210 µm while *Ecn. s. groenlandicus* is considered a large species with a body length between 270–390 µm in females); cuticular tubercles (smooth in the new species, frequently with a central point in *Ecn. s. groenlandicus*); adult claw formula (7,7,7,6 in the new species, 8–10 claws on legs I–II and 7–9 claws on legs IV in *Ecn. s. groenlandicus*) and shape of the tip of cephalic cirri (tufted in the new species and conical in *Ecn. s. groenlandicus*).

Habitat characteristics and distribution: All specimens were collected from intertidal beach sand at Playa Negra in Puerto Viejo on the Caribbean coast.

***Echiniscoides ritavargasae* sp. nov. Bartels, Fontoura, Mioduchowska and Kaczmarek**

Sites 23, 24, 25, 33 and 34 (Tables I and II, Figure 1)

Figures 15–17, Table IX

Material examined and type repository: Holotype, adult female (slide CRII-45-28). Allotype, adult male (slide CRII-63-8) and 28 other paratypes: 18 females (slides CRI-43-4, CRI-43-5, CRI-43-6, CRI-45-1, CRI-45-2, CRI-45-6, CRI-45-17, CRII-45-20, CRII-45-21, CRII-45-27, CRII-46-7, CRII-59-1, CRII-60-6, CRII-60-7, CRII-60-20, CRII-60-28, CRII-63-4, CRII-63-15); two males (slides CRI-45-12, CRI-45-16); three second instar larvae (slides CRI-43-7, CRI-45-5, CRII-60-17) and four first instar larvae (slides CRI-44-8, CRI-44-21, CRI-45-9, CRII-45-25) and one specimen of undetermined sex (slide CRI-45-19). The type material (holotype and paratypes) is deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal. Specimens analysed for DNA were labelled Ech1–3, Ech5, Ech10 and the exuviae are mounted on slides and deposited in the collection at the Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań. The SEM stub is also deposited in that collection. Additional specimens are at the Department of Biology, Warren Wilson College, Asheville, North Carolina, USA, and the Museo de Zoología, Universidad de Costa Rica.

Type locality: Golfo de Santa Elena at Playa Santa Elena, Guanacaste, Costa Rica (Pacific Ocean) (10°55′ 29.2′′N, 085°49′ 06.5′′W) from barnacles on an intertidal rocky beach. Other material collected from Punta Pochote and Playa Toyosa (Tables I and II).

Etymology: This species is named in honour of MSc. Rita Vargas-Castillo, for her work at the Zoology Museum, Universidad de Costa Rica, and contributions to crustacean biodiversity studies.

Specific diagnosis: Medium-sized *Echiniscoides* species with dark-brown eyespots and dorsal cuticle sculptured with small granules particularly evident on the posterior region of the body; anteriorly, the cuticle is wrinkled or even smooth. Ventral cuticle smooth. Variable number of anisonych claws on legs (in adults from 6–11 on legs I–III and from 7–10 on leg IV), with 9,9,9,9 as the most common claw configuration. Buccal tube short (< 44 µm long). Short cirri *A* and *E* (<12 µm long) with annulated bases. Papillary primary clavae with terminal hole and lens-shaped secondary clavae. Internal and external cirri with swollen bases and tufted distal tips. Sensory organs on all legs: papillae with pointed tip on legs I; spine-like with enlarged bases and thin distal portions on legs II–III; papillae with a terminal hole on leg IV. Six cell rosette-like female gonopore. Male gonopore a simple oval.

Description of the holotype (measurements and statistics in Table IX): Female with cylindrical body (Figures 15 and 16a). Dark-brownish eyespots present. Dorsal cuticle sculptured with small circular granules (major granules about 1.0–1.2 µm in diameter) (Figure 16a,b). Cuticular granulation more evident in the posterior portion of the body, and poorly visible under light microscopy. Anteriorly, the dorsal cuticle is wrinkled but almost smooth. A few dorsal patches present, arranged symmetrically and interpreted as muscle attachment points. Ventral cuticle smooth.

Internal and external cephalic cirri with conical bases and tufted distal tips, of similar length (Figure 16c). Tips of cephalic cirri ~50% of the total length. Median cephalic cirrus absent. Lateral cirri *A* and *E*

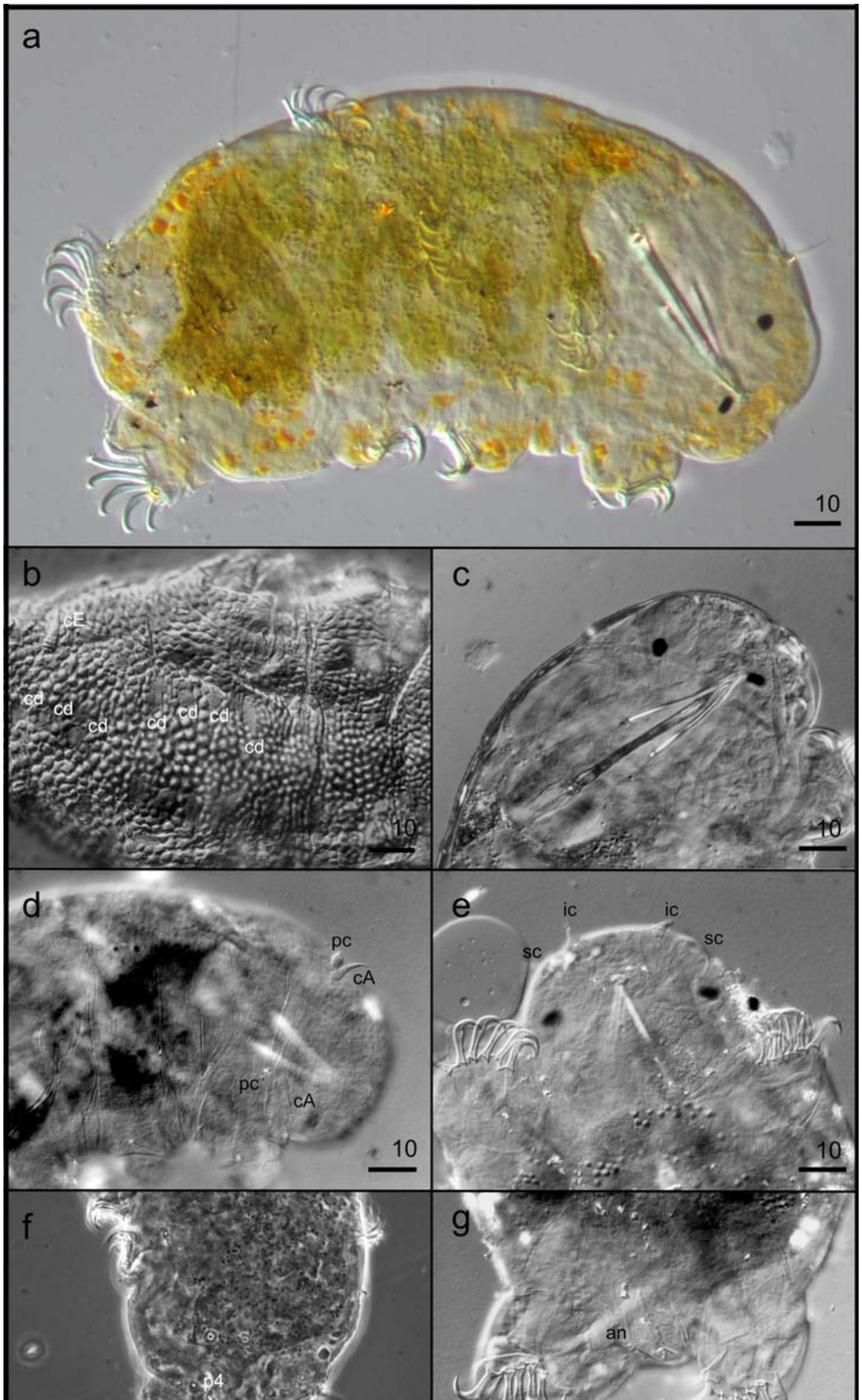

Figure 14. *Echiniscoides costaricensis* sp. nov. **a**. Habitus, holotype. DIC, focus merged. **b**. Posterior dorsal surface showing cuticular sculpture (specimen CRI-15-7). DIC. **c**. Buccal apparatus of holotype. DIC. **d**. Anterior section, dorsal aspect (specimen CRII-15-11). DIC. **e**. Anterior section, ventral aspect (specimen CRII-15-11). Note internal cirri with tufted tips. DIC. **f**. Posterior section showing legs III and IV (specimen CRII-15-11). PhC. **g**. Posterior section, ventral aspect (specimen CRII-15-11). DIC. an = anus, cA = cirrus *A*, cd = cuticular discs, cE = cirrus *E*, ic = internal cirrus, pc = primary clava, sc = secondary clava, p4 = leg IV sensory organ. Scale bars in µm.

Table IX. Measurements (in µm) of selected morphological structures of the holotype, allotype and paratypes of *Echiniscoides ritavargasae* sp. nov.

Characters	Holotype Female CRII-45.28	Allotype Male CRII-63.8	Females mean ± sd (range); n	Males mean ± sd (range); n	Juveniles 2nd instar mean ± sd (range); n	Juveniles 1st instar mean ± sd (range); n
Body length	246	156	200.3 ± 31.9 (148–253);18	149 ± 6.2 (141–156);3	128.3 ± 13.5 (110–142);3	107 ± 19.0 (87–134);4
Body width	113.2	66.8	89.9 ± 15.6 (63.9–119);18	65.4 ± 2.8 (61.5–67.8);3	63.3 ± 7.4 (52.9–69.1);3	71.0 ± 14.7 (35.3–48.4);4
Buccal tube length	41.8	35.1	38.0 ± 3.4 (33.3–43.8);17	32.0 ± 2.4 (29.3–35.1);3	28.4 ± 0.9 (28.0–29.7);3	23.0–27.6;2
Placoid length	9.9	9.7	9.6 ± 1.15 (8.0–12.5);15	8.8 ± 1.23 (7.1–9.7);3	7.8 ± 0.75 (7.3–8.9);3	4.6–6.6;2
Internal cirri	4.6	4.4	4.1 ± 0.77 (2.6–5.3);8	3.8 ± 0.49 (3.2–4.4);3	3.2–3.3;2	3.1;1
External cirri	4.9	3.8	4.3 ± 0.72 (2.5–5.2);14	3.5 ± 0.29 (3.1–3.8);3	3.1–3.2;2	3.3 ± 0.46 (2.7–3.8);3
Cirri *A*	10.1	8.1	8.6 ± 1.62 (6.2–11.4);11	6.6 ± 1.31 (4.9–8.1);3	5.9–6.9;2	4.2–4.6;2
Primary clava	4.7	3.8	4.2 ± 0.70 (3.0–5.2);13	3.7 ± 0.19 (3.4–3.8);3	2.8–3.3;2	2.8–3.2;2
Secondary clava*	13.6	9.7	9.8 ± 1.87 (7.7–13.6);15	8.6 ± 1.22 (6.9–9.7);3	7.3–9.4;2	6.2 ± 1.37 (4.7–8.0);3
Cirri *E*	10.3	7.8	8.8 ± 1.34 (7.0–11.7);16	7.8;1	7.1;2	5.1–9.2;2
Leg I sensory org.*	3.3	?	3.4 ± 0.52 (2.6–4.2;5	?	2.8;1	2.4;1
Leg II sensory org.	7.5	5.0	7.6 ± 0.05 (7.5–7.6);3	5.0;1	4.3–4.5;2	?
Leg III spine	7.5	6.2	6.8 ± 1.08 (5.2–7.9);12	4.5–6.2;2	5.1–5.6;2	4.1–4.6;2
Leg IV papilla	4.2	?	4.0 ± 0.39 (3.3–4.7);13	?	3.4 ± 0.59 (2.6–3.9);3	2.3;1
Legs I–III:						
Internal claw	9.9	7.6	9.0 ± 1.15 (6.3–11.1);18	7.2 ± 0.99 (5.8–8.1);3	7.1 ± 0.54 (6.5–7.8);3	5.7 ± 0.79 (4.7–6.6);3
External claw	6.8	3.7	5.9 ± 0.84 (3.7–7.2);19	4.1 ± 0.79 (3.4–5.2);3	4.2 ± 0.48 (3.8–4.9);3	3.3 ± 0.17 (3.1–3.5);3
Leg IV:						
Internal claw	12.3	8.7	10.6 ± 1.18 (8.1–12.3);16	7.6 ± 1.35 (5.7–8.7);3	7.9 ± 1.22 (6.4–9.4);3	7.3 ± 1.39 (5.6–9.0);3
External claw	8.6	5.2	7.1 ± 0.89 (6.0–8.6);15	5.2 ± 0.69 (4.3–6.0);3	4.7 ± 0.47 (4.4–5.4);3	4.7 ± 1.00 (3.6–6.0);3
Gonopore*	8.9	4.9	7.6 ± 0.84 (6.1–8.9);12	4.2 ± 0.52 (3.7–4.9);3	–	–
Gonopore–anus distance	34.9	13.7	28.1 ± 4.40 (22.4–35.6);9	13.7–13.8;2	–	–
Claw formula: right	9,9,9,8	9,8,8,9	9.1,9.3,9.2,8.7	8.5,8.0,8.5,8.7	9.3,9.3,9.3,8.3	7.7,7.3,8.0,7.7
left	9,9,9,9	9,9,9,9	9.1,8.3,8.6,8.6	8.3,8.7,8.7,8.7	9.5,9.5,9.5,8.7	8.3,8.3,8.0,7.0

Note: Body width was measured between legs III–IV. * indicates diameter. Excluding the holotype and allotype, numbers of claw formula are mean values.

short, both with annulated base and pointed flagellum (Figure 16a,b).

Papillary primary clavae with terminal hole and a van der Land's organ at the base (Figure 16a). Slightly protruded lens-shaped secondary clavae (Figure 16c). Subterminal mouth without mouth plates (Figure 16c). Buccal tube (buccal tube length/body length ratio = *0.17*) rigid and wide (2.5 µm) (Figure 16g). Stylets, ending in large furcae at the anterior edge of the pharyngeal bulb, with stylet sheaths (stylet sheaths ~24.9 µm long); stylet supports not visible. Pharyngeal bulb contains placoids (~9.9 µm long).

Legs with sensory organs (Figures 15 and 16a,b,d-f): papillae with pointed tip on legs I; spine-like with enlarged bases and distal thin blunt portions on legs II–III (but with slightly thinner base on legs II); papillae with terminal hole on legs IV.

Nine claws on legs I–III, while on legs IV there were 8 on the right side and 9 on the left side. Claws, as typical of the genus, are anisonych (smaller/longer claw ratio is *0.69* on legs I–III and *0.70* on legs IV).

The gonopore is a six-lobed rosette (Figure 16f). As typical of the genus, the anus is trilobed, with two lateral lobes and a short posterior lobe.

Remarks: Males are similar to females in qualitative characters, except their gonopore is a simple oval and located nearer the anus. In quantitative characters, males appear to be smaller than females, however, this needs to be confirmed by future examination of more

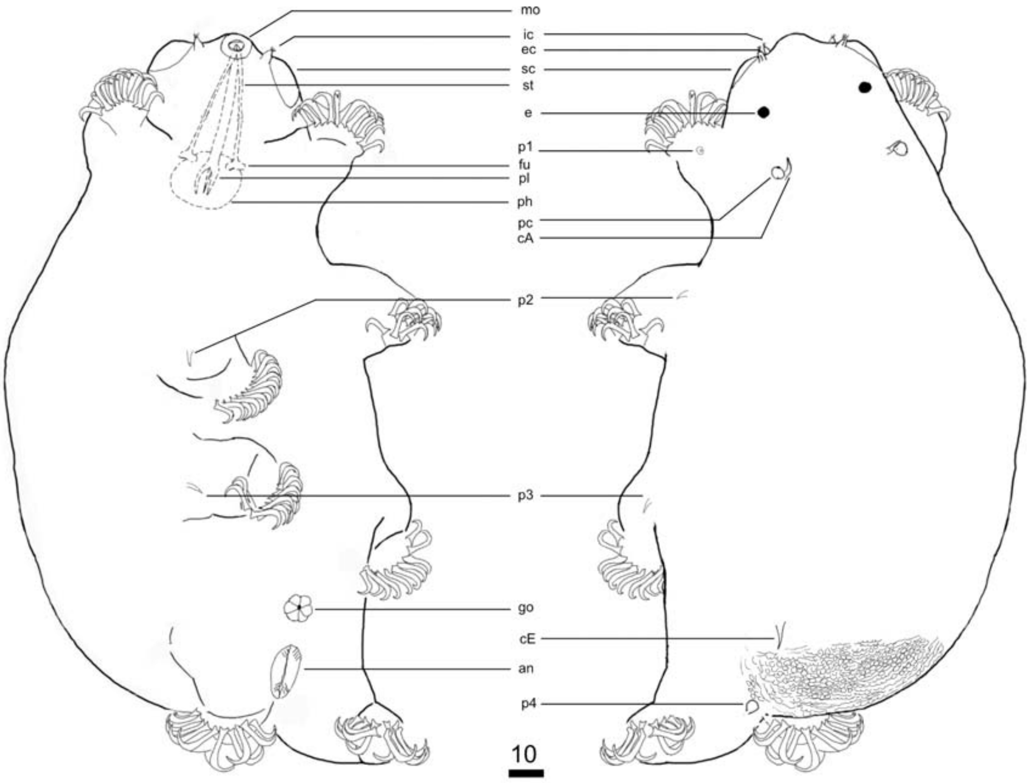

Figure 15. Ventral and dorsal aspects of *Echiniscoides ritavargasae* sp. nov. an = anus, cA = cirrus *A*, cE = cirrus *E*, e = eye, ec = external cirrus, f = furca, go = gonopore, ic = internal cirri, mo = mouth, pc = primary clava, ph = pharynx, pl = placoids, p1–p4 = sensory organs on legs I–IV, sc = secondary clavae, st = stylet. Dorsal cuticular pattern shown only for terminal segment. Scale bar in µm.

males. In adults, the number of claws is highly variable, from 8–11 on legs I–III, and from 7–10 on legs IV. The most common configuration is 9,9,9,9. In juveniles the number of claws is slightly reduced (7–10 and 9–10 on legs I–III, and 7–9 and 8–9 on leg IV, respectively, in first and second instar larvae). In one first instar larvae an unusual number of claws on one leg I (0 claws) and one leg IV (4 claws) was observed, while opposite legs had claw numbers in the normal range. One adult female exhibited six claws on one leg III, and in another individual claws were lacking on one leg III, again with normal claw numbers on the opposite legs. These numbers, considered abnormal, were not used for comparative purposes or evaluation of mean values.

Differential diagnosis: Only two other known *Echiniscoides* species, *Echiniscoides hoepneri* Kristensen and Hallas, 1980 and *Echiniscoides wyethi* Perry and Miller, 2015, both from the Atlantic Ocean, do not have a dome-shaped papilla (sensory organ) on leg II. This new species is easy to distinguish from them because:

- *Ecn. hoepneri* has a smooth cuticle, leg II sensory organs are shaped like setae, and has a much longer buccal tube (69 µm long vs 42 µm in similar-sized individuals).
- *Ecn. wyethi* has a smooth cuticle, leg II sensory organs are pointed papillae and has a much longer buccal tube (65 µm long vs 42 µm in similar-sized individuals).

Given that in some specimens of *Ecn. ritavargasae* sp. nov. the sensory organs on legs I and II are difficult to observe, we compared the new species with other species and subspecies with granulated cuticles: *Ecn. rugostellatus*, *Ecn. sigismundi groenlandicus*, *Echiniscoides s. verrucariae* de Zio Grimaldi, Gallo d'Addabbo and Pientanza, 2000 and *Echiniscoides s. porphyrae* de Zio Grimaldi, Gallo d'Addabbo and Pientanza, 2000. The new species can be distinguished from these because:

- *Ecn. rugostellatus* has cephalic cirri with shorter tufted tips (one-third of the total cirri length in *Ecn. rugostellatus* and ~one-half of the total cirri length in the new species) and has many large dorsal cuticular discs in a unique pattern compared to the new species (see Figures 15a,b here and figure 1 in Perry et al. 2018).

- *Ecn. sigismundi groenlandicus* has larger body length (270– 390 µm in females), lacks tufted cephalic cirri and, in some populations the cuticular sculpture includes cuticular tubercles which frequently have a central point.
- *Ecn. s. verrucariae* lacks tufted cephalic cirri and has larger internal claws (always > 12.0 and 15.0 µm in adult males and females, respectively).
- *Ecn. s. porphyrae* lacks tufted cephalic cirri and has a different morphology of the male gonopore (surrounded by a horseshoe-shaped thickening).

Mitochondrial *COI* gene region and phylogenetic analyses: All the obtained *COI* sequences of *Ecn. ritavargasae* sp. nov. were unique and distinct from those deposited in GenBank (see Supp. Table SII). Four nucleotide substitutions were detected, resulting in five haplotypes. The length of the *COI* gene (GenBank: MW046189–MW046193) was 646–665 bp, and no stop codons, insertions or deletions were observed.

For mtDNA data, 56 sequences (54 haplotypes) of *Echiniscoides*, *Isoechiniscoides* and *Neoechiniscoides* species were compared to analyse *Ecn. ritavargasae* sp. nov. phylogenetically. The alignment of the *COI* gene produced 425 characters, 242 of which were variable. The phylogenetic analysis (Figures 17, S1) showed that the new species was clustered together with *Echiniscoides* sp. from Panama City (GenBank: JQ689571.1) and *Echiniscoides* sp. from Costa Rica (GenBank: JQ689560.1). The p-distance between *Ecn. ritavargasae* sp. nov. haplotypes was 0.2%–0.5%. The p-distances between *COI* sequences of all compared populations/species ranged from the most similar (6.6% for *Echiniscoides* sp. from Costa Rica (GenBank: JQ689560.1)) to least similar (23.8% for *Echiniscoides* sp. from Faroe Islands (GenBank: JF437856.1)), with an average distance of 18.4%.

The p-distances for *Ecn. ritavargasae* sp. nov. and other *Echiniscoides* from the nearest localities (Faurby et al. 2012) indicate a very close evolutionary relationship. *Echiniscoides* sp. in Costa Rica was collected in the Pacific Ocean only 160 km from the type locality for *Ecn. ritavargasae* sp. nov., and the *Echiniscoides* from the Pacific Ocean in Panama City was ~715 km away. The p-distance between *Ecn. ritavargasae* sp. nov. and these two is only 6.6% and 8.1%, respectively, which suggests that both of those haplotypes could belong to our new species, though this requires verification based on morphology and ITS sequences. The similarity of these three Central American Pacific *Echiniscoides* populations is clear because COI p-distances between other *Echiniscoides* species (and tardigrade species in general) is most often more than 12% (Guidetti et al. 2019; Grobys et al. 2020; Roszkowska et al. 2020; Stec et al. 2020).

Habitat characteristics and distribution: This species is widespread on intertidal barnacles in Golfo de Santa Elena on the Pacific coast. Other specimens were also found with limpets, sabellarid tubes, and in one intertidal beach sample.

Class: Eutardigrada Richters, 1926 (amended by Morek et al. 2020)
Order: Apochela Schuster, Nelson, Grigarick and Christenberry 1980 (amended by Morek et al. 2020)
Family: Milnesiidae Ramazzotti, 1962 (amended by Morek et al. 2020)
Genus: *Milnesium* Doyère, 1840 (amended by Morek et al. 2020)

***Milnesium* sp.**

Site 35 (Tables I and II, Figure 1)

Material examined: Two specimens, a female and a presumed juvenile. Specimens deposited in the collection of tardigrades at the Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Morphological comments: The female (474 µm long), with no modified claws, with brownish eyes and transparent body. The dorsal cuticle is smooth without granulation, pseudopores or pseudoplates. Mouth opening surrounded by six peribuccal papillae and six peribuccal lamellae. Two lateral cephalic papillae, slightly shorter than peribuccal papillae. Buccal tube wide and almost cylindrical (*pt* of anterior and posterior width *48.6* and *44.9*, respectively; *pt* of standard width *47.3*). Stylets inserted in the buccal tube at 73.6% of its length. Elongated pear-shaped pharyngeal bulb without placoids. Claws of the *Milnesium* type: primary branches with clearly visible accessory points. Secondary branches with basal spurs (claw configuration [3-3]-[3-3], according to formula from Michalczyk et al. 2012). Spurs on secondary branches of external and posterior claws very small, normally developed on internal and anterior claws. Secondary branches with rounded basal thickenings, slightly ragged. Cuticular bars under all claws present on all legs.

With a body length of 255 µm, the second specimen (presumed juvenile) is very similar to the female in the majority of characters (e.g. eyes, smooth cuticle, buccal apparatus). However, this specimen exhibits a different claw configuration by lacking basal spurs on the secondary branches of all claws (claw configuration [2-2]-[2-2]). Some small morphometric differences can

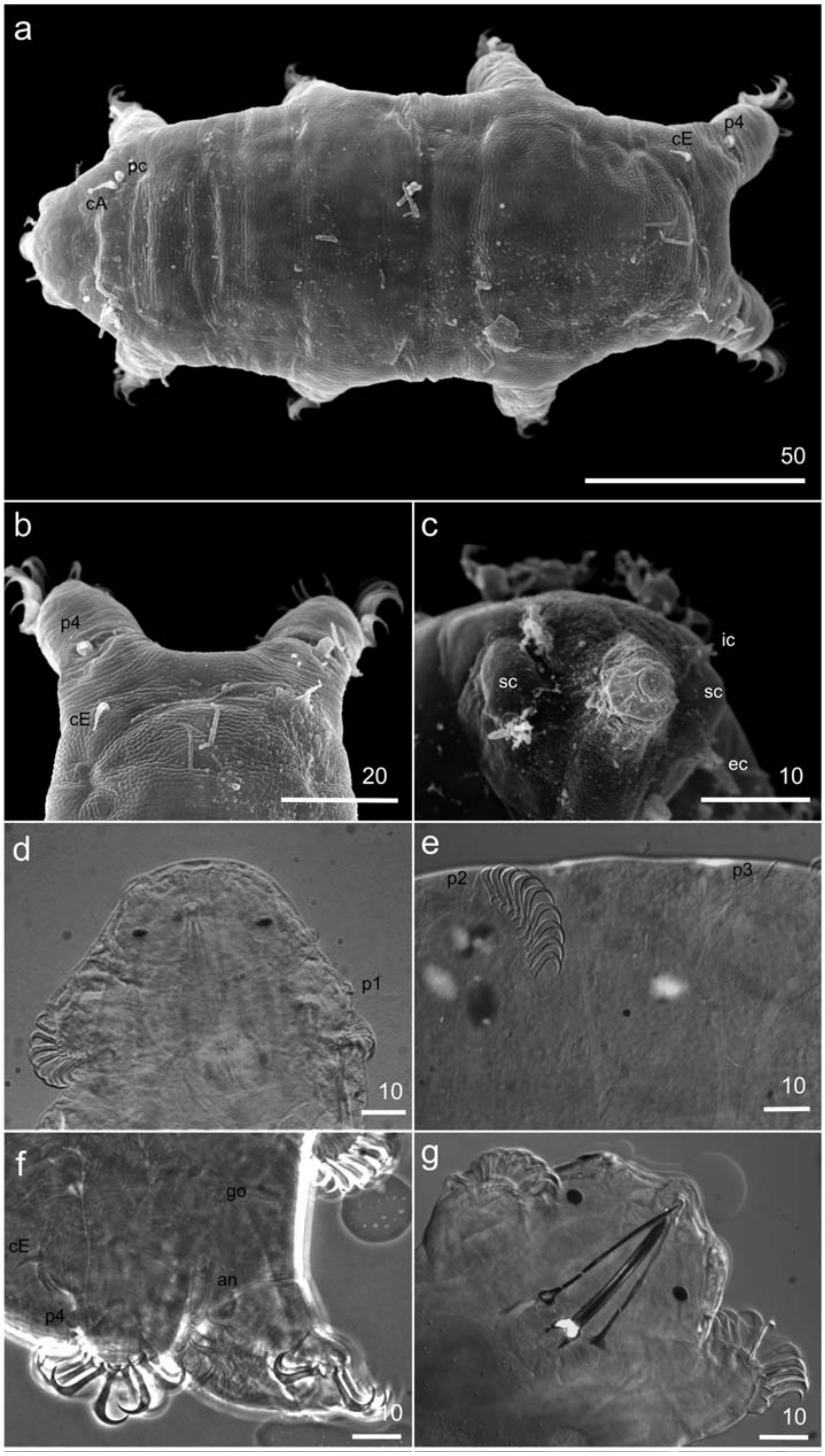

Figure 16. *Echiniscoides ritavargasae* sp. nov. **a**. Habitus, dorsal view. SEM. **b**. Posterior dorsal detail showing cuticular sculpture. SEM. **c**. Mouth and anterior cephalic appendages. Note tufted tips on internal and external cirri. SEM. **d**. Anterior section of CRI-45-5. DIC. **e**. Leg II with claws and leg III of holotype. DIC. **f**. Posterior ventral aspect of CR-45-17. PhC, focus merged. **g**. Buccal apparatus of CR-45-17. DIC, focus merged. an = anus, cA = cirrus *A*, cE = cirrus *E*, ec = external cirrus, go = gonopore, ic = internal cirrus, p1-–4 = leg sensory organs, pc = primary clava, sc = secondary clava. Scale bars in µm.

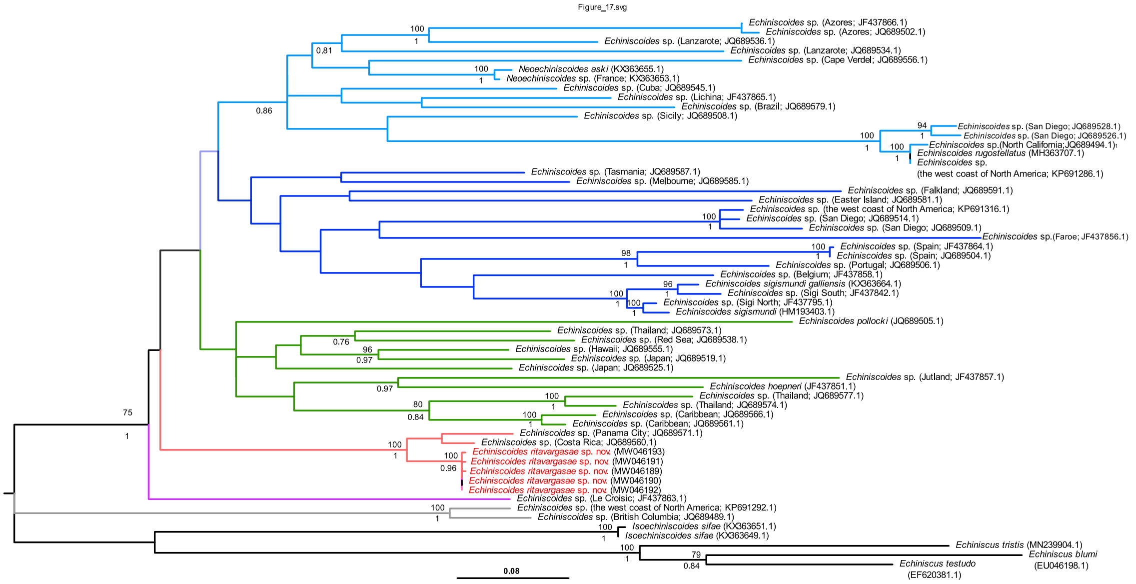

Figure 17. The phylogenetic position of *Echiniscoides ritavargasae* sp. nov. based on COI sequences in the Maximum Likelihood and the Bayesian tree with the GTR + G + I evolution model. The supporting bootstrap values (lower than 70 were deleted) are given above branches, values of posterior probabilities (PP) are presented below the branches (nodes with PP < 0.80 were collapsed). The GenBank accession numbers of all the sequences applied are given in Table SIII. Coloured lines indicate clades/subclades, and our new species is indicated with red font).

be observed (e.g. slightly longer *pt* of primary branches on claws of legs I–II, which is *44.9–46.3* in this specimen and *37.9–38.4* in the female). Based on the small size of the specimen and the similarities in buccal apparatus morphometrics (i.e. the stylet support insertion point at 73.9% of its length), and the ontogenetic variability in claw morphology, it is probable that this specimen is a juvenile from the same species as the female. Ontogenetic variability in the presence/absence of basal spurs on hatchlings and juveniles has been reported in some *Milnesium* species (see Meyer and Hinton 2012; Morek et al. 2016; Moreno-Talamantes et al. 2019; Surmacz et al. 2020). Moreover, body lengths of specimens with 474 and 255 µm are compatible with *Milnesium* adult and juvenile lengths, respectively (see Surmacz et al. 2019). As illustrated for other *Milnesium* species (e.g. *Milnesium variefidum* Morek, Gąsiorek, Stec, Blagden and Michalczyk, 2016), the small morphometric differences observed between our two specimens fall within an acceptable range of measurements that include different developmental stages of the same species.

Based on the assumption that both specimens collected in this study belong to the same species, the adult specimen should be compared with *Milnesium shilohae* Meyer, 2015. Among the 42 known *Milnesium* species described until now (Degma et al. 2009–2020), this species is the only one that also has a claw configuration [3-3]-[3-3] in adults with spurs on internal-external and anterior-posterior branches with markedly different lengths. The specimen from Costa Rica differs from *Mil. shilohae* by having stylet supports inserted more anteriorly (at 73.6% of the buccal tube length vs 75.7–77.7% in *Mil. shilohae*) and shorter spurs (*pt = 7.0–7.5* and *pt = 8.0–15.2* for the larger spur in the specimen from Costa Rica and in *Mil. shilohae*, respectively). The shorter spurs are also much smaller in the specimen from Costa Rica (*pt* ≤ *2.5* and *pt = 3.5–5.9* in posterior claws of leg IV in the specimen from Costa Rica and in *Mil. shilohae*, respectively).

In another species, *Milnesium pseudotardigradum* Surmacz, Morek and Michalczyk, 2019, although usually having a typical claw configuration [2-3]-[3-3], some specimens may have additional spurs, smaller than the regular ones, exhibiting a [3-3]-[3-3] configuration. However, the specimen from Costa Rica differs from these *Mil. pseudotardigradum* specimens in having shorter spurs (*pt = 7.0–7.5* vs *pt* mean values *12.6–19.5*) and shorter claws (e.g. primary branches of claws IV *pt = 46.1* and *51.6* for the anterior and posterior claws in the specimen from Costa Rica vs *pt* mean values of *59.6* and *63.7* in *Mil. pseudotardigradum*).

Therefore, specimens from Costa Rica may belong to a known species with the observed differences explained by intrapopulation variability, similar to *Mil. shilohae*, or to an undescribed species. With only a single adult specimen and a presumed juvenile, intrapopulation morphometric differences as well as ontogenetic variability cannot be assessed. Therefore, further specimens need to be collected to assign the specimens to a known *Milnesium* species or to allow a formal description of a new species.

Habitat characteristics and distribution: These specimens were found in intertidal beach sand at Golfo de Santa Elena, Playa Cuajiniquil, on the Pacific coast. A *Milnesium* with enlarged Malpighian glands was found in intertidal beach sand in France by R.M. Kristensen (pers. comm.), and he believes it is adapted for marine conditions. Playa Cuajiniquil is an estuary and it is possible that this is a limnoterrestrial species washed onto the beach by the nearby stream. Malpighian glands were not visible in studied specimens.

Discussion

In this study we found 19 species of marine tardigrades and described seven species new to science. These numbers were the result of sampling multiple habitats, intertidal and subtidal zones, and both Caribbean and Pacific coasts. However, it is important to recognize the paucity of specimens from our subtidal samples. While 24 subtidal samples were taken (10 in the Caribbean and 14 in the Pacific), none from the Pacific and just six from the Caribbean yielded tardigrades. Only 25 individuals and two species were found in those six Caribbean samples. We expect that many more species remain to be discovered in subtidal habitats on both coasts. The reason for the sparse yield in Caribbean subtidal samples is largely due to the limited sampling we were able to perform there. Sea conditions were too rough for boats and scuba diving, so we were limited to very shallow samples that could be reached by wading or snorkelling from shore. In contrast, subtidal samples collected in the Pacific were from a variety of habitats and depths. The total lack of marine tardigrades in these Pacific subtidal samples was quite surprising, especially since other meiofauna were abundant in our samples (also see Guzmán et al. 1987; Vargas 1988). There are three possible explanations for the lack of subtidal tardigrades in these Pacific samples. One possibility is that marine tardigrades cannot exist in the seasonally fluctuating conditions created by upwellings. This seems unlikely since a diverse tardigrade fauna was found in another area with upwellings (Kristensen et al. 2015). Upwellings cause many water chemistry changes and have profound impacts on meiofauna community structure (Giere 2009), so another possibility is that tardigrade populations are lost during upwellings then re-colonize during more benign conditions. Finally, and perhaps more likely, it is possible that tardigrades migrate vertically within the substrate as a function of temperature changes. Harris (1972) found such a pattern for a number of non-tardigrade meiofauna in intertidal habitats. If tardigrades move down in the sediment during upwellings, they may have been inaccessible by the grab sampler. Additional sampling is planned to assess these three hypotheses.

Sabellarid polychaete tubes

While barnacles are commonly sampled in intertidal marine tardigrade research, one unusual habitat we sampled revealed more diversity. Barnacle tardigrade communities were dominated by *Ecn. ritavargasae* sp. nov. and contained only one additional species, *Arc. murilloi* sp. nov. In comparison, intertidal sabellarid tubes (Family Sabellariidae, 'reef-building tube worms' or 'sandcastle worms' or 'honeycomb worms') proved to be a rich source of tardigrades yielding five species, including *Bat. homocercus* sp. nov. and *Sty. vargasi* sp. nov., which were only found on this substrate. While serpulid polychaete tubes (Family Serpulidae, 'fan worms' or 'feather duster worms') have been reported to host tardigrades (Hansen et al. 2012; Kristensen et al. 2012), to our knowledge only one previous study (Ataide et al. 2014) has reported tardigrades from sabellarid polychaetes, but the tardigrades were identified only to phylum level. Sabellarids sometimes form extensive sandy reefs, and these may be important habitats for future investigations of marine tardigrades. Furthermore, anhydrobiosis, while common in terrestrial and limnoterrestrial tardigrades, is only known in a few intertidal marine tardigrades (Møbjerg et al. 2011; Jørgensen and Møbjerg 2015; Hygum et al. 2016). Anhydrobiosis would be interesting to investigate in *Bat. homocercus* sp. nov., *Sty. vargasi* sp. nov. and others found here with intertidal sabellarids, which are partially or completely exposed to air during tidal cycles.

Trans-isthmus of Central America species distributions

Lessios (2008) discussed mechanisms driving trans-isthmus species distributions. Some trans-isthmus species or sibling species (i.e. geminate species) evolved before the final closure of the isthmus. For others, colonisation events could have occurred after the closure, either around North or South America (admittedly unlikely for tropical species) or recently via the Panama Canal. Lessios (2008) used 2.8 mya as an estimate for the final closure, but others have suggested that this occurred in successive waves and could date back further to as much as 10 mya (Knowlton et al. 1993; Montes et al. 2015).

We know very little about the timing of marine tardigrade speciation events, nor dispersal mechanisms. They do not have a planktonic dispersal stage, and other mechanisms of dispersal are largely unconfirmed. Nelson et al. (2018) mention rafting on algae or other flotsam, marine snow, and accidental transport via ships as possible dispersal mechanisms, and rafting has been documented for one marine tardigrade (Bartels et al. 2015b). Dispersal by wind has been documented in limnoterrestrial tardigrades (Nelson et al. 2018), and it is certainly possible for some intertidal marine tardigrades since some of them, like their limnoterrestrial relatives, are able to undergo anhydrobiosis, as mentioned above. Additionally, dispersal by other animals is possible. There is some evidence that birds transport limnoterrestrial tardigrades on their legs with particles of sand or mud, in their feathers, with nest building material or in their intestines (Mogle et al. 2018; Kaczmarek et al. 2018b; Robertson et al. 2020), but again, phoresy has yet to be reported for marine tardigrades.

Trans-isthmus of Central America distributions have been reported for only a few meiofaunal groups: tardigrades (Faurby et al. 2012), nemerteans (Leasi and Norenburg 2014; Leasi et al. 2016), Proseriata flatworms (Scarpa et al. 2015; Curini-Galletti et al. 2019), kinorhynchs (Pardos et al. 2016) and caecid microsnails (Egger et al. 2020). The previous study of tardigrades focused on the very common intertidal marine tardigrade genus *Echiniscoides*. As part of a worldwide study, Faurby et al. (2012) collected *Echiniscoides* from the Pacific coast of Costa Rica and both the Pacific and Caribbean coasts of Panama. Based on *COI* and 28S rRNA results, four haplotypes were recovered from these locations, two from the Pacific and two from the Caribbean. The haplotypes on each side of the isthmus were very similar, while there was great divergence between Caribbean and Pacific haplotypes. This is intriguing because Faurby et al. (2011) reported two evolutionarily independent units of *Echiniscoides* that had trans-Atlantic distributions. The isthmus, apparently, is a better isolating mechanism for *Echiniscoides* than the Atlantic Ocean. Faurby et al. (2012) also suggested that two 'clusters' (Cuba versus Pacific Panama/Costa Rica and Caribbean Panama versus Thailand 2) might demonstrate trans-Panama vicariance. Each of these is a sister cluster and, in other words, might be considered geminate species. Morphological analyses of these populations were not reported.

Only three of the 19 species discovered in our survey were encountered on both Caribbean and Pacific coasts. Other species that appear to be restricted to a single coast may actually have a broader distribution, but perhaps were not encountered due to the lack of appropriate habitats on both coasts. Algae were not sampled on the Pacific coast, barnacles were rare in the Caribbean, and sabellarid polychaetes were not sampled in the Caribbean. It is possible that additional sampling will turn up more overlapping species, especially in subtidal samples.

The three species found on both coasts were *Hal. r. remanei*, *Rai. colurus* and *Stg. gourbaultae*. *Halechiniscus r. remanei* is very common and, based on morphological data, is thought to have a cosmopolitan distribution (Kaczmarek et al. 2015; Bartels et al. 2015a). Therefore, it seems unlikely that their trans-isthmus distribution is the result of vicariance or recent dispersal across the isthmus. *Stygarctus gourbaultae* previously was known only from the Caribbean and Florida (Renaud-Mornant 1981a, 1984; Renaud-Mornant et al. 1983; Renaud-Mornant and Gourbault 1981, 1984; Kristensen and Higgins 1984a; Hansen et al. 2012). Given the rather restricted known range of this species, the occurrence of *Stg. gourbaultae* on both coasts could suggest relatively recent dispersal. *Raiarctus colurus* has been recorded from the Atlantic Ocean (Renaud-Mornant 1981a; Hansen et al. 2001; Jørgensen et al. 2010), the Mediterranean (Grimaldi de Zio et al. 1983; D'Addabbo Gallo et al. 1987; Grimaldi de Zio et al. 2003; Accogli et al. 2011) and the Caribbean (Renaud-Mornant 1981a; Renaud-Mornant and Gourbault 1981, 1984), but never before in the Pacific. However, the taxonomy of this genus, based on just a few characters, is very problematic and the occurrence of several cryptic/pseudocryptic species assigned to *Rai. colurus* cannot be excluded. This species should be investigated further to determine the degree of evolutionary divergence between Pacific and Caribbean populations. Other potential geminate species include the two *Batillipes* species and the *Tanarctus* species. Both of these appear to have very restricted ranges and seem to be very closely related. A moleclular/morphological phylogenetic analysis and a molecular clock calculation could reveal a range of morphological and molecular divergences, driven by vicariance in some cases and dispersal in others, and modified by stabilizing or divergent evolution. In short, marine tardigrades of the Isthmus of Central America could be a model for meiofauna evolution and a natural experiment in speciation and dispersal.

Conclusion

This is an initial survey of marine tardigrades for Costa Rica, and our sampling was limited by weather and time constraints. Still, we found high diversity with a minimum of 37% of species new to science. *Milnesium* sp. and possibly others may prove to be new to science as well when more material can be collected. The ratio of new species discovered to total species found is indicative of both the rich, hidden biological wonders of Costa Rica and our extreme ignorance of this group of animals. Several intriguing questions of meiofaunal ecology and biogeography have been raised by our findings, candidate geminate species have been identified, and we hope this might stimulate interest in a poorly studied group in an understudied region.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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