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New record of *Batillipes dandarae* Santos, Rocha, Gomes Jr. & Fontoura, 2017 (Tardigrada, Heterotardigrada) from the southeast Brazilian coast

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Abstract. We present new records of the tardigrade *Batillipes dandarae* Santos, Rocha, Gomes Jr. & Fontoura, 2017 from the north coast of São Paulo state in southeastern Brazil. The species was identified using both light and scanning electron microscopy. This report has a biogeographic importance since we extend this species' geographic range to two distinct marine ecoregions. The new records are also the first from outside the species' type locality.

Keywords. Batillipedidae, distribution patterns, new occurrence, tardigrades

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Introduction

The phylum Tardigrada, popularly known as water bears, are microinvertebrates ca. 70–1200 µm in length which are distributed worldwide and commonly found in various terrestrial and marine and freshwater aquatic habitats (Nelson 2002; Nelson et al. 2015; Fontoura et al. 2017). The phylum comprises more than 1460 species (Degma et al. 2023) which are accommodated in two classes, the Eutardigrada Richters, 1926 and Heterotardigrada Marcus, 1927. The latter class is traditionally divided into the orders Echiniscoidea Richters, 1926 and Arthrotardigrada Marcus, 1927 (Fontoura et al. 2017); however, recent molecular studies and morphological evidence indicates that Arthotardigrada are paraphyletic and this taxon has been subsequently rejected on grounds that it is not monophyletic (Fujimoto et al. 2017; Grollmann et al. 2023). The marine heterotardigrade family Batillipedidae Ramazzotti, 1962 contains only the genus *Batillipes* Richters, 1909 (Fontoura et al. 2017; Santos et al. 2019a).

Batillipes species are adapted to interstitial life in subtidal sandy environments; they have four- or six-toed paws with digits and terminal adhesive disks on their digits (Santos et al. 2018, 2019a). This genus was considered to be monotypic for about four decades—*Batillipes mirus* Richters, 1909 is the type species—but it currently contains 39 formally described species, including the most recently described *Batillipes kalami* Vishnudattan, Rubal & Nandan, 2023. Among these species, *Batillipes dandarae* Santos, Rocha, Gomes Jr. & Fontoura, 2017 was recently described from material collected from shallow-subtidal sediments of the northeastern coast of Brazil—Ponta do Sal Beach (02°48′18″S, 041°43′51″W), Piauí state; Forte Orange Beach (07°48′44″S, 034°50′37″W), Pernambuco state (Santos et al. 2017); and Patacho Beach (09°11′40″S, 035°18′27″W), Alagoas state. Our aim of this study is to report *B. dandarae* for the first time from outside its type locality and to analyze specimens using scanning electron microscopy for the first time.

Methods

Samples of sandy sediment from the intertidal zone (15 cm deep at low tide, about 1.5 L) were collected on 16 December 2021 at Sununga Beach and 17 December 2021 on the beach of the Oceanographic Institute of the University of São Paulo. Both beaches are in the city of Ubatuba, on the north coast of the state of São Paulo (Fig. 1). The collected material was transferred to buckets and taken to the laboratory, being kept with constant aeration and temperature around 20 °C to maximize the survival of the animals in the laboratory.

Small amounts of sediment were mixed with 7% seawater MgCl2 in Petri dishes and under a Zeiss Stemi 2000 stereomicroscope, tardigrades were sorted and isolated alive from sand. In total, four individuals were sampled, and one individual was mounted on a permanent slide with Fluoromount aqueous (F4680 Sigma-Aldrich) medium and studied with a Zeiss Axio Imager M2 light microscope equipped with differential interference contrast (DIC) and an AxioCam MRC5 digital video camera. Images and measurements were taken using ZEN lite v. 2.5 imaging software. Three individuals were dehydrated through a graded series of ethanol (70%, 80%, 90%, 95%, 100% twice, 5 min each) (Abolafia 2015) and subsequently brought to the critical-point Bal-Tec CPD 030, mounted on aluminum stubs, and sputter coated with palladium gold using a Sputter Coater SCD-050. The observations of these specimens were carried out under a JEOL JSM 5800LV SEM at the Universidade Estadual de Campinas (Unicamp).

Light-microscopy photomicrographs are available at the Museu de diversidade Biológica, Unicamp, under access number ZUEC PIC 821. SEM photomicrographs are available under access numbers ZUEC PIC 822–824. The map (Fig. 1) showing the location of the type locality and new records was made using QGIS v. 2.8.2 (Quantum GIS Development Team 2020).

Results

Phylum Tardigrada Doyère, 1840
Class Heterotardigrada Marcus, 1927
Family Batillipedidae Ramazzotti, 1962
Genus *Batillipes* Richters, 1909

Batillipes dandarae Santos, Rocha, Gomes Jr. & Fontoura, 2017

Figures 2, 3

New records (Fig. 1). BRAZIL – **São Paulo** • Ubatuba, Sununga Beach; 23°30′32″S, 045°07′57″W; 16.XII.2021; M.P. da Silva leg; 2 sex indeterminate (indet.), photographic records ZUEC PIC 822 and 823 • Ubatuba, beach of the Oceanographic Institute of the University of São Paulo; 23°30′00″S, 45°07′06″W; 17.XII.2021; M.P.

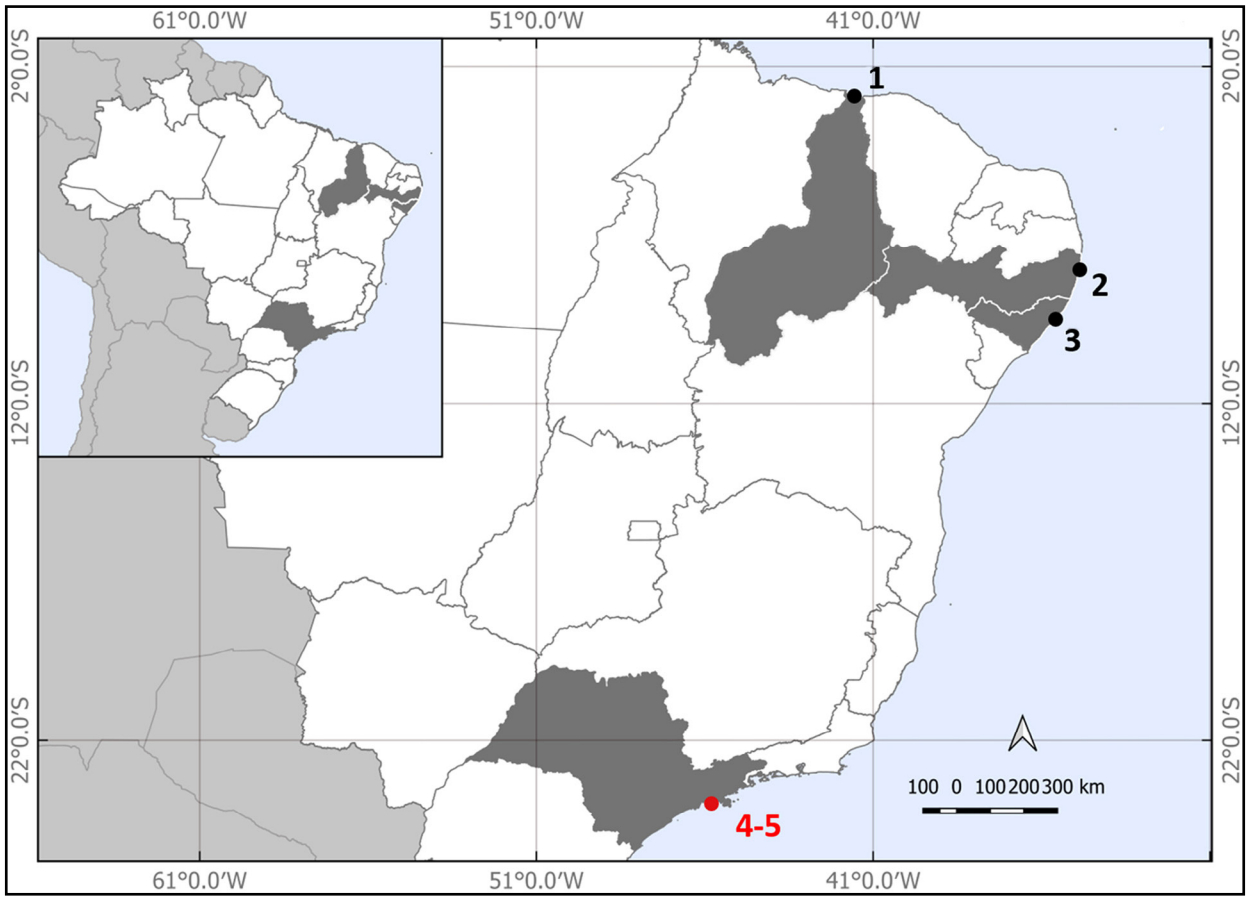

Figure 1. Map of northeastern and southeastern Brazil showing sample localities of *Batillipes dandarae*. The red circle denotes the new localities (3 = Sununga Beach; 4 = Oceanographic Institute), and the black circles are localities from Santos et al. (2017) (1 = Patacho Beach; 2 = Forte Orange Beach; 3 = Gunga Beach).

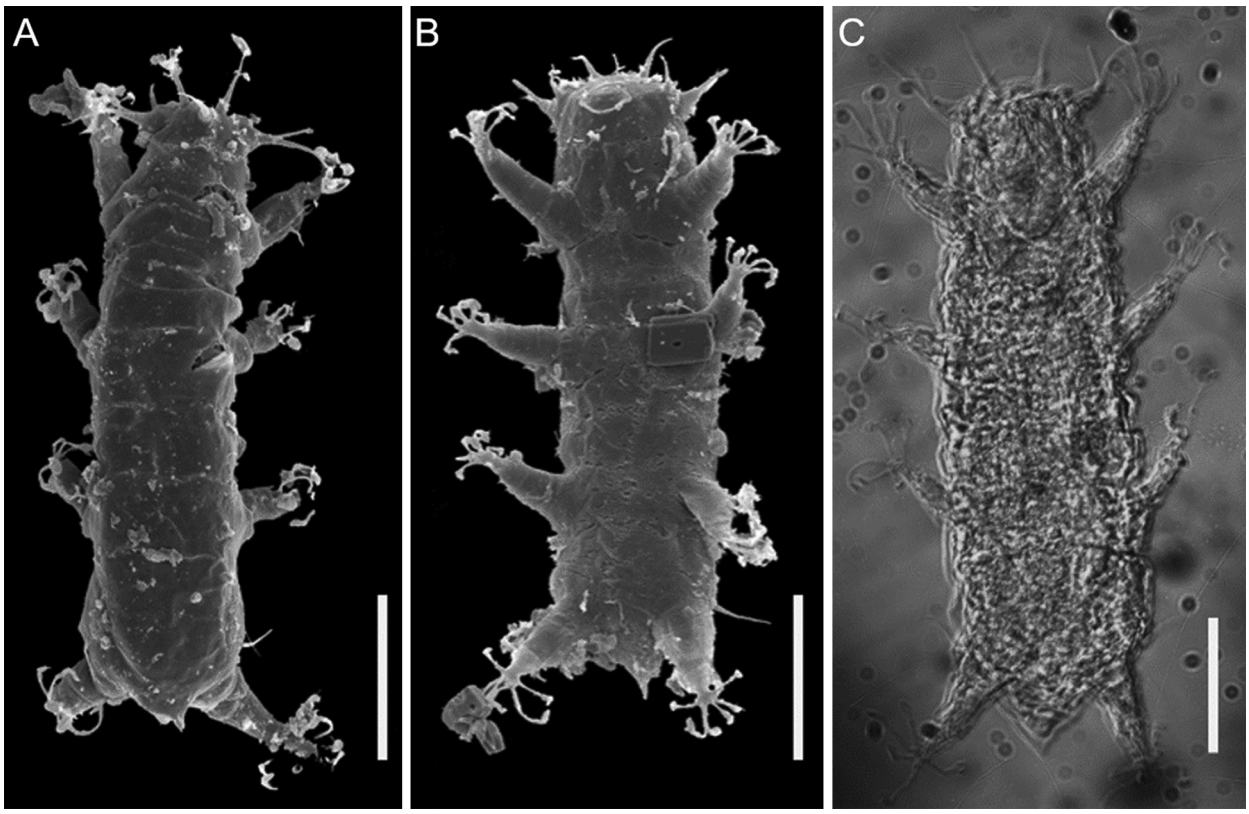

Figure 2. *Batillipes dandarae.* **A.** SEM photomicrographs of habitus (dorsal view). **B.** SEM photomicrographs (ventral view) of habitus. **C.** DIC photomicrographs (ventral view) of habitus. Scale bars = 50 µm.

da Silva leg; 1 ♀; photographic record ZUEC PIC 824.

Identification. *Batillipes dandarae* is characterized by having medial toes 3 and 4 on leg IV of different lengths, and these also different from toes 1 and 2; lateral cuticular processes on the body are lacking, and the pointed triangular caudal appendage varies considerably in shape and size among specimens. Leg I shows the leg spine (pI) inserted on the posterior part of the leg and turning frontwards (Santos et al. 2017).

Our specimens (Figs. 2, 3) share the same morphological characters reported by Santos et al. (2017) in the original description of *B. dandarae*. We noticed that the pointed triangular caudal appendage and sensory spine insert into the posterior part of leg I facing forward (Fig. 3A–D). Furthermore, evident lateral processes between legs III and IV were not present (Fig. 2), and the cuticle had small pillars distributed uniformly on the dorsal body (Fig. 3F). This last character has not been observed in any other *Batillipes* species (Santos et al. 2017). The anus structure, when observed by SEM, it is similar to that previously described, but more delicate than the "plate aspect" observed in optical microscopy (Fig. 3E; see original description). This impression may be due to the flattening effect of mounting specimens on slides compared to a three-dimensional view in SEM.

The measurements of the specimen observed under light microscopy are closely similar to those of the holotype (Table 1). Only some structures, such as the primary clava and some fingers, show some difference (slightly bigger), but this is probably due to variations common in this species (Santos et al. 2017).

Discussion

In the meiofauna, even a few meters can bring significant sedimentological and ecological changes that impact the presence or absence of meiofaunal organisms (Giere 2009). New records of species far from their type locality are highly important for understanding and expanding the distribution and diversity of Brazilian meiofauna (Maria et al. 2016a, 2016b). However, there are challenges that hinder our understanding of meiofaunal biodiversity, especially among marine tardigrades (Fontoura et. al. 2017); older descriptions based on fewer morphological features and the absence of deposited type series in museum collections contribute to these taxonomic challenges (Fonseca et al. 2018; Santos et al. 2018, 2019b; Garraffoni et al. 2019). In this study, we expand the distribution of *Batillipes dandarae*, which previously was known only from its type locality in northeastern Brazil (Santos et al. 2017; de Barros 2020), but we now have it from the southeastern Brazilian coast. Thus, the geographic distribution is increased southward by 2,471 km.

There was already evidence of *B. dandarae* on the southeast Brazilian coast. According to Santos et al. (2019b), records of *B. tubernatis* Pollock, 1971 from the northeastern coast of Brazil are instead *B. dandarae* and *B. potiguarensis* Santos, Rocha, Gomes Jr. & Fontoura, 2017. Likewise, Santos et al. (2019b)

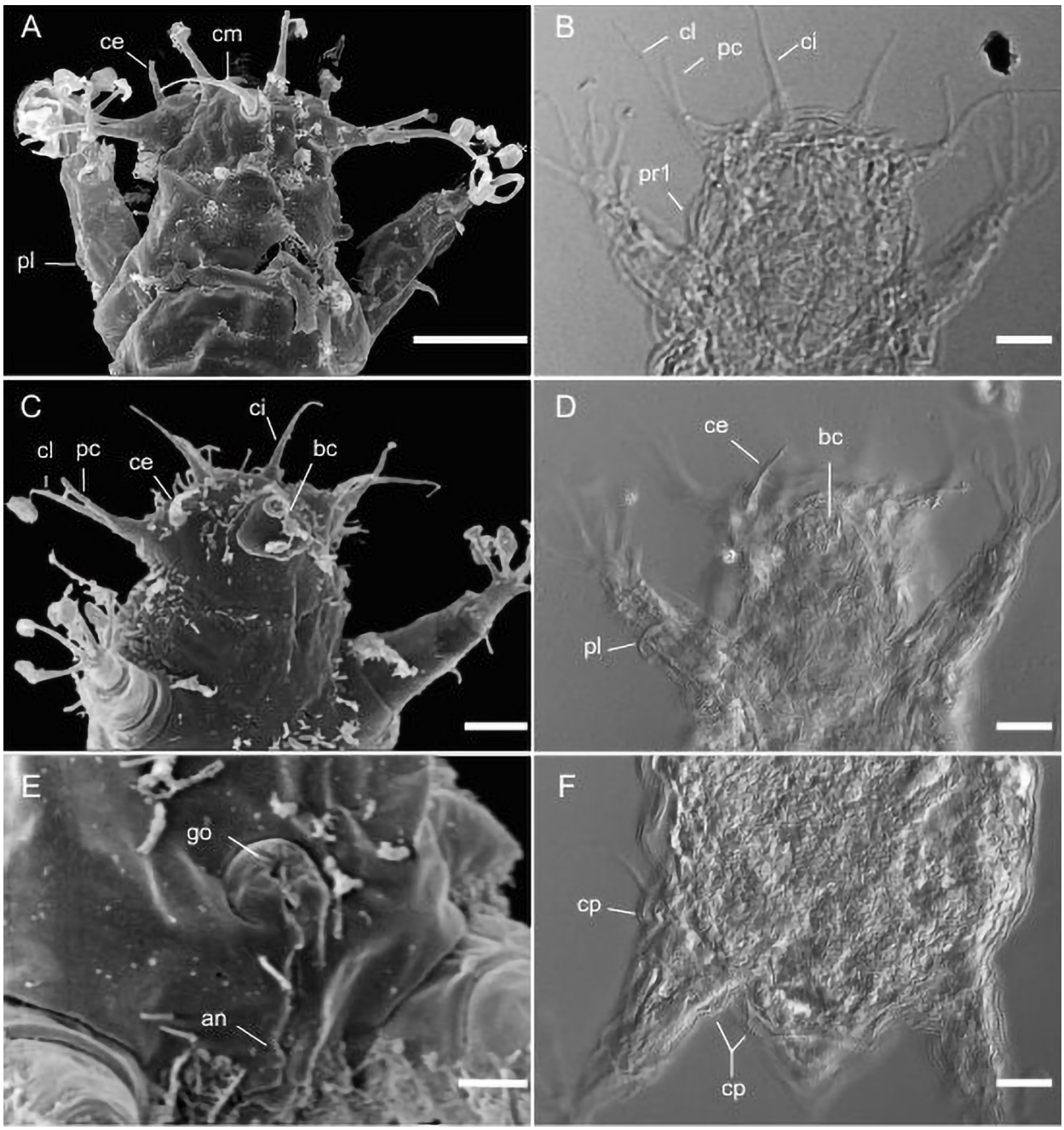

Figure 3. *Batillipes dandarae.* **A.** SEM photomicrograph showing dorsal view of the head showing the median cirrus (cm), leg I of a paratype showing the leg spine (pl) inserted on the posterior part of the leg and turning frontwards , the external cephalic cirrus (ce). **B.** DIC photomicrograph of anterior portion dorsal of the body showing cephalic appendages (ci = internal cirrus; cl = lateral cirrus), clavae (pc = primary clava) and first body projection (pr1). **C.** SEM photomicrograph of anterior portion ventral of the body showing cephalic appendages (ci = internal cirrus; cl = lateral cirrus; ce = the external cephalic cirrus), clavae (pc = primary clava) and the buccal cone (bc = buccal cone). **D.** DIC photomicrograph showing anterior portion of the ventral of the body showing the buccal cone, leg I of a paratype showing the leg spine (pl) inserted on the posterior part of the leg and turning frontwards. **E.** SEM photomicrograph of posterior portion ventral of the body showing gonopore (go) and anus (an); **F.** DIC photomicrographs, posterior portion ventral of the body showing the finely punctated cuticle comprised of small pillars (cp). Scale bars: A= 50 µm; B, D, F= 20 µm; C, E= 10 µm.

suspected that Höfling-Epiphanio's (1972) record of *B. tubernatis* from São Paulo state, may be *B. dandarae*. However, they could not assign it with certainty due to the incomplete description provided by Höfling-Epiphanio (1972).

We were unable to perform a molecular analysis due to the limited number of specimens found in our study. The four individuals sampled were used to guarantee the quality of the morphological description and were used for optical microscopy and SEM. Furthermore, Santos et al. (2017) did not provide molecular data on this species, so we would not have been able to make a comparison that could confirm genetic similarity with type material.

Table 1. Measurements (in µm) of selected morphological features of *Batillipes dandarae* specimens. ? = not measured.

Structure	Present study (*n* = 1)	Holotype (Santos et al. 2017)
Body length (BL)	207	204
Body width	56	68.9
BL without caudal apparatus	194	194
Internal cirri	21.3	23.2
External cirri	13.1	16.1
Lateral cirri A	26.1	28.2
Primary clavae	19.9	13.3
Leg IV sense organ	11.4	12.4
Leg I Toe 1	?	13.4
Toe 2	?	4.7
Toe 3	15.3	16.2
Toe 4	10.8	10.2
Toe 5	?	17.9
Toe 6	10	12.7
Caudal apparatus	13.9	8.9
Leg IV Toe 1	14.1	14.4
Toe 2	10.8	?
Toe 3	16.4	10.1
Toe 4	?	12.7
Toe 5	15.4	?
Toe 6	12.4	?

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Author Contributions

Conceptualization: ES, ARSG. Data curation: MPS, ES, ARSG. Formal analysis: MPS, ES. Investigation: MPS, ES, ARSG. Methodology: MPS, ES, ARSG. Project administration: ARSG. Supervision: ARSG. Visualization: MPS, ES, ARSG. Writing – original draft: MPS, ARSG. Writing – review and editing: MPS, ES, ARSG.

References

Abolafia J (2015) A low-cost technique to manufacture a container to process meiofauna for scanning electron microscopy. Microscopy Research and Technique 78: 771–776. https://doi.org/10.1002/jemt.22538

de Barros RC (2020) Tardigrades research in Brazil: an overview and updated checklist. Arquivos de Zoologia 51: 1–11. https://doi.org/10.11606/2176-7793/2020.51.01

Degma P, Guidetti R (2023) Actual checklist of Tardigrada species. 42nd edition. https://doi.org/10.25431/11380_1178608

Doyère MLF (1840) Mémorie sur les tardigrades Annales des Sciences Naturelles. Serie 2 (Zoologie) 14: 269–362.

Fonseca G, Fontaneto D, Di Domenico M (2018) Addressing biodiversity shortfalls in meiofauna. Journal of Experimental Marine Biology and Ecology 502: 26–38. https://doi.org/10.1016/j.jembe.2017.05.007

Fontoura P, Bartels PJ, Jørgensen A, Kristensen RM, Hansen JG (2017) A dichotomous key to the genera of the marine heterotardigrades (Tardigrada). Zootaxa 4294: 1–45. https://doi.org/10.11646/zootaxa.4294.1.1

Fujimoto S, Jørgensen A, Hansen JG (2017) A molecular approach to arthrotardigrade phylogeny (Heterotardigrada, Tardigrada). Zoologica Scripta 46: 496–505. https://doi.org/10.1111/zsc.12221

Garraffoni ARS, Kieneke A, Kolicka M, Corgosinho PH, Prado J, Nihei SS, Freitas AVL (2019) ICZN Declaration 45: a remedy for the nomenclatural and typification dilemma regarding soft-bodied meiofaunal organisms? Marine Biodiversity 49: 2199–2207. https://doi.org/10.1007/s12526-019-00983-7

Giere O (2009) Meiobenthology: the microscopic motile fauna of aquatic sediments. Second edition. Springer-Verlag, Berlin, Germany, 527 pp. https://doi.org/10.1007/978-3-540-68661-3

Grollmann MM, Jørgensen A, Møbjerg N (2023) *Actinarctus doryphorus* (Tanarctidae) DNA barcodes and phylogenetic reinvestigation of Arthrotardigrada with new *A. doryphorus* and Echiniscoididae sequences. Zootaxa 5284: 351–363. https://doi.org/10.11646/zootaxa.5284.2

Höfling-Epiphanio E (1972) Ocorrência de *Batillipes mirus* Richters, 1909 e *B. tubernatis* Pollock, 1971 (Tardigrada) no litoral brasileiro. Ciência e Cultura 24 (6): 358–359.

Marcus E (1927) Zur Anatomie und Ökologie mariner Tardigraden. Zoologische Jahrbücher Abteilung für Systematik, Ökologie und Geographie der Tiere 53: 487–558.

Maria TF, Vanaverbeke J, Vanreusel A, Esteves AM (2016a) Sandy beaches: state of the art of nematode ecology. Anais da Academia Brasileira de Ciências 88: 1635–1653. https://doi.org/10.1590/0001-3765201620150282

Maria TF, Wandeness AP, Esteves AM (2016b) State of the art of the meiofauna of Brazilian sandy beaches. Brazilian Journal of Oceanography 64: 17–26. https://doi.org/10.1590/S1679-875920160946064sp2

Nelson DR (2002) Current status of the Tardigrada: evolution and ecology. Integrative and Comparative Biology 42: 652–659. https://doi.org/10.1093/icb/42.3.652

Nelson DR, Guidetti R, Rebecchi L (2015) Phylum Tardigrada. In: Thorp J, Rogers DC (Eds.) Ecology and general biology: Thorp and Covich freshwater invertebrates. Academic Press, London, UK, 347–380. https://doi.org/10.1016/b978-0-12-385026-3.00017-6

Pollock LW (1971) On some British marine Tardigrada including two new species of *Batillipes*. Journal of the Marine Biological Association of the United Kingdom 51: 93–103. https://doi.org/10.1017/S0025315400006482

Quantum GIS Development Team (2020) Quantum GIS

geographic information system. Open Source Geospatial Foundation Project. http://www.qgis.org/.

Ramazzotti G (1962) Il Phylum Tardigrada. Memorie dell'Istituto Italiano di Idrobiologia 14: 1–595.

Richters F (1909) Tardigraden-Studien. Bericht der Senckenbergische Naturforschende Gesellschaft in Frankfurt am Main 40 (1–2): 28–49, pls. 1, 2.

Richters F (1926) Tardigrada. In: W Kükentahl, T Krumbach (Eds.) Handbuch der Zoologie. Vol. 3. Walter de Gruyter & Co., Berlin & Leipzig, Germany, 58–61

Santos E, da Rocha CM, Gomes-Jr E, Fontoura P (2017) Three new *Batillipes* species (Arthrotardigrada: Batillipedidae) from the Brazilian coast. Zootaxa 4243: 483–502. https://doi.org/10.11646/zootaxa.4243.3.4

Santos E, Rubal M, Veiga P, da Rocha CM, Fontoura P (2018) *Batillipes* (Tardigrada, Arthrotardigrada) from the Portuguese coast with the description of two new species and a new dichotomous key for all species. European Journal of Taxonomy 425: 1–32. https://doi.org/10.1080/17451000.2015.1024133

Santos E, Veiga P, Rubal M, Bartels PJ, da Rocha CM, Fontoura P (2019a) *Batillipes pennaki* Marcus, 1946 (Arthrotardigrada: Batillipedidae): deciphering a species complex. Zootaxa 4648: 549–567. https://doi.org/10.11646/zootaxa.4648.3.9

Santos E, Rubal M, Veiga P, Bartels PJ, da Rocha CM, Fontoura P (2019b) On the distribution of *Batillipes tubernatis* Pollock, 1971 (Arthrotardigrada: Batillipedidae) in the Atlantic Basin. Marine Biodiversity 49 (2): 621–631. https://doi.org/10.1007/s12526-017-0834-9

Vishnudattan N, Rubal M, Nandan SB (2023) A new species of *Batillipes* (Arthrotardigrada: Batillipedidae) from the mid littoral zone of the southeast coast of India. Zootaxa 5346: 163–172. https://doi.org/10.11646/zootaxa.5346.2.4