

# A closer look at *Batillipes dicrocercus* and new records of *Batillipes potiguarensis* (Tardigrada: Arthrotardigrada) from the Brazilian coast with comments on intra- and interspecific variability

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**ABSTRACT.** *Batillipes* specimens collected in the Southwestern Atlantic Ocean, on the Brazilian Coast (Campos Basin and Potiguar Basin) with a bifurcated caudal appendage similar to *B. dicrocercus* Pollock, 1970 were assigned to *B. potiguarensis* Santos, da Rocha, Gomes Jr & Fontoura, 2017. This species was described as having a roundish caudal protrusion but, as shown in this study, it exhibits intraspecific variability of the morphology of the caudal apparatus. The examination of specimens of *B. dicrocercus* from the type locality (Massachusetts, U.S.A.) and the analysis of the original description and two subsequent emended descriptions, one from the Gulf of Mexico (U.S.A.) and another from the Mediterranean Sea, used for comparison, revealed important differences among them concerning important taxonomic characters, suggesting that what was considered a single species should now be split into three different species. Therefore, an emended diagnosis of *B. potiguarensis* and comments on the geographic distribution of *B. dicrocercus* are provided, clarifying the taxonomic status of the two species.

**KEYWORDS.** Batillipedidae, Biogeography, meiofauna, Atlantic Ocean, Taxonomy.

**RESUMO.** Considerações sobre *Batillipes dicrocercus* e novos registros de *Batillipes potiguarensis* (Tardigrada: Arthrotardigrada) da costa brasileira, com foco na variabilidade intra- e interespecífica. Exemplares do gênero *Batillipes* provenientes do sudoeste do Oceano Atlântico na Costa Brasileira (Bacia de Campos e Bacia Potiguar), apresentando um apêndice caudal bifurcado muito semelhante ao de *B. dicrocercus* Pollock, 1970, foram identificados como pertencendo a *B. potiguarensis* Santos, da Rocha, Gomes Jr & Fontoura, 2017, uma espécie caracterizada por exibir uma protrusão caudal arredondada. A identificação baseou-se, como demonstrado neste estudo, que em *B. potiguarensis* a morfologia do aparato caudal apresenta variabilidade intraespecífica. O exame de exemplares de *B. dicrocercus* da localidade-tipo (Massachusetts, E.U.A.) e a análise da descrição original e duas descrições subsequentes, uma baseada em exemplares do Golfo do México (U.S.A.) e outra em exemplares do Mar Mediterrâneo, efetuadas para comparação, revelou diferenças importantes no que diz respeito a caracteres taxonômicos relevantes. Este resultado sugere que aquelas descrições correspondem não a uma, mas sim a três espécies distintas. Assim, neste trabalho, completa-se a diagnose de *B. potiguarensis* e tecem-se comentários sobre a distribuição geográfica de *B. dicrocercus*, contribuindo para o esclarecimento do status taxonômico destas espécies.

**PALAVRAS-CHAVE.** Batillipedidae, Biogeografia, meiofauna, Oceano Atlântico, Taxonomia.

The interstitial heterotardigrade genus *Batillipes* was erected with the description of *Batillipes mirus* Richters, 1909 from the Baltic Sea (Northeastern Atlantic Ocean). Currently, 36 species are known (KRISTENSEN & MACKNESS, 2000; GUIDETTI & BERTOLANI, 2005; DEGMA & GUIDETTI 2007; DEGMA *et al.*, 2009-2018) from which eight were recorded from Brazil (SANTOS *et al.*, 2017). One of these species, *Batillipes dicrocercus* was described by POLLOCK (1970) from the Northwest Atlantic Ocean. The description was based on specimens collected in intertidal sand at 5–25 cm depth, from Crane's Beach, Penzance Point, Woods Hole, Massachusetts, United States of America. Subsequently, the

species has been recorded from many other localities and reported from different types of sand not only in the intertidal but also in the subtidal zone at depths of about 80–100 m (*cf.* DA ROCHA *et al.*, 2013; KACZMAREK *et al.*, 2015): from the Atlantic Ocean (Gulf of Mexico by MCKIRDY, 1975; Brazil by DA ROCHA *et al.*, 2009, 2013; Galicia, North of Spain by VEIGA *et al.*, 2009); Mediterranean Basin (by D'ADDABBO GALLO *et al.*, 1978, 2007; GRIMALDI DE ZIO *et al.*, 1980, 1983; MAUCCI & DURANTE-PASA, 1984; GALLO D'ADDABBO *et al.* 2000; DE ZIO GRIMALDI & D'ADDABBO GALLO, 2001; DE ZIO GRIMALDI *et al.* 2003; ACCOGLI *et al.*, 2011); Baltic Sea (HUMMON, 1994), and more recently (MILLER *et al.*,

2014) from the Pacific Ocean, the only record outside the Atlantic Basin.

The main attributes characterizing *B. dicrocercus* are the presence of a furcate caudal appendage and prominent lateral body projections between legs III and IV (POLLOCK, 1970). Traditionally, the morphology of the caudal apparatus was the main distinguishing trait used in the taxonomy of the genus *Batillipes* (cf. RAMAZZOTTI & MAUCCI, 1983) but, nowadays, it is recognized that within the genus, the morphology of this character exhibits considerable intraspecific variability (e.g. MCKIRDY, 1975; KRISTENSEN, 1978; MORONE DE LUCIA *et al.*, 1988; GALLO D'ADDABBO *et al.*, 2000). Thus, and despite emended descriptions provided by MCKIRDY (1975) and GALLO D'ADDABBO *et al.* (2000), imprecisions still subsist and the probability of misidentification concerning *B. dicrocercus* is high. The record from the Baltic Sea (HUMMON, 1994) was questioned by ZAWIERUCHA *et al.* (2015) and attributed to *B. noerrevangi* Kristensen, 1978, and the erroneous report of MAUCCI & DURANTE-PASA (1984) for the Balearic Sea based on a drawing of a *Batillipes* with a furcate caudal appendage formerly identified as *B. mirus* Richters, 1909 by RODRIGUEZ-RODA (1947, 1952) (see RUBAL *et al.*, 2017) are examples of misidentifications.

In having a bifurcate caudal appendage and prominent fourth lateral body projections, *Batillipes* specimens from the Brazilian Coast were also formerly identified as *B. dicrocercus* (DA ROCHA *et al.*, 2009, 2013). However, during a recent survey of marine tardigrades in the Brazilian states of Rio de Janeiro and Rio Grande do Norte mixed populations of *Batillipes* specimens exhibiting both a bifurcate caudal appendage or a roundish caudal protrusion as observed in *B. potiguarensis* Santos, da Rocha, Gomes Jr & Fontoura 2017, were collected. This fact demanded a deeper morphological study of *B. potiguarensis*, a species recently described from Brazil (SANTOS *et al.*, 2017), in order to know if in this species the caudal apparatus also displays intraspecific variability or if the *Batillipes* from this collection belong to two distinct species. Moreover, a comparative study with *B. dicrocercus* was also necessary because the specimens with furcate caudal apparatuses could be attributed to this species, as it happened in the previously mentioned studies, or could belong to an undescribed species.

Thus, a closer look based on specimens of *B. dicrocercus* from the type locality has been carried out. On one hand, this study allowed us to assign the Brazilian specimens to *B. potiguarensis*. On the other hand, it revealed important differences concerning reliable taxonomic characters, such as toe arrangement pattern, toe-disc shape, shape and number of lateral body projections, morphology of sensorial appendages and cuticular sculpture, among the original description of *B. dicrocercus* (POLLOCK, 1970) and the two subsequent emended descriptions by MCKIRDY (1975) and GALLO D'ADDABBO *et al.* (2000), suggesting that what was considered a single species should now be split into three different species. Therefore, an emended diagnosis of *B. potiguarensis* and comments on the geographic distribution

of *B. dicrocercus* are provided, clarifying the taxonomic status of the two species.

## MATERIAL AND METHODS

Tardigrades of the genus *Batillipes* were obtained from subtidal sediment samples collected in 2009 by box corer in two localities of the Brazilian Coast (Southwestern Atlantic Ocean): Campos Basin (22°10'S, 40°20'W), Rio de Janeiro State, at 25 m depth, and Potiguar Basin (04°37'S, 36°45'W), Rio Grande do Norte State, at 5 m depth. In both localities, the sediment type was represented by bioclastic and litoclastic sands. Samples were preserved in 4% buffered formaldehyde for later study. At the laboratory, sediments were washed and sieved through a 40 µm mesh sieve. Tardigrades were sorted under a dissecting microscope and transferred to microslides with coverslips. Specimens were permanently mounted in pure glycerine with formalin (ca. 1%) or in glycerol that, after a period of several days to evaporate to glycerine, were sealed with nail varnish. Measurements, given in micrometers (µm), and photomicrographs were made under 100x oil immersion, using a Phase Contrast Microscope (PHC) and a Differential Interference Contrast Microscope (DIC), both equipped with digital cameras and using Zen Imaging Software.

For comparison, a paratype and two specimens of *B. dicrocercus* from Pollock's collection (from the type locality: Crane's Beach, Penzance Point, Woods Hole, Massachusetts, U.S.A., deposited in the Department of Biology, Warren Wilson College, Asheville, U.S.A.); specimens attributed to this species from some Northeast Brazilian States [Pernambuco, Ceará and Rio Grande do Norte (deposited in the collection of tardigrades – UFRPE, Brazil)], and the holotype and paratypes of *B. potiguarensis* (deposited in the collection of the Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal) were examined. In addition, the original description of *B. dicrocercus* (POLLOCK, 1970) and subsequent emended descriptions by MCKIRDY (1975), based on specimens from the Gulf of Mexico, Florida (U.S.A.), and by GALLO D'ADDABBO *et al.* (2000), based on specimens from Tremiti Island, Adriatic Sea (Mediterranean Sea) were also used.

## RESULTS

### *Batillipes potiguarensis* Santos, da Rocha, Gomes Jr & Fontoura, 2017

Material examined: 35 specimens (18 females, 5 males and 12 juveniles) collected in Campos Basin; and 29 specimens (11 females, 5 males and 13 juveniles) collected in Potiguar Basin, both stations located on the Brazilian Coast.

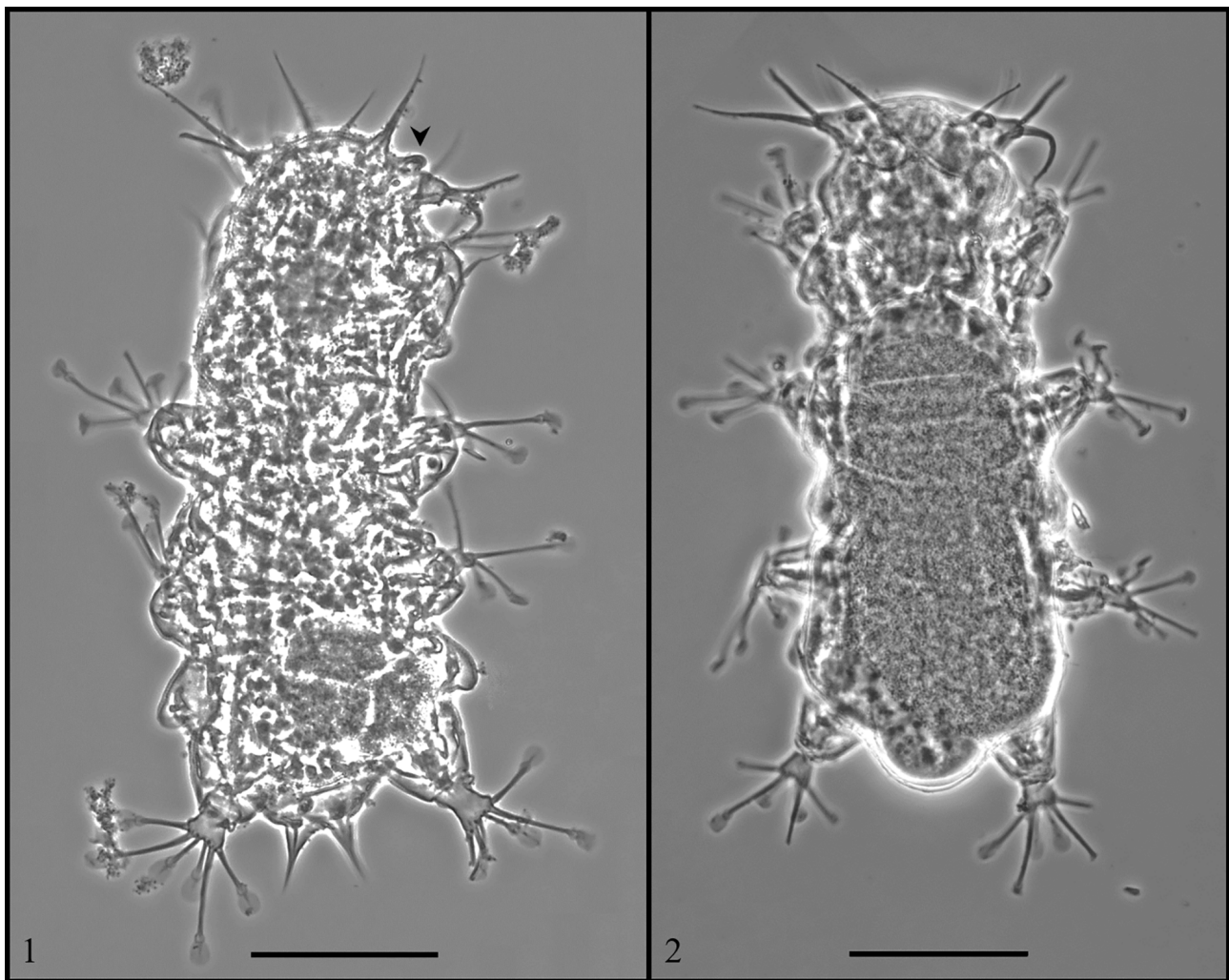
Description of the examined specimens. *Batillipes* specimens with distinct head separated from the body by a neck constriction. Scapular region well developed, protruding laterally at the level of the first pair of legs. Body length (caudal apparatus not included) between 105-257 µm (Fig.

1). Four pairs of blunt ventrolateral body processes are present: a lateral expansion between the head and the first pair of legs; two small lateral processes between the first three pairs of legs and well-developed blunt lateral processes between legs III and IV (Figs 1, 2). Occasionally, the blunt lateral body projections can present a small protruding tip. In some specimens, the lateral projection between the first and second pair of legs is not visible. The caudal apparatus exhibits high variability: in the majority of the specimens it is bifurcated, composed of two spines inserted in a swollen base (Figs 1, 3). In some specimens, the spines are two short spikes of about 9.7  $\mu\text{m}$ , while other specimens exhibit two longer spines, reaching 23.8  $\mu\text{m}$ . In a few specimens the caudal apparatus is asymmetric and only a single small spike is present (Fig. 4, 5) or it is restricted to a conspicuous semicircle-shaped protrusion without any spikes or spines at all (Fig. 2, 6).

Dorsal cuticle punctated, with evident transverse folds (Fig. 7). Dorso-lateral punctation larger (about 7-10 pillars / 10  $\mu\text{m}$ , and each pillar with about 1  $\mu\text{m}$  high) and more widely spaced than mid-dorsal punctation (about 12-

15 pillars / 10  $\mu\text{m}$ ) (Fig. 7). In the lateral processes between legs III and IV the cuticle is punctated. The ventral surface, also with transverse folds, is more finely punctated.

Head bearing eleven cephalic appendages. The median cirrus, with cirrophore. Internal cirri inserted dorsally on the frontal edge of the head with cirrophores. External cirri with indistinct cirrophores, inserted more ventrally, near the lateral cirri *A* and primary clavae. The lateral cirrus is located dorsally in relation to the unconstricted, but wrinkled, tubular primary clava. These two appendages share a common pedestal. A van der Land's organ is present at the base of the primary clava that exhibits a terminal pore. In the frontal edge of the head, well-developed papillary secondary clavae are visible (Fig. 1, arrowhead). All cephalic cirri, including external cirri, and lateral cirri have an optically dense dot (dark when observed under PHC) near their swollen tips. Indentations between the external cephalic cirrus and the pedestal bearing the primary clava and lateral cirrus were not seen.



Figs 1,2. *Batillipes potiguarensis* Santos, Rocha, Gomes Jr & Fontoura, 2017: 1, specimen from our samples with a bifurcated caudal appendage. The arrowhead shows the well-developed secondary clava; 2, holotype with a rounded caudal protrusion. Scale bars: 50  $\mu\text{m}$ .

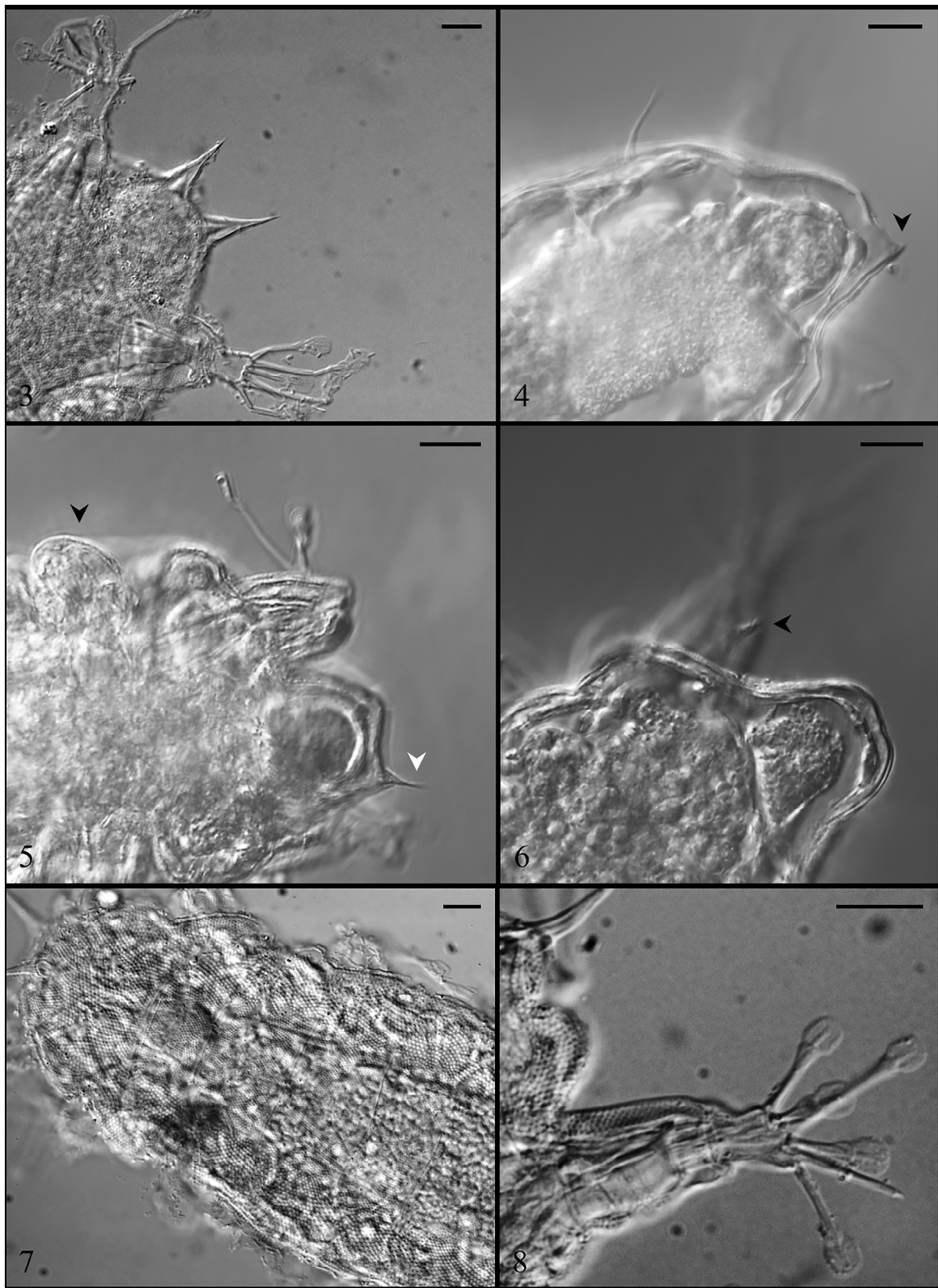


Fig. 3-8. *Batillipes potiguarensis* Santos, Rocha, Gomes Jr & Fontoura, 2017: 3, detail of a bifurcated caudal appendage; 4, posterior portion of the body, showing an asymmetric caudal appendage with one short spike only (arrowhead); 5, posterior portion of the body showing an asymmetric caudal appendage with one short spine only (white arrowhead). The black arrowhead shows the blunt lateral projection between legs III–IV; 6, caudal appendage without spines. The sense organ on leg IV is visible (arrowhead); 7, dorsal cuticle with visible folds and punctation not uniformly distributed; 8, detail of the first leg showing the oblique tarsus and spatula-like toe discs. Scale bars: 10  $\mu$ m.

Eye spots not observed. Ventral mouth opening in a protruded cone. Ovoid pharyngeal bulb. Placoids not visible after slide mounting.

Sense organs with swollen tips on legs I and IV and spines on legs II and III. They are similar in length in the first three pair of legs (rarely sense organ on leg I is shorter than on legs II and III). The sense organ on leg IV is consistently the shortest and divided into a cirrophore and a distal portion with a basal van der Land's organ (Fig. 6, arrowhead). Sharply pointed cirri *E* are present (Fig. 4).

Telescopic legs with long toes with spatula-like suction discs with straight frontal edges and with short and thin braces (Figs 1, 8). The frontal edge of tarsi is oblique in the first three pair of legs, with toes inserted at different levels (Fig. 8). In these three first pairs of legs, toe 2 is the shortest, toes 3 and 5 are the longest and toes 1, 4 and 6 are medium sized (Fig. 1). In the fourth pair of legs, medial toes 3 and 4 are of equal lengths (toe arrangement pattern A, according to SANTOS *et al.*, 2018). Toes 2 and 5 are the longest and similar to each other; toes 1 and 6, also similar to each other, are of intermediate length (Figs 1, 2, 8).

Tab. I. Measurements (in  $\mu\text{m}$ ) of selected morphological structures of specimens of *Batillipes potiguarensis* Santos, Rocha, Gomes Jr & Fontoura, 2017 (SD, Standard deviation. Range refers to the smallest and largest measured specimen/structure; N, number of specimens/structures measured).

STRUCTURES	Females Adults This study	Males adults This study	Juveniles 6-digit This study	Females SANTOS <i>et al.</i> , 2017
	Mean $\pm$ SD (Range); N	Mean $\pm$ SD (Range); N	Mean $\pm$ SD (Range); N	Mean $\pm$ SD (Range); N
Body length (BL)	195.3 $\pm$ 30.41 (146.3-243.3); 29	200.4 $\pm$ 38.65 (140.7-261.4); 10	207.5 $\pm$ 33.70 (125.4-281.2); 25	184.5 $\pm$ 18.04 (152.2-201.1); 6
BL without caudal apparatus	179.3 $\pm$ 31.03 (130.0-228.1); 29	182.6 $\pm$ 38.23 (126.6-243.4); 10	191.4 $\pm$ 34.55 (105.8-257.0); 25	–
Body width	67.5 $\pm$ 13.61 (48.7-99.7); 21	66.4 $\pm$ 14.02 (53.9-88.9); 8	71.9 $\pm$ 15.21 (48.2-100.4); 15	64.5 $\pm$ 11.26 (47.9-78.1); 6
Median cirrus	18.1 $\pm$ 4.36 (10.4-27.0); 21	20.5 $\pm$ 4.12 (14.7-26.1); 7	20.0 $\pm$ 3.17 (11.3-26.6); 20	16.0 $\pm$ 4.20 (10.4-20.5); 4
Internal cirri	20.4 $\pm$ 3.24 (13.3-25.7); 25	20.1 $\pm$ 4.18 (14.8-25.6); 10	21.9 $\pm$ 3.85 (12.2-27.8); 23	20.5 $\pm$ 1.93 (17.0-22.5); 6
External cirri	17.2 $\pm$ 3.09 (10.6-21.6); 25	16.8 $\pm$ 2.74 (12.6-20.0); 8	17.4 $\pm$ 2.85 (11.8-21.9); 24	16.0 $\pm$ 3.26 (12.6-20.1); 5
Lateral cirri <i>A</i>	33.0 $\pm$ 5.14 (24.0-43.3); 23	31.4 $\pm$ 8.30 (20.0-46.0); 9	32.2 $\pm$ 5.41 (23.0-44.2); 21	28.8 $\pm$ 4.27 (22.7-35.8); 6
Primary clavae	17.9 $\pm$ 3.07 (13.0-22.6); 27	17.7 $\pm$ 5.13 (10.0-26.1); 9	18.8 $\pm$ 3.07 (12.0-24.9); 23	16.5 $\pm$ 2.26 (13.4-19.4); 6
Secondary clavae	4.9 $\pm$ 0.66 (3.8-6.5); 27	4.5 $\pm$ 1.00 (3.1-6.6); 8	5.2 $\pm$ 0.92 (2.8-6.8); 20	5.1 $\pm$ 1.05 (3.7-6.2); 5
Cirri <i>E</i>	19.3 $\pm$ 3.62 (14.2-25.4); 14	19.0 $\pm$ 4.17 (12.3-25.3); 6	17.6 $\pm$ 5.25 (8.7-27.3); 14	20.3 $\pm$ 2.27 (17.0-22.8); 5
Leg I sense organ	9.8 $\pm$ 2.11 (5.1-14.0); 24	10.1 $\pm$ 2.45 (7.6-13.8); 7	10.1 $\pm$ 1.79 (7.0-12.8); 21	10.0 $\pm$ 0.93 (8.3-10.9); 6
Leg II sense organ	10.8 $\pm$ 2.85 (5.7-18.6); 21	9.7 $\pm$ 3.01 (6.9-14.9); 7	10.7 $\pm$ 3.28 (6.7-19.0); 21	9.8 $\pm$ 2.51 (7.3-13.5); 5
Leg III sense organ	10.6 $\pm$ 2.14 (7.0-14.5); 16	10.2 $\pm$ 3.12 (6.7-14.2); 6	10.5 $\pm$ 3.63 (6.3-17.2); 17	10.6 $\pm$ 2.64 (7.2-13.1); 4
Leg IV sense organ	8.9 $\pm$ 1.65 (6.1-11.9); 26	7.4 $\pm$ 1.15 (5.8-9.2); 8	9.4 $\pm$ 1.60 (6.0-12.3); 22	8.4 $\pm$ 1.9 (6.6-11.1); 6
Body projection 1	7.0 $\pm$ 1.41 (4.2-9.5); 27	6.2 $\pm$ 2.04 (3.4-8.5); 8	7.5 $\pm$ 1.58 (5.0-10.8); 23	6.0 $\pm$ 1.62 (3.2-7.8); 6
Body projection 2	6.3 $\pm$ 2.13 (3.3-11.4); 22	5.0 $\pm$ 1.65 (3.5-7.7); 7	6.3 $\pm$ 0.97 (5.0-8.8); 18	4.4 $\pm$ 1.47 (2.5-6.1); 4
Body projection 3	9.0 $\pm$ 2.43 (5.2-15.1); 27	7.7 $\pm$ 2.50 (5.1-12.7); 7	9.3 $\pm$ 1.73 (6.2-12.3); 22	5.5 $\pm$ 1.30 (3.8-7.4); 5
Body projection 4	10.4 $\pm$ 2.11 (6.2-14.7); 27	10.5 $\pm$ 2.9 (6.6-16.7); 9	10.2 $\pm$ 2.44 (6.4-15.0); 25	9.0 $\pm$ 1.52 (6.6-10.8); 5
Body projection 4 width	15.0 $\pm$ 3.22 (10.2-23.8); 26	12.2 $\pm$ 3.68 (8.5-19.3); 8	15.8 $\pm$ 3.12 (10.5-21.9); 23	–
Caudal apparatus (spines)	17.2 $\pm$ 2.65 (9.7-23.0); 27	17.8 $\pm$ 3.34 (13.5-23.8); 10	18.3 $\pm$ 4.01 (13.3-31.7); 22	–
Legs I-III toe 1	13.4 $\pm$ 2.73 (9.8-22.2); 20	13.5 $\pm$ 3.56 (9.7-18.6); 7	14.3 $\pm$ 2.26 (10.0-18.1); 21	10.9 $\pm$ 0.50 (10.3-11.5); 4
Toe 2	9.2 $\pm$ 1.99 (6.8-13.1); 14	9.0 $\pm$ 2.47 (5.0-11.0); 7	10.0 $\pm$ 2.06 (5.7-14.7); 19	8.2 $\pm$ 1.83 (6.5-10.8); 4
Toe 3	21.4 $\pm$ 3.10 (17.2-28.2); 20	19.7 $\pm$ 4.98 (14.6-27.1); 7	22.3 $\pm$ 3.40 (13.7-28.1); 23	18.6 $\pm$ 1.06 (17.6-19.7); 4
Toe 4	13.3 $\pm$ 1.87 (11.7-18.1); 17	12.6 $\pm$ 3.81 (8.5-16.8); 7	13.3 $\pm$ 2.52 (6.7-17.0); 22	11.7 $\pm$ 1.93 (9.5-14.2); 4
Toe 5	24.2 $\pm$ 3.76 (18.2-31.6); 18	25.0 $\pm$ 6.02 (18.2-32.9); 7	25.2 $\pm$ 3.69 (17.0-31.8); 23	21.8 $\pm$ 1.43 (20.6-23.4); 3
Toe 6	16.6 $\pm$ 2.52 (12.3-21.0); 17	16.5 $\pm$ 2.88 (12.3-20.5); 7	17.1 $\pm$ 2.00 (12.2-20.7); 21	14.0 $\pm$ 1.38 (12.4-15.0); 3
Leg IV toe 1	18.6 $\pm$ 2.81 (14.6-23.6); 20	18.8 $\pm$ 2.80 (16.0-23.8); 6	19.8 $\pm$ 2.46 (16.7-24.3); 18	– (17.5-20.4); 2
Toe 2	26.9 $\pm$ 4.09 (19.2-33.5); 23	28.0 $\pm$ 4.62 (22.1-35.2); 6	28.7 $\pm$ 5.37 (12.8-39.1); 18	– (25.6); 1
Toe 3	14.7 $\pm$ 2.84 (8.1-20.1); 21	15.0 $\pm$ 2.93 (11.6-19.8); 6	16.0 $\pm$ 2.11 (11.0-20.0); 16	13.3 $\pm$ 2.70 (10.3-15.5); 3
Toe 4	14.6 $\pm$ 2.67 (8.5-19.0); 21	14.9 $\pm$ 2.97 (12.2-20.0); 6	16.0 $\pm$ 2.80 (11.5-24.1); 16	13.4 $\pm$ 2.60 (10.6-15.7); 3
Toe 5	28.1 $\pm$ 4.45 (18.0-34.3); 22	26.6 $\pm$ 3.02 (22.8-30.0); 5	28.8 $\pm$ 3.39 (20.0-33.4); 19	– (25.7-27.5); 2
Toe 6	18.9 $\pm$ 2.10 (16.0-22.4); 20	18.3 $\pm$ 2.72 (15.4-22.7); 6	19.2 $\pm$ 2.48 (15.3-24.0); 17	18.8 $\pm$ 1.01 (17.1-19.1); 3
Gonopore-Anus	11.6 $\pm$ 1.93 (8.9-16.0); 21	5.1 $\pm$ 2.11 (3.4-10.4); 9	–	9.0 $\pm$ 2.21 (6.6-11.1); 5

In females, the gonopore is rosette-shaped delimited by four punctate cuticular platelets, two anterior and two posterior, forming a groove running toward the anus. Males have a circular gonopore with a cuticular crescent shaped fold.

Four-toed larvae were not found.

Morphometric data are provided in Table I.

## DISCUSSION

Based on the bifurcated aspect of caudal apparatuses, the examined specimens from Brazil could be easily confused with *B. dicrocercus*. The original description of *B. dicrocercus*, based on specimens from the Northwest Atlantic Ocean (Massachusetts, U.S.A.), is very incomplete and the main trait used to distinguish the species was, in fact, the bifurcated caudal apparatus (POLLOCK, 1970). The scarce information provided in that description and the high intra and interspecific variability of the morphology of the caudal apparatus observed in *Batillipes* (MCKIRDY, 1975; GALLO D'ADDABBO *et al.*, 2000; KRISTENSEN & MACKNESS, 2000) justified the subsequent search for a reliable diagnosis. With

this goal, MCKIRDY (1975) provided an emended diagnosis of *B. dicrocercus* based on specimens recorded from the Gulf of Mexico (Florida, U.S.A.) and, later on, GALLO D'ADDABBO *et al.* (2000) redescribed the species using specimens from the Adriatic Sea (Mediterranean Sea). However, a closer look at those emended descriptions, the examination of specimens from the type locality, including a paratype of *B. dicrocercus*, and their comparison with the Brazilian specimens, performed in the present study, showed that these are actually four different *Batillipes* species that can be distinguished by reliable taxonomic characters (Tab. II).

Specimens from the Gulf of Mexico examined by MCKIRDY (1975), from now on designated *B. dicrocercus sensu* McKirdy, are very different from the specimens from the Northwestern Atlantic (Massachusetts) described by POLLOCK (1970), here designated *B. dicrocercus sensu stricto* (Fig. 9), and clearly belong to a different unnamed species. Contrary to *B. dicrocercus sensu stricto*, the species described by MCKIRDY (1975) has frayed cephalic cirri and leg IV sense organs; fourth foot with medial toes of different lengths (toe 4 longer than toe 3, being toe 3 the

Tab. II. Differences and similarities among populations attributed to *Batillipes dicrocercus* Pollock, 1970 and *B. potiguarensis* Santos, Rocha, Gomes Jr & Fontoura, 2017 (MED, Mediterranean Sea; NEA, Northeast Atlantic; NWA, Northwest Atlantic; SWA, Southwest Atlantic). For quantitative data the values refer to mean  $\pm$  standard deviation.

	<i>Batillipes dicrocercus</i>			<i>Batillipes potiguarensis</i>
	<i>sensu stricto</i> POLLOCK, 1970	<i>sensu</i> MCKIRDY, 1975	<i>sensu</i> GALLO D'ADDABBO <i>et al.</i> , 2000	This study
Ecoregion,	NWA, cold temperate	NWA, warm temperate	NEA, MED, temperate	SWA, tropical
Country	U.S.A.	U.S.A.	Italy	Brazil
State/Coastal region	Massachusetts	Florida (Gulf of Mexico)	Adriatic Sea/Tritimi Island	Rio Grande Norte/Rio Janeiro
Zone	Intertidal	Intertidal	Subtidal	Subtidal
	N=7	N=6	N=9	N=64
Body length	167.5 $\pm$ 9.06	150.3 $\pm$ 10.29	158.3 $\pm$ 24.51	184.5 $\pm$ 33.52
Body shape	increasing posteriorly	increasing posteriorly	increasing posteriorly	scapular protruded
Cephalic appendages	single tipped	frayed	single tipped	single tipped
Median cirrus length	23.7 $\pm$ 5.40	26.7 $\pm$ 3.80	23.4 $\pm$ 3.01	19.3 $\pm$ 3.92
Internal cirri length	22.2 $\pm$ 1.73	26.0 $\pm$ 3.80	24.1 $\pm$ 5.56	20.9 $\pm$ 3.67
External cirri length	13.7 $\pm$ 2.44	15.31 $\pm$ 1.79	19.9 $\pm$ 3.22	17.2 $\pm$ 2.90
Lateral cirri A length	33.0 $\pm$ 9.07	47.1 $\pm$ 4.47	34.9 $\pm$ 8.07	32.2 $\pm$ 5.97
Primary clavae length	15 $\pm$ 2.07	19.8 $\pm$ 1.57	17.6 $\pm$ 4.53	18.2 $\pm$ 3.41
Secondary clavae	not observed	?	kidney-shaped	papillary
Cirri E length	17.9 $\pm$ 3.67	20.5 $\pm$ 1.41	26.4 $\pm$ 2.70	18.5 $\pm$ 4.40
Dorsal papillae on legs	absent	present	absent	absent
Leg sense organs (relative size)	I < II < III < IV	I < II < III < IV	III < II $\leq$ I < IV	I $\approx$ II $\approx$ III > IV
Sense organ Leg IV (length)	23.3 $\pm$ 3.69	35.0 $\pm$ 2.45	17.8 $\pm$ 4.60	8.9 $\pm$ 1.67
Leg IV sense organ	single tipped	with distal threads	single tipped	single tipped
Lateral process on leg IV	present	absent	absent	absent
Projections between legs I–III	barely visible, conical	? (probably absent)	conspicuous, conical with point	present, blunt
Projection between legs III–IV	acute conical	present	conical with blunt pointed tip	blunt, a few with a pointed tip
Caudal spikes length	17.3 $\pm$ 2.79	17.4 $\pm$ 1.57	15.9 $\pm$ 4.34	17.7 $\pm$ 3.31
Caudal spike base	swollen	moderately swollen	not swollen	swollen
Toes 3, 4 on legs IV	toe 3 = 4	toe 3 < 4	toe 3 = 4	toe 3 = 4
Toe disc shape	circular	circular	oval	spatula-like
Tarsi on legs I–III	straight	?	straight	oblique
Cuticular punctation	uniformly distributed	dorso-lateral > cephalic and caudal	uniformly distributed	dorso-lateral > mid-dorsal
	12–13/10 $\mu$ m	8–9/10 $\mu$ m > 10–11/10 $\mu$ m	?	7–10/10 $\mu$ m > 11–15/10 $\mu$ m
Female gonopore (platelets)	?	?	2 posterior	2 anterior and 2 posterior

most cephalic, corresponding to toe arrangement pattern B, subgroup B1, according to SANTOS *et al.* 2018); dorsal cuticle not uniformly punctated (mid-dorsal cuticle with smaller punctations than cephalic and caudal dorsal areas); and a small dorsal papilla is present on each leg. In addition, an evident indentation located on the anterior edge of the head, between external cirri and pedestals bearing primary clava and lateral cirrus A, is visible in *B. dicrocercus sensu* McKirdy, and lateral processes on legs IV are absent; while in *B. dicrocercus sensu stricto* the indentation in the frontal edge of the head is barely visible or absent (Fig. 10), and a lateral process is present on the lateral surface of legs IV [this lateral process, although represented in the original drawing, was not described by POLLOCK (1970), but its presence was confirmed in this study, see Fig. 11]. Although not considered a relevant distinguishing character, specimens from Florida have slightly longer cephalic appendages (Tab. II) as emphasized by MCKIRDY (1975). The two species share the same body shape and both exhibit toes with circular

discs. On the other hand, in both species leg sense organs increase in length from legs I to IV, where they are particularly long (Fig. 11). The female gonopore was not described for the specimens from Massachusetts nor for the specimens from Florida. Unfortunately, in the reexamined specimens from Pollock's collection, the gonopore was not visible. Therefore, this important taxonomic character cannot be used for comparison.

Despite having a similar morphometry, qualitative morphological characters of *B. dicrocercus sensu* Gallo D'Addabbo *et al.* from the Adriatic Sea (GALLO D'ADDABBO *et al.*, 2000), do not match with *B. dicrocercus sensu stricto* either. The presence of peculiar kidney-shaped secondary clavae (secondary clavae are indistinct in *B. dicrocercus sensu stricto*, see Fig. 10); long toes with elongated ovoid suction discs; conspicuous lateral conical body projections between all pairs of legs (very small and difficult to see, when present, between the first three pairs of legs in *B. dicrocercus sensu stricto*); evident indentation in the frontal edge of the head;

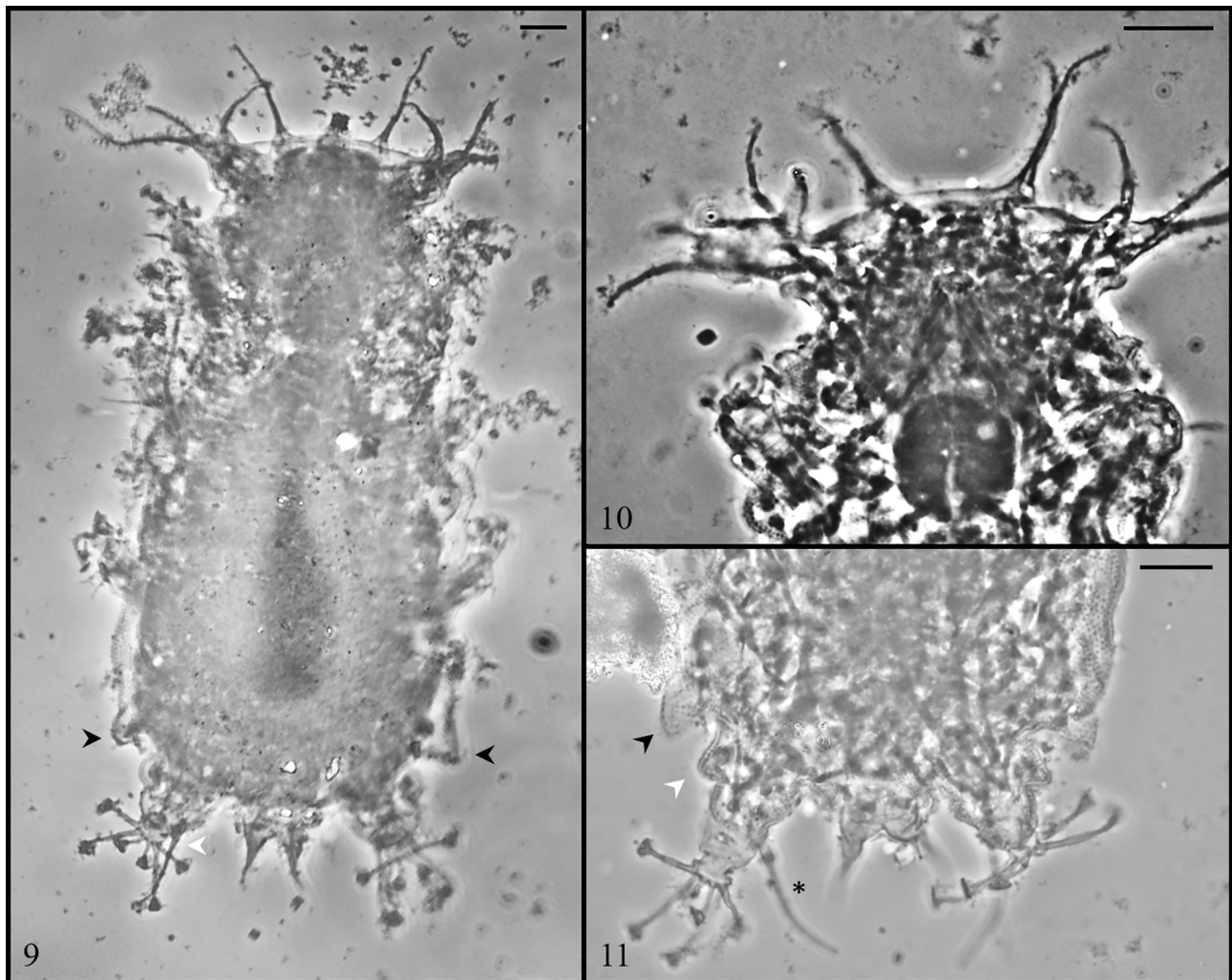


Fig. 9-11. *Batillipes dicrocercus* Pollock, 1970: 9, paratype. Conical body projections between legs III–IV (black arrowhead) and sense organ on leg IV (white arrowhead) are visible; 10, anterior region of a specimen showing the head and cephalic appendages. 11, posterior region of the body showing long sense organ on leg IV (asterisk), conical body projections between legs III–IV (black arrowhead) and lateral processes on legs IV (white arrowhead). Scale bars: 10  $\mu$ m.

and the lack of lateral processes on legs IV are differences strong enough to prevent the assignment of the specimens from the Adriatic Sea to *B. dicrocercus*. Moreover, despite variability and contrary to *B. dicrocercus sensu stricto*, in *B. dicrocercus sensu* Gallo D'Addabbo *et al.* caudal apparatuses can display many different aspects (they can have one, two or even more points, see fig. 2A in GALLO D'ADDABBO *et al.*, 2000) and are not inserted into a swollen base.

Obviously, *B. dicrocercus sensu* McKirdy and *B. dicrocercus sensu* Gallo D'Addabbo *et al.* are also different species that can be easily distinguished by toe disc shape, relative toe lengths on leg IV, morphology of cephalic appendages, presence of secondary clavae, distribution of the dorsal cuticle punctation and development and shape of lateral body projections (see Tab. II).

It is interesting to note that the specimens on which the three above referred descriptions were based were found in very different ecological contexts, reinforcing the probability they belong to different species: *B. dicrocercus sensu stricto* in the intertidal from the cold temperate Northwest Atlantic; *B. dicrocercus sensu* McKirdy also in the intertidal but from the warm temperate Northwest Atlantic, in the transition to the tropical Atlantic; and *B. dicrocercus sensu* Gallo D'Addabbo *et al.* in the subtidal from the temperate Mediterranean Sea.

Subtidal specimens examined in this study from Rio de Janeiro and Rio Grande do Norte states, tropical Southwest Atlantic, are similar to specimens previously recorded by DA ROCHA *et al.* (2009, 2013) in the Brazilian states of Pernambuco, Ceará, and also Rio Grande do Norte, and attributed to *B. dicrocercus*. However, despite having a bifurcated caudal apparatus inserted in a swollen base, they cannot be assigned to any of the above descriptions attributed to *B. dicrocercus*. These specimens differ from the specimens from Massachusetts, Florida and Adriatic Sea in having a different body shape due to their protruded scapular region; evident papillary secondary clavae; very short sensorial organs on leg IV (despite longer body size) that are shorter than the sensorial spines on the other legs; oblique tarsi on the first three pairs of legs, and spatula-like toe discs (Tab. II, Figs 1, 2). In addition, Brazilian specimens can be distinguished from: (1) *Batillipes dicrocercus sensu stricto* by the absence of lateral processes on legs IV, by the different-shaped lateral projections that are blunt, and by having the mid-dorsal cuticular punctation smaller than dorsolateral; (2) *Batillipes dicrocercus sensu* McKirdy in having single tipped cephalic sensorial appendages, different-shaped lateral projections that are blunt, middle toes on leg IV equal in length, caudal apparatus inserted in a more developed swollen base, and by the absence of papillae on legs; (3) *Batillipes dicrocercus sensu* Gallo D'Addabbo *et al.* in having the mid-dorsal cuticular punctation smaller than the dorsolateral punctation, different-shaped lateral projections that are blunt (conical with a distal point in *B. dicrocercus sensu* Gallo D'Addabbo *et al.*), and a different female gonopore that is delimited by four punctate cuticular platelets (only two in *B. dicrocercus sensu* Gallo D'Addabbo *et al.*).

Given that the specimens recorded from Brazil do not correspond to *B. dicrocercus*, we initially hypothesized that they might belong to a new undescribed species. However, when comparing the morphology of these specimens with specimens of other known species, a remarkable similarity with *B. potiguarensis* was noted (see Tab. I for morphometric data). The only difference is the caudal apparatus that in the original description of *B. potiguarensis* was noted as a conspicuous semicircle-shaped protrusion (14–23 µm long) and not bifurcated (Fig. 2). The oblique shape of tarsi on the three first pairs of legs (also present in *B. spinicauda* Gallo D'Addabbo, Sandulli & de Zio Grimaldi, 2005) and dorsolateral punctations larger than mid-dorsal punctations observed in the specimens with bifurcated caudal apparatuses, were characters previously neglected by SANTOS *et al.* (2017) in the original description, but they are also present in *B. potiguarensis* as confirmed in this study. On the other hand, specimens of different gender and age (males were not known until now), with and without bifurcated caudal appendage, occurred simultaneously in time and space in samples from the Potiguar Basin (all specimens collected in Campos Basin had bifurcated caudal apparatuses). Otherwise, all other traits were identical among these specimens.

Ontogenetic variability of the caudal apparatus in *Batillipes* species has been reported by several authors (GRIMALDI DE ZIO & D'ADDABBO GALLO, 1975; MCKIRDY, 1975; KRISTENSEN, 1978; MORONE DE LUCIA *et al.*, 1988; GALLO D'ADDABBO *et al.*, 2000; KRISTENSEN & MACKNESS, 2000) and although a prominent key character in some species (*e.g.* *B. phreaticus* Renaud-Debyser, 1959 and *B. bullacaudatus* McGinty & Higgins, 1968), it should be used with caution for systematic purposes. As recommended by MCKIRDY (1975) and GALLO D'ADDABBO *et al.* (2000), the institution of new species should be supported in several stable traits and not in only one highly intraspecifically variable character such as the caudal apparatus. Thereby, specimens from Brazil, examined in this study, with a bifurcated caudal apparatus, with two short or long spines, sometimes displaying asymmetry, having only one short spike, or with a conspicuous more or less semicircle-shaped caudal protrusion without any spines at all, are assigned to *B. potiguarensis*, and the following emended diagnosis is provided.

Specific diagnosis of *Batillipes potiguarensis* (emended): *Batillipes* with tubular undivided primary clavae and well developed papillary secondary clavae. Cephalic cirri with swollen distal tips. Sensorial spines on all legs. Sensorial organ on leg IV very short. Legs terminated by long toes with spatula-like suction discs. Medial toes (toes 3 and 4 of legs IV of equal lengths). Three first pairs of legs with oblique tarsi with digits inserted at different levels. A distinct head separated from the body by a neck constriction. Scapular region well-developed, protruding laterally at the level of the first pair of legs. Small lateral blunt processes between legs I–III. Well-developed blunt processes between legs III and IV. Caudal apparatus exhibiting high variability: it can be bifurcated, composed of two spines inserted in a swollen



base, in some specimens it is asymmetric with only a single small spike, or it can be a prominent more or less semicircular caudal projection without any spines. Cuticle finely but not uniformly punctated, dorsolateral punctation larger than mid-dorsal punctation, with transverse folds. Rosette-like female gonopore delimited by four punctate cuticular platelets. Males with circular gonopore with crescent-shaped cuticular fold.

Given this study and including the correction of the records of DA ROCHA *et al.* (2009, 2013), the distribution range of *B. potiguarensis*, up till now only known from the Southwestern Atlantic Ocean, from the Brazilian states of Rio Grande do Norte and Alagoas, has been expanded in the Northern Brazilian Coast to the states of Ceará and Pernambuco, and to the Southern Coast to the state of Rio de Janeiro.

The clarification of the taxonomic status of *B. dicrocercus* Pollock, 1970 (*sensu stricto*) has consequences for its geographic distribution. Taking into account the need to confirm the only record outside the Atlantic Basin, from the Pacific Ocean (California, U.S.A.), based on one single specimen (MILLER *et al.*, 2014), the distribution of the species is restricted to the Northwestern Atlantic Ocean (Massachusetts, U.S.A.).

Specimens of the only record from the North of Spain that, as reported by VEIGA *et al.* (2009) were identified using the redescription of GALLO D'ADDABBO *et al.* (2000), and all the records from the Mediterranean Sea should be assigned to the unnamed species described by GALLO D'ADDABBO *et al.* (2000). Therefore, this unnamed species is widely distributed in the Mediterranean Basin (Adriatic Sea, Balearic Sea, Ionian Sea and Tyrrhenian Sea) and in the Northeast Atlantic Ocean (South European Shelf). We suggest that until this species is described it should be indicated as *Batillipes dicrocercus sensu* Gallo D'Addabbo *et al.*

The distribution of the unnamed species described by MCKIRDY (1975) is restricted to the Gulf of Mexico (Florida, U.S.A.). We suggest that until this species is described it should be indicated as *Batillipes dicrocercus sensu* McKirdy.

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