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First record of the box crab *Cryptosoma balguerii* (Desbonne in Desbonne and Schramm, 1867) (Brachyura: Calappidae) from São Paulo, Brazil revealed by DNA markers

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ABSTRACT

The present study provided information extending the known geographical distribution of the calappid crab *Cryptosoma balguerii* (Desbonne in Desbonne and Schramm, 1867). We provide new data that extends the range of this species in the western Atlantic farther to the south in the Brazilian region, State of São Paulo and check its identification by molecular tools within *Cryptosoma* and other calappid crabs. New records of marine species provide a baseline for a better understanding of the biodiversity of ecological provinces. This work is an example of the importance of an exhaustive and continuous investigation on the biodiversity of subtidal rocky bottoms.

KEYWORDS

South Atlantic, Calappids, Biodiversity, Range extension, Molecular markers

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Introduction

Family Calappidae De Haan, 1833 is a group of marine crabs with distribution extending throughout the tropical and subtropical regions of the world (Bellwood, 1996). The phylogenetic relationships among *Calappa* Weber 1795, *Cryptosoma* Brullé, 1837 and *Cycloes* de Haan, 1841, *Paracyclois* Miers, 1886 and *Cyclozodion* Williams and Child, 1988, with a close proximity between *Cryptosoma* and *Cycloes,* were indicated based on morphological characters (Bellwood, 1998) and recently by molecular data (Ewers-Saucedo *et al.*, 2016). An obscure relationship was solved by a taxonomic revision which designated the genus name *Cryptosoma* for all the Atlantic species of the *Cycloes-Cryptosoma* complex, and *Cycloes* for all Indo-Pacific species (Galil and Clark, 1996).

The nomenclature of the genus *Cryptosoma* and *Cycloes* was reviewed. From this, *Cryptosoma* has four recognized species: *Cr. bairdii* (Stimpson, 1860), *Cr. balguerii* (Desbonne in Desbonne and Schramm, 1867), *Cr. cristatum* Brullé, 1937, and *Cr. garthi* Galil and Clark, 1996 (Galil and Clark, 1996; Ng *et al.*, 2008) and *Cycloes* is composed by *Cy. granulosa* De Haan, 1837 and *Cy. marisrubri* Galil and Clark, 1996 (Galil and Clark, 1996; Ng *et al.*, 2008). After these reviews, all western Atlantic records of *Cycloes bairdii* were attributed to *Cryptosoma balguerii* (Galil and Clark, 1996). Herein, we provide new data that extends the geographical distribution of *Cryptosoma balguerii* to the coast of the state of São Paulo (Brazil).

Material and Methods

During an exhaustive period of sampling along the coast of São Paulo state as part of a long-term project on marine biodiversity (Mantelatto *et al.*, 2018), a single adult calappid crab was collected in Ubatuba region in October 2019 by using a fishing boat with two double-rig nets, at 26 m depth. Specimen was kept alive, anesthetized and frozen on ice to be photographed after the collection in order to record the color pattern. After that, the crab was stored in 80% ethanol and deposited in the Coleção de Crustáceos do Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (CCDB/FFCLRP/USP): CCDB 6417. The crab was identified according to specific and current literature (Galil and Clark, 1996). We measured the carapace length (CL) and carapace width (CW) with a vernier caliper (0.01 mm) from the posterior to the anterior margin.

Molecular protocols

To carry out molecular identification, DNA was extracted from the muscle tissue of pereopods using two distinct methods, salting-out method (Miller *et al.*, 1988) and Chelating Ion Exchange Resin (Chelex VR 100) (Estoup *et al.*, 1996). Details on methods can be found in Mantelatto *et al.* (2018).

We performed polymerase chain reactions (PCR) on fragments of the mitochondrial 16S rDNA (16S) and cytochrome oxidase subunit I (COI) with appropriate primers 16S: 16L29 (5'-YGCCTGTTTATCAAAAACAT-3') and H9/16S-br (5'- CCGGTCTGAACTCAGATC ACGT-3) (Palumbi *et al.*, 1991; Klaus *et al.*, 2006); COI: COH6 (5'-TADACTTCDGGRTGDCCAAARAAYCA-3') and COL6b (5'-ACAAATCATAAAGATATYGG-3') (Schubart and Huber, 2006).

Successful PCR products were purified and sent for sequencing to the Departamento de Tecnologia da Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV), Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP).

All sequences were confirmed by sequencing both strands (forward and reverse directions). The consensus sequences ware obtained using the BioEdit 7.2.5 software (Hall, 1999). Primer regions and non-readable parts at the beginning of the sequences were omitted. All obtained sequences were deposited in the GenBank database.

Molecular data analysis

Sequences were compared to the sequence database collection known as GenBank using the basic local alignment tool (BLAST) as implemented on the NCBI website. In order to check the identification, we entered each sequence using the nblast section of BLAST and searched for the more similar sequences presented in the general sequence database. Once the search was completed, we confirmed that the results were organized by the closest identity.

We used a previous phylogenetic hypothesis (Bellwood, 1996; 1998; Ewers-Saucedo *et al.*, 2016) in order to place *Cr. balguerii* within the context of

Calappidae, and phylogenetic reconstructions were carried out using Maximum Likelihood (ML) with a concatenated analysis of the both genes. Both alignments were created and combined using the Clustal W with the BioEdit interface using default parameters (Thompson *et al.*, 1994), posteriorly in Gblocks version 0.91b. (Castresana, 2000; Talavera and Castresana, 2007). Specimens whose sequences were not available (COI and/or 16S), we substituted by missing data ("?") in the data matrix. Details about DNA sequences from other calappids used as a comparative group and the outgroup (*Hepatus pudibundus* (Herbst, 1785) - Aethridae), can be seen in Tab. 1.

ML was performed with RAxML (Randomised Accelerated Maximum Likelihood) using default parameters proposed by Stamatakis (2014), implemented at the CIPRES portal (Miller *et al.*, 2010). The model of DNA evolution assumed GTR+G+I as default parameters for RAxML. GTR-based models are adopted in RAxML because GTR is the most common and general model for real-world DNA analysis; it is more desirable to implement and optimize this model instead of offering a plethora of distinct models which are only special cases of this model (Stamatakis, 2016). Branch support was measured by the bootstrap method (1000 bootstrap pseudoreplicates), showing all confidence values above 50% as suggested by Ewers-Saucedo *et al.* (2016).

RESULTS

Molecular data context

Outgroup (represented by *H. pudibundus*) was chosen according to the phylogeny of box crab genera proposed by Bellwood (1996; 1998). These authors had tested several outgroups such as Xanthidae MacLeay, 1838 and Portunidae Rafinesque, 1815, but a close relationship between the Calappidae and the Hepatidae Stimpson, 1871 was found, suggesting a well-supported sister group association (Bellwood, 1996).

Cryptosoma was well supported with 76% of branch support; it is sister group of *Cycloes* (bootstrap 97). The specimen of *Cr. bairdii* from Gulf of Panama is sister group of the other species (bootstrap 76). *Cryptosoma cristatum* is sister species of the branch with *Cr. balguerii* and *Cr. bairdii* from Florida (bootstrap 90). *Cryptosoma balguerii* is sister species of *Cr. bairdii* from Florida, USA (bootstrap 77). *Cryptosoma balguerii* from São Paulo coast was positioned together with other specimens from St. John Island, Virgin Islands (KU853989; KU853990) (bootstrap 57) (Fig. 1).

SYSTEMATICS

Section Eubrachyura Saint Laurent, 1980

Superfamily Calappoidea De Haan, 1833

Family Calappidae De Haan, 1833

Cryptosoma balguerii (Desbonne in Desbonne and Schramm, 1867)

(Fig. 2)

Mursia balguerii Desbonne in Desbonne and Schramm, 1867: 52, pl. IV, fig. 20.

Material examined. 1 ♀ (CL 31.57 mm; CW 34.00 mm), CCDB 6417, Brazil, São Paulo, Ubatuba, offshore, 23°27'43.21"S and 45°00'9.59"W colls. D. Rosa and F. Zara, 23.x.2019.

Description. Carapace slightly wider than long, regularly convex, surface densely granulate; branchial ridges bearing prominent tubercles diminishing in size posteriorly. Front with two teeth with subtriangular median sinus. Anterolateral margin slightly scalloped, irregularly granulate, denticulated. Lateral spine short and slightly curved. Merus of cheliped with two teeth, distal tooth keel-like, proximal tooth bluntly triangular. Upper margin of chela with two bicuspidate proximal lobes; external surface of chela coarsely granulate, separated by rows of granules; medially a granulate line, proximal-most granule most prominent. Upper palm face with prominent teeth. Dactylar stridulating band with transverse ridges. Fourth pair of pereopods flatter than the others. (modified from Galil and Clark, 1996; Melo 1996).

Color pattern. The carapace is whitish with many small bright red or crimson spots, especially anteriorly, spines and tubercles white. Chelipeds whitish, spotted and banded with bright scarlet red; inside white with

Table 1. Collection details for *Cryptosoma balguerii* (Desbonne in Desbonne and Schramm, 1867) and species of Calappidae De Haan, 1833 used in the present study. Voucher refers to specimen and respective catalog numbers from museum collections. Location refers to the region of collection of the specimen. 16S and COI refer to the DNA fragments and respective numbers of NCBI Genbank accession. Abbreviations: CCDB = Coleção de Crustáceos do Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto; FLMNH = Florida Museum of Natural History; USNM = United States National Museum of Natural History (Smithsonian Institution); ZMK = Zoological Museum Kiel; 16S = 16S rDNA; COI = cytochrome oxidase subunit 1.

Species	Voucher	Location	16S	COI	Sequence source
Calappidae De Haan, 1833					
Acanthocarpus alexandri Stimpson, 1871	FLMNH 9401	Florida, USA	KU206578	KU853963	Ewers-Saucedo *et al.*, 2016
Calappa ocellata Holthuis, 1958	CCDB 5917	Ubatuba, São Paulo, Brazil	MF490170	MF490072	Mantelatto *et al.*, 2018
Calappula saussurei Rathbun, 1898	FLMNH 8082	Gulf of Panama, Panama	KU206607	KU853986	Ewers-Saucedo *et al.*, 2016
Cryptosoma bairdii (Stimpson, 1860)	FLMNH 6675	Florida, USA	–	KU853987	Ewers-Saucedo *et al.*, 2016
Cryptosoma bairdii	FLMNH 8126	Gulf of Panama, Panama	–	KU853988	Ewers-Saucedo *et al.*, 2016
Cryptosoma balguerii (Desbonne in Desbonne and Schramm, 1867)	FLMNH 4635	St John Island, Virgin Islands	–	KU853989	Ewers-Saucedo *et al.*, 2016
Cryptosoma balguerii	FLMNH 4610	St John Island, Virgin Islands	–	KU853990	Ewers-Saucedo *et al.*, 2016
Cryptosoma balguerii	CCDB 6417	Ubatuba, São Paulo, Brazil	present study	present study	present study
Cryptosoma cristatum Brullé, 1837	ZMK 2570	Angola	KU206616	–	Ewers-Saucedo *et al.*, 2016
Cycloes granulosa De Haan, 1837	KC3082	–	EU920917	–	Toon *et al.*, 2009
Mursia cristiata H. Milne Edwards, 1837	USNM 1192394	–	KU206617	–	Ewers-Saucedo *et al.*, 2016
Mursia cristiata	-	–	–	KU853991	NCBI GenBank
Paracyclois atlantis Chace, 1939	USNM 1071602	Colombia	KU206618	–	Ewers-Saucedo *et al.*, 2016
Platymera gaudichaudii H. Milne Edwards, 1837	USNM 1192407	–	KU206620	–	Ewers-Saucedo *et al.*, 2016
Platymera gaudichaudii	FLMNH 8128	Bay of Panama, Panama	–	KU853992	Ewers-Saucedo *et al.*, 2016
Aethridae Dana, 1852 (Outgroup)					
Hepatus pudibundus (Herbst, 1785)	CCDB 1445	São Vicente, São Paulo, Brazil	MF490141	MF490086	Mantelatto *et al.*, 2018

large dark red spot at distal end of manus; tips of digits and teeth of the dorsal crest of manus white; carpus with two red spots. Pereopods whitish with bright yellow dactylus, with bands of red and purple, and purplish red margins on the merus (Fig. 2).

Distribution. Western Atlantic – USA (North Carolina, Florida), Gulf of Mexico, Bermuda, Antilles, Colombia, Venezuela, Brazil (Fernando de Noronha Islands, Amapá, Pará, Maranhão, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia, Espírito

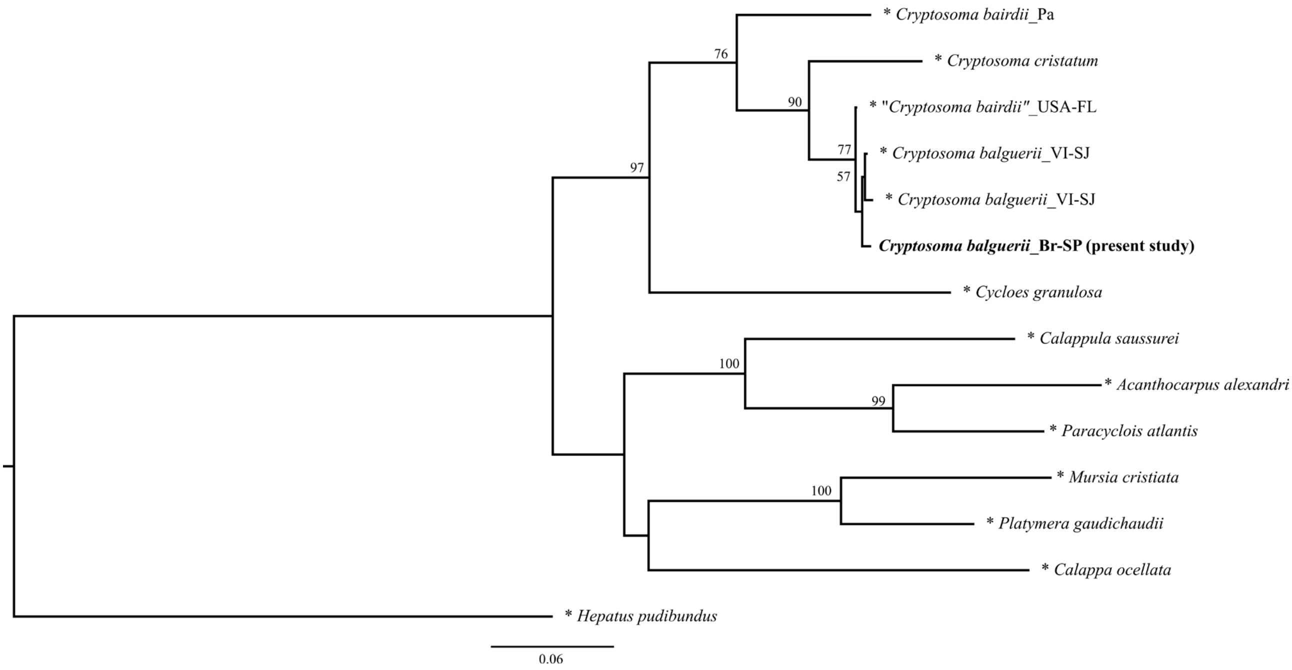

Figure 1. Phylogram based on Maximum Likelihood analysis of partial fragments of 16S and COI to place *Cryptosoma balguerii* from São Paulo (Brazil) within the context of Calappidae based on sister genera. GenBank sequences are marked with an asterisk (*); Br-SP: São Paulo State, Brazil; Pa: Gulf of Panama; USA-FL: Florida, United States of America; VI-SJ: St John Island, Virgin Islands; quoted species name suggests misidentification; bootstrap values under 50% are not shown.

Figure 2. *Cryptosoma balguerii* (Desbonne in Desbonne and Schramm, 1867). Dorsal view, 1 ♀, CC 31.57 mm, CCDB 6417. Scale bar: 12 mm. Photo: Raquel C. Buranelli.

Santo, Rio de Janeiro, São Paulo) (Coelho and Ramos, 1972; Galil and Clark, 1996; Melo and Veloso, 2005; Coelho Filho, 2006; Serejo *et al.*, 2006; Almeida and Coelho, 2008; present study).

Habitat. Rocky bottom, sand, mud, coral fragments and shell gravel (Melo and Veloso, 2005) in shallow waters less than 54 m (Galil and Clark, 1996; Bellwood, 1998; Coelho Filho, 2006).

Remarks. As far as we know, this is the first time that *Cr. balguerii* is reported in the State of São Paulo and confirmed by morphological and molecular data. It can be identified by the presence of a row of granules on the anterior-most sternal plate, which is considered the autapomorphy of the genus (Bellwood, 1998). It is a new name attributed to the western Atlantic records of *Cycloes bairdii* Stimpson, 1860, a species found along the Brazilian coast (Galil and Clark, 1996). Previously reported to Brazilian coast as *Cr. bairdii* was noted by Coelho and Ramos (1972), Melo (1996) and Barros and Pimentel (2001). Sequences accession number (GenBank): CCDB 6417 - COI (MN241497) and 16S (MN239506).

DISCUSSION

After more than 30 years working exhaustively in the decapod biodiversity of Ubatuba region, one of us (FLM) was surprised with the capture of this species in shallow waters. This is the first occurrence of *Cr. balguerii* to São Paulo, specifically in Ubatuba, which extends its distribution to the southeast Brazilian coast, being the second calappid that occurs in São Paulo — the first one was *Calappa gallus* (Herbst, 1803) with previous records in Ubatuba (Mantelatto and Fransozo, 2000; Bertini *et al.*, 2004; 2010) and Ubatumirim (Bertini *et al.*, 2004; Fransozo *et al.*, 2012).

There are some non-exclusive reasons for the increasing records of new species and extension of distributions reported over the last few years for decapod species in Brazilian waters, such as the increased number of studies on crustaceans, as well as a larger research program and laboratories focused on marine biodiversity (Mantelatto *et al.*, 2001; 2018). As on the São Paulo coast, other calappids were also collected from Bahia to Rio de Janeiro with low species richness. In addition to *Cr. balguerii*, *Acanthocarpus bispinosus* A. Milne-Edwards, 1880 and *Calappa gallus* (Herbst, 1803) were also sampled (Serejo *et al.*, 2006).

As well as reported here for *Cr. balguerii*, additional new records of decapod crustaceans along the western Atlantic were reported before. This is the case for *Microphrys antillensis* Rathbun, 1920 and *Inachoides forceps* A. Milne-Edwards, 1879 on the São Paulo coast (Camargo *et al.*, 2010). The extension of their geographical distributions was probably a delayed record, because the previous southern limit for these crabs was just north of Ubatuba and São Sebastião Island (Ilhabela), with no biogeographical barriers that could prevent its dispersal (Camargo *et al.*, 2010). We sampled one female of *Cr. balguerii,* which is considered rare and/or accidental because this species is difficult to find due to its cryptic behavior; it lives buried in rocky, sand, and mud bottoms (Melo and Veloso, 2005).

As a major finding, we noted that the relationship between *Cryptosoma* and the other genera did recapitulate some of the relationships inferred from morphological traits (Bellwood, 1998) and other molecular data (Ewers-Saucedo *et al.*, 2016). As expected, our dataset has positioned *Cr. balguerii* with other specimens of the same species, confirming its identity.

Furthermore, there were two specimens of *Cr. bairdii* from Florida (KU853987) and Gulf of Panama (KU853988) that were positioned in different regions in the phylogram: the first one was sister group to the other species of *Cryptosoma* and the latter was within *Cr. balguerii* (Fig. 1). From this, we presented the specimen/sequence from Florida with quotation marks and based on the well supported branch value which joined it with *Cr. balguerii* (bootstrap 77), we hypothesize a possible misidentification case. Thus, it is necessary to verify the morphological identity of the vouchers (FLMNH 6675 - Florida, FLMNH 8126 - Panama) and eventually perhaps a correction by the authors (Ewers-Saucedo *et al.*, 2016) who have deposited it in GenBank database.

In the present study, the branch support values were enough to confirm the presence of *Cr. balguerii* on the São Paulo coast. *Cryptosoma* is a genus with few representatives (four species), belonging to a morphologically and ecologically diverse family whose phylogenetic relationship are complex (Bellwood, 1996; 1998; Ewers-Saucedo *et al.*, 2016); the inclusion of other missing genera may better clarify the position of *Cryptosoma* and, as a consequence, increase the support value. Furthermore, we only sampled *Cr. balguerii* from two localities, which may influence these values due to the low number of specimens.

We obtained branch support values that confirmed the sister relationship between *Cycloes* and *Cryptosoma*, which is similar to the result of Ewers-Saucedo *et al.* (2016), although the focus of their study was the family-level. However, it is noteworthy that inferences about the internal relationship of *Cryptosoma* and its sister group is also not part of the scope of the present study.

The São Paulo coast encompasses about 57% of the Brazilian coastal diversity, being considered a region with a high biodiversity of decapods due to the variety of habitats found along its coast (Mantelatto *et al.*, 2018). The Ubatuba region, in the northeast of São Paulo, has been pointed out as mainly responsible for this biodiversity, primarily because of its latitudinal position, abiotic characteristics, and different habitats (Almeida *et al.*, 2018).

Finally, range extensions of decapod crustaceans have been recorded in the São Paulo coast (*e.g.*, Mantelatto *et al.*, 2001; Cobo *et al.*, 2002; Camargo *et al.*, 2010; Almeida *et al.*, 2018; Terossi *et al.*, 2018), including the present study. These findings are relevant to understanding the environment and community dynamics (Cobo *et al.*, 2002), as well as in terms of biogeographic and taxonomic studies.

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