A multifaceted comparison between the spider crabs *Epialtus bituberculatus* and *Epialtus brasiliensis* (Brachyura: Majoidea: Epialtidae) in the neotropical areas of the western Atlantic: morphology, morphometry and DNA markers

Múltiples facetas en la comparación entre los cangrejos araña *Epialtus bituberculatus* y *Epialtus brasiliensis* (Brachyura: Majoidea: Epialtidae) en las zonas neotropicales del Atlántico occidental: morfología, morfometría y marcadores de ADN

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The morphological characters used to distinguish the two species of the crab genus *Epialtus* that occur in the neotropical areas of the Brazilian coast, *E. bituberculatus* and *E. brasiliensis*, are variable, casting doubt on their taxonomic status. Three approaches were chosen in order to resolve this uncertainty: morphological, molecular analysis and combined phylogenetic analysis with genetic data and morphometry. A molecular phylogenetic analysis was performed to test whether these two species can be separated at the molecular level (using the two mitochondrial genes 16S and COI – DNA barcode). The genetic distances and phylogenetic trees showed that *E. brasiliensis* fell within the clade of *E. bituberculatus*. We suggest that there is one species in Brazil and the presence or absence of the spine on the ventral surface of the propodus of the ambulatory pereiopods (a key diagnosing character) appears to be uninformative. However, all the analysis showed that specimens of *Epialtus* can be separated in three clades corresponding to three geographic regions: Caribbean Sea, Venezuela and Brazil. We conjectured the existence of three cryptic species that evolved recently.

http://zoobank.org/urn:lsid:zoobank.org:pub:611E18BF-4CD6-40C8-B57E-01F2FA62E841

Keywords: DNA barcode; total evidence; cryptic species; molecular taxonomy; morphology

Los caracteres morfológicos utilizados para distinguir las dos especies de cangrejo del género *Epialtus* que se presentan en la región neotropical de la costa brasileña, *E. bituberculatus* y *E. brasiliensis* son variables, poniendo en duda su estado taxonómico. Tres enfoques se eligieron con el fin de resolver esta incertidumbre: morfológico, análisis molecular y análisis filogenético combinado con los datos genéticos y morfometría. Se realizó un análisis filogenético molecular para comprobar si estas dos especies se pueden separar en el nivel molecular (usando los dos genes mitocondriales 16S y COI – Código de barras del ADN). Las distancias genéticas y los árboles filogenéticos evidenciaron que *E. brasiliensis* se positionó dentro del clado de *E. bituberculatus*. Sugerimos que hay solamente una especie en Brasil y la presencia o ausencia de la espina dorsal en la superficie ventral de la propodia de los pereiopodos ambulatorios (un carácter diagnóstico clave) no parece ser informativo. Sin embargo, todo el análisis mostró que los especímenes de *Epialtus* se pueden separar en tres clados que corresponden a tres regiones geográficas: Mar del Caribe, Venezuela y Brasil. Suponemos que son tres especies crípticas que evolucionaron recientemente.

Palabras clave: Código de barras del ADN; evidencia total; especies crípticas; taxonomía molecular; morfología

Introduction

The family Epialtidae MacLeay, 1838, includes 76 genera of marine crabs living from the intertidal zone to the deep sea. Among them, *Epialtus* H. Milne Edwards, 1834, contains 11 valid species.[1] Members of this genus can be found on both sides of the American continent: on the Pacific side, they can be found from southern California to Chile, while on the Atlantic coast, they are distributed from northern Florida (USA) to Rio Grande do Sul (Brazil).[2–4]

*Epialtus bituberculatus* H. Milne Edwards, 1834, is the only species that has been reported to both coasts of the American continent.[2,3] This species was first described from the eastern Pacific,[5] but has never been reported again along this coast. In fact, a collecting expedition to find this species in the intertidal zone in the
eastern Pacific was unsuccessful, and like Garth,[6] we now have serious doubts that the original type locality is correct. We follow Rathbun [2], Garth [3] and Powers [7] in restricting the range of this species to the western Atlantic coast. Recently, this species was removed from the Chilean decapod fauna.[8]

The two species of *Epialtus* reported for the Brazilian coast with overlapping distributions are *E. bitu­berculus*, which can be found from Florida (USA) to Santa Catarina (Brazil)[2,7,9–15] and *E. brasiliensis* Dana, 1852 which occurs on the Atlantic coast of Colombia, Venezuela and Brazil (Ceará, Espirito Santo to Rio Grande do Sul).[9,11,12,15–17] However, there is a gap in the distribution of *E. brasiliensis* from Rio Grande do Norte to Sergipe, an area where this species has never been found.[9,17,18] Furthermore, the only report of this species occurrence in Salvador (Bahia, Brazil) [19] has been challenged based on its apparently disjointed geographical distribution and with no other records from Bahia.[20] Thus, the only confirmed record of its occurrence in northern Brazil is Ceará.[12,16] In addition, the geographic distribution of *E. brasiliensis* is discontinuous because it occurs separately in Colombia and Venezuela [9,11], in Ceará (Brazil) [16] and in the south and southeast regions of Brazil.[15] These patterns of occurrence raise questions about whether these gaps are due to a lack of faunal surveys,[14] a misidentification of specimens that are morphologically similar to the sympatric species *E. bitu­berculus* (present study) and/or because *E. brasiliensis* is a morphologically variable species but part of a species complex. Sharing similar habitats can result in morphological similarities due to convergence, which makes it more difficult to infer evolutionary relationships.

*Epialtus bitu­berculus* and *E. brasiliensis* are sympatric in the southern hemisphere. They inhabit the intertidal zone to 10 m depth and can be frequently found associated with sea grasses and algae on hard substrates or sandy bottoms.[9,17,21] The larval morphologies of the two species are very similar,[22] and adults of both species have two tubercles on the gastric region and a simple rostrum.[2,9] They can be differentiated by the carapace subpentagonal and rostrum with rounded extremity in *E. bitu­berculus* and carapace subhexagonal and rostrum equilaterally triangular in *E. brasiliensis*; ambulatory per­eiopods of *E. brasiliensis* are stouter than those of *E. bitu­berculus* and the last three pairs also very short with a spine on the ventral surface of the propodus.[2,9,23]

In this study, a multifaceted approach composed of morphological characters, molecular phylogenetic analysis and combined phylogenetic analysis was used in order to clarify the taxonomic positions of *E. bitu­berculus* and *E. brasiliensis*. For the combined analysis, we used numerical methods (traditional morphometric) with genetic data as a complement to the morphological and molecular data-sets.

Material and methods

**Morphological data collection**

For the taxonomic revision and to compare *Epialtus bitu­berculus* with *E. brasiliensis*, we selected 17 morphological characters that have been used in species descriptions [2,3,5,9,23]; and we added another nine characters for both sexes and across a size range (Table 1).

The examined material was deposited in the Crustacean Collection of the Department of Biology (CCDB), Faculty of Philosophy, Sciences and Letters at Ribeirão Preto (FFCLRP), University of São Paulo (USP), Ribeirão Preto, São Paulo State, Brazil. Additional specimens were obtained on loan from the following collections: Federal University of Pernambuco (DOUFPE – Recife, Pernambuco, Brazil); National Museum of Rio de Janeiro (MNRJ – Rio de Janeiro, Rio de Janeiro, Brazil); Federal University of Rio Grande do Sul (UFRGS – Porto Alegre, Rio Grande do Sul, Brazil); University of Louisiana-Lafayette, Zoological Collections (ULLZ – Lafayette, Louisiana, USA); Universidad Nacional Autónoma de México (UNAM – Mexico City, Mexico); and Museo de Zoología, Universidad de Costa Rica (UCR – San Pedro, San José, Costa Rica). We measured the carapace length (CL = from the posterior to the anterior margin, including the rostrum) with a vernier caliper (0.01 mm) of each specimen. This standard measure is used to indicate the size of the specimens and facilitate comparisons with previous published results, between genus and among age groups.

**Genetic data collection**

Molecular analysis was used as a complement to the morphological data-set. Most of the specimens used for the molecular analysis were deposited in the CCDB/FFCLRP/USP. Complementary specimens were obtained on loan from crustacean collections (Table 2). Partial fragments of the large ribosomal subunit (16S mtDNA) and the Cytochrome oxidase I (COI – the barcode region) were used as genetic markers. Our methodology followed the protocols used by Mantelatto et al. [24], adjusted as follows.

Genomic DNA was retrieved from the muscle tissue of pereiopods from male specimens if possible, although muscle was extracted from females or eggs when necessary.[24] Fragments of DNA were amplified by polymerase chain reaction (PCR) using specific primers, 16SF (5′ – TATTTTGACCGTGAAGGTA – 3′) and 16SR (5′ – ATTAAAGGTCCAGACCATC – 3′) [25]; COH6 (5′ – TADACTTCDGRTGDCCAAAR­AAYCA – 3′) and COL6b (5′ – ACAATCATAAAGA­TATYGG – 3′).[26] Thermal cycling was performed with the following profiles: for 16S, initial denaturation for
5 min at 95°C, followed by 40 cycles of 45 s at 95 °C, 45 s at 58.5 °C and 1 min at 72 °C, with a final extension of 3 min at 72 °C; and for COI, initial denaturation for 2 min at 94 °C, followed by 35 cycles of 30/45 s at 94 °C, 30/45 s at 46–50 °C and 1 min at 72 °C, with a final extension of 2 min at 72 °C. All products were stained with GelRed™ (Biotium, Inc.; 3159 Corporate Place, Hayward, California 94545, USA) and visualized under UV light in 1% agarose gels with a co-migrating 1000-bp ladder molecular weight marker to confirm the correct amplification.

Successful PCR products were purified using SureClean® following vendor protocols and sequenced with the ABI Big Dye® Terminator Mix (Applied Biosystems, Table 1. Morphological characters chosen for the taxonomic revision of the Brazilian species of Epialtus.

| Characters                                      | Description                                                                 |
|------------------------------------------------|-----------------------------------------------------------------------------|
| Carapace                                       | Shape; distribution and type of setae and tubercles                          |
| Rostrum                                        | Shape; type of setae                                                        |
| Orbits*                                        | Angle in relation to the rostrum                                            |
| Eyes                                           | Presence or absence of setae                                                |
| Preorbital tooth                               | Presence or absence; type of setae                                          |
| Postorbital tooth                              | Presence or absence                                                         |
| Hepatic Region*                                | Shape; presence or absence of setae                                         |
| Gastric and cardiac regions                    | Presence or absence of setae and tubercles; distribution and type of setae |
| Brachial Region*                               | Presence or absence of teeth; type of setae                                 |
| Intestinal Region*                             | Presence or absence of setae and tubercles; distribution and type of setae |
| Basal article of antenna                       | Shape; articles position in relation to the rostrum; distribution and type of setae |
| Chelipeds                                      | Size; presence or absence of gap between fingers                            |
| Ischium*; merus; carpus; propodus; dactylus     | Presence or absence of setae, tubercles, crest and teeth; type of setae     |
| Pereopods                                      | Size; distribution and type of setae                                        |
| Ischium*; merus*; carpus*; propodus; dactylus  | Shape; presence or absence of setae, lobe, tubercles, spines; distribution and type of setae |
| Thoracic sternum*                              | Shape; position; presence or absence of setae                               |
| Abdominal somites                              | Degree of fusion; shape; presence or absence of setae                      |
| Male first pleopod*                            | Shape                                                                        |

*New characters analyzed.

Table 2. Specimens used for the molecular analysis with respective site of collection, museum catalog number and GenBank accession numbers.

| N (16S/COI) | Species                                      | Locality               | Catalog Number | GenBank Number |
|-------------|----------------------------------------------|------------------------|----------------|----------------|
| (3/–)       | Epialtus bituberculatus H. Milne Edwards,    | Puerto Viejo, Costa Rica | UCR 1038       | KC695753       |
|             | 1834                                          | Bocas del Toro, Panama | CCDB 917       | KC695754       |
| (–4)        |                                               | Isla Margarita, Venezuela | CCDB 2429     | KC695755       |
| (5/5)       |                                               | La Restinga, Venezuela | CCDB 3634     | KC695756       |
| (7/6)       |                                               | Pernambuco, Brazil     | CCDB 3813     | KC695758       |
| (–7)        |                                               | Pernambuco, Brazil     | CCDB 3813     | KC695759       |
| (6/8)       |                                               | Bahia, Brazil          | CCDB 2426     | KC695757       |
| (8/9)       |                                               | Rio de Janeiro, Brazil | CCDB 3861     | KC695759       |
| (9/11)      |                                               | Sao Paulo, Brazil      | CCDB 2441     | KC695760       |
| (–10)       |                                               | Sao Paulo, Brazil      | CCDB 2431     | KC695761       |
| (10/–)      | Epialtus brasiliensis Dana, 1852             | Bocas del Toro, Panama | ULLZ 10755    | KC695761       |
| (12/13)     |                                               | Sao Paulo, Brazil      | CCDB 2373     | KC695763       |
| (11/12)     |                                               | Sao Paulo, Brazil      | CCDB 2432     | KC695762       |
| (2/2)       | Epialtus dilatatus A. Milne-Edwards, 1878    | Bocas del Toro, Panama | CCDB 975     | KC695752       |
| –           | Acanthonyx petiverii H. Milne Edwards, 1834   | Sao Paulo, Brazil      | CCDB 760      | KC695764       |
| (1/1)       | Taliepus nuttallii (Randall, 1840)           | California, United States of America | Uninformed | EU682828       |

Notes: CCDB: Crustacean Collection of the Department of Biology, FFCLRP, University of Sao Paulo; UCR: Coleccion Nacional de Crustáceos, Universidad de Costa Rica; ULLZ: University of Louisiana at Lafayette, Zoological Collection. N: numbers that make the correspondence between specimens used in the genetic distance tables and trees.
850 Lincoln Centre Drive, Foster City, California 94404, USA) in an ABI Prism 3100 Genetic Analyzer® (Applied Biosystems automated sequencer; Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA). Both strands of all sequences were sequenced and a consensus sequence was obtained using the computational program BioEdit 7.2.5.[27] Sequences were aligned using Clustal W with an interface in BioEdit using default parameters.[28]

Genetic distances calculated with the program Mega5 [29] were used to compare *E. bituberculatus* with *E. brasiliensis* and congeners. Tree building was performed using maximum likelihood (ML) [30] and executed in the program randomized accelerated maximum likelihood (RAxML) [31] as implemented on the online platform Cipres (Cyberinfrastructure for Phylogenetic Research).[32] The model of DNA evolution assumed GTR+G+I as default parameters for RAxML. Only bootstrap (1000 replicates) confidence values >50% were reported.

For the 16S and COI analysis, sequences from GenBank of *Taliepus nuttallii* (Randall, 1840) and *Acanthonyx petiverii* H. Milne Edwards, 1834 were used as the out-groups because they are phylogenetically close to the genus *Epialtus.*[25]

Then, we grouped specimens according to geographic distribution (Caribbean, Venezuela and Brazil) and calculated the genetic distances again with the aid of the program Mega5 in order to compare with the ML tree.

**Traditional morphometrics data collection**

Eight body measurements, four from carapace and four from third pereopod (Table 3), were taken from the 12 specimens whose DNA was sequenced for both genes. All dimensions were measured to the nearest 0.1 mm using a caliper rule or under a light stereomicroscope. Individuals with injured or missing appendages were excluded. These measurements were first used for traditional morphometrics, and then they were added to molecular data on a combined analysis.

| Abbreviation | Definition                        |
|--------------|-----------------------------------|
| Carapace     | CLR Carapace length with rostrum  |
|              | CL Carapace length without rostrum|
|              | CW Carapace width                  |
| Pereopod 3   | RL Rostrum length                 |
|              | LPD Length of the dactylus        |
|              | LPP Length of the propodus        |
|              | LPC Length of the carpus          |
|              | LPM Length of the merus           |

Carapace length is a standard measure which can be used to indicate the size of the specimens and facilitate comparisons with previous published results. However, we chose an alternative approach with principal components analysis using the first component (PC1) as a general descriptor of organisms’ size [33] and the residual method to adjust the size through the residual of the relationship between PC1 (in this study, carapace length with rostrum) and each of the measures (Table 3). Most of the variation attributed to the size is removed in the analysis, allowing a better approach in changes in the general shape of the body of organisms.[34,35]

The software PAST 3.08 [36] was used for the regression between PC1 and each measure; residual was obtained and used in phylogenetic analysis.[34,35] The residual includes values below zero that were changed with the addition of 10 units, and transformed in logarithm (=LOG10) to standardize the data.[34] These changes did not affect the comparison since the data retained the same structure.

**Combined phylogenetic analysis**

The residual table along with the molecular alignment data (16S and COI separated) was used for building a combined matrix in the notepad, according to a specific script (data not show), and it was analyzed in the software TNT 1.1 (Tree Analysis Using New Technology) [37]. This software permits the use of continuous characters with values ranging from 0 to 65 with up to three decimal places, and analyzing them without making them discrete characters.[38]

Heuristic search using new technology (New Technology Search) aims to analyze the largest number of possible trees and to find the most parsimonious tree, TBR algorithm (Tree bisection and reconnection; implemented in TNT) randomly performs rearrangements of branches from the initial trees, searching by chance a combination of branches that produces an even shorter tree than the other found.[39]

In a more refined stage, sector search and tree drifting were used to isolate and analyze each part of the most parsimonious trees independently, which means an increase in effectiveness to find shorter trees even in analyses of matrices that could theoretically be analyzed only for heuristic search.[37,39] Branches of possible groupings not supported by synapomorphies were collapsed to prevent formation of monophyletic false groups; gaps (‘–’) in the molecular matrix were considered missing entries. Resampling method (1000 replicates, probability 33) was used as branch support because it is the most recommended for continuous data using TNT.[37,38]

Three different values of implied weighing (*k* = 3, *k* = 7 and *k* = 10) of characters were chosen; we
calculated the consistency index (CI) to measure the number of homoplastic events of a determined character or clademogram [33] and the retention index to indicate the proportion of autapomorphies and homoplasies related to the total steps.[40]

Results

Taxonomic assignments

We obtained a total of 97 specimens of Epialtus bituberculatus (44 males, 36 females, 17 ovigerous females) and 33 samples of E. brasiliensis (9 males, 8 females, 16 ovigerous females). Due to the morphological similarity between the two species and the lack of a distinguishing character, we could not separate them into two species. Between the two species and the lack of a distinguish-

16 ovigerous females). Due to the morphological similarity between the two species and the lack of a distinguishing character, we could not separate them into two entities and our first hypothesis that there were two valid species could not be corroborated. Thus, based only in morphological data, we present a re-description of E. bituberculatus because of its older description,[5] combining the groups (present study), and with a detailed discussion in the Remarks section.

Superfamily Majoidea Samouelle, 1819
Family Epialtidae MacLeay, 1838
Subfamily Epialtinae MacLeay, 1838
Genus Epialtus H. Milne Edwards, 1834

Epialtus bituberculatus H. Milne Edwards, 1834 (Figure 1(A) and (B))

Epialtus bituberculatus H. Milne Edwards, 1834: 345; pl. 15: fig. 11.

Epialtus affinis Stimpson, 1859: 50.

Epialtus brasiliensis Dana, 1852: 132.

Epialtus bituberculatus – Rathbun 1894: 67; 1901: 60; 1925: 148, pl. 45: figs. 3, 4; Garth 1958: 228; Fausto-Filho 1970: 59; Powers 1977: 42; Abele & Kim 1986: 38, 164 (key); Lima Júnior et al. 2010: 71, 72; Melo 1996: 174, fig. 1; Hernández-Aguilera et al. 1997: 61; Marcano & Bolaños 2001: 75; Almeida & Coelho 2008: 192.

Epialtus brasiliensis – Moreira 1901: 66; Rathbun 1925: 149, fig. 55, pl. 220: fig. 1; Fausto-Filho 1970: 59; Vélez 1977: 118, fig. 8; Melo 1996: 175; Marcano & Bolaños 2001: 75; Melo 2008: 5.

Epialtus brasiliensis – Fausto-Filho 1966: 33.

Type locality: Chile.[2,5,41] The holotype is supposedly in the Muséum National d'Histoire Naturelle, Paris (France).[2,5] At this stage, we studied the original description and its plates but not the specimen.

Material examined:

MEXICO, Quintana Roo, Cozumel: 2 males (CL 9.9 and 10.9 mm), 1 female (CL 6.08 mm), 1 ovigerous female (CL 6.08 mm), 15/I/1985, coll. J.C. Nates, J.L. Villalobos & A. Cantu (UNAM 3710); Quintana Roo, José María Morelos: 1 male (CL 5.2 mm), 1 ovigerous female (CL 6.2 mm), 27/I/1987, coll. not available (UNAM 21051); Quintana Roo, Solidaridad: 2 males (CL 5.7 and 12.5 mm), 1 ovigerous female (CL 7.4 mm), 26/VI/1988, coll. not available (UNAM 9578); BELIZE, Danginga, Pelican Beach Resort: 1 male (CL 11.0 mm), 1 female (CL 7.8 mm), undated, coll. D.L. Felder (ULLZ 12624); Danginga, Pelican Beach Resort: 1 ovigerous female (carapace damaged), 10/V/2006, coll. S. Fredericq (ULLZ 6695); COSTA RICA, Puerto Viejo: 1 male (CL 7.9 mm), 2 ovigerous females (CL 7.3 and 7.4 mm), 12/II/1981, coll. D. Moron (UCR 1038); PANAMA, Bocas del Toro: 1 male (CL 7.9 mm), 2 females (CL 3.1 and 5.9 mm), 1 ovigerous female (CL 5.9 mm), 09/VIII/2004, coll. D.L. Felder (ULLZ 10755); Bocas del Toro, Playa Puach: 1 male (CL 7.0 mm), 1 female (CL 4.7 mm), 05/VIII/2011, coll. F.L. Mantelatto (CCDB 917); VENEZUELA, Isla Margarita, Boca Chica: 4 males (CL 3.1–7.8 mm), 3 females (CL 3.8–6.1 mm), 3 ovigerous females (CL 6.3–8.0 mm), 03/XI/2010, coll. R. Lopez (CCDB 2429); Playa Valdez: 1 ovigerous female (CL 7.6 mm), 27/VII/2006, coll. F.L. Mantelatto & L. Pileggi (CCDB 1786); BRAZIL, Rio Grande do Norte, Potiguar Basin: 1 male (CL 4.7 mm), 23/XI/2003, coll. not available (DOUFPE 13874); Rio Grande do Norte, Potiguar Basin: 4 males (CL 5.5–6.1 mm), 6 females (CL 3.9–6.3 mm), 23/XI/2003, coll. not available (DOUFPE 13890); Rio Grande do Norte, Potiguar Basin: 1 female (CL 4.9 mm), 1 ovigerous female (CL 6.0 mm), 23/XI/2003, coll. not available (DOUFPE 13899); Pernambuco, Recife, Boa Viagem Beach: 6 males (CL 5.1–12.4 mm), 5 females (CL 4.7–5.6 mm), 06/IV/2012, coll. F.L. Mantelatto (CCDB 3813); Pernambuco, Piedade Beach: 2 males (CL 6.6 and 11.0 mm), 24/VI/1986; coll. A.L. Castro, P. Coelho & G. Melo (MNRJ 4476); Pernambuco, Santo Aleixo Island: 2 males (CL 4.1 and 7.1 mm), 06/II/2007, coll. A.O. Almeida (DOUFPE 13525); Bahia, Abrolhos, Viçosa Reef: 1 male (CL 9.5 mm), 23/VIII/1994, coll. G. Franco & C. Serejo (MNRJ 4475); Bahia, Ilhéus, Badusca Beach: 1 ovigerous female (CL 6.9 mm), 06/VI/2010, coll. F.L. Mantelatto, F.L. Carvalho & L.G. Pileggi (CCDB 2426); Espírito Santo, Ipiri Beach: 2 males (CL 3.6 and 3.9 mm), 2 females (CL 3.6 and 6.5 mm), 19/VI/2012, coll. F.C. Carvalho, R. Robles & D. Peiró (CCDB 4067); Rio de Janeiro, Niterói, Itaipu Beach: 2 males (CL 2.4 and 3.6), 18/VI/2012, coll. I. Cardoso et al. Turma Decapoda 2012 – Disciplina MNRJ (CCDB 4140); Rio de Janeiro, Paraty, Jurujurim Beach: 1 ovigerous female (CL 7.7 mm), 24/IV/2012, coll. I.C. Leone, M. Negri & A.F. Tamburus (CCDB 3861); São Paulo, Ubatuba, Flamengo Bay: 9 males (CL 3.3–15 mm), 7 females (CL 3.2–7.1 mm), 2 ovigerous females (CL 8.7 and 9.4 mm), 12/V/2010, coll. F.L. Mantelatto (CCDB 2441); São Paulo, Ubatuba, Itaguaí Beach: 1 male (CL 6.2 mm), 6 females (CL 5.0–7.8 mm), 17/II/2010, coll. F.L. Mantelatto (CCDB 2431); Paraná, Matinhos, Caiobá, Mansa Beach: 1 female (CL 4.3 mm), 10/II/2002, coll. F.L. Mantelatto & E.C.
Mossolin (CCDB 2263); Santa Catarina, Florianópolis, Ponta Norte, Sambaqui Beach: 1 male (CL 5.6 mm), 1 ovigerous female (CL 6.7 mm), 16/IV/2007, coll. F.L. Mantelatto, L.G. Pileggi, L.S. Torati & E.C. Mossolin (CCDB 1887).

Material examined as *E. brasiliensis* and synonymized here under *E. bituberculatus*: PANAMA, Bocas del Toro: 1 ovigerous female (CL 5.36 mm), 09/VIII/2004, coll. D.L. Felder (ULLZ 10755); BRAZIL, Rio de Janeiro, Saquarema, Lage de Itaúna: 1 female (CL 8.00 mm), 31/V/2012, coll. I. Cardoso (CCDB 4141); São Paulo, Ubatuba: 3 males (CL 11.8–12.8 mm), 11 ovigerous females (CL 7.2–9.0 mm), XII/1995, coll. F.L. Mantelatto & E.K. Correa (CCDB 437); São Paulo, Ubatuba, Itaguá Beach: 2 males (CL 9.7 and 10.8 mm), 7 females (CL 6.4–7.5 mm), 17/II/2010, coll. F.L. Mantelatto (CCDB 2432); São Paulo, Ubatuba, Vermelha do Sul Beach: 1 male (CL 10.58 mm), 1 ovigerous female (CL 8.64 mm), 30/I/1984, coll. F.R. Marcozès (CCDB 2433); Santa Catarina, Garopaba: 2 males (CL 7.6 and 9.5 mm), 1971, coll. not available (UFRGS 034); Rio Grande do Sul, Torres: 1 male (CL 13.96 mm), 3 ovigerous females (CL 9.7–11.6 mm), 08/XII/1981, coll. not available (UFRGS 580).

Comparative material: *Epialtus dilatatus* – PANAMA, Bocas del Toro: 1 ovigerous female (CL 8.7 mm), 05/VIII/2011, coll. F.L. Mantelatto (CCDB 975); *E. dilatatus* form *elongata* – COSTA RICA, Puerto Vargas: 3 males, 1 female (CL1.6–3.8 mm), 19/XI/1999, coll. not available (UCR 2312-19); *E. longirostris* – COSTA RICA, Puerto Viejo: 1 male, 1 ovigerous female (CL 7.35 and 5.3 mm), 18/V/1980, coll. not available (UCR 1275-002); *E. sulcirostris* – COSTA RICA, Isla del Caño: 1 female, 2 ovigerous females (CL 1.6–5.6 mm), 11/IV/1971, coll. not available (UCR 389).

**Diagnosis:** Simple rostrum; carapace subpentagonal in both genders; cardiac region slightly elevated; absence (Figure 1(C)) or presence (Figure 1(D)) of proximal spine on the propodus ventral surface in the last three ambulatory pereopods.

**Redescription:** Carapace smooth, subpentagonal; 2 lateral lobes, 1 hepatic, 1 branchial separated by a concave border. Rostrum triangular or longer than broad; extremity rounded. Orbits absent; orbital region with lateral angles obtuse. Eyes small, mobile and partially hidden by carapace margin. Preorbital teeth absent or minute, not pointed, setae with variable length and thickness, sometimes absent. Postorbital teeth absent or minute, not pointed. Hepatic region with 2 lateral lobes slightly pronounced, curved upwards, smooth or with setae with length and thickness variable along margins. Gastric region smooth, with 2 small protogastric tubercles, smooth or with tufts of short/long setae. Cardiac region smooth, slightly elevated. Branchial region with 2
small teeth, not pointed, smooth or with short/long setae. Intestinal region smooth. Basal article of antenna subtriangular; following 2 articles cylindrical, hidden underneath rostrum in males, almost hidden in females; second and third articles formed by tufts of short/long setae on inner margin. Chelipeds of adult male strong, larger than first pair of ambulatory pereopods. In females, smaller or same size as first pair of ambulatory pereopods. Merus enlarged in 3 lobes in distal end, 1 central, 2 lateral lobes; inner margin of merus carinated in males larger than 8.0 mm. Inner margin of carpus lightly carinated, 2 crests on dorsal surface in males; inner margin not carinated in females, 2 crests on dorsal surface, 1 lobe on each lateral margin. Propodus enlarged in the lateral margins and high in the upper margin in females, smaller or same size as males; less enlarged and less high in females.

Fixed in the lateral margins and high in the upper margin in surface, 1 lobe on each lateral margin. Propodus enlarged in the lateral margins and high in the upper margin in males; less enlarged and less high in females. Fixed finger and dactylus almost entirely gaping in male, almost closed in female; fingers with ventral surface dentate and tufts of setae near distal end; dactylus (movable finger) with a large tooth in the middle of ventral surface. Ambulatory pereopods slender, subcylindrical; posteri-orly decreasing in size. Merus with 3 lobes in distal end of first and second pereopods. Carpus shorter than merus, subtriangular in last 2 ambulatory pereopods. Propodus with 1 or 2 tufts of setae on ventral surface, 1 central, 1 near distal end; lightly dilated at outer distal end. Proximal spine on ventral margin of propodus in last 3 ambulatory pereopods may be present. Dactylus with 2 rows of minute spines intercalated with fine setae on ventral surface. Fourth and fifth abdominal somites united in male; fourth, fifth and sixth in female. Male first pleopod terminally truncate; coarse and less salient terminally, with a subtriangular lobe.

Remarks: Epialtus bituberculatus was described by Milne Edwards [5] for the coast of Chile and made the type species of the genus, but the Chilean location has been shown to be an error.[6, p.31] All subsequent records for the species have been from the Atlantic, [2,3,7,41,42] and this species has not been registered in Chilean decapod fauna since its original description.[8] It seems that E. bituberculatus was first recorded in western Atlantic as E. affinis Stimpson, 1859 in Indian River (Florida, USA), described through material from the Smithsonian Institution [43] and synonymized by Rathbun.[2] The type of E. affinis was probably lost and pointed out as not extant.[2] In Brazil, E. bituberculatus was recorded for the first time in Pernambuco, but it was mentioned with doubts and as ‘brasilensis form’.[44, p.67] However, the type locality of E. brasiliensis is Guanabara Bay, Rio de Janeiro, Brazil [23,45] and the holotype of this species has most likely been lost.[2, p.149] These facts make difficult the comparison of material analyzed herein with the typical representative of the specimen of the species, but finally we analyzed only a figure of Milne Edwards.[5] Thus, the designation of clades was problematic because in fact we do not know which entity we are dealing with. Finally, we suggested names and explain why we chose them (see Discussion).

Material of E. bituberculatus examined herein matches with the original description,[5] but that description is short and not detailed. In general, the specimens analyzed by us were similar to the original illustration [5, plate 15, figure 11], but even the smaller individuals and females have longer legs, mainly the first pereopod. Furthermore, merus and propodus in all pereopods are longer (Figure 1(A) and (B)) than the specimen in original description [5, plate 15, figure 11]. In the present study, the description of the adult male was identical to the material examined by Rathbun [2], but she did not mention females and juveniles. Moreover, we observed variation in the size of the rostrum (Figure 2) and in the presence or absence of teeth in the chelipeds fingers, which differs from the Rathbun’s material [2, plate 45, figure 3].

In the original description, Dana [23] observed a small, closely apressed tooth behind the eyes and noted that the rostrum was entire and nearly equilaterally triangular in E. brasiliensis. In the material analyzed, we observed that the shape of rostrum was variable and ranged from triangular to longer than broad (Figure 2). In the carapace of E. brasiliensis, the anterior margin of the hepatic lobe is transverse,[2] but we observed that this angle can be oblique in females and may be shorter in males.

The shape of carapace and rostrum were two of the main traits used to distinguish species of Epialtus, according to Rathbun [2, p.147, figure 53] as emphasized in the figure of her study. However, the specimens of E. bituberculatus and E. brasiliensis analyzed in the present study showed a wide variation in the length/shape of the rostrum (Figure 2) and in the carapace shape (Figure 3). Therefore, these characters are not sufficient to distinguish E. bituberculatus from E. brasiliensis. Nonetheless, these characters allow the distinction of these two species from other species of the genus; E. dilatatus, E. hiltoni, E. minimus and E. peruvianus present deeply bilobed carapace and a bifid rostrum.[2–4]

The ambulatory legs of E. brasiliensis are relatively stouter than those of E. bituberculatus and with the last three pairs very short.[2] However, in this study, both species have slender ambulatory legs that were subcylindrical and posteriorly decreasing in size. Epialtus brasiliensis is morphologically very similar to E. bituberculatus and differs mainly in the presence of a spine on the propodus in last three ambulatory pereopods, which we now know is a variable character (Figure 4). Finally, there are no reliable morphological characters that can be used to separate the two species or patterns that justified the genetic separation (Figures 5 and 6), the low genetic distances between E. bituberculatus and E. brasiliensis (Tables 4and 5) or the combined phylogenetic analysis (Figure 7).
The male first pleopod has been described for *Epialtus minimus* and *E. sulcirostris*.[3] so we added it here as a comparative character within the genus; it has also been extensively used in other studies on American majoids.[3,46] The male first pleopod of *E. bituberculatus* and *E. brasiliensis* was the same and similar to that of *E. sulcirostris* but different from *E. minimus*.[3, plate O, figures 5 and 6]

**Genetic comparison**

Thirteen specimens were used for molecular analysis, consisting of 9 samples of *Epialtus bituberculatus*, 3 individuals of *E. brasiliensis* and 1 specimen of *E. dilatatus* (Table 2). Sequences of 16S and COI contained 410-429 and 560-640 basepairs, respectively.

The divergence between the external group *T. nuttalii* and genus *Epialtus* ranged from 20.7 to 23.6% for
Figure 3. Carapace shape of epiialtid crabs: (A) ‘Epialtus bituberculatus’, male CL 6.8 mm (CCDB 2431, São Paulo, Brazil); (B) ‘E. bituberculatus’, ovigerous female CL 6.9 mm (CCDB 2426, Bahia, Brazil); (C) ‘E. bituberculatus’, male CL 6.9 mm (CCDB 2429, Venezuela); (D) ‘E. bituberculatus’, ovigerous female CL 7.8 (CCDB 3861, Rio de Janeiro, Brazil); (E) ‘E. bituberculatus’, male CL 6.9 mm (CCDB 917, Panama – Atlantic coast); (F) ‘E. bituberculatus’, male CL 9.7 mm (CCDB 2441, São Paulo, Brazil); (G) ‘E. bituberculatus’, male CL 11.5 mm (CCDB 3813, Pernambuco, Brazil); (H) ‘E. bituberculatus’, male CL 10.8 mm (CCDB 3634, Venezuela); (I) ‘E. bituberculatus’, male CL 8.9 mm (CCDB 2441, São Paulo, Brazil); (J) ‘E. brasiliensis’, male CL 10.9 (CCDB 2373, São Paulo, Brazil); (L) ‘E. brasiliensis’, male CL 10.7 mm (CCDB 2432, São Paulo, Brazil); and (M) ‘E. brasiliensis’ 13.2 mm (CCDB 0437, São Paulo, Brazil).

Notes: Scale bars: 2 mm (A, B, C, D, E); 5 mm (F, G, H, I, J, L, M). Species with name in inverted commas refers to the original identification.
Figure 4. Last three pereopods of epialtid crabs: (A) ‘Epialtus bituberculatus’ (CCDB 2431, São Paulo, Brazil); (B) ‘E. bituberculatus’ (CCDB 2426, Bahia, Brazil); (C) ‘E. bituberculatus’ (CCDB 2429, Venezuela); (D) ‘E. bituberculatus’ (CCDB 3861, Rio de Janeiro, Brazil); (E) E. bituberculatus (CCDB 917, Panama – Atlantic coast); (F) ‘E. bituberculatus’ (CCDB 2441, São Paulo, Brazil); (G) ‘E. bituberculatus’ (CCDB 3813, Pernambuco, Brazil); (H) ‘E. bituberculatus’ (CCDB 3634, Venezuela); (I) E. brasiliensis (CCDB 2373, São Paulo, Brazil); and (J) E. brasiliensis (CCDB 2432, São Paulo, Brazil).

Notes: Scale bars: 1 mm (A, B, C, D, E, F, H); 2 mm (I, J); (G: 2, 1, 1 mm). Species with name in inverted commas refers to the original identification.
Figure 5. Phylogram obtained from ML analysis of 16S mtDNA sequences for Brazilian species of the genus *Epialtus*. Showing three clades: (A) Caribbean; (B) Venezuela; and (C) Brazil. Notes: ATL: Atlantic coast; BA: Bahia; Br: Brazil; CR: Costa Rica; Pa: Panama; SP: São Paulo; PE: Pernambuco; RJ: Rio de Janeiro; USA: United States of America; and Ve: Venezuela. Numbers below the branches are significance values for 1000 bootstraps; values ≤50% are not shown. Numbers in the right are specimens in the Table 4. All the species with names in inverted commas refer to the original identification.

Figure 6. Phylogram obtained from ML analysis of COI sequences for Brazilian species of the genus *Epialtus*. Showing three clades: (A) Caribbean; (B) Venezuela; and (C) Brazil. Notes: ATL: Atlantic coast; BA: Bahia; MX: Mexico; SP: São Paulo; PE: Pernambuco; RJ: Rio de Janeiro; and USA: United States of America. Numbers below the branches are significance values for 1000 bootstraps; values ≤50% are not shown. Numbers in the right are specimens in the Table 5. All the species with names in inverted commas refer to the original identification.
Table 4. Genetic divergence matrix of the partial sequences of the 16S gene among the specimens of genus *Epialtus*.

|       | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1. Taliepus nuttallii _USA* | 0.0   |       |       |       |       |       |       |       |       |       |       |       |
| 2. *E. dilatatus* _Panama_ | 20.7  | 0.0   |       |       |       |       |       |       |       |       |       |       |
| 3. *E. bituberculatus* _Costa Rica_ATL_ | 23.6  | 17.0  | 0.0   |       |       |       |       |       |       |       |       |       |
| 4. *E. bituberculatus* _Panama_ATL_ | 23.6  | 17.0  | 0.0   | 0.0   |       |       |       |       |       |       |       |       |
| 5. *E. bituberculatus* _Venezuela_ | 23.1  | 17.3  | 4.9   | 4.9   | 0.0   |       |       |       |       |       |       |       |
| 6. *E. bituberculatus* _BA-Brazil_ | 22.7  | 17.3  | 4.6   | 4.6   | 3.0   | 0.0   |       |       |       |       |       |       |
| 7. *E. bituberculatus* _PE-Brazil_ | 22.7  | 17.3  | 4.6   | 4.6   | 3.0   | 0.0   | 0.0   |       |       |       |       |       |
| 8. *E. bituberculatus* _RJ-Brazil_ | 22.7  | 16.9  | 4.6   | 4.6   | 3.0   | 0.3   | 0.3   | 0.0   |       |       |       |       |
| 9. *E. bituberculatus* _SP-Brazil_ | 22.7  | 16.9  | 4.6   | 4.6   | 3.0   | 0.3   | 0.3   | 0.0   | 0.0   | 0.0   | 4.6   | 0.0   |
| 10. *E. brasiliensis* _Panama_ATL_ | 23.6  | 20.3  | 0.3   | 0.3   | 5.3   | 4.6   | 4.6   | 4.6   | 4.6   | 0.0   |       |       |
| 11. *E. brasiliensis* _SP-Brazil_ | 22.7  | 16.9  | 4.6   | 4.6   | 3.0   | 0.3   | 0.3   | 0.0   | 0.0   | 4.6   | 0.0   | 0.0   |
| 12. *E. brasiliensis* _SP-Brazil_ | 22.7  | 16.9  | 4.6   | 4.6   | 3.0   | 0.3   | 0.3   | 0.0   | 0.0   | 4.6   | 0.0   | 0.0   |

*GenBank Sequence, (1) EU682828; ATL: Atlantic coast; BA: Bahia; USA: United States of America; SP: São Paulo; PE: Pernambuco; and RJ: Rio de Janeiro.

Table 5. Genetic divergence matrix of the partial sequences of the COI gene among the specimens of genus *Epialtus*.

|       | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1. Taliepus nuttallii _USA* | 0.0   |       |       |       |       |       |       |       |       |       |       |       |       |
| 2. *E. dilatatus* _Panama_ATL_ | 23.9  | 0.0   |       |       |       |       |       |       |       |       |       |       |       |
| 3. *E. bituberculatus* _Panama_ATL_ | 18.2  | 19.7  | 0.0   |       |       |       |       |       |       |       |       |       |       |
| 4. *E. bituberculatus* _Venezuela_ | 17.6  | 19.9  | 9.3   | 0.0   |       |       |       |       |       |       |       |       |       |
| 5. *E. bituberculatus* _Venezuela_ | 17.6  | 20.1  | 9.3   | 0.4   | 0.0   |       |       |       |       |       |       |       |       |
| 6. *E. bituberculatus* _PE-Brazil_ | 19.5  | 19.8  | 8.4   | 6.9   | 6.9   | 0.0   |       |       |       |       |       |       |       |
| 7. *E. bituberculatus* _PE-Brazil_ | 19.2  | 19.3  | 8.6   | 6.7   | 6.7   | 0.6   | 0.0   |       |       |       |       |       |       |
| 8. *E. bituberculatus* _BA-Brazil_ | 19.2  | 19.8  | 8.2   | 6.7   | 6.7   | 0.2   | 0.4   | 0.0   |       |       |       |       |       |
| 9. *E. bituberculatus* _RJ-Brazil_ | 19.0  | 21.1  | 7.7   | 6.7   | 6.7   | 1.8   | 2.0   | 1.7   | 0.0   |       |       |       |       |
| 10. *E. bituberculatus* _SP-Brazil_ | 19.2  | 20.8  | 7.9   | 6.9   | 6.9   | 2.0   | 2.2   | 1.8   | 0.2   | 0.0   |       |       |       |
| 11. *E. bituberculatus* _SP-Brazil_ | 19.2  | 21.3  | 7.9   | 6.9   | 6.9   | 2.0   | 2.2   | 1.8   | 0.2   | 0.4   | 0.0   |       |       |
| 12. *E. brasiliensis* _SP-Brazil_ | 19.0  | 21.1  | 7.7   | 6.7   | 6.7   | 1.8   | 2.0   | 1.7   | 0.0   | 0.2   | 0.2   | 0.0   |       |
| 13. *E. brasiliensis* _SP-Brazil_ | 19.0  | 21.1  | 7.7   | 6.7   | 6.7   | 1.8   | 2.0   | 1.7   | 0.0   | 0.2   | 0.2   | 0.0   | 0.0   |

*GenBank Sequence, (1) EU682873; ATL: Atlantic coast; BA: Bahia; USA: United States of America; SP: São Paulo; PE: Pernambuco; and RJ: Rio de Janeiro.

Figure 7. (A) Parsimonious tree obtained from combined analysis of continuous data (eight measurements of traditional morphometrics) and genetic data (16S and COI). (B) Details of the southeast (SE) clade for parameter $k = 7$; (C) Details of the SE clade for parameter $k = 10$.

Notes: ATL: Atlantic coast; BA: Bahia; Br: Brazil; NE: Northeast Region; Pa: Panama; PE: Pernambuco; RJ: Rio de Janeiro; SP: São Paulo; and Ve: Venezuela. Numbers below the branches are significance values for 1000 resampling, probability 33. The arrow indicates differences in the values of branch support compared with the whole tree.
16S (Table 4) and from 17.6 to 23.9% for COI (Table 5). Within *Epialtus*, the genetic distances ranged from 0 to 17.4% and 0 to 21.3% for 16S and COI, respectively. Interspecific distances between *E. bituberculatus* and *E. brasiliensis* ranged from 0 to 5.3% for 16S and 0 to 9.3% for COI. For 16S, the intraspecific distances ranged from 0 to 4.6% (Table 4). For COI, intraspecific values for *E. bituberculatus* and *E. brasiliensis* ranged from 0.2 to 9.3% and 0 to 0.2%, respectively (Table 5).

The phylogram constructed from the ML for 16S and COI showed a clear separation between *E. dilatatus* and the two species that are the focus of this study, but could not distinguish *E. bituberculatus* and *E. brasiliensis* from each other (Figures 5 and 6) as some specimens of *E. brasiliensis* were positioned within the group of *E. bituberculatus*. Overall, three distinct groups were observed: the first (upper part of phylogram) was formed by specimens from Caribbean, hereafter referred to as group A – *Epialtus bituberculatus*; the second clade consisted of specimens from Venezuela, hereafter group B – *Epialtus* n. sp.; and the third one, group C – *Epialtus brasiliensis*, contained specimens from Brazil (Figures 5 and 6). All of the species with names in inverted commas refer to the original identification. All branches were well supported with bootstrap values above 50% (see Figures 5 and 6).

Divergences among clades observed in ML tree were 4.7% between group A (Caribbean) and group B (Venezuela) for both 16S and COI; 4 and 4.1% for 16S and COI between groups A and C, respectively; and 3.2% for 16S and 3.4% for COI between groups B and C.

**Discussion**

The topology and small interspecific genetic distances (16S: 0 and 5.3%; COI: 0 and 9.3%) showed that *E. brasiliensis* falls within the group of *E. bituberculatus*. According to the genetic similarities among specimens in the ML tree, we infer that our initial hypothesis of two valid species was not supported and the main character (a spine in the propodus of ambulatory legs) that supposedly has been used to separate the two species and the others characters we had examined were not informative enough to support the two taxa, as they are quite variable. This genetic divergence is lower than the 5–24% interspecific genetic distance threshold in DNA barcoding for crustaceans.[47] Furthermore, it can be use to clarify cases of deep genetic divergence among individuals grouped as a single species that may indicate overlooked species, along with conventional taxonomic approaches.[47]

Both *E. bituberculatus* and *E. brasiliensis* have similar habitats, coexisting on the same algae.[9,21,48, pers. obs.] The spine of ambulatory leg was conspicuous in all specimens that we called *E. brasiliensis*, but we observed that they also varied in size or can be reduced. This spine may be the result of environmental selection as the ambulatory legs are used to cling onto algae or even rocks.[9,17,49,50, pers. obs.] and this habit perhaps required a means of attachment afforded by subchelate legs.[2] However, if the absence of the spine could be occasionally caused by accidental loss, evidences of damage or a tubercle or a bump should still remain, but none was observed. This morphological difference could be the expression of intraspecific variability[51] or we suppose it is a plastic character and the presence or absent can be at random.

Genetic distance and color can be used for species recognition. Recently, they have unmasked a cryptic hermit crab species *Clibanarius symmetrius* (Randall, 1840) that differs from a related species *C. vittatus* (Bosc, 1802) in color pattern of pereiopods with a genetic divergence ranging from 5.18 to 7.29%.[52] However, from *E. bituberculatus* and *E. brasiliensis*, we cannot infer about color because it varies according to the substrate or algae they inhabit[9,11] from dark green, brown and yellow[16] to sometimes with white spots in queliped (pers. obs.).

Analogous morphological and molecular analyses on decapods can be used to support our assertion. Pérez-Barros et al. [53] observed little genetic distance between sympatric squat lobsters *Munida gregaria* and *M. subrugosa* (0 and 0.2% for 16S and COI, respectively), which led the authors to synonymize them and suggest that they were morpho-species of a single taxon, and may be one of the plausible explanation for the *Epialtus* case reported here. In addition, some morpho-species that lack
genetic differentiation could either be due to recent speciation or extensive hybridization.[54] For the tree-climbing mangrove crab *Aratus pisonii* from the same ocean (Jamaica, Hispaniola, Brazil, Costa Rica and Ecuador), no consistent morphological differences and limited genetic differentiation were found among the studied populations.[55] However, based on the morphologies of male pleopod and on genetic data (16S: 3.0–3.5% and 28S: 0.2–0.3%), populations from different oceans are distinct and required the description of a new species, *A. pacificus*, as the sister species of *A. pisonii*. Unfortunately, we did not find morphological characters to justify genetic clusters observed here. If they are different species that arise recently, old traits could not be used to recognize and describe them.

The classification suggested here also found support in the larval morphology. The superfamily Majoidae presents two zoeal stages and one megalopa, and the characters are largely used to understand taxonomic and evolutionary relationships within this group.[22,56–58] Similarities in the larval traits of the two species of *Epialtus* provide additional support to the genetic data from the branch of Brazilian specimens. The zoeae I of *E. bituberculatus* closely resemble the zoeae I of *E. brasiliensis* in several aspects: quantity and pattern of spines and setae on carapace, presence and position of tubercles in abdomen, quantity of setae maxillule and identical patterns of setae on scaphognathite, first and second maxillipeds.[22,57] They share one feature (an unilobed coxal endite of the maxilla) with *E. dilatatus* and are different in the other characters.[22,56] The larval duration aspect is not frequently discussed by authors and some papers do not provide this information.[57] Majids are characterized by advanced development, in which the young hatch as zoeae, but in a state more developed than in other families. This is characterized by fewer stages as well as shorter duration of stages.[59] The duration of the larval stages of *E. brasiliensis* was 26 days, approximately as long as that of *E. dilatatus* and *E. bituberculatus* reared under similar conditions.[22,56,57] This time can be considered short; if in natural conditions larvae remain less time in the plankton stage, this potentially limits the dispersion of the group to other localities, which partially explains why the Caribbean is separated from Brazil.

These conditions and similarities in larval traits are consistent with the genetic similarity observed for our samples in group C (Figures 5 and 6) and therefore provide additional evidence of a single species of *Epialtus* in Brazil. Hence, we suggest that there is only one species in the Brazilian coast that is genetically distinct from the one in the Caribbean and Venezuela and we proposed to nominate it as *E. brasiliensis* because of its type locality is Rio de Janeiro.[23]

The Caribbean and Brazilian groups were strongly supported by both the genetic distances (16S: 4.6 and 5.3%; COI: 7.9 and 8.6%) and bootstrap values (16S: 93 and 88, respectively; COI: 98 from Brazil branch). This is in agreement with the 2–3% genetic distance which is often postulated as the threshold for recognizing separate species in DNA barcoding,[60,61] including decapod crustaceans.[47] The distinction is likely because of geographical distances, intraspecific variability or perhaps the Amazon barrier. The Amazon is a potential impediment to dispersal in the coastal waters because of the freshwater and silt it deposits at the mouth, where the sea water is characterized by greatly reduced salinity and increased turbidity.[62] The coastal and algae-substrate marine cidean shrimp *Hippolyte obliquimanus* Dana, 1852 can be separated into Caribbean and Brazilian groups, but there are no morphological differences between them, and authors believed that the distinction could be explained by the Amazon barrier.[63] However, we did not observe morphological differences of species of *Epialtus* that support this separation that was justified by the combined analysis clustered with the molecular clades. Thus, identification should be based possibly by their geographical distribution. Here, we suggest the Caribbean group as *E. bituberculatus* because it is the older species, although its type locality has been erroneously referred to Chile [6]; all remaining occurrences were for the western Atlantic, [2,3,7,10,11,41] including the Caribbean.[12–15]

Specimens from Venezuela fell along a different branch, which could be a distinct species, but at this phase, we did not find morphological diagnosing characters due the low number of specimens available for analysis. On the other hand, there are also species with only little or no morphological differences, which can only be distinguished by genetic differences; and this is of particular interest if there are cryptic species.[54] This difference may be due to the barrier formed by the rivers in Venezuela and Brazil,[63] geographical distance, variability within the population or the distinctness of the Venezuelan stock which suggests that three cryptic species evolved recently. Specimens of the foliate kelp crab *Mimulus foliatus* Stimpson (1860) have variable lengths of the rostral horns and the supra-orbital angles varied with age and sex, but they observed that the structure of the first pleopod of male fell within the range of species variation of the genus *Pugettia* Dana, 1851 and justified the change of *M. foliatus* to this genus with a new name *Pugettia foliata* (Stimpson, 1860).[46] Negri et al. [64] found that the specimens of *Clibanarius vitatus* (Bosc, 1802) were separated into two subgroups, one from Brazil and another from the Gulf of Mexico with high molecular divergences (COI and 16S genes) between these two subgroups, suggesting the existence of a cryptic species conformed under the name *C. vitatus*. 
Although Epialtus affinis is synonymous of E. bituberculatus,[2] we did not resurrect this name to designate specimens from Venezuela because E. affinis was recorded only in Florida (USA) [43], the type was probably lost [2] and unfortunately, we have no specimens available from this region for molecular comparison. Furthermore, descriptions of E. longirostris and E. sulcirostris species show they are similar to E. bituberculatus and E. affinis in general characters of carapace, but differ in the shape and length of rostrum and chelipeds. [65] Both were synonyms of E. bituberculatus [42], but they were validated by the same author in 1925, and E. sulcirostris occurs only in some localities of the Gulf of California. [2,3] Thus, we propose that specimens from Venezuela are a new species and are not named so far due to the lack of morphological material and genetic analyses.

The five sympatric species of Thenus Leach, 1815 are remarkably homogenous in appearance, but with significant genetic divergence; a combination of live color patterns and morphometric ratios was used for species discrimination. [66] These authors emphasized T. australiensis and T. orientalis as the most difficult to distinguish within the genus using morphological characters, even though they are clearly distinct genetically (divergence of 2%). This low interspecific divergence clearly indicates a recent evolutionary split between these two sister taxa [66] as well as in the different genetic groups (Caribbean, Venezuela and Brazil) of Epialtus. Thus, we suggest that these groups have not had enough time to evolve morphological traits to distinguish them from each other; or the environmental selection favors the same phenotype even in different geographical areas. [67] If the phenotype is appropriate or near the optimum, there will be pressure to its maintenance and not for its change. [67,68]

First, there were two species of Epialtus that occur in Brazil, E. bituberculatus and E. brasiliensis, with a main character (the spine on the propodus’ ventral surface in the ambulatory pereopods) previously used to separate them from each other. However, molecular findings and combined phylogenetic analysis showed that E. brasiliensis fell within the branch of E. bituberculatus. Furthermore, based on the genetic differences and combined analysis, there were three groups: Caribbean sea, Venezuela and Brazil. Since we observed no phenotypic differentiation to validate such separation at this time, we propose a genetic and geographical new classification for the three groups: the Caribbean species is E. bituberculatus; specimens from Venezuela are a different and probably a new species; and there is a single species in Brazil, which is E. brasiliensis. Possibly, we have three recent/ cryptic species for which we have not found robust morphological characters to support. Finally, we suggest including other DNA sequences to increase the number of E. bituberculatus and E. brasiliensis specimens and populations, including species from Pacific, and new analyses with other genes to justify this separation and provide evidence for a separate species in Venezuela.

Author contribution
The authors contributed equally to the general idea, data collection, analysis, interpretation, writing and critically revising the paper for intellectual content.

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References
[1] Ng PKL, Guinot D, Davie PJF. Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. Raffles Bull Zool. 2008;17:1–286.
