



# A new species of freshwater crab genus *Fredius* Pretzmann, 1967 (Crustacea: Brachyura: Pseudothelphusidae) from a naturally isolated orographic forest enclave within the semiarid Caatinga in Ceará, northeastern Brazil

Liviano C. Santos<sup>1,2</sup>, Marcos Tavares<sup>3</sup>, José R.F. Silva<sup>1</sup>, Marcelo Cervini<sup>4</sup>, Allysson P. Pinheiro<sup>2</sup> and William Santana<sup>2,5</sup>

<sup>1</sup>Programa de Pós-Graduação em Ecologia e Recursos Naturais, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil

<sup>2</sup>Laboratório de Crustáceos do Semiárido (LACRUSE), Universidade Regional do Cariri, Crato, Ceará, Brazil

<sup>3</sup>Museum of Zoology, University of São Paulo, São Paulo, Brazil

<sup>4</sup>Departamento de Ciências Biológicas, Universidade Estadual Sudoeste da Bahia, Jequié, Bahia, Brazil

<sup>5</sup>Laboratory of Systematic Zoology (LSZ), Universidade do Sagrado Coração, Bauru, São Paulo, Brazil

## ABSTRACT

A new species of freshwater crab, *Fredius ibiapaba*, is described and illustrated from a mid-altitude forested patch in Ipú (Ibiapaba plateau, Ceará, northeastern Brazil), between 635 to 782 m. The new species can be separated from its congeners by the morphology of its first gonopod: proximal half remarkably swollen, sloping abruptly downwards distally to a nearly right-angular shoulder; mesial lobe much smaller than cephalic spine; cephalic lobe moderately developed; auxiliary lobe lip, delimiting field of apical spines, protruded all the way to distal margin of auxiliary lobe. Comparative 16S rDNA sequencing used to infer the phylogenetic placement of *Fredius ibiapaba* n. sp. revealed that it is the sister taxon of *F. reflexifrons*, a species which occurs allopatrically in the Amazon and Atlantic basin's lowlands (<100 m). *Fredius ibiapaba* n. sp. and *F. reflexifrons* are highly dependent upon humidity and most probably were once part of an ancestral population living in a wide humid territory. Shrinking humid forests during several dry periods of the Tertiary and Quaternary likely have resulted in the fragmentation of the ancestral humid area and hence of the ancestral crab population. *Fredius reflexifrons* evolved and spread in a lowland, humid river basin (Amazon and Atlantic basins), whilst *F. ibiapaba* n. sp. evolved isolated on the top of a humid plateau. The two species are now separated by a vast intervening area occupied by the semiarid Caatinga

Submitted 6 December 2019

Accepted 27 May 2020

Published 29 June 2020

Corresponding author  
Allysson P. Pinheiro,  
allysson.pinheiro@urca.br

Academic editor  
Tony Robillard

Additional Information and  
Declarations can be found on  
page 18

DOI 10.7717/peerj.9370

© Copyright  
2020 Santos et al.

Distributed under  
Creative Commons CC-BY 4.0

OPEN ACCESS

**Subjects** Biodiversity, Taxonomy, Zoology, Freshwater Biology

**Keywords** Fredius, Refuges, Brejos, Ibiapaba, Ipú, Amazon, Zoogeography

## INTRODUCTION

Cumulative evidence from many independent sources argue in favor of the mid-altitude forested patches in northeastern Brazil being remnants of a once much larger humid forest, connected to both the Amazonian and Atlantic rainforests during the moister periods (e.g., [Andrade-Lima, 1982](#); [Cartelle & Hartwig, 1996](#); [De Vivo, 1997](#); [Ab'Saber, 2000](#); [Auler et al., 2004](#); [Carnaval & Bates, 2007](#); [Carmignotto, De Vivo & Langguth, 2012](#); and references therein). These humid forest refuges ([Figs. 1A–1D](#)), naturally isolated by the vast surrounding semiarid Caatinga ([Figs. 1F, 1G](#)), are indeed known to harbor many woody plant and animal species (fossil and Recent) that are also found or are closely related to species occurring allopatrically in the Amazonian and Atlantic rainforests.

Here we describe and illustrate a new species of a freshwater pseudothelphusid crab, *Fredius ibiapaba* n. sp., from a humid forest refuge in Ipú (Ibiapaba plateau, Ceará, northeastern Brazil), between 665 to 782 m ([Figs. 1A–1D](#)). Evidence from a phylogenetic analysis using 16S rDNA is presented for a sister taxa relationship between *Fredius ibiapaba* n. sp. and *F. reflexifrons* (Ortmann, 1897), a species occurring allopatrically in the Amazonian humid lowlands. Previous hypothesis on the phylogenetic relationships of *F. reflexifrons* and the possible evolutionary scenario that led to the emergence of the sister taxa *Fredius ibiapaba* n. sp. and *F. reflexifrons* are discussed.

## MATERIALS & METHODS

### Procedures with material examined

The specimens were collected using license permission from the Sistema de Autorização e Informação em Biodiversidade (SISBIO #29615) of the Brazilian Ministry of Environment (MMA). The studied specimens are deposited in the collections of the INPA (Instituto Nacional de Pesquisas da Amazônia, Manaus), MZUSP (Museu de Zoologia, Universidade de São Paulo, Brazil) and LACRUSE (Laboratório de Crustáceos do Semiárido). Other acronyms: SMF (Naturmuseum Senckenberg) and CCDB (Coleção de Crustáceos do Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto). Measurements: cl (carapace length, taken along the carapace axis to the posterior median margin) and cw (carapace width, taken at the widest point), in millimeters (mm). Dates are written in the format day.month.year, with months in lower-case Roman numerals. Abbreviations are as follows: G1, G2, first and second gonopods, respectively. Mxp3, third maxilliped. The terminology used in the description of the G1 essentially follows ([Rodríguez & Pereira, 1992](#); [Rodríguez & Campos, 1998](#)) ([Fig. 2](#)).

### Molecular data analysis

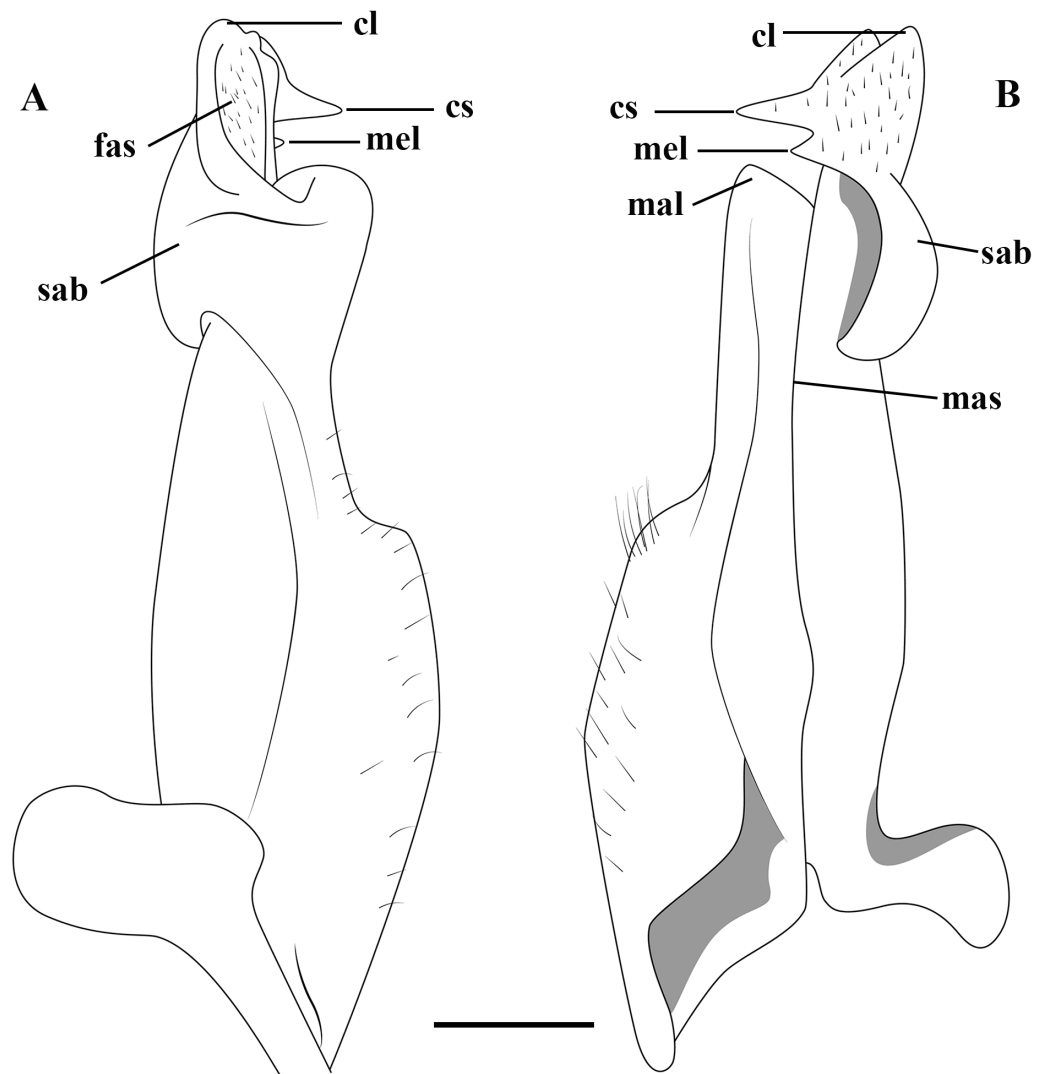
DNA extraction, amplification and sequencing: muscle tissue samples were obtained from the pereopods or pleon of *Fredius ibiapaba* n. sp., *F. buritizatis* Magalhães & Mantellato in [Magalhães et al., 2014](#), and *Prionothelphusa eliasi* Rodriguez, 1980. At the Laboratório de Biologia Molecular da Universidade Estadual do Sudoeste da Bahia- LBM/UESB a small region of the 16S rDNA gene was extracted with Wizard<sup>®</sup> Genomic DNA Purification Kit (Promega), amplified in a 12,5 µl final volume reaction with 2,5 mM de MgCl<sub>2</sub>



**Figure 1** Sítio Caranguejo, Ipú, Ceará, 04°18'50"S, 40°44'47"W, 729 m high, type locality of *Fredius ibiapaba* n. sp. (A–E) Mid-altitude, naturally isolated, humid forested patch nested within the vast semi-arid Caatinga domain. Note in (E) burrow (arrow) of *Fredius ibiapaba* n. sp. among the leaf litter. (E–F) Lowland, surrounding semiarid Caatinga forest. (E) View from above from Ipú. (F) Detail of a dry-stream channel.

Full-size DOI: 10.7717/peerj.9370/fig-1

(Invitrogen), 0,05 mM de dNTP (Invitrogen), buffer 1x (Invitrogen –10xPCR Buffer: 200mM Tris-HCl (pH 8.4), 500mM KCl), 1U de taq platinum (Invitrogen) and 0,3 $\mu$ M of each primer. The PCR conditions were: one cycle at 94 °C, 60 s; five cycles at 94 °C, 60 s; 45 °C, 40 s and 72 °C, 60 s; and 35 cycles at 94 °C, 60 s; 51 °C, 40 s and 72 °C; 60 s; a final extension of five minutes at 72 °C was performed. The primers used were 16Sar (5'-CCGGTCTGAACTCAGATCACGT-3') and 16Sbr (5'-CGCCTGTTTATCAAAAACAT-3') (Palumbi et al., 1991). PCR products were purified using a polietilenoglicol (PEG) 20% and sequenced in an ABI Prism 3100 Genetic Analyzer<sup>®</sup> (Applied Biosystems) at the



**Figure 2** (A–B) Semi-diagrammatic view of the first male gonopod in abdominal and sternal views, respectively, with the terminology used in the descriptions. Cl, cephalic lobe; cs, cephalic spine; fas, field of apical spines; mal, marginal lobe; mas, marginal suture; mel, mesial lobe; sab, subapical bulge.

Full-size [DOI: 10.7717/peerj.9370/fig-2](https://doi.org/10.7717/peerj.9370/fig-2)

Departamento de Tecnologia da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Jaboticabal. Sequencing reaction was performed with Big Dye v3.1 (Applied Biosystems), prepared with 4,75  $\mu$ l ultrapure water, 1,5  $\mu$ l BigDye 5 $\times$ buffer, 0,75  $\mu$ l BigDye terminator Mix, 2  $\mu$ l primer (0,8 pmol) and 1  $\mu$ l of Purified PCR product. Sequence conditions were: one minute at 96  $^{\circ}$ C; 35 cycles of 15 s at 96  $^{\circ}$ C; 15 s at 50  $^{\circ}$ C and 2 min at 60  $^{\circ}$ C. Both, forward and reverse sequence strands were obtained and the consensus generated by the software BioEdit 7.0.5 (Hall, 2005). The identities of the final sequences were confirmed with a BLAST (Basic Local Alignment Search Tool) on GenBank database. Additional comparative sequences were retrieved from GenBank (Table 1).

**Table 1** Species of *Fredius* Pretzmann, 1967, *Prionothelphusa* Rodriguez, 1980 and *Trichodactylus* Latreille, 1828 used in the phylogenetic analyses, with respective sample locality, GenBank accession number and catalogue number of the voucher specimen.

Species	Locality	GenBank accession numbers	Catalogue number
<i>Fredius buritizatis</i>	Ji-Paraná, Rondônia, Brazil	JN402376	INPA 1891
<i>Fredius buritizatis</i>	Ji-Paraná, Rondônia, Brazil	JN402377	CCDB 342
<i>Fredius buritizatis</i>	Chupinguaia, Rondônia, Brazil	MN787136	LACRUSE002
<i>Fredius denticulatus</i>	Serra do Navio, Amapá, Brazil	JN402372	INPA 582
<i>Fredius estevisi</i>	Posto Indígena Parafuri, Roraima, Brazil	JN402379	INPA 839
<i>Fredius fittkai</i>	Aldeia Balawa-ú, Amazonas, Brazil	JN402373	INPA 1330
<i>Fredius platyacanthus</i>	Comunidade Paapi-ú, Roraima, Brazil	JQ414023	INPA 841
<i>Fredius ibiapaba</i> n. sp.	Sítio Caranguejo, Ipu, Ceará, Brazil	MN787135	LACRUSE001
<i>Fredius reflexifrons</i>	Rio Chumucuí, Bragança, Pará, Brazil	JN402378	INPA 1512
<i>Fredius stenolobus</i>	Rio Tawadu, Bolívar, Venezuela	JN402374	INPA 833
<i>Fredius stenolobus</i>	Aldeia Palimi-ú, Rio Uraricoera, Roraima, Brazil	JN402375	INPA 848
<i>Prionothelphusa eliasi</i>	Japurá, Vila Bittencourt, Amazonas, Brazil	MN787137	LACRUSE003
<i>Trichodactylus dentatus</i>	Bahia, Brazil	FM208777	SMF 32763

Phylogenetic analyses: substitution saturation in 16S rDNA was tested using the saturation index implemented in DAMBE 5 (Xia, 2013). The sequences were grouped and edit in BioEdit and aligned using the ClustalW interface (Thompson, Higgins & Gibson, 1994). *Prionothelphusa eliasi* (Pseudothelphusidae) and *Trichodactylus dentatus* H. Milne Edwards, 1853 (Trichodactylidae) were chosen as outgroups. The best-fit model HKY + G was selected using jModeltest 2.1.7 (Darriba et al., 2012). This model was used to generate Maximum Likelihood gene trees in MEGA 6.06 (Tamura et al., 2013). Branch support values were calculated using bootstrap analyses with 1,000 replicates (Felsenstein, 1985). Only nodes with bootstrap support greater than 50 are shown on the phylogenetic tree. Nucleotide divergence estimated from pairwise distance was calculated in MEGA 6.06 with the same best-fit model (Table 2).

### Registration of nomenclatural act

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: [urn:lsid:zoobank.org:pub:0925982D-7441-120 4256-9856-A553987956A6]. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

**Table 2** Pairwise distance matrix from the portion of the mitochondrial 16S rRNA based on ~560 bp.

	1	2	3	4	5	6	7	8	9	10	11	12
1 <i>Fredius ibiapaba</i> n. sp.	–	–	–	–	–	–	–	–	–	–	–	–
2 <i>Fredius reflexifrons</i>	0,04	–	–	–	–	–	–	–	–	–	–	–
3 <i>Fredius burutzatilis</i>	0,10	0,07	–	–	–	–	–	–	–	–	–	–
4 <i>Fredius burutzatilis</i>	0,11	0,08	0,02	–	–	–	–	–	–	–	–	–
5 <i>Fredius burutzatilis</i>	0,11	0,08	0,02	0,00	–	–	–	–	–	–	–	–
6 <i>Fredius denticulatus</i>	0,12	0,09	0,08	0,08	0,08	–	–	–	–	–	–	–
7 <i>Fredius stenolobus</i>	0,10	0,07	0,07	0,06	0,06	0,10	–	–	–	–	–	–
8 <i>Fredius stenolobus</i>	0,10	0,07	0,07	0,06	0,06	0,10	0,00	–	–	–	–	–
9 <i>Fredius estevisi</i>	0,11	0,07	0,07	0,06	0,06	0,09	0,02	0,02	–	–	–	–
10 <i>Fredius fittkai</i>	0,09	0,07	0,07	0,07	0,07	0,09	0,08	0,08	0,08	–	–	–
11 <i>Fredius platyacanthus</i>	0,10	0,07	0,06	0,06	0,06	0,09	0,02	0,02	0,02	0,08	–	–
12 <i>Prionothelphusa eliasi</i>	0,16	0,13	0,12	0,12	0,12	0,13	0,12	0,12	0,12	0,13	0,12	–
13 <i>Trichodactylus dentatus</i>	0,22	0,19	0,21	0,21	0,21	0,22	0,21	0,21	0,22	0,20	0,21	0,22

## RESULTS

Family Pseudothelphusidae Ortmann, 1893

Genus *Fredius* Pretzmann, 1967

*Fredius ibiapaba* n. sp. (Figs. 3A–3E; Figs. 4A–4C; Fig. 5A, 5C; Figs. 6A–6D; Figs. 7A–7E)

*Fredius reflexifrons* –Magalhães et al., 2005: 94, fig 1 –Santos et al., 2020: 3.

**Type material.** Holotype, Ceará, Ipú, Sítio Caranguejo, 04°18'50"S, 40°44'47"W, 729 m, xii.2017, male cl 36 mm, cw 53 mm (MZUSP 39710). Paratypes: Same data as holotype, male cl 34 mm, cw 48 mm (MZUSP 39169); Ceará, Ipú, Sítio Gameleira, 04°17'17"S, 40°44'44"W, 665 m, 5.i.2018, female cl 35 mm, cw 49 mm (MZUSP 39171); Ceará, Ipú, Sítio Santa Cruz, 04°19'40"S, 40°45'09"W, 782 m, 10.x.2014, male cl 32 mm, cw 48 mm (MZUSP 39167); Ceará, Ipú, Sítio Santa Cruz, 04°19'40"S, 40°45'09"W, 782 m, 23.iv.2015, female cl 31 mm, cw 44 mm (MZUSP 39168); Ceará, Ipú, Sítio Ipuçaba, 798 m, 27.xii.2017, male cl 41.2 mm, cw 62.6 mm (MZUSP 39742). Ceará, Ipú, Sítio Gameleira, quintal do Kindó, 04°17'42"S, 40°44'43"W, L.C. Cruz, J.G. Araújo, H.S. Mattos and J.E.P Araújo coll., 665 m, 01.v.2018, 3 males, cl 35.5 mm, cw 52.2 mm, cl 37.7 mm, cw 56.6 mm, cl 32.2 mm, cw 46.6 mm (LACRUSE 259). Ceará, Ipú, Sítio Santa Cruz, 04°19'40"S 40°45'09"W, L.C. Cruz coll., 782 m, 23.iv.2015, 2 males, cl 28.7 mm, cw 42.4 mm, cl 31.5 mm, cw 46.0 mm, 1 female, cl 37.7 mm, cw 55, one mm (LACRUSE 216).

**Non-type material.** Ceará, Viçosa do Ceará, Fonte do Caranguejo, 03°33'43.2"S, 41°5'09.6"W, M. Pereira coll., 24. vi. 2004, 2 males (INPA 1382).

**Comparative material.** *Fredius fittkai* (Bott, 1967): Guyana - Potaro-Siparuni, Rio Kuribrong, 05°22'35"N, 59°33'4"W, P. Bernardo and B. Newman coll., 28.ix.2010, male,



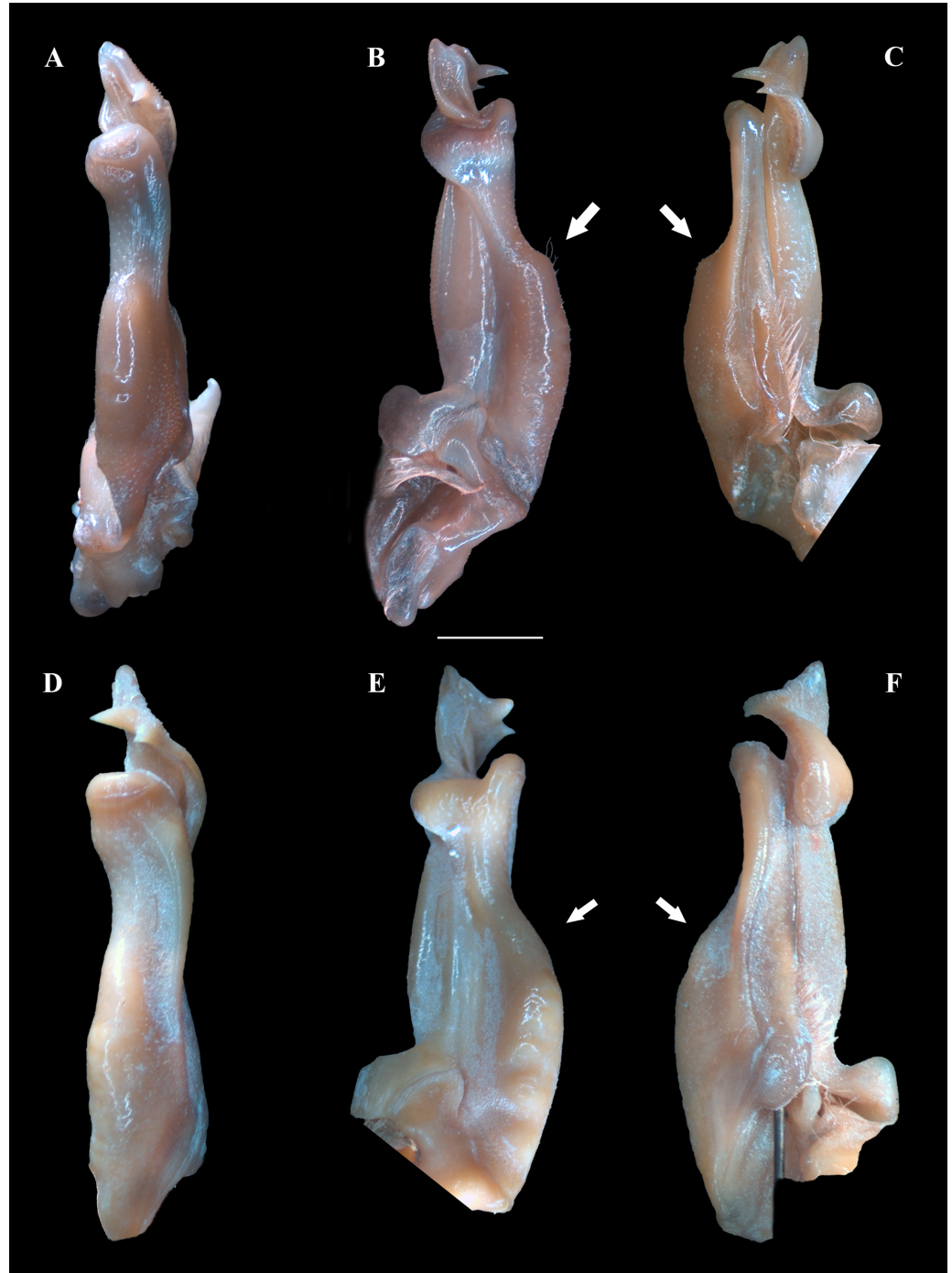
**Figure 3** *Fredius ibiapaba* n. sp., male cl 36 mm, cw 53 mm (MZUSP 39710). (A–B) Habitus, dorso and ventral views, respectively. (C) Cephalothorax, frontal view. (D–E) Right and left chelipeds in lateral view, respectively. Scales: A–E, 10 mm.

Full-size DOI: 10.7717/peerj.9370/fig-3

cl 47.1 mm, cw 66.9 mm (MZUSP 24497). *Fredius reflexifrons* (Ortmann, 1897): Brazil - Amapá, Serra do Navio, Serra do Veado, Projeto Diversitas Neotropica, M. Tavares coll. 7.v.1994, male, cl 37 mm, cw 52 m (MZUSP 19922). Amapá, Rio Jari, montante, Cachoeira Santo Antônio, M. Jegú and J. Zuanon coll., 9-26.vi.1981, 2 males, cl 42 mm, cw 57.7 mm, and cl 53 mm, cw 73.8 mm (MZUSP 13178). Amapá, Serra do Navio/ Serra do Veado, 07.v.1994, male (INPA 583). Amapá, Laranjal, 16.i.2012, male (INPA 2125). Amazonas, Manaus, Reserva do Km 41, 02°26'56"S, 59°46'13"W, male (INPA 889). Amazonas, Manaus, Reserva Ducke, 22.ii.1986, male (INPA 368). Amazonas, Manaus, 11.vii.2001, male (INPA 850). Amazonas, Iranduba, Sítio Anaíra, 03°10'39"S, 60°07'39"W, 12.ix.1999, male (INPA 852). Pará, Santarém, Com. Santa Rosa, male (INPA 1254). Pará, Rio do Peixe Boi, 01°11'30"S, 47°18'54"W, E. Matos and A. Henriques Jr coll., 03.iii.1995, male (INPA 851). Pará, Bragança, Rio Chumucuí, S. Alves coll., 12. xi. 2004, male (INPA 1512). Peru: Rio Apiacu, Departamento Loreto, Boris Malkin coll., 15-25.iv.1966, male, cl 31 mm, cw 42.5 mm (MZUSP 6389). *Fredius denticulatus* (H. Milne Edwards, 1853): Brazil - Rio Amapari, Serra do Navio, AP, Projeto Diversitas Neotropica, no 151, M. Tavares coll., 30.iv.1994, C. Magalhães det. 16.ii.1996, male cl 45 mm, cw 62 mm (MZUSP 16294).

**Type locality.** Sítio Caranguejo, Ipú, Ceará, 04°18'50"S, 40°44'47"W, 729 m.

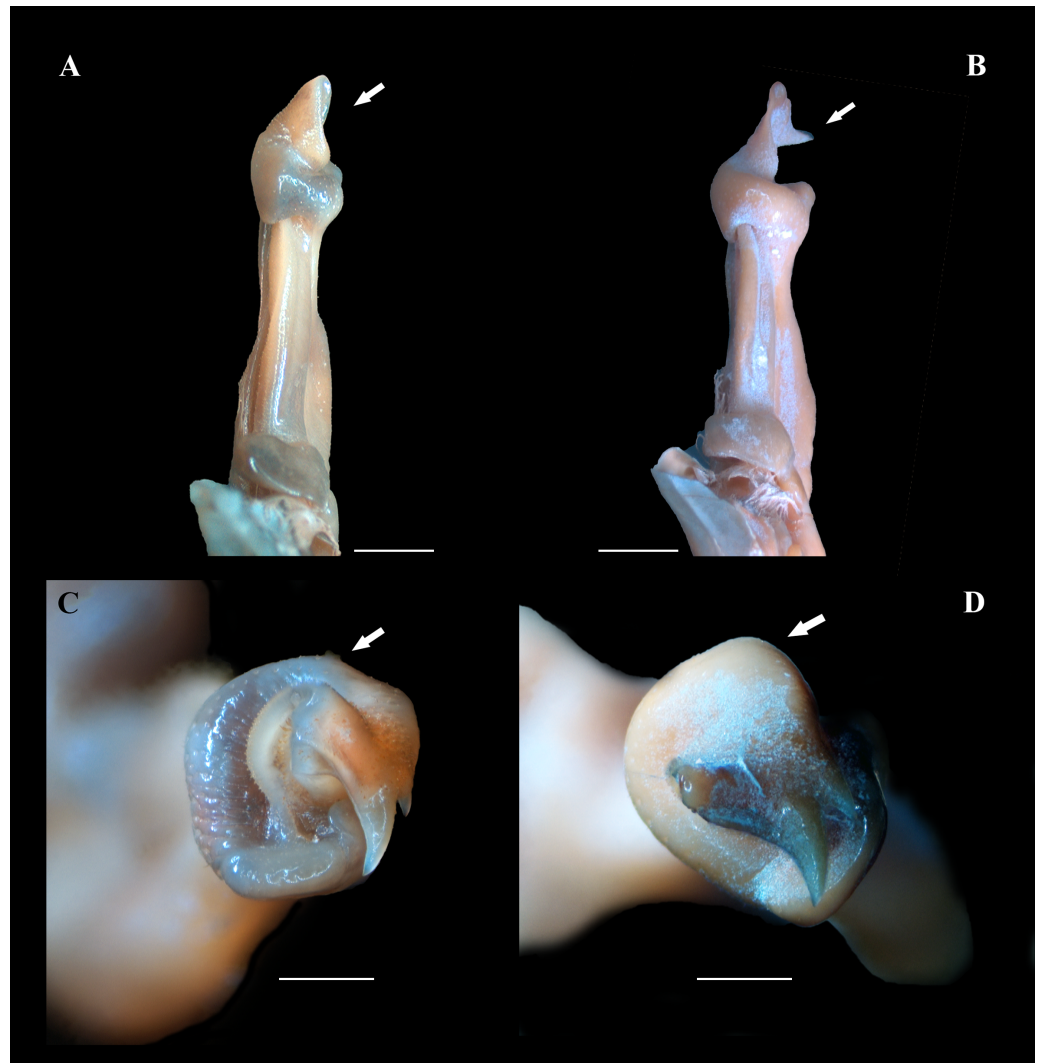
**Distribution.** Currently known from Ipú, Ibiapaba plateau, Ceará, northeastern Brazil, in mid-altitude forests between 665 to 798 m.



**Figure 4** (A–F) Right male first gonopod (G1) in pleonal (tilted left), lateral and mesial views from A–C and D–F, respectively. (A–C) *Fredius ibiapaba* n. sp., holotype, male cl 36 mm, cw 53 mm (MZUSP 39710). (D–F) *Fredius reflexifrons* (Ortmann, 1897), male cl 73.8 mm, cw 53 mm (MZUSP 13178). Note in (B, C) the G1 remarkably swollen, sloping abruptly downwards anteriorly to a nearly right-angular shoulder (arrow), and in (E, F) the G1 shoulder clearly more gently sloping distally (arrow). Scales: A–F, two mm.

Full-size  DOI: [10.7717/peerj.9370/fig-4](https://doi.org/10.7717/peerj.9370/fig-4)



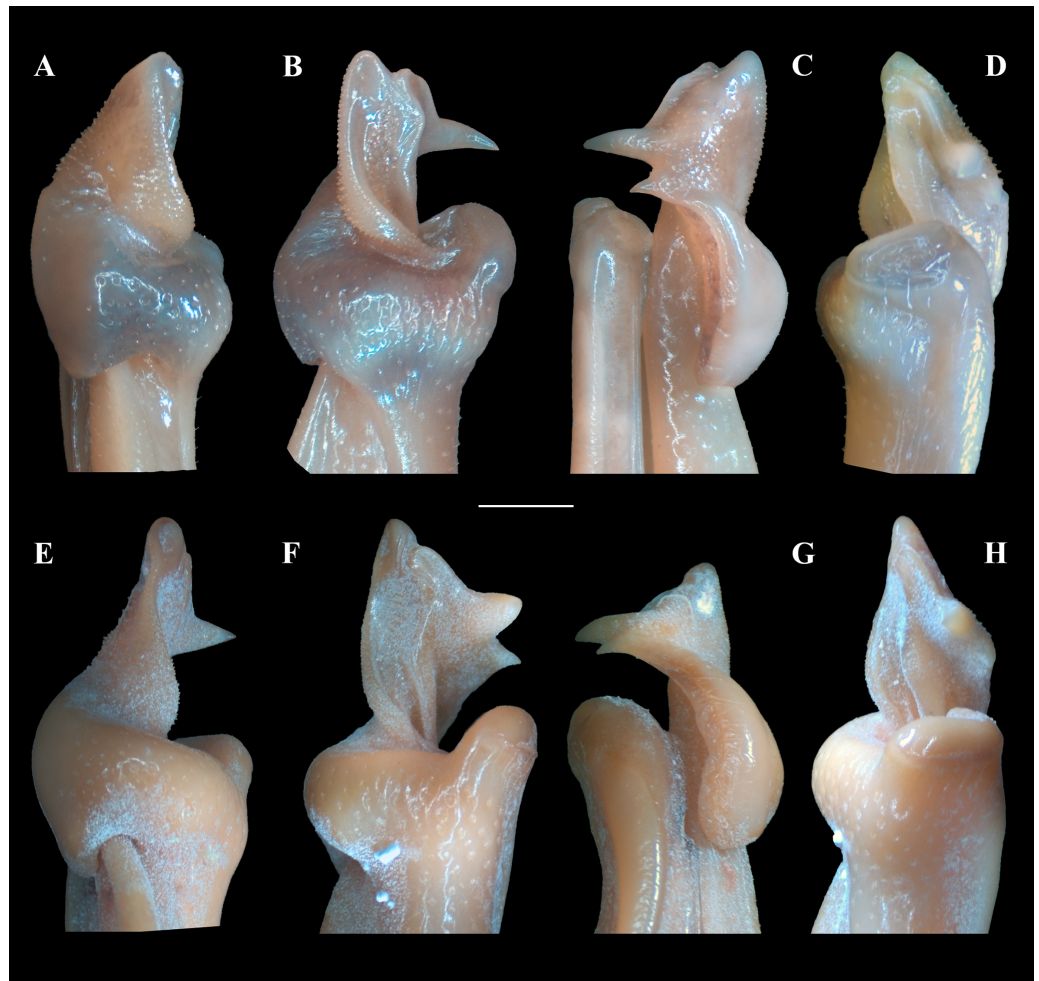


**Figure 5** (A–D) Right male first gonopod (G1) in sternal and apical views from A to B and C to D, respectively. (A, C) *Fredius ibiapaba* n. sp., holotype, male cl 36 mm, cw 53 mm (MZUSP 39710). (B, D) *Fredius reflexifrons* (Ortmann, 1897), male cl 73.8 mm, cw 53 mm (MZUSP 13178). Note in (A) and (C) the G1 apex much less tilted so that the mesial lobe is not visible in sternal view (arrow), and the subapical bulge markedly less swollen (arrow), respectively. Note the opposite in (B) and (D). Scales: A–B, two mm; C–D, one mm.

Full-size DOI: 10.7717/peerj.9370/fig-5

**Etymology.** The specific epithet is a noun in apposition taken from the Tupi language word for plateau, “yby’ababa”, ibiapaba.

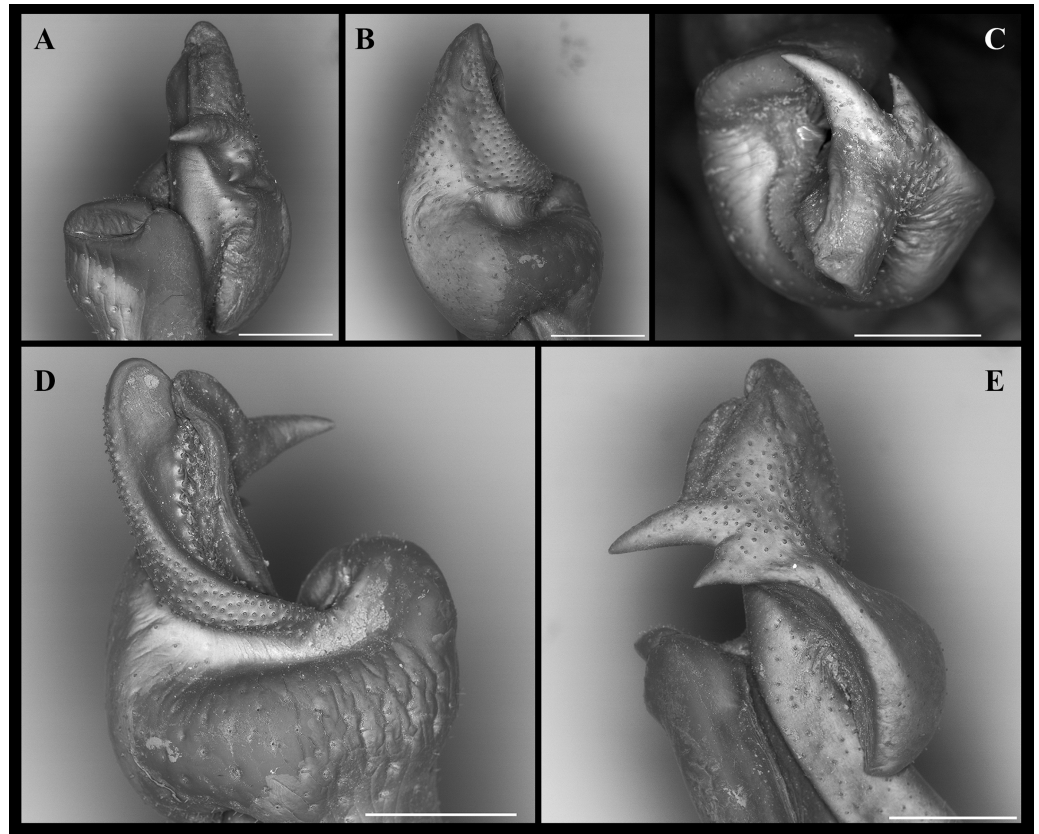
**Diagnosis.** G1 robust, proximal half remarkably swollen, sloping abruptly downwards anteriorly to a nearly right-angular shoulder (Figs. 4B, 4C); mesial lobe much smaller than cephalic spine (Figs. 4B, 4C; 5C, 5D; 7A, 7C, 7E); cephalic lobe somewhat broad, rounded apically (Fig. 4A); auxiliary lobe lip, delimiting field of apical spines, protruded all the way to distal margin of auxiliary lobe (Figs. 4A, 4B; 6B; 7D).



**Figure 6** (A–H) Right male first gonopod (G1) in sternal, lateral, mesial, and pleonal views from A–D and E–H, respectively. (A–D) *Fredius ibiapaba* n. sp., holotype, male cl 36 mm, cw 53 mm (MZUSP 39710). (E–H) *Fredius reflexifrons* (Ortmann, 1897), male cl 73.8 mm, cw 53 mm (MZUSP 13178). Scales: A–H, one mm.

Full-size  DOI: [10.7717/peerj.9370/fig-6](https://doi.org/10.7717/peerj.9370/fig-6)

**Description of the holotype.** Carapace transversally ovate (Fig. 3A), widest at midlength (cw/cl, 1.51); dorsal surface smooth, slightly convex, regions ill-defined. Gastric pits minute, very close to each other. Cervical grooves shallow, nearly straight, poorly indicated, distal ends reaching to anterolateral margin. Front deflexed, almost straight in dorsal view, entire, marked with row of very small granules; front lower border carinate, with an almost indistinct sinus medially in frontal view; postfrontal lobules obsolete; median groove between postfrontal lobules faint (Figs. 3A, 3C). Upper orbital margin with row of very faint granules; lower margin minutely denticulate; exorbital angle marked by obtuse tooth, followed posteriorly by faint notch (Fig. 3C). Carapace anterolateral margin semicircular in outline, fringed by minute denticles; posterolateral margins almost straight, strongly convergent, smooth (Figs. 3A, 3C). Epistomial margin with minute granules; epistomial



**Figure 7** *Fredius ibiapaba* n. sp., paratype, male cl 41.2 mm, cw 62.6 mm (MZUSP 39742). Scanning electron microscopy of the first right male gonopod. (A) mesial (tilted right), (B) sternal, (C) apical, (D) lateral, and (E) mesial views. Scales: A–E, one mm.

Full-size  DOI: [10.7717/peerj.9370/fig-7](https://doi.org/10.7717/peerj.9370/fig-7)

tooth broadly triangular, deflexed (Fig. 3C). Suborbital and subhepatic regions of carapace smooth; pterygostomial region densely pubescent around buccal cavity (Figs. 3B, 3C).

Mxp3 palp slender, long, reaching slightly beyond articulation of merus and ischium when folded. Merus markedly operculiform. Posterior half of mesial margin of merus and mesial margin of ischium with conical teeth (Fig. 3C). Exopod short, 0.28 times length of lateral margin of ischium, devoid of flagellum. Efferent branchial channel opening subcircular (Fig. 3C).

Chelipeds moderately heterochelous, right cheliped larger than left one (Figs. 3E, 3F). Major cheliped merus subtriangular in cross-section; lateral surface smooth, with irregular row of small tubercles of different sizes along dorsal surface; mesial surface smooth, slightly concave to fit lateral sides of carapace; mesial lower margin with row of conical teeth slightly increasing in size distally; lateral lower margin with row of small teeth. Carpus smooth dorsally; mesial margin with row of small, irregular teeth and strong, acute spine about midlength of margin. Palm moderately swollen, smooth on lateral and mesial surfaces, with minute granules on rounded dorsal and ventral faces. Dactylus in process of regeneration.

Cutting margin of dactylus and fixed finger both with larger teeth interspersed with smaller ones. Fingers not gaping when closed, tips not crossing. Minor cheliped similar in shape.

Thoracic sternal suture 2/3 complete, distinct; sternal suture 3/4 interrupted, visible only laterally (Fig. 3B); sternal sutures 4/5 and 5/6 interrupted, ending just before reaching midline of thoracic sternum; sternal sutures 6/7 and 7/8 complete. Midline of thoracic sternum deeply incised in sternites VII and VIII.

All pleonal segments free. Lateral margins of male telson slightly concave, tip rounded (Fig. 3B).

G1 robust (Figs. 4A–4C), proximal half remarkably swollen, sloping abruptly downwards distally to a nearly right-angular shoulder (Fig. 4B, 4C). Subapical bulge moderately developed around lateral and sternal sides (Figs. 4B; 5A, 5B; 6A). Marginal suture straight (Fig. 4C). Marginal lobe truncate, projected distally beyond pleonal surface, junction with marginal lobe marked by distinct depression. Mesial lobe much smaller than cephalic spine, showing as triangular, acute spine, pointing to pleonal direction (Figs. 4A–4C, 5B; 5C; 6A–6C). Cephalic spine very strong, acuminate at tip, pointing to mesial direction (Figs. 4A–4C, 5B; 5C; 6A–6C). Cephalic lobe prominent, blunt, tip rounded, with several spinules along lateral, mesial and sternal sides (Fig. 4A, 4B; 6A, 6B). Auxiliary lobe much shorter than cephalic lobe in pleonal view, separated from it by distinct depression, their junction forming lateral channel running distally in almost straight direction before ending in inward curve subterminally (Figs. 4A; 6A). Field of apical spines large, open, flattened, elongated, ear-shaped, provided with small spinules, delimited by lateral and pleonal lips of apex (Fig. 4A, 4B; 5B; 6A, 6A).

G2 slightly longer than G1; very slender, tapering distally progressively, distal part moderately flattened, with somewhat dense, minute spinules along sternal side.

**Remarks.** *Fredius ibiapaba* n. sp. is herein assigned to the genus *Fredius*, whose diagnostic characters (Rodríguez, 1982; Rodríguez & Pereira, 1992) are readily recognized in the new species, namely, exopod of mxp3 short, about 0.3 times length of outer margin of ischium with G1 widest at base (Figs. 4B, 4C); marginal lobe simple, ending in an inverted cup-shaped elongation at base of field of apical spines; subapical bulge covering lateral and sternal sides; field of apical spines large, open, flattened, ear-shaped, with small scattered spinules at proximal sternal border (Figs. 4A–4C; 5B; 6A, 6B).

The new species morphologically resembles *Fredius denticulatus*, *F. fittkai*, *F. reflexifrons* and *F. ykaa* (Magalhães, 2009), in that the gonopod cephalic spine is much more developed than the mesial lobe (see Magalhães & Rodríguez, 2002: 679, fig. 1; 683, fig. 2, respectively; Rodríguez & Campos, 1998: 766, fig. 2O, 2P) (Fig. 4A, 4C; 5B; 6A, 6C), whereas other species either have the gonopod cephalic spine little larger than the mesial lobe (*F. stenolobus* Rodríguez & Suárez, 1994, and *F. adpressus* Rodríguez & Pereira, 1992), or have it much shorter than the mesial lobe (e.g., *F. buritizatis*, *F. platyacanthus* Rodríguez & Pereira, 1992, and *F. estevi* Rodríguez, 1966), or have the cephalic spine and the mesial lobe similar in size (e.g., *F. granulatus* Rodríguez & Campos, 1998), and *F. chaffanjonii* Rathbun, 1905) (see Magalhães et al., 2014 and references therein).

*Fredius ibiapaba* n. sp. stands apart from *Fredius denticulatus*, *F. fittkai*, *F. reflexifrons* and *F. ykaa* in having the G1 proximal half remarkably swollen on the pleonal side, sloping

abruptly downwards distally to a nearly right-angular shoulder (Figs. 4B, 4C), whereas in the latter four species the G1 shoulder is clearly more gently sloping distally (Figs. 4E, 4F).

*Fredius ibiapaba* n. sp. closely resembles *F. reflexifrons*, but the following characters derived from G1 distinguish the new species from the latter species: (1) in having the auxiliary lobe lip, delimiting the field of apical spines, protruded all the way to the distal margin of the auxiliary lobe (Figs. 4A, 4B), whereas in *F. reflexifrons* the lip fades away well before reaching the distal margin of the lobe (Figs. 4D, 4E); (2) the subapical bulge markedly less swollen (Figs. 5A, 5C) and the G1 apex much less tilted so that the mesial lobe is not visible in sternal view (Fig. 5A), in contrast to *F. reflexifrons* (Fig. 5B, 5D, respectively). Also, in *F. ibiapaba* n. sp. the distal margin of the cephalic lobe is blunt (Figs. 4A, 6A), whereas in *F. reflexifrons* it tapers progressively to a distinct narrower tip (Figs. 4D, 6D).

*Fredius ibiapaba* n. sp. further differs from *F. ykaa* in that the G1 shoulder is high and robust (Figs. 4B, 4C), whilst in *F. ykaa* the G1 shoulder is remarkably lower; it can be easily further differentiated from *F. denticulatus* in that its G1 caudal lobe lacks a field of spines spirally twisted to a transverse position (viz., [Rodríguez & Campos, 1998](#)) and from *F. fittkaui* in having the G1 cephalic spine straight and sharply acuminate, whereas in *F. fittkaui* it is curved and round tipped. Morphological differentiation between female specimens is difficult.

## DISCUSSION

### Phylogenetic analysis

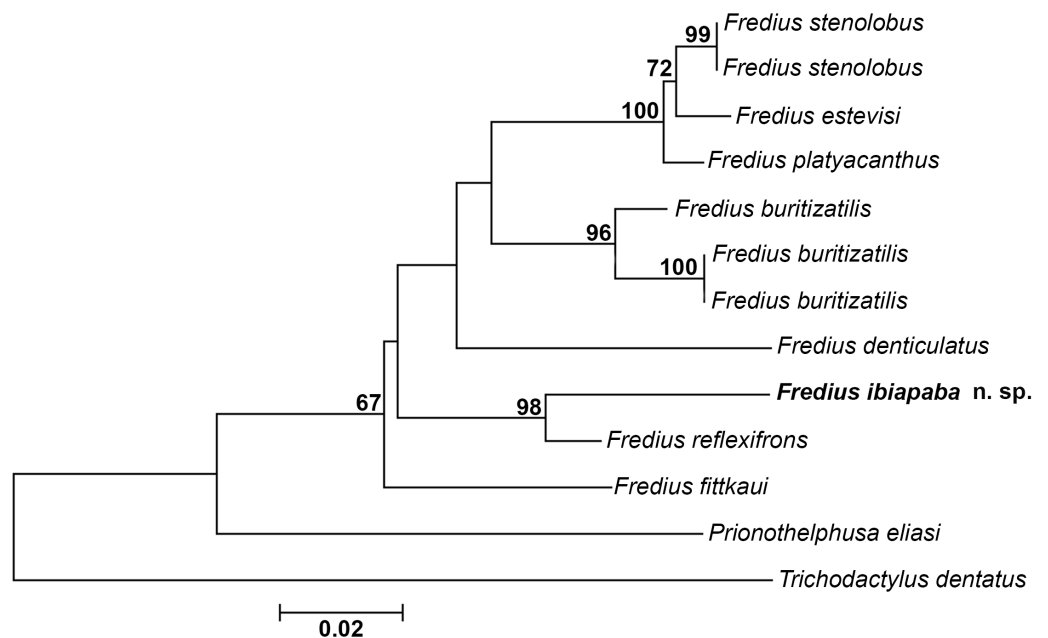
The mitochondrial loci 16S was successfully amplified and sequenced for *Fredius buritizatis*, *F. ibiapaba* n. sp., and *Prionothelphusa eliasi*. Additional sequences used were retrieved from GenBank (Table 1). Bootstrap support values are shown on nodes of the phylogenetic tree (Fig. 8). The sister species relationships between *Fredius reflexifrons* and the new species is well supported by high bootstrap value. The close morphological similarity between the two species also supports such relationship.

The divergence rates between *Fredius reflexifrons* and *F. ibiapaba* n. sp. (4%) is higher than between *F. estevisi* x *F. stenolobus*, *F. platyacanthus* x *F. stenolobus* and *F. platyacanthus* x *F. estevisi* all with 2% of divergence (Table 2). Morphology and molecular data hence provide evidences for the differentiation between *F. ibiapaba* n. sp. and *F. reflexifrons*.

A survey of the pseudothelphusids described from 1840 to 2004 ([Yeo et al., 2008](#)) showed that the curve of described species is still far from being asymptotic. And indeed, new species are still being discovered either by collecting in new biomes (e.g., *F. buritizatis* from a palm swamp known as “buritizal”), or by revisiting the taxonomy of widely disjunct species for testing as to their conspecific identity, such as *F. ibiapaba* n. sp. and *F. reflexifrons*.

### Zoogeographical notes

*Fredius* currently consists of 14 species (Table 3), distributed over a vast territory, which encompass five main river basins ([Rodríguez & Campos, 1998](#); [Magalhães et al., 2014](#)): (1) the Orinoco River basin; (2) the Essequibo-Cuyuni River basin; (3) the Amazon River basin; (4) the Madeira River basin and its tributary (Machado River); and (5) the Atlantic rivers



**Figure 8** Phylogeny inferred from the partial mitochondrial DNA sequence of the 16S rDNA gene.

Note the sister taxon relationship between *Fredius ibiapaba* n. sp. and *F. reflexifrons* (Ortmann, 1897).

Full-size [DOI: 10.7717/peerj.9370/fig-8](https://doi.org/10.7717/peerj.9370/fig-8)

basin, a coastal drainage of small rivers in northern South American (Guyana, Suriname and French Guiana) discharging directly into the Atlantic Ocean.

*Rodríguez & Pereira (1992)* performed a cladistic analysis of *Fredius* and suggested that *F. reflexifrons* and *F. adpressus* were sister species. The purported clade *F. reflexifrons* / *F. adpressus* was presumably supported by three putative synapomorphies: (1) [G1] mesial lobe attached to back of auricular lobe; (2) basal denticle of mesial lobe present; and (3) subapical bulge well developed.

Later, however, *Rodríguez & Campos (1998)* reviewed the previous data and performed a new analysis in which they decided that character 1 (mesial lobe attached to back of auricular lobe) was no longer tenable and hence was eliminated from the new analysis. They also realized that the basal denticle of the mesial lobe was indeed present in *F. adpressus* (character 2), but was absent in all other *Fredius* species. They further concluded that the subapical bulge was actually “reduced” in *F. adpressus* and “strongly developed” in *F. granulatus*, *F. reflexifrons*, *F. fittkauii*, and *F. denticulatus*, so that these latter two characters were also removed from the new analysis. Therefore, the putative sister taxon relationship between *F. reflexifrons* and *F. adpressus* dissolved. *Rodríguez & Campos (1998)* put forward, instead, the hypothesis that *F. reflexifrons* was sister to *F. fittkauii*, not to *F. adpressus*, based on the assumption that *F. reflexifrons* and *F. fittkauii* synapomorphically share the cephalic lobe distal margin armed with several spinules. However, as found here, this character is more widely distributed being also found in *F. ibiapaba* n. sp. and, therefore, cannot be used to argue for the sister taxon relationship between *F. reflexifrons* and *F. fittkauii*.

**Table 3** Geographic and altitudinal distributions for the species of *Fredius* Pretzmann, 1967.

Species	Country	Environment	Altitude (m)	References
<i>F. ykaa</i> Magalhães, 2009	Brazil (Amazon River basin)	Lowland streams	36 to 73	Magalhães, 2009
<i>F. adpressus</i> Rodríguez & Pereira, 1992	Venezuela (Orinoco River basin)	Lowland streams	100	Rodríguez & Pereira, 1992
<i>F. beccarii</i> Coifmann, 1939	Brazil, Guyana, Venezuela, Suriname (Essequibo-Cuyuni Rivers basin)	Streams (igarapés)	50 to 752	Rodríguez & Campos, 1998; Cumberlidge, Alvarez & Villalobos, 2014; Mora-Day, Magalhães & Souki, 2009; Magalhães et al., 2014; Zanetti, Castro & Magalhães, 2018
<i>F. buritizatis</i> Magalhães & Mantellato in Magalhães et al., 2014	Brazil (Madeira River basin)	Buritizal (palm) fields	150	Magalhães et al., 2014
<i>F. chaffanjonii</i> Rathbun, 1905	Venezuela (Orinoco River basin)	River's headwaters and mid-courses	105–300	Rodríguez & Pereira, 1992
<i>F. cuaeensis</i> Suárez, 2015	Venezuela (Orinoco River basin)	Highland streams	950	Suárez, 2015
<i>F. denticulatus</i> (H. Milne Edwards, 1853)	Brazil, Suriname, French Guiana (Amazon and Atlantic river basins)	Streams (igarapés) and along river margins	70 to 400	Rodríguez & Pereira, 1992; Rodríguez & Campos, 1998; Magalhães et al., 2005; Magalhães, 2009; Cumberlidge, Alvarez & Villalobos, 2014; Magalhães et al., 2014
<i>F. estevisi</i> Rodríguez, 1966	Brazil, Venezuela (Amazon and Atlantic rivers basins)	River's headwaters and streams	446 to 944	Mora-Day, Magalhães & Souki, 2009
<i>F. fittkai</i> Bott, 1967	Brazil, Venezuela, Guyana (Amazon and Atlantic rivers basins)	Streams (igarapés) and along river margins	151 to 500	Rodríguez & Campos, 1998; Magalhães & Rodríguez, 2002; Cumberlidge, Alvarez & Villalobos, 2014; Magalhães et al., 2014; Zanetti, Castro & Magalhães, 2018
<i>F. granulatus</i> Rodríguez & Campos 1998	Colombia (Amazon River basin)	Lowlands	180 to 200	Rodríguez & Campos, 1998; Cumberlidge, Alvarez & Villalobos, 2014; Cumberlidge, Alvarez & Villalobos, 2014; Zanetti, Castro & Magalhães, 2018;
<i>F. platyacanthus</i> Rodríguez & Pereira, 1992	Brazil, Venezuela (Atlantic rivers basin)	Streams (igarapés) and mountain areas	106 to 1229	Rodríguez & Pereira, 1992; Cumberlidge, Alvarez & Villalobos, 2014; Magalhães et al., 2014; Zanetti, Castro & Magalhães, 2018
<i>F. reflexifrons</i> Ortmann, 1897	Brazil, Venezuela, Suriname, French Guiana, Peru, Guyana (Amazon and Atlantic rivers basins)	Lowland streams	37 to 200	Magalhães & Rodríguez, 2002; Magalhães et al., 2005; Cumberlidge, Alvarez & Villalobos, 2014

(continued on next page)

Table 3 (continued)

Species	Country	Environment	Altitude (m)	References
<i>F. stenobus</i> Rodríguez & Suárez, 1994	Brazil, Venezuela (Orinoco River basin)	Streams in rocky areas	65 to 1020	Rodríguez & Campos, 1998; Magalhães & Pereira, 2007; Cumberlandidge, Alvarez & Villalobos, 2014; Magalhães et al., 2014; Zanetti, Castro & Magalhães, 2018
<i>Fredius ibiapaba</i> n. sp.	Brazil (Orographic forest enclaves)	Burrows among the leaf litter, alongside little streams and water ponds inside forest stands or directly on the humid forest floor	665 to 782	Present study



*Magalhães et al. (2014)* performed a distance analysis based on 16S rRNA, in which *F. reflexifrons* was recovered as the sister taxa to (*F. fittkauii* (*F. denticulatus* (*F. granulatus* (*F. buritizatis* (*F. platyacanthus* (*F. denticulatus* (*F. stenolobus*)))))). The discovery of *F. ibiapaba* n. sp. revealed, however, that it is actually the sister taxa of *F. reflexifrons*, as shown by a comparative 16S rDNA sequencing used to infer the phylogenetic placement of *Fredius ibiapaba* n. sp., with *F. fittkauii* recovered as the sister taxa to the remaining species (Fig. 8).

The distribution range of *Fredius ibiapaba* n. sp. is very narrow and currently restricted to a humid enclave, a small mid-altitude forested patch in Ipú (Ceará, northeastern Brazil, Figs. 1A–1E), nested within the vast semiarid Caatinga domain (Figs. 1F, 1G). The orographic forest enclaves, such as Ipú, are typically located along the slopes of plateaus, between 600 and 1,100 m, hence high enough to receive rainfall of more than 1,200 mm year<sup>-1</sup> of Atlantic origin (*Tabarelli & Santos, 2004* and references therein). These enclaves are regionally known as “Brejos” (or “Brejos de altitude” or even “Brejos nordestinos”) (*Andrade-Lima, 1982; Silva & Casteletti, 2003; Tabarelli & Santos, 2004*). *Fredius ibiapaba* n. sp. inhabits the mid-highlands of the Ibiapaba plateau, between about 635 to 782 m, where it digs burrows among the leaf litter, alongside little streams and water ponds inside forest stands or directly on the humid forest floor (Fig. 1E). In contrast, *F. reflexifrons* is widely distributed in the Amazon basin’s lowlands (<100 m) from as far west as Peru (Ampyacu River, a tributary of the Amazonas River) to as far east as the Atlantic basin (French Guiana) (*Magalhães, 2003*). It is found in burrows alongside the “igarapés” (streams) or digs its burrows on the humid forest floor (*Magalhães & Rodríguez, 2002*). *Magalhães et al. (2005)* misidentified the specimens from the mid-highlands of the Ibiapaba plateau with *F. reflexifrons* and explained its presence in Ibiapaba by a migration “... eastwards as far as Serra de Ibiapaba” during the expansion of the humid tropical forest.

*Fredius ibiapaba* n. sp. and *F. reflexifrons* are highly dependent upon humidity and our view is that they most probably were once part of an ancestral population living in a wide humid territory. The shrinking humid forests during several dry periods of the Tertiary and Quaternary (*Katzer, 1933; Andrade-Lima, 1953; Bigarella, Andrade-Lima & Riehs, 1975; Ab’Saber, 1977; Bigarella & Andrade-Lima, 1982; Andrade-Lima, 1982; Clapperton, 1993; Thomas, 2000; Haffer, 2001; Haffer & Prance, 2002*) likely have resulted in the fragmentation of the ancestral humid area and hence of the ancestral crab population, which was split into two sister species. *Fredius reflexifrons* evolved and spread in a lowland, humid river basin and is now widely distributed, whilst *F. ibiapaba* n. sp. evolved isolated on the top of a humid plateau (Figs. 1A–1E). The two species are now separated by a vast intervening area occupied by the semiarid Caatinga (Figs. 1F, 1G).

The expansion and shrinkage of mountain, floodplain, and gallery forests, associated to complex topography are known to have affected flora and fauna (*Vanzolini, 1970; Vanzolini & Williams, 1970; Vuilleumier, 1971; Andrade-Lima, 1982; Teixeira, Nacinovic & Tavares, 1986; Haffer, 1969; Haffer, 2001; Haffer & Prance, 2002; Santos et al., 2007; Leite et al., 2016*). *Andrade-Lima (1982)* provided a number of examples of plant species that are now confined to the Brejos, isolated from the surrounding, widely distributed Caatinga.

He found two floristic components in these refuges on the top of hills, one whose species and genera have mostly originated from the southeastern flora, lies further inland in the states of Alagoas and Rio Grande do Norte; and a second one in the humid mid highlands closer to the coast, especially between Pernambuco and the border of Ceará and Piauí states (referred to as the Pernambuco Centre by [Santos et al. \(2007\)](#)), in which the Amazonian flora are better represented ([Andrade-Lima, 1982](#)). [Santos et al. \(2007\)](#) found strong bootstrap support for a close floristic relationship between the Pernambuco Centre and Amazonian localities.

It has long been known that a number of freshwater fish species inhabiting the Brejos have their closest relationships with those from the Amazonian Basin ([Géry, 1969](#); [Paiva, 1978](#); [Weitzman & Weitzman, 1982](#); [Ploeg, 1991](#); [Vari, 1991](#); [Menezes, 1996](#); [Rosa & Groth, 2004](#)). More recently, [Pinheiro & Santana \(2016\)](#) described a new species of freshwater crab genus *Kingsleya* Ortmann, 1897 (also a Pseudothelphusidae), from a Brejo about 750 m in Arajara district, municipality of Barbalha, Ceará. Previously to their discovery *Kingsleya* was known from nine species inhabiting the Amazonian lowlands ([Pedraza & Tavares, 2015](#)).

## ACKNOWLEDGEMENTS

We are thankful to Célio Magalhães (Instituto Nacional de Pesquisas da Amazônia) and Rafael Lemaitre (National Museum of Natural History, Smithsonian Institution) for granting access to their respective collections. We are in debt to Waltécio de Oliveira Almeida (Universidade Regional do Cariri) for providing access to optical equipment and laboratory space and to Jessica Colavite (Universidade Estadual Paulista “Júlio de Mesquita Filho”) for the help during figure preparations. This work greatly benefited from the comments of Célio Magalhães, Tomoyuki Komai (Natural History Museum and Institute, Chiba) and an anonymous reviewer.

## ADDITIONAL INFORMATION AND DECLARATIONS

### Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) [2013/01201–0], by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior –Brasil (CAPES) –Finance code 001 (fellowship #88887.169169/2018-00 to William Santana and grant Proequipamentos #775705/2012 to Allysson Pinheiro), Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP # BP3-0139-00166.01.00/18 to Allysson Pinheiro and fellowship to William Santana) and the Financiadora de Inovação e Pesquisa (FINEP) (#1015/13). CNPq (303122/2016-1) provided funding studies on the taxonomy of decapod crustaceans. The Universidade Regional do Cariri (URCA), Universidade do Sagrado Coração (USC) and MZUSP provided logistic support. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors:

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): 2013/01201–0.

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior –Brasil (CAPES) –Finance code 001: #88887.169169/2018-00, #775705/2012.

Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico: FUNCAP # BP3-0139-00166.01.00/18.

Financiadora de Inovação e Pesquisa (FINEP): #1015/13.

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq): 303122/2016-1.

Universidade Regional do Cariri (URCA).

Universidade do Sagrado Coração (USC).

Museu de Zoologia, Universidade de São Paulo (MZUSP).

### Competing Interests

The authors declare there are no competing interests.

### Author Contributions

- Livanio C. Santos conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Marcos Tavares analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- José R.F. Silva and Marcelo Cervini performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Allysson P. Pinheiro conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- William Santana analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

### Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

The biological material was collected using license permission from the Sistema de Autorização e Informação em Biodiversidade (SISBIO #29615) of the Brazilian Ministry of Environment (MMA).

### DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The partial mitochondrial sequences of the 16S rDNA gene of *Fredius ibiapaba* (MN787135), *Fredius buritizatis* (MN787136) and *Prionothelphusa eliasi* (MN787137) are available at GenBank.

### Data Availability

The following information was supplied regarding data availability:

Raw data is available as a [Supplemental File](#).

## New Species Registration

The following information was supplied regarding the registration of a newly described species:

Publication LSID: urn:lsid:zoobank.org:pub:0925982D-7441-4256-9856-A553987956A6.

Fredius ibiapaba LSID: urn:lsid:zoobank.org:act:FAE32D6B-89D2-4820-834C-A06F4D27A7F3.

## Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.9370#supplemental-information>.

## REFERENCES

- Ab'Saber AN. 1977.** Espaços ocupados pela expansão dos climas secos na América do Sul. por ocasião dos períodos glaciais quaternários. *Paleoclimas* 3:1–19.
- Ab'Saber AN. 2000.** Spaces occupied by the expansion of bry climates in South America during the Quaternary ice ages. *Revista do Instituto Geológico* 21(1–2):71–78  
DOI 10.5935/0100-929X.20000006.
- Andrade-Lima DD. 1953.** Notas sobre a dispersão de algumas espécies vegetais no Brasil. *Anais da Sociedade de Biologia de Pernambuco* 11(1):25–49.
- Andrade-Lima D. 1982.** Present day forest refuges in northeastern Brazil. In: Prance GT, ed. *Biological diversification in the tropics*. New York: Columbia University Press 245–254.
- Auler AS, Wang X, Edwards RL, Cheng H, Cristalli PS, Smart PL, Richards DA. 2004.** Quaternary ecological and geomorphic changes associated with rainfall events in presently semi-arid northeastern Brazil. *Journal of Quaternary Science* 19(7):693–701  
DOI 10.1002/jqs.876.
- Bigarella JJ, Andrade-Lima D. 1982.** Paleoenvironmental changes in Brazil. In: Prance GT, ed. *Biological diversification in the tropics*. New York: Columbia University Press 27–40.
- Bigarella JJ, Andrade-Lima D, Riehs PJ. 1975.** Considerações a respeito das mudanças paleoambientais na distribuição de algumas espécies vegetais e animais no Brasil. *Anais da Academia Brasileira de Ciências* 47:411–464.
- Carmignotto AP, De Vivo MD, Langguth A. 2012.** In: Patterson BD, Costa LP, eds. *The history and geography of recent Neotropical mammals*. Chicago: University of Chicago Press, 307–350.
- Carnaval AC, Bates JM. 2007.** Amphibian DNA shows marked genetic structure and tracks Pleistocene climate change in northeastern Brazil. *Evolution: International Journal of Organic Evolution* 61(12):2942–2957 DOI 10.1111/j.1558-5646.2007.00241.x.
- Cartelle C, Hartwig WC. 1996.** A new extinct primate among the Pleistocene megafauna of Bahia, Brazil. *Proceedings of the National Academy of Sciences of the United States of America* 93(13):6405–6409 DOI 10.1073/pnas.93.13.6405.

- Clapperton CM. 1993.** Nature of environmental changes in South America at the Last Glacial Maximum. *Palaeogeography, Palaeoclimatology, Palaeoecology* **101**(3–4):189–208 DOI [10.1016/0031-0182\(93\)90012-8](https://doi.org/10.1016/0031-0182(93)90012-8).
- Cumberlidge N, Alvarez F, Villalobos JL. 2014.** Results of the global conservation assessment of the freshwater crabs (Brachyura, Pseudothelphusidae and Trichodactylidae): the Neotropical region, with an update on diversity. *ZooKeys* **457**:133 DOI [10.3897/zookeys.457.6598](https://doi.org/10.3897/zookeys.457.6598).
- Darriba D, Taboada GL, Doallo R, Posada D. 2012.** jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**(8):772.
- De Vivo M. 1997.** Mammalian evidence of historical change in the Caatinga semiarid vegetation of northeastern Brazil. *Journal of Comparative Biology* **2**(1):65–73.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**(4):783–791 DOI [10.1111/j.1558-5646.1985.tb00420.x](https://doi.org/10.1111/j.1558-5646.1985.tb00420.x).
- Géry J. 1969.** The freshwater fishes of South América. In: Fittkau EJ, Iles J, Klinge H, Schwabe GH, Sioli H, eds. *Biogeography and ecology in South America*. Volume 2. The Hague: Dr. W. Junk Publishers 828–848.
- Haffer J. 1969.** Speciation in Amazonian forest birds. *Science* **165**:131–137 DOI [10.1126/science.165.3889.131](https://doi.org/10.1126/science.165.3889.131).
- Haffer J. 2001.** Hypotheses to explain the origin of species in Amazônia. In: Vieira IC, Silva JMC, Oren DC, D’Incao MA, eds. *Diversidade Biológica e Cultural da Amazônia*. 4. Belém: 5–118.
- Haffer J, Prance GT. 2002.** Impulsos climáticos da evolução na Amazônia durante o Cenozóico: sobre a teoria dos Refúgios da diferenciação biótica. *Estudos Avançados* **16**(46):175–206 DOI [10.1590/S0103-40142002000300014](https://doi.org/10.1590/S0103-40142002000300014).
- Hall T. 2005.** iEdit biological sequence alignment editor. Version 7.0.4. Carlsbad: Ibis Therapeutics.
- Katzer F. 1933.** Geologia do Estado do Pará. *Boletim do Museu Paraense Emílio Goeldi de História Natural e Etnografia* **9**:1–270.
- Leite YLR, Costa LP, Loss AC, Rocha RG, Batalha-Filho H, Bastos AC, Quaresma VS, Fagundes V, Paresque R, Passamani M, Pardini R. 2016.** Neotropical forest expansion during the last glacial period challenges refuge hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* **113**(4):1008–1013 DOI [10.1073/pnas.1513062113](https://doi.org/10.1073/pnas.1513062113).
- Magalhães C. 2003.** Brachyura: Pseudothelphusidae Trichodactylidae. *Manual de Identificação dos Crustáceos Decápodos de Água Doce Brasileiros* **14**:3–297.
- Magalhães C. 2009.** A new species of freshwater crab of the genus *Fredius* Pretzmann, 1967 from the middle Amazon River basin, Brazil (Crustacea: Decapoda: Pseudothelphusidae). *Proceedings of the Biological Society of Washington* **122**(1):81–86 DOI [10.2988/08-35.1](https://doi.org/10.2988/08-35.1).
- Magalhães C, Abrunhosa FA, Pereira MDO, Melo MA. 2005.** New records of *Fredius denticulatus* (H. Milne-Edwards, 1853) and *F. reflexifrons* (Ortmann, 1897), and the eastern limits of the distribution of pseudothelphusid crabs (Crustacea: Decapoda) in Brazil. *Acta Amazonica* **35**(1):93–96 DOI [10.1590/S0044-59672005000100014](https://doi.org/10.1590/S0044-59672005000100014).

- Magalhães C, Pereira G. 2007.** Assessment of the decapod crustacean diversity in the Guayana Shield region aiming at conservation decisions. *Biota Neotropica* 7(2):111–124.
- Magalhães C, Rodríguez G. 2002.** The systematic and biogeographical status of *Fredius reflexifrons* (Ortmann, 1897) and *Fredius fittkauii* (Bott, 1967) (Crustacea: Brachyura: Pseudothelphusidae) from the Amazon and Atlantic Guianas River basins. *Acta Amazonica* 32(4):677–689.
- Magalhães C, Sanches VQA, Pileggi LG, Mantelatto FL. 2014.** Morphological and molecular characterization of a new species of *Fredius* (Decapoda, Pseudothelphusidae) from Rondônia, southern Amazonia, Brazil. In: Yeo DCJ, Cumberlidge N, Klaus S, eds. *Advances in freshwater decapod systematics and biology. Crustacean Monographs 19*. Leiden: Brill, 296 p.
- Menezes NA. 1996.** Methods for assessing freshwater fish diversity. In: Bicudo CE deM, Menezes NA, eds. *Biodiversity in Brazil: a first approach*. São Paulo: CNPq, 289–295.
- Mora-Day J, Magalhães C, Souki MEL. 2009.** Lista Sistemática de los macroinvertebrados colectados durante el RAP Alto Cuyuní 2008, Estado Bolívar, Venezuela. In: Lasso CA, Señaris JC, Rial A, Flores AL, eds. *Evaluación Rápida de la Biodiversidad de los Ecosistemas Acuáticos de la Cuenca Alta del Río Cuyuní, Guayana Venezolana*. Washington, D.C.: Conservation International, 89–105.
- Paiva M. 1978.** Ictiofauna e as grandes represas brasileiras. *Revista Dae* 38(116):49–57.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991.** *The Simple Fool's Guide to PCR, Version 2*. Honolulu: University of Hawaii Zoology Department, 45 pp.
- Pedraza M, Tavares M. 2015.** A new species of freshwater crab of the genus *Kingsleya* Ortmann, 1897 (Crustacea: Brachyura: Pseudothelphusidae) from Amazonia, Brazil. *Zootaxa* 4032(4):444–450 DOI 10.11646/zootaxa.4032.4.9.
- Pinheiro AP, Santana W. 2016.** A new and endangered species of *Kingsleyia* Ortmann, 1897 (Crustacea: Decapoda: Brachyura: Pseudothelphusidae) from Ceará, northeastern Brazil. *Zootaxa* 4171(2):365–372 DOI 10.11646/zootaxa.4171.2.9.
- Ploeg A. 1991.** Revision of the South American cichlid genus *Crenicichla* Heckel, 1840, with descriptions of fifteen new species groups, phylogeny and biogeography (Pisces, Perciformes, Cichlidae). Doctoral dissertation, Universiteit van Amsterdam, Netherland.
- Rodríguez G. 1982.** Les crabes d'eau douce d'Amérique. Famille des Pseudothelphusidae. In: *Faune Tropicale*. 22. Paris: ORSTOM, 224 p.
- Rodríguez G, Campos MR. 1998.** A cladistic revision of the genus *Fredius* (Crustacea: Decapoda: Pseudothelphusidae) and its significance to the biogeography of the Guianan lowlands of South America. *Journal of Natural History* 32(5):763–775 DOI 10.1080/00222939800770391.
- Rodríguez G, Pereira G. 1992.** New species, cladistic relationships, and biogeography of the genus *Fredius* (Decapoda: Brachyura: Pseudothelphusidae) from South America. *Journal of Crustacean Biology* 12(2):298–311 DOI 10.2307/1549082.

- Rosa RS, Groth F. 2004.** Ictiofauna dos ecossistemas de brejos de altitude de Pernambuco e Paraíba, Brejos de Altitude em Pernambuco e Paraíba: História Natural, Ecologia e Conservação. *Série Biodiversidade* **9**:201–210.
- Santos AMM, Cavalcanti DR, Silva JMCD, Tabarelli M. 2007.** Biogeographical relationships among tropical forests in north-eastern Brazil. *Journal of Biogeography* **34**(3):437–446 DOI [10.1111/j.1365-2699.2006.01604.x](https://doi.org/10.1111/j.1365-2699.2006.01604.x).
- Santos LC, Nascimento WM, Matos HS, Pinheiro AP, Silva JRF. 2020.** The distribution of the freshwater crab *Fredius reflexifrons* (Ortmann, 1897) (Brachyura, Pseudothelphusidae) in an Environmental Protection Area of the Planalto da Ibiapaba, Northeastern Brazil. *Anais da Academia Brasileira de Ciências* **92**(1):e20180814 DOI [10.1590/0001-3765202020180814](https://doi.org/10.1590/0001-3765202020180814).
- Silva JMC, Casteletti CHM. 2003.** Status of the biodiversity of the Atlantic Forest of Brazil. In: Galindo-Leal C, Câmara IG, eds. *The Atlantic Forest of South America: biodiversity status, threats, and outlook*. Washington D.C.: CABS and Island Press, 43–59.
- Suárez H. 2015.** Six new species of freshwater crabs from Pantepui, Venezuela (Crustacea: Decapoda: Pseudothelphusidae). *Anartia* **25**:64–94 [2013].
- Tabarelli M, Santos AMM. 2004.** Uma breve descrição sobre a história natural dos brejos nordestinos. *Brejos de Altitude em Pernambuco e Paraíba, História Natural, Ecologia e Conservação* **9**:17–24.
- Tamura K, Stecher G, Peterson D, Filipiski A, Kumar S. 2013.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**(12):2725–2729 DOI [10.1093/molbev/mst197](https://doi.org/10.1093/molbev/mst197).
- Teixeira DM, Nacinovic JB, Tavares MS. 1986.** Notes on some birds of northeastern Brazil. *Bulletin of the British Ornithologists' Club* **106**:70–74.
- Thomas MF. 2000.** Late Quaternary environmental changes and the alluvial record in humid tropical environments. *Quaternary International* **72**(1):23–36 DOI [10.1016/S1040-6182\(00\)00018-5](https://doi.org/10.1016/S1040-6182(00)00018-5).
- Thompson JD, Higgins DG, Gibson TJ. 1994.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**(22):4673–4680 DOI [10.1093/nar/22.22.4673](https://doi.org/10.1093/nar/22.22.4673).
- Vanzolini PE. 1970.** Zoologia sistemática, geografia e a origem das espécies. Série Teses e Monografias, 3, Instituto de Geografia, Universidade de São Paulo, 56 p.
- Vanzolini PE, Williams EE. 1970.** South American anoles: geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). *Arquivos de Zoologia* **19**:1–298 DOI [10.11606/issn.2176-7793.v19i1-2p1-176](https://doi.org/10.11606/issn.2176-7793.v19i1-2p1-176).
- Vari R. 1991.** Systematics of the neotropical characiform genus *Steindachnerina* Fowler (Pisces: Ostariophysi). *Smithsonian Contributions to Zoology* **507**:1–118.
- Vuilleumier BS. 1971.** Pleistocene changes in the fauna and flora of South America. *Science* **173**(3999):771–780 DOI [10.1126/science.173.3999.771](https://doi.org/10.1126/science.173.3999.771).
- Weitzman SH, Weitzman M. 1982.** Biogeography and evolutionary diversification in the Neotropical freshwater fishes, with comments on the refuge theory. In: Prance G.T.,

ed. *Biological Diversification in the Tropics*. New York: Columbia University Press, 403–422.

- Xia X. 2013.** DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* **30(7)**:1720–1728 DOI [10.1093/molbev/mst064](https://doi.org/10.1093/molbev/mst064).
- Yeo DCJ, Ng PKL, Cumberlidge N, Magalhães C, Daniels SR, Campos MR. 2008.** Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. *Hydrobiologia* **595**:275–286 DOI [10.1007/s10750-007-9023-3](https://doi.org/10.1007/s10750-007-9023-3).
- Zanetti F, Castro PMD, Magalhães C. 2018.** Freshwater crabs (Decapoda: Brachyura: Pseudothelphusidae, Trichodactylidae) from the state of Roraima, Brazil: species composition, distribution and new records. *Nauplius* **26**:e2018011 DOI [10.1590/2358-2936e2018011](https://doi.org/10.1590/2358-2936e2018011).