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

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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 anteriorly 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.

Introduction

Cumulative evidences 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, 2012; and references therein). These humid forest refuges (Figure 1A–D), naturally isolated by the vast surrounding semiarid Caatinga (Figure 1F, G), 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 mid-altitude forest enclave in Ipú (Ibiapaba plateau, state of Ceará, northeastern Brazil), between 665 to 782 m (Figure 1A–D). Evidences from a phylogenetic analysis using 16S rDNA are presented for a sister taxon 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

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Comment [1]: distally [in opposed to “proximal”, as used in the beginning of the sentence]

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Comment [2]: It doesn’t seem to me that this character is useful for separating the new species from its congeners, particularly from *F. reflexifrons*. If the mesial lobe is compared to the cephalic spine (Figs. 4B,c x 4E,F and 6C x 6G) disregarding the more pronounced tilt of the apex in *F. reflexifrons*, the size of the mesial lobe is quite similar in both taxa. Furthermore, check both structures in Fig. 1A, B in Magalhães & Rodríguez (2002) or in Figs. 16, 17 de Magalhães (1986), where one can notice that the mesial lobe in *F. reflexifrons* could also be considered “much smaller than cephalic spine”. In addition, in *F. ykaa* the mesial lobe is distinctly smaller than the cephalic spine as well (see Magalhães, 2009: 83, fig. 1D,F).

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Comment [3]: I believe that this excerpt can be removed from the Abstract since it seems to me to be somewhat detailed and more appropriate for the Discussion. I suggest adding to the end of this Abstract something like this: *The affinities of the new species are inferred based on a genetic distance analysis made from the partial sequencing of the 16S rDNA gene from mitochondrial DNA and notes on its zoogeography are presented*.

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Comment [4]: genetic distance [?].

relationships of *F. reflexifrons* and the possible evolutionary scenario that led to the emergence of the sister taxons *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) and MZUSP (Museu de Zoologia, Universidade de São Paulo, Brazil). Measurements: cl, carapace length, taken along the carapace axis to its posterior median margin, 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 follows [cite literature] and is referred in the figure 2.

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Comment [5]: Measurements for carapace width (cw) were also presented. Please, include the meaning of this abbreviation

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Molecular data analysis

DNA extraction, amplification and sequencing: Muscle tissue samples were obtained from the pereopods or abdomen of *Fredius ibiapaba* n. sp., *F. buritizatilis* Magalhães & Mantelatto 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® Genomic DNA Purification Kit (Promega), amplified in a 12,5 µl final volume reaction with 2,5 mM de MgCl2 (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 0,3µM of each primer. The PCR conditions were: one cycle at 94°C, 60 sec; five cycles at 94°C, 60 sec;

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45°C, 40 sec and 72°C, 60 sec; and 35 cycles at 94°C, 60 sec; 51°C, 40 sec and 72°C; 60 sec; 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® (Applied Biosystems) at the 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 µl ultrapure water, 1,5 µl BigDye 5x buffer, 0,75 µl BigDye terminator Mix, 2 µl primer (0,8 pmol) and 1 µl of Purified PCR product. Sequence conditions were: one minute at 96°C; 35 cycles of 15 sec at 96°C; 15 sec at 50°C and 2 minutes at 60°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).

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 et al., 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).

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Comment [6]: The analysis carried out was of genetic distance between some representatives of the genus for which the authors had material available for sequencing. For a phylogenetic analysis, all known (or, at at least, most of the) representatives of the genus should be included and, in the case of a molecular study, it would be much more appropriate to use other genes.

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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.

Results

Pseudothelphusidae Ortmann, 1893

Fredius Pretzmann, 1967

Fredius ibiapaba n. sp. (Figures 3A-E; 4A-C; 5A, C; 6A-D; 7A-E)

Type material. Holotype, male cl 36 mm, cw 53 mm (MZUSP 39710), Brazil, Ceará, Ipú, Sítio Caranguejo, 04°18'50"S, 40°44'47"W, 729 m, xii.2017. Paratypes: male cl 34 mm, cw 48 mm (MZUSP 39169), same data as holotype; female cl 35 mm, cw 49 mm (MZUSP 39171), Sítio Gameleira, Ipú, Ceará, 04°17'17"S, 40°44'44"W, 665 m, 5.i.2018; male cl 32 mm, cw 48 mm (MZUSP 39167), Sítio Santa Cruz, Ipú, Ceará, 04°19'40"S, 40°45'09"W, 782 m, 10.x.2014; female cl 31 mm, cw 44 mm (MZUSP 39168), Sítio Santa Cruz, Ipú,

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Comment [8]: Synonymic list?

Magalhães et al. (2005) published two records of pseudothelphusid crabs that they, based only on the morphology of G1, determined as being *Fredius reflexifrons*: one lot from Viçosa do Ceará (2 males, INPA 1382) and another from Ipú, Sítio Santa Cruz (4 males, INPA 1318), both on the Ibiapaba plateau. The latter is the type-locality of *F. ibiapaba* sp. n., while the former is located approximately 120 km N-NW from the type-locality. In this situation, it is highly possible that the specimens studied by Magalhães et al. (2005) are co-specific with the new taxon. If so, the determination of Magalhães et al. (2005) should be included in the synonymic list of *F. ibiapaba*, since there would have already been a previous mention of the present new taxon.

As this reviewer is one of the authors of the 2005 paper, I can explain that, based only on the morphology of G1 and without taking into account molecular data, we then consider the subtle differences to be insufficient to recognize such populations as a distinct taxon. The present manuscript, however, completely ignores the material studied by Magalhães et al. (2005) and does not compare the specimens from lots INPA 1318 and 1382 with those studied in the present manuscript. This issue is even more striking if we take into account that (a) the article by Magalhães et al. (2005) was cited in this study (although in another context); and (b) the senior author should have examined this material when visiting the INPA collection (as mentioned in the acknowledgments).

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Comment [9]: I recommend to quote the localities always from the largest geographical unit to the least: Country, State/Department/Province, Municipality, locality, geographical coordinates. Please, do it for all records listed in material examined.

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Ceará, 04°19'40"S, 40°45'09"W, 782 m, 23.iv.2015; male cl 41.2 mm, cw 62.6 mm (MZUSP 39742), Sítio Ipuçaba, Ipú, Ceará, 27.x11.2017.

Comparative material. *Fredius fittkaui* (Bott, 1967): male, cl 47.1 mm, cw 66.9 mm (MZUSP 24497), Guyana, Potaro-Siparuni, Rio Kuribrong, 05°22'35"N, 59°33'4"W, P. Bernardo & B. Newman coll., 28.ix.2010. *Fredius reflexifrons* (Ortmann, 1897): male, cl 31 mm, cw 42.5 mm (MZUSP 6389), Peru, Departamento Loreto, Rio Apiacu, Boris Malkin coll., 15-25.iv.1966. Male cl 37 mm, cw 52 m (MZUSP 19922), Brazil, Amapá, Serra do Navio, Serra do Veado, M. Tavares coll. (Projeto Diversitas Neotropica), 7.v.1994. 2 males, cl 57.7 mm, cw 42 mm and cl 73.8 mm, cw 53 mm (MZUSP 13178), Brazil, Amapá, Rio Jari, upstream from Cachoeira Santo Antônio, M. Jegú & J. Zuanon coll., 9-26.vi.1981. Male (INPA 583), Brazil, Amapá, Serra do Navio/Serra do Veado, coll. unknown, 07.v.1994. Male (INPA 2125), Brazil, Amapá, municipality of Laranjal do Jari, 00°34'43.6"N, 52°38'40.1"W, V.T. de Carvalho coll., 16.i.2012. Male (INPA 889), Brazil, Amazonas, Manaus, Reserva Florestal ZF 3 (INPA/PDBFF), 02°26'56"S, 59°46'13"W. Male (INPA 368), Brazil, Amazonas, Manaus, Reserva Florestal Ducke (INPA), 02°58'05"S, 59°55'49"W, M. Yamakoshi coll., 22.ii.1986. Male (INPA 850), Brazil, Amazonas, Manaus, Reserva Florestal Ducke (INPA), L. Schilsari coll., 11.vii.2001. Male (INPA 852), Brazil, Amazonas, municipality of Iranduba, Sítio Anaíra, 03°10'39"S, 60°07'39"W, G.M. dos Santos coll., 12.ix.1999. Male (INPA 1254), Brazil, Pará, Santarém, Comunidade Santa Rosa, Team from Faculdades Integradas do Tapajós coll., 03.iv.1999. Male (INPA 851), Brazil, 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 1512), Brazil, Pará, Bragança, Rio Chumucuí, S. Alves coll., 12.xi.2004. *Fredius denticulatus* (H. Milne Edwards, 1853): male cl 45 mm, cw 62 mm (MZUSP 16294), Brazil, Amapá, Rio Amapari, Serra do Navio, M. Tavares coll. (Projeto Diversitas Neotropica, n°151), 30.iv.1994.

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Comment [10]: The material of the lots INPA 1318 and 1382 must be compared with the type material of the new species to check if they are co-specific and, if so, to list the determination of Magalhães et al. (2005) in its synonymic list.
If the senior author did not examine these specimens during his visit to the INPA's collection, then he could (a) ask them on loan for a detailed examination; (b) judge from the morphology of the G1 figured in Magalhães et al. (2005); or (c) consider, in the synonymic list, the determination of Magalhães et al. (2005) as doubtful (with a question mark).
In any case, the records presented by Magalhães et al. (2005) for *Fredius reflexifrons* from the same localities of the *F. ibiapaba* sp. n. type series should not be ignored.

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

Distribution. Only recorded from the type locality to date.

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Comment [12]: This is not true! The paratypes were collected in other places near the type locality. Please, remove.

Etymology. The specific epithet is a noun in apposition taken from the Tupi language word for plateau, "yby'ababa".

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Diagnosis. G1 robust, proximal half remarkably swollen, sloping abruptly downwards anteriorly 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.

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Comment [13]: This is not diagnostic, as commented above.

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Comment [14]: The word "moderately" is quite subjective, especially if used outside a comparative context. I recommend: *cephalic lobe somewhat broad, rounded apically*".

Description of the holotype. Carapace transversally ovate (Figure 3A), widest at middle (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 papillae; front lower border carinate, with an almost indistinct sinus medially in frontal view; postfrontal lobules obsolete; median groove between postfrontal lobules faint. Upper orbital margin with row of very faint papillae; lower margin minutely denticulate; exorbital angle marked by obtuse tooth, followed posteriorly by faint notch. Carapace anterolateral margin semicircular in outline, fringed by minute denticles; posterolateral margins almost straight, strongly convergent, smooth. Epistomial margin with minute papillae; epistomial tooth broadly triangular, deflexed (Figure 3C). Suborbital and subhepatic regions of carapace smooth; pterygostomial region densely pubescent around mouthparts (Figures 3B, C).

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 (Figure 3B). Exopod short, 0.28 times length of lateral margin of ischium, devoid of flagellum. Efferent branchial channel opening subcircular (Figure 3B).

Chelipeds moderately heterochelous, right cheliped larger than left one (Figure 3E, F). Major cheliped merus subtriangular in cross-section; lateral surface smooth, 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 sides, with minute papillae on dorsal and ventral rounded faces. Dactylus regenerated. 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 (Figure 3B); sternal sutures 4/5and 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 abdominal segments free. Lateral margins of male telson slightly concave, tip rounded (Figure 3B).

G1 robust, proximal half remarkably swollen, sloping abruptly downwards anteriorly to a nearly right-angular shoulder (Figure 4B, C). Subapical bulge moderately developed around lateral and sternal sides (Figures 4B; 5A, B; 6A). Marginal suture straight (Figure 4C). Marginal lobe truncate, projected distally beyond abdominal surface, junction marked by distinct depression. Mesial lobe much smaller than cephalic spine, showing as

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triangular, acute spine, pointing to abdominal direction (Figures 4A, C, 5B; C; 6A, C). Cephalic spine very strong, acuminate at tip, pointing in mesial direction (Figures 4A–C, 5B; C; 6A–C). Cephalic lobe prominent, truncate, tip rounded, with several very small spines along lateral, mesial and sternal sides (Figures 4A, B; 6A, B). Auxiliary lobe much shorter than cephalic lobe in abdominal view, separated from it by distinct depression, their junction forming lateral channel running distally in almost straight direction before ending in inward curve subterminally (Figures 4A; 6A). Field of apical spines large, open, flattened, elongated, ear-shaped, provided with small spinules, delimited by lateral and abdominal lips of apex (Figures 4A, B; 5B; 6A, B).

G2 slightly longer than G1 when abdomen folded beneath cephalothorax; very slender, progressively tapering distally, 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 (Rodriguez, 1982; Rodriguez & Pereira, 1992) are readily recognized in the new species, namely, exopod of mxp3 short, about 0.3 times length of outer margin of ischium. G1 widest at base (Figure 4B, C); 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 (Figures 4A–C; 5B; 6A, B).

The new species morphologically resembles *Fredius denticulatus*, *F. fittkaui*, *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, P) (Figure 4A, C; 5B; 6A, C), whereas other species either have the gonopod cephalic spine little larger than the mesial lobe (*F. stenolobus* Rodríguez & Suárez, 1994 and *F. adpressus* Rodriguez & Pereira,

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Comment [17]: pointing in OR pointing to OR pointing towards [?]

Célio Magalhães 21/1/2020 11:18
Comment [18]: I don't think "truncate" (shorten something by cutting off the top or the end) is a good word here; better remove it.

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Comment [19]: spinules [?]

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Comment [20]: Following the recommendations by Ng (1994) (https://lkcnhm.nus.edu.sg/app/uploads/2017/06/42rbz509-513.pdf) and Dubois (2008) (https://www.mapress.com/zootaxa/2008/f/z01771p068f.pdf), the author's names should be written exactly as spelled in the original publication. The name of Gilberto Rodríguez sometimes appeared with ï" (without accent), sometimes with "í" (with accent) in his publications.

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1992), or have it much shorter than the mesial lobe (e.g., *F. buritizatilis* Magalhães & Mantelatto in Magalhães et al., 2014, *F. platyacanthus* Rodriguez & Pereira, 1992, and *F. estevi* Rodriguez, 1966), or have the cephalic spine and the mesial lobe similar in size (e.g., *F. granulatus* Rodríguez & Campos, 1998, *F. chaffanjoni* (Rathbun, 1905) (see Magalhães et al., 2014 and references therein).

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Fredius ibiapaba n. sp. stands apart from *Fredius denticulatus*, *F. fittkaui*, *F. reflexifrons* and *F. ykaa* in having the G1 proximal half remarkably swollen on the abdominal side, sloping abruptly downwards anteriorly to a nearly right-angular shoulder (Figures 4B, C), whereas in the latter four species the G1 shoulder is clearly more gently sloping distally (Figure 4E, F).

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The closest morphological resemblance of *Fredius ibiapaba* n. sp. is with *F. reflexifrons* of which it additionally differs (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 (Figure 4A, B), whereas in *F. reflexifrons* the lip fades away well before reaching the distal margin of the lobe (Figure 4D, E); (2) the subapical bulge markedly less swollen (Figures 5A, C); and (3) the G1 apex much less tilted so that the mesial lobe is not visible in sternal view (Figure 5A), in contrast to *F. reflexifrons* (Figures 5B, D, respectively). Also, in *F. ibiapaba* n. sp. the distal margin of the cephalic lobe is truncate (Figure 4A, 6A), whereas in *F. reflexifrons* it tapers progressively to a distinct narrower tip (Figure 4D, 6D).

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Comment [22]: I can't see quite like that. I would say "slightly before" at most.

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Comment [23]: See Comment [18} above. I would recommend "rounded".

Fredius ibiapaba n. sp. further differs from *F. ykaa* in that the G1 shoulder is high and robust (Figures 4B, C), 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.

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Discussion

Genetic distance analysis

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The mitochondrial loci 16S was successfully amplified and sequenced for *Fredius buritizatilis*, *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 dendrogram of genetic distance (Figure 8). *Fredius ibiapaba* n. sp. is very well supported (98) as a sister taxon to *F. reflexifrons* reflecting the close morphological resemblance between the two species.

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Comment [24]: Magalhães et al. (2014) also presented a dendrogram of genetic distance for some species of *Fredius*. It would be appropriate a few comments on how they are comparable.

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The divergence rates between *Fredius reflexifrons* and *F. ibiapaba* n. sp. (4%) is higher than those between *F. estevisi* x *F. stenolobus*, *F. platyacanthus* x *F. stenolobus* and *F. platyacanthus* x *F. estevisi* all with of 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. buritizatilis* from a palm swamp known as "buritizal"), or by revisiting the taxonomy of widely disjunct species for testing as to their conspecific status, such as *F. ibiapaba* n. sp. and *F. reflexifrons*.

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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

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Comment [27]: Two species are invalid in Table 3 and should be removed.

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

Rodriguez & 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 the sister taxon 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*.

The discovery of *F. ibiapaba* n. sp. revealed that it is actually the sister group of *F. reflexifrons*, as shown by a comparative 16S rDNA sequencing used to infer the phylogenetic placement of *Fredius ibiapaba* n. sp. (Figure 8). The distribution range of *Fredius ibiapaba* n. sp. is very narrow and currently restricted to a humid enclave, a small

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mid-altitude forested patch in Ipú (Ceará, northeastern Brazil, Figure 1A–E), nested within the vast semiarid Caatinga domain (Figure 1F, G). The orographic forest enclaves, such as Ipú, are typically located along the slopes of plateaus, between 600 and 1100 m, hence high enough to receive rainfall of more than 1200 mm year^{-1} of Atlantic origin (Tabarelli et al., 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 (Figure 1E).

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Comment [29]: A somewhat similar discussion has already been made in Magalhães et al. (2005) and I believe it should somehow be mentioned here.

In contrast, *F. reflexifrons* is widely distributed in the Amazon basin's lowlands (< 100 m) from as far west as Peru (Ampyiacu River, a tributary of the Amazonas River) to as far east as the Atlantic basin (French Guiana) (Magalhães, 2003; Magalhães et al., 2005). It is found in burrows alongside the “igarapés” (streams) or digs its burrows on the humid forest floor (Magalhães & Rodríguez, 2002).

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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. The shrinking humid forests during several dry periods of the Tertiary and Quaternary (Katzer, 1933; Andrade-Lima, 1953; Bigarella et al., 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 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 (Figure 1A–E). The two species are now separated by a vast intervening area occupied by the semiarid Caatinga (Figure 1F, G).

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 et al., 1986; Haffer, 1969; 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.

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It has long been known that a number of freshwater fish species inhabiting the Brejos have their closest relationships with 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, state of Ceará. Previously to their discovery *Kingsleya* was known from nine species inhabiting the Amazonian lowlands (Pedraza & Tavares, 2015).

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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 with 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. We also thank the Universidade Regional do Cariri (URCA), Universidade do Sagrado Coração (USC) and MZUSP for the logistic support.

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Comment [33]: I am not sure if the PeerJ rules demand that the references regarding the taxonomic authorities should be included in the References list. If so, please add Bott (1967); H. Milne Edwards (1853); Ortmann, 1893, 1897; Pretzmann (1967); Rodríguez & Suárez (1994); Rodríguez (1966); and Rodriguez (1980) to the list.

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Captions for the figures and tables

Figure 1. (A–E) Mid-altitude, naturally isolated, humid forested patch nested within the vast semiarid Caatinga domain. Sítio Caranguejo, Ipú, Ceará, 04°18'50" S, 40°44'47"W, 729 meters high, type locality of *Fredius ibiapaba* n. sp. 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.

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.

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Figure 3. (A–D) *Fredius ibiapaba* n. sp., male, holotype, cl 36 mm, cw 53mm (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.

Figure 4. (A–F) First right male gonopod (G1) in abdominal (tilted left), lateral and mesial views from A–C and D–F, respectively. (A–C) *Fredius ibiapaba* n. sp., male, holotype, cl 36 mm, cw 53mm (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).

Figure 5. (A–D) First right male gonopod (G1) in sternal and apical views from A to B and C to D, respectively. (A, C) *Fredius ibiapaba* n. sp., male, holotype, cl 36 mm, cw 53mm (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).

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

Figure 7. (A–E) *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 in mesial (tilted right), sternal, apical, lateral, and mesial views. Scales: A–E, 1 mm.

Figure 8. Dendrogram of genetic distance 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).

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

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

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

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

Species	**Country**	**Environment**	**Altitude (m)**	**References**
F. ykaa Magalhães, 2009	Brazil (Amazon River basin)	Lowland streams	36 to 73	Magalhães, 2009
F. adpressus Rodriguez & Pereira, 1992	Venezuela (Orinoco River basin)	Lowland streams	100	Rodriguez & Pereira, 1992
F. beccarii (Coifmann, 1939)	Brazil, Guyana, Venezuela, Suriname (Essequibo-Cuyuni Rivers basin)	Streams (igarapés)	50 to 752	Rodríguez & Campos, 1998; Cumberlidge et al., 2014; Mora-Day et al., 2009; Magalhães et al., 2014; Zanetti et al., 2018
F. buritizatilis Magalhães & Mantelatto, 2014	Brazil (Madeira River basin)	Buritizal (palm) fields	150	Magalhães et al., 2014
F. chaffanjoni (Rathbun, 1905)	Venezuela (Orinoco River basin)	River's headwaters and mid-courses	105-300	Rodriguez & Pereira, 1992
F. convexa (Rathbun, 1898)	Costa Rica	Highland streams	770	Smalley, 1964
F. cuaoensis Suárez, 2015	Venezuela (Orinoco River basin)	Highland streams	950	Suárez, 2015
F. cuyunis (Pretzmann, 1967)	British Guyana (Cuyuní River)	Lowlands	around 100	WoRMS, 2019
F. denticulatus (H. Milne Edwards, 1853)	Brazil, Suriname, French Guiana (Amazon and Atlantic river basins)	Streams (igarapés) and along river margins	70 to 400	Rodriguez & Pereira, 1992; Rodríguez & Campos, 1998; Magalhães et al., 2005; Magalhães, 2009; Cumberlidge e

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				al., 2014; Magalhães et al., 2014
F. estevisi (Rodriguez, 1966)	Brazil, Venezuela (Amazon and Atlantic rivers basins)	River's headwaters and streams	446 to 944	Mora-Day et al., 2009
F. fittkaui (Bott, 1967)	Brazil, Venezuela, Guyana (Amazon and Atlantic rivers basins)	Streams (iIgarapés) and along river margins	151 to 500	Rodríguez & Campos, 1998; Magalhães & Rodríguez, 2002; Cumberlidge et al., 2014; Magalhães et al., 2014; Zanetti et al., 2018
F. granulatus Rodríguez & Campos 1998	Colombia (Amazon River basin)	Lowlands	180 to 200	Rodríguez & Campos, 1998; Cumberlidge et al., 2014
F. platyacanthus Rodriguez & Pereira, 1992	Brazil, Venezuela (Atlantic rivers basin)	Streams (igarapés) and mountain areas	106 to 1229	Rodriguez & Pereira, 1992; Cumberlidge e al., 2014; Magalhães et al., 2014; Zanetti e al., 2018
F. reflexifrons (Ortmann, 1897)	Brazil, Venezuela, Suriname, French Guaiana, Peru, Guyana (Amazon and Atlantic rivers basins)	Lowland streams	37 to 200	Magalhães & Rodríguez, 2002; Magalhães et al., 2005; Cumberlidge e al., 2014
F. stenolobus Rodríguez & Suárez, 1994	Brazil, Venezuela (Orinoco River basin)	Streams in rocky areas	65 to 1020	Rodríguez & Campos, 1998; Magalhães & Pereira 2007; Cumberlidge e al., 2014; Magalhães

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				et al., 2014; Zanetti et al., 2018
Fredius ibiapaba 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 to782	Present study