Parasitology

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Molecular features of *Probopyrus* sp. (Isopoda: Bopyridae) from Brazilian Amazonia and the parasitism of inland populations of *Macrobrachium amazonicum* (Decapoda: Palaemonidae)

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Research Article

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Abstract

Bopyrid isopods of the genus *Probopyrus* are well-known parasites of freshwater prawns of the genus *Macrobrachium*. The parasitism of coastal populations of *Macrobrachium amazonicum* by *Probopyrus bithynis*, for example, has been documented since the late 1980s. Despite this, molecular data on different populations are not available for any *Probopyrus* species. The present study is the first to describe *Probopyrus* populations from distinct regions of the Amazon basin based on sequences of two genes, the mitochondrial cytochrome oxidase C subunit I (COI) and the nuclear 18S ribosomal DNA (18S rDNA) gene. The analyses indicated the presence of two *Probopyrus* species, each parasitizing either the coastal or the inland populations of *M. amazonicum*. The results indicated the potential use of the COI barcode for the identification of *Probopyrus* species. We discuss the potential implications of the findings for the taxonomy of *Probopyrus bithynis* and other species of the genus *Probopyrus*.

Introduction

Crustaceans are a diverse group of organisms, including commercially important species such as shrimp, lobsters and crabs, in addition to a variety of parasitic species associated with both vertebrate and invertebrate hosts. Parasitic crustaceans include the isopods of the superfamilies Bopyroidea and Cryptoniscoidea, which are specialized for the parasitism of other crustaceans (Williams and Boyko, 2012). The family Bopyridae is a highly diversified group of isopods, with more than 600 species in nine subfamilies (Boyko *et al.*, 2013). One bopyrid genus, *Probopyrus*, includes isopods that are typically ectoparasites in the branchial chamber of paleomonid prawns (Masunari *et al.*, 2000). The species of this genus include *P. bithynis* Richardson, 1904, *P. buitendijki* (Horst, 1910), *P. floridensis* Richardson, 1904, *P. markhami* Román-Contreras, 1996, *P. pacificensis* Román-Contreras, 1993 and *P. pandalicola* (Packard, 1879), which are known to parasitize prawns of the genera *Macrobrachium*, *Palaemon* and *Palaemonetes* (Lemos de Castro, 1974; Masunari *et al.*, 2000; Román-Contreras, 2004; Brinton and Curran, 2015*a*; Gopalakrishnan *et al.*, 2017; Ribeiro *et al.*, 2019; de Barros *et al.*, 2021).

Macrobrachium amazonicum Heller, 1862 is a freshwater prawn with a wide distribution in South America, and is the native prawn species with the most widespread occurrence in the inland waters of Amazonia (Odinetz-Collart and Moreira, 1993). Despite being endemic to the Amazon region (Odinetz-Collart, 1991), *M. amazonicum* is also found in the basins of the Paraná and São Francisco rivers (Bialetzki *et al.*, 1997; Sampaio *et al.*, 2007), as well as many other hydrographic basins in South (Kensley and Walker, 1982; Melo, 2003; Valencia and Campos, 2007) and Central America (Vergamini *et al.*, 2011).

Macrobrachium amazonicum is the definitive host of *P. bithynis*, and a number of studies have focused on the relationship between these two species. Odinetz-Collart (1990), for example, found evidence of a stable interaction between the two species, supported by data on the infestation rates and life cycle of the host, based on specimens collected on the lower Tocantins River, in the Brazilian state of Pará, given that the body length of the female isopods correlated positively with that of the prawn host. More recently, Corrêa *et al.* (2018) described histopathological alterations in the gills of *M. amazonicum* specimens collected from the lower Amazon River, in Pará state, caused by *P. bithynis* infestation. These authors concluded that the alterations were consistent with the ingestion of the branchial tissue by *P. bithynis*, which would have a negative impact on the respiratory capacity of the host. Infestation by *Probopyrus* females may also induce the castration of the host, the feminization of the males (Beck, 1980), reduction of the development of the nutritional conditions of the host (de Barros *et al.*, 2021), and even predator–prey interactions, through the reduction in

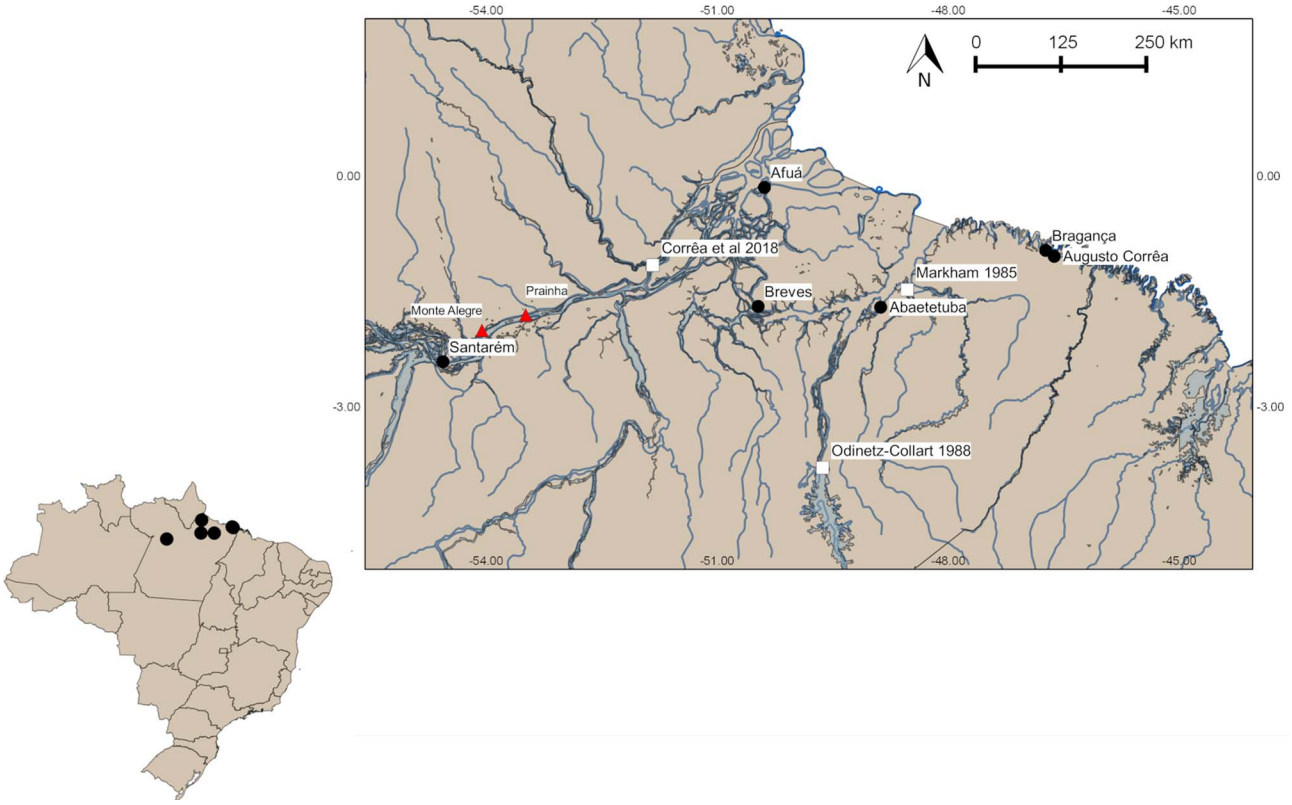

Fig. 1. Location of the sampling points in the inland and coastal regions of Brazilian Amazonia (Black circles), previous records of *Probopyrus bithynis* (white squares) and geographic boundaries of the continental and coastal genetic lineages of the host *Macrobrachium amazonicum* (red triangles). More details are presented in the discussion.

the capacity of the host to camouflage itself, leaving it more susceptible to predation (Brinton and Curran, 2015*b*).

While these records of bopyrid parasitism on *M. amazonicum* all refer to specimens collected in the coastal region of the Amazon (Maciel and Valenti, 2009), no evidence has been found of this phenomenon, up to now, in the inland waters of the Amazon basin. The present study not only provides records of the occurrence of *Probopyrus* sp. parasitism in specimens of *M. amazonicum* collected in areas that are approximately 650 km from the mouth of the Amazon River, but also reports on molecular analyses that indicate distinct host–parasite relationships between the inland and coastal regions of the Amazon basin. The implications of these findings for the taxonomy of some *Probopyrus* species are also discussed.

Materials and methods

Study area

The study area includes both coastal and inland regions of Brazilian Amazonia. Inland, specimens were collected on the left margin of the Amazon River in the municipality of Santarém, Pará (Brazil), in an area of várzea swamp known as Pixuna do Tapará (02°24.98′ S, 54°33.9′ W). Local prawn fishermen indicated the presence of large numbers of parasitized *M. amazonicum* in this area. In the coastal region, specimens of *M. amazonicum*, both with and without parasites, were collected from the municipalities of Abaetetuba, Afuá, Augusto Corrêa, Bragança and Breves (Fig. 1), all located in the Brazilian state of Pará.

Sampling

In the Santarém region, prawns were collected in a type of trap known locally as the *covo*, which is used by the local shrimpers. These traps are made of semi-fixed frames of wood or iron (2 m × 1.5 m) covered with a wire or nylon mesh, and set in the direction of the current. There is a lateral rectangular opening at each extremity, large enough to allow individual prawn to enter the structure, where they are trapped (Castro e Silva and Cavalcante, 1994). In the coastal region, the prawns were collected using baited traps known locally as the *matapí* (see Maciel and Valenti, 2009). Each parasitized prawn was placed in an individual plastic bag to avoid losing or mixing the parasites. In the laboratory, the parasites were removed from each host and preserved in 1.5-ml microtubes containing 100% ethanol.

Molecular analyses

The total genomic DNA of the host was obtained from abdominal tissue using the ammonium acetate protocol of Bruford *et al.* (1998). The DNA of the parasites was obtained from the males using the QIAamp DNA Investigator kit (QIAGEN), following the maker's instructions. The sample included 28 parasites and 29 *M. amazonicum* specimens (Table 1). Part of the sample material was deposited in the scientific collection of the Museum of Biological Diversity – Zoology (Museu de Diversidade Biológica – área Zoologia, MDBio – Zoologia) of the Campinas State University (Universidade Estadual de Campinas – UNICAMP) with the ZUEC CRU 4381, 4382 and 4383 vouchers. Information on new vouchers also can be obtained from the authors or from the museum's curatorship.

The extracted DNA was processed by polymerase chain reaction (PCR) to isolate and amplify different regions of the cytochrome oxidase C subunit I (COI) gene. The A and F primers (Palumbi and Benzie, 1991) were used to amplify the COI of *M. amazonicum*, while the HCO2198 and LCO1490 primers (Folmer *et al.*, 1994) were used for the parasites.

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Table 1. Number of specimens of the host (*Macrobrachium amazonicum*) and parasite (*Probopyrus* sp.) analysed in the present study at each sampling point.

	Augusto Corrêa	Abaetetuba	Breves	Afuá	Santarém	Total
Parasite	10	4	4	2	8	28
Host	5	4	8	3	9	29

Table 2. Results of the BLAST search of the 18S rRNA sequences of the *Probopyrus* specimens obtained in the present study

Species	Sequence ID	Score	E-value	Identity	Gap	Reference
Probopyrus pacificiensis	AF255683	1786	0.0	973/976 (99%)	1/976 (0%)	Dreyer and Wägele (2001)
Probopyrus pandalicola	EU848422	1696	0.0	965/985 (98%)	13/985 (1%)	Cho (2012)
Probopyrus buitendijki	KF765767	1681	0.0	961/984 (98%)	9/984 (0%)	Boyko *et al.* (2013)

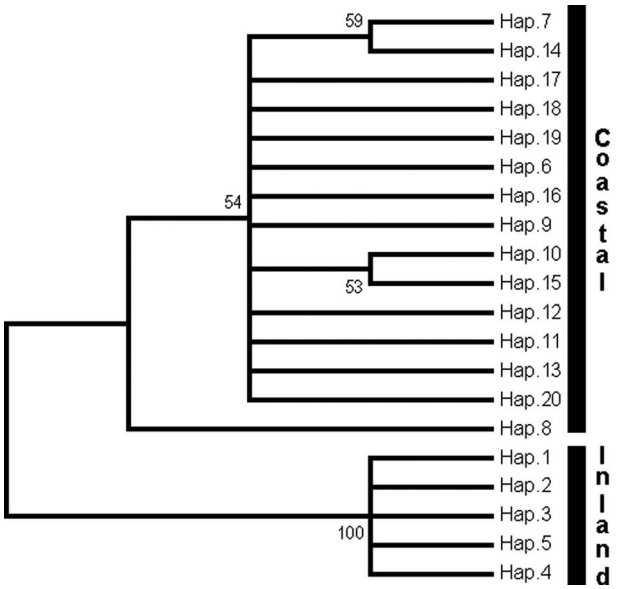

Fig. 2. Neighbour-joining tree obtained from the COI sequences showing the relationships among haplotypes 1–20 retrieved from the *Probopyrus* parasites collected in Brazilian Amazonia. The values above the nodes represent the bootstrap significance of the clades. Bootstrap values of less than 50 are not shown here.

The molecular identification of the parasites was based on sequences of the 18S rDNA gene, obtained using the 'ai' and 'bi' primers of Whiting *et al.* (1997). Despite being a highly conserved region, this is the gene with the largest number of bopyrid sequences deposited in GenBank. Given this, samples of parasites were selected randomly from each sampling locality. Similarly, the COI database of the parasites included two sequences of *P. pandalicola* (GenBank id: MH087672 and MK308333).

The quality of the extracted DNA and the PCR products was evaluated by electrophoresis in 1% agarose gel to which GelRed (Biotium) was added. The sequences were obtained using an ABI 3500 (Applied Biosystems) automatic sequencer with the Big Dye 3.1 kit (Applied Biosystems), following the maker's instructions. The sequencing reactions were run in both directions using the PCR primers.

Data analysis

The sequences obtained were aligned in CodonCode Aligner v7.1.2 (CodonCode Corporation) for the visualization and editing of reading errors. Three databases were compiled, one for the hosts (COI) and two for the parasites (COI and 18S rDNA).

The number of haplotypes (unique sequences) was obtained from these three databases using DNAsp v5.10.1 (Librado and Rozas, 2009). For the hosts, a haplotype network was constructed in PopART (Leigh and Bryant, 2015), based on the median joining networks method (Bandelt *et al.*, 1999). In the case of the parasites, a neighbour-joining tree was constructed in MEGA v7.0 (Kumar *et al.*, 2016) using the p distance model. This program was also used to obtain the mean genetic (p) distances between the populations. The 18S rDNA sequences were used for a basic local alignment search tool (BLAST) search (MegaBLAST), with a similarity of at least 98% being considered valid, with e-values near or equal to zero.

Results

18s rDNA

An 18S rDNA sequence of 975 base pairs (bps) was obtained from ten bopyrid individuals (four from Santarém, two from Abaetetuba and Augusto Corrêa, and one each from Afuá and Breves). All the sequences were 100% identical and the BLAST search demonstrated that all the parasites collected from both study areas belong to the genus *Probopyrus*, with sequences closest to the species *Probopyrus pacificiensis*, *P. pandalicola* and *P. buitendijki* (Table 2).

COI gene

The COI database for the parasites included 28 sequences of 603 bps, which included 20 haplotypes, considering all the populations analysed. Haplotypes 1–5 were recorded in individuals from Santarém (inland population), while haplotypes 6–20 were recorded exclusively in the coastal populations (Fig. 2).

These findings were further corroborated by the mean genetic distances. The parasite sequences from Santarém are approximately 16% different from those of the other localities (Table 3), whereas the differences between the coastal populations ranged from only 0.41% (between Breves and Afuá) to 0.81% between Abaetetuba and Augusto Corrêa. In comparison with the sequences of *P. pandalicola*, the genetic distance was 16.68% from the Santarém population, and up to 18.37%, in the case of the population of Abaetetuba.

The 29 COI sequences of *M. amazonicum* included 10 haplotypes, of which haplotypes 1–4 were recorded exclusively in Santarém, and haplotypes 5–10 were found only in the coastal populations (Fig. 3). The sequences from Santarém were also approximately 3% different from those of the other localities, with extremely low variation (0.1–0.3%) being found among the coastal populations (Table 3).

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Table 3. Mean genetic distances (p distance in %) estimated from the COI sequences of *Probopyrus* (dark grey, lower) and *Macrobrachium amazonicum* (light grey, upper) between the sampling localities in eastern Brazilian Amazonia

	P. pandalicola	Santarém	Afuá	Abaetetuba	Breves	Augusto Corrêa
P. pandalicola	(0.0)[a]					
Santarém	16.68	(0.0; 0.1)	3.2	3.3	3.2	3.1
Afuá	18.32	16.31	(0.0; 0.0)	0.1	0.1	0.2
Abaetetuba	18.37	16.23	0.66	(1.0; 0.2)	0.2	0.3
Breves	18.20	16.27	0.41	0.73	(0.0; 0.3)	0.3
Augusto Corrêa	18.29	16.30	0.53	0.81	0.60	(1.0; 0.3)

[a]The values within parentheses represent the intra-population variation of the host and parasite, respectively.

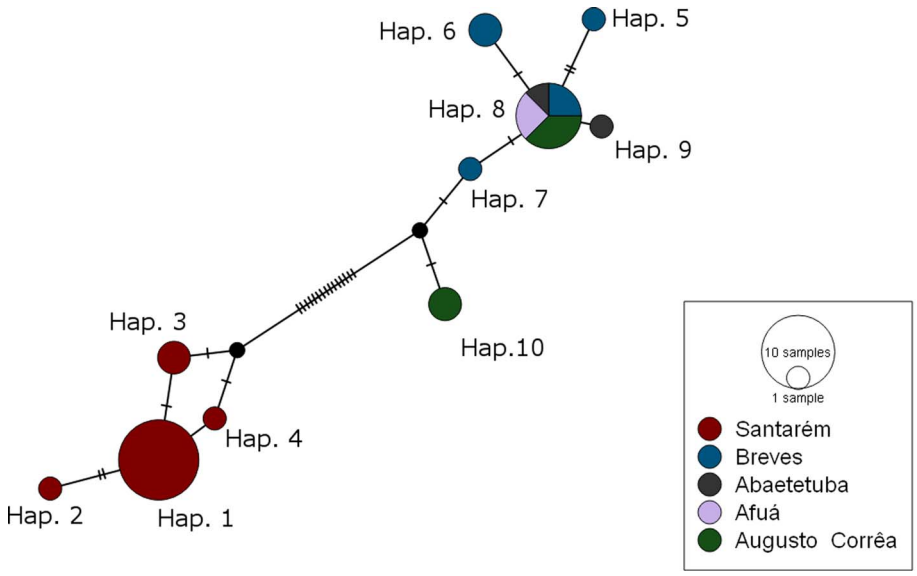

Fig. 3. Haplotype network based on COI sequences of the host *Macrobrachium amazonicum* collected in Brazilian Amazonia.

Discussion

The present study provides the first evidence of the infestation of an inland population of *M. amazonicum* by a parasite of the genus *Probopyrus*. The molecular analyses also revealed that the coastal and inland populations of *M. amazonicum* are parasitized by different *Probopyrus* species.

Parasites of the genus *Probopyrus* have a well-established relationship with a number of different representatives of the family Palaemonidae, in particular, the prawns of the genus *Macrobrachium* (Markham, 1985; Saito *et al.*, 2010). In the specific case of *M. amazonicum*, records of infestation by *P. bithynis* are restricted to the coastal region of the Amazon basin, in areas near the Tucuruí hydroelectric dam on the Tocantins River, which is approximately 300 km from the Atlantic Ocean (Odinetz-Collart, 1988, 1990). More recently, records of this parasitism were obtained from the lower Amazon, near the community of Maruim, in the municipality of Gurupá, around 400 km from the Atlantic Ocean, in the Brazilian state of Pará (Corrêa *et al.*, 2018). The inland population analysed here is from Santarém, approximately 650 km from the ocean (see Fig. 1).

In the case of the earliest record, Odinetz-Collart (1988) observed parasitized prawns only downstream from the Tucuruí dam, and none from the reservoir itself, and a similar lack of parasitism in prawn specimens collected from the region of Manaus (Central Amazonia) and the Ucayali River in Peru (Odinetz-Collart, 1990). Given the evidence, this author concluded that bopyrid parasites are limited by the distribution of the brackish water copepods that act as intermediate hosts. In the laboratory, Dale and Anderson (1982) found that *Acartia tonsa* Dana, 1849 was the intermediate host of *P. bithynis*, even in the presence of other copepods present in the mixed zooplankton cultures experiment made by the authors. *Acartia tonsa* is a common calanoid copepod found in estuarine environments (Figueroa *et al.*, 2020). Given this, it is very likely that *A. tonsa* acts as an intermediate host in the coastal populations of *Probopyrus* analysed in the present study. This does not apply, however, to the parasites collected in the inland area (Santarém), which must rely on a different calanoid copepod intermediate host, still unidentified.

In addition to the fact that this is a freshwater region, and thus outside the geographic range of *A. tonsa*, the analysis of the COI sequences indicated a clear separation (with no gene flow) of the parasites collected in Santarém from those obtained in the coastal municipalities (Afuá, Abaetetuba, Augusto Corrêa and Breves), with a mean genetic distance (16.2%) that is consistent with the presence of two distinct *Probopyrus* species parasitizing *M. amazonicum*. The lack of gene flow between the populations of the definitive host found in Santarém and the coastal areas (Abaetetuba, Augusto Corrêa, Afuá and Breves) further reinforces this conclusion. While still preliminary, the few available genetic data clearly indicate a lack of gene flow between the coastal and inland populations of *M. amazonicum* (Vergamini *et al.*, 2011; Iketani *et al.*, 2021). Iketani *et al.* (2021) obtained COI sequences from a number of *M. amazonicum* populations distributed along the length of the Amazon River, and found that the coastal group was restricted to the lower Amazon, below Prainha (approximately 550 km from the Atlantic Ocean), while the inland populations were located upriver from the municipality of Monte Alegre (around 620 km from the Atlantic) (see Fig. 1).

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Sequences of COI are widely used for the molecular identification of species using the DNA barcode approach proposed by Hebert *et al.* (2003), with many studies applying this method successfully in crustaceans, in particular, those of the order Decapoda (Lefébure *et al.*, 2006; Costa *et al.*, 2007; da Silva *et al.*, 2011; Raupach and Radulovici, 2015). Raupach and Radulovici (2015) reviewed the literature from the period between 2003 and 2014 and found 164 papers on the DNA barcode of crustaceans, although only six of these studies focused on the order Isopoda. Given that the largest interspecific genetic distances found in decapod crustaceans range from 20.92% (da Silva *et al.*, 2011) to 22.66% (Costa *et al.*, 2007), and those in isopods, from 12.01% to 27.17% (see S1 table of Raupach *et al.*, 2015), the divergence of up to 16.31% observed in the present study between the inland (Santarém) and coastal populations (Afuá, Abaetetuba, Breves and Augusto Corrêa) of *Probopyrus* parasites infesting *M. amazonicum* would appear to be consistent with the presence of two distinct parasite species in the two regions (Fig. 2 and Table 3). By contrast, the level of genetic differentiation (0.41–0.81%) between the coastal *Probopyrus* populations is only slightly higher than that observed in *M. amazonicum* (0.1–0.3%), which indicates the occurrence of gene flow between the populations of both parasites and hosts in this coastal sector.

The morphology of *Probopyrus* has been the subject of considerable controversy in recent decades. In an analysis of the morphology of the cryptoniscus larvae of *Probopyrus bithynis*, *P. pandalicola* and *P. floridensis*, Dale and Anderson (1982) concluded that the morphological characteristics of these larvae were sufficient to confirm the validity of the three species, which, despite their morphological similarities as adults, can be distinguished based on larval morphometric parameters. However, the *Probopyrus* species from the Western Atlantic was synonymized with *P. pandalicola* by Markham (1985) based solely on adult morphology. At the present time, *P. bithynis*, *P. pandalicola* and *P. floridensis* are all considered to be valid by the World Register of Marine Species (WoRMS, 2021).

Based on the available data (Odinetz-Collart, 1988, 1990; Corrêa *et al.*, 2018), it would be reasonable to assume that the species found in the coastal populations is *P. bithynis*, given that the inland population appears to represent a new species of *Probopyrus*. *Probopyrus bithynis* presents differences in the larval morphology that can be used to distinguish this species from other *Probopyrus* taxa (see Dale and Anderson, 1982), in addition to some of the traits of the adult female (see Ribeiro *et al.*, 2019). As larvae were not collected in the present study and the research team has limited practical experience with the morphology of *Probopyrus* species, it was not possible to provide a more conclusive diagnosis of the morphology of the specimens collected here. Given this, it is necessary to describe the morphological characteristics of the material collected in the present study. It is also necessary to expand the number of sampling points, especially in the inland region and to conduct analyses to determine the prevalence and other parameters of the population dynamics of the parasites and their hosts.

Ribeiro *et al.* (2019) recently recorded the occurrence of *P. cf. pandalicola* in the Brazilian state of Bahia and provided insights into the morphology of the females and distinctions from the description of Markham (1985), while also emphasizing the need for molecular data and the analysis of the larval morphology for more reliable identification of the species. Ribeiro *et al.* (2019) also reinforce the need for molecular data to delimit the different species of the group and determine whether *P. pandalicola* is a single, widely dispersed species or a complex of cryptic species. The use of COI sequences was suggested by the authors and the present work has done that by analysing COI sequences obtained from different populations of *Probopyrus* species.

Few molecular data are available on the parasites of the family Bopyridae, and up to now, most studies have sequenced the 18S rDNA gene, which has a much lower mutation rate than COI. Dreyer and Wägele (2001) used the sequences of this gene to evaluate the phylogenetic relationships of the family Bopyridae, while Boyko *et al.* (2013) expanded the dataset to the superfamilies Bopyroidea and Cryptoniscoidea. In the specific case of the genus *Probopyrus*, 18S rDNA sequences are available for *P. pacifiensis* (Dreyer and Wägele, 2001), *P. buitendijki* (Boyko *et al.*, 2013) and *P. pandalicola* (Cho, 2012), in addition to those described in the present study. Wu *et al.* (2015) evaluated the taxonomic resolution of the 18S gene in copepods and determined that the limit between the intra- and inter-specific similarity was close to 100%. In the authors' words this value 'is unrealistic when attempting to achieve a high rate of successful identification, owing to potential PCR or sequencing errors'. From this perspective, the levels of similarity observed in the BLAST search run in the present study (Table 3) are of limited interpretative value, indicating only that the sequences analysed all belong to representatives of the genus *Probopyrus*.

While the 18S rDNA gene is not useful for the molecular identification of species, the COI sequences presented here indicate that this marker can be extremely valuable, not only for the molecular identification of species, but also for the study of the population genetics of *Probopyrus* species, which should contribute to a better understanding of the taxonomy and phylogenetic relationships within the genus. These data will, in turn, provide important insights for the understanding of the parasite–host relationships involving these isopods.

Data

Nucleotide sequences obtained in this paper are available in the GenBank database under accession numbers MZ686260 (*Probopyrus*, 18S rRNA) MZ687054 – MZ687073 (*Probopyrus*, COI) and MZ674502 – MZ674511 (*M. amazonicum*, COI).

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Author contributions. GI conceived and designed the study. CRM and RISP conducted the field sampling. RISP and GI gathered data and wrote the article.

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Conflict of interest. None.

Ethical standards. Not applicable.

References

Bandelt H-J, Forster P and Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37–48.

Beck JT (1980) The effects of an isopod castrator, *Probopyrus pandalicola*, on the sex characters of one of its caridean shrimp hosts, Palaemonetes paludosus. *The Biological Bulletin* **158**, 1–15.

Bialetzki A, Nakatani K, Baumgartner G and Bond-Buckup G (1997) Occurrence of *Macrobrachium amazonicum* (Heller) (Decapoda, Palaemonidae) ln Leopoldo's inlet (Ressaco do Leopoldo), upper Paraná river, Porto Rico, Paraná, Brazil. *Revista Brasileira de Zoologia* **14**, 379–390.

Boyko CB, Moss J, Williams JD and Shields JD (2013) A molecular phylogeny of Bopyroidea and Cryptoniscoidea (crustacea: Isopoda). *Systematics and Biodiversity* **11**, 495–506.

https://doi.org/10.1017/S0031182021001657 Published online by Cambridge University Press

Brinton BA and Curran MC (2015*a*) The effect of temperature on synchronization of brood development of the bopyrid isopod parasite *Probopyrus pandalicola* with molting of its host, the daggerblade grass shrimp Palaemonetes pugio. *The Journal of Parasitology* **101**, 398–404.

Brinton BA and Curran MC (2015*b*) The effects of the parasite *Probopyrus pandalicola* (Packard, 1879) (Isopoda, Bopyridae) on the behavior, transparent camouflage, and predators of *Palaemonetes pugio* Holthuis, 1949 (Decapoda, Palaemonidae). *Crustaceana* **88**, 1265–1281.

Bruford MW, Hanotte O, Brookfield JFY and Burke T (1998) Multilocus and single-locus DNA fingerprinting. In Hoelzel AR (ed.), *Molecular Genetic Analysis of Populations: A Practical Approach*. Oxford, UK: IRL Press, pp. 287–336.

Castro e Silva SMM and Cavalcante PPL (1994) *Perfil do setor lagosteiro nacional*. Brasilia: IBAMA.

Cho S-M (2012) Development of a denaturing high-performance liquid chromatography (DHPLC) assay to detect parasite infection in grass shrimp *Palaemonetes pugio*. *Fisheries and Aquatic Sciences* **15**, 107–115.

Corrêa LL, Sousa EMO, Silva LVF, Adriano EA, Oliveira MSB and Tavares-Dias M (2018) Histopathological alterations in gills of Amazonian shrimp *Macrobrachium amazonicum* parasitized by isopod *Probopyrus bithynis* (Bopyridae). *Diseases of Aquatic Organisms* **129**, 117–122.

Costa FO, deWaard JR, Boutillier J, Ratnasingham S, Dooh RT, Hajibabaei M and Hebert PDN (2007) Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 272–295.

Dale WE and Anderson G (1982) Comparison of morphologies of *Probopyrus bithynis*, *P. floridensis*, and *P. pandalicola* larvae reared in culture (Isopoda, Epicaridea). *Journal of Crustacean Biology* **2**, 392–409.

da Silva JM, Creer S, Dos Santos A, Costa AC, Cunha MR, Costa FO and Carvalho GR (2011) Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca). *Public Library of Science ONE* **6**, e19449.

de Barros MSF, da Silva Neto LS and Calado TCdS (2021) First record of parasitism by *Probopyrus pandalicola* (Isopoda, Bopyridae) on the freshwater prawn *Macrobrachium acanthurus* (Decapoda, Palaemonidae) and ecological interactions. *Journal of Parasitic Diseases* **45**, 273–278.

Dreyer H and Wägele J-W (2001) Parasites of crustaceans (Isopoda: Bopyridae) evolved from fish parasites: molecular and morphological evidence. *Zoology* **103**, 157–178. doi:0944-2006/01/103/03-04-157

Figueroa NJ, Figueroa DF and Hicks D (2020) Phylogeography of *Acartia tonsa* Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus *Acartia* Dana, 1846. *Marine Biodiversity* **50**, 23.

Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Gopalakrishnan A, Raja K, Trilles JP, Rajkumar M, Rahman MM and Saravanakumar A (2017) Bopyrid isopods parasitizing on the cultured fresh water prawn, *Macrobrachium malcolmsonii* in South India. *Journal of Parasitic Diseases* **41**, 93–96.

Hebert PDN, Cywinska A, Ball SL and deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Biological Sciences/The Royal Society* **270**, 313–321.

Iketani G, Pimentel L, Torres EdS, Rêgo PSd and Sampaio I (2021) Mitochondrial heteroplasmy and pseudogenes in the freshwater prawn, *Macrobrachium amazonicum* (Heller, 1862): DNA barcoding and phylogeographic implications. *Mitochondrial DNA Part A* **32**, 1–11.

Kensley B and Walker I (1982) Palaemonid shrimps from the Amazon Basin, Brazil (Crustacea: Decapoda: Natantia). *Smithsonian Contributions to Zoology* **362**, 1–28. doi:10.5479/si.00810282.362

Kumar S, Stecher G and Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Lefébure T, Douady CJ, Gouy M and Gibert J (2006) Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. *Molecular Phylogenetics and Evolution* **40**, 435–447.

Leigh JW and Bryant D (2015) POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**, 1110–1116. doi: 10.1111/2041-210X.12410

Lemos de Castro A (1974) Crustaceos isopodos epicarideos do Brasil. IX. Genero *Probopyus* Giard e Bonnier. *Revista Brasileira de Biologia* **34**, 209–217.

Librado P and Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics (Oxford, England)* **25**, 1451–1452.

Maciel CR and Valenti WC (2009) Biology, fisheries, and aquaculture of the Amazon River Prawn *Macrobrachium amazonicum*: a review. *Nauplius* **17**, 61–79.

Markham JC (1985) A review of the bopyrid isopods infesting caridean shrimps in the northwestern Atlantic Ocean, with special reference to those collected during the Hourglass Cruises in the Gulf of Mexico. *Memoirs of the Hourglass Cruises* **VII**, 1–156.

Masunari S, Da Silva Castagini A and Oliveira E (2000) The population structure of *Probopyrus floridensis* (Isopoda, bopyridae), a parasite of *Macrobrachium potiuna* (Decapoda, palaemonidae) from the Perequê River, Paranaguá basin, southern Brazil. *Crustaceana* **73**, 1095–1108.

Melo GAS (2003) *Manual de Identificação dos Crustacea Decapoda de Água Doce do Brasil*. São Paulo: Loyola.

Odinetz-Collart O (1988) Aspectos ecológicos do camarão *Macrobrachium amazonicum* (Heller, 1862) no baixo Tocatins (PA-Brasil). *Memoria de la Sociedad de Ciencias Naturales La Salle* **48**(Suppl), 341–353.

Odinetz-Collart O (1990) Interactions Entre Le Parasite *Probopyrus bithynis* (Isopoda, Bopyridae) Et L'Un De Ses Hôtes, La Crevette *Macrobrachium Amazonicum* (Decapoda, Palaemonidae). *Crustaceana* **58**, 258–269.

Odinetz-Collart O (1991) Tucurui dam and the population of the prawn *Macrobrachium amazonicum* int the Lower Tocantins (Pa-Brazil): a four-year study. *Archiv fur Hydrobiology* **122**, 213–227.

Odinetz-Collart O and Moreira L (1993) Potencial pesqueiro de *Macrobrachium amazonicum* na Amazônia Central (Ilha do Careiro): variação da abundância e do comprimento. *Amazoniana* **12**, 399–413.

Palumbi SR and Benzie J (1991) Large mitochondrial DNA differences between morphologically similar penaeid shrimp. *Molecular Marine Biology and Biotechnology* **1**, 27–34.

Raupach MJ and Radulovici AE (2015) Looking back on a decade of barcoding crustaceans. *ZooKeys* **2015**, 53–81.

Raupach MJ, Barco A, Steinke D, Beermann J, Laakmann S, Mohrbeck I, Neumann H, Kihara TC, Pointner K, Radulovici A, Segelken-Voigt A, Wesse C and Knebelsberger T (2015) The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. *PLoS One* **10**, e0139421.

Ribeiro FB, Horch AP and Williams JD (2019) New occurrences and host records for two species of parasitic isopods (Isopoda, Cymothoida, Bopyridae) associated with caridean shrimps (Decapoda, Caridea) from Brazil. *Journal of Natural History* **53**, 2437–2447.

Román-Contreras R (2004) The genus *Probopyrus* Giard and Bonnier, 1888 (Crustacea: Isopoda: Bopyridae) in the eastern Pacific with seven new records for Mexico. *Contribuciones al Estudio de los Crustáceos del Pacífico Este* **3**, 153–168.

Saito N, Shokita S and Naruse T (2010) A new species of freshwater bopyrid, *Probopyrus iriomotensis* (Crustacea: Isopoda), parasitizing *Macrobrachium* spp.(Crustacea: Decapoda), from Iriomote Island, Ryukyu Islands, Southwestern Japan. *Species Diversity* **15**, 169–183.

Sampaio CMS, Silva RR, Santos JA and Sales SP (2007) Reproductive cycle of *Macrobrachium amazonicum* females (Crustacea, Palaemonidae). *Brazilian Journal of Biology* **67**, 551–559.

Valencia DM and Campos MR (2007) Freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea: Decapoda: palaemonidae) of Colombia. *Zootaxa* **1456**, 1–44.

Vergamini FG, Pileggi LG and Mantelatto FL (2011) Genetic variability of the Amazon River prawn *Macrobrachium amazonicum* (Decapoda, Caridea, Palaemonidae). *Contributions to Zoology* **80**, 67–83.

Whiting MF, Carpenter JC, Wheeler QD and Wheeler WC (1997) The strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology* **46**, 1–68.

Williams JD and Boyko CB (2012) The global diversity of parasitic isopods associated with crustacean hosts (Isopoda: Bopyroidea and Cryptoniscoidea). *PLoS One* **7**, e35350.

WoRMS Editorial Board (2021) World Register of Marine Species. Available from https://www.marinespecies.org at VLIZ (accessed 2021-07-01). doi:10.14284/170

Wu S, Xiong J and Yu Y (2015) Taxonomic resolutions based on 18S rRNA genes: a case study of subclass Copepoda. *PLoS One* **10**, e0131498.

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