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TWO IN ONE: REPORT OF TWO EPIBIONTS GROUPS ON THE Callinectes bocourti A. MILNE-EDWARDS, 1879 (DECAPODA: PORTUNIDAE) FROM THE AMAZON PROVINCE

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Abstract

Case studies reporting crustaceans acting as basibionts for a wide range of encrusting invertebrates are widely observed in the literature. For swimming crabs of the genus *Callinectes*, these commensal interactions are commonly observed in estuaries and coastal zones worldwide. Despite the broad distribution of *Callinectes bocourti* A. Milne-Edwards, 1879 in Amazon province, the occurrence of epibiosis for this species in the area is rarely reported. Herein, we report the double occurrence of epibiont groups: the mollusks *Ostrea puelchana* d'Orbigny, 1842 and *Sphenia fragilis* (H. Adams & A. Adams, 1854) and the barnacles *Amphibalanus improvisus* (Darwin, 1854) and *Chelonibia testudinaria* (Linnaeus, 1758) from the new host *C. bocourti*, collected from the Amazon province, Brazil. The specimens of *C. bocourti* showing epibionts were collected manually in low tide, with a baited trap called "puçá", in the region of Curuçá River mouth, state of Pará (0°40'3,705"S, 047°54"43,405"W). We observed five specimens of *C. bocourti* showing double cases of epibiosis, composed by mollusks and crustaceans sharing the same area. In addition, here, we expand the range extension of the mollusk *O. puelchana* and we register the presence of the invasive species barnacle *A. improvisus* from the state of Pará, being this last species, reported as parasite adhered in carapace and branchial chambers of the host.

Keywords: Mollusk. Barnacle. Commensal interaction. Non-native species. New host interaction.

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1 Introduction

Epibiosis is the main mechanism used by marine benthic sessile organisms in search of settlement for their development on hard substrates (ABELLÓ et al., 1990; NEGREIROS-FRANSOZO et al., 1995; EMPARANZA et al. 2011). For the attachment in hard bottoms, the individuals present a wide range of morphological and behavioral adaptations, enabling survival in the aquatic environment (WAHL, 2008; DOLDAN et al., 2012). However, in aquatic habitats, hard substrate is limited or it exhibits high competition for space, especially in estuarine and coastal areas, where soft sediments and high levels of sedimentation are common (WAHL, 2008; LIMA et al., 2017).

As adaptation to fixation in environments with high hydrodynamics and sedimentation levels, fouling organisms use the body structures of other groups for adherence (MCDERMOTT, 2006; RIBEIRO et al., 2016). This is widely observed for mollusks and crustaceans (barnacles), and also for other invertebrates and vertebrates (EMPARANZA et al., 2011; GÓNGORA-GÓMEZ et al., 2015). In the commensal interaction, mollusks and barnacles are broadly reported as epibionts in several invertebrate groups such as crabs, sponges, echinoderms, corals, ascidians and in vertebrates adhered in whales, dolphins, manatees and turtles (LEWIS, 1978; LIMA et al., 2017).

The commercial swimming crabs of the family Portunidae Rafinesque, 1815 are commonly found in estuarine and coastal zones around the world, being a source of food and livelihood for several families at different levels of fishing activity (MORUF; LAWAL-ARE, 2017; CORDEIRO et al., 2021). The presence of epibiosis in species of the genus *Callinectes* Stimpson, 1860 was reported by Ganon and Wheatl (1992), Negreiros-Fransozo et al. (1995), Key Jr. et al. (1999), Santos and Bueno (2002) and Mantelatto et al. (2003), especially with barnacles adhered on carapace, chelipeds and ambulatory legs. According to Marin and Belluga (2005), Lima et al. (2017) and Alves-Júnior et al. (2021), this fixation favors the supply of food for the epibiont, based on the fact that the basibiont is vagile, on the other hand, for the host, it can be harmful because of the extra weight and friction in the water.

According to Farrapeira (2010), one of most representative epibiont barnacle in literature is *Chelonibia testudinaria* (Linnaeus, 1758), followed by the invasive species *Amphibalanus improvisus* (Darwin, 1854), which is reported along the Brazilian waters fixed in a wide range of organisms, covering invertebrates and vertebrates.

In addition, the bivalve mollusk *Ostrea puelchana* d'Orbigny, 1842 was observed as epibiont in some crustaceans such as *Callinectes exasperatus* (Gerstaecker, 1856) and *Eriphia gonagra* (Fabricius, 1781) (LIMA et al. 2011; ALVES-JÚNIOR et al., 2021). However, the mollusk species *Sphenia fragilis* (H. Adams & A. Adams, 1854) is not reported in the literature as epibiont in decapods, being recorded, in Brazil, from Pará to Paraná, on mussel and barnacle beds along the intertidal zone. Herein, we report the double occurrence of epibionts groups; the barnacles *C. testudinaria* and *A. improvisus* and the mollusks *O. puelchana* and *S. fragilis* in the new host *Callinectes bocourti* A. Milne-Edwards, 1879, collected from the Amazon province, Brazil; additionally, extending the range extension of one barnacle and two mollusk species for the northern Brazil.

2 Material and Methods

Study Area

The specimens of *C. bocourti* and their epibionts were manually collected in low tide, with a baited trap called "puçá", in Santa Maria River, Iriritеua comunity, located in the municipality of Curuçá, state of Pará (0°40'3,705"S, 047°54"43,405"W) (Figure 1), between January and May 2023. The region is a fluvial-marine plain, in the region of Curuçá River mouth, influenced by Curuçá, Mocajuba and Maripanema Rivers and subjected to sedimentation process and macro-tidal range. The collection was authorized by the "*Sistema de Autorização e Informação em Biodiversidade*" (SISBIO Number 44915-3), "*Instituto Chico Mendes de Conservação da Biodiversidade*" (ICMBio), Ministry of Environment, from the Federative Republic of Brazil.

Laboratory Procedures

After collected, the individuals showing epibiosis were sorted out, frozen and carried to the Carcinology Laboratory of *Universidade Federal Rural da Amazônia* (UFRA), where they were photographed, measured in carapace width (CW), carapace length (CL) and wet weight (W) (swimming crabs), largest and smallest diameter (Ø) (barnacles); and the mollusks the valve length (VL). The total area of the carapace and chelipeds was counted as 100%, and the area occupied in centimeters by each epibiont was evaluated and assigned a percentage of host occupancy following the methodology of Lima et al. (2017). Species identification followed Melo (1996) for the swimming crabs, while the barnacles identification followed Young (1999); and the mollusk species according to Rios (2009). The specimens were then fixed in 70% ethanol and deposited at the Carcinological Collection of Carcinology Laboratory of UFRA.

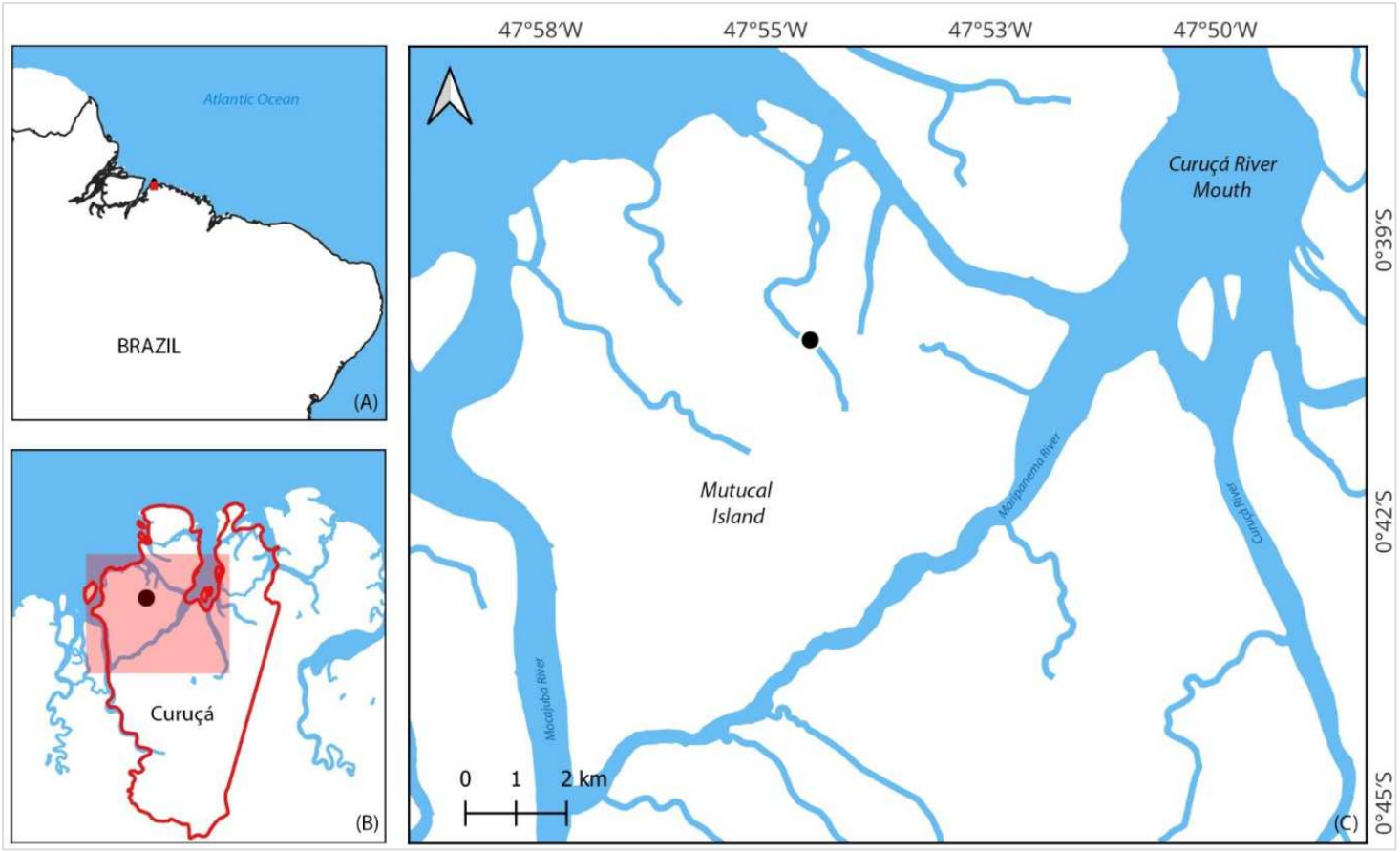

Figure 1. Map of the study area, **A.** Northern Brazilian coast. **B.** Municipality of Curuçá, state of Pará. **C.** Sampling point (black circle) in Santa Maria River.

3 Results and Discussion

We collected five specimens of *C. bocourti* (CL: 5.81-6.30 cm; CW 11.5 - 14.12 cm; W 87.2 - 216.76 g) showing epibiosis composed by two barnacles species: *C. testudinaria* (Ø: 1.34 - 3.16 cm) and *A. improvisus* (Ø: 0.60 - 0.88 cm). We observed 73 individuals of *C. testudinaria*, covering ~47% of carapace and ~18% of chelipeds (Figures 2A-B; 3A-B; 4 F-G-H-I-J); 11 individuals of *A. improvisus*, covering ~16% of the carapace area, and ~53% of the branchial chambers (Figures 2C - 3C-D; 4 A-B-C-D-E). In addition, we observed 4 specimens of *O. puelchana* (VL: 1.12 - 2.44 cm) adhered only in chelipeds, represented by ~28% of the chelipeds region (Figures 2B-D), while 9 specimens of the bivalve *S. fragilis* (VL: 0.69 - 0.97 cm) were represented adhered only to the carapace, with ~3% of total area occupied (Figure 2E).

The presence of epibiosis in swimming crabs may be associated with its free horizontal and vertical migration through the water column and in non-consolidated substrata, which turns the epibionts able to spread out in the aquatic environment (KEY Jr. et al. 2021; ALVES-JÚNIOR et al., 2022).

The occurrence of epibiosis in other semi terrestrial crustaceans, as *Ucides cordatus* (Linnaeus, 1763), *E. gonagra* or fiddler crabs of the genera *Uca* Leach, 1814, *Minuca* Bott, 1953 and *Leptuca* Bott, 1973 from Brazil are rare or non-existent, due the exposition of epibiont to air, high temperatures, sun and desiccation, which can lead the epibiont to death (GILI et al., 1993; ALVES-JÚNIOR et al., 2021).

The occurrence of epibionts in swimming crabs as *C. bocourti* may expand the geographic distribution for these sessile animals. They occupy different environments, increasing the species' occurrence area and distribution along estuarine and marine habitats; their larvae have a greater reach and greater food supply along different habitats (LIMA et al., 2017; ALVES-JÚNIOR et al., 2021; 2022; KEY Jr. et al. 2021). Another positive feature of epibiosis is the ability of the sessile epibiont to escape from predation, for the case of the barnacles and mollusks which are predated by puffer fish, budion, octopus, sea stars or other crabs (KEY Jr. et al. 1997; O'CONNOR; NEWMAN, 2001).

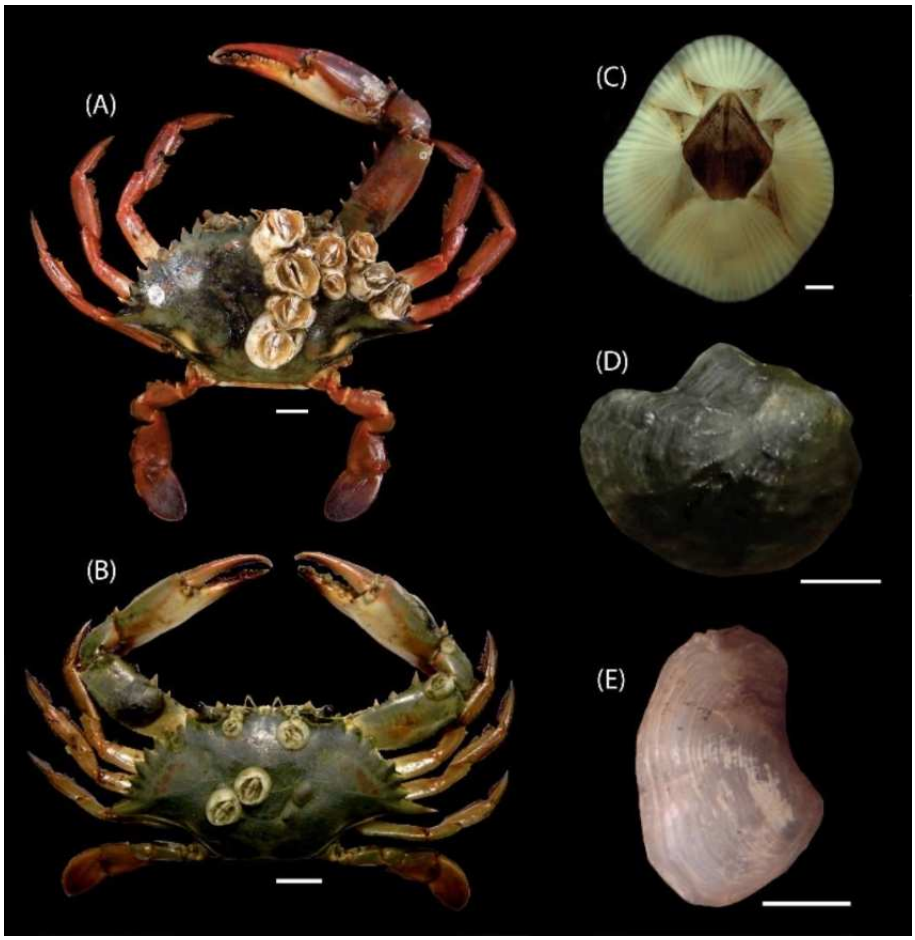

Figure 2. A-B. The swimming crab *Callinectes bocourti* A. Milne-Edwards, 1879 showing epibiosis by *Chelonibia testudinaria* (Linnaeus, 1758); **B.** *Ostrea puelchana* d'Orbigny, 1842 adhered to the left cheliped of *C. bocourti*. **C.** *Amphibalanus improvisus* (Darwin, 1854) in highlighted view. **D.** *Ostrea puelchana* in highlighted view. **E.** *Sphenia fragilis* (H. Adams & A. Adams, 1854) in highlighted view. Scale bars= swimming crabs = 20 mm; barnacle = 1 mm; mollusks = 5 mm.

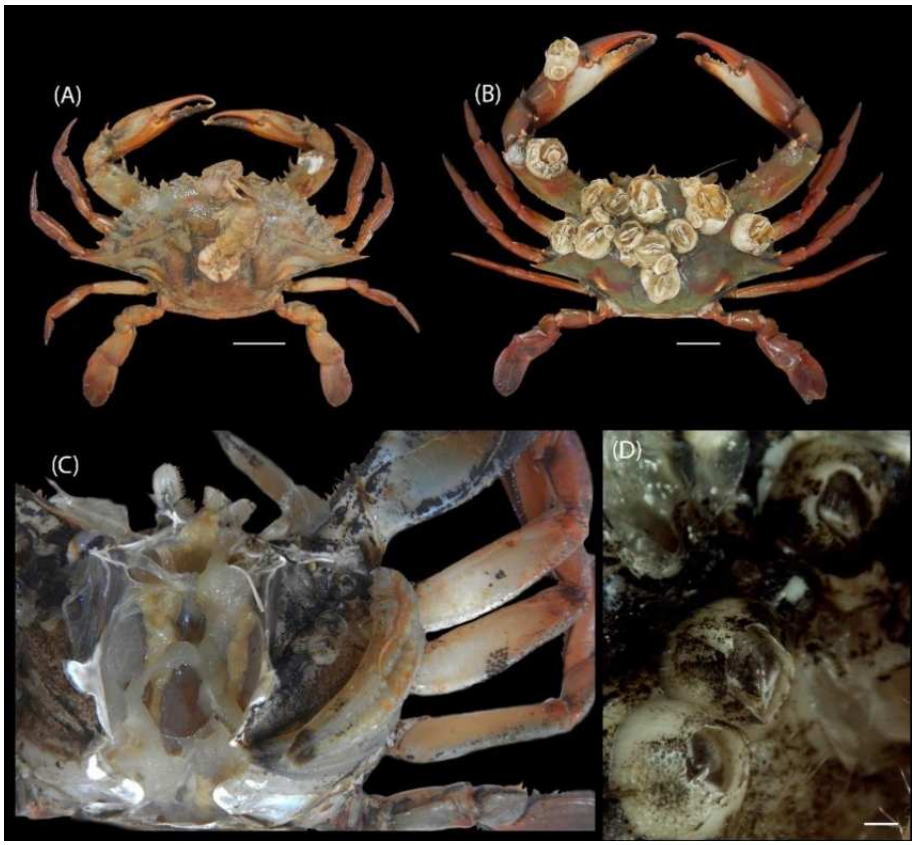

Figure 3. A. The swimming crab *Callinectes bocourti* A. Milne-Edwards, 1879 showing double epibiosis promoted by the barnacle *Amphibalanus improvisus* (Darwin, 1854) and the mollusk *Ostrea puelchana* d'Orbigny, 1842. B. High infestation by *Chelonibia testudinaria* (Linnaeus, 1758) in *C. bocourti*. C. Branchial chamber of *C. bocourti* indicating *A. improvisus* as endoparasite. D. *Amphibalanus improvisus* as endoparasite in highlighted view. Scale bars = swimming crabs = 20 mm.

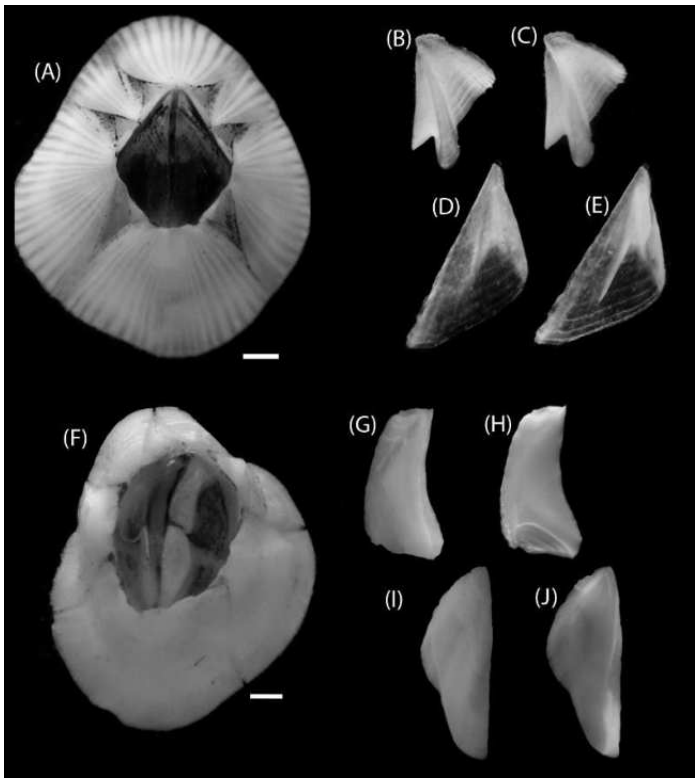

Figure 4. A. *Amphibalanus improvisus* (Darwin, 1854) view of the shell, top view. B. Tergum, exterior view. C. Tergum, internal view. D. Scutum, exterior view. E. Scutum, internal view. F. *Chelonibia testudinaria* (Linnaeus 1758), view of the shell, top view. G. Tergum, exterior view. H. Tergum, internal view. I. Scutum, exterior view. J. Scutum, internal view. Scale = 1 mm.

As previously observed, the crustacean carapace acts as a hard substrata in aquatic environments, favoring the presence and attachment of epibionts, however, the crustacean species have mechanisms to avoid the adhesion, such as carapace cleaning using oral appendages and chelipeds or burying in the sediment or galleries (BAUER 1989, BECKER; WAHL, 1996; COSTA et al., 2010). In studies provided by Abelló et al. (1990), Lima et al. (2017) and Alves-Júnior et al. (2021; 2022), the crustacean carapace acts only as a temporary substrata for the fixation of epibionts, due the process of ecdysis (molting), in which the old carapace is discarded to the environment carrying the epibionts, which are often destroyed by the action of waves, currents, sedimentation or predators. In specimens of *C. testudinaria* observed herein (Figure 2A), the largest sizes can be related to the high growth rates promoted by the epibionts, specifically to rapidly reach the reproductive period and spread of the planktonic larvae to different regions (due the mobility of swimming crab), before the molting period of the basibiont.

The presence of *A. improvisus* adhered in branchial chambers may be considered a parasitism, due to the damage caused by the fixation of barnacle species and its growth among the gills (Figures 3C-D). The adhesion in this area may be associated to high level of gasses and the water flux promoted by the respiration of basibiont, however, this action damages the gill chamber, reducing the basibiont breathing rate according to the growth of the barnacle (GANON; WHEATLY, 1992; POULIN, 2007).

The presence of *O. puelchana* in the northern region of Brazil (state of Pará), represents the northernmost record of this species, which has been reported in Rio Grande do Sul, Paraná, Espírito Santo, Pernambuco and Paraíba. The bivalve species *Sphenia fragilis* was reported in a few localities such as Pará, Rio Grande do Sul, Santa Catarina and Paraná (RIOS, 2009).

For the barnacle species *C. testudinaria* the report from the Amazon province was performed by Farrapeira (2010) and Alves-Júnior et al. (2022), adhered in a large range of epibionts as swimming crabs, lobsters, rocks forming barnacle beds, mangroves roots and piers.

The high risk to native Amazon biodiversity is the presence in the state of Pará of invasive species *A. improvisus*, which was reported as non-native species from Brazilian waters by Farrapeira (2006), in samples performed along the state of Pernambuco, and in additional reports covering the states of Maranhão to Pernambuco by Farrapeira (2010), adhered in a wide range of crustacean hosts as prawns and crabs, especially in the swimming crabs *Callinectes bocourti*, *C. danae*, *C. exasperatus* and *C. larvatus* (see complete list in Farrapeira, 2010). The species *A. improvisus* is native to the Indo-Pacific regions, being transported to Brazil through ship's hulls and/or ballast water.

The report of the barnacle *A. improvisus* in the Amazon province is an alert for the bioinvasion in the region, being necessary studies for assessing the invasion rate, and the environmental and trophic damage to the Amazon natural environments.

4 Conclusions

We highlight the occurrence of new epibionts for the swimming crab *C. bocourti*, through adhesion of the barnacles *A. improvisus* and *C. testudinaria* (indicating the occurrence of *A. improvisus* in the gill chamber of *C. bocourti*); and the mollusks species *O. puelchana* and *S. fragilis*. Additionally, warning about the risk of invasion of the barnacle *A. improvisus* in the Amazon province.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

FAAJ, DEGM and BSM conceived the research ideas and designed the study and writing the manuscript; JAF, AMCK and IHAC species identification, first draft of this manuscript and revisions along the main text.

DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this study.

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