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# Confirmed by integrative taxonomy first and unusual occurrence of the exotic shrimp *Neocaridina davidi* (Caridea: Atyidae) in Brazil

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## ABSTRACT

A shrimp specimen of the genus *Neocaridina* Kubo 1938, originally from Asia, was collected for the first time in a natural marine environment in Pernambuco, Brazil. This represents the first record of this genus in the South American continent. Morphological characteristics and mitochondrial and nuclear DNA sequences (COI, 16S, 18S, and H3) of the specimen were compared with other species of *Neocaridina*, enabling its identification as *Neocaridina davidi*. Finding this freshwater species native to mainland China in a marine environment was surprising. Based on prior findings in other locations, the most likely hypothesis for this unusual occurrence is that the shrimp may have been released intentionally or unintentionally into the environment by an aquarium hobbyist.

**Keywords:** Alien species; Aquarism; Decapoda; Ornamental invertebrates; South Atlantic.

## Confirmada pela taxonomia integrativa a primeira e incomum ocorrência do camarão exótico *Neocaridina davidi* (Caridea: Atyidae) no Brasil

## Resumo

Um exemplar de camarão do gênero *Neocaridina* Kubo 1938, originário da Ásia, foi coletado pela primeira vez em ambiente marinho natural em Pernambuco, Brasil. Ele representa o primeiro registro desse gênero no continente sul-americano. As características morfológicas e as sequências de DNA mitocondrial e nuclear (COI, 16S, 18S e H3) do espécime foram comparadas com as de outras espécies de *Neocaridina*, possibilitando sua identificação como *Neocaridina davidi*. Foi surpreendente encontrar essa espécie de água doce, nativa da China continental, num ambiente marinho. Com base em descobertas anteriores em outros locais, a hipótese mais provável para essa ocorrência incomum é que o camarão pode ter sido solto intencionalmente, ou não no meio ambiente por um aquarista.

**Palavras-chave:** Espécies alienígenas; Aquarismo; Decápode; Invertebrados ornamentais; Atlântico Sul.

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## INTRODUCTION

Bioinvasion is considered the second major cause of decreased biodiversity, which can lead to changes in the structure and function of ecosystems, increasing biotic homogenization (Coradin and Tortato, 2006). Some species can negatively impact their new ecosystems by competing for resources, decreasing biodiversity, altering habitats, and acting as vectors of pathogens and hosts of ectosymbiotic species, which can transfer their hosts to native species (Gherardi, 2007; Emde et al., 2016; Patoka et al., 2016; Ohtaka et al., 2017). Among the leading causes of bioinvasion, there are the actions of human beings as protagonists in the increase of global species exchange (Gozlan et al., 2010; Havel et al., 2015). Introductions can be unintentional, including transport through shipping channels and ballast water (Nentwig, 2007), or intentional, such as trade in aquarium animals, which have contributed to introducing exotic species around the world (Heerbrandt and Lin, 2006; Lipták and Vitázková, 2015; Patoka et al., 2016; Chucholl and Wendler, 2017).

As a result, species of commercial interest have appeared in several non-native regions. Many of these species have become increasingly popular in the aquarium industry (Patoka et al., 2016; Levitt-Barmats et al., 2019). Freshwater shrimp of the genus *Neocaridina* Kubo 1938, native to China, Japan, Korea, Vietnam, and Taiwan, is one such example (Levitt-Barmats et al., 2019). In particular, *Neocaridina davidi* (Bouvier, 1904), whose taxonomy is complex, has been documented in the literature under several synonyms and divisions of species into subspecies (Yixiong, 1996; Liang, 2002, 2004; Shih and Yixiong, 2007), and remains without a precise definition.

*Neocaridina davidi* belongs to the family Atyidae (De Haan, 1849), the largest family of the infraorder Caridea that inhabit freshwater bodies, from fast-moving mountain streams to slow and oligohaline waters (De Grave et al., 2008; De Grave and Fransen, 2011; Schoolmann and Arndt, 2018). A small proportion of this family's members are amphidromous (Dudgeon, 1985; Heerbrandt and Lin, 2006; Vogt, 2013), but most are confined to freshwater and characterized by abbreviated or direct development by suppression of larva. This is especially evident in the genera *Caridina* (H. Milne Edwards, 1837), and *Neocaridina* (Shih and Yixiong, 2007; Lai and Shy, 2009; Karge and Klotz, 2013; Vogt, 2013; Pantaleão et al., 2015). *Neocaridina davidi*, popularly known as red cherry shrimp or RCS, has a small size, and bright coloration that includes different shades of red, yellow, and blue, making it attractive among freshwater aquarists (Tomas et al., 2020). This species was previously reported as invasive in the United States of America, Japan, Israel, and Europe (Klotz et al., 2013; Jabłońska et al., 2018; Levitt-Barmats et al., 2019; Weiperth et al., 2019).

The objective of the present study was to report the unprecedented and unusual record to *N. davidi* collected on the coast of the Brazilian state of Pernambuco, confirmed by morphological and molecular analyses.

## MATERIALS AND METHODS

On February 14, 2022, during exploratory sampling activities for different purposes of the Biota-FAPESP INTERCRUSTA Thematic Project, three trawls were carried out along of Candeias beach, in Jaboatão dos Guararapes, PE (08°12'37"S; 34°54 '12"W) (Fig. 1), using a shrimp boat with double bottom trawls. Along with banks of filamentous algae, one live specimen of a different morphology was sampled, frozen, and conserved in 100% ethyl alcohol. We followed the methods of Englund and Yixiong (1999) and Klotz et al. (2013) for identification and measured the carapace length with a caliper (0.01 mm). Specimens from Brazil were obtained and analyzed under federal permits (permanent licenses for FLM and FJZ for collection of zoological material 11777-2 and 34587 MMA/ICMBio/SISBIO, respectively), and for genetic access SISGEN CEA7CD5 and AE6029A). The specimen was deposited in the Crustacean Collection of the Biology Department of the Faculty of Philosophy, Sciences, and Letters of Ribeirão Preto, Universidade de São Paulo: CCDB 6963.

### *Molecular protocols and analysis*

Molecular procedures (extraction, amplification, purification, and sequencing) followed the protocols described by Mantelatto et al. (2018). DNA was extracted from the specimen's abdominal muscle tissue for molecular identification using the Chelating Ion Exchange Resin method (Chelex VR 100) (Estoup et al., 1996). The base pair region of the mitochondrial 16S rRNA (Schubart et al., 2000) and cytochrome c oxidase subunit 1 (COI) (Schubart and Huber, 2006), and nuclear 18S (Medlin et al., 1988) and H3 (Colgan et al., 1998), gene of the extracted DNA, were amplified using polymerase chain reaction (Sambrook et al., 1989).

Sequences were compared to the GenBank genomic database using the basic local alignment search tool (BLAST), implemented on the National Center for Biotechnology Information website. To verify identification, we entered each sequence using nucleotide BLAST (BLASTn) and looked for the most similar sequences in the general sequence database.

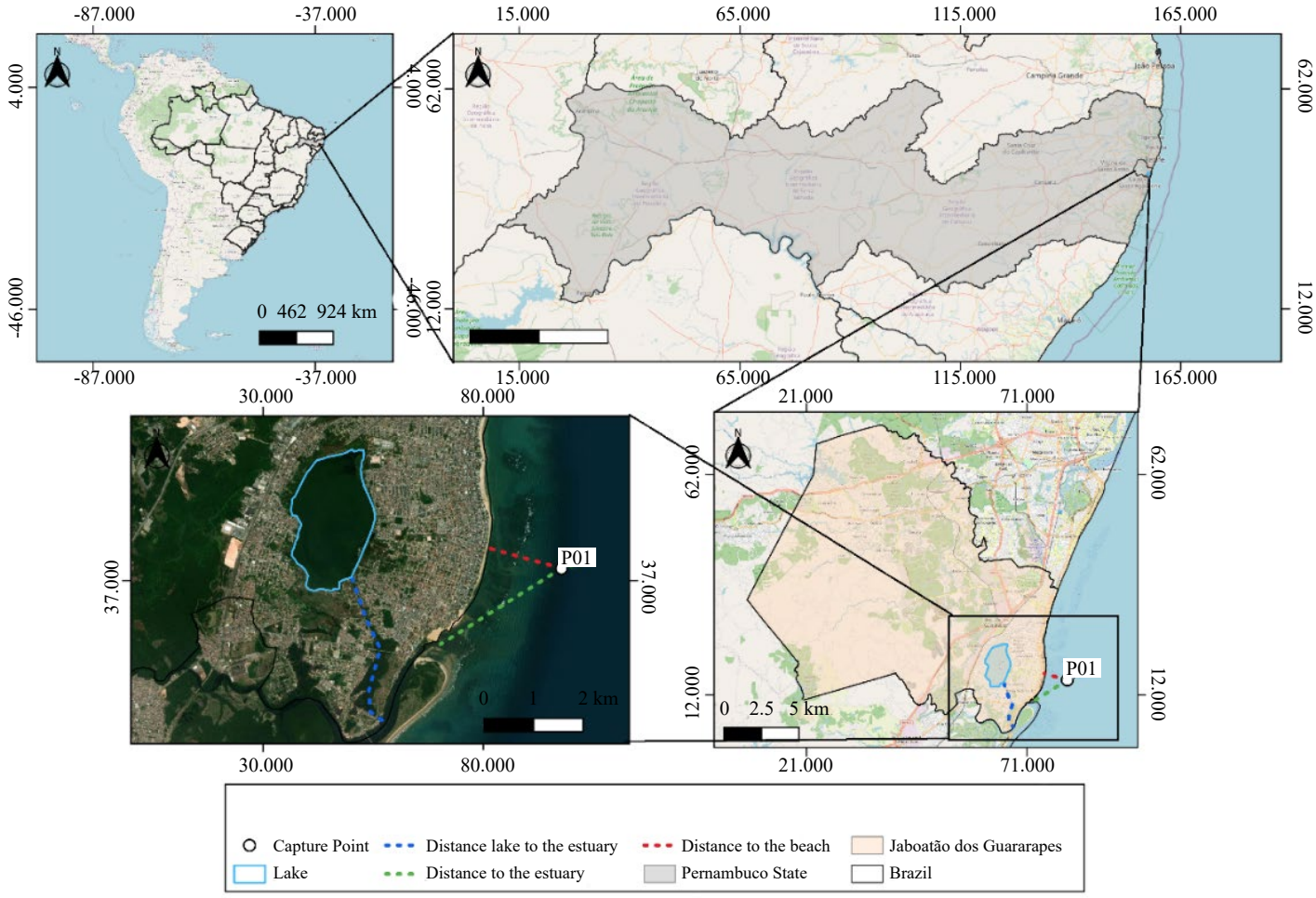


**Figure 1.** Collection site (P01) and the nearest freshwater bodies in Jaboatão dos Guararapes, Pernambuco, Brazil.

After completing the search, we confirmed that the results were organized by the closest identity.

Maximum likelihood phylogenetic analyses were performed using the IQ-TREE program (Miller et al., 2010) with the COI gene due to the greater number of sequences available from GenBank for comparison. Other molecular markers (16S, 18S, and H3) were used to confirm the specimen's identity and increase the number of sequences in the GenBank database, helping future research. The evolutionary model that best fit the data was determined by the IQ-TREE according to the Bayesian information criterion (Luo et al., 2010) and used for tree inference. Branch support was evaluated by ultrafast bootstrap with 1,000 replicates. The choice of the outgroup (represented by *Caridina formosae* Hung et al., 1993) was conducted according to the phylogeny proposed by Shih and Cai (2007). To confirm the similarity between the sequences generated and those available in GenBank, distance matrix analyses were performed in the MEGA X software (Kumar et al., 2018).

## RESULTS

The results of our robust morphological and genetic analyses confirmed the specimen's identification as *N. davidi* living in the marine environment of the Brazilian territory (Fig. 2). Our phylogenetic analysis from the COI marker revealed the positioning of our specimen to be close (genetic distance = 0,00%) to *N. davidi* specimens from Poland and Canada with high branch support. Unfortunately, this is the only marker available for specimens from other localities in the GenBank data base that allowed a phylogenetic comparison. The sequences accession number (GenBank) generated from the adult female specimen (CCDB 6963) are: COI (PP718677), 16S (PP716860), 18S (PP716861), and H3 (SUB14399993).

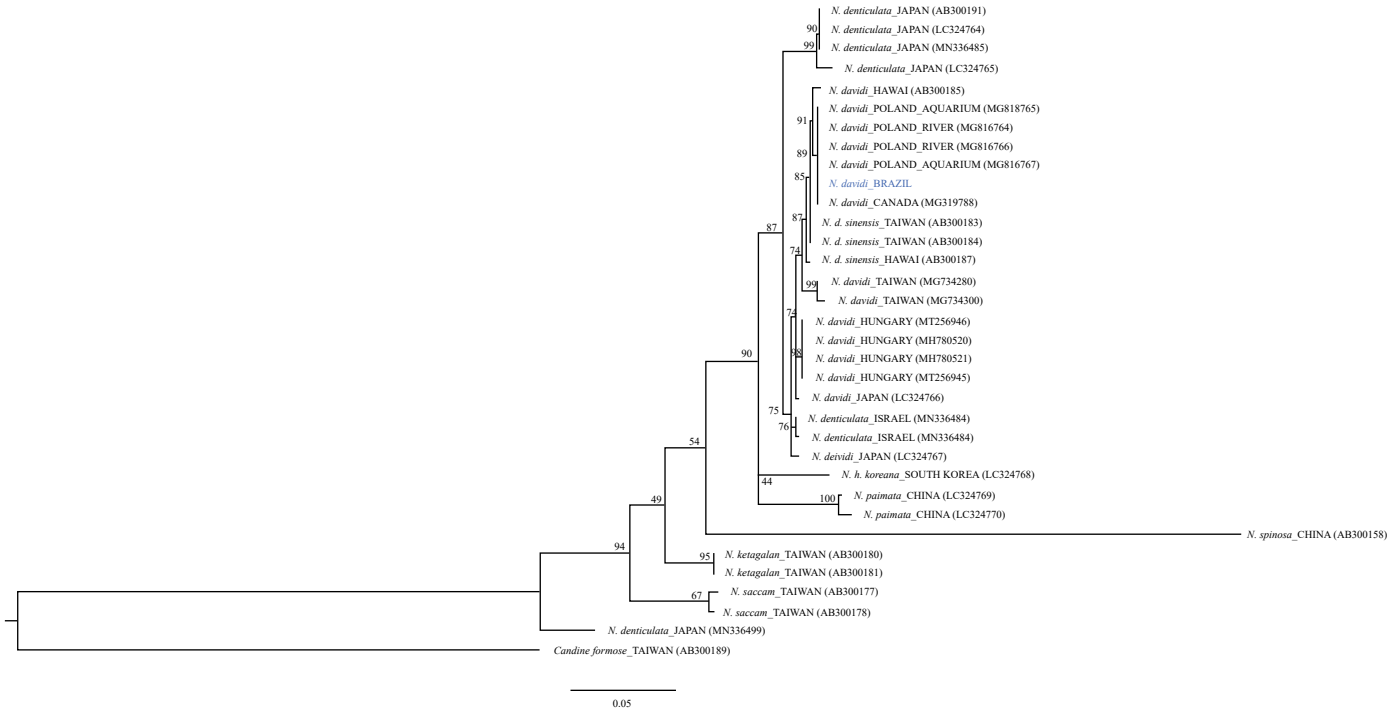


**Figure 2.** Maximum likelihood phylogenetic tree for the COI gene of species of the genus *Neocaridina* distributed in the world. Bootstrap values are indicated on the nodes.

## Systematics

Infraorder Caridea Dana, 1852

Superfamily Atyoidea De Haan, 1849

Family Atyidae De Haan, 1849

*Neocaridina* Kubo, 1938

*Neocaridina davidi* (Bouvier, 1904) (Figs. 1–3) (Fig. 3)

Material examined. 1 ♀, CL 6.52 mm, CCDB 6963, Brazil, Pernambuco, Jaboatão dos Guararapes, 08°12'37"S; 34°54'12"W, colls. Bochini, G.L.; Rios, A.S; Teles, J.N.; Garcia-Bento, M.A. & F.J. Zara, 14.ii.2022.

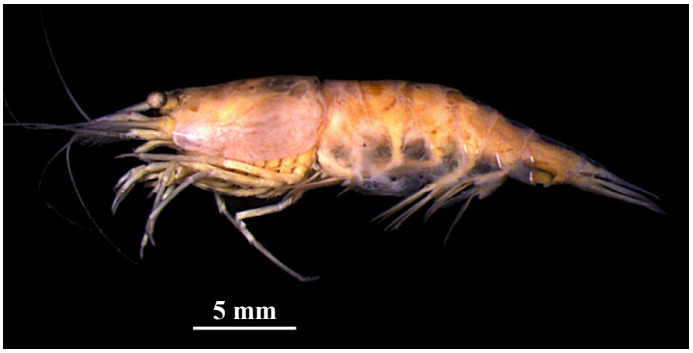


**Figure 3.** *Neocaridina davidi* (Bouvier, 1904) collected in Jaboatão dos Guararapes, Pernambuco, Brazil. Adult female specimen (CCDB 6963). Source: G. L. Bochini (2022).

## DISCUSSION

This work is the first report of the occurrence of an exotic shrimp, *N. davidi*, in South America in a natural and coastal marine environment. This finding in a marine environment is unusual and unprecedented as the specimen is typically a freshwater species. Depending on the recording area (Fig. 1), it can be conjectured that the species may have originated from the lagoon environment, which is 3.16 km away from the sampled site, or from the estuary, which is located 1.58 km away. Unfortunately, the opportunity to conduct samplings in these nearby places to assess the presence of other specimens has not yet arisen. The nearby lagoon system poses challenges in terms of access and makes trawling practically impossible. Additionally, the belief that freshwater habitats could serve as a source for the spread of *N. davidi* in coastal environments is not held. This condition is understood to be more related to a physiological or osmoregulatory mechanism rather than being a step to reach marine environments. This rationale is the basis for advocating future experiments in this and other fields in the discussion, as recently carried out by Carvalho-Batista et al. (2023), that investigated the preference of *N. davidi* for types of shelter and concluded that the java moss *Vesicularia sp*. was the most used shelter, supporting our finding of the specimens inhabiting banks of filamentous algae.

To evaluate the potential existence of these populations, the sporadic recording of this species encourages future monitoring to assess the establishment of this shrimp variety in natural environments, particularly in coastal inland waters and those having connectivity with the marine environment. Because it is a globally invasive species, special attention to protecting the native biodiversity of Brazil should be made. The fact that only one specimen was sampled does not make the finding any less worrying, as similar results have already been reported, mainly in relation to invasive decapod species, when the first records in Brazilian waters occurred with few individuals (Mantelatto and Dias, 1999; Almeida et al., 2012; Tamburus et al., 2019). Furthermore, based on our past experience in the field and using previous literature on the survey of the region's decapod fauna, we are convinced of this record, mainly due to the use of the precise analyses that were carried out.

Concern about the invasion of *N. davidi* and other shrimps with similar characteristics in South America has been described previously (Pantaleão et al., 2017). It was demonstrated that the species has prolific reproduction capabilities, allowing it to reach similar population densities as in native areas (Nur and Christianus, 2013). In addition, the small size of this species makes it easily confused with the juvenile forms of other shrimp and challenging to identify in nature to accomplish control. Besides, although the species has a life cycle entirely in freshwater, it is reportedly also tolerant in high salinity (Nur and Christianus, 2013), and investigators have cultivated newly hatched larvae in a slightly saline (5–10 ppt) environment. These results detailing the plasticity and survival of larvae may indicate their ability to live in a saline environment, at least during the larval phase. It is known that this species is commercialized in Brazil and Argentina (Pantaleão et al., 2017), and the possibility of intentional or unintentional disposal in the natural environment is almost inevitable.

Among invasive species, freshwater crustaceans represent a growing portion compared to marine species (Patoka et al., 2016). Introducing exotic species can negatively impact the host community, directly, or indirectly, affecting the functioning of the ecosystem and biodiversity, even without an established population, as they are potential transmitters of pathogens (Simberloff et al., 2013). Thus, the concern is not restricted only to the competitive potential with native species, but also because *Neocaridina* can be accompanied by symbiotic worms (Niwa et al., 2005; Niwa and Ohtaka, 2006; Klotz et al., 2013), which increases the potential for these worms to be introduced into Brazilian aquatic systems. There are reports that the introduction of the parasitic fungus *Microsporidia* in Germany occurred through *N. davidi*. This fungus preferentially lives in brackish and marine waters and has become a critical threat to the shrimp farming industry, particularly in Southeast Asia (Simberloff et al., 2013; Kim et al., 2022; Schneider et al., 2022).

In the case of newly introduced North American crayfish, these species are carriers of the crayfish pest, which infects European crayfish species and negatively influences indigenous European ecosystems (Aquiloni et al., 2011; Pârvulescu et al., 2012). In the United States of America and Canada, the invasion of the crustacean *Mysida*, popularly known as skunk shrimp, altered different freshwater ecosystems in both countries (Spencer et al., 1991).

The most plausible hypothesis explaining the introduction of the unusual species into Brazilian waters is via trade in ornamental animals. There were strong indications obtained in our phylogenetic analysis that the introduction route is through organisms from Europe. Trade in aquarium ornamentals in Turkey comes with the important risk of invasion mechanisms in the post-import stages (Turkmen and Karadal, 2012). *N. davidi* is also considered invasive in other regions, such as Chinese waters, where they replace other shrimp species.

On the coast of Pernambuco, several exotic marine species have already been reported. The caridean shrimp, *Athanas dimorphus* (Ortmann, 1894), originating from the Indo-Pacific, has the Porto de Suape as a possible introduction area (Almeida et al., 2015). Another exotic crustacean species found in the region is the Indo-Pacific crab *Charybdis hellerii* (Milne-Edwards, 1867) (Coelho and Santos, 2003). With introduction routes already mapped to Brazil, this later is considered invasive, with well-established populations in almost all of Brazil (Dineen et al., 2001; Mantelatto and Garcia, 2001; Negri et al., 2018).

The record of yet another exotic species in Brazilian waters is cause for alarm. Considering the capacity of *N. davidi* for reproduction and dispersal, concern lies in the potential for an increase in abundance in a short time and damage to the native fauna that is still obscure and difficult to predict. It is hoped that this work will inspire further research to find the focus of the dispersion of this species in the Brazilian territory to facilitate mitigation measures. In addition, future investigation on osmoregulatory mechanisms may elucidate the physiological capacity of adults of this species to occupy marine environments.

## CONFLICT OF INTEREST

Nothing to declare.

## DATA AVAILABILITY STATEMENT

All research data/codes are available in the text. Generated DNA sequences have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and their accession number is available throughout the text.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Bochini GL; Rios AS; Teles JN; Zara FJ; Mantelatto FL; **Data curation:** Bochini GL; Rios AS; Teles JN; Zara FJ; Mantelatto FL; **Formal analysis:** Bochini GL; **Writing – original draft:** Bochini GL; **Writing – review & editing:** Bochini GL; Rios AS; Teles JN; Zara FJ; Mantelatto FL; **Final approval:** Bochini GL; Rios AS; Teles JN; Zara FJ; Mantelatto FL.

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