

First record of *Kryptolebias hermaphroditus* Costa, 2011 (Cyprinodontiformes: Rivulidae) in the extreme north Atlantic Forest mangroves, Rio Grande do Norte state, Brazil

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Abstract: This study records the first occurrence of *Kryptolebias hermaphroditus* Costa, 2011 in canals or perennial salty water pools in two mangroves of the Rio Grande do Norte state, in the estuaries of Ceará-Mirim River and Curimataú River. Due to its hermaphroditism and self-fertilization, a unique feature among vertebrates, this species is very important for many biological areas. *Kryptolebias hermaphroditus* depends on favorable conditions for their development, therefore, human impacts such as shrimp farming and removal of native vegetation from mangroves may affect populations of this species in northeastern Brazil.

Key words: Mid-Northeastern Caatinga Ecoregion, estuary, coastal basin

Kryptolebias Costa, 2004 correspond to a monophyletic genus (Vermeulen and Hrbek 2005), composed by seven species of non-annual rivulids, inhabiting an area that extends from Florida, EUA, passing through Central America until South American coast (Taylor 2012), recorded up to São Paulo in Brazil (Costa 2011). Although the genus exhibits a confusing taxonomy, which resulted in the recent change of names of two species, *K. caudomarginatus* (Seegers, 1984) as a synonym of *K. ocellatus* (Hensel, 1868); and the species previously identified as *K. ocellatus* was redescribed as *K. hermaphroditus* Costa, 2011 (Costa 2011). Among the genus, the *K. marmoratus* group is a well-supported clade consisting of three valid nominal species, *K. marmoratus* (Poey, 1880), *K. ocellatus* and *K. hermaphroditus* (Costa 2011). These species inhabit brackish water channels associated with mangroves (Costa 2011), where they could become stuck to plant stems during low tide (Costa 2009), due to cutaneous respiration when out of water (Grizzle and Thiyagarajah

1987). Among these, *K. marmoratus* and *K. hermaphroditus* are characterized by having a unique reproductive system: hermaphroditism with self-fertilization, assigned only to these two sister species of vertebrates. While *K. marmoratus* populations are composed of hermaphrodites and few males, with distribution from Southeastern USA to the Guianas, *K. hermaphroditus* is only known from southeastern Brazil populations, which functional females are not known and males are absent or very rare (Costa 2006). However, reports on these species east to Venezuela are scarce (Taylor 2012), including north and northeastern Brazil.

During recent collections, between 2011 and 2014, in coastal basins of northeastern Brazil, the occurrence of *K. hermaphroditus* (Figure 1) was recorded in the estuaries of Ceará-Mirim River (UFRN 2539, five hermaphrodites, 26.6–32.3 mm SL, 05°40'25.9"S, 035°14'14.5"W, 9 April 2014) and Curimataú River (UFRN 2542, five hermaphrodites, 25.4–36.8 mm SL, 06°21'32.5" S, 035°01'54.8" W, 9 April



Figure 1. *Kryptolebias hermaphroditus* from the Curimataú River mangrove, Baía Formosa municipality, Rio Grande do Norte state. Photo by Marcelo Brito.

Table 1. Records of *Kryptolebias hermaphroditus* in Brazilian coastal basin estuaries according to this study, online museum specimens, and literature (ordered from north to south). Acronyms: CZNC – Coleção Zoológica Norte Capixaba, MBML – Museu de Biologia Professor Mello Leitão, MCP-Peixes – Universidade Católica do Rio Grande do Sul, MZUSP – Museu de Zoologia da Universidade de São Paulo, UFRJ – Universidade Federal do Rio de Janeiro, UFRN – Universidade Federal do Rio Grande do Norte. SMSP – Serra do Mar State Park.

| Voucher | Locality | Reference |
|------------------|---|--------------------------------|
| UFRN 2539 | Ceará-Mirim river, Extremoz, Rio Grande do Norte | This study |
| UFRN 2542 | Curimataú river, Baía Formosa, Rio Grande do Norte | This study |
| MCP-Peixes 12820 | Mundaú lagoon mangrove, Maceió, Alagoas | Species Link (2014) |
| MZUSP 64340 | Mundaú lagoon, Maceió, Alagoas | Species Link (2014) |
| Uncatalogued | São Mateus river, Conceição da Barra, Espírito Santo | Sarmiento-Soares et al. (2014) |
| CZNC 334 | Preto do Sul river, São Mateus, Espírito Santo | Sarmiento-Soares et al. (2014) |
| MBML 8660 | Stream at Sauna beach, Conceição da Barra, Espírito Santo | Sarmiento-Soares et al. (2014) |
| MZUSP 38369 | Iguaba Grande, Araruama, Rio de Janeiro | Species Link (2014) |
| UFRJ 6863 * | Piracão river, Guaratiba, Rio de Janeiro | Costa (2011) |
| MZUSP 38335 | Mangaratiba, Rio de Janeiro | Species Link (2014) |
| Uncatalogued | Fazenda river, Picinguaba SMSP, Ubatuba, São Paulo | Tatarenkov et al. (2011) |
| MZUSP103633 | Rio Jurubatuba, Santos, São Paulo | Mattox and Iglesias (2010) |
| MZUSP 38362 | Forte beach, Florianópolis, Santa Catarina | Species Link (2014) |

*Holotype.

2014) (Table 1), both medium-sized basins disembodying on the eastern shore of Rio Grande do Norte state. These drainages are inserted in the hydrographic ecoregion of Mid-Northeastern Caatinga (MNC, *sensu* Albert et al. 2011), that comprises the basins between Parnaíba River and São Francisco River, covering a small area of Piauí, most of the area of Ceará, and the states of Rio Grande do Norte and Paraíba, in addition to parts of Pernambuco and Alagoas (Rosa et al. 2003).

Collections were taken with hand sieves sized 50 × 60 cm and a 1 mm mesh, under permit #30532-1/2011 issued by ICMBio/SISBIO (Instituto Chico Mendes de Conservação da Biodiversidade/Sistema de Autorização e Informação em Biodiversidade). The collected specimens were anesthetized with dilute ethanol and fixed in a 10% formalin solution, subsequently transferred to an 70% alcoholic solution and deposited in the ichthyologic collection of Universidade Federal do Rio Grande do Norte. Part of the collected specimens was fixed directly in 96% ethanol for molecular analysis. All individuals collected were identified as *Kryptolebias hermaphroditus* through the genus identification key proposed by Costa (2009), in order to present populations composed only by hermaphrodites, 1 to 4 teeth in vomer and color pattern with a row of small silvery or pale golden spots on the dorsal portion of the flank (Costa 2011). All specimens presented a female appearance, composed by a pale color pattern and well-developed black spot on the caudal-fin base, typical of the hermaphroditic individuals of the genus (Costa et al. 2010).

According to specialized scientific literature, *K. hermaphroditus* is restricted to the southeastern Brazil, in the states of Espírito Santo (Sarmiento-Soares et al. 2014), Rio de Janeiro and São Paulo, often sympatric to *K. ocellatus* (Mattox and Iglesias 2010, Costa et al. 2010, Costa 2011; Table 1). However, some populations of Panamá, Cuba and Puerto Rico are genetically more similar to *K.*

hermaphroditus than *K. marmoratus* (Tatarenkov et al. 2011). Thus, it is likely that *K. hermaphroditus* is widely distributed along the Neotropical coastal drainages. Based on online databases, there are three records of the species outside of southeastern Brazil: a material collected in the state of Santa Catarina in 1966 (MZUSP 38362), and two from a mangrove in Alagoas, in the southern portion of MNC ecoregion, dated 1988 (MCP-Peixes 12820 and MZUSP 64340) (Species Link 2014). The new records extend the geographical distribution of the species in approximately 400 km, with the Ceará-Mirim River estuary representing the northernmost locality where the species was collected (Figure 2). The scarcity of records of *K. hermaphroditus* in northeastern Brazil may be due to improper use of equipment in collections (Davis et al. 1990), the lack of collection in specific biotopes, as shallow mangrove channels (Taylor 2012), or even the lack of ichthyofauna studies in the region.

In the basins sampled in Rio Grande do Norte, *Kryptolebias hermaphroditus* was only found in shady stretches of mangroves. In the estuary of Ceará-Mirim River with mangrove area of 3.15 km², the killifishes were captured in pools with shallow clear water, muddy bottom with litter, inserted in the middle estuarine portion, characterized by the interaction area between marine and continental waters (Schettini 2002), lined with white mangrove *Laguncularia racemosa* (L.) Gaertn (Figure 3A). Along with *K. hermaphroditus*, were collected: *Poecilia vivipara* Bloch & Schneider, 1801; *Guavina guavina* (Valenciennes, 1837); *Eleotris pisonis* (Gmelin, 1789) and juvenile *Mugil* sp., in addition to decapod crustaceans such as *Goniopsis cruentata* (Latreille, 1803); *Uca maracoani* (Latreille, 1802–1803) and *Cardisoma guanhumii* Latreille, 1825. In the mangrove from the basin of Curimataú River, the one with the largest area in Rio Grande do Norte state (Maia et al. 2006), *K. hermaphroditus* was also found in the middle portion of the estuary, mainly between the aerial

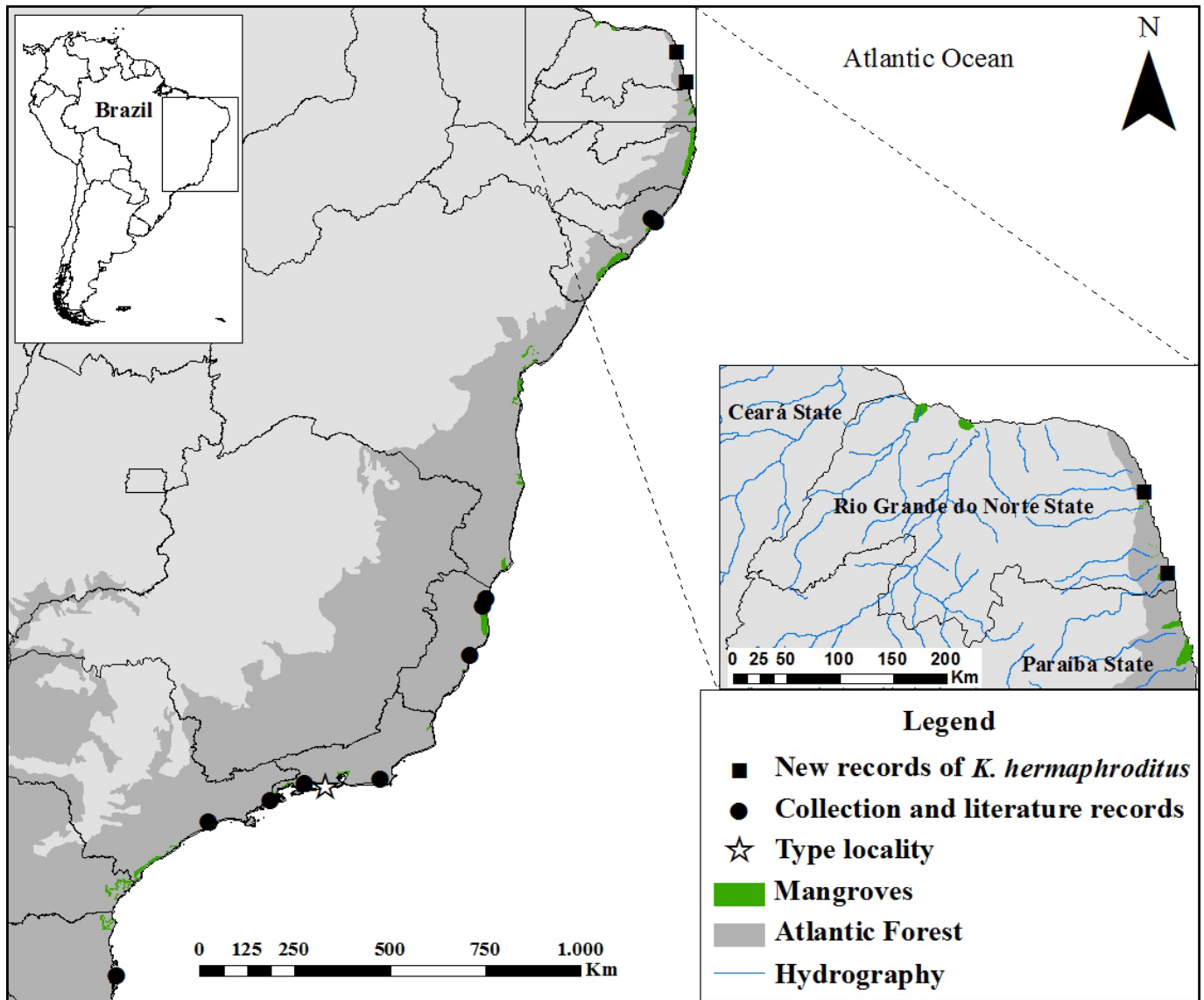


Figure 2. Geographical distribution of *Kryptolebias hermaphroditus* with literature and online collection specimens (circles) in Brazil, including the new records (squares) in Rio Grande do Norte state.

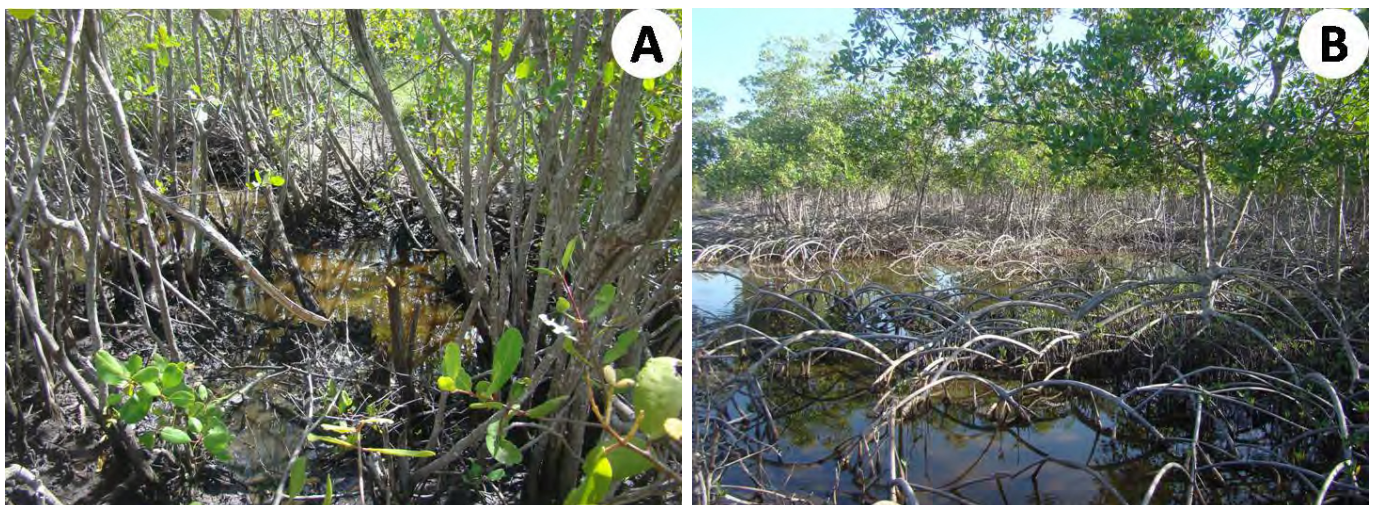


Figure 3. Mangrove at Ceará-Mirim River in Extremoz municipality (A) and Curimataú River in Baía Formosa municipality (B) in Rio Grande do Norte state, northeastern Brazil.

roots of red mangrove *Rhizophora mangle* L., showing similar physical characteristics to Ceará-Mirim River (Figure 3B). These mangroves are inserted in Semiárid Northeastern Coast (*Costa Nordeste Semiárida*), which stretches from the west of Ceará state to the Itamaracá Island in Pernambuco state, and contains approximately 60% Brazilian shrimp farming area (Maia et al. 2006).

According to Taylor (1992) *K. marmoratus* specimens are commonly found in burrows of *C. guanhumi* crabs. However, among mangroves in Rio Grande do Norte, the only fish species observed inside *C. guanhumi* burrows were individuals of the gobiid *G. guavina* (measuring between 44.1 and 94.1 mm SL, UFRN 088) in Ceará-Mirim River, a potential predator for *K. hermaphroditus*.

While northern populations of *K. marmoratus* are composed only by hermaphrodites, populations in low latitudes are generally composed by males and hermaphrodites, although males are rare (Ellison et al. 2012). Concerning *K. hermaphroditus*, no males or molecular evidence of sexual reproduction has been found among populations from southeastern Brazil (Tatarenkov et al. 2011). As the occurrence of males seems to be directly related to warmer temperatures, the development of males and sexual reproduction is more probable in populations of northeastern Brazil.

Although physiologically tolerant to natural environmental changes related to the tidal regime in mangroves, the rivulids from the *K. marmoratus* group are extremely sensitive to environmental changes (Earley et al. 2012). Thus, activities that cause changes in salinity and temperature of the water, caused mainly by the removal of the native vegetation, may reduce the number of individuals of the species (Davis et al. 1990).

Estimations indicates that a third of the mangroves in the world was lost during the last 50 years due to several direct human activities, in addition to climate change and sea level rise (Ellison et al. 2012). Brazilian federal law considers the mangroves as permanent preservation areas (*áreas de preservação permanente*, APP), however detailed mappings of these ecosystems are scarce (Lacerda et al. 2006), and the same applies to ichthyofauna inventories. Northeastern Brazil estuaries show various environmental problems such as nutrient overload, contamination by pathogens and toxic chemicals, changes in freshwater flow, habitat and biodiversity loss, resulting from disorderly coastal occupation and use of these areas for shrimp farms and salt evaporation ponds (Maia et al. 2006). Thus, effective conservation of mangroves is extremely necessary because they are important areas for feeding, refuge and reproduction of a large diversity of resident and migratory species (Maia et al. 2006).

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