Geographic distribution and conservation of seasonal killifishes (Cyprinodontiformes, Rivulidae) from the Mid-Northeastern Caatinga ecoregion, northeastern Brazil

Yuri Gomes Abrantes1, Lucas Silva de Medeiros1, Ana Beatriz Alves Bennemann1, Diego de Medeiros Bento1,2, Francisco Keilo Teixeira3, Carla Ferreira Rezende3, Telton Pedro Anselmo Ramos4, Sergio Maia Queiroz Lima1

1 Laboratório de Ictiologia Sistemática e Evolutiva, Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil
2 Centro Nacional de Pesquisa e Conservação de Cavernas – CECAV/ICMBio, Brazil
3 Laboratório de Ecologia de Ecosistemas Aquáticos, Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, CE, Brazil
4 Laboratório de Sistemática e Morfologia de Peixes, Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, João Pessoa, PB, Brazil

Corresponding author: Yuri Gomes Abrantes (yuriabrantes177@gmail.com)

Abstract
The Rivulidae fish family, which includes Neotropical seasonal killifishes, is one of the most diverse taxonomic groups in the aquatic systems of Caatinga in Brazil. Cynolebias and Hypsolebias genera, with 20 and 35 endemic species, respectively, concentrate the greatest diversity of rivulid species in the semiarid. Sixty-eight years after the first records of annual killifishes in the Mid-Northeastern Caatinga ecoregion (MNCE), only four valid species have been sampled in this area. Here we combined bibliographic surveys and recent samplings to investigate the distribution of seasonal rivulids in MNCE. Twenty-one records were obtained, nine of which are new localities, expanding the distribution of three species: Hypsolebias martinsi, H. antenori and Cynolebias microphthalmus.
Hypsolebias longignatus is still only known from its type locality in Ceará, near the Environmental Protection Area in Pacoti River, and has not been sampled ever since its description in 2008. Among the four species present in MNCE, H. antenori is the only species occurring within the limits of a conservation unit in the Furna Feia National Park. Anthropogenic impacts were observed in most temporary habitats visited, which ranged from river channel to small ponds in cave entrances. All records are found in coastal basins that discharge in the northern coast of the MNCE, in Ceará and Rio Grande do Norte states, which are crucial for the conservation of the Caatinga’s killifishes. The results also evidenced the importance of karstic habitats in the Jandaíra Formation as potential biotopes for seasonal fish in MNCE. This information must be used to update the conservation status of these species and highlight the importance of strategies for preserving the Caatinga’s temporary aquatic habitats, which should be considered for environmental licensing purposes.

Keywords
Dry lands, endangered species, Karstic areas, Neotropical freshwater fishes, São Francisco interbasin water transfer, temporary habitats

Introduction

Among the freshwater fishes better adapted to the extreme hydrological regime of the semiarid regions are the seasonal killifishes of the Rivulidae family. These species live in seasonal ponds filled by rain that dry in a part of the year, when the eggs deposited on the substrate survive due to developmental and metabolic diapauses (Murphy and Collier 1997; Berois et al. 2016). In the semiarid Caatinga, composed of four freshwater ecoregions, the genera Cynolebias Steindachner, 1876 and Hypsolebias Costa, 2006 are represented by 20 and 35 valid species, respectively, with highest richness in the São Francisco ecoregion (Lima et al. 2017; Costa 2017; Costa et al. 2018a, b, c).

Seasonal killifishes were first recorded in the Caatinga by Myers (1952) while sampling in the Jaguaribe river basin in the Mid-Northeastern Caatinga ecoregion (MNCE) (Lima et al. 2017). This ecoregion is located between the Parnaíba and São Francisco river basins and includes small to medium-sized drainages, most of them intermittent (Lima et al. 2017). Due to its particular hydrological regime, MNCE has been historically considered as a scientifically neglected region for freshwater fish surveys (Lévêque et al. 2008). However, recent studies have pointed out that the MNCE’s ichthyofauna is relatively rich, consisting of around 90 species, approximately 30% endemic (Lima et al. 2017; Berbel-Filho et al. 2018). Historical negligence in MNCE explains why so far only four freshwater fish species are considered threatened in the ecoregion, besides many human-induced impacts on aquatic habitats, most of them related to the water shortage (Lima et al. 2017, ICMBio 2018).

For the MNCE, only four valid species of seasonal killifish are known, namely Cynolebias microphthalmus Costa & Brasil, 1995, Hypsolebias antenori (Tulipano, 1973), H. martinsi Britzke, D. T. B. Nielsen & C. de Oliveira, 2016 and H. longignatus (Costa, 2008), all endemic to this ecoregion (Tulipano 1973; Costa 1995; 2008; Britz-
ke et al. 2016; Berbel-Filho et al. 2018). Among these species, only *H. longignatus* is currently on the Brazilian red list of threatened fish species, in the vulnerable category, due to the risk of habitat loss to agricultural activities within its restricted geographic distribution (ICMBio 2018). However, the condition of *H. martinsi* was not assessed because it was described after the last published evaluation (MMA 2014).

Describing the geographic distribution of species can broaden knowledge of regional biodiversity and contribute to developing conservation policies (Lévêque et al. 2008). Although many rivulid species have been described from the Caatinga in past decades, conservation initiatives for these species and their environments have advanced at a lower rate when compared to the human-induced changes in the ecosystem. Extensive deforestation for agriculture and cattle raising, road construction, hydrological change projects (e.g. dams, landfills) and urban area expansion are among the changes threatening seasonal killifish (Costa 2002, ICMBio 2013). These threats can be particularly problematic in the MNCE’s drainages that receive waters from the ongoing transposition of the São Francisco river, as changes in the groundwater levels may affect the diapause cycle of the seasonal killifishes (Costa 2002).

Based on recent fieldwork and records obtained in the ichthyological literature and collections, the aim of this study was to explore the geographical distribution of the seasonal killifishes in the MNCE, describe their habitat and indicate conservation strategies to effectively protect these endemic species.

**Methodology**

In this study, we analyzed seasonal killifish records in the northern portion of the MNCE, between the coastal basins of the Icaraiçinho river in Ceará State (CE) to the Apodi-Mossoró river in the Rio Grande do Norte State (RN). Inserted in an area also known as ‘Depressão Sertaneja Setentrional’, where prolonged drought periods are common, the rainy season extends from October to April, with an average annual rainfall around 500 to 800 mm (Velloso et al. 2002 Silva et al. 2017a). This area has extensive rocky outcrops belonging to the Jandaíra Formation, which is considered the largest area of Phanerozoic carbonate outcrops in Brazil (Bezerra et al. 2007). Since the first record of killifishes in this area, extensive surveys have been carried out in the drainages of the Jandaíra formation, including a recent created federal conservation unit, the Furna Feia National Park (FFNP) (Brasil 2012). With an area of 84.94 km², located between a small coastal basin and the Apodi-Mossoró river, the FFNP mainly protects endemic plants and animals of the Caatinga, including oceanic relicts and invertebrate troglobitic species (Bento et al. 2013).

Field expeditions were performed during or after the rainy season from June to July 2018 and from February to June 2019. The specimens were captured at daytime by active fishing with a hand net (40 × 30 cm). Each sampling site had its attributes (geographical coordinates, depth, substrate, vegetation, threats, etc.) noted, and was photographed and explored extensively to guarantee all species in the temporary
habitat were recorded. To estimate the size of the puddles (length, width and total area), we created polygons using satellite images from the Google Earth Pro software (version 7.3.2.5776). All specimens collected were anesthetized in eugenol alcoholic solution (10 mL of eugenol diluted in 90 mL of ethyl alcohol), according to the protocol proposed by Lucena et al. (2013). Subsequently, some specimens were fixed in formaldehyde 4%, for seven days and preserved in 70% alcohol, according to the curation procedures of Malabarba and Reis (1987), except for some specimens or fin-clips preserved in ethanol 99% for molecular studies. The collections were made under permit 30532-1/2011 and 54274-2/2018 of the Instituto Chico Mendes de Conservação da Biodiversidade/Sistema de Autorização e Informação em Biodiversidade (ICMBio/SISBIO).

The specimens were identified using the descriptions or re-descriptions of the species (Costa 2001, 2007; Britzke et al. 2016). The material is deposited at the fish collection of the Universidade Federal do Rio Grande do Norte (UFRN) (Table 1). Additional records were obtained from the bibliography (Costa 2001, 2007, 2008, Britzke et al. 2016; Costa et al. 2018c; Oliveira et al. 2018) and online fish collections database search at SpeciesLink (http://www.splink.org.br/), Global Biodiversity Information Facility (GBIF) (https://www.gbif.org/) and NEODAT II (http://www.mnrj.ufrj.br/search). We compiled the data from the occurrence of the four valid seasonal killifish species of the MNCE. Acronyms of institutional depository follow Sabaj (2016): MZUSP: Museu de Zoologia da Universidade de São Paulo, São Paulo; MNRJ: Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro; UFRJ: Laboratório de Sistemática e Evolução de Peixes Teleósteos; UFRN: Universidade Federal do Rio Grande do Norte, Natal; ZUEC: Museu de Zoologia da Universidade Estadual de Campinas “Adão José Cardoso”, Campinas.

All localities were listed and described in Table 1 and indicated in the map from north to south and from west to east (Fig. 1). Ecological attributes of the visited sampling sites are described in Table 2. Altitudes were obtained by plotting geographic coordinates in Google Earth Pro software (version 7.3.2.5776). However, some vouchers found in the online databases were partially lacking critical information, such as geographic coordinates. In this case, it was only possible to estimate the geographic position according to the description of the location using the GeoNames online tool (http://geonames.org).

Results

Among 21 records, nine were from primary data (UFRN vouchers) and 12 were from literature and databases, which are distributed in seven MNCE basins (Fig. 1; Table 1). From the secondary data, nine were compiled based on the literature and three compiled using online databases (SpeciesLink and GBIF). The primary data included nine records, eight of which are new and expanded the geographic distribution of three of the four known species of the MNCE, _Cynolebias microphthalmus_, _Hypsolebias antenori_ and _H. martinsi_ (Fig. 2), with an altitude ranging from 12 to
Table 1. Sampled sites and records of Hypsolebias antenori, H. martinsi, H. longignatus and Cynolebias microphthalmus. * Localities in the Furna Feia National Park.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Species</th>
<th>Basin/UF</th>
<th>Geographic Coordinate</th>
<th>Altitude</th>
<th>Voucher</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H. martinsi</td>
<td>Icaraizinho/CE</td>
<td>03°11’04”S, 39°44’58”W</td>
<td>16 m</td>
<td>ZUEC 10791</td>
<td>Britzke et al. (2016)</td>
</tr>
<tr>
<td>2</td>
<td>H. martinsi</td>
<td>Mundaú/CE</td>
<td>03°24’22”S, 39°44’25”W</td>
<td>40 m</td>
<td>UFRN 5414</td>
<td>This study</td>
</tr>
<tr>
<td>3</td>
<td>H. martinsi</td>
<td>Mundaú/CE</td>
<td>03°19’55”S, 39°30’14”W</td>
<td>20 m</td>
<td>UFRN 5413</td>
<td>This study</td>
</tr>
<tr>
<td>4</td>
<td>H. longignatus</td>
<td>Pacoti/CE</td>
<td>03°53’48”S, 38°24’17”W</td>
<td>13 m</td>
<td>UFRJ 8764</td>
<td>Costa (2008)</td>
</tr>
<tr>
<td>5</td>
<td>H. antenori</td>
<td>Choró/CE</td>
<td>04°13’24”S, 38°28’37”W</td>
<td>60 m</td>
<td>MZUSP 38342</td>
<td>Costa (2007)</td>
</tr>
<tr>
<td>6</td>
<td>H. antenori</td>
<td>Jaguaribe/CE</td>
<td>04°35’13”S, 38°05’23”W</td>
<td>40 m</td>
<td>UFRJ 4873</td>
<td>Costa (2007)</td>
</tr>
<tr>
<td>7</td>
<td>H. antenori</td>
<td>Jaguaribe/CE</td>
<td>04°47’43”S, 38°03’30”W</td>
<td>30 m</td>
<td>MNRI 19465</td>
<td>GBIF</td>
</tr>
<tr>
<td>8</td>
<td>H. antenori</td>
<td>Jaguaribe/CE</td>
<td>04°54’44”S, 37°54’57”W</td>
<td>48 m</td>
<td>MZUSP 14755</td>
<td>SpeciesLink</td>
</tr>
<tr>
<td>9</td>
<td>H. antenori</td>
<td>Jaguaribe/CE</td>
<td>05°10’00”S, 38°05’00”W</td>
<td>30 m</td>
<td>UFRJ 4860, 4878</td>
<td>Costa (2001, 2007)</td>
</tr>
<tr>
<td>10</td>
<td>H. antenori/C. microphthalmus</td>
<td>Jaguaribe/CE</td>
<td>05°11’05”S, 38°06’18”W</td>
<td>30 m</td>
<td>UFRJ 4864</td>
<td>Costa (2008)</td>
</tr>
<tr>
<td>11</td>
<td>H. antenori</td>
<td>Jaguaribe/CE</td>
<td>05°09’25”S, 37°30’15”W</td>
<td>110 m</td>
<td>UFRN 5226</td>
<td>This study</td>
</tr>
<tr>
<td>12*</td>
<td>H. antenori</td>
<td>Córrego da Mata/RN</td>
<td>05°04’15”S, 37°27’44”W</td>
<td>30 m</td>
<td>UFRN 5624</td>
<td>This study</td>
</tr>
<tr>
<td>13*</td>
<td>H. antenori</td>
<td>Córrego da Mata/RN</td>
<td>05°10’09”S, 37°32’12”W</td>
<td>100 m</td>
<td>UFRN 5581, 5582</td>
<td>This study</td>
</tr>
<tr>
<td>14</td>
<td>H. antenori/C. microphthalmus</td>
<td>Apodi-Mossoró/RN</td>
<td>05°10’54”S, 37°23’46”W</td>
<td>30 m</td>
<td>UFRN 5626, 5623</td>
<td>This study</td>
</tr>
<tr>
<td>15</td>
<td>H. antenori/C. microthalmus</td>
<td>Apodi-Mossoró/RN</td>
<td>05°13’29”S, 37°21’31”W</td>
<td>12 m</td>
<td>UFRJ 4862</td>
<td>Costa (2001)</td>
</tr>
<tr>
<td>16</td>
<td>H. antenori</td>
<td>Apodi-Mossoró/RN</td>
<td>05°15’00”S, 37°20’00”W</td>
<td>50 m</td>
<td>MZUSP 38343</td>
<td>Costa (2001)</td>
</tr>
<tr>
<td>17</td>
<td>C. microphthalmus</td>
<td>Apodi-Mossoró/RN</td>
<td>05°33’45”S, 37°42’36”W</td>
<td>90 m</td>
<td>UFRN 5627</td>
<td>This study</td>
</tr>
<tr>
<td>18</td>
<td>H. antenori</td>
<td>Apodi-Mossoró/RN</td>
<td>05°33’46”S, 37°39’54”W</td>
<td>70 m</td>
<td>UFRN 4700</td>
<td>This study</td>
</tr>
<tr>
<td>19</td>
<td>H. antenori</td>
<td>Apodi-Mossoró/RN</td>
<td>05°59’35”S, 37°82’67”W</td>
<td>120 m</td>
<td>UFRN 5625</td>
<td>Oliveira et al. (2018)</td>
</tr>
<tr>
<td>20</td>
<td>H. antenori</td>
<td>Apodi-Mossoró/RN</td>
<td>05°35’31”S, 37°49’35”W</td>
<td>120 m</td>
<td>UFRN 5625</td>
<td>This study</td>
</tr>
</tbody>
</table>

120 m in relation to sea level (Table 1). The temporary habitats usually consisted in sun-exposed, shallow muddy waters and ponds, usually with abundant vegetation and muddy or sandy substrate, varying from 178 m² to 27,800 m². A few exceptions were the smaller clear water pools over a rocky bottom in the Jandaíra Formation in which a few specimens of H. antenori were found (Table 2). The species geographic distributions are described in clockwise order from north to south. Besides samplings along the Ceará coastal plains, none of them was in the Pacoti river basin and H. longignatus was not recorded.

Previously known only from its type locality, herein we report two new records of H. martinsi at the Mundaú River basin (Fig. 1, localities 1, 2 and 3) (Fig. 3A, B). In the locality 2, the temporary pond was adjacent to a road containing private
Table 2. Ecological data of the new localities of occurrence of the species of seasonal killifish, *Cynolebias microphthalmus* (Cm), *Hypsolebias antenori* (Ha) and *H. martinsi* (Hm).

<table>
<thead>
<tr>
<th>Locality</th>
<th>Length (m)</th>
<th>Width (m)</th>
<th>Area (m²)</th>
<th>Depth (cm)</th>
<th>Water Substrat</th>
<th>Aquatic vegetation</th>
<th>Vegetal cover</th>
<th>Species Impacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>24</td>
<td>9</td>
<td>178</td>
<td>72</td>
<td>Clear Sand</td>
<td>Submerse</td>
<td>Present</td>
<td>Hm Corn and bean plantation, bovines</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>8</td>
<td>205</td>
<td>45</td>
<td>Muddy Sand</td>
<td>Emerging</td>
<td>Present</td>
<td>Hm Corn and bean plantation, bovines and ovines</td>
</tr>
<tr>
<td>12</td>
<td>46</td>
<td>30</td>
<td>1340</td>
<td>42</td>
<td>Muddy Mud</td>
<td>Floating</td>
<td>Absent</td>
<td>Ha None (Furna Feia National Park)</td>
</tr>
<tr>
<td>13</td>
<td>72</td>
<td>38</td>
<td>1670</td>
<td>50</td>
<td>Clear Sand</td>
<td>Emerging</td>
<td>Absent</td>
<td>Ha None (Furna Feia National Park)</td>
</tr>
<tr>
<td>14</td>
<td>127</td>
<td>110</td>
<td>7246</td>
<td>36</td>
<td>Muddy Mud</td>
<td>Emerging</td>
<td>Present</td>
<td>Cm/Ha Sorghum and cassava plantation, ovines</td>
</tr>
<tr>
<td>15</td>
<td>336</td>
<td>150</td>
<td>27800</td>
<td>120</td>
<td>Muddy Mud</td>
<td>Absent</td>
<td>Present</td>
<td>Cm/Ha Organic waste, ovines, urban allotment project</td>
</tr>
<tr>
<td>18</td>
<td>31</td>
<td>20</td>
<td>501</td>
<td>193</td>
<td>Muddy Sand</td>
<td>Floating</td>
<td>Present</td>
<td>Ha Banana and papaya plantations, ovines</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>25</td>
<td>Clear Rock</td>
<td>Absent</td>
<td>Absent</td>
<td>Ha None</td>
</tr>
<tr>
<td>21</td>
<td>15</td>
<td>3</td>
<td>50</td>
<td>15</td>
<td>Clear Rock</td>
<td>Absent</td>
<td>Absent</td>
<td>Ha Disorderly visitation and lime mining</td>
</tr>
</tbody>
</table>

Figure 1. Map of seasonal killifish sampling sites in the Mid-Northeastern Caatinga ecoregion, northeastern Brazil. Light blue lines indicate hydrography. The dark gray area indicates the Jandaíra karstic formation, while the green shapes show the limits of conservation units. Stars represent type localities, triangles show new records and circles represent literature and database records. In blue *Hypsolebias martinsi*, yellow *H. longignatus*, red *H. antenori*, white *Cynolebias microphthalmus*, and pink syntopy of *H. antenori* and *C. microphthalmus*. 
Figure 2. Seasonal killifish species from the Mid-Northeastern Caatinga ecoregion, northeastern Brazil. Live specimens of *Cynolebias microphthalmus* male (A) and female (B) collected in May 2019 in Apodi-Mossoró river basin (locality 14), Rio Grande do Norte State; live specimens of *Hypsolebias antenori* male (C) and female (D) collected in June 2018 in Córrego da Mata coastal basin (locality 12), Furna Feia National Park, Rio Grande do Norte State; and preserved specimens of *H. martinsi* male (E) and female (F) collected in February 2019 in Mundaú river basin (locality 2), Ceará State.

Properties used for agriculture and livestock activities. Locality 3 lies along the middle course of the Cruxati river, near Itapipoca municipality in CE. At this site a few specimens were caught in the main river channel, possibly carried out from adjacent ponds. Besides sand extraction from the river floodplains (Fig. 4A), the region also has large rural estates where the land is used for corn and bean plantations or bovine and ovine raising (Table 2). These records expand the distribution of the species 24 km to the south and 32 km east from the previous record of the Icaraizinho river basin, in the Amontada municipality in CE.

*Hypsolebias antenori* has the highest number of localities of occurrences (n = 15), widely distributed between the Choró in CE and Apodi-Mossoró river basins.
Six of these localities are new records, expanding the known distribution of this species in the Apodi-Mossoró river basin and in the Córrego da Mata, a small coastal basin to the west, in RN. Of these occurrences, two (localities 12 and 13) are in the FFNP (Fig. 5A, B), and two in the rocky outcrops of the Jandaíra formation (localities 19 and 21) in an area known as Rosário and Soledade rocky formations (Fig. 1 and Fig. 3C, D) that suffer from disorderly visitation and limestone mining activities (Table 2). The presence of the species in some localities might be accidental, and the killifishes were possibly carried by torrential rainwater, as in the artificial well built for water storage (locality 18, Fig. 5C, D) surrounded by banana and papaya plantations and goats (Table 2), and a pond in a cave entrance of the Rosário rocky formation (locality 19).

Finally, we provide two new records (localities 14 and 15, Fig. 1) of *C. microphthalmus* in temporary ponds 24 km and 14 km at the northwest of the type locality, in the Apodi-Mossoró river basin (Fig. 5E, F). This species was found in syntopy with *H. antenori* in both sampling sites. The locality 14 is in a rural settlement mainly involved in sorghum and cassava plantations and ovine raising, and the locality 15 is on a rural estate in a temporary waterlogged area with intense anthropogenic activity.
Figure 4. Environmental impacts identified near the localities of the seasonal killifishes in the Mid-Northeastern Caatinga ecoregion, northeastern Brazil. (A) Sand removal along the Cruxati river near locality 3 of Hypsolebias martinsi in Mundaú river basin, Itapipoca municipality in Ceará State; (B) silting in temporary pond (locality 14), and (C) construction of a real estate development near locality 15 of Cynolebias microphthalmus and H. antenori in Apodi-Mossoró river basin, Mossoró municipality in Rio Grande do Norte State.

near the pond (Table 2). The identified impacts were: soil erosion, plastic debris, organic waste and the implementation of an urban allotment project in the region that might negatively affect the temporary ponds and riverine floodplains (Fig. 4B, C).

Discussion

Britzke et al. (2016) suggested that new species of Hypsolebias might occur in the coastal basins of MNCE. This might be valid for specimens from the Mundaú river basin, due to subtle morphological differences regarding its identity. However, most diagnostic features are based on dimorphic characters of males, and only two small males from this basin were sampled, making it difficult to determine if they belong to a distinct species. Thus, we suggest considering it as H. martinsi since it shares most of the diagnostic characters. Just as the H. antenori complex was dismembered into different species, some cryptic species may be recognized in MNCE (Britzke et
Yuri Gomes Abrantes et al. 2016). A detailed integrative analysis using both molecular and morphological data, including more specimens, especially males, may confirm the identity of the species from the Mundaú river basin. Moreover, the occurrence of the critically endangered non-annual rivulid, *Anablepsoides cearensis* (Costa & Vono, 2009) in the coastal plains of this basin (Teixeira et al. 2017, ICMBio 2018) corroborates it as an extremely high priority area for the Caatinga’s fish conservation (MMA 2018).

*Hypsolebias antenori* is the seasonal killifish with the widest known geographical distribution in MNCE and possibly in the whole Caatinga biome. Although it

**Figure 5.** Seasonal killifish locations during rainy and dry seasons in Apodi-Mossoró river basin, Rio Grande do Norte State. (A, B) *Hypsolebias antenori* temporary pond (locality 12) in Córrego da Mata coastal basin in Furna Feia National Park, and (C, D) artisanal well (locality 18) in Felipe Guerra municipality, and (E, F) *Cynolebias microphthalmus* and *H. antenori* temporary pond (locality 14) in Mossoró municipality.
does not appear on the red lists, its presence in the FFNP is probably the first of a protected killifish population in MNCE. To date, none of the four MNCE’s endangered freshwater fish species were recorded in conservation units (Silva et al. 2017b). The FFNP was created in 2012 and does not yet have a management plan, nor any fish inventory until this study. In the few water bodies within the limits of the conservation unit, only two species, *H. antenori* and the “piaba” *Astyanax bimaculatus* Linnaeus, 1758 were recorded. Although the park is located 12 km from the localities of *C. microphthalmus*, this species was not found in the FFNP.

The presence of *H. antenori* in the rocky outcrops of Phanerozoic carbonates on the left bank of the Apodi-Mossoró river, at the Soledade Formation (locality 20) was already reported by Oliveira et al. (2018). These occurrences allow us to identify that *H. antenori* establishes its life cycle in different habitats (rocky outcrops including cave entrances) to those in which seasonal Caatinga killifish are usually found (Costa 2002). This area also harbors a rich biodiversity of troglobitic invertebrates, some of them endemic to the Jandaíra formation (Ferreira et al. 2010; Fišer et al. 2013; Bento et al. 2016).

The vulnerable *H. longignatus* is only known from its type material and locality (Costa 2008) (Fig.1 Locality 4), close to the limits of the Rio Pacoti Environmental Protection Area (RPEPA). This protection area was created in 2000, without an updated management plan, which is still in progress (https://uc.socioambiental.org/). The lack of efforts to conserve the remaining coastal plains and mangroves in this area have resulted in water pollution, environmental degradation and real estate speculation (Nascimento and Carvalho 2003). Thus, future inventories in the temporary ponds of the RPEPA should be carried out to evaluate the distribution of *H. longignatus*. Here, we recommend that the limits of conservation units should be expanded to include the type locality of *H. longignatus*. Additionally, field investigations in this region should be prioritized to obtain new records. A review of the material from the locality 5, in the Choró river basin and close to the type locality of *H. longignatus*, is also recommended, although it was identified as *H. antenori* (UFRJ 4864).

Known from the Jaguaribe (CE) to the Apodi-Mossoró (RN) river basins, *C. microphthalmus* has not been collected since 1999 in CE (Costa 2001). Although Nascimento et al. (2015), Costa et al. (2018c) and Oliveira et al. (2018) collected *H. antenori* in CE and RN, they did not collect *C. microphthalmus*. In RN this species has not been found since 1972 (Costa 2001) and the new occurrences might be useful to address conservation strategies of this large killifish species. In both recently-sampled localities (14 and 15) it occurred in sympatry with *H. antenori*, as was previously reported in the Jaguaribe river basin (Costa 2001; Costa 2007). These localities should also be especially considered in future conservation actions, mainly due to the rarity of *C. microphthalmus* records. The surrounding area close to the previously known localities of *C. microphthalmus* was altered for irrigated fruit activities, which can reduce groundwater levels and possibly impact its water quality through contamination with pesticides and fertilizers, potentially compromising the aquatic biota. It is noteworthy that the Jaguaribe and Apodi-Mossoró river basins are recipients of the São Francisco river water transfer project (Berbel-
Filho et al. 2016), and changes in the water regime can negatively affect seasonal fish living in the floodplain of these basins (Costa 2002).

Although only *H. longignatus* is currently evaluated as vulnerable according to IUCN criteria (ICMBio 2018), the reduced geographic distribution and rarity in recent field studies suggest that *H. martinsi* and *C. microphthalmus* might be included in the threatened category, as well as *H. longignatus*, which could be included in a more severe category. None of the MNCE’s rivulids were selected as targets of the National Action Plan for the conservation of endangered rivulid fish, which include 14 Caatinga’s species (ICMBio 2013). Considering that two localities of *H. antenori* are protected by the FFNP, conservation actions should be implemented to expand the limits of the conservation units in the region, aiming to protect MNCE’s rivulids. Additional activities, such as initiatives for environmental education activities, as well as greater awareness of the owners of the private areas with rivulid records are required. Ordering and limiting visits to areas subject to tourism (cave habitats in Jandaíra formation) is vital. Also urgently needed are specific studies on seasonal killifish for new ventures installation (environmental licensing) as highways, and wind farms. Real estate speculation on areas of killifish records should be avoided and, finally, the establishment of some of these areas in Private Reserves of Natural Heritage (MMA 2018) is a pressing requirement.

**Conclusion**

The identification of the rocky outcrops of the Jandaíra Formation as seasonal killifish habitats helps to integrate actions to conserve underground and surface ecosystems and protect aquatic and terrestrial biota. The western portion of RN has the largest number of caves in the state. Some fish species were recorded in the pools and caves of the region, which provide a refuge for the Caatinga’s ichthyofauna. The main human impacts on the caves of the Jandaíra Formation are related to disorderly visitation and mining (Ferreira et al. 2010). The restricted geographical distribution of most annual killifishes in the basins of the MNCE and the high rates of habitat loss make these endemic species preferential candidates for protection and conservation (ICMBio 2013). The new records on MNCE and the data compiled in this study indicate the fundamental role of the region between the west of CE to the west of RN for the conservation of seasonal killifishes of the ecoregion and might support future evaluations on the conservation status of these species, as well as the creation of small private conservation units in the new recorded areas.

**Acknowledgements**

The authors are grateful to the Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV/ICMBio) crew, especially to Iatagan Freitas and Uilson Paulo for support in the fish collection in the Parque Nacional da Furna Feia and in the Jandaíra formation. To Maria Rita Duarte, Tamara Maciel and Daniel Pontes from the Laboratório de Ecologia de Ecossistemas Aquáticos da Universidade Federal do Ceará
(LEAA/UFC) for helping in the collections in the Mundaú river basin. To Arthur Antunes and Gutenberg Nunes for support in field logistics in the collections in the Mossoró river basin, and to the staff of Laboratório de Ictiologia e Sistemática Evolutiva (LISE) of the UFRN who contributed in the field activities. SMQL receives CNPq productivity research grant (313644/2018-7), FKT is grateful to CAPES for PhD scholarship (Nº Proc 1487767), LSM is grateful to CAPES for the Master scholarship (Nº Proc 1798425), and TPAR is grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

References


