

RESEARCH ARTICLE

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

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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 Icaraizinho 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

Locality	Species	Basin/UF	Geographic Coordinate	Altitude	Voucher	Reference
1	H. martinsi	nartinsi Icaraizinho/ 03°11'04"S, 39°44'58"W 16 m		16 m	ZUEC 10791	Britzke et al.
		CE				(2016)
2	H. martinsi	Mundaú/CE	03°24'22"S, 39°44'25"W	40 m	UFRN 5414	This study
3	H. martinsi	Mundaú/CE	03°19'55"S, 39°30'14"W	20 m	UFRN 5413	This study
4	H. longignatus	Pacoti/CE	03°53'48"S, 38°24'17"W	13 m	UFRJ 8764	Costa (2008)
5	H. antenori	Choró/CE	04°13'24"S, 38°28'37"W	60 m	MZUSP	Costa (2007)
					38342	
6	H. antenori	Jaguaribe/CE	04°35'13"S, 38°05'23"W	40 m	UFRJ 4873	Costa (2007)
7	H. antenori	Jaguaribe/CE	04°47'43"S, 38°03'30"W	30 m	MNRJ 19465	GBIF
8	H. antenori	Jaguaribe/CE	05°07'02"S, 38°22'53"W	48 m	MZUSP	SpeciesLink
		-			14755	
9	H. antenori	Jaguaribe/CE	04°54'44"S, 37°54'57"W	17 m	MNRJ 4542	GBIF
10	H. antenori/C.	Jaguaribe/CE	05°10'00"S, 38°05'00"W	30 m	UFRJ 4860,	Costa (2001,
	microphthalmus	-			4878	2007)
11	H. antenori	Jaguaribe/CE	05°11'05"S, 38°06'18"W	30 m	UFRJ 4864	Costa (2008)
12*	H. antenori	Córrego da	05°02'55"S, 37°30'15"W	110 m	UFRN 5226	This study
		Mata/RN				
13*	H. antenori	Córrego da	05°04'15"S, 37°27'44"W	30 m	UFRN 5624	This study
		Mata/RN				
14	H. antenori/C.	Apodi-	05°10'09"S, 37°32'12"W	100 m	UFRN 5581,	This study
	microphthalmus	Mossoró/RN			5582	
15	H. antenori/C.	Apodi-	05°10'54"S, 37°23'46"W	30 m	UFRN 5626,	This study
	microphthalmus	Mossoró/RN			5623	
16	H. antenori	Apodi-	05°13'29"S, 37°21'31"W	12 m	UFRJ 4862	Costa (2001)
		Mossoró/RN				
17	C. microphthalmus	Apodi-	05°15'00"S, 37°20'00"W	50 m	MZUSP	Costa (2001)
		Mossoró/RN			38343	
18	H. antenori	Apodi-	05°33'45"S, 37°42'36"W	90 m	UFRN 5627	This study
		Mossoró/RN				
19	H. antenori	Apodi-	05°33'46"S, 37°39'54"W	70 m	UFRN 4700	This study
		Mossoró/RN				
20	H. antenori	Apodi-	05°59'35"S, 37°82'67"W	120 m	UFRJ 5625	Oliveira et al.
		Mossoró/RN				(2018)
21	H. antenori	Apodi-	05°35'31"S, 37°49'35"W	120 m	UFRN 5625	This study
		Mossoró/RN				

Table 1. Sampled sites and records of *Hypsolebias antenori*, *H. martinsi*, *H. longignatus* and *Cynolebias microphthalmus*. * Localities in the Furna Feia National Park.

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

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

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).

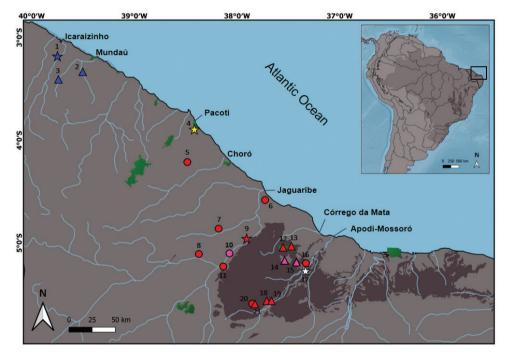


Figure 1. Map of seasonal killifish sampling sites in the Mid-Northeastern Caatinga ecoregion, northeastern Brazil. Light blue lines indicate hydrography. The dark gray area indicates the Jandaíra karstic formation, while the green shapes show the limits of conservation units. Stars represent type localities, 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*.

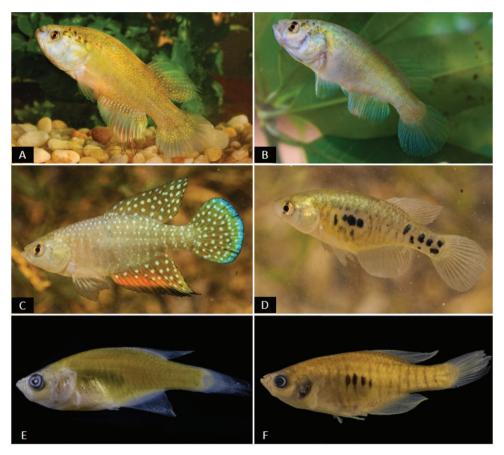


Figure 2. Seasonal killifish species from the Mid-Northeastern Caatinga ecoregion, northeastern Brazil. Live specimens of *Cynolebias microphthalmus* male (**A**) and female (**B**) collected in May 2019 in Apodi-Mossoró river basin (locality 14), Rio Grande do Norte State; live specimens of *Hypsolebias 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



Figure 3. Atypical habitats of seasonal killifish in the Mid-Northeastern Caatinga ecoregion, northeastern Brazil. (**A**) Main channel of the Cruxati river basin (locality 2) and (**B**) partially shaded temporary pond (locality 3) of *Hypsolebias martinsi* in the Mundaú river basin floodplains, Itapipoca municipality in Ceará State. (**C**) Details of *H. antenori* temporary pond in the rocky outcrops (**D**) of Lajedo Soledade (locality 21) in the Jandaíra karstic formation in the Apodi-Mossoró river basin, Apodi municipality in Rio Grande do Norte State.

in RN (Fig.1). 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. microph-thalmus* 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

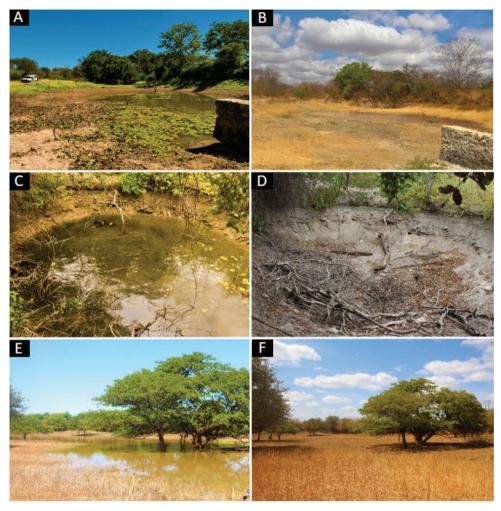


Figure 5. Seasonal killifish locations during rainy and dry seasons in Apodi-Mossoró river basin, Rio Grande do Norte State. (**A**, **B**) *Hypsolebias antenori* temporary pond (locality 12) in Córrego da Mata coastal basin in Furna Feia National Park, and (**C**, **D**) artisanal well (locality 18) in Felipe Guerra municipality, and (**E**, **F**) *Cynolebias microphthalmus* and *H. antenori* temporary pond (locality 14) in Mossoró municipality.

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

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.

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References

- Bento DM, et al. (2013) Parque Nacional da Furna Feia: o parque nacional com a maior quantidade de cavernas do Brasil. In: Rasteiro MA (Ed.) Congresso Brasileiro de Espeleologia, 32, 2013. Barreiras. Anais Campinas SBE 2013, 31–43.
- Bento DM, Ferreira RL, Prous X, Souza-Silva M, Bellini BC, Vasconcellos A (2016) Seasonal variations in cave invertebrate communities in the semiarid Caatinga, Brazil. Journal of Caves and Karst Studies 78(2): 61–71. https://doi.org/10.4311/2015LSC0111
- Berbel-Filho WM, Martinez PA, Ramos TP, Torres RA, Lima SMQ (2016) Inter-and intrabasin phenotypic variation in two riverine cichlids from northeastern Brazil: Potential eco-evolutionary damages of Sao Francisco interbasin water transfer. Hydrobiologia 766(1): 43–56. https://doi.org/10.1007/s10750-015-2440-9
- Berbel-Filho WM, Ramos TP, Jacobina UP, Maia DJ, Torres RA, Lima SMQ (2018) Updated checklist and DNA barcode-based species delimitations reveal taxonomic uncertainties among freshwater fishes from the mid-north-eastern Caatinga ecoregion, northeastern. Brazil Journal of Fish Biology 93(2): 311–323. https://doi.org/10.1111/jfb.13758
- Berois N, Arezo MJ, Papa NG, Chalar C (2016) Life cycle, reproduction, and development in annual fishes: cellular and molecular aspects. In: Berois N, García G, de Sá RO (Eds) Annual Fishes: life history strategy, diversity, and evolution. Boca Ratón, CRC Press, 33–46. https://doi.org/10.1201/b19016-4
- Bezerra FH, Takeya MK, Sousa MO, Nascimento AF (2007) Coseismic reactivation of the Samambaia fault, Brazil. Tectonophysics 430(1–4): 27–39. https://doi.org/10.1016/j. tecto.2006.10.007
- Brasil (2012) Decreto de 5 de junho de 2012. Dispõe sobre a criação do Parque Nacional da Furna Feia, nos municípios de Baraúna e Mossoró, Estado do Rio Grande do Norte. Diário Oficial [da] República Federativa do Brasil, Brasília, DF.
- Britzke R, Nielsen DT, Oliveira C (2016) Description of two new species of annual fishes of the *Hypsolebias antenori* species group (Cyprinodontiformes: Rivulidae), from Northeast Brazil. Zootaxa 4114(2): 123–138. https://doi.org/10.11646/zootaxa.4114.2.2
- Costa WJEM (1995) Pearl killifishes: The Cynolebiatinae. Systematics and Biogeography of a Neotropical annual fish subfamily (Cyprinodontiformes: Rivulidae). T.F.H. Publications, Inc, Neptune City, 128. https://doi.org/10.2307/1446910
- Costa WJEM (2001) The Neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. Ichthyological Exploration of Freshwaters 12(4): 333–383.

- Costa WJEM (2002) Peixes anuais brasileiros: diversidade e conservação. Editora UFPR, Curitiba, 238.
- Costa WJEM (2007) Taxonomic revision of the seasonal South American killifish genus *Simpsonichthys* (Teleostei: Cyprinodontiformes: Aplocheiloidei: Rivulidae). Zootaxa 1669(1): 1–134. https://doi.org/10.11646/zootaxa.1213.1.1
- Costa WJEM (2008) *Simpsonichthys longignatus*, a new seasonal killifish of the *S. flammeus* group from the Pacoti River basin, northeastern Brazil (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 19(2): 155–159.
- Costa WJEM (2017) Description of two endangered new seasonal killifish species of the genus *Cynolebias* from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes, Aplocheilidae). Zoosystematics and Evolution 93(2): 333–341. https://doi. org/10.3897/zse.93.20906
- Costa WJEM, Amorim PF, Mattos JLO (2018a) Cryptic species diversity in the *Hypsolebias* magnificus complex, a clade of endangered seasonal killifishes from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes, Aplocheilidae). ZooKeys 777: 141–158. https://doi.org/10.3897/zse.94.29718
- Costa WJEM, Amorim PF, Mattos JLO (2018b) Diversity and conservation of seasonal killifishes of the *Hypsolebias fulminantis* complex from a Caatinga semiarid upland plateau, São Francisco River basin, northeastern Brazil (Cyprinodontiformes, Aplocheilidae). Zoosystematics and Evolution 94(2): 495–504. https://doi.org/10.3897/zse.94.29718
- Costa WJEM, Amorim PF, Mattos JLO (2018c) Synchronic historical patterns of species diversification in seasonal aplocheiloid killifishes of the semi-arid Brazilian Caatinga. PLoS One 13(2): 1–10. https://doi.org/10.1371/journal.pone.0193021
- Ferreira RL, Prous X, Oliveira LF, Souza-Silva M (2010) Fauna subterrânea do Estado do Rio Grande do Norte: Caracterização e impactos. Revista Brasileira de Espeleologia 1(1): 25–51.
- Fišer C, Zagmajster M, Ferreira RL (2013) Two new Amphipod families recorded in South America shed light on an old biogeographical enigma. Systematics and Biodiversity 11(2): 117–139. https://doi.org/10.1080/14772000.2013.788579
- ICMBio SEDPD (2013) Sumário Executivo do Plano de Ação Nacional para a Conservação dos Peixes Rivulídeos Ameaçados de Extinção. Brasília: Instituto Chico Mendes de Conservação da Biodiversidade.
- Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (2018) Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume VI – Peixes. In: Instituto Chico Mendes de Conservação da Biodiversidade (Ed.) Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. ICMBio, Brasília, 1234.
- Lévêque C, Oberdorff T, Paugy D, Stiassny MLJ, Tedesco PA (2008) Global diversity of fish (Pisces) in freshwater. In: Balian EV, Lévêque C, Segers H, Martens K (Eds) Freshwater Animal Diversity Assessment. Developments in Hydrobiology, Dordrecht, 545–567. https://doi.org/10.1007/978-1-4020-8259-7_53
- Lima SMQ, Ramos TPA, Silva MJ, Rosa RS (2017) Diversity, distribution, and conservation of the Caatinga fishes: advances and challenges. In: Da Silva, JMC, Leal, IR and Tabarelli M (Eds) Caatinga: the largest tropical dry forest region in South America. Springer, Cham, 97–131. https://doi.org/10.1007/978-3-319-68339-3_4

- Lucena CAS, Calegari BB, Pereira EHL, Dallegrave E (2013) O uso de óleo de cravo na eutanásia de peixes. Boletim Sociedade Brasileira de Ictiologia 105: 20–24.
- Malabarba LR, Reis RE (1987) Manual de técnicas para a preparação de coleções zoológicas. Sociedade Brasileira de Zoologia 36: 1–14.
- MMA [Ministério do Meio Ambiente] (2014) Portaria N° 445, de 17 de dezembro de 2014. Lista de peixes e invertebrados aquáticos da fauna brasileira ameaçada de extinção. Diário Oficial da União, Brasília/DF, seção 1, n. 98, 86.
- MMA [Ministério do Meio Ambiente] (2018) Portaria Nº 463, de 18 de dezembro de 2018. Áreas Prioritárias para a Conservação, Utilização Repartição de Benefícios da Biodiversidade Prioritárias para a Biodiversidade. Diário Oficial da União, Brasília/DF, seção 1, n. 243, 160. http://areasprioritarias.mma.gov.br/2-atualizacao-das-areas-prioritarias
- Murphy WJ, Collier GE (1997) A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): The role of vicariance and the origins of annualism. Molecular Biology and Evolution 14(8): 790–799. https://doi.org/10.1093/oxfordjournals.molbev.a025819
- Myers GS (1952) Annual fishes. Aquarium Journal 23: 125-141.
- Nascimento FR, Carvalho O (2003) Gerenciamento ambiental em unidade de conservação: Bacia hidrográfica como estudo de caso. Geografia (Londrina) 12(2): 111–130.
- Nascimento WS, Yamamoto ME, Chellappa NT, Rocha O, Chellappa S (2015) Conservation status of an endangered annual fish *Hypsolebias antenori* (Rivulidae) from Northeastern Brazil. Brazilian Journal of Biology 75(2): 484–490. https://doi.org/10.1590/1519-6984.19113
- Oliveira JF, Ramos TPA, Oliveira VRT, Filgueira RF, Peretti D (2018) First record of an annual endemic fish from a semiarid environment in northeastern Brazil. Neotropical Biology and Conservation 13(3): 249–253. https://doi.org/10.4013/nbc.2018.133.07
- Sabaj MH (2016) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. American Society of Ichthyologists and Herpetologists, Washington, DC.
- Silva JMC, Barbosa LCF, Leal IR, Tabarelli M (2017a) The Caatinga: Understanding the Challenges. In: Silva JMC, Leal IR, Tabarelli M (Eds) Caatinga. Springer, Cham, 3–19. https://doi.org/10.1007/978-3-319-68339-3_1
- Silva MJ, Neves CP, Ramos TPA, Miriam MP, Lima SMQ (2017b) Efetividade das unidades de conservação para a ictiofauna das bacias hidrográficas envolvidas na transposição do rio São Francisco. In: Monteiro RF (Ed.) Pesquisas em unidades de conservação no domínio da Caatinga: subsídios à gestão. Editora da Universidade Federal do Ceará, Fortaleza, 181–205.
- Teixeira FK, Ramos TPA, Paiva REC, Távora MA, Lima SMQ, Rezende CF (2017) Ichthyofauna of Mundaú river basin, Ceará State, Northeastern Brazil. Biota Neotropica 17(1): 1–10. https://doi.org/10.1590/1676-0611-bn-2016-0174
- Tulipano J (1973) *Cynolebias antenori*. Killie Notes. American Killifish Association 6(11): 23–24. [silva]
- Velloso AL, Sampaio EVSB, Pareyn FGC (2002) Ecorregiões: propostas para o bioma caatinga; resultados do seminário de planejamento ecorregional da caatinga. Seminário de Planejamento Ecorregional da Caatinga. TNC/APNE Recife, Aldeia-Pernambuco, 76.