## Correspondence

# Expanding the known range of *Caecilia tentaculata* (Amphibia: Gymnophiona) to relict mountain forests in northeastern Brazil: linking Atlantic forests to the Amazon?

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Caecilians of the family Caeciliidae RAFINESQUE, 1814 comprise two genera, Caecilia LINNAEUS, 1758, with 33 species, and Oscaecilia TAYLOR, 1968, with nine species (WILKINSON et al. 2011). They occur in the eastern parts of the Andes, from Costa Rica in Central America to the south of Bolivia in South America (MACIEL & HOOGMOED 2011, FROST 2015). Both genera occur in Brazil, represented by Caecilia armata DUNN, 1942, C. gracilis SHAW, 1802, C. marcusi WAKE, 1985, C. tentaculata LINNAEUS, 1758, and Oscaecilia hypereumeces TAYLOR, 1968 (MACIEL & HOOG-MOED 2011, SBH 2014). Caecilia, with the largest number of species, is distributed from Panama to southern Bolivia in areas with rainforest (FROST 2015). The type species is Caecilia tentaculata LINNAEUS, 1758, described already in the 'Systema Naturae' with 'America' as its type locality. According to NUSSBAUM & HOOGMOED (1979), the holotype was lost and two new specimens from Surinam (without specific locality data) were referred to as syntypes by DUNN (1942). However, according to DUELLMAN (2012), apparently the type was recently located. This species is found in terrestrial habitats and is categorized as 'Least Concern' by the International Union for Conservation of Nature (IUCN 2016). Until now, Caecilia tentaculata has been regarded as having a distribution linked to the Amazon forest habitat, but here we expand the known distribution to two isolated 'brejos-de-altitude', relict mountain forests surrounded by dry Caatinga scrubland in northeastern Brazil. Isolated 'brejos-de-altitude' have sometimes been considered a biogeographical link between the coastal Atlantic forest and Amazonian forests. Finding *C. tentaculata* in the area might therefore suggest that it is likely present also in Atlantic forest habitats. Herein we confirm the species' identification using both morphological and molecular characters and use environmental modelling to identify if the environmental conditions of this region are suitable to promote the distribution of *C. tentaculata*.

In the state of Ceará, five separate 'brejos-de-altitude' occur, including the Massif of Baturité (4°05'-4°40' S, 38°30'-39°10' W) and the Maranguape Mountain (3°54'-4°03' S, 38°32'-38°40' W), where our specimens were found. They are separated by a gap of about 45 km. Four specimens were collected at both these sites (Fig. 1, Appendix 1) during herpetological surveys, anaesthetised and subsequently killed by injections with lidocaine hydrochloride, fixed with 90% ethanol or 10% formol, and deposited in the Collection of Herpetology of the Universidade Federal of Ceará (CHUFC). Specimens were identified according to TAYLOR (1968) and MACIEL & HOOGMOED (2011) by assessing diagnostic morphological characters.

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To further confirm their specific identity, and to resolve genetic diversity between samples from widely separated geographic regions, two specimens, CHUFC A6775 from Maranguape Mountain and CHUFC A6543 from the Massif of Baturité, were sequenced for a region of the mitochondrial 12S rRNA gene by means of Polymerase Chain Reaction (PCR) amplification, using the primers 12Sa (CTGGGATTAGATACCCCACTAT) / 12Sb (GAGGGT-GACGGGGGGGGGTGTGT) from Kocher et al. (1989). DNA from tissue samples (muscle or liver) was extracted using standard high-salt protocols (SAMBROOK et al. 1989). The PCR protocol was based on a 20-µl reaction mix containing 4.0 µl of Blend MasterMix (Hot Fire), 0.5 µl of 10 pmol primers each, 2.0 µl of DNA, and distilled water making up the balance, following HARRIS et al. (1998). The reaction mix was amplified using the following cycles: 94°C (13 min), 35 × [94°C (30 sec), 47°C (45 sec) and 72°C (1 min)], and 72°C (10 min). Positive and negative checks were run with each reaction. Both positive products were sent for sequencing to Beckman Coulter Genomics. The new sequences were submitted to the GenBank database under accession number KY745902.

The potential model of the *C. tentaculata* distribution was assessed using sampling points in databases (Global Biodiversity Information Facility – GBIF 2016, IUCN 2016) and literature (DUNN 1942, MACIEL & HOOGMOED 2011), using Maxent software for species habitat modeling. Fifty-two points of non-redundant occurrence were plotted across South America, with a precision of 10' ( $18.6 \times 18.6$ =  $344 \text{ km}^2$ ), and posteriorly five non-correlated environmental variables were projected on the mesh and used as predictors of the distribution of the studied species based on its ecology (mean annual temperature, temperature seasonality, precipitation, precipitation seasonality, and precipitation over the warmest quarter). The environmental variables used were downloaded from WorldClim (2016). We also constructed a correlation matrix between all variables and selected only those that were not highly correlated (r < 0.9). The performance of the niche model was evaluated according to the curve between specificity (lack of commission errors) and sensitivity (lack of omission error) and AUC, that is, the point that minimizes the relationship between sensitivity and specificity of the model, assessing their ability to correctly predict the occurrence of the species. The AUC value ranges from 0 to 1, with values > 0.75 being considered significant (ELITH et al. 2006, PHILLIPS & DUDÍK 2008). This analysis was performed with R platform (R Core Team 2016).

BORGES-NOJOSA (2006) recorded two specimens of Gymnophiona from the Massif of Baturité, but due to their poor state of preservation, listed these erroneously as *Chthonerpeton* aff. *arii*, a more aquatic caecilian species usually known from open areas (CASCON & LIMA-VERDE 1994). Diagnostic morphological characters (following TAYLOR 1968, MACIEL & HOOGMOED 2011) of the newly collected specimens clearly indicated that these specimens represented Caecilia tentaculata, a terrestrial forest species: in particular the visible eyes, the uniform grey or blue natural colouration, total body length (up to 850.0 mm), eve-tentacle distance (ET) greater than the distance between nostril-tentacle (NT), total length 30 times larger than the body diameter, and numbers of primary annuli (PA) (112-131) and secondary grooves (SG) (12-54). A 368bp fragment of the 12S rRNA gene was successfully sequenced from each of the two specimens (CHUFC A6543 and A6775). These proved identical for this fragment, but showed five differences (all transition changes) from the sequence of C. tentaculata from GenBank (KF540146) from Kaw in French Guiana (SAN MAURO et al. 2014). Such a low level of differentiation (1.4%) is typical of intraspecific variation; the next most similar sequence (C. gracilis, KF540147) differed by 9%. Limited genetic diversity across

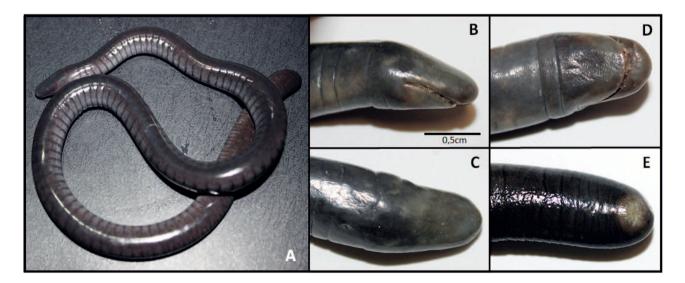


Figure 1. Specimens of *Caecilia tentaculata*: CHUFC A6773, live specimen showing the natural coloration (A); and CHUFC A6774, lateral (B), dorsal (C), and ventral (D) views of the head, and cloacal opening (E). Photos: D. M. BORGES-NOJOSA.

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Table 1. Morphometric (in mm) and meristic data of *Caecilia tentaculata* from Ceará state. Legend: TL – total length; BW – body width at mid-body; HL – head length measured between tip of snout and posterior edge of skull; HW – head width; ET – distance between eye and tentacular aperture; NT - distance between nostril and tentacle; PMT – premaxillary-maxillary teeth; PPT – prevomerine-palatine teeth; DT – dentary teeth; ST – splenial (inner mandibular) teeth; PA – primary annuli; SG – secondary grooves (= primary annuli bearing secondary grooves); Notes: CHUFC A6477 – immature female; CHUFC A6543 – incomplete specimen.

Character	Locality / Voucher Number						
	Massif of Baturité		Maranguape Mountain				
	A6477	A6478	A6479	A2601	A6773	A6774	A6775
Eyes	visible	visible	visible	visible	visible	visible	visible
Coloration	uniform	uniform	uniform	uniform	uniform	uniform	uniform
TL	286.0	268.1	200.3	405.0	363.4	362.6	321.8
BW	9.1	5.6	6.5	_	9.6	9.6	9.8
Proportion TL/BW	31.4	47.7	30.7	-	37.9	37.9	33.0
HL	9.5	10.3	8.5	11.9	12.7	11.6	11.1
HW	7.0	6.6	6	7.8	8.57	8.3	9.3
ET	3.5	4.1	4.0	4.1	4.3	4.5	5.0
NT	1.8	1.2	1.8	1.3	1.9	2.3	2.0
PMT	11	21	19	15	15	16	20
PPT	14	15	13	17	13	16	20
DT	15	19	17	16	16	17	15
ST	2	3	4	3	4	3	4
PA	125	134	121	131	123	122	114
SG	26	23	15	22	20	16	20

large geographic distances has been reported in another but not more closely related caecilian, Ichthyophis bombayensis TAYLOR, 1960, from India (GOWER et al. 2007) and was suggested to indicate recent genetic continuity possibly associated with variables such as a relatively large clutch size. However, as ichthyophiids have an aquatic larval stage (SAN MAURO et al. 2014, KUPFER et al. 2016) that will likely contribute to the dispersal whereas caeciliids are directdevelopers lacking a larval stage, dispersal processes in C. tentaculata are different. Detailed comparisons between *C. tentaculata* from across its entire distribution range may shed further light on how environmental or other variables might be linked to genetic continuity. MACIEL & HOOG-MOED (2011) defined three OTUs (Operational Taxonomic Units) with head length and distance between nostrils as the most discriminant characters in C. tentaculata, although these were based on limited differentiation and had only 60% correctly classified. Based on the few samples available from the "brejos-de-altitude" it is not possible to infer the OTU.

The new record extend the known species range by about 850 km to the east of previously known localities (Viseu, State of Pará; Nova Vida, State of Maranhão) (MA-CIEL & HOOGMOED 2011) (Fig. 2), and add another typical Amazon forest species to the herpetofauna of the brejosde-altitude in Ceará, similar to *Amphisbaena anomala* (BARBOUR, 1914) (BORGES-NOJOSA & CARAMASCHI 2003). These records, in association with other species that have a distribution corridor Amazon-'brejos'-Atlantic, such as the snakes *Lachesis muta* (LINNAEUS, 1766) (BORGES-NOJ- JOSA & LIMA-VERDE 1999), Sibon nebulatus (LINNAEUS, 1758), Imantodes cenchoa (LINNAEUS, 1758) (NASCIMENTO & LIMA-VERDE 1989), Drymoluber dichrous (PETERS, 1863) (BORGES-NOJOSA & LIMA 2001), and the lizard Cercosaura o. ocellata WAGLER, 1830 (BORGES-NOJOSA & CARAMASCHI 2003), support the hypothesis of the Amazon and Atlantic Forest being biogeographically linked. Interestingly, although there is a very small region corresponding to the Brejos, which the model identifies as being appropriate (p > 0.6), the environmental conditions required by the species currently seem to be missing from the Atlantic Forest (Fig. 2). However, this does not by default invalidate the hypothesis that the species was widely distributed in past periods such as the Pleistocene during which climatic conditions facilitated rainforests to expand. The model for the current distribution of the species had a good efficiency rating (AUC = 0.823). The variables that contributed most to it are 'seasonality temperature' (40.6%) and 'precipitation in the warmest quarter' (47%).

The 'brejos-de-altitude' are humid, elevated regions that are isolated within the dry region of Caatinga scrubland. With altitudes ranging between 400 and 1,000 m, they are typically covered with relict forest vegetation from adjacent humid forests (ANDRADE-LIMA 1982, PRANCE 1982, SAN-TOS et al. 2006) with different climatic conditions. They contain a unique mix of flora and fauna of three different biogeographic regions, Amazonian, Atlantic forest, and dry Caatinga (ANDRADE-LIMA 1966, VANZOLINI 1981, SANTOS et al 2006, BORGES-NOJOSA & CARAMASCHI 2003). Various endemic amphibians and reptiles have been reported from

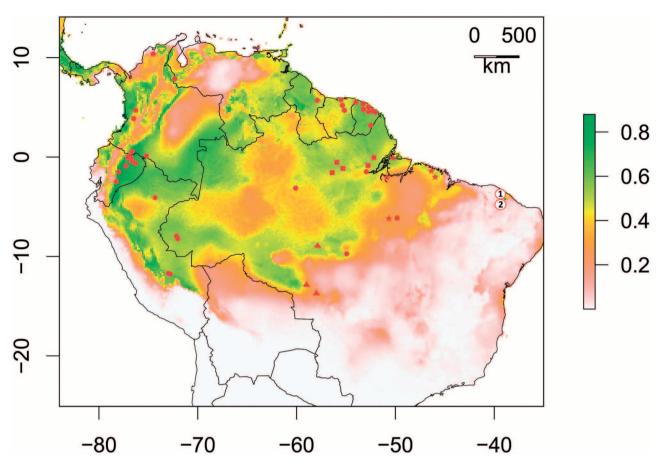


Figure 2. Map of ecological niche modelling for *Caecilia tentaculata*. Squares, triangles and stars are Operational Taxonomic Units (MACIEL & HOOGMOED 2011), circles other localities (GBIF 2016), and numbers are new records (1 – Maranguape Mountain; 2 – Massif of Baturité; specimens of CHUFC) used for modelling. The Maxent default output depicts probability values ranging from 0 to 1, where higher values indicate higher environmental suitability and therefore a higher probability of species occurrence (PHILLIPS & DUDÍK 2008).

here, almost all of which are categorized as 'Threatened' as per IUCN criteria due to the relatively small expanse of the 'brejos' and ongoing environmental destruction. These include amphibians such as Adelophryne baturitensis and A. maranguapensis HOOGMOED, BORGES & CASCON, 1994 (VU), Rhinella casconi Roberto, Brito & Thomé, 2014, the lizards Copeoglossum arajara (REBOUÇAS-SPIEKER, 1981), Leposoma baturitensis Rodrigues & Borges, 1997 (EN), and the snake Atractus ronnie Passos, Fernandes & BORGES-NOJOSA, 2007 (EN) (MMA 2014). The areas of 'brejos' in the state of Ceará are considered 'areas of highest priority', as they represent an Atlantic Forest relict (MMA 2000, BRASIL 2004). Our new records confirm that the 'brejos' contain a mix of florae and faunae of both Amazonian and Atlantic origins. Finding C. tentaculata, about 850 km from the nearest known population, highlights not only the uniqueness of the 'brejos', but also that new and detailed herpetological surveys are urgently needed to comprehensively understand the ancient link between the Amazon and the Atlantic forests, two of the most biodiverse regions of the world.

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## Appendix 1

*Caecilia tentaculata* (8 specimens) Brazil: Ceará: Serra de Maranguape, Maranguape Municipality: CHUFC A2601 (s/NC); CHUFC A6773 (B086); CHUFC A6774 (B284); CHUFC A6775 (B285), Maciço de Baturité, Pacoti Municipality: CHUFC A6477 (DMB916); CHUFC A6478 (DMB483); CHUFC A6479 (DMB684); CHUFC A6543 (BAT03).