

ORIGINAL ARTICLE

Record of the rare Caribbean mud eel, *Pythonichthys sanguineus* (Heterenchelyidae, Anguilliformes), in the region of the Amazon Reef

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ABSTRACT

As they spend most of their time buried in the substrate and are not a fishery resource, heterenchelyids are seldom seen. These eels are characterized by their greatly reduced eyes, which are covered by semi-transparent skin, the absence of a pectoral fin or lateral line, and no pores on the head or body. *Pythonichthys sanguineus* is a particularly poorly-known species, with only eight scientific records from Cuba, Puerto Rico, Colombia, Venezuela, Guyana, and Suriname. The present study is based on six adult specimens of *P. sanguineus* captured by vessels of the shrimp trawling fleet along the northern Brazilian coast, in the vicinity of the Amazon Reef. We provide meristic, morphometric and DNA barcoding data. These findings provide insights into the distribution of the species off the northern Brazilian coast and contribute to the discussion about the southern limit of the Greater Caribbean fauna.

KEYWORDS: fisheries, conservation, Brazilian northern coast, new record, systematics

Registro da rara enguia de lama do Caribe, *Pythonichthys sanguineus* (Heterenchelyidae, Anguilliformes) na região dos Recifes da Amazônia

RESUMO

Heterenchelídeos passam a maior parte do tempo enterrados no substrato e raramente são vistos, não representando recursos pesqueiros importantes. Essas enguias são caracterizadas por seus olhos muito reduzidos e cobertos por pele semi-transparente, ausência de nadadeiras peitorais ou linha lateral, e ausência de poros na cabeça ou no corpo. *Pythonichthys sanguineus* é uma espécie particularmente pouco conhecida, com apenas oito registros em coleção zoológica para Cuba, Porto Rico, Colômbia, Venezuela, Guiana e Suriname. O presente estudo é baseado em seis espécimes adultos de *P. sanguineus* capturados pela frota industrial de arrasto de camarão-rosa na costa norte do Brasil, ao largo dos Recifes da Amazônia. Apresentamos dados merísticos, morfométricos e de DNA barcoding dos exemplares examinados. O registro fornece informações importantes sobre a distribuição da espécie na costa norte do Brasil, e contribui para a discussão sobre o limite sul da fauna do Grande Caribe.

PALAVRAS-CHAVE: pesca, conservação, costa norte do Brasil, novo registro, sistemática

INTRODUCTION

The order Anguilliformes includes 938 species in 19 families and 159 genera (Nelson *et al.* 2016). The marine mud eels of the family Heterenchelyidae, a sister group to the Myrocongridae and Muraenidae (Inoue *et al.* 2010), are a small group of two genera, *Panturichthys* Pellegrin 1913 and *Pythonichthys* Poey 1868, found in the Mediterranean, tropical Atlantic, and eastern Pacific (Nelson *et al.* 2016). As they spend most of their time buried in the substrate, heterenchelyids are seldom seen, not representing a fishery resource (Smith 2015). These eels, which

can reach a total length of 1 m, are characterized by their greatly reduced eyes, which are covered by semi-transparent skin, the absence of a pectoral fin or lateral line and no pores on the head or body (Smith 1989).

At least four species of *Pythonichthys* are known to exist. *Pythonichthys microphthalmus* (Regan 1912) and *Pythonichthys macrurus* (Regan 1912) are found in the Eastern Atlantic (EA), while *Pythonichthys asodes* Rosenblatt & Rubinoff 1972 occurs in the Eastern Pacific (EP), and *Pythonichthys sanguineus* Poey 1868 is the only species found in the Western

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Atlantic (WA) (Figure 1a). Smith (1989) described the history and diversity of the heterenchelyids and redescribed the osteology of *P. asodes* and *P. macrurus*. Eagderi and Adriaens (2010) also described the osteology of these species, Blache (1977) described the leptocephalus larvae from the Gulf of Guinea. *Pythonichthys sanguineus*, a senior synonym of *Heterenchelys biaggii* Böhlke (1956) (*sensu* Smith 1989; Smith *et al.* 2012) is the least well known species of the genus, and nothing is known of its biology (Smith 1989, 2002; Smith *et al.* 2012). Only eight records of adult specimens of *P. sanguineus* are found in zoological collections (in 10 collection lots), representing six different localities. Two specimens are known from Cuba (MCZ 32805 and USNM 111340) and three from Puerto Rico, including the type specimens of *Heterenchelys biaggii* (FMNH 61775, ANSP 73872 and FMNH 61774). From South America, five specimens are available from Venezuela (four specimens, MBUCV 293 and one specimen from the Fernandez Yopez's collection), and one each from Colombia (UMML 222150), Guyana (BMNH 1961.8.31.99) and Suriname (NSMT 40060), while the 11 specimens examined by Poey (1868) have been lost (Faloh-Gandarilla *et al.* 2016).

Considering the general lack of data on the fish diversity of the northern coast of Brazil (Marceniuk *et al.* 2013; Marceniuk *et al.* 2017), the Centro de Pesquisa e Gestão de Recursos Pesqueiros do Litoral Norte (CEPNOR), together with

taxonomists from the Museu Paraense Emílio Goeldi (MPEG), in Belém, Brazil, has obtained specimens from the industrial shrimp otter trawling fleet based in the Brazilian state of Pará, in a concentrated effort to expand the scientific inventory of the marine fish diversity of the region. This sampling effort has resulted in the collection of six specimens of *P. sanguineus*. Meristic, morphometric and DNA barcoding data are presented (cataloged specimens), and provide important insights into the distribution and diversity of the genus in the western Atlantic.

MATERIAL AND METHODS

The present report on *Pythonichthys sanguineus* is based on three adult specimens collected in Amapá state on April 28, 2017 and March 2-3, 2018 (cataloged, Figure 1b,c) and other three adult specimens captured on March 5, 2018 (not cataloged, Figure 1d,e), all captured by vessels of the shrimp trawling fleet. The cataloged specimens were deposited in the zoological collections of Museu Paraense Emílio Goeldi (MPEG 35269, 493 mm TL (total length), 2°19'42"N, 48°36'46"W, 50 m), Acervo Zoológico da Universidade Santa Cecília (AZUSC 5314, 650 mm TL, 2°19'39"N, 48°26'35"W, 50 m), and the fish collection of Laboratório de Biologia e Genética de Peixes of Universidade Estadual Paulista Júlio de Mesquita Filho (LBP 26612, 431 mm TL, 1°52'45"N, 48°16'32"W, 44 m) (see Material Examined). The specimens were identified based on the original description and subsequent references (Smith 1989, 2002, Smith *et al.* 2012), and measured according to Smith (1989). All measurements were taken using either a digital calliper (precision 0.01 mm) or a ruler (precision 1 mm) for dimensions over 200 mm. The data on the occurrence of *P. sanguineus* were obtained from the zoological collection records available at <http://www.fishnet2.net> and in Smith (1989).

Molecular analysis

Molecular identification of the specimens was done according to the DNA barcoding protocol (Hebert *et al.* 2003) and deposited in the BOLD system (Ratnasingham and Hebert 2007) and in Genbank (<https://www.ncbi.nlm.nih.gov/genbank>). A partial COI sequence (578 bps) was obtained from a single *P. sanguineus* specimen deposited at Museu Paraense Emílio Goeldi (MPEG 35269), and deposited in the Genbank database (accession number: MG833002) and BOLD (RMRBR001-18). For comparison, COI sequences of additional 57 anguilliforms were obtained from NCBI (see Supplementary Material, Appendix S1). These sequences were derived from a phylogenetic study that analyzed the mitochondrial genomes of all 19 anguilliform families (Inoue *et al.* 2010) and represent an almost complete sample of Anguilliformes at genus level.

The molecular assays were conducted at the Laboratory of Fish Biology and Genetics at Universidade Estadual Paulista Júlio de Mesquita Filho in Botucatu, São Paulo, Brazil. Total genomic DNA was isolated from muscular

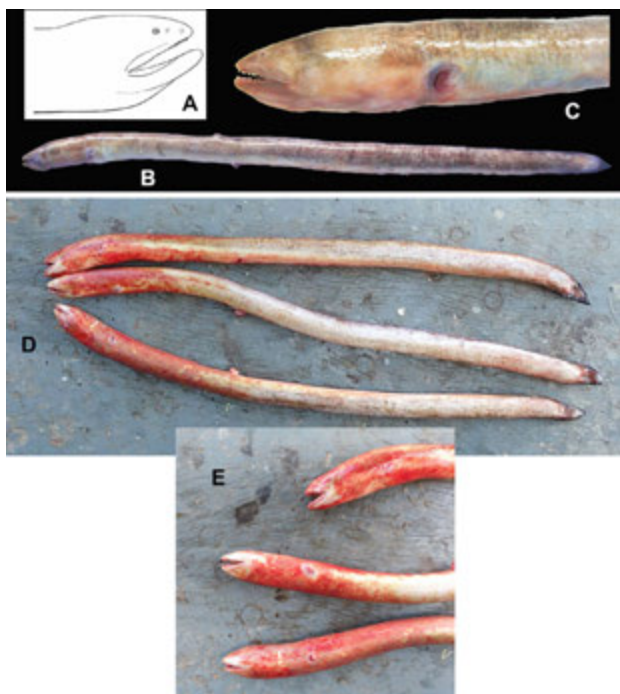


Figure 1. *Pythonichthys sanguineus*, body and head in lateral view. A. Holotype drawn by Poey (1868); B-C. Cataloged Brazilian specimen (MPEG 35269); D-E. Non-cataloged Brazilian specimen. This figure is in color in the electronic version.

tissue using an automated glass fiber protocol (Ivanova *et al.* 2008). The fragment at the 5' end of the mitochondrial COI gene was amplified by PCR using the primers FishF1 and FishR1 (Ward *et al.* 2005). The reactions were carried out in a 12.5 µL reaction volume containing 1.25 µL of 10 × PCR buffer, 0.375 µL MgCl₂ (50 mM), 0.5 µL of the dNTPs (2 mM), 0.25 µL of each primer (5 µM), 0.2 µL of 1.25 U Taq platinum DNA polymerase, and 1 µL of the DNA template (200 ng), and 8.675 µL of ultrapure water. The PCR cycle was as follows: 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 54°C for 45 s, 72°C for 1 min, and then 72°C for 5 min. The PCR products were visualized in a 1% agarose gel and purified using ExoSAP-IT (USB Europe GmbH, Staufen, Germany) at 37°C for 60 min followed by 15 min at 80°C. The sequencing reactions were conducted using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, California, USA) according to the manufacturer's instructions. The sequences were read in both the forward and reverse directions, and the primers used for sequencing were the same as those used for the PCR reaction. Finally, the labeled sequences were assessed using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, California, USA).

Bidirectional sequences were assembled using Geneious v.5.6 (Kearse *et al.* 2012) to obtain consensus sequences and to check for indels or stop codons. Indels or stop codons, which may indicate the amplification of nuclear mitochondrial DNA segment (NUMTs), were absent from all sequences. Genetic distances between and within taxonomic groups were calculated using the Kimura 2-parameter (K2P) substitution model (Kimura 1980) in MEGA 7 (Kumar *et al.* 2016). The best model of nucleotide substitution was GTR+G+I as calculated in MEGA 7 (Kumar *et al.* 2016). The maximum likelihood tree was obtained in MEGA 7 (Kumar *et al.* 2016) using the GTR+G+I model, with node supports estimated by 1000 nonparametric bootstrap replicates (Felsenstein 1985).

RESULTS

Pythonichthys sanguineus Poey 1868

Pythonichthys sanguineus Poey 1868: 265 (original description); Uyeno and Sasaki in Uyeno *et al.* 1983:101 (Suriname); Smith 1989:52 (taxonomic considerations); Cervigón 1991:29 (Venezuela); Aguilera 1998:46 (Venezuela); Smith 2002:694 (Western Central Atlantic); Smith *et al.* 2012:492 (Caribbean); Faloh-Gandarilla *et al.* 2016:28 (Cuba).

Heterenchelys biaggii Böhlke 1956:8, Fig. 2A (original description, Puerto Rico).

Diagnosis

Some authors have expressed doubts about the distinction between the genera *Pythonichthys* and *Panturichthys* (Rosenblatt and Rubinoff 1972). The identification of the

specimens analyzed here as *Pythonichthys* was supported by the diagnostic characters proposed by Blache (1968): the absence of a longitudinal cutaneous ridge on the upper surface of the head (*vs.* present in *Panturichthys*), the absence of the fleshy fold located posteriorly on the inner edge of the maxilla (*vs.* present in *Panturichthys*), the presence of a gap between the vomerine and ethmoid dentition (*vs.* absent in *Panturichthys*), the inner row of maxillary teeth is approximately equal in length to the outer row (*vs.* shorter in *Panturichthys*), the pectoral girdle originates dorsally (*vs.* on or slightly anterior to the pectoral waist in *Panturichthys*), head and trunk 22–36% TL (*vs.* 12–20% TL in *Panturichthys*, Table 1), and 109–134 vertebrae (*vs.* 141–227 in *Panturichthys*, Table 1).

While counts of vertebrae and proportional measurements can be used to distinguish *P. sanguineus* from *P. macrurus* (EA) and *P. asodes* (EP), these parameters are highly similar to those observed in *P. microphthalmus* (EA). *Pythonichthys sanguineus* can nevertheless be distinguished from *P. microphthalmus* by the number of vomerine tooth rows (2–3), in contrast with a single row in *P. microphthalmus*, and 3–5 maxillary and mandibular tooth rows (*vs.* 2 rows in *P. microphthalmus*). The two species are also differentiated clearly by the molecular data (see below).

Molecular analysis

Maximum likelihood analysis showed that the specimen analyzed here clusters with *Pythonichthys microphthalmus*, with a bootstrap support of 60% (Figure 2), corroborating the assignment of our specimens to the genus *Pythonichthys*. The K2P genetic distance between *P. sanguineus* and *P. microphthalmus* was 25.0 ± 1.9%, with 89/578 positions being different between species, corroborating our hypotheses that these are in fact different species.

Morphological measurements

The morphometric and meristic parameters of the specimens analyzed in here did not exceed the amplitude of values presented in the original description or subsequent studies (Table 1).

Color Pattern

Scharpf and Lazara (2017) refer to the coloration of fresh *Pythonichthys sanguineus* as blood-red. The living specimens (Figure 1d,e) presented the same coloration described by Scharpf and Lazara (2017), while the fresh specimen was light gray, with a brownish anterior dorsal region extending to approximately the anus, and a pale belly (Figure 1b,c). The distal portions of the dorsal and anal fins were blackened posteriorly from the median region of the body.

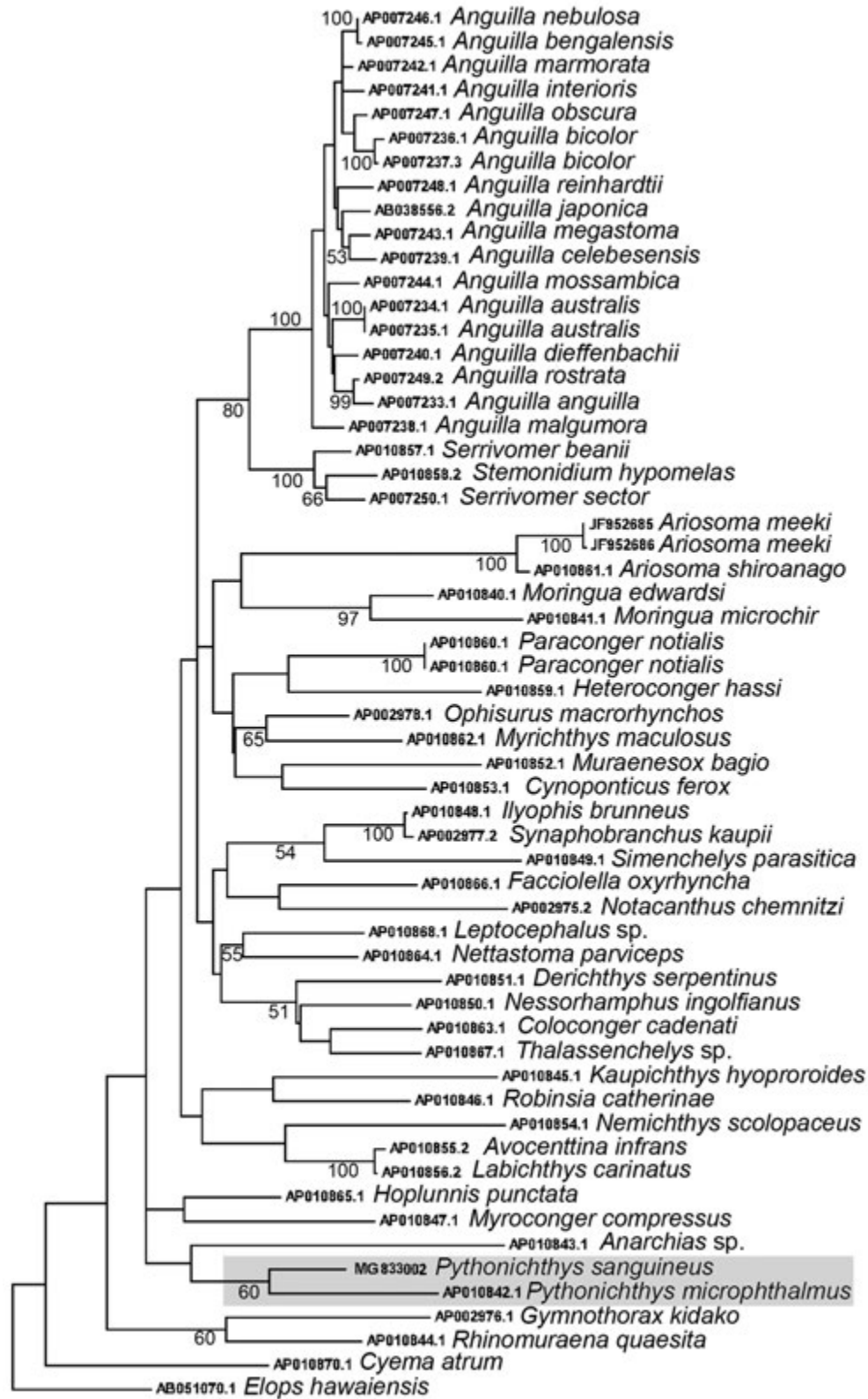


Figure 2. Maximum likelihood tree of the Anguilliformes species analyzed, using GTR+G+I genetic model. Bootstrap values greater than 50 are shown. Species of the genus *Pythonichthys* are highlighted in gray.

Table 1. Morphometric and meristic data of three specimens of *Pythonichthys sanguineus* captured in the vicinity of the Amazon Reef and other geographic areas (Smith, 1989), expressed in millimeters (mm), percent of total length (TL%) or percent of standard length (SL%).

	MPEG 35269	AZUSC 5314	LBP 26612	Amazon Reef specimens	Other geographic areas
				Total length %	Total length %
Total length	493	650	431		
Preanal length	173.2	223.0	142.0	33.0–35.1	33–37
Predorsal length	61.5	71.0	49.0	10.9–12.5	10–15
Head length	59.7	70.0	48.7	10.8–12.1	11–13
Trunk length	116.3	153.0	93.0	21.6–23.6	
Tail length	317.0	427.0	295.0	64.3–68.5	
Depth at anus	20.2	29.3	16.9	3.9–4.5	3
Depth at gill opening	20.9	26.7	18.0	4.1–4.2	4–6
				Standard length %	Standard length %
Snout length	9.0	9.6	7.9	13.7–16.2	15–19
Interorbital	7.5	11.6	6.9	12.6–16.6	12–15
Interbranchial	16.4	15.7	9.5	19.5–27.5	15–25
Gill opening	5.1	11.3	5.0	8.5–16.1	9–14
Jaw length (snout to rictus)	19.5	25.0	15.3	31.4–35.7	27–36
Eye diameter	1.3	1.7	1.5	2.2–3.1	2–5
Total vertebrae number	110				110–111
Predorsal vertebrae number	8				8–9
Preanal vertebrae number	36				36–38
Caudal vertebrae number	74				
Vomerine teeth rows			2		
Maxillary teeth rows			3		
Mandibular teeth rows		3 anterior and 4 posterior			
Maxillary teeth length (internal and external rows)		same size			

Distribution

Tropical Western Atlantic Ocean, from Cuba to Brazil (Figure 3). The present study extends the occurrence of the Atlantic mud eels from Suriname to Amapá state in northern Brazil.

Doubtful records from the Brazilian coast

The occurrence of *Pythonichthys sanguineus* in Brazilian waters was reported by Miranda-Ribeiro (1919). This record was based on the observation (no specimen collected) of an eel at Trindade Island, off the eastern coast of Brazil, which was identified as *Pythonichthys sanguineus* based on its intense red coloration. This record was considered doubtful by Simon *et al.* (2013) and was not accepted by Pinheiro *et al.* (2015). Hudson Pinheiro (personal information) confirmed that the species does not occur at Trindade Island (Figure 3).

Biology, habitat, depth, and presumed schooling pattern

There is very little information available for this species, that inhabits muddy substrates of reefs and rocky areas (Smith 2015). *Pythonichthys sanguineus* has previously been recorded

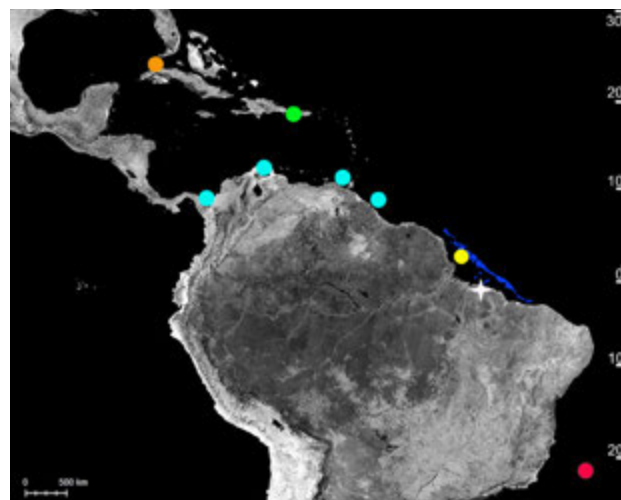


Figure 3. Records of *Pythonichthys sanguineus* in the western Atlantic. Type-locality of *Pythonichthys sanguineus* = orange; type-locality of *Heterenchelys biaggii* = green; non-type specimens = light blue; Brazilian specimens = yellow; doubtful records = red; Pirabas Formation (Aguilera *et al.* 2014) = white star; Amazon Reef = dark blue. This figure is in color in the electronic version.

at depths of 12–55 m. The cataloged specimens were captured on a gravel bottom, at a depth of between 44 and 50 m, at 2°19'42"N, 48°36'46"W to 1°52'45"N, 48°16'32"W (Figure 3), and the non cataloged specimens were captured on a gravel bottom, at a depth of 70 m, at 2°26'73"N, 48°41'52"W (Figure 3), both sites in the vicinity of the Amazon Reef (Moura *et al.* 2016; Francini-Filho *et al.* 2018).

Fisheries and conservation

Otter trawling is practiced worldwide, and is characterized by the largest discarded catches, causing profound impacts on the environment through the destruction of bottom substrates and the elimination of benthic organisms such as coral reefs (Stobutzki *et al.* 2001). In this context, the capture of the mud eel specimens reported here always occurred after the end of the closure periods determined by the government to protect shrimp stocks. No other specimens were caught after the beginning of the shrimping season, indicating that the fishing activity may have influence on the occurrence of the species in the fishing zone, as this pattern was observed during the two sampling seasons. This question clearly requires further investigation, given the well-known impact of otter trawling on benthic communities.

DISCUSSION

Pythonichthys sanguineus is the only species of the genus found in the tropical western Atlantic, and is known from localities in the Caribbean and northern South America, being recognized as part of the typical Greater Caribbean fauna (*sensu* Robertson and Cramer 2014). The present record of *P. sanguineus* from the vicinity of the Amazon Reef (Moura *et al.* 2016; Francini-Filho *et al.* 2018) is relevant to the discussion on the range of the southern limit of the Greater Caribbean fauna, which has been inferred by different authors to reach the region from the Gulf of Paria in Venezuela to Suriname (Petruich 2013) or specific points on the Brazilian coast (Robins 1971), an imprecision that stems from the lack of any prominent physical barriers in the region. In this context, the Amazonian-Orinoco plume, formed by the rearrangements of the hydrographic basins of the Amazon (Shepard *et al.* 2010) and the Orinoco (Rod 1981), during the middle to late Miocene (7.2 mya) (Figueiredo *et al.* 2009; Hoorn *et al.* 2017), has been recognized as an important barrier to the dispersal of coral reef fishes of the Caribbean and Brazil (Bernal and Rocha 2011).

Aguilera *et al.* (2014) described *Pythonichthys pirabasensis* based on a fossil from the Aquitanian period of the early Miocene (20.4–23.0 mya), from the Pirabas Formation of Atalaia Beach (municipality of Salinópolis, in the northern Brazilian state of Pará) and concluded that the current interruption of the shallow marine bioprovince, found off the Amazon delta cannot be perceived in the fish fauna of the Pirabas Formation, which is closely related to that of the

Gatunian/proto-Caribbean bioprovince (Petuch 1982, 2013; Landau *et al.* 2008). The shift of the mouth of the Amazon River, close to its present day location, has terminated the carbonate cycle of the Pirabas Formation, pushing the Pirabas fish fauna northwards, including *Pythonichthys* and the congrid genus *Paraconger* Kanazawa 1961, also captured with the *Pythonichthys* specimens described herein (A.P. Marceniuk, unpublished data).

Our record of *Pythonichthys sanguineus* indicates that the shift in the location of the Amazon plume may not have displaced the fauna of the Pirabas Formation further north, but only modified the distribution of these lineages from the coastal zone further offshore, to an area where environmental conditions are more similar to those of the Pirabas Formation of the early Miocene (Aquitanian). This area coincides with the recently-discovered coral reefs off the mouth of the Amazon. The fauna associated with the reefs is still poorly-known, but is possibly closely related to those of the Pirabas Formation and the present-day Caribbean (Robertson and Cramer 2014).

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SUPPLEMENTARY MATERIAL

(only available in the electronic version)

ROTUNDO *et al.* Record of the rare Caribbean mud eel, *Pythonichthys sanguineus* (Heterenchelyidae, Anguilliformes), in the region of the Amazon Reef

Appendix S1. GenBank system accession numbers of the sequences of *Pythonichthys sanguineus* and 57 other anguilliforms used in the present study.

Species	BOLD Accession #
<i>Anarchias</i> sp.	AP010843.1
<i>Anguilla anguilla</i>	AP007233.1
<i>Anguilla australis</i>	AP007234.1
<i>Anguilla australis</i>	AP007235.1
<i>Anguilla bengalensis</i>	AP007245.1
<i>Anguilla bicolor</i>	AP007236.1
<i>Anguilla bicolor</i>	AP007237.3
<i>Anguilla celebesensis</i>	AP007239.1
<i>Anguilla dieffenbachii</i>	AP007240.1
<i>Anguilla interioris</i>	AP007241.1

Appendix S1. Continued.

Species	BOLD Accession #
<i>Anguilla japonica</i>	AB038556.2
<i>Anguilla malgumora</i>	AP007238.1
<i>Anguilla marmorata</i>	AP007242.1
<i>Anguilla megastoma</i>	AP007243.1
<i>Anguilla mossambica</i>	AP007244.1
<i>Anguilla nebulosa</i>	AP007246.1
<i>Anguilla obscura</i>	AP007247.1
<i>Anguilla reinhardtii</i>	AP007248.1
<i>Anguilla rostrata</i>	AP007249.2
<i>Ariosoma meeki</i>	ABFJ241-07
<i>Ariosoma meeki</i>	ABFJ242-07
<i>Ariosoma shiroanago</i>	AP010861.1
<i>Avocettina infans</i>	AP010855.2
<i>Coloconger cadenati</i>	AP010863.1
<i>Cyema atrum</i>	AP010870.1
<i>Cynoponticus ferox</i>	AP010853.1
<i>Derichthys serpentinus</i>	AP010851.1
<i>Elops hawaiiensis</i>	AB051070.1
<i>Facciolella oxyrinchya</i>	AP010866.1
<i>Gymnothorax kidako</i>	AP002976.1
<i>Heteroconger hassi</i>	AP010859.1
<i>Hoplunnis punctata</i>	AP010865.1
<i>Ilyophis brunneus</i>	AP010848.1
<i>Kaupichthys hyoproroides</i>	AP010845.1
<i>Labichthys carinatus</i>	AP010856.2
<i>Leptocephalus</i> sp.	AP010868.1
<i>Moringua edwardsi</i>	AP010840.1
<i>Moringua microchir</i>	AP010841.1
<i>Muraenesox bagio</i>	AP010852.1
<i>Myrichthys maculosus</i>	AP010862.1
<i>Myroconger compressus</i>	AP010847.1
<i>Nemichthys scolopaceus</i>	AP010854.1
<i>Nessorhamphus ingolfianus</i>	AP010850.1
<i>Nettastoma parviceps</i>	AP010864.1
<i>Notacanthus chemnitzii</i>	AP002975.2
<i>Ophichthus altipennis</i>	ABFJ240-07
<i>Ophisurus macrorhynchus</i>	AP002978.1
<i>Paraconger notialis</i>	AP010860.1
<i>Pythonichthys micropthalmus</i>	AP010842.1
<i>Pythonichthys sanguineus</i>	MG833002
<i>Rhinomuraena quaesita</i>	AP010844.1
<i>Robinsia catherinae</i>	AP010846.1
<i>Serrivomer beanii</i>	AP010857.1
<i>Serrivomer sector</i>	AP007250.1
<i>Simenchelys parasitica</i>	AP010849.1
<i>Stemonidium hypomelas</i>	AP010858.2
<i>Synaphobranchus kaupii</i>	AP002977.2
<i>Thalassenchelys</i> sp.	AP010867.1



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