Zoologica Scripta

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

Akawaio penak, a new genus and species of Neotropical electric fish (Gymnotiformes, Hypopomidae) endemic to the upper Mazaruni River in the Guiana Shield

Javier A. Maldonado-Ocampo, Hernán López-Fernández, Donald C. Taphorn, Calvin R. Bernard, William G. R. Crampton & Nathan R. Lovejoy

Submitted: 4 March 2013
Accepted: 29 July 2013
doi:10.1111/zsc.12035

Maldonado-Ocampo, J.A., López-Fernández, H., Taphorn, D.C., Bernard, C.R., Crampton, W.G.R. & Lovejoy, N.R. (2014). *Akawaio penak*, a new genus and species of Neotropical electric fish (Gymnotiformes, Hypopomidae) endemic to the upper Mazaruni River in the Guiana Shield. —*Zoologica Scripta*, *43*, 24–33.

Akawaio penak, a new genus and species, is described from the upper Mazaruni River, Guyana. The new species is diagnosed from all other species of Hypopomidae by several anatomical traits. The phylogenetic affinities of the new genus were inferred using data from one nuclear (rag2) and two mitochondrial (COI and cyt *b*) genes. The phylogenetic analyses indicate that *Akawaio* is the sister taxon of a clade that includes *Brachyhypopomus*, *Hypopomus*, *Microsternarchus* and *Racenisia*. These results provide evidence for the phylogenetic composition of Hypopomidae supported by previous molecular studies and support the position of the Steatogenini (*Hypopygus* + *Steatogenys*) as the sister group of *Rhamphichthys* + *Gymnorhamphichthys*. The description of this new electric knifefish increases the total number of endemic genera and species in the upper Mazaruni, a region that is suffering freshwater habitat degradation as consequence of gold-mining activities.

Corresponding author: *Javier A. Maldonado-Ocampo, Carrera 7 N° 43-82, Bogotá, DC, Colombia. E-mail: maldonadoj@javeriana.edu.co*

Javier A. Maldonado-Ocampo, Unidad de Ecología y Sistemática (UNESIS), Departamento de Biología, Facultad de Ciencias, Pontificia Universidad Javeriana, Carrera 7 N° 43-82, Bogotá, DC, Colombia. E-mail: maldonadoj@javeriana.edu.co

Hernán López-Fernández, Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, ON, Canada M5S 2C6 and Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON, Canada M5S 3B2. E-mail: hernanl@rom.on.ca

Donald C. Taphorn, 1822 North Charles Street, Belleville, IL, 62221, USA. E-mail: taphorn@gmail.com

Calvin R. Bernard, Centre for the Study of Biodiversity, University of Guyana, Georgetown, Guyana. E-mail: calrber@gol.net.gy

William G. R. Crampton, Department of Biology, University of Central Florida, Orlando, FL, 32816-2368, USA. E-mail: crampton@mail.ucf.edu

Nathan R. Lovejoy, Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON, Canada M5S 3B2 and Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON, Canada M1C 1A4. E-mail: lovejoy@utsc.utoronto.ca

Introduction

The Mazaruni River of Guyana runs from its origin in the remote Pakaraima Mountains to its confluence with the Cuyuni River near the town of Bartica. The upper Mazaruni runs through the eastern edge of the Guiana Shield escarpment and is separated from the lower portion of the drainage by a series of waterfalls that create a formidable barrier for fish movement. The rivers and streams of the upper Mazaruni drain the eastern 'Pantepui' region of the Guiana Shield and remained almost completely unexplored

until very recently. The remoteness and extraordinary biodiversity of the Guiana Shield highlands make this region of significant evolutionary and ecological interest (e.g. Rull 2005, 2007; Vari & Ferraris 2009; Salerno *et al.* 2012). However, the absence of detailed faunal surveys has limited our understanding of biological diversity and conservation challenges in the region (Alofs *et al.* 2013).

Since 2008, the Royal Ontario Museum (ROM) and the University of Guyana (UG) have made several expeditions to explore the fish fauna of the upper Mazaruni. These expeditions and ongoing ROM research programmes have made it clear that this region hosts an astonishing level of endemic fish diversity (Alofs *et al.* 2013). Indeed, 11 endemic species have now been described from the upper Mazaruni, as well as at least three endemic genera. The endemic genera represent a cross-section of the Neotropical fish fauna, including the cichlid *Mazarunia* (Perciformes; Kullander 1990; López-Fernández *et al.* 2012), the lebiasinid *Derrhamia* (Characiformes; Géry & Zarske 2002), the loricariid *Paulasquama* (Siluriformes; Armbruster & Taphorn 2011) and possibly the crenuchid *Skiotocharax* (Characiformes), (Presswell *et al.* 2000 and see López-Fernández *et al.* 2012).

In 2008 and 2011, ROM/UG expeditions collected specimens of a gymnotiform electric knifefish that could not be identified based on existing taxonomy. Here, we describe these specimens as a new genus and species of the family Hypopomidae based on characters of external morphology. We also provide a molecular phylogenetic analysis of the superfamily Rhamphichthyoidea (Hypopomidae + Rhamphichthyidae) and comment on the phylogenetic placement of the new genus within this clade.

Materials and methods

Morphological data

Morphological diagnosis is based on data from the original descriptions of other Hypopomidae, from Sullivan (1997), and comparison with cleared and stained (cs) *Hypopomus artedi* specimens. Measurements were taken as point-to-point linear distances using digital callipers with a precision of 0.1 mm as follows: total length (TL) – distance from the tip of the snout to the end of the caudal filament; length to end of anal fin (LEA) – the distance from the tip of the snout to the end of the base of the anal fin; anal-fin base – the distance between the bases of the first and last rays of the anal fin; preanal-fin distance – the distance from the tip of the snout to the base of the first anal-fin ray; snout to anus – the distance from the tip of the snout to the anterior margin of the anus; greatest body depth – the greatest vertical extent of the body at the beginning of anal fin; head length (HL) – the distance from the tip of the snout to the posterodorsal angle of the opercular bone; head depth at eye – the head depth measured at the centre of the eye; head depth at occiput – vertical distance at nape to ventral body border; head width – the head width measured at the opercular region; snout length – the distance from the tip of the snout to the anterior margin of the eye; postorbital distance – the distance from the posterior margin of the eye to the posterodorsal limit of the opercular bone; eye diameter – the horizontal width of the eye; interorbital width – the minimum width between the dorsal margins of the orbits; posterior naris to snout – the distance from the anterior border of the naris to the tip of the snout; posterior naris to eye – the distance from the posterior border of the naris to the anterior margin of the eye; branchial opening – the height of the opening measured along the vertical; mouth length – the distance from the tip of the snout to the rictus of the mouth; pectoral-fin length – the distance from the base of the dorsalmost ray of the pectoral fin to the distalmost point on the margin of the fin; prepectoral-fin distance – the distance from the tip of the snout to the base of the dorsalmost pectoral-fin ray.

Anal and pectoral-fin ray counts were taken using a dissecting microscope with transmitted backlight. Vertebral counts were taken from radiographs, excluding the first four vertebra that make up the weberian apparatus. Osteological data were taken from cleared and stained (cs) specimens following Taylor & Van Dyke (1985). Institutional abbreviation followed Fricke & Eschmeyer (2012).

Molecular data

Taxon sampling. A total of 23 individuals representing nine genera and 12 species of Rhamphichthyoidea were used in this study (Table 1). Additionally, seven individuals were included as out-groups representing four species from the families Apteronotidae (one sp.), Gymnotidae (one sp.) and Sternopygidae (two spp.; Table 1). Specimens were collected in the field by the authors and colleagues. Muscle tissues and or fin clips were sampled and stored in either 95–100% ethanol or a buffered solution consisting of 20% DMSO and 0.25 M EDTA at pH 8, saturated with NaCl.

DNA extraction, PCR amplification and sequencing. DNA sequences of two mitochondrial genes [cytochrome *b* (cyt *b*) and cytochrome *c* oxidase subunit I (COI)] and one nuclear gene [recombination activating gene subunit 2 (rag2)] were obtained. Total genomic DNA from muscle or fin-clip samples was isolated using DNeasy Tissue Kits (QIAGEN, Hilden, Germany). Whenever possible, two specimens per terminal taxon were sequenced to confirm sequence identity. Primers for all loci sequenced in this study are listed in Table 2. The amplifications were performed for each locus as follows: COI, a total volume of 25 μL, containing 2.5 μL of 10× reaction buffer, 1.5 μL of dNTP mix at 10 mM each, 1 μL of each primer at 10 μM,

Table 1 List of specimens included in study

Species	Specimen number	Voucher specimen	Locality (Country, Drainage)	Genbank accession number		
				Rag2	COI	Cytb
Brachyhypopomus brevirostris	2617	UF 116556	Peru, Nanay	GQ862536	KF533324	GQ862588
B. brevirostris	7019	UF 177359	Suriname, Commewijne	KF533301	KF533325	KF533280
Brachyhypopomus occidentalis	8780	MCNG (uncataloged specimen)	Venezuela, Maracaibo	KF533302	KF533326	KF533281
Brachyhypopomus pinnicaudatus	2121	MCP 45281	Brazil, Amazonas	KF533303	KF533327	KF533282
B. pinnicaudatus	2122	MCP 45433	Brazil, Amazonas	KF533304	KF533328	KF533283
Brachyhypopomus sp.PALE	8783	MECN-DP 001496	Ecuador, Esmeraldas	KF533305	KF533329	KF533284
B. sp. PALE	2432	UF 148572	Ecuador, Palenque	GQ862539	KF533330	GQ862591
Hypopomus artedi	2232	ANSP 179505	Guyana, Mazaruni	GQ862585	KF533331	GQ862637
H. artedi	2233	AUM 35574	Guyana, Mazaruni	KF533306	KF533332	KF533285
Hypopygus lepturus	8798	MNRJ 33615	Brazil, Xingú-Tapajós	KF533307	KF533333	KF533286
H. lepturus	8799	MNRJ 33615	Brazil, Xingú-Tapajós	–	KF533334	KF533287
Akawaio penak	8795	ROM 83884	Guyana, Mazaruni	KF533308	KF533335	KF533288
A. penak	8796	ROM 83884	Guyana, Mazaruni	KF533309	KF533336	KF533289
Microsternarchus bilineatus	2137	MCP 45480	Brazil, Amazonas	–	–	KF533290
M. bilineatus	2138	MCP 45463	Brazil, Amazonas	KF533310	–	KF533291
Racenisia fimbriipinna	2339	UF 177352	Venezuela, Orinoco	KF533311	KF533337	KF533292
R. fimbriipinna	2340	UF 177352	Venezuela, Orinoco	KF533312	KF533338	KF533293
Steatogenys elegans	8807	INPA 28860	Brazil, Negro	KF533313	KF533339	KF533294
S. elegans	8808	INPA 28877	Brazil, Negro	KF533314	KF533340	–
Gymnorhamphichthys rondoni	2153	MCP 46936	Brazil, Amazonas	KF533315	–	–
G. rondoni	2154	MCP 46936	Brazil, Amazonas	KF533316	–	–
Rhamphichthys rostratus	8825	LFCE 0802210711A_MOU46	Brazil, Negro	KF533317	KF533341	KF533295
R. rostratus	8827	MNRJ 33659	Brazil, Negro	KF533318	KF533342	–
Apteronotus albifrons	8687	MNRJ 33618	Brazil, Xingú-Tapajós	KF533319	KF533343	KF533296
Gymnotus carapo	2006	UF 131129	Peru, Amazonas	GQ862549	KF533344	GQ862601
G. carapo	2007	UF 131129	Peru, Amazonas	GQ862550	KF533345	GQ862602
Eigenmannia virescens	9703	STRI 02154	Peru, Amazonas	KF533320	KF533346	KF533297
E. virescens	9704	STRI 02154	Peru, Amazonas	KF533321	KF533347	KF533298
Sternopygus macrurus	9051	MZUSP 99361	Brazil, Tapajos	KF533322	KF533348	KF533299
S. macrurus	9054	ANSP 189024	Brazil, Negro	KF533323	KF533349	KF533300

Table 2 Primers used for amplification and sequencing of the COI, cyt *b* and rag2 genes

Primer	Primer sequence (5′–3′ direction)	Original reference
COI	F: BOL-COIfishF1 TCAACYAATCAYAAAGATATYGGCAC	Fish-Bol
	R: BOL-COIfishR1 ACTTCYGGGTGRCCRAARAATCA	Fish-Bol
cyt *b*	F: GLUDG.L CGAAGCTTGACTTGAARAACCAYCGTTG	Palumbi *et al.* (1991)
	R: CytbR CTCCGATCTTCGGATTACAAG	Palumbi *et al.* (1991)
rag2	F: RAG2GY-F ACAGGCATCTTTGGKATTCG	Lovejoy *et al.* (2010)
	R: RAG2R6 TGRTCCARGCAGAAGTACTTG	Lovejoy & Collette (2001)

0.2 μL of *Taq* DNA polymerase and 2 μL of DNA; Cyt *b*, a total volume of 25 μL, containing 2.5 μL of 10× reaction buffer, 2 μL of dNTP mix at 10 mM each, 1 μL of each primer at 10 μM, 0.2 μL of *Taq* DNA polymerase and 2 μL of DNA; RAG2, a total volume of 25 μL, containing 2.5 μL of 10× reaction buffer, 2 μL of dNTP mix at 10 mM each, 1 μL of each primer at 10 μM, 0.2 μL of *Taq* DNA polymerase and 2 μL of DNA. Thermal cycling conditions for each locus were as follows: COI, 94 °C denaturing step for 2 min, 35 cycles of 94 °C for 30 s, 52 °C for 40 s, 72 °C for 1 min, followed by a final 72 °C extension cycle of 5 min; Cyt *b*, 95 °C denaturing step for 30 s, 35 cycles of 95 °C for 30 s, 50 °C for 1 min, 72 °C for 90 s, followed by a final 72 °C extension cycle of 5 min; rag2, 95 °C denaturing step for 2 min, 38 cycles of 95 °C for 30 s, 48–50 °C for 1 min, 72 °C for 90 s, followed by a final 72 °C extension cycle of 5 min. PCR products were purified using the Montage PCR purification kit (Millipore, Billerica, MA, USA). Samples were then sequenced using either an ABI 377 or ABI 3730 Genetic Analyzer, using dideoxynucleotide dye terminators (BigDye version 3.1; Applied Biosystems, Foster City, CA, USA). Primers used for PCR amplifications were also used for sequencing. The DNA sequences were edited and assembled using Geneious Pro 5.5.6 (Drummond *et al.* 2012). Alignment was performed using the clustal w algorithm as implemented

in Geneious Pro 5.5.6. Alignments of coding sequences were visually evaluated by aligning their amino acid sequences in Geneious Pro 5.5.6, to ensure that no stop codons were present. GenBank accession numbers for all sequences are listed in Table 1.

Phylogenetic analysis. A total of 2925 aligned bp from the three gene fragments were analysed. Parsimony analysis of the concatenated alignment of mitochondrial and nuclear markers was conducted in paup* (Swofford 2002), using the heuristic search algorithm with 1000 replicates (random addition of taxa; TBR branch swapping; hold = 10). Gaps were treated as missing data. Bootstrap values (Felsenstein 1985) were calculated in paup* using the Heuristic search option (1000 bootstrap replicates; 100 random taxon addition replications; TBR branch swapping; hold = 10). Trees were rooted with *Apteronotus albifrons*. For Bayesian inference, model selection was performed in model test (Posada & Crandall 1998), implemented in HyPhy 1.0b (Kosakovsky *et al.* 2005) using the Akaike information criterion. Bayesian inference was performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Two independent analyses were performed, each composed of four Markov chains with default heating values. Markov chains were run for 20 million generations, sampling trees and parameters every 1000 generations. Data were partitioned by gene, all parameters were unlinked and default priors were used. The GTR + G + I model was used for all partitions. Convergence was assessed with likelihood vs. generation time plots using the sump command in MrBayes and by examination of average standard deviation of split frequencies and potential scale reduction factors. We conservatively estimated that burn-in had been reached within 5 million generations, and we discarded these. The remaining trees from each run were combined, and the frequency of clade occurrence is used to represent the posterior probability of clades.

Akawaio gen. n.

Diagnosis. *Akawaio* is diagnosed from all other genera in the family Hypopomidae by (i) coracoid with a foramen (Fig. 1; vs. coracoid without a foramen in other hypopomids); (ii) opercle with concave posterior margin (Fig. 2; vs. opercle with trapezoidal posterior margin in other hypopomids); (iii) one ossified basibranchial (vs. two in *Hypopomus* and basibranchials not ossified in other hypopomids; Fig. 3); (iv) absence of postpectoral or humeral accessory electric organs (vs. presence in *Hypopygus* and *Steatogenys*); (v) 14 total pectoral-fin rays (ii–iii) (shared with ranges of some *Brachyhypopomus* species; vs. 17–22 in *Hypopomus*, 10–12 in *Microsternarchus* and 13 in *Racenisia*); (vi) 173–199 anal-fin rays (shared with ranges of few *Brachyhypopomus* species and *Microsternarchus*; vs. 217–237 in *Hypopomus*, 193–224 in *Racenisia* and 193–279 in most *Brachyhypopomus* species); (vii) snout length 24.7–32.6% HL (vs. 36–41% in *Hypopomus*, 32–37% in *Microsternarchus* and 31% in *Racenisia*); (viii) five pectoral radials, in some individuals four partially fused to five (shared with *Hypopomus* vs. 2–4 in other hypopomids); (ix) mesopterygoid with short or reduced ascending process (Fig. 2; shared with *Microsternarchus* vs. mesopterygoid with long ascending process in other hypopomids); (x) mesocoracoid bridge absent (shared with most *Brachyhypopomus* species, *Microsternarchus* and *Racenisia* vs. present in *Hypopomus*, *Brachyhypopomus brevirostris* and *Brachyhypopomus bullocki*); (xi) 17 precaudal vertebrae (shared with some *Brachyhypopomus* species vs. 15–16 in *Hypopomus*, 13–14 in *Microsternarchus* and 19 in *Racenisia*).

Etymology. This genus is named in honour of the Akawaio Amerindians that populate the region of the upper Mazaruni and to recognize their valuable help while studying the fishes of their lands. To be regarded as a masculine noun.

Akawaio penak *sp. n. (Fig. 4)*.

Holotype. CSBD 1654 (208 mm TL), Guyana, Zone 7, Kamarang, Mazaruni River, 05°56′10.1″N 60°36′53.8″W, 24 April 2008, H. López-Fernández, D.C. Taphorn, E. Liverpool & C. Thierens.

Paratypes. ROM 83884 (2, 189–190 mm TL, 1 C&S, 190 mm TL), same data as the holotype; ROM 83866 (1, 158 mm TL), Guyana, Zone 7, Kamarang, Mazaruni River, Membaru creek, 5°55′34″N 60°35′26.8″W, 23 April 2008, H. López-Fernández, D.C. Taphorn & E. Liverpool; ROM 89519 (4, 160–210 mm TL), Guyana, Zone 7, Mazaruni River, river channel on right bank, just downstream of confluence with Kukui River (Jawalla), tailings beach, 5°40′37.38″N 60°28′55.74″W, 6 March 2012, H. López-Fernández, D.C. Taphorn, E. Liverpool, S. Refvik, J. Enright & K. Kramer; ROM 89554 (8, 164–188 mm TL), Guyana, Zone 7, Mazaruni River, main channel on a mine tailings beach just downstream from Abbou Creek on left bank, 5°42′30.45″N 60°21′39.56″W, 4 March 2010, D.C. Taphorn, E. Liverpool, H. López-Fernández & S. Refvik; ROM 89708 (2, 185 mm TL), Guyana, Zone 7, Mazaruni River, right bank, across the channel from Kelly Kramer's house, 5°51′59.5″N 60°37′14.5″W, 12 March 2010, E. Liverpool & D.C. Taphorn; ROM 89740 (2, 172–217 mm TL), Guyana, Zone 7, Mazaruni River, channel on left bank, downstream from Kamarang, 1 km upstream from Membaru, 5°55′1.77″N 60°36′13.49″W, 13 March 2010, H. López-Fernández, D.C. Taphorn, E. Liverpool, S. Refvik, J. Enright & K. Kramer; ANSP 193352 (ex. ROM 89554; 2, 164–166 mm TL), same data as ROM 89554.

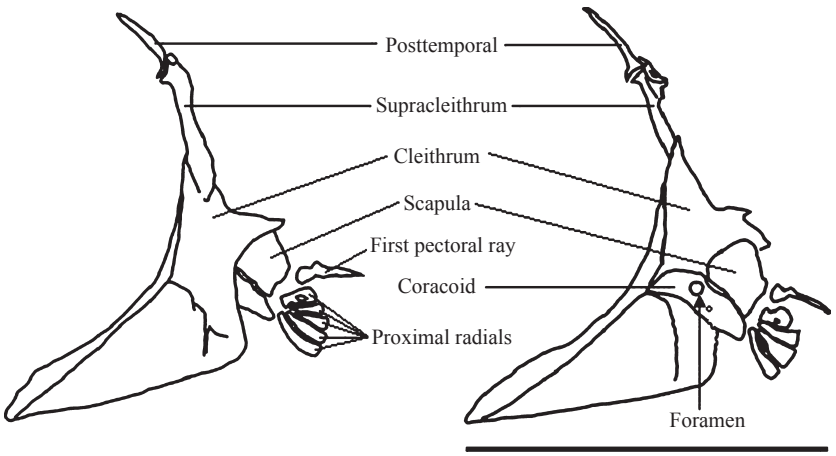

Fig. 1 Ventral gill arches in dorsal view. bh, basihyal; hb, hypobranchial; bb, basibranchial; cb, ceratobranchial; ep, epibranchial. ROM 83884 (190 mm TL).

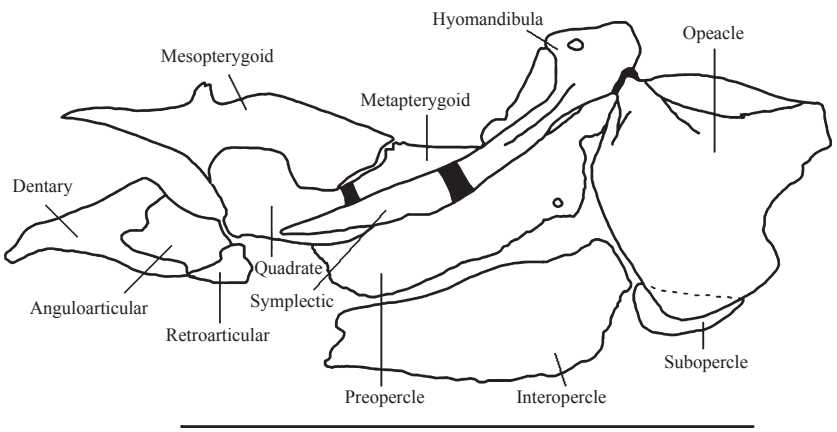

Fig. 2 Suspensorium and opercular bones from the right side in lateral view. ROM 83884 (190 mm TL).

Etymology. The species name is from the Akawaio name 'penak', which is apparently used unambiguously for this species. To be regarded as a masculine noun in apposition.

Diagnosis. As for genus, by monotypy.

Description. Morphometric and meristic data are presented in Table 3. Body long and compressed. Highest body depth located posterior to anal-fin origin. Dorsal profile of body straight or slightly convex. Head laterally compressed, widest at opercular region and deepest at occiput region. Profile nearly straight. Eyes small. Mouth subterminal, lower jaw included in upper jaw. Jaws edentulous. Snout long. Posterior naris closer to eye than to tip of snout. Gill opening straight, from above pectoral fin to front of pectoral-fin base. Pectoral-fins broad, rounded. Five branchiostegal rays. Coracoid with foramen. Mesocoracoid absent. No scapular foramen. Posttemporal and supracleithrum not fused. Anal-fin long. Body covered with thin cycloid scales except for scaleless fins and head. First anterior perforated scale of lateral line above pectoral-fin origin and lateral line extending to caudal filament tip.

Coloration in alcohol. General coloration uniform dusky brown, countershaded (with dorsal surface darker). Dark brown pigment along rays of pectoral and anal fins, membranes hyaline. Posterior opercular edge and membrane white; lips and tip of lower jaw white, isthmus whitish grey, rest of head of general colour (Fig. 4). Some individuals (>190 mm TL) with brownish blotches on white background on ventral half of body along caudal half of anal-fin length or over most of the body (Fig. 4).

Ecological notes. *Akawaio penak* was captured both in the main channel and in tributaries of the upper Mazaruni

© 2013 The Norwegian Academy of Science and Letters, **43**, 1, January 2014, pp 24–33

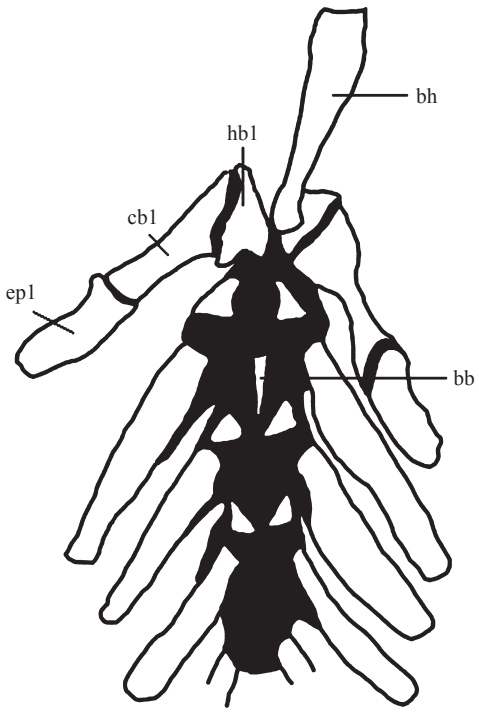

Fig. 3 Left pectoral girdle, lateral view and right pectoral girdle, medial view. Black arrow indicating coracoid foramen. ROM 83884 (190 mm TL). bb, basibranchial; bh, basihyal; cb, ceratobranchial; hb, hypobranchial; ep, epibranchial.

Table 3 Morphometric and meristic data for *Akawaio penak*

Morphometrics	Holotype	Range	Mean	*n*
Total lenght (TL)	208	158–217		21
Lenght to end of anal fin (LEA)	171.0	129–175		21
Head length (HL)	23.7	18.9–25.4		21
Caudal filament length (% TL)	17.8	14.6–22.3	19.2	21
Anal-fin base (% TL)	66.8	58.6–68.0	63.7	21
Percents of LEA (%)				
Head length	13.9	13.5–15.9	15.0	21
Snout to occiput	10.5	11.1–14.7	12.8	21
Preanal-fin distance	18.2	17.8–21.6	19.4	21
Snout to anus	9.3	8.9–12.1	10.5	21
Body depth	11.1	9.5–14.6	11.3	21
Caudal filament length	21.6	17.7–28.8	24.0	21
Pectotal fin lenght	6.7	5.8–7.7	6.9	21
Percents of HL (%)				
Snout length	29.3	24.7–32.6	29.5	21
Gape	17.7	16.0–24.1	19.9	21
Orbital diameter	9.1	6.4–13.8	10.2	21
Inteorbital distance	8.9	7.4–13.3	10.0	21
Postorbital distance	65.7	58.2–69.7	62.3	21
Posterior naris-eye	7.5	4.3–9.2	6.6	21
Snout to posterior naris	21.8	19.2–26.1	22.6	21
Branchial opening	28.8	18.4–33.9	24.3	21
Head depht at occiput	62.0	53.9–64.6	58.4	21
Head depth at eye	76.1	72.0–96.5	85.1	21

Meristics	Holotype	Range	Mode	*n*
Anal-fin rays	187	173–199	179	21
Pectoral-fin rays	14	ii–iii	14	21
Precaudal vertebrae	17	17	17	6

River. Specimens from the Mazaruni River main channel and from Membaru Creek were captured at night on beaches resulting from artificial accumulation of sand and pebbles that are the by-product of gold-mining dredging. These individuals were captured at depths of no more than 1 m and were presumably feeding. In contrast, the Waruma River specimens were captured during the day in hiding places in the banks of a shallow backwater pool. Specimens in the main channel were exposed to slow current in a relatively calm but open beach without structure, whereas the Membaru and Waruma specimens were found in habitats with structure consisting of some submerged woody debris and vegetation. In all three localities where the species was found, the water was black to reddish black with pH ranging from 4.4 to 4.8, temperature of 22–23.5 °C and conductivity <10 μS.

Distribution. *Akawaio penak* is presently known only from the main channel of the upper Mazaruni River, the mouth of the Kamarang River and Waruma Creek: a small tributary of the Kako River, which in turn is a major tributary of the upper Mazaruni. This disjunct distribution suggests that *A. penak* has a broader distribution within the upper Mazaruni drainage, but further collections are necessary to ascertain how widespread the species is (Fig. 5).

Phylogenetic relationships. Our parsimony-based phylogenetic analysis yielded two equally parsimonious trees of 3950 steps (Fig. 6). Bayesian inference provided a very similar, but more resolved topology (not shown). In the parsimony analysis, the relationships among the genera *Brachyhypopomus*, *Hypopomus*, *Microsternarchus* and *Racenisia* were unresolved, while in the Bayesian analysis, the clade (*Hypopomus*, (*Microsternarchus*, *Racenisia*)) was the sister clade of *Brachyhypopomus*. The analyses also differed in the relationships among some out-group taxa. In both analyses, *Akawaio* is well supported as the sister group of a clade that includes *Brachyhypopomus*, *Hypopomus*, *Microsternarchus* and *Racenisia*. Both analyses support a sister group relationship between (*Gymnorhamphichthys* + *Rhamphichthys*) and (*Hypopygus* + *Steatogenys*).

Discussion

The discovery and description of *A. penak* brings the total number of endemic genera in the upper Mazaruni to at

Fig. 4 *Akawaio penak*, holotype CSBD 1654 (208 mm TL), Guyana, Zone 7, Kamarang, Mazaruni River.

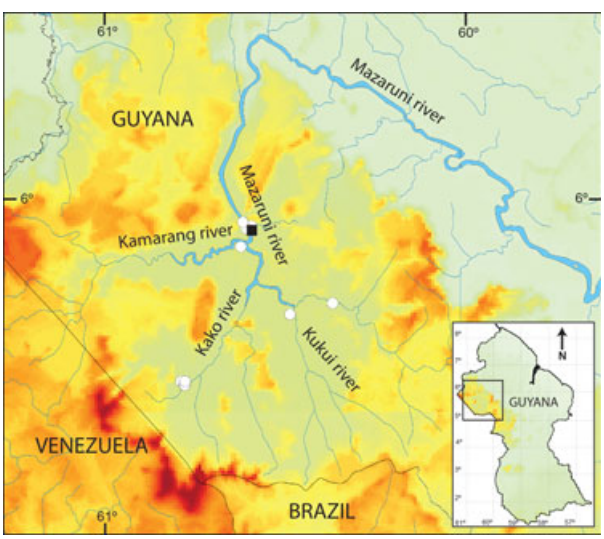

Fig. 5 Map of the upper Mazaruni River basin showing collection localities and known distribution of *A. penak*. Black square indicates the type locality.

least four. The clades containing these endemic genera now include the cichlids, characiforms, armoured catfishes and electric knifefishes – a taxonomic cross-section of the Neotropical ichthyofauna as a whole. This level of endemicity, and the phylogenetic position of the upper Mazaruni endemics *A. penak* and the cichlid genus *Mazarunia*, suggests that lineages in this region share a long history of geographic isolation from other nearby river systems. *Akawaio* appears to be sister to all other hypopomids, and *Mazarunia* forms a strongly supported clade with the circum-Guiana Shield endemic genus *Guianacara* (López-Fernández *et al.* 2010). Recent molecular dating found a possible age for *Mazarunia* of approximately 11–33 Ma (López-Fernández *et al.* 2013). If this age is indicative of the upper Mazaruni fauna in general, it suggests that some lineages may have been isolated since the Oligocene. This scenario is compatible with the idea that the Mazaruni used to flow directly into the Atlantic until the Miocene-Pliocene (Lujan & Armbruster 2011).

Our molecular phylogenetic analysis provides an improved understanding of the evolutionary relationships within the

© 2013 The Norwegian Academy of Science and Letters, **43**, 1, January 2014, pp 24–33

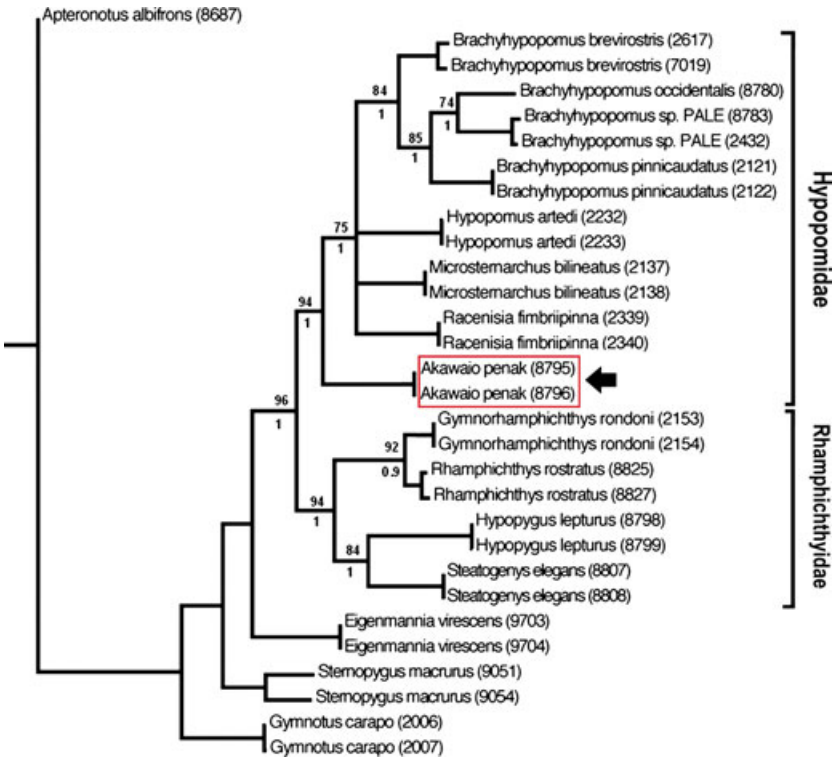

Fig. 6 Phylogenetic relationships among Rhamphichthyoidea, showing the placement of *Akawaio penak* as sister to the rest of the Hypopomidae (black arrow). Tree shown is a strict consensus of two equally parsimonious trees, based on a combined parsimony analysis of nuclear (rag2) and mitochondrial (cytochrome *b* and cytochrome *c* oxidase subunit I) genes. Branch lengths calculated using ACCTRAN optimization. Numbers below nodes denote bootstrap support values based on 1000 replicates, and Bayesian posterior probabilities are listed above nodes. Support values not shown for intraspecific nodes.

gymnotiform superfamily Rhamphichthyoidea (comprising the families Hypopomidae and Rhamphichthyidae). Our analysis includes representatives from all but one (*Iracema*) of the currently recognized rhamphichthyoid genera, is based on both nuclear and mitochondrial gene sequences and provides strong support for many clades (Fig. 6).

The Rhamphichthyoidea is well supported as a monophyletic group by both morphological (Triques 1993; Albert & Campos da-Paz 1998; Albert 2001) and molecular analyses (Alves-Gomes *et al.* 1995; Sullivan 1997; Schmitt 2005), including the present study. However, the relationships between genera, and the composition of the two included families, Hypopomidae and Rhamphichthyidae, are less certain. In particular, morphological studies and molecular studies have disagreed on the composition of the two families. Morphological studies have generally included *Brachyhypopomus*, *Racenisia*, *Hypopomus*, *Microsternarchus*, *Hypopygus* and *Steatogenys* within the family Hypopomidae, while placing *Rhamphichthys* and *Gymnorhamphichthys* in the family Rhamphichthyidae (Mago-Leccia 1994; Albert & Campos da-Paz 1998; Albert 2001; Albert & Crampton 2003; however, see Triques 1993 for an alternative arrangement in which the genus *Parapygus* [now considered a junior synonym of *Hypopomus*] is placed as sister taxon to a group comprising *Gymnorhamphichthys* + *Rhamphichthys*, while *Hypopomus* is placed as sister taxon to a group comprising *Hypopygus* + *Steatogenys*). In contrast, molecular genetic investigations have resolved a smaller Hypopomidae clade comprising *Brachyhypopomus*, *Racenisia*, *Hypopomus* and *Microsternarchus*, while *Hypopygus* and *Steatogenys* (the tribe Steatogenini, *sensu* Albert 2001) are grouped with *Rhamphichthys* and *Gymnorhamphichthys* in the Rhamphichthyidae (Alves-Gomes *et al.* 1995; Sullivan 1997; Schmitt 2005; Arnegard *et al.* 2010). In support of a phylogenetic placement of the Steatogenini within the Rhamphichthyidae, Cardoso *et al.* (2011) noted that the diploid numbers of *Hypopygus* and *Steatogenys* species are identical to species of *Rhamphichthys* (Rhamphichthyidae) but distinct from those of species of *Hypopomus* and *Brachyhypopomus* (Hypopomidae).

Our findings (Fig. 6) provide additional support for the phylogenetic arrangement supported by previous molecular studies and strongly support the close relationship between Steatogenini and *Rhamphichthys* plus *Gymnorhamphichthys*. In addition, our results indicate that *Akawaio* should be added to the family Hypopomidae as the sister taxon to all

other genera in this clade. The total number of species within Hypopomidae (from which the Steatogenini are removed) is now 15, including *A. penak*. These species are distributed in five genera, four of them monotypic (*Akawaio*, *Hypopomus*, *Microsternarchus* and *Racenisia*) and one containing 11 species (*Brachyhypopomus*).

Akawaio penak presents one of the most restricted distributions of any species within the family Hypopomidae. As with other fish endemic to the upper Mazaruni river, the small range means that habitat degradation is especially threatening. Unfortunately, gold mining has escalated in the upper Mazaruni over the last decade (Hammond *et al.* 2007; Howard *et al.* 2011). Gold mining has profound effects on freshwater ecosystems (e.g. increasing sediment load) and the functional structure of fish assemblages (Miller *et al.* 2003; Brosse *et al.* 2011) and currently represents a severe conservation threat to endemic taxa such as *A. penak*.

Comparative material. *Hypopomus artedi*: FMNH 50189 (6, 225–336 mm TL, two cs), New River drainage, head of Itabu Creek, Guyana. CSBD F700 (2, 291–308 mm TL, one cs), Burro Burro River, Deer falls between Deer Creek and Water Dog falls. CSBD F838 (1, cs, 135 mm TL), same data of CSBD F700.

Acknowledgements

For museum and technical support and/or loan of material, we thank Erling Holm, Don Stacey, Mary Burridge and Marg Zur (ROM), Mary Anne Rogers (FMNH), Marcelo Rocha (INPA) and Fernando Forero and Carlos Montaña (IAvH-P). JAMO is grateful to TWAS-CNPq Postgraduate Fellowships programme for support during this project. For their invaluable help in the field, we are grateful to Elford Liverpool, Shannon Refvik, June Enright, Clydon Thierens and Kelly, George and Mary Kramer. We are grateful to the Akawaio communities of Kamarang, Kako, Jawalla and Phillipai in the upper Mazaruni for granting us permission to do research in their lands. Permits for field collections in the Mazaruni were granted by the Environmental Protection Agency of Guyana. Tissue samples donated at MCP were exported from Brazil under Material Transfer Agreement MTA #02/2008-PUCRS. Funding for this research was provided by an NSERC Discovery Grant to HLF, NSF Grant DEB 0516831 to K.O. Winemiller, R.L. Honeycutt and HLF, a Schad Conservation Grant from the Royal Ontario Museum to HLF, NSF Grants DEB-0614334 and DEB-1146374 to WGRC, and an NSERC Discovery Grant to NRL. This is publication number 8 in collaboration between the Royal Ontario Museum and the Centre for the Study of Biodiversity, University of Guyana, to describe the fishes of Guyana.

References

Albert, J. S. (2001). Species diversity and phylogenetic systematics of American knifefishes (Gymnotiformes, Teleostei). *Miscellaneous Publications of the Museum of Zoology, University of Michigan*, *190*, 1–127.

Albert, J. S. & Campos da-Paz, R. (1998). Phylogenetic systematics of gymnotiformes with diagnosis of 58 clades: a review of available data. In L. R. Malabarba, R. E. Reis, R. P. Vari, C. A. S. Lucena & Z. M. S. Lucena (Eds) *Phylogeny and Classification of Neotropical Fishes* (pp. 419–446). Porto Alegre: Edipucrs.

Albert, J. S. & Crampton, W. G. R. (2003). Family Hypopomidae. In R. E. Reis, S. O. Kullander & C. J. Ferraris Jr (Eds) *Checklist of the Freshwater Fishes of South and Central America* (pp. 494–496). Porto Alegre: Edipucrs.

Alofs, K. M., Liverpool, E. A., Taphorn, D. C., Bernard, C. R. & López-Fernández, H. (2013). Mind the (information) gap: the importance of exploration and discovery for assessing conservation priorities for freshwater fish. *Diversity and Distributions*, in press.

Alves-Gomes, J. A., Ortí, G., Haygood, M., Meyer, A. & Heiligenberg, W. (1995). Phylogenetic analysis of the South American electric fishes (Order Gymnotiformes) and the evolution of their electrogenic system: a synthesis based on morphology, electrophysiology and mitochondrial sequence data. *Molecular Biology and Evolution*, *12*, 298–318.

Armbruster, J. W. & Taphorn, D. C. (2011). A new genus and species of weakly armored catfish from the upper Mazaruni River, Guyana (Siluriformes: Loricariidae). *Copeia*, *2011*, 46–52.

Arnegard, M. E., Zwickl, D. J., Lu, Y. & Zakon, H. H. (2010). Old gene duplication facilitates origin and diversification of an innovative communication system – twice. *Proceedings of the National Academy of Sciences of the USA*, *107*, 22172–22177.

Brosse, S., Grenouillet, G., Gevrey, M., Khazraie, K. & Tudesque, L. (2011). Small-scale gold mining erodes fish assemblage structure in small neotropical streams. *Biodiversity and Conservation*, *20*, 1013–1026.

Cardoso, A. L., Pieczarka, J. C., Feldberg, E., Rodrigues Milhomem, S. S., Moreira-Almeida, T., Silva, D. S., Silva, P. C. & Nagamachi, C. Y. (2011). Chromosomal characterization of two species of genus *Steatogenys* (Gymnotiformes: Rhamphichthyoidea: Steatogenini) from the Amazon basin: sex chromosomes and correlations with Gymnotiformes phylogeny. *Reviews in Fish Biology and Fisheries*, *21*, 613–621.

Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. & Wilson, A. (2012). Geneious v5.5.6. Available via http://www.geneious.com/.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*, 783–791.

Fricke, R. & Eschmeyer, W. N. (2012). A guide to fish collections in the Catalog of Fishes. Available via http://research.calacademy.org/redirect?url=http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Géry, J. & Zarske, A. (2002). *Derhamia hoffmannorum*, gen. et. sp. n. – a new pencil fish (Teleostei, Characiformes, Lebiasinidae), endemic from the Mazaruni River in Guyana. *Zoologische Abhandlungen; Staatliches Museum fur Tierkunde in Dresden*, *52*, 35–47.

Hammond, D. S., Gond, V., de Thoisy, B., Forget, P. M. & DeDijn, B. P. E. (2007). Causes and consequences of a tropical forest

gold rush in the Guiana Shield, South America. *Ambio*, *36*, 661–670.

Howard, J., Trotz, M. A., Thomas, K., Omisca, E., Chiu, H. T., Halfhide, T., Akiwumi, F., Michael, R. & Stuart, A. M. (2011). Total mercury loadings in sediment from gold mining and conservation areas in Guyana. *Environmental Monitoring and Assessment*, *179*, 555–573.

Kosakovsky, S. L., Frost, S. D. W. & Muse, S. V. (2005). HyPhy: hypothesis testing using phylogenies. *Bioinformatics*, *21*, 676–679.

Kullander, S. O. (1990). *Mazarunia mazarunii* (Teleostei: Cichlidae), a new genus and species from Guyana, South America. *Ichthyological Exploration of Freshwaters*, *1*, 3–14.

López-Fernández, H., Winemiller, K. O. & Honeycutt, R. L. (2010). Multilocus phylogeny and rapid radiations in Neotropical cichlid fishes (Perciformes: Cichlidae: Cichlinae). *Molecular Phylogenetics and Evolution*, *55*, 1070–1086.

López-Fernández, H., Taphorn, D. C. & Liverpool, E. A. (2012). Phylogenetic diagnosis and expanded description of the genus *Mazarunia* Kullander, 1990 (Teleostei: Cichlidae) from the upper Mazaruni River, Guyana, with description of two new species. *Neotropical Ichthyology*, *10*, 465–486.

López-Fernández, H., Arbour, J. H., Winemiller, K. O. & Honeycutt, R. L. (2013). Testing for ancient adaptive radiations in Neotropical cichlid fishes. *Evolution*, *67*, 1321–1337.

Lovejoy, N. R. & Collette, B.B. (2001). Phylogenetic relationships of New World needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. *Copeia*, *2001*, 324–338.

Lovejoy, N. R., Lester, K., Crampton, W. G. R., Marques, F. P. L. & Albert, J. S. (2010). Phylogeny, biogeography, and electric signal evolution of neotropical knifefishes of the genus Gymnotus (Osteichthyes: Gymnotidae). *Molecular Phylogenetics and Evolution*, *54*, 278–290.

Lujan, N. K. & Armbruster, J. W. (2011). The Guiana Shield. In J. S. Albert & R. E. Reis (Eds) *Historical Biogeography of Neotropical Freshwater Fishes* (pp. 211–224). Berkeley, CA: University of California Press.

Mago-Leccia, F. (1994). *Electric Fishes of Continental Water of Continental Waters of America*. Caracas: Fundación para el Desarrollo de las Ciencias Físicas, Matemáticas y Naturales.

Miller, J. R., Lechler, P. J. & Bridge, G. (2003). Mercury contamination of alluvial sediments within the Essequibo and Mazaruni River basins, Guyana. *Water*, *Air*, *and Soil Pollution*, *148*, 139–166.

Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L. & Grabowski, G. (1991). *The Simple Fools Guide to PCR*. Honolulu: University of Hawaii Press.

Posada, D. & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*, *14*, 817–818.

Presswell, B., Weitzman, S. & Bergquist, T. (2000). *Skiotocharax meizon*, a new genus and species from Guyana with discussion of its relationships (Characiformes: Crenuchidae). *Ichthyological Exploration of Freshwaters*, *11*, 175–192.

Ronquist, F. & Huelsenbeck, J. P. (2003). MRBAYES 3: bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574.

Rull, V. (2005). Biotic diversification in the Guayana highlands: a proposal. *Journal of Biogeography*, *32*, 921–927.

Rull, V. (2007). The Guayana highlands: a promised (but threatened) land for ecological and evolutionary science. *Biotropica*, *39*, 31–34.

Salerno, P. E., Ron, S. R., Señaris, J. C., Rojas-Runjaic, F. J. M., Noonan, B. P. & Cannatella, D. C. (2012). Ancient tepui summits harbor young rather than old lineages of endemic frogs. *Evolution*, *66*, 3000–3013.

Schmitt, R. (2005). *Filogeografia de* Hypopygus lepturus *Hoedeman, 1962 (Gymnotiformes: Rhamphichthyidae) ao longo do médio rio Negro, Amazônia*. Unpublished MSc Thesis. Instituto Nacional de Pesquisas da Amazônia/Universidade Federal do Amazonas, Manaus, Amazonas.

Sullivan, J. P. (1997). *A phylogenetic study of the Neotropical hypopomid electric fishes (Gymnotiformes: Rhamphichthyoidea)*. Unpublished PhD Dissertation. Duke University, Durham, NC.

Swofford, D. L. (2002). *PAUP – Phylogenetic Analysis Using Parsimony. Ver. 4. 0. [Computer Software and Manual]*. Suderland, MA: Sinauer Associates, Inc. Publishers.

Taylor, W. R. & Van Dyke, G. C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, *9*, 107–119.

Triques, M. L. (1993). Filogenia dos gêneros de Gymnotiformes (Actinopterygii, Ostariophysi), com base em caracteres esqueléticos. *Comunicações do Museu de Ciências e Tecnologia da PURCS*, *Série Zoologia*, *6*, 85–130.

Vari, R. P. & Ferraris, C. J. (2009). Fishes of the Guiana Shield. *Bulletin of the Biological Society of Washington*, *17*, 9–18.