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Fluviphylax gouldingi and *F. wallacei*, two new miniature killifishes from the middle and upper Rio Negro drainage, Brazilian Amazon

(Teleostei, Cyprinodontiformes, Cyprinodontoidei)

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Fluviphylax gouldingi spec. nov. from the upper Rio Negro drainage and *F. wallacei* spec. nov. from the middle Rio Negro drainage, Rio Amazonas basin, northern Brazil, are described. *Fluviphylax gouldingi* is distinguished from all other species of *Fluviphylax* in having a sharp ventral process in the opercle, a narrow subopercle and interopercle, a filamentous second pelvic-fin ray, absence of mesethmoid and the presence of an orange bright blotch on the preorbital region of both males and females. *Fluviphylax wallacei* differs from all congeners in having a sexual dimorphic pattern of the cephalic lateral line system and by the presence of a pointed dorsal fin in males. *Fluviphylax gouldingi* and *F. wallacei* differ from all congeners by the presence of a slender retroarticular, a triangular opercle, an orange colouration on the posterior region of the pectoral fin in males and the head free neuromasts placed at the same level of head surface. The genus *Fluviphylax* is diagnosed by the presence of eye extremely large, absence of vomer, absence of interarcual cartilage, absence of interhyal, derived shape of maxilla, basihyal cartilage enlarged, reduction of caudalfin rays and cephalic sensory system, and a unique colour pattern, consisting of melanophores concentrated on dorsal and ventral midlines of body.

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Introduction

The genus *Fluviphylax* Whitley, 1965 comprises miniature oviparous killifish species distributed over the Rio Amazonas and Orinoco basins, occurring in clear and black water streams and lakes (Costa 1996, Bragança & Costa 2018). Despite including one of the smallest Amazon fish species, little attention has been directed to the genus, with few studies directed to its morphology, ecology and taxonomy (Roberts 1970, 1972, Goulding et al. 1988, Weitzman & Vari 1988, Costa 1996, Costa & Le Bail 1999). The first taxonomic revision was published four decades after the first species being described, revealing an until then unknown diversity for the genus (Costa 1996). *Fluviphylax pygmaeus* (Myers & Carvalho, 1955), the genus type species, from the lower Rio Madeira drainage was redescribed, and three new species were described: *F. obscurus* Costa, 1996, known from the middle and upper Rio Negro drainage, *F. zonatus* Costa, 1996 from the lower Rio Negro drainage and *F. simplex* Costa, 1996 known from the Rio Solimões and Rio Amazonas drainage. In the same publication a new diagnosis for *Fluviphylax* was provided on the basis of fifteen synapomorphies, including the following character states: eye extremely large,

miniaturization, absence of vomer, absence of interarcual cartilage, absence of interhyal, absence of fourth ceratobranchial teeth, derived shape of maxilla, fifth ceratobranchial anterior portion folded laterally, basihyal cartilage enlarged, posttemporal scythe-shaped, opercle anterodorsal portion with a narrow process, reduction of caudal-fin rays, anal-fin rays and cephalic sensory system, and a unique colour pattern. The last *Fluviphylax* species to be described was *F. palikur* Costa & Le Bail, 1999, known from the Rio Oiapoque drainage.

Historically, the genus *Fluviphylax* has been considered closely related to the African procatopodins, because of a similar pattern of fin position (Myers & Carvalho 1955, Roberts 1970), and further morphology based phylogenetic analysis also supported a close relationship between the African procatopodins and *Fluviphylax* that were grouped in a more comprehensive Poeciliidae, including also the American livebearers (Parenti 1981, Costa 1996, Ghedotti 2000). However, the first molecular studies to include all poeciliid lineages revealed a paraphyletic assemblage, in which the American poeciliines are sister to the Anablepidae, *Fluviphylax* is sister to the Poeciliinae and Anablepidae, and the African poeciliids are sister to the Old World Valenciidae and Aphaniidae (Pollux et al. 2014, Helmstetter et al. 2016, Reznick et al. 2017, Bragança et al. 2018, Bragança & Costa 2018). Following the recent molecular studies, a new classification for taxa previously grouped in the Poeciliidae were proposed: the American poeciliines were considered the only members of the Poeciliidae, *Fluviphylax* species were grouped in the Fluviphylacidae and the African procatopodins were placed in the Procatopodidae (Bragança et al. 2018).

Recently, the first molecular analysis including all five nominal species and three undescribed species of *Fluviphylax* was published, providing the first insight into the evolution and diversification patterns of the genus in the Rio Amazonas and Orinoco basins (Bragança & Costa 2018). In the present study, almost two decades after the description of *F. palikur*, two new species from the middle and upper Rio Negro are described, based mostly on material recently collected in expeditions directed to improve the knowledge on the taxonomy and distribution of *Fluviphylax* in the Amazon. This study also provides an updated diagnosis for *Fluviphylax*.

Material and methods

Data on life colour pattern were based both on direct examination of live specimens during collections and numerous photographs of live individuals, at least two males and one female for each collection, taken in aquaria within ten hours after collection. Specimens were captured by active collection right after the visualization of the small shoals on the surface of the water, through the use of small mesh dip nets (1.5 mm × 0.5 mm). Specimens were euthanized in tricain mesylate (TMS), a powder easily soluble in water, used for anaesthesia, sedation, or euthanasia of fish (Neiffer & Stamper 2009) and preserved in formalin. The colour pattern of preserved specimens was also analysed. Measurements and counts follow Costa (1988). Measurements are presented as percentages of standard length (SL) except for subunits of head length (HL). Measurements were obtained by digital calliper and taken through observation made using a microscope. Osteological studies were made on cleared and stained specimens (c&s) prepared according to Taylor & Van Dyke (1985). Nomenclature for frontal squamation follows Hoedeman (1956) and that for head sensory canals Gosline (1949), except for the posterior section of supraorbital canal, here called the posterior infraorbital canal.

The examined specimens are deposited in the following institutions: CICCAA, Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais da Universidade Federal do Maranhão, Chapadinha; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; NRM, Naturhistoriska Riksmuseet, Stockholm; UFRJ, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro. The acronym EPA refers to the Expedição Permanente da Amazônia, a scientific cooperative effort between Museu Paraense Emílio Goeldi (MPEG), Instituto Nacional de Pesquisas da Amazônia (INPA) and the Museu de Zoologia da Universidade de São Paulo (MZUSP) coordinated by Paulo Vanzolini during the late 1960's and 1970's.

Comparative material

Fluviphylax pygmaeus: MNRJ 14160 (ex-MNRJ 4126), 7 syntypes, 13.1–15.0 mm SL; lagoon at Borba, Rio Madeira; A. Parko, 1943. – UFRJ 9120, 86, 7.8–12.7 mm SL; UFRJ 10108, 6, 10.4–12.1 mm SL (c&s); Borba, Igarapé Jatuarãna, Rio Madeira, 04°24'16"S 59°32'48"W; P. Bragança et al., 6 Nov. 2012. – UFRJ 9201, 27, 8.2–12.6 mm SL; UFRJ 9247, 5 (c&s); Igarapé Puxurizal, about 10 km following the road from Borba to Rio Mapiá Grande, 04°28'27"S 59°35'18"W; P. Bragança et al., 6 Nov. 2012.

Fluviphylax zonatus: MZUSP 49207, holotype, 15.4 mm SL; Anavilhanas archipelago, about 02°34'39"S 60°53'39"W; M. Goulding, 21 Nov. 1979. – MZUSP 29367, 132, 3 (c&s) paratypes, 9.4–15.3 mm SL; Anavilhanas archipelago, about 02°34'39"S 60°53'39"W; M. Goulding, 21 Nov. 1979. – UFRJ 7954, 18, 15.2–17.1 mm SL; beach in Igarapé da Freguesia a tributary of the left bank of Rio Negro, about 8 km from Novo Airão, 2°39'32"S 60°59'46"W; P. Bragança & P. Amorim, 28 Jan. 2011. – UFRJ 8874, 4 (c&s); beach in Igarapé da Freguesia a tributary of the left bank of Rio Negro, about 3 km from Novo Airão, 2°37'47"S 60°58'37"W; P. Bragança & P. Amorim, 28 Jan. 2011. – UFRJ 7960, 14, 14.4–15.6 mm SL; UFRJ 8873, 5 (c&s); igarapé behind Lua beach close

to Manaus, Município de Manaus, 3°01'47"S 60°08'23"W; P. Bragança & P. Amorim, 1 Feb. 2011.

Fluviphylax obscurus: MZUSP 49207, holotype, 17.3 mm SL; pool in island, Município de Barcelos, about 0°56'39"S 62°55'39"W; M. Goulding, 29 Feb. 1980. – MZUSP 29374, 7 paratypes, 12.0–13.3 mm SL; pool in island, Município de Barcelos, about 0°56'39"S 62°55'39"W; M. Goulding, 29 Feb. 1980. – MZUSP 29372, 36 (4 c&s) paratypes, 9.4–14.1 mm SL; central pool in Buiu-Açu island, near Rio Urubaxi, about 0°30'39"S 64°54'39"W; M. Goulding, 06 Feb. 1980. – MZUSP 29370, 14 paratypes, 7.5–14.8 mm SL; Rio Negro just below Rio Daraá, about 0°27'39"S 64°45'39"W; M. Goulding, 17 Feb. 1980. – UFRJ 9124, 23, 10.3–13.1 mm SL; UFRJ 9246, 5 (c&s); beach in island of the Mariuá archipelago in Rio Negro basin, near Barcelos, Município de Barcelos, 0°50'34"S 62°58'52"W; P. Bragança et al., 18 Nov. 2012. – UFRJ 9125, 5, 10.4–13.0 mm SL; UFRJ 10107, 2 (c&s); beach in island of the Mariuá archipelago, near Barcelos, Município de Barcelos, 0°56'15"S 62°56'21"W; P. Bragança et al., 18 Nov. 2012.

Fluviphylax simplex: MZUSP 49209, holotype, 14.3 mm SL; MZUSP 7817, 114 paratypes, 9.6–14.2 mm SL; Igarapé of Lago José-Açu, Município de Parintins, about 02°40'39"S 56°40'39"W; EPA, 11–12 Dec. 1967. – UFRJ 5373, 360, 5.7–13.4 mm SL; UFRJ 5374, 11 (c&s); Lago Máximo margin, Amazon basin, Município de Parintins, about 02°42'S 56°40'W; C. A. de Figueiredo & C. Codeço, 14 Sep. 1996. – UFRJ 9824, 21; margin of Lago Zé-Açu, near Bom Socorro community, Município de Parintins, about 02°40'S 56°40'W; C. A. de Figueiredo & C. Codeço, 13 Sep. 1996.

Fluviphylax palikur: MNHN 1998-0471, 24 paratypes, 6.9–11.6 mm SL; Rio Taparabu, a right tributary of the Rio Oiapoque, Juminán, Município de Oiapoque, 03°58'35"N 51°41'06"W; P.-Y. Le Bail, 12 Sep. 1997. – NRM 28302, 2 paratypes, 13.6–13.9 mm SL; igarapé at Aldeia Cunene, Rio Oiapoque drainage, Juminán, Município de Oiapoque, 04°01'08"N 51°37'06"W; S. Kullander & F. Fang, 28 Mar. 1994. – UFRJ 8824, 22, 6.9–11.8 mm SL; UFRJ 8877, 5 (c&s); igarapé on the right bank of Rio Oiapoque, Município de Oiapoque, 03°59'28"N 51°41'40"W; P. Bragança & E. Henschel, 29 Jul. 2012. – UFRJ 8828, 3, 9.1–9.8 mm SL; igarapé on the right bank of Rio Oiapoque after crossing Vila de Taparabu, Município de Oiapoque, 04°3'8"N 51°38'1"W; P. Bragança & E. Henschel, 31 Jul. 2012. UFRJ 8876, 7(c&s); pool near tower 6 of AMCEL cellulose company area, Rio Araguari drainage, Município de Ferreira Gomes, 0°50'52"N 51°04'42"W; C. Gama & D. Halboth, 2 Jun. 2002.

Results

Fluviphylax gouldingi, spec. nov.
= *Fluviphylax* sp. A in Bragança & Costa (2018)
Figs 1–2

Holotype. UFRJ 9275, male, 13.2 mm SL; Brazil: Estado do Amazonas: Município de São Gabriel da Cachoeira: igarapé near Monte Cristo community, a tributary of Rio Vaupés drainage, upper Rio Negro drainage, Rio Amazonas basin, 0°05'23"N 67°22'05"W, altitude about

Table 1. Morphometric data of *Fluviphylax gouldingi* and *F. wallacei*.

	Fluviphylax gouldingi			*F. wallacei*		
	holotype	paratypes		holotype	paratypes	
	UFRJ 9275	males	females	UFRJ 9080	males	females
Standard lenght (mm)	13.3	11.3–14.3	10.6–13.9	12.8	11.4–14.8	11.2–12.6
Percent of standard lenght						
Body depth	15.7	13.7–15.7	14.1–17.2	16.9	15.3–18.4	17.5–20.2
Caudal peduncle depth	9.0	8.2–9.7	7.9–9.2	10.1	9.6–10.6	10.3–11.7
Pre-dorsal lenght	74.9	73.8–77.3	72.3–78.9	73.5	73.2–76.4	76.2–80.6
Pre-pelvic lenght	46.1	45.3–50.7	43.6–48.8	47.0	45.5–47.0	48.3–50.1
Lenght of dorsal-fin base	9.7	9.7–10.9	8.1–11.3	10.2	10.2–11.3	9.4–11.2
Lenght of anal-fin base	15.4	15.1–17.7	12.3–15.7	18.6	15.7–18.6	13.4–16.7
Caudal-fin lenght	34.2	34.1–37.7	32.4–36.7	35.2	32.9–35.9	35.4–39.5
Pectoral-fin lenght	19.1	18.8–23.9	18.1–22.9	20.4	19.7–22.9	22.8–26.8
Pelvic-fin lenght	31.2	26.9–31.2	12.9–15.6	27.0	21.0–27.0	15.6–17.7
Head lenght (mm)	3.2	2.6–3.4	2.6–3.4	3.1	2.8–3.6	3.0–3.3
Percent of head lenght						
Head depth	52.8	53.2–58.0	50.7–56.2	52.7	49.0–56.5	57.4–61.0
Head width	71.3	71.3–77.7	70.2–76.5	71.4	68.4–78.6	74.7–80.0
Snout lenght	22.0	15.6–22.0	17.6–20.5	16.8	16.8–20.6	17.7–20.3
Lower jaw lenght	7.2	5.9–7.5	6.5–8.0	9.2	8.2–10.7	8.0–8.4
Eye diameter	52.8	47.3–52.8	47.0–53.4	48.2	48.2–52.7	48.7–54.6

Fig. 1. *Fluviphylax gouldingi*, UFRJ 9275, holotype, 13.3 mm SL.

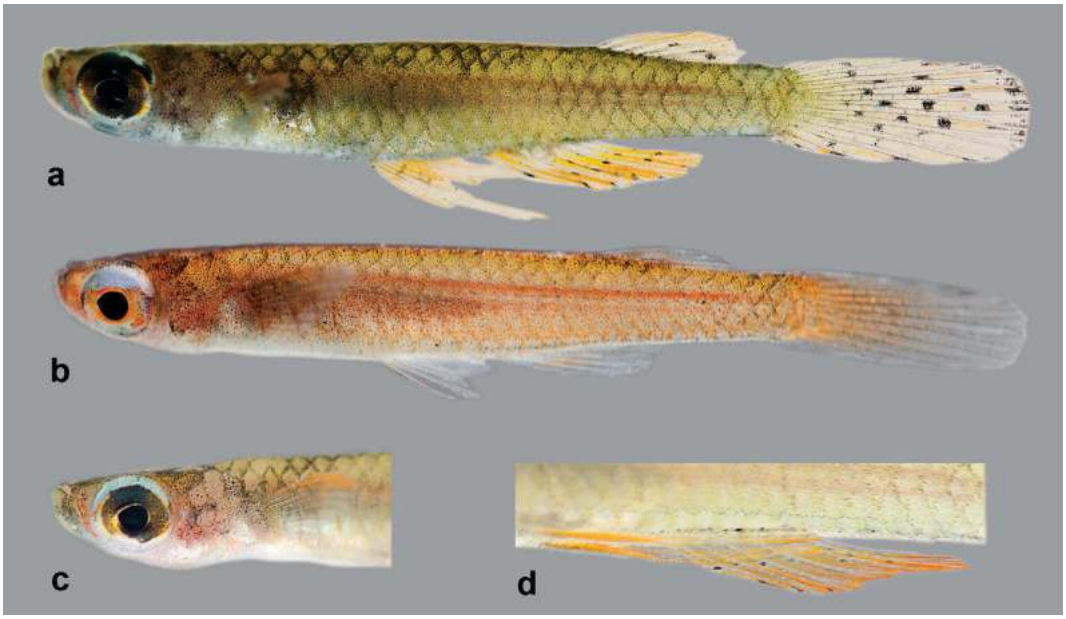

Fig. 2. *Fluviphylax gouldingi*: **a.** UFRJ 9126, paratype, male, 13 mm SL; **b.** UFRJ 9121, paratype, female, 11.8 mm SL; **c, d.** UFRJ 9126, paratype, male, 13.3 mm SL; Brazil: Amazonas, São Gabriel da Cachoeira, Rio Vaupés drainage.

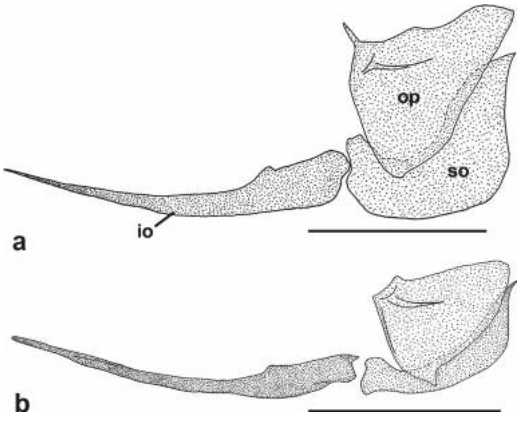

Fig. 3. Opercular suspensorium, lateral view: **a.** *Fluviphylax pygmaeus*; **b.** *Fluviphylax gouldingi*. Abbreviations: **io**, interopercle; **op**, opercle; **so**, subopercle.

90 m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 12 Nov. 2012.

Paratypes. UFRJ 9126, 15, 9.3–13.4 mm SL; UFRJ 9245, 4 (c&s), 8.0–11.5 mm SL; collected with holotype; UFRJ 9121, 59, 7.2–13.3 mm SL; UFRJ 9276, 6 (c&s), 8.3–12.5 mm SL. Brazil: Estado do Amazonas: Município de São Gabriel da Cachoeira: upper Rio Negro drainage: Lago Tiburiari on Igarapé do Tiburiari near Trovão community a tributary of Rio Vaupés drainage, 0°04'27"N 67°24'31"W, altitude about 80 m; P. H. N. Bragança et al., 12 Nov. 2012. – MZUSP 109617, 4 (1 c&s), 10.8–13.7 mm SL; Estado do Amazonas: Município de Santa Isabel do Rio Negro: upper Rio Negro: rocky bank on left margin of Rio Neuixi drainage close to its confluence with Rio Negro, 0°21'45"S 65°04'13"W; Toledo-Piza et al., 8 Feb. 2011. – MZUSP 109622, 3, 14.1–14.6 mm SL; Open area in the igapó forest, right margin of Rio Aiuanã drainage, 0°33'05"S 64°55'9"W; Toledo-Piza et al., 9 Feb. 2011.

Diagnosis. *Fluviphylax gouldingi* is distinguished from all other congeners by having a sharp ventral process on the opercle (Fig. 3a) (vs. absence; Fig. 3b); a subopercle narrow (Fig. 3a) (vs. wide; Fig. 3b); an interopercle narrow (Fig. 3a) (vs. wide; Fig. 3b); a filamentous second pelvic-fin ray (Figs 2, 4g) (vs. not filamentous; Fig. 4h–i); all cephalic lateral line system opened in males and females (Fig. 5a,f) (vs. closed; Fig. 5b–e,g); mesethemoid absent (Fig. 6b) (vs. present; Fig. 6a) and by the presence of an orange bright blotch on the preorbital region of both males and females (Fig. 2a–c) (vs. absent). It is similar to *F. wallacei* and distinguished from all other congeners by having a slender retroarticular (Fig. 7b) (vs. deep; Fig. 7a); a triangular opercle (Fig. 3a) (vs. scale shaped; Fig. 3b); an orange colouration on the posterior region of the pectoral fin in males (Fig. 2a,c) (vs. hyaline); and the head neuromasts not placed inside shallow groves (Fig. 5a,f) (vs. in shallow grooves; Fig. 5c,d). Other character states not unique but useful to identify *F. gouldingi* are: second and fourth pharyngobranchial tooth plates not enlarged (Fig. 8b) (vs. enlarged; Fig. 8a); absence of teeth on second pharyngobranchial plate (Fig. 8b,c) (vs. presence; Fig. 8a); ventral process of posttemporal short (Fig. 9a) (vs. long; Fig. 9b); rostral cartilage present (Fig. 7a,b) (vs. absent); anterodorsal process of opercle absent (Fig. 3a) (vs. present; Fig. 3b) and an elongate and pointed anal fin reaching vertical to dorsal-fin tip (Fig. 4f) (vs. short and rounded; Fig. 4e).

Description

Morphometric data are presented in Table 1. Maximum recorded adult size 14.6 mm SL. Dorsal profile of body approximately straight. Ventral profile slightly convex from lower jaw to end of anal-fin base, nearly straight on caudal peduncle. Anterior portion of body cylindrical, about as wide as deep, becoming compressed behind anal-fin origin.

Dorsal fin rounded, its origin in vertical just behind anal-fin base or between penultimate and last anal-fin rays. Anal fin long and pointed in males, tip in vertical through dorsal-fin tip, short and rounded in females, tip reaching vertical through end of dorsal-fin base. Caudal fin slender. Pectoral fin rounded, its posterior margin reaching vertical just behind pelvic-fin base. Pelvic fin long in males, second pelvic-fin ray filamentous, reaching posterior to anal-fin base; short in females, tip reaching between urogenital opening and anal-fin origin. Pelvic-fin bases medially separated by interspace broader than width of each pelvic-fin base. Dorsal-fin rays 5–6; anal-fin rays 7–8; caudal-fin rays 15–18; pectoral-fin rays 10–12; pelvic-fin rays 6.

Frontal squamation G-patterned. Head free neuromasts placed over the body. Cephalic lateral line system: anterior portion of supraorbital sensory canal open, with one neuromast, posterior portion open,

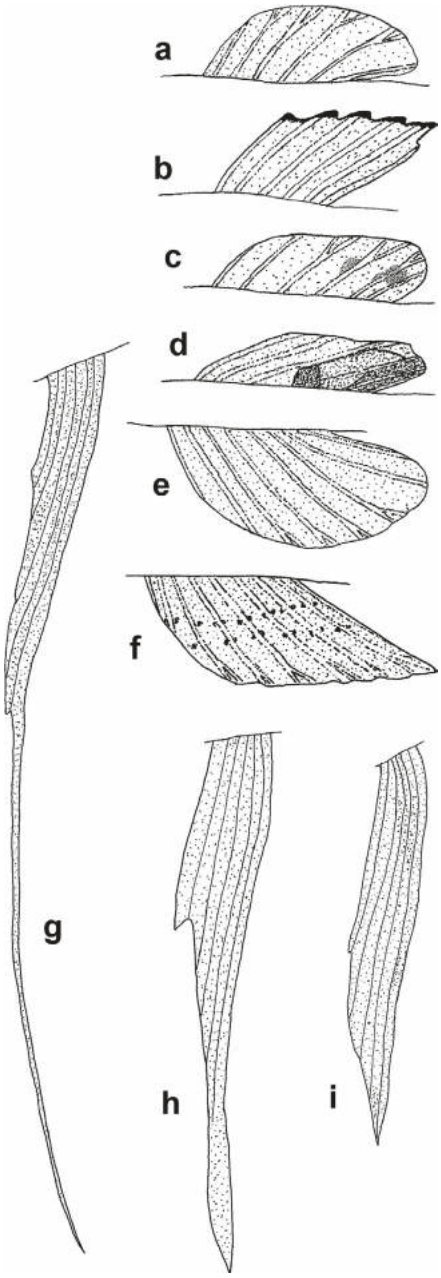

Fig. 4. Dorsal fin: **a.** *Fluviphylax obscurus*; **b.** *F. wallacei*; **c.** *F. gouldingi*; **d.** *F. palikur*. Anal fin: **e.** *F. simplex*; **f.** *F. wallacei*. Pelvic fin: **g.** *F. gouldingi*; **h.** *F. wallacei*; **i.** *F. palikur*.

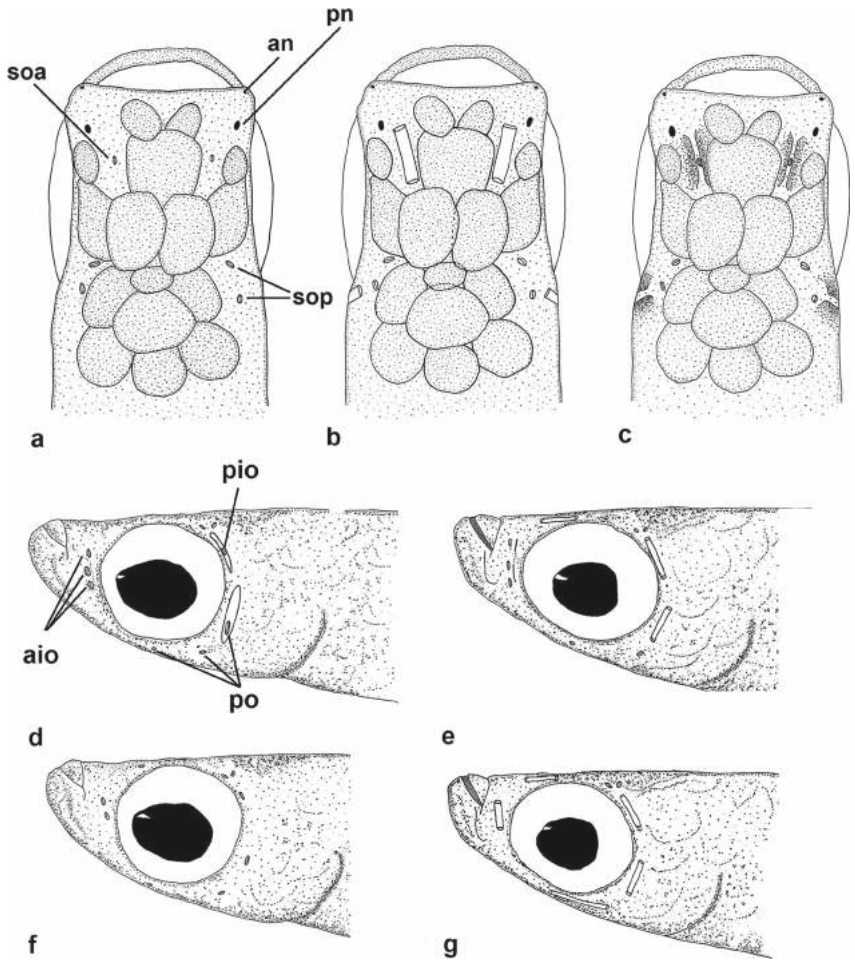

Fig. 5. Cephalic pores dorsal and lateral view: **a.** *F. gouldingi*; **b.** *F. obscurus*; **c.** *F. simplex*; **d.** *F. simplex*; **e.** *F. pygmaeus*; **f.** *F. gouldingi*; **g.** *F. obscurus*. Abbreviations: **aio**, anterior infra-orbital channel; **an**, anterior nostril; **pio**, posterior infra-orbital channel; **pn**, posterior nostril; **po**, preopercular channel; **soa**, supraorbital channel anterior portion; **sop**, supraorbital channel posterior portion.

with two exposed neuromasts; anterior infra-orbital canal open, with two neuromasts; median portion of infra-orbital region with series of six to eight minute neuromasts; posterior infra-orbital canal open, with one neuromast; preopercular canal open in dorsal and ventral portions; mandibular canal represented by single neuromast in vertical through corner of mouth. In juveniles, all canals open. Longitudinal series of scales 25–26; transverse series of scales 6.

Osteology. Mesethmoid absent (Fig. 6b). Premaxillary and dentary teeth well developed. Rostral cartilage present. Retroarticular slender (Fig. 7b). Opercle triangular, sharp ventral process present, its anterodorsal process absent. Subopercle narrow. Interopercle narrow (Fig. 3a). Posttemporal scythe-shaped, ventral process short (Fig. 9a); dorsal process of cleithrum sharp and long. Anterior process of fifth ceratobranchial short and folded laterally (Fig. 10a). Second and fourth pharyngobranchial tooth plates not enlarged. Second pharyngobranchial teeth absent (Fig. 8b). Total vertebrae 27–28, 12–13 precaudal and 14–15 caudal. First proximal radial of dorsal-fin between neural spine of vertebrae 16 and 17. First proximal radial of anal-fin between pleural rib of vertebrae 10 and 12. Gill rakers on first branchial arch 10. Branchiostegal rays 4–5.

Colouration in alcohol. Overall colouration of body pale brownish yellow with minute chromatophores sparsely distributed (Fig. 1). Brown chromatophores on dorsum of head. Melanophores

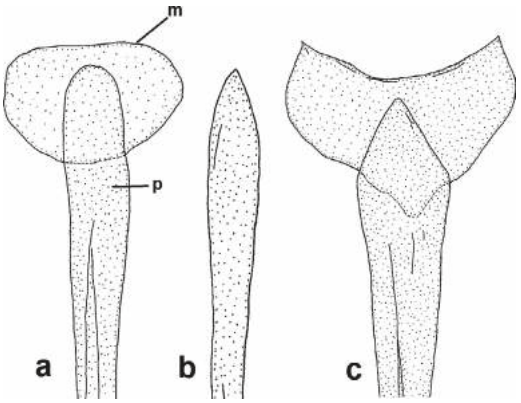

Fig. 6. Parasphenoid anterior margin and mesethmoid, ventral view: **a.** *Fluviphylax zonatus*; **b.** *Fluviphylax gouldingi*; *Fluviphylax palikur*. Abbreviations: **m**, mesethmoid; **p**, parasphenoid.

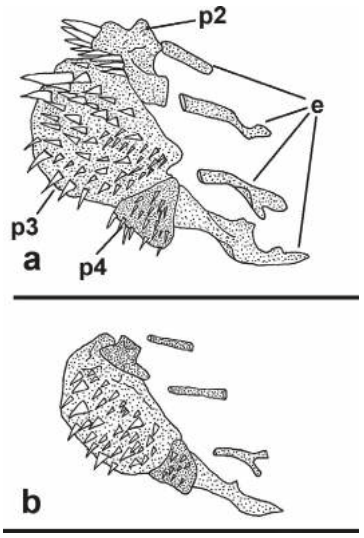

Fig. 8. Dorsal branchial arches, ventral view: **a.** *F. zonatus*; **b.** *F. gouldingi*. Abbreviations: **e**, epibrachials; **p**, pharyngobranchials.

on nape and along dorsal midline between nape and caudal-fin base. Ventrum scarcely pigmented. Pale brown chromatophores along whole mid-body line of flank, more visible on posterior region, on caudal peduncle. Melanophores forming a longitudinal stripe on ventral midline, between pelvic-fin base and caudal-fin base. Head overall colouration yellowish brown. Brown chromatophores on dorsum of head. Minute faintly pigmented and sparsely distributed chromatophores on jaws and opercular region. Iris silver, darker close to pupil; dark pigment concentrated on dorsal margin of eye. All fins hyaline, melanophores sparsely concentrated only on fins membranes and along fin rays; melanophores forming small black spots on distal portion of males dorsal fin; a black margin on males caudal fin and black dots on males anal and caudal fins.

Colouration in life. Males. Side of body light brownish grey (Fig. 2a,c,d). Silvery blue blotch on scapular girdle and on flank just posterior to pectoral fin. Dorsum brown. Venter white between head and region anterior to urogenital opening and

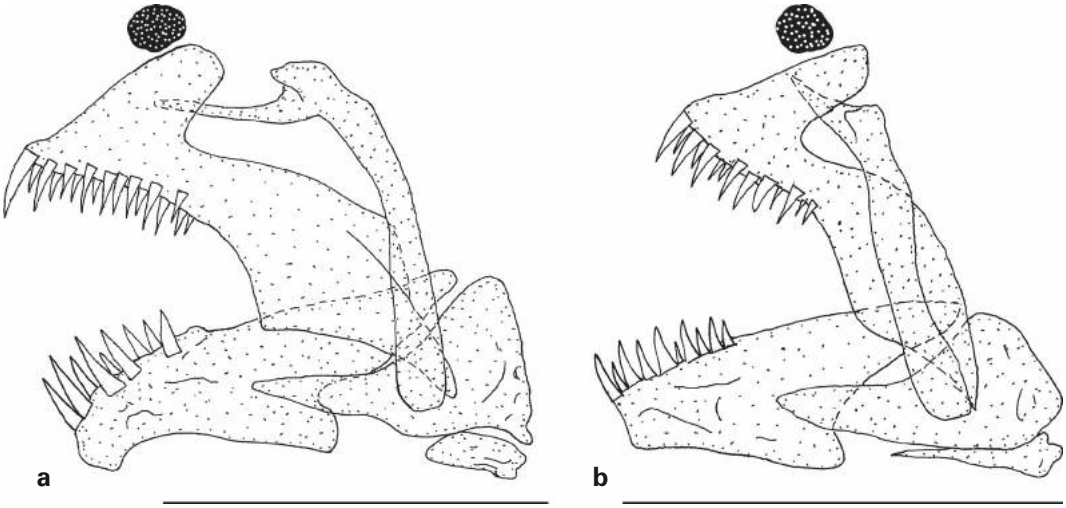

Fig. 7. Jaws, lateral view: **a.** *Fluviphylax zonatus*; **b.** *Fluviphylax gouldingi*. Abbreviations: **aa**, anguloarticular; **ar**, anguloreticular; **d**, dentary; **m**, maxilla; **p**, premaxilla; **rc**, rostral cartilage.

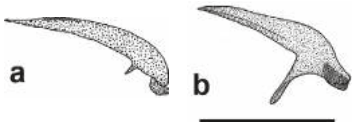

Fig. 9. Posttemporal, lateral view: **a.** *F. gouldingi*; **b.** *F. zonatus*. Abbreviations: **p**, posttemporal; **s**, supracleithrum.

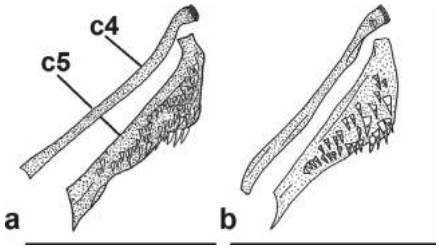

Fig. 10. Fourth and fifth ceratobranchials, ventral view: **a.** *F. zonatus*; **b.** *F. palikur*. Abbreviations: **c**, ceratobranchials.

brownish grey just anterior to urogenital opening to caudal peduncle. Side of head greyish pink, dorsal portion light orange. Iris orange. Eye bright silvery blue on dorsal portion with an orange pigmentation extending to eyes anterior margin. Jaws brownish grey. Orange pigmentation on preorbital area. Dorsal fin pale orange, with 2–3 black dots; orange distal margin. Anal fin orange with 12–15 black dots. Caudal fin light orange with black dots; black distal margin. Pectoral fin hyaline; orange distal margin. Pelvic fin bright orange.

Females. Side of body light orangish brown (Fig. 2b). Dorsum orange. Jaws brownish orange. Dorsal fin hyaline, to orangish hyaline on anterior portion of dorsal-fin rays. Anal fin hyaline, pale orange pigmentation on anterior anal-fin rays.

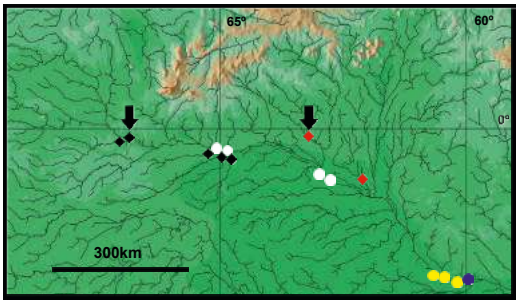

Fig. 11. Geographic distribution of *Fluviphylax gouldingi* (◆), *F. wallacei* (◈), *F. obscurus* (○), *F. simplex* (●), *F. zonatus* (◌), arrows indicate type localities.

Caudal fin orange, distal portion hyaline. Pectoral fin hyaline, basal portion orangish hyaline. Pelvic fin hyaline.

Distribution and habitat. Known from four main river drainages of the upper and middle Rio Negro drainage: Vaupés, Urubaxi, Neuixi and Aiuanã (Fig. 11). Habitat data are available only from two localities in the Rio Vaupés drainage (Fig. 12a). *Fluviphylax gouldingi* was collected close to the deadwood in a shallow, slow flowing, high transparency black-water stream, about 30–50 cm deep, in a dense vegetation area and in Lago Tiburiari, an open vegetation area connected with the Rio Vaupés through a straight canal (Fig. 12b). In the small stream they were found in small shoals of about 3–5 individuals swimming near the surface, whereas in the Lago Tiburiari they formed larger shoals of about 20 individuals. The miniature scoloplacid, *Scoloplax dolicholophia* Schaefer, Weitzman & Britski, 1989 was found sympatrically.

Etymology. Named in honor to the American ichthyologist Michael Goulding for his collection efforts in the

Fig. 12. **a.** Rio Vaupés drainage near Monte Cristo community; **b.** Lago Tiburiari, type locality of *F. gouldingi*.

Fig. 13. *Fluviphylax wallacei*, UFRJ 9080, holotype, 13.3 mm SL.

Fig. 14. *Fluviphylax wallacei*: **a.** UFRJ 9081, paratype, male, 13.5 mm SL; **b.** UFRJ 9081, paratype, juvenile male, 11.7 mm SL; **c.** UFRJ 9081, paratype, female, 11.4 mm SL. Brazil: Amazonas, Barcelos, Rio Aracá drainage.

Amazon, especially in the Rio Negro, and for his many ecological studies on the Neotropical ichthyofauna.

Remarks. The new species also occurs in the lower Rio Urubaxi drainage, close to its confluence with the Rio Negro, where it is sympatric to *Fluviphylax obscurus*. Unfortunately, due to the bad conditions of the material, it was not possible to securely identify each of the 140 specimens present on the lot MZUSP 29376.

***Fluviphylax wallacei*, spec. nov.**
= *Fluviphylax* sp. B in Bragança & Costa (2018)
Figs 13–14

Holotype. UFRJ 9080, male, 12.8 mm SL; Brazil: Estado do Amazonas: Município de Barcelos: Igarapé Mauaú near Romão community, Rio Aracá drainage, middle Rio Negro drainage, 0°20'31"S 62°56'30"W, altitude about 30 m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 18 Nov. 2012.

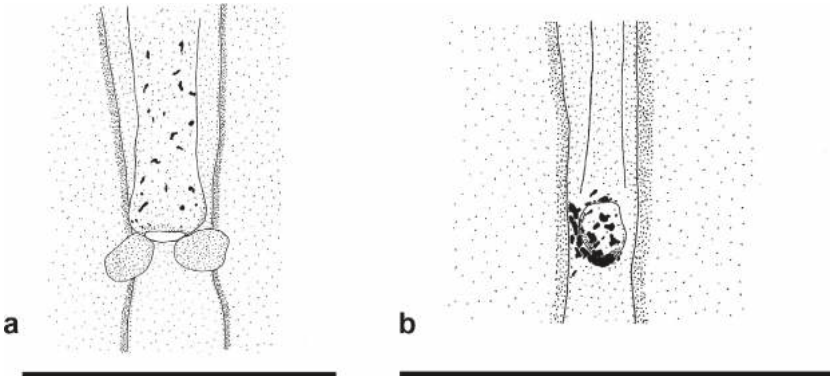

Fig. 15. Urogenital papillae sexual dimorphism: **a.** male of *F. wallacei*; **b.** female of *F. wallacei*.

Paratypes. UFRJ 9081, 84, 7.3–13.5 mm SL; UFRJ 9082, 6 (c&s), 10.2–12.4 mm SL; CICCAA 00280, 10, 10.4–12.7 mm SL collected with holotype. Brazil: Estado de Roraima: Muncípio de Caracaraí: MZUSP 112556, 4, 9.2–14.2 mm SL; MZUSP 112525, 14 (2 c&s), 7.8–14.4 mm SL; Igarapé do Campo near Caicubi community, tributary of Rio Jufari drainage, Middle Rio Negro drainage, 1°04'01"S 62°07'40"W; O. Oyakawa et al., 28 Aug. 2011.

Diagnosis. *Fluviphylax wallacei* is distinguished from all other congeners by having a sexual dimorphic pattern on cephalic lateral line system, in which males have whole opened sensory canals (Fig. 5a,f) and females have the anterior section of supra-orbital, posterior infra-orbital and preopercular canal dorsal portion closed (Fig. 5b,e) and by a pointed dorsal fin in males (Fig. 4b) (vs. rounded; Fig. 4a,c,d). It is similar to *F. gouldingi* and distinguished from all other congeners by having a slender retroarticular (Fig. 7b) (vs. deep; Fig. 7a) and by the presence of a triangular opercle (Fig. 7b) (vs. scale-like; Fig. 7a). It is further distinguished from all its congeners except *F. gouldingi* by having the head free neuromasts not placed in shallow grooves (Fig. 5a,f) (vs. in shallow grooves; Fig. 5c,d) and presence of orange colouration on the posterior region of the pectoral fin in males (Fig. 14) (vs. absent). Other character states not unique but useful to identify *Fluviphylax wallacei* are: presence of two to four black bars on the anterior portion of the flank in preserved male (Fig. 13) (vs. absent; Fig. 1); anal fin elongate and pointed, reaching vertical to dorsal-fin tip in males (Fig. 14) (vs. short and rounded); male pelvic-fin reaching base of fifth anal-fin ray (Fig. 4h) (vs. reaching base of third anal-fin ray; Fig. 4i); ventral process of posttemporal short (Fig. 9a) (vs. long; Fig. 9b); mesethmoid rounded (Fig. 6a) and presence of sexual dimorphism in urogenital papillae morphology (Fig. 15a,b) (vs. absent).

Description

Morphometric data are presented in Table 1. Maximum recorded adult size 14.4 mm SL. Dorsal profile of body approximately straight. Ventral profile convex from lower jaw to end of anal-fin base, nearly straight on caudal peduncle. Anterior portion of body cylindrical, about as wide as deep, becoming compressed behind anal-fin origin.

Dorsal fin long and pointed in males, short and rounded in females, its origin at vertical just behind anal-fin base or between penultimate and last anal-fin ray. Anal fin long and pointed in males, tip at vertical through dorsal-fin tip, short and rounded in females, its tip reaching vertical through end of dorsal-fin base. Caudal fin slender. Pectoral fin rounded, posterior margin reaching vertical between urogenital opening and pelvic-fin base. Pelvic fin long in males, tip reaching vertical through base of fifth anal-fin ray, short in females, tip reaching between urogenital opening and anal-fin origin. Pelvic-fin bases medially separated by interspace broader than width of each pelvic-fin base. Dorsal-fin rays 5–7; anal-fin rays 7–9; caudal-fin rays 15–19; pectoral-fin rays 10–11; pelvic-fin rays 6.

Frontal squamation G-patterned. Head free neuromasts placed over the body. Cephalic lateral line system: anterior portion of supraorbital canal opened in males with one exposed neuromast and closed in females, with two pores, posterior portion open in both, with two exposed neuromasts; anterior infra-orbital canal open, with two or three exposed neuromasts; median portion of infra-orbital region with six to eight minute neuromasts; posterior infra-orbital canal opened in males with a single neuromast and closed in females, with two pores; preopercular dorsal and ventral portion canals opened in males with three neuromasts; dorsal portion of preopercular canal closed in females, with two pores, ventral

portion opened with one neuromast; mandibular canal represented by single neuromast in vertical through corner of mouth (Fig. 3a,b,e,f). In juveniles, all canals open. Urogenital papillae sexual dimorphic. Longitudinal series of scales 24–26; transverse series of scales 6.

Osteology. Mesethmoid rounded (Fig. 6a). Premaxillary and dentary teeth well developed. Rostral cartilage present. Retroarticular slender (Fig. 7b). Opercle triangular, its anterodorsal process absent (Fig. 3b). Posttemporal scythe-shaped, ventral process short (Fig. 9a); dorsal process of cleithrum sharp and long. Anterior process of fifth ceratobranchial short and folded laterally (Fig. 10a). Second and fourth pharyngobranchial tooth plates not enlarged. Second pharyngobranchial teeth, 0–4 (Fig. 8b) Total vertebrae 27–28, 12–13 precaudal and 15 caudal. First proximal radial of dorsal-fin between neural spine of vertebrae 15 and 17. First proximal radial of anal-fin between pleural rib of vertebrae 10 and 11. Gill rakers on first branchial arch 11. Branchiostegal rays 4–5.

Colouration in alcohol. Overall colouration of body pale brownish yellow with minute dark chromatophores, forming a reticulate pattern; in males chromatophores more concentrated in zones of anterior portion of body, usually forming three to four transverse dark bars (Fig. 13). Melanophores on nape and along dorsal midline between nape and caudal-fin base. Ventrum scarcely pigmented. Dark brown chromatophores along whole mid-body line of flank, more visible on posterior region, between anal-fin origin and caudal peduncle. Melanophores forming a longitudinal stripe on ventral midline, between pelvic-fin base and caudal-fin base. Head overall colouration light brown. Dark brown chromatophores on dorsum of head. Small dark chromatophores on jaws, snout and opercular region. Iris silver, lighter close to pupil; dark pigment concentrated on dorsal margin of eye. All fins hyaline, melanophores sparsely concentrated on fins membranes and along fin rays; males with patches of melanophores forming small black spots on anal fin and caudal fin distal portion and posterior border of anal-fin and caudal-fin with a black margin.

Colouration in life. Males. Side of body pale orange (Fig. 14a,b). Metallic bronze line on middle of flank, extending from posterior limit of head to caudal peduncle. Dorsum light orange. Venter white to bluish white between head and region anterior to urogenital opening, with metallic blue ventral line between point just anterior to urogenital opening and caudal peduncle. Side of head orangish pink, dorsal portion orange. Jaws pale orange. Iris orange. Eye bright silver on dorsal portion. Dorsal fin pale orange, base hyaline; black distal margin. Anal fin orange with 6–7 black dots, base hyaline. Caudal fin orange to orangish yellow on its base; black distal margin. Pectoral fin pale orange. Pelvic fin pale orange, basal region hyaline.

Females. Side of body brownish grey (Fig. 14c). Dorsum brownish grey. Jaws orangish brown. Dorsal fin hyaline, to orangish hyaline on anterior dorsal-fin rays. Anal fin hyaline, black pigmentation on anterior anal-fin rays. Caudal fin hyaline, basal portion orangish hyaline. Pectoral fin hyaline, basal portion orangish hyaline. Pelvic fin hyaline, black pigmentation along first ray.

Distribution and habitat. Known from two localities in the middle Rio Negro drainage, the Igarapé Mauaú in the Rio Aracá subdrainage and the Igarapé do Campo in the Rio Jufari subdrainage (Fig. 12). In the Igarapé Mauaú, *F. wallacei* was collected in a shallow area, about 30–70 cm deep, in a slow flowing high transparency black-water stream, with sandy beaches and leaf litter areas on the bottom near stream margin. It was found forming shoals of about 15–20 individuals swimming near the surface (Fig. 16). *Fluviphylax* was found sympatrically with the Hemiramphidae *Hyporhamphus brederi* (Fernández-Yépez, 1948).

Etymology. Named in honour of the British naturalist Alfred Russel Wallace (1823–1913) for his collection effort in the Amazon, especially in the Rio Negro drainage. Wallace explored the Rio Negro (1849–1852) collecting many fish species unknown at that time. Unfortunately, when he was returning to Europe, an accidental fire resulted in the sinking of the ship and all specimens were lost. Only some drawings of the fish fauna remained.

Discussion

Costa (1996) published the unique revision and morphology based phylogenetic analysis on the genus, describing three new species and proposing fifteen synapomorphic characters diagnosing it. However, considering the information taken from recently collected specimens of *F. simplex*, *F. zonatus*, *F. obscurus*, *F. pygmaeus*, *F. palikur* and the new species herein described, an updated diagnosis is needed. Therefore, *Fluviphylax* is presently diagnosed by the presence of eye extremely large, absence of vomer, absence of interarcual cartilage, absence of interhyal, derived shape of maxilla, basihyal cartilage enlarged, reduction of caudal-fin rays and cephalic sensory system, and a unique colour pattern, consisting of melanophores concentrated on dorsal and ventral midlines of body. Some of these characters are also seen in some African procatopodid genera, but they are considered to have been independently involved,

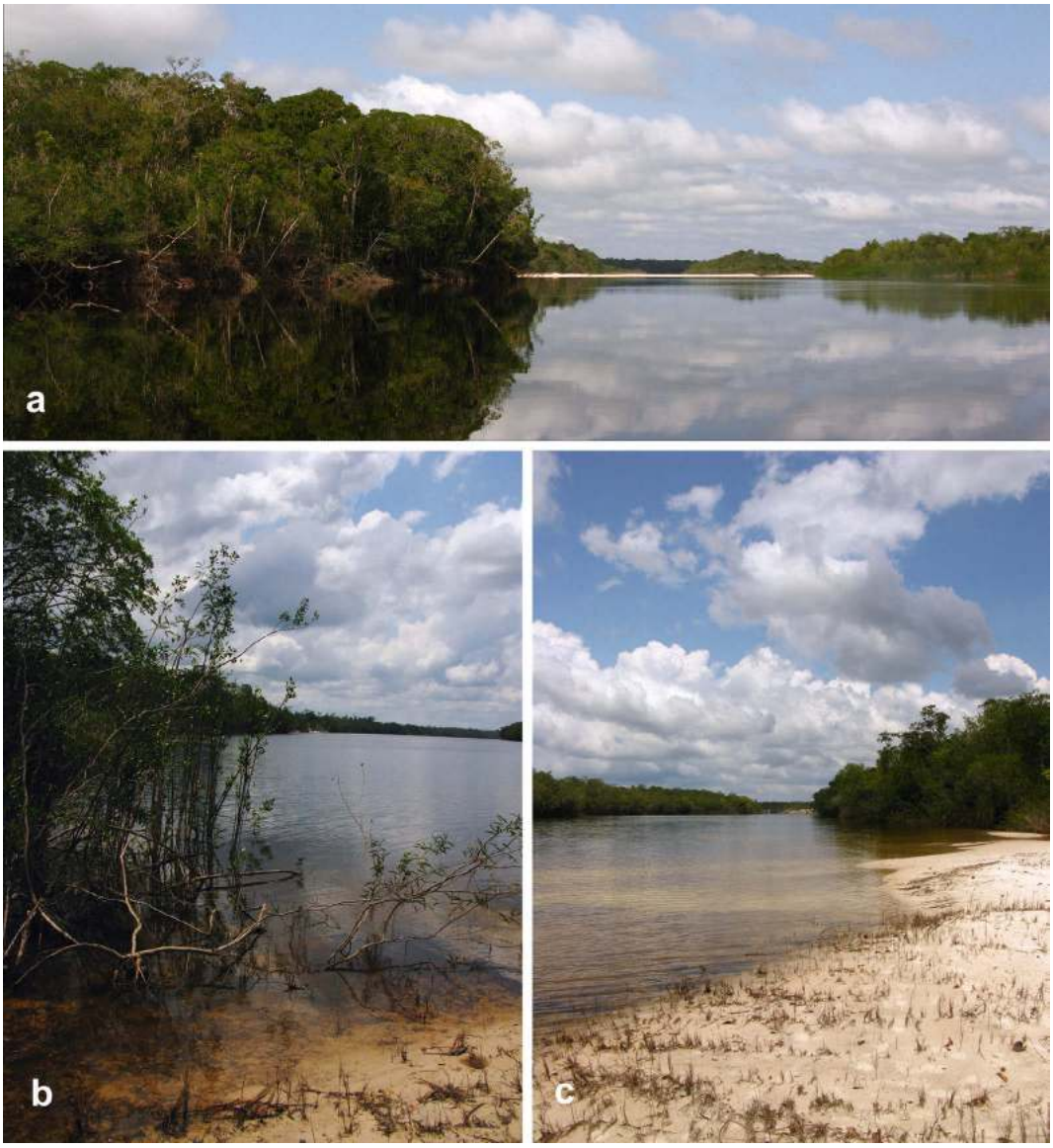

Fig. 16. **a.** Rio Aracá drainage; **b, c.** sand beach in Igarapé Mauaú, type locality of *F. wallacei*.

following recent molecular analysis that do not support a sister relationship between *Fluviphylax* and the procatopodids (Bragança et al. 2018, Bragança & Costa 2018). Among the African procatopodids, some species of *Congopanchax*, *Hylopanchax* and *Micropanchax* lack the vomer and interarcual cartilage, present extremely large eyes and a similar colour pattern on dorsal and ventral midlines. Other characters that were originally proposed as synapomorphic for *Fluviphylax* are not anymore considered as such. The presence of a distinct narrow process in the anterior portion of the opercle is not seen in *F. gouldingi* and *F. wallacei*, and the presence of teeth in the fourth ceratobranchial was recorded in some specimens of *F. simplex* and *F. palikur* (Fig. 10b), indicating that this plesiomorphic condition may be retained at least in part of this species. The present study agrees with Costa & Le Bail (1999), that considered *F. palikur* the sister species to all remaining species due to the retention of plesiomorphic character states like the

presence of more anal fin rays, more scales on longitudinal series and more vertebrae. There were also found other characters uniquely seen in *F. palikur*: anterior margin of parasphenoid lozenge-shaped (Fig. 6c); teeth of third pharyngobranchial and fifth ceratobranchial claw shaped with an adjacent lobe close to teeth apical region (Fig. 16); anterior process of anguloarticular truncate; anguloarticular ventral process present; dentary robust; notch on dorsal portion of entopterygoid present (Fig. 17).

Fluviphylax gouldingi and *F. wallacei* are easily distinguished from the other congeners by the presence of a slender retroarticular, a triangular opercle, the head neuromasts not placed in shallow grooves and by the presence of an orange colouration on the posterior region of the pectoral fin in males. These shared morphological and osteological characters suggest that the new species are probably closely related. However, Bragança & Costa's (2018) analysis has not recognized a sister species relationship between *F. gouldingi* and *F. wallacei*, but defined three main lineages among *Fluviphylax* species: a basal lineage including only *F. palikur*, a second lineage including only *F. gouldingi* and a third lineage with the remaining *Fluviphylax* species. According to Bragança & Costa (2018), *F. wallacei* is sister to *F. zonatus*.

In addition to the aforementioned character states, *Fluviphylax gouldingi* differs from all its congeners by the presence of a sharp ventral process on the opercle, a narrow subopercle, a narrow interopercle, a filamentous second pelvic-fin ray, an opened cephalic lateral line system in males and females and

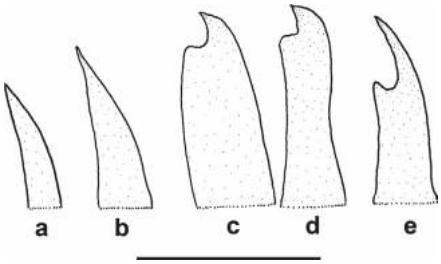

Fig. 17. Fifth ceratobranchial and third pharyngobranchial teeth shape: **a–b.** *F. simplex*; **c–e.** *F. palikur*.

the absence of a mesethmoid, supporting its placement in a distinct lineage. Also the presence of an orange bright blotch on the preorbital region in both males and females is unique for *F. gouldingi*. At last, *Fluviphylax wallacei* is distinguished from the other *Fluviphylax* species in having a sexual dimorphic pattern of the cephalic lateral line system and by the presence of a pointed dorsal fin in males.

According to the literature only three species were known to occur in the Rio Negro drainage: *F. zonatus* and *F. obscurus* were described from the lower and the middle/upper Rio Negro, respectively (Costa 1996); and *F. simplex* was reported to occur on the lower section of this drainage (Souza et al. 2011). *Fluviphylax gouldingi* and *F. wallacei* were described from tributaries in the middle and in the upper Rio Negro drainage, increasing the number of *Fluviphylax* species know to occur in this river drainage.

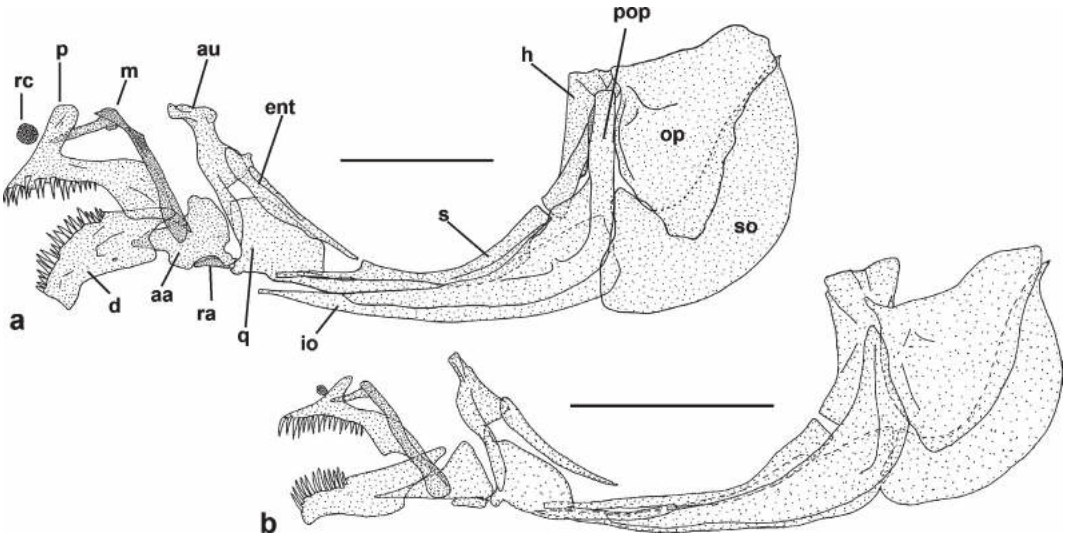

Fig. 18. Jaws and suspensorium: **a.** *F. palikur*; **b.** *F. obscurus*. Abbreviations: **aa**, anguloarticular; **au**, autopterotic; **d**, dentary; **ent**, entopterygoid; **h**, hyomandibula; **io**, interopercle; **m**, maxilla; **op**, opercle; **p**, premaxilla; **pop**, preopercle; **q**, quadrate; **ra**, retroarticular; **rc**, rostral cartilage; **s**, sympletic; **so**, subopercle.

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References

Bragança, P. H. N. & Costa, W. J. E. M. 2018. Time-calibrated molecular phylogeny reveals a Miocene–Pliocene diversification in the Amazon miniature killifish genus *Fluviphylax* (Cyprinodontiformes: Cyprinodontoidei). Organism, Diversity & Evolution. doi:10.1007/s13127-018-0373-7.

– – , Amorim, P. F. & Costa, W. J. E. M. 2018. Pantanodontidae (Teleostei, Cyprinodontiformes), the sister group to all other cyprinodontoid killifishes as inferred by molecular data. Zoosystematics and Evolution 94(1): 137–145.

Costa, W. J. E. M. 1988. Sistemática e distribuição do complexo de espécies *Cynolebias minimus* (Cyprinodontiformes, Rivulidae), com a descrição de duas espécies novas. Revista Brasileira de Zoologia 5: 557–570.

– – 1996. Relationships, monophyly and three new species of the neotropical miniature poeciliid genus *Fluviphylax* (Cyprinodontiformes: Cyprinodontoidei). Ichthyological Exploration of Freshwaters 7(2): 111–130.

– – & Le Bail, P. Y. 1999. *Fluviphylax palikur*: a new Poeciliid from the Rio Oiapoque Basin, Northern Brazil (Cyprinodontiformes: Cyprinodontoidei), with comments on miniaturization in *Fluviphylax* and other neotropical freshwater fishes. Copeia 1999: 1027–1034.

Ghedotti, M. J. 2000. Phylogenetic analysis and taxonomy of the poecilioid fishes (Teleostei: Cyprinodontiformes). Zoological Journal of the Linnean Society 130: 1–53.

Goulding, M., Leal-Carvalho, M. & Ferreira, E. 1988. Rio Negro,rich life in poor water. Amazonian diversity and foodchain ecology as seen through fish communities. the Netherlands: SPB Academic Publishing.

Gosline, W. A. 1949. The sensory canals of the head in some cyprinodont fishes, with particular reference to the genus *Fundulus*. Occasional Papers of the Museum of Zoology from University of Michigan 519: 1–17.

Helmstetter, A. J., Papadopulos, A. S. T., Igea, J., Van Dooren, T. J. M., Leroi, A. M. & Savoleinen, V. 2016. Viviparity stimulates diversification in an order of fish. Nature Communications 7: 11271. doi:10.1038/ncomms 11271

Hoedeman, J. J. 1956. Die bisher beschriebenen Formen und Arten der Gattung *Rivulus* Poey. Aquarium Terrarium 1956: 199–202.

Myers, G. S. & Carvalho, A. 1955. Notes on the classification and names of cyprinodont fishes. Tropical Fish Magazine 4: 7.

Neiffer, D. L. & Stamper, M. A. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. Institute for Laboratoy Animal Research 50: 343–360.

Parenti, L. R. 1981. A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bulletin of the American Museum of Natural History 168: 335–557.

Pollux, B. J. A., Meredith, R. W., Springer, M. S., Garland, T. & Reznick, D. N. 2014. The evolution of the placenta drives a shift in sexual selection in livebearing fish. Nature 513: 233–236.

Reznick, D. N., Furness, A. I., Meredith, R. W. & Springer, M. S. 2017. The origin and biogeographic diversification of fishes in the family Poeciliidae. PLoS ONE 12(3): e0172546. doi:10.1371/journal.pone.0172546

Roberts, T. R. 1970. Description, osteology, and relationships of the Amazonian cyprinodont fish *Fluviphylax pygmaeus*. Breviora 347: 1–28.

– – 1972. Ecology of the fishes in the Amazon and Congo basins. Bulletin of the Museum of Comparative Zoology 143: 117–147.

Souza, E. R., Ribeiro, L. B., Feldberg, E., Farias, I. P., Hrbek, T. & Gross, M. C. 2011. Comparative cytogenetics of two of the smallest Amazonian fishes: *Fluviphylax simplex* Costa, 1996 and *Fluviphylax zonatus* Costa, 1996 (Cyprinodontiformes, Poeciliidae). Comparative Cytogenetics 5: 411–422.

Taylor, W. R. & Dyke, V. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–109.

Weitzman, S. H. & Vari, R. P. 1988. Miniaturization in South America freshwater fishes: an overview and discussion. Proceedings of the Biological Society of Washington 101: 444–465.

Whitley, G. P. 1965. Some fish genera scrutinized. Proceedings of the Royal Zoological Society of New South Wales 1964/65: 25–26.