Universidade Federal do Rio de Janeiro

Instituto de Biologia

Programa de Pós graduação em Biodiversidade e Biologia Evolutiva

Species Delimitation and Taxonomic Revision of the miniaturized poeciliid genus *Fluviphylax* Whitley, 1965 (Cyprindontiformes: Poeciliidae: Procatopodinae)

Pedro Henrique Negreiros de Bragança

Rio de Janeiro, 2014

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Pedro Henrique Negreiros de Bragança

Master thesis presented to the Programa de Pós Graduação em Biodiversidade e Biologia Evolutiva, for the obtainment of the Master degree in Biodiversidade e Biologia Evolutiva – by the Instituto de Biologia da Universidade Federal do Rio de Janeiro

Advisor: Prof. Dr. Wilson José Eduardo Moreira da Costa

Rio de Janeiro

January 2014

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_____

Prof. Dr. Carlos Augusto Assumpção de Figueiredo

_____

Prof. Dr. Carlos Eduardo Guerra Schrago

_____

Prof. Dr. João Alves de Oliveira

_____

Prof. Dra. Michelle Regina Lemos Klautau

_____

Prof. Dr. Renner Luiz Cerqueira Baptista

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"What is life without one's hard-earned gold?"

Rathern than live a man like one of these,

I'd be an Indian here, and live content

To Fish, and hunt, and paddle my canoe,

And see my children grow, like young wild fawns,

In health of body and in peace of mind,

Rich without wealth, and happy without gold!"

Alfred Russel Wallace (march, 1851)

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Abstract

Species Delimitation and Taxonomic Revision of the miniaturized poeciliid genus *Fluviphylax* Whitley, 1965 (Cyprindontiformes: Poeciliidae: Procatopodinae)

Pedro Henrique Negreiros de Bragança

Advisor: Wilson José Duardo Moreira da Costa

Abstract of the Master thesis submitted to the Programa de Pós Graduação em Biodiversidade e Biologia Evolutiva, Instituto de Biologia, da Universidade Federal do Rio de Janeiro - UFRJ, as part of the requirements needed to obtain the title of Master in Biodiversidade e Biologia Evolutiva.

The diversity of the miniaturized genus, *Fluviphylax*, is approached by two distinct species delimitation methods: a tree-based method and a character-based method. The character based method identified 14 distinct lineages within the genus, the already known *F. palikur*, *F. simplex*, *F. pygmaeus*, *F. obscurus* and *F. zonatus* and nine new putative species. The COI gene haplotype tree analyses comprised both maximum parsimony (MP) and maximum likelihood (ML) methods. The tree-based approach delimited six species groups herein called: *F. palikur*, *F. simplex*, *F. pygmaues*, *F. obscurus*, *F. zonatus* and *Fluviphylax sp.* B. The MP haplotype tree identified 13 lineages, ten of these corresponds to species delimited through the character-based method. The ML haplotype tree also recognized thirteen lineages but only seven corresponds to the species delimited through the character-based method. The ten PAA

putative species recovered by the MP haplotype tree are considered valid species. The distinct lineages from the *zonatus* species group were not recognized due to discrepancies in the specie limits between PAA and the haplotype trees. The genus is diagnosed by the eye extremely large, vomer absent, dorsal process of the maxilla greatly reduced, interarcual cartilage absent, interhyal absent, basihyal cartilage enlarged, caudal-fin rays 17-20, cephalic sensory system reduced and colour pattern consisting of melanophores concentrated on the dorsal and ventral midlines of body.

Key-words: amazon, biodiversity, procatopodins, fishes, systematics.

Rio de Janeiro

January - 2014

Resumo

Delimitação de Espécies e revisão taxonômica do gênero miniaturizado de poecilídeo *Fluviphylax* (Cyprinodontiformes: Poeciliidae: Procatopodinae).

Pedro Henrique Negreiros de Bragança

Orientador: Wilson José Duardo Moreira da Costa

Resumo da Dissertação de Mestrado submetida ao Programa de Pós Graduação em Biodiversidade e Biologia Evolutiva, Instituto de Biologia, da Universidade Federal do Rio de Janeiro - UFRJ, como parte dos requisitos necessários para obtenção do título de Mestre em Biodiversidade e Biologia Evolutiva.

A diversidade do gênero mininaturizado, *Fluviphylax,* é abordado através de dois métodos distintos de delimitação de espécies: um método baseado em caracteres e outro na construção de árvores. O método baseado em caracteres identificou 14 linhagens distintas no gênero, os já descritos, *F. palikur*, *F. simplex*, *F. pygmaeus*, *F. obscurus, F. zonatus* e supostas nove novas espécies. As árvores de haplótipos para o gene COI compreenderam tanto análises de máxima parcimônia (MP) como de máxima verossimilhança (MV). O método baseado na construção de árvores delimitou seis grupos de espécies: *F. palikur*, *F. simplex*, *F. pygmaues*, *F. obscurus*, *F. zonatus* e *Fluviphylax sp.* B. A árvore de haplótipos de MP identificou 13 linhagens, dez dessas correspondendo a espécies delimitadas pelo método baseado em caracteres. A árvore de haplótipos de MV também reconheceu 13 linhagens, porém apenas sete correspondem a

espécies demilitadas através do método baseado em caracteres. As dez supostas espécies indicadas pelo PAA e recuperadas pela árvore de hapolótipos de MP são consideradas válidas. As distintas linhagens do grupo de espécies *zonatus* não foram reconhecidas devido aos limites de espécies incongruentes entre o PAA e as árvores de haplótipos. O gênero é diagnosticado pelo olho extremamente grande, vômer ausente, processo dorsal da maxila muito reduzido, cartilagem interarcual ausente, interhial ausente, cartilagem basial expandida, 17-20 raios na nadadeira caudal, sistema cefálico sensorial reduzido e melanóforos concentrados no dorso e no ventre do corpo.

Palavras chave: amazônia, biodiversidade, procatopodíneos, peixes, sistemática.

Rio de Janeiro

Janeiro - 2014

Summary

Illustrations list

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Abbreviations and acronyms list

Institutions and research expedition

UFRJ - Universidade Federal do Rio de Janeiro

MZUSP - Museu de Zoologia da Universidade de São Paulo

IEPA - Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá

MCP - Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul

MHNLS - Museo de História Natural La Salle

MNRJ - Museu Nacional do Rio de Janeiro

EPA - Expedição Permanente da Amazônia

Analysis

PAA - Population aggregation analysis

MP - Maximmum parsimony

ML - Maximum likelihood

Osteology

The osteological structures abbreviations are present in the figures legends.

Introduction

The familiy Poeciliidae comprises small killifishes that mainly inhabits freshwater environments widespread over the African and American continents, grouped in three subfamilies, Poeciliinae, Aplocheilichthyinae and Procatopodinae, according to the most recent classification proposed (Ghedotti, 2000). The subfamily Poeciliinae is restricted to the American continent with about 220 species in 28 genera and can be easily diagnosed by the presence of a copulatory organ in males, the gonopodium, and by the occurrence of viviparity or ovoviparity in all members of the subfamiliy, execept in *Tomeurus gracilis* Eigenmann 1907, a facultative viviparous species (Rosen & Bailey, 1963; Lucinda, 2003). The subfamily Aplocheilichthyinae contains only *Aplocheilichthys spilauchen* (Duméril, 1861) with a broad distribution along Africa West Coast brackish water environments. The subfamiliy Procatopodinae is a diverse assemblage of oviparous killifishes, also called lampeyes, primarily inhabiting freshwater environments, subdivided in two tribes, the Procatopodini and the Fluviphylacini. The Procatopodini are spread over the African continent with more than 100 species placed in nine genera, whereas the tribe Fluviphylacini contains only one genus, *Fluviphylax*, restricted to the Amazon (Ghedotti, 2000).

Fluviphylax was briefly described in 1955 as *Potamophylax* Myers & Carvalho, 1955. Some remarkable characters such as the small size, the extremely large eyes, the highset pectoral fin and the dorsal fin posteriorly positioned were suggested as evidence of a close relationship with the african lampeyes (Myers & Carvalho, 1955). Later, Whitley (1965) proposed *Fluviphylax* as a replacement name because *Potamophylax* was preoccupied in Insecta. Roberts (1970) redescribed *Fluviphylax pygmaeus* providing relevant information about the species biology, osteology, external

morphology and ecology. The material examined by Roberts (1970) included part of the type series and material recently collected by the Expedição Permanente da Amazônia (EPA) from lower and middle Amazon. Roberts (1970) considered *F. pygmaeus* as a geographically widespread Amazon taxon and also suggested a close relationship between *Fluviphylax* and the African Procatopodinae (Aplocheilichthyinae sensu Parenti, 1981) or to the American Fundulinae, first erecting the subfamily Fluviphylacinae.

Parenti (1981) proposed a new classification for the order Cyprinodontiformes based on cladistic methods, leading to several modifications relative to previous classifications that were based mainly on arbitrary criteria relative to the occurrence of viviparity and structures associated with internal fertilization. In previous classifications, the family Poeciliidae was restricted to the Poeciliinae (sensu Parenti, 1981; Costa, 1996; Costa, 1998; Ghedotti, 2000) and the family Cyprinodontidae grouped all oviparous Cyprinodontiformes, including the African poeciliids and the Neotropical genus *Fluviphylax*. The new classification turned the Poeciliidae more inclusive, for comprising two additional subfamilies, the Aplocheilichthyinae and the Fluviphylacinae.

Fluviphylax remained as a monotypic genus until Costa (1996) described three new species: *F. obscurus*, from middle and upper Negro river basin, *F. zonatus*, to the lower Negro river between Manaus and Anavilhanas and *F. simplex* from the Solimões-Amazon river, and considered *F. pygmaeus* as restricted to the Madeira river basin. Costa (1996) also proposed a new classification for the Poeciliidae, consisting of two tribes, Aplocheilichthyini and Fluviphylacini, within the Aplocheilichthyinae. Costa & Le Bail (1999) described *Fluviphylax palikur* as the smallest known species in the order

Cyprinodontiformes, from Oiapoque river basin, in which the largest specimens did not exceed 14.0 mm standard lenght.

A phylogenetic analysis of the superfamily Poecilioidea based on morphological characteres was proposed by Ghedotti (2000) leading to the present Poeciliidae classification. The African poeciliids and the genus *Fluviphyax*, previously members of the Aplocheilichthyinae were moved to the subfamily Procatopodinae Fowler, 1916 and the subfamily Aplocheilichthyinae become a monotypic taxon with only *Aplocheilichthys spilauchen*. According to Ghedotti (2000), the previous use of the subfamily name Aplocheilichthyinae Myers (1928) for a group composed of all African lampeyes and the genus *Fluviphylax* was in error because Procatopodinae Fowler (1916) has priority.

Historically, the first naturalists to explore the Neotropical ichthyofauna did not collect nor pay attention to the small sized or miniature species, probably due to the difficulty in collection and preservation of the specimens or to the belief that the small fishes were juveniles (Roberts, 1984; Weitzman & Vari, 1987; Weitzman & Vari, 1988; Costa & Le Bail, 1999). These particular challenges, in addition to the complexity in studying miniaturized species, may have contributed to the late description and few studies on the genus.

Haken & Wake (1993) defined miniaturization as the development of extremely small body size in adults. The miniaturization process occurs through changes in developmental parameters such as the growth rate, and the time in with growth begins and ends. However, the resulting patterns of miniaturization are more easily observed and studied than the intricate aspects of this developmental process (Hanken, 1993). Miniaturization among vertebrates is commonly found in fish, amphibians and reptiles and is generally associated with three effects, mainly over the skeleton: reduction and

structural simplification, increased morphological variability and morphological novelties (Hanken, 1993).

The reduction or structural simplification is the most common effect of miniaturization resulting in the reduction and loss of many bone elements due to changes in the degree of mineralization or even to the non ossification of cartilaginous precursors (Hanken & Wake, 1993). Weitzman & Vari (1988) reported many cases of miniaturization among the Neotropical ichthyofauna leading to the reduction of laterosensory system, number of vertebrae, body scales and fin rays, and in the sculpturing on the surface bones of the head. The reduction and structural simplification can even lead to complete bone loss or poorly ossified structures (Johnson & Brothers, 1993; Britz & Kottelat, 2003). Increased osteological variability in the adult skeleton frequently involves late forming structures that have an precociously truncated development or an alteration of skeletal patterning in early development structures. This processes results in intraespecific and even intraindividual (right -left asymmetry) variation in bone shape, size and in some cases absence or presence of the bone. This intraespecific variation may obscure interespecific differences in adult morphology turning the morphological study of miniature taxa challenging (Hanken & Wake, 1993). Hanken & Wake (1993) also reported miniaturization often related to the evolution of morphological novelties and many examples have been found, especially in the study of Southeast Asian freshwater fishes of the order Cypriniformes (Kottelat et. al, 2006; Britz et. al, 2009)

Some authors pointed problems caused by the inclusion of miniature taxa in phylogenetic analysis like the broad occurrence of reversals and paedomorphic parallelism. Weitzman & Vari (1987) mention that one of the greatest difficulties is to differentiate between plesiomorphic character states and paedomorphic conditions

because in many cases plesiomorphies are restored by a paedomorphic process. In addition, paedomorphic parralelism may indicate non-monphyletic assemblages as a result of homoplastic characters derived from convergent evolution (Schaefer et. al, 1989).

Miniaturization also has great impact over the ecology of the species. Roberts (1972) hypothesized that miniaturization among freshwater fishes in the Amazon river basin was primarily a response to biotic pressures. Small sized fishes would have access to food resources unexploited by adults of larger species and probably avoid them from being attacked by predaceous fishes (Roberts, 1972). Later, Goulding et al (1988) explored many ecological aspects of the Negro river basin ichthyofauna as its diversity, community development, trophic interactions and organization, corroborating some of Robert's hypotheses. The stomach content of *Fluviphylax* suggested feeding habits primarily based on algae and terrestrial invertebrates, but no information about the availability of these food items among larger species have been reported (Goulding et al, 1988). In addition, among the feeding habits of 25 piscivore fishes analyzed, *Fluviphylax* specimens have been reported as a prey for only one predator species, corroborating Robert's hypothesis.

Although studies dealing with ecology, miniaturization, biology and diversity of the genus *Fluviphylax*, those are occasional and little extensive. The small size of *Fluviphylax* and the need for extreme care during field collections probably make it an overlooked taxon, but not less attractive. Main issues such as the distribution and diversity of one of the smallest vertebrates in the world are still unknown. A recent increase in the practical implementation and theoretical development of species delimitation methods turns it a main issue over the research and description of *Fluviphylax* diversity.

Under the multidisciplinary approach of integrative taxonomy, the utilization of independent methods for species delimitation improves rigor in the identification of putative lineages. The establishment of more rigorous procedures in species delimitation is of great concern once the main issues in taxonomy are the identification and description of species in a world adversely noted by the loss of biodiversity. The present study provides a new sight over *Fluviphylax* diversity through the implementation of two distinct species delimitation methods: a character-based method and a molecular tree-based method. The study also includes information about the distribution of the identified lineages. The species distribution is of great concern once that species range may have a great importance in further biogeographical, ecological and conservation studies in the Amazon.

Material and Methods

Material

The specimens are deposited in the following institutions: IEPA, Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá, Macapá; MCP, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre; MHNLS, Museo de História Natural La Salle, Caracas; MNRJ, Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Rio de Janeiro; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; UFRJ, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro.

Morphological data

Morphological data were obtained from specimens fixed in formalin for a period of 10 days, and then transferred to 70% ethanol. 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 between the moment right after collection and less than 10 hours after that. The colouration pattern of preserved specimens was also analyzed. Measurements and counts follow Costa (1988). Measurements are presented as percentages of standard length (SL) except for subunits of head length (HL). 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 follows Gosline (1949), except for the posterior section of supraorbital canal, here called post-orbital canal, following Costa (1996). When the figures do not contain information about scale size, the scale bar represents 1millimiter (mm).

DNA extraction, amplification and sequencing

Molecular data were obtained from specimens fixed in absolute alcohol just after collection, and later preserved in the same solution. Total genomic DNA was extracted from muscle tissue of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To amplify the fragment of the mitochondrial DNA, the primers LCO1490, HCO2198 (Folmer et al., 1994) were used, which are specific for the mitochondrial gene Cytochrome c oxidase subunit I (COI). Polymerase chain reaction (PCR) was performed in 15µl reaction mixtures containing 5 x Green Go Taq Reaction Buffer

(Promega, Fitchburg, WI, USA), 3.2 mM MgCl2, 1µM of each primer, 75ng of total genomic DNA, 0.2 mM of each dNTP and 1 U of Taq polymerase. The thermocycling profile was as follows: (1) 1 cycle of 4 min at 94°C; (2) 35 cycles of 1 min at 92°C, 1min at 47-50°C and 1 min at 72°C; and (3) 1 cycle of 4 min at 72°C. In all PCRs, negative controls without DNA were used to check contaminations. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems, Foster City, CA, USA). Cycle sequencing reactions were performed in 10µl reaction volumes containing 1µl BigDye 2.5, 1.55µl 5 x sequencing buffer (Applied Biosystems), 2µl of the amplified products (10-40 ng) and 2µl primer. The thermocycling profile was (1) 35 cycles of 10s at 96°C, 5 s at 54°C and 4min at 60°C. The sequencing reactions were purified and denatured, and the samples were run on an ABI 3130 Genetic Analyzer. Sequences were edited using MEGA 5 (Tamura et al. 2011).

Species concept and species delimitation

The unified species concept proposed by de Queiroz (2007) is herein followed. It assumes that alternative species concepts shares a common conceptual definition disconnected with any particular operational criteria. The common element shared between all species concepts is that species are considered lineages united through gene flow. According to de Queiroz (2007) this is the only necessary property of a species and the secondary properties are instead different lines of evidence (operational criteria) relevant to assessing lineage separation (species delimitation).

Test of species delimitation among populations of *Fluviphylax* were according with two distinct methods: the Population Aggregation Analysis method (Davis & Nixon, 1992) and the tree-based method proposed by Wiens & Penkrot (2002). The Population Aggregation Analysis method is a character-based method, in which species are delimitated based on unique combination of morphological characters present in one or more populations. This method relies on the assumption that at least one character state that do not overlap between populations is evidence of absence of gene flow, indicating the existence of two distinct species. Character analysis focuses mainly on the colour pattern in life and preserved specimens, external morphology and osteology. The preserved colour pattern in all examined material was analyzed; life colour pattern was observed only in material collected by P. H. N. de Bragança through the direct examination of live specimens during collections, and numerous photographs of live individuals taken in the field. The method was applied to all collected and examined populations. The results of PAA involves the description of the PAA informative characters, formatted following Sereno (2007), the characterization of the putative lineages (focal species) and a character matrix with the exclusive characters combination for each identified lineage.

The second method is based on the direct inspection of phylogenetic analysis trees based on molecular data having as terminals specimens with known geographical information and at least two individuals of each focal species. The putative species recognized by PAA are herein reported as the focal species of the tree-based delimitation method and according with the resulting topology the species hypothesis will be corroborate or rejected. Terminals clustered in high supported clades and with concordant geographical distributions are considered evidence of absence of gene flow with other terminal taxa whereas, individuals from the same population failing to cluster

are evidence of genetic flow with other populations. This method allows recognition of species as both exclusive and non exclusive lineages. An exclusive lineage is identified when the haplotypes of the same focal species come together in a single cluster. When the haplotypes of the focal species cluster with haplotypes of another species it is recognized as a non exclusive species. Species may be either distinct or even morphologically diagnosable from each other but still present nonexclusive gene genealogies. This may be usual when a species with a large geographic range and a large population size gives rise to a distinct peripheral isolate species with a much smaller distribution range, such that the latter species quickly becomes exclusive, whereas the former species does not (Graybeal, 1995; Wiens & Penkrot, 2002).

Phylogenetic analyses comprised both maximum parsimony (MP) and maximum likelihood (ML) methods. MP was performed with TNT 1.1 (Goloboff et al., 2008), using the ‘new technology’ search. Branch support of the MP tree was assessed by bootstrap analysis, using a heuristic search with 1000 replicates under the TNT 'new technology' search, but saving a maximum of 1000 trees in each random taxon addition replicate. ML was run in MEGA 5 (Tamura et al., 2011), under the best nucleotide substitution model previously determined by MEGA; the Hasegawa-Kishino-Yano model with discrete Gamma distribution and invariant sites (Hasegawa et al.,1985) was indicated as the best-fit model of sequence evolution. The ML analysis was performed with random-starting parameters and using a random-starting tree; branch support was calculated with 1000 nonparametric bootstrap replicates using the same settings.

The tree-based method was applied to individuals from all lineages found in PAA method except in a population restricted to Orinoco basin due to the absence of material avaiable for molecular analyses. The outgroup species sequences were obtained in Genbank; they include: the Poeciliinae *Cnesterodon decemmaculatus* (GenBank

JX111729.1), *Poecilia caucana* (GenBank JX968687.1), *Poecilia petenensis* (GenBank EU751941.1) and the Procatopodinae *Lacustricola huteraui* (GenBank AY356594.1).

Euthanasia

The specimens were euthanize in tricain mesylate (TMS), a white powder easily soluble in water, used for anesthesia, sedation, or euthanasia of fish. It is a muscle relaxant and acts by blocking action potentials leading to no sensory input or muscle contractions.

Results

Species delimitation through Population Aggregation Analysis (PAA)

The application of the population aggregation analysis, resulted in the characterization of fourteen distinct *Fluviphylax* lineages through the comparative analysis of 63 morphological characters. All nominal species, *F. pygmaeus*, *F. simplex*, *F. zonatus*, *F. obscurus* and *F. palikur*, were recognized as independent lineages and nine new lineages were identified. Informative characters are bellow listed with further information about their states and distribution among the identified lineages. A matrix showing the distribution of all informative characters is presented in Appendix I.

- **PAA informative characters description and analysis**

Neurocranium and lachrymal bone

1. Mesethmoid: **(0)** absent (Fig. 1E); **(1)** present (Fig. 1A-D) (new character). The mesethmoid is ossified in all lineages, except in *Fluviphylax sp.* B, in which the mesethmoid is absent.

2. Mesethmoid, size: **(0)** minute (Fig. 1D); **(1)** large (Fig.1A-C,E) (new character). A minute mesethemoid, with a width smaller than parasphenoid anterior extremity, is uniquely seen in *Fluviphylax sp.* D. A large mesethmoid, with a greater width than parasphenoid anterior extremity is observed in other all other *Fluviphylax.*

3. Mesethmoid, shape: **(0)** rounded (Fig. 1C,D); **(1)** subtriangular (Fig 1, A,B) (Costa, 1996: fig. 11). The mesethemoid is rounded in most *Fluviphylax* lineages. A subtriangular mesethemoid is present in *F. simplex*, *Fluviphylax sp.* F and *Fluviphylax sp.* A. Costa (1996) reported a subtriangular mesethemoid for *F. pygmaeus*, but in the present study, the analyzed material has a rounded mesethemoid.

4. Parasphenoid, anterior margin, shape: **(0)** straight (Fig. 1A, C-E); **(1)** spatulate, preapical margin laterally expanded (Fig. 1B) (new character). The spatulate parasphenoid is present only in *Fluviphylax sp.* A.

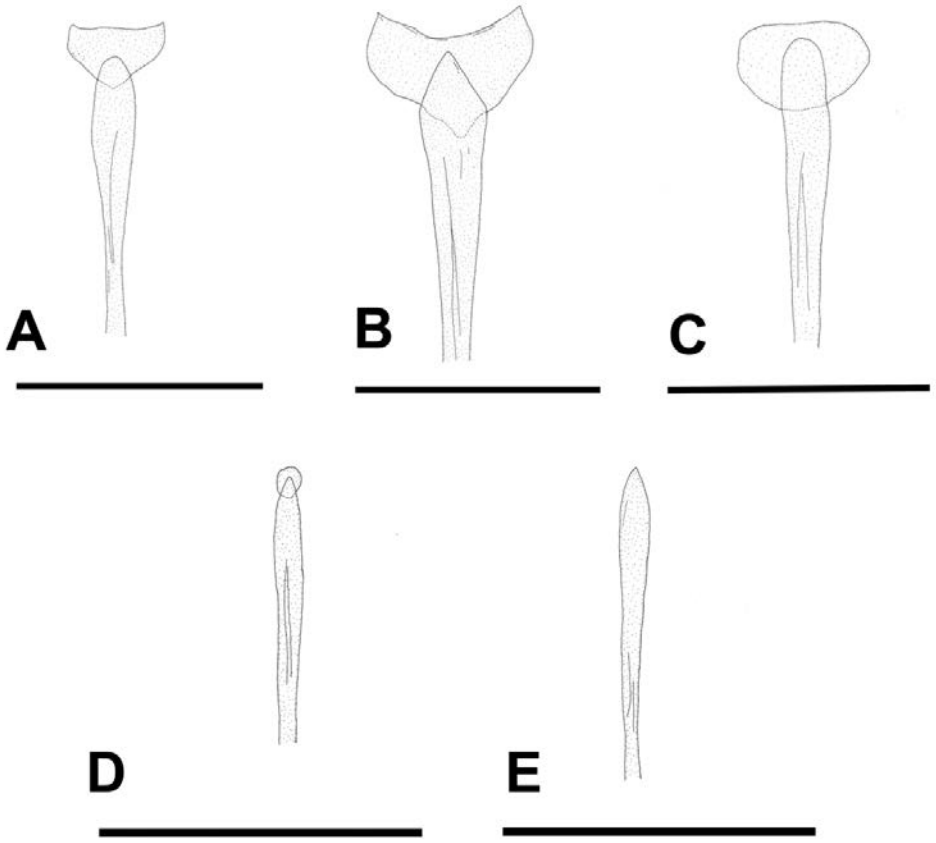

Fig 1. Anterior part of parasphenoid and mesethmoid. **A:** *Fluviphylax simplex*; **B:** *Fluviphylax sp.* A; **C:** *Fluviphylax obscurus*; **D:** *Fluviphylax sp.* D; **E:** *Fluviphylax sp.* B; m, mesethmoid; p, parasphenoid.

5. Lachyrmal, dorsal lobe, indentation: **(0)** absent (Fig. 2B); **(1)** present (Fig. 2A) (new character). A dorsal lobe indentation in the lachrymal bone is uniquely seen in *Fluviphylax sp.* H.

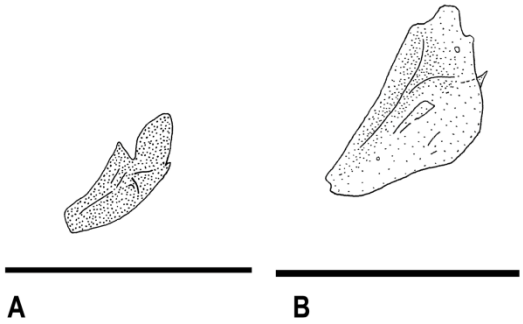

Fig 2. Lachyrmal bone. **A:** *Fluviphylax sp.* H; **B:** *Fluviphylax zonatus*.

Hyoid and branchial arch

6. Branchiostegal rays, number: **(0)** four (Fig. 3A); **(1)** five (Fig. 3B) (Costa, 1996). Costa (1996) considered the possession of four branchiostegal rays a unique condition present only in *Fluviphylax pygmaeus*. However, this condition is also observed in *Fluviphylax sp.* I and a polymorphic condition is seen in *Fluviphylax sp.* B, *Fluviphylax sp.* C and in *Fluviphylax obscurus.*

7. Fifth ceratobranchial, anterior process, shape: **(0)** folded laterally (Fig. 4A) ; **(1)** straight or slightly curved laterally (Fig. 4B)(Costa & Le Bail, 1999: fig. 3). The anterior process of the fifth ceratobranchial is folded laterally in all *Fluviphylax* lineages except in *Fluviphylax palikur* and it is polymorphic in *Fluviphylax sp.* A.

8. Second pharyngobranchial, tooth plate, development: **(0)** rudimentary (Fig. 6A); **(1)** well developed (Fig. 6B-C) (Costa & Le Bail, 1999: fig. 2). The second pharyngobranchial tooth plate is generally a rudimentary structure not overlapping most dorsal portion of the third pharyngobranchial, but in *Fluviphylax palikur*, *Fluviphylax zonatus*, *Fluviphylax sp.* A, *Fluviphylax sp.* E and *Fluviphylax sp.* G this structure is well developed, overlapping most dorsal portion of the third pharyngobranchial.

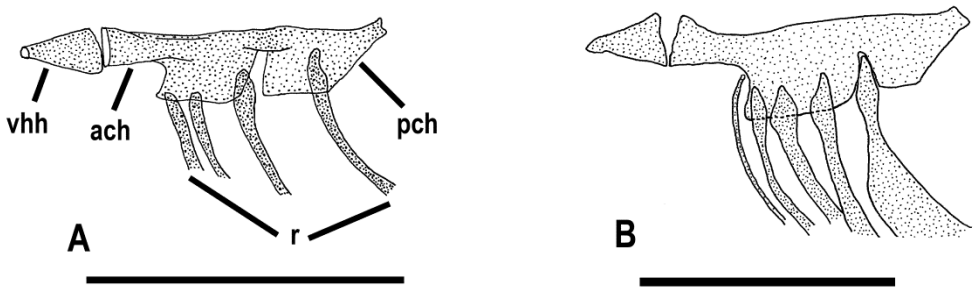

Fig 3. Hyoid arch and branchiostegal rays. **A:** *Fluviphylax pygmaeus*; **B:** *Fluviphylax zonatus*; vhh, ventral hypohyal; ach, anterior ceratohyal; pch, posterior ceratohyal; r, branchiostegal rays.

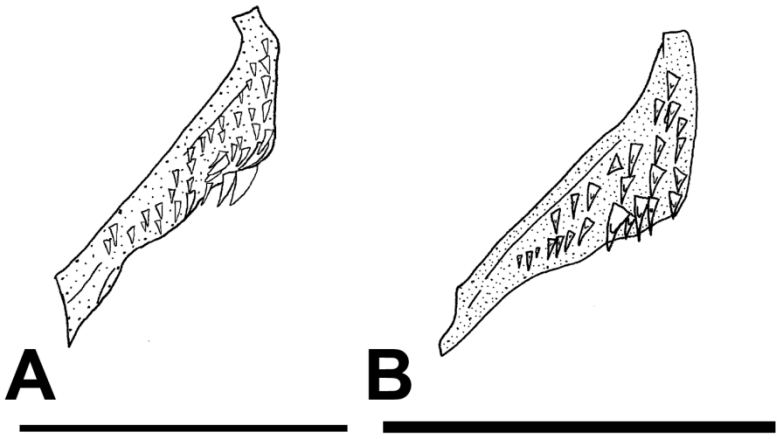

Fig 4. Fifth ceratobranchial. A: *F. zonatus*; B: *Fluviphylax sp.* A

9. Third pharyngobranchial and fifth ceratobranchial, teeth, expanded lobe adjacent to tooth tip: **(0)** absent (Fig. 5A-B); **(1)** present (Fig. 5C-E) (new character). The teeth in the third and fifth ceratobranchial tooth plates are pointed and conical in all *Fluviphylax* lineages, except in *Fluviphylax palikur* and *Fluviphylax sp.* A that have an adjacent lobe close to the teeth tip, resembling a claw.

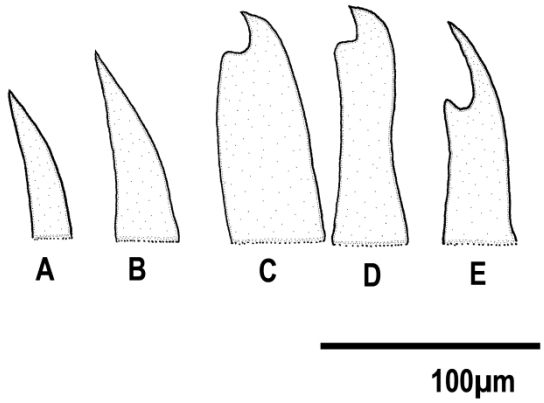

Fig 5. Third pharyngobranchial teeth. **A-B:** *Fluviphylax zonatus*; **C-E:** *Fluviphylax sp* A.

10. Fourth pharyngobranchial, tooth plate, development: **(0)** rudimentary (Fig. 6A); **(1)** well developed (Fig. 6B-C) (new character). The fourth pharyngobranchial tooth plate usually is a rudimentary structure with few minute teeth, but in *Fluviphylax palikur*, *Fluviphylax zonatus*, *Fluviphylax sp.* A, *Fluviphylax sp.* E and *Fluviphylax sp.* G, this structure is well developed with many well developed teeth.

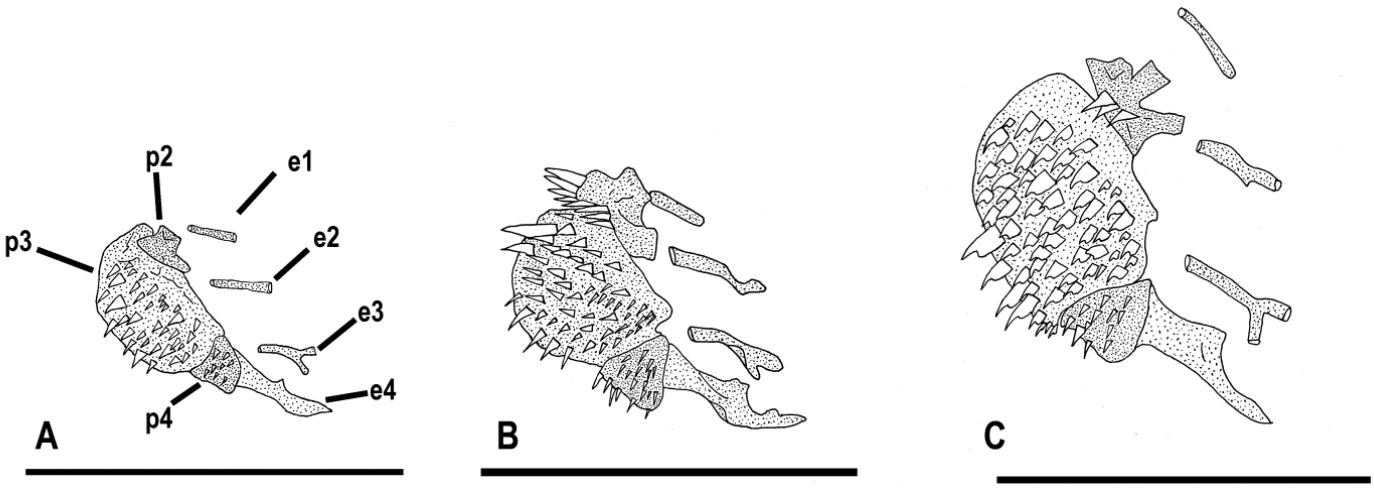

Fig 6. Left dorsal portion of branchial arches, ventral view. **A:** *Fluviphylax sp.* B; **B:** *Fluviphylax sp.* G; **C:** *Fluviphylax sp.* A; e 1-4, epibranchials 1-4; p2-4, pharyngobranchial 2-4.

Pectoral fin and girdle

11. Posttemporal, shape: **(0)** scythe-shaped (Fig. 7A-B); **(1)** straight (Fig. 7C) (Costa, 1996). Costa (1996) considered a scythe-shaped posttemporal as a synapomorphy of *Fluviphylax*, but Costa & Le Bail (1999) did not mention the shape of the posttemporal bone of *F. palikur*. However both *F. palikur* and *Fluviphylax sp.* A have a straight posttemporal bone.

12. Posttemporal, ventral process, lenght: **(0)** short , not reaching exoccipital bone (Fig. 7B-C); **(1)** long, reaching exoccipital bone (Fig. 7A) (Costa, 1996: fig. 9). A short posttemporal ventral process not reaching the exoccipital bone is observed in *Fluviphylax pygmaeus*, *Fluviphylax simplex*, *Fluviphylax palikur*, *Fluviphylax sp*. A, *Fluviphylax sp.* B, *Fluviphylax sp.* C, *Fluviphylax sp.* F and *Fluviphylax sp.* I. A long ventral process of the posttemporal reaching exoccipital bone is present in *Fluviphylax zonatus*, *Fluviphylax obscurus*, *Fluviphylax sp.* D, *Fluviphylax sp.* E, *Fluvihylax sp.* G and *Fluviphylax sp.* H.

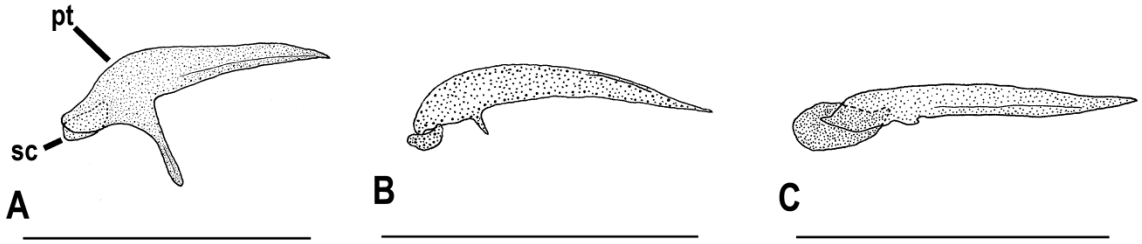

Fig 7. Posttemporal and supracleithrum bone. **A:** *Fluviphylax sp* G; **B:** *F. pygmaeus*; **C:** *Fluviphylax sp.* A; sc, supracleithrum; pt, posttemporal.

Jaw suspensorium and opercular apparatus

13. Opercle, anterodorsal process: **(0)** absent (Fig. 8A) ; **(1)** present (Fig. 8B-C) (Costa, 1996: fig. 10). The presence of an anterodorsal process in the opercle was considered by Costa (1996) as diagnostic for *Fluviphylax*. However among the new analyzed lineages this apomorphic condition is not found in *Fluviphylax sp.* B, *Fluviphylax sp.* C, *Fluviphylax sp.* H and is polymorphic in *Fluviphylax sp.* A and in *Fluviphylax sp.* D.

14. Opercle, anterodorsal process, lenght: **(0)** short (Fig. 8C); **(1)** long (Fig.8B) (Costa, 1996). A long anterodorsal process of opercle occurs in *Fluviphylax pygmaeus*, but *Fluviphylax simplex* and *Fluviphylax sp.* E are polymorphic for this character.

15. Opercle, sharp ventral process: **(0)** absent (Fig. 8B-C) ; **(1)** present (Fig. 8A) (new character). A sharp ventral process of opercle is present only in *Fluviphylax sp.* B.

16. Interopercle, width: **(0)** narrow (Fig. 8A); **(1)** wide (Fig. 8B-C) (new character). A narrow interopercle is uniquely seen in *Fluviphylax sp.* B.

17. Subopercle, width: **(0)** narrow (Fig. 8A); **(1)** wide (Fig. 8B-C) (new character). A narrow subopercle is uniquely seen in *Fluviphylax sp.* B.

18. Preopercle, rounded flange: **(0)** absent (Fig. 9A); **(1)** present (Fig. 9B) (new character). The preopercular rounded flange is backward oriented overlapping opercle anterior margin and it is only seen in *Fluviphylax sp.* F

19. Autopalatine notch, development: **(0)** reduced (Fig. 10A); **(1)** well developed

(Fig. 10B) (new character). A well developed autopalatine notch is present only in *Fluviphylax sp*. A.

20. Rostral cartilage: **(0)** absent (Fig. 11D); **(1)** present (Fig. 11A-C) (Costa, 1996: fig.6). The presence of the rostral cartilage is observed in all lineages whereas the absence of this structure is observed only in *Fluviphylax simplex*, *Fluviphylax pygmaeus*, *Fluviphylax sp.* F and *Fluviphylax sp.* I.

21. Anguloarticular, anterior process, shape (new character): **(0)** pointed (Fig. 11A,B,D) ; **(1)** truncate (Fig. 11C). A truncate anguloarticular anterior process is seen only in *Fluviphylax palikur* and *Fluviphylax sp.* A.

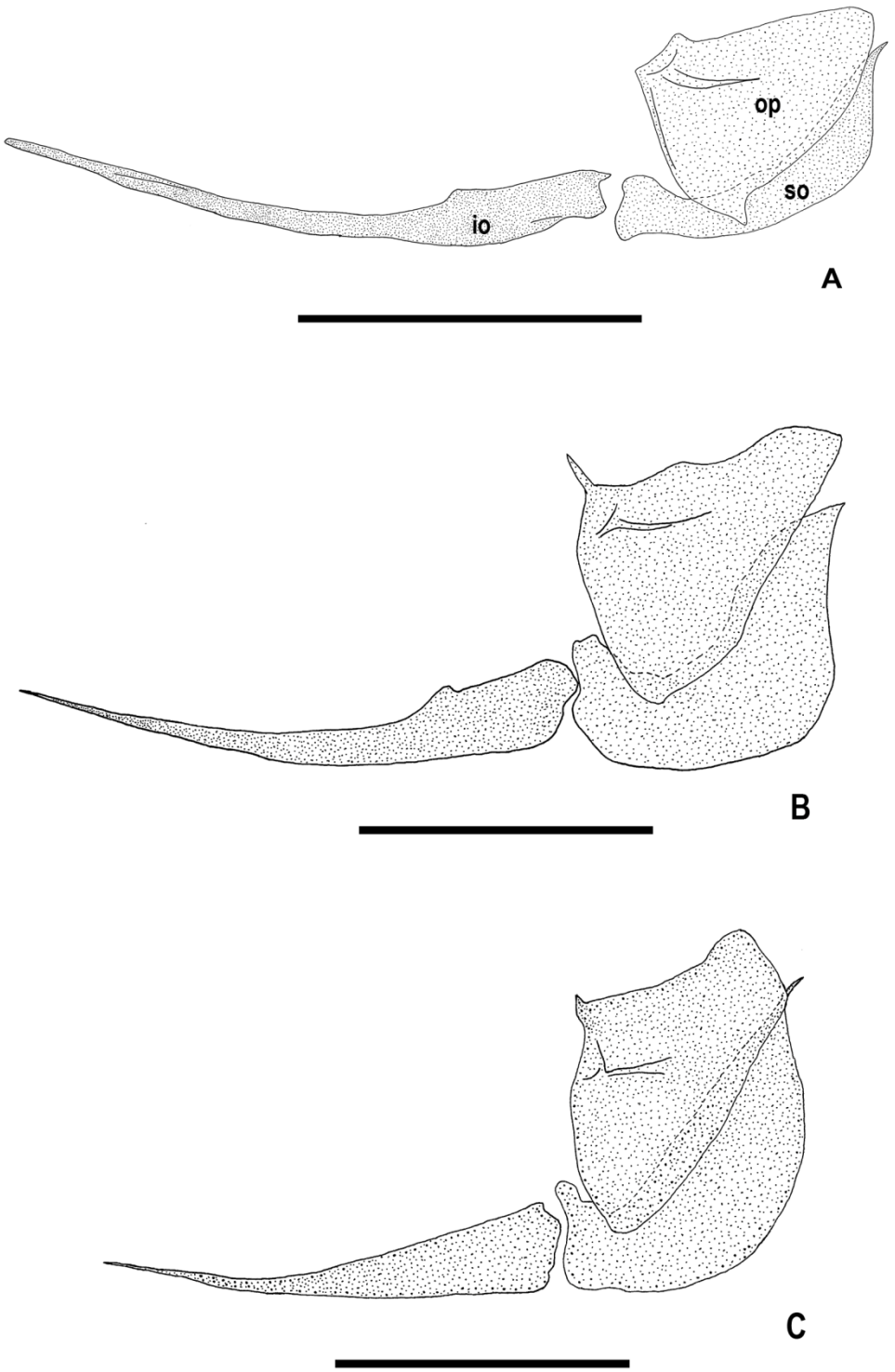

Fig 8. Opercular apparatus. **A:** *Fluviphylax sp.* B; **B:** *Fluviphylax pygmaeus*; **C:** *Fluviphylax sp.* F; op, opercle; so, subopercle; io, interopercle.

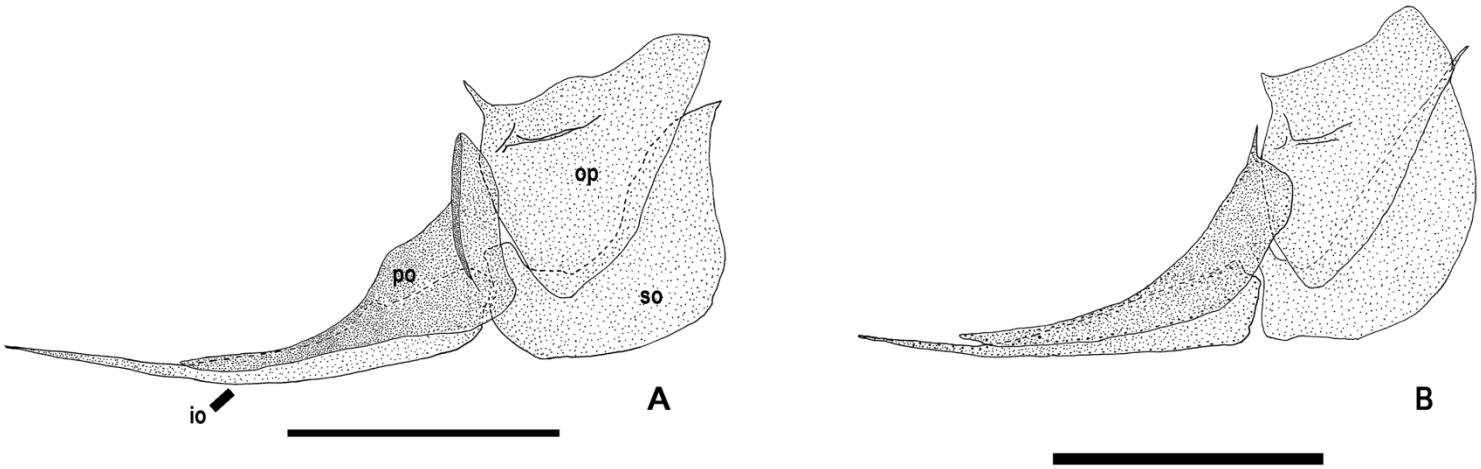

Fig 9. Opercular apparatus. **A:** *F. pygmaeus*; **B:** *Fluviphylax sp.* F; op, opercle; so, subopercle; io, interopercle; po, preopercle.

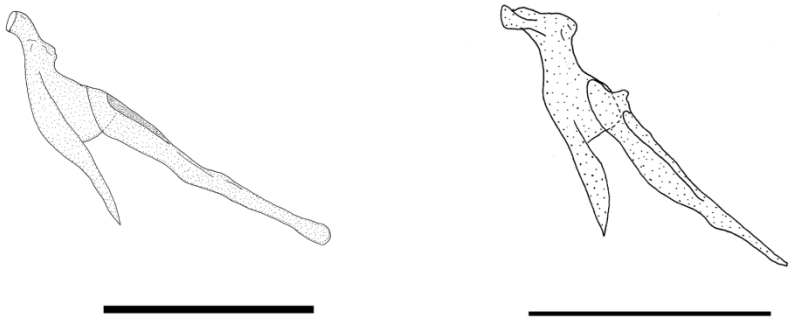

Fig 10. Autopalatine. **A:** *Fluviphylax obscurus*; **B:** *Fluviphylax sp.* A

22. Anguloarticular, ventral process: **(0)** absent (Fig. 11A,B,D); **(1)** present (Fig. 11C) (new character). The presence of a ventral process in the anguloarticular is uniquely seen in *Fluviphylax palikur* and in *Fluviphylax sp.* A.

23. Retroarticular, shape: **(0)** rectangular (Fig. 11A,C,D); **(1)** straight and pointed (Fig. 11B) (new character). All *Fluviphylax* lineages have a rectangular retroarticular bone except *Fluviphylax sp.* B and *Fluviphylax sp.* C that have a straight and pointed retroarticular.

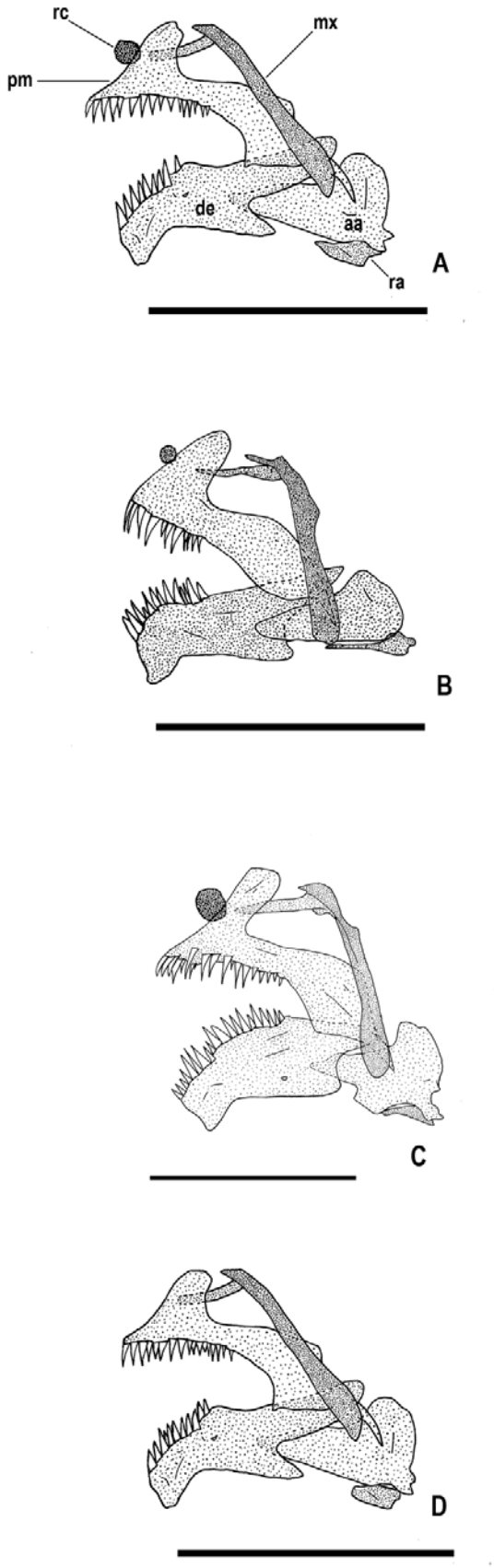

Fig 11. Jaws. **A:** *Fluviphylax zonatus*; **B:** *Fluviphylax sp.* C; **C:** *Fluviphylax sp.* A; **D:** *Fluviphylax pygmaeus*; rc, rostral cartilage; pm, premaxilla; mx, maxilla; de, dentary; aa, anguloarticular; ra, retroarticular.

24. Dentary, shape: **(0)** thin (Fig. 11A,B,D); **(1)** robust (Fig. 11C) (new character). A robust dentary is uniquely seen in *Fluviphylax sp.* A.

25. Dentary, teeth raws : **(0)** one; **(1)** two ; **(2)** three (new character). All *Fluviphylax* lineages have two teeth raws on dentary, except *Fluviphylax sp.* A with three teeth raws and *Fluviphylax palikur* with only one teeth raw on dentary. A polymorphic condition was recorded for *F. obscurus* with two and three teeth raws on dentary.

26. Premaxilla, teeth raws: **(0)** one ; **(1)** two; **(2)** three (new character). All *Fluviphylax* lineages have two teeth raws on premaxilla, except *Fluviphylax sp.* A with three teeth raws and *Fluviphylax palikur* and *Fluviphylax sp.* F with only one teeth raw on premaxilla. A polymorphic condition was recorded for *Fluviphylax sp.* D and *Fluviphylax sp.* H with one and two teeth raws on premaxilla.

Fins

27. Males, caudal-fin, filament: **(0)** absent (Fig. 12B-D); **(1)** present (Fig. 12A) (new character). The presence of a filamentous caudal-fin in males occurs only in *Fluviphylax sp.* A. The filaments are prolongations of caudal fin dorsal and ventral margins.

28. Males, pelvic-fin, length excluding the filamentous portion: **(0)** short, reaching second to third anal fin ray (Fig. 13A-B); **(1)** long, reaching fifth anal-fin ray (Fig. 13C-E) (new character). The male pelvic fin length are usually short, reaching between second to third anal fin ray whereas in *Fluviphylax pygmaeus*, *Fluviphylax sp.* B, *Fluviphylax sp.* C and *Fluviphylax sp.* H it is long.

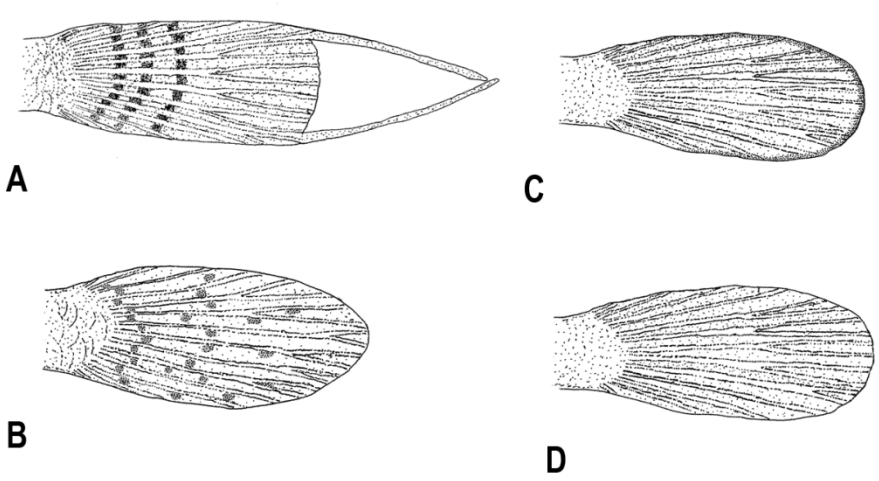

Fig 12. Caudal fin. **A:** *Fluviphylax sp.* A; **B:** *Fluviphylax simplex*; **C:** *Fluviphylax obscurus*; **D:** *Fluviphylax sp.* E.

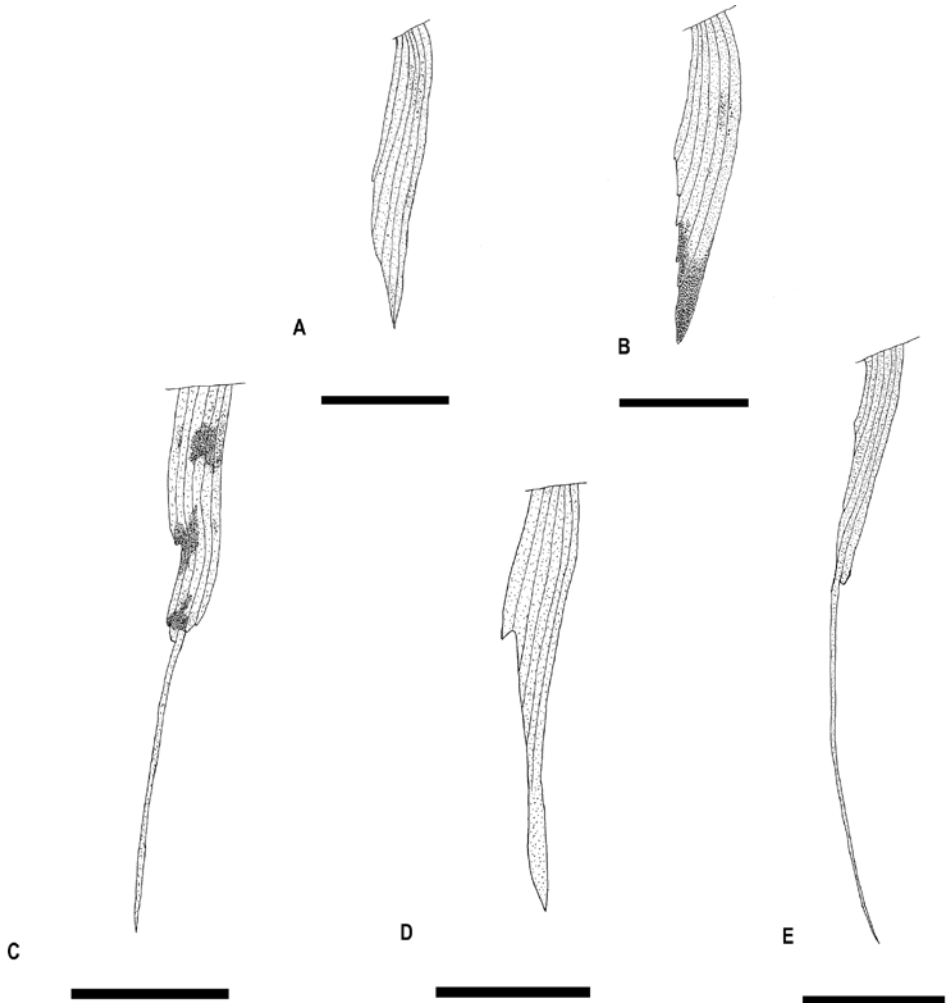

Fig 13. Pelvic fin. **A:** *Fluviphylax sp.* A; **B:** *Fluviphylax obscurus*; **C:** *Fluviphylax pygmaeus*; **D:** *Fluviphylax sp.* C; **E:** *Fluviphylax sp.* B.

29. Pelvic-fin, second ray, filament: **(0)** absent (Fig. 13A,B,C,D) ; **(1)** present (Fig. 13E) (modified from Costa, 1996). The presence of a filamentous second pelvic-fin ray occurs only in *Fluviphylax sp*. B.

30. Pelvic-fin, third ray, filament: (0) absent (Fig. 13A,B,D,E); (1) present (Fig. 13C)(new character). The presence of a filamentous third pelvic-fin ray occurs only in *Fluviphylax pygmaeus*.

31. Dorsal-fin, shape: **(0)** rounded (Fig. 14B-D) ; **(1)** triangular (Fig. 14A) (new character). All *Fluviphylax* lineages have a rounded dorsal fin except *Fluviphylax sp.* C that have a triangular dorsal fin.

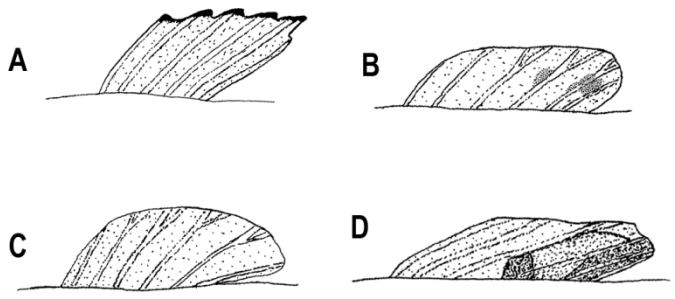

Fig 14. Dorsal fin. **A:** *Fluviphylax sp.* C; **B:** *Fluviphylax sp.* B; **C:** *Fluviphylax sp.* E; **D:** *Fluviphylax sp.* A.

32. Males, anal fin, shape: **(0)** rounded (Fig.15B,D); **(1)** triangular, pointed (Fig. 15C); **(2)** rectangular (Fig. 15A) (modified from Costa, 1996). A rectangular anal fin is uniquely seen in male of *Fluviphylax palikur* and *Fluviphylax sp.* A, whereas the triangular and pointed anal fin occurs only in *Fluviphylax pygmaeus*, *Fluviphylax sp.* B, *Fluviphylax sp.* C and *Fluviphylax sp.* H.

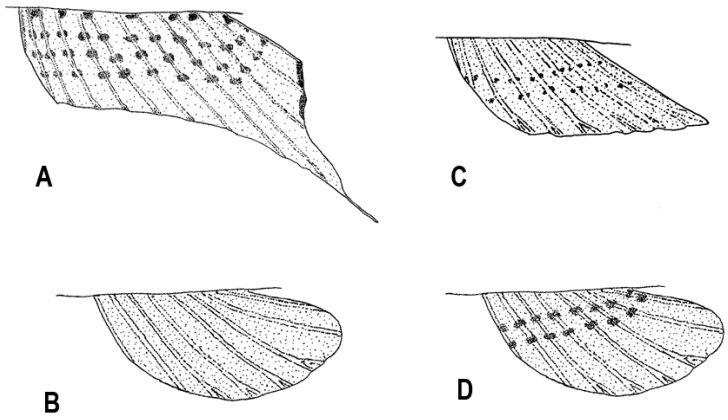

Fig 15. Anal fin. **A:** *Fluviphylax sp.* A; **B:** *Fluviphylax sp.* E; **C:** *Fluviphylax sp.* C; **D:** *Fluviphylax zonatus.*

33. Anal-fin, filament (new character): **(0)** absent (Fig. 15B-D); **(1)** present (Fig. 15A). A filamentous anal fin is present only in *Fluviphylax sp*. A

34. Anal-fin rays, number: **(0)** 7-10 anal fin rays ; **(1)** 12-15 anal fin rays (Costa & Le Bail, 1999). All *Fluviphylax* lineages have between 7-10 anal fin rays except *Fluviphylax sp.* A and *Fluviphylax palikur* with 12-15 anal fin rays.

Cephalic sensory system

35. Preorbital canal: **(0)** close (Fig.17D); **(1)** open (Fig17A-C) (new character). A closed preorbital canal is present in *Fluviphylax obscurus*, *F. palikur*, *Fluviphylax sp.* A and *Fluviphylax sp.* H. A polymorphic condition was observed in *Fluviphylax zonatus*.

36. Postorbital canal, preopercular canal upper section and supraorbital canal anterior section : **(0)** close (Fig. 16B; 17B,D); **(1)** open (Fig. 16A,C; 17 A,C) (new character). An opened preopercular canal upper section, postorbital canal and supraorbital canal anterior section occurs only in *Fluviphylax sp.* B and Fluviphylax sp. I This character is polymorphic for *Fluviphylax simplex.* The lineage *Fluviphylax sp.* C was scored (?) because it presents an sexual dimorphic pattern over the cephalic sensory system.

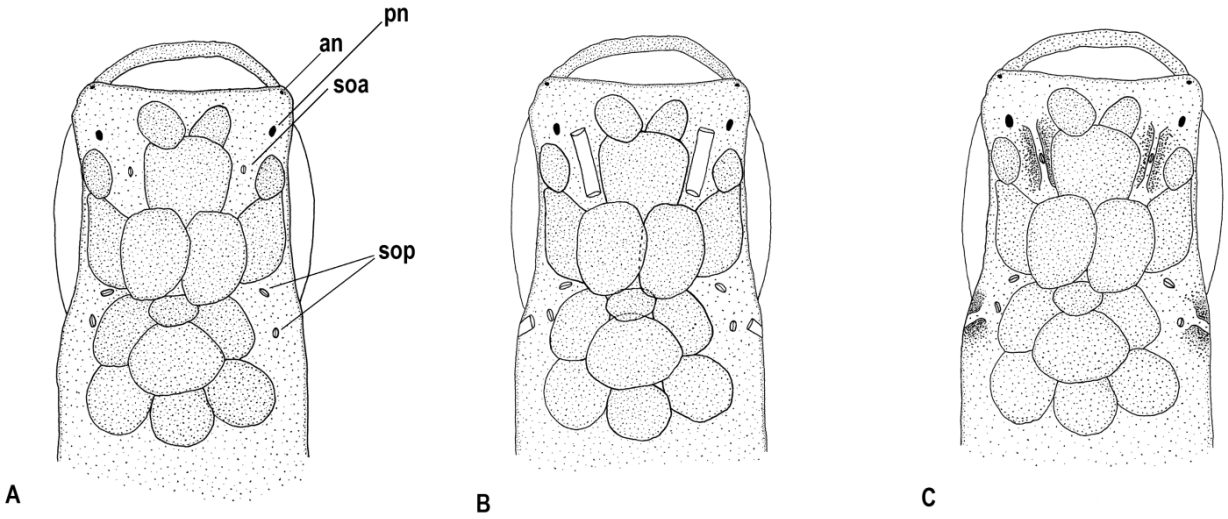

Fig 16. Diagrammatic representation of head dorsal view. **A:** *Fluviphylax sp.* B; **B:** *F. obscurus*; **C:** *F. simplex*; an, anterior nostril; pn, posterior nostril; soa, supraorbital canal anterior portion;sop, supraorbital canal posterior portion.

37. Postorbital canal, preopercular canal upper section and supraorbital canal anterior section, sexual dimorphic pattern : **(0)** absent; **(1)** present (new character). A dimorphic cephalic sensory system occurs only in *Fluviphylax sp.* C. The male have an opened preopercular canal upper section, postorbital canal and supraorbital canal anterior section whereas the female presents all structures closed.

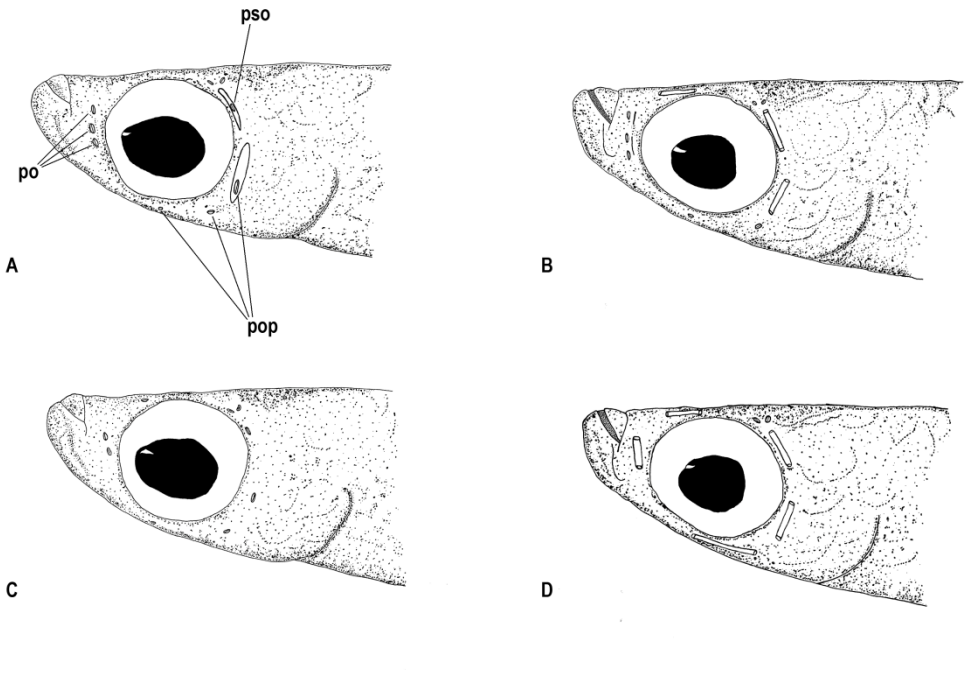

Fig 17. Diagrammatic representation of head lateral view. A: *Fluviphylax simplex*; **B:** *F. pygmaeus*; **C:** *Fluviphylax sp.* B; **D:** *F. obscurus*; pso, postorbital canal; pop, preopercular canal; po, preorbital canal.

38. Preopercular canal lower section: **(0)** close (Fig. 17D); **(1)** open (Fig. 17A-C) (new character). A closed preopecular canal lower section occurs only in *Fluviphylax obscurus* and *Fluviphylax sp.* A. Polymorphism have been observed in *Fluviphylax zonatus*, *Fluviphylax sp.* G and *Fluviphylax sp.* H.

39. Free neuromasts, placement: **(0)** in shallow grooves (Fig. 16C; Fig. 17 A); **(1)** over body surface (Fig. 16A; Fig. 17C) (new character). Free neuromasts are generally placed inside shallow grooves but in *Fluviphylax sp.* B and *Fluviphylax sp.* C the neuromasts are placed over body surface.

Colour patterns

40. Males, flank dorsoanterior portion, bright cooper blotches: **(0)** absent; **(1)** present (Fig. 41) (new character). The presence of bright cooper blotches on anterior portion of flank is uniquely seen in *Fluviphylax sp.* E.

41. Males, flank anterior portion, bright green: (0) absent; (1) present (Fig. 49) (new character). A bright green anterior portion of flank is only seen in *Fluviphylax sp.* I.

42. Males, flank of preserved specimens, dark bars: **(0)** absent (Fig.18C); **(1)** present (Fig. 18A,B) (Costa, 1996). The presence of dark bars over the flank in male is observed in *F. zonatus*, *F. pygmaeus*, *F. obscurus*, *Fluviphylax sp.* C, *Fluviphylax sp.* G, *Fluviphylax sp.* E and *Fluviphylax sp.* I. The absence of dark bars over the flank is seen in *F. simplex*, *F. palikur*, *Fluviphylax sp.* A, *Fluviphylax sp.* B and *Fluviphylax sp.* F. Because of the poor preservation of *Fluviphylax sp.* H this character was coded (?).

43. Males, flank of preserved specimens, dark bars, number: **(0)** 2 - 5 (Fig. 18A) ; **(1)** 6 or more (Fig. 18B) (Costa, 1996). The presence of 6 or more dark bars over the flank of preserved males is uniquely seen in *F. zonatus*.

Fig 18. Left size lateral view. **A:** *Fluviphylax sp.* C; **B:** *Fluviphylax zonatus*; **C:** *Fluviphylax sp.* B.

44. Males, caudal peduncle, yellowish white bars: **(0)** absent (Fig. 19A); **(1)** present (Fig. 19B) (new character). The presence of yellowish white bars over caudal peduncle is uniquely observed in *F. palikur* and *Fluviphylax sp.* A.

45. Urogenital papillae sexual dimorphism: **(0)** absent (Fig. 21E) ; **(1)** present (Fig. 21A-D) (new character). A sexual dimorphic urogenital papillae is observed only in *Fluviphylax palikur*, *Fluviphylax sp.* A and *Fluviphylax sp.* C.

46. Preorbital region, bright orange blotch: **(0)** absent (Fig. 20A) ; **(1)** present (Fig. 20B) (new character). A bright orange blotch on preorbital region is uniquely observed in *Fluviphylax sp.* B.

47. Males, dorsum, bright blue reticulate pattern: **(0)** absent; **(1)** present (Fig. 26) (new character). A bright blue reticulate pattern over male dorsum is present only in *Fluviphylax simplex*.

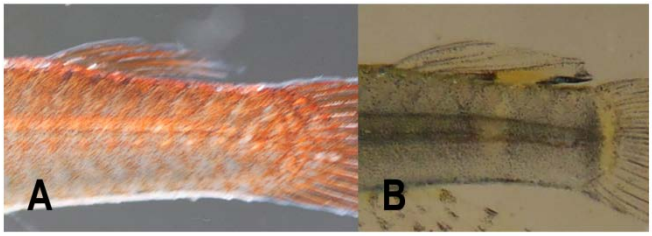

Fig 19. Dorsal fin and caudal peduncle. **A:** *Fluviphylax sp.* D; **B:** *Fluviphylax sp.* A.

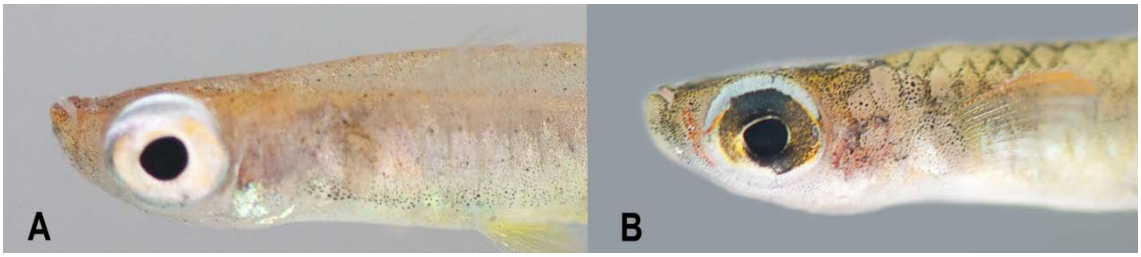

Fig 20. Head lateral view. **A:** *Fluviphylax obscurus*; **B:** *Fluviphylax sp.* B.

48. Males, dorsal-fin margin, black zone: **(0)** absent (Fig. 14B-D) ; **(1)** present (Fig. 14A) (new character). A black zone in male dorsal fin margin is uniquely seen in *Fluviphylax obscurus*, *F. pygmaeus* and is polymorphic in *Fluviphylax sp.* C.

49. Males, dorsal-fin, black dots : **(0)** absent (Fig. 14 A,C,D) ; **(1)** present (Fig. 14B) (new character). The presence of black dots in male dorsal fin occurs only in *Fluviphylax zonatus* and *Fluviphylax sp.* B.

50. Males, dorsal-fin posterior region, yellow and black blotch: (0) absent (Fig. 19A) ; (1) present (Fig. 19B) (Costa & Le Bail, 1999). The presence of a yellow and black blotch on male dorsal fin is observed only in *Fluviphylax palikur* and *Fluviphylax sp.* A.

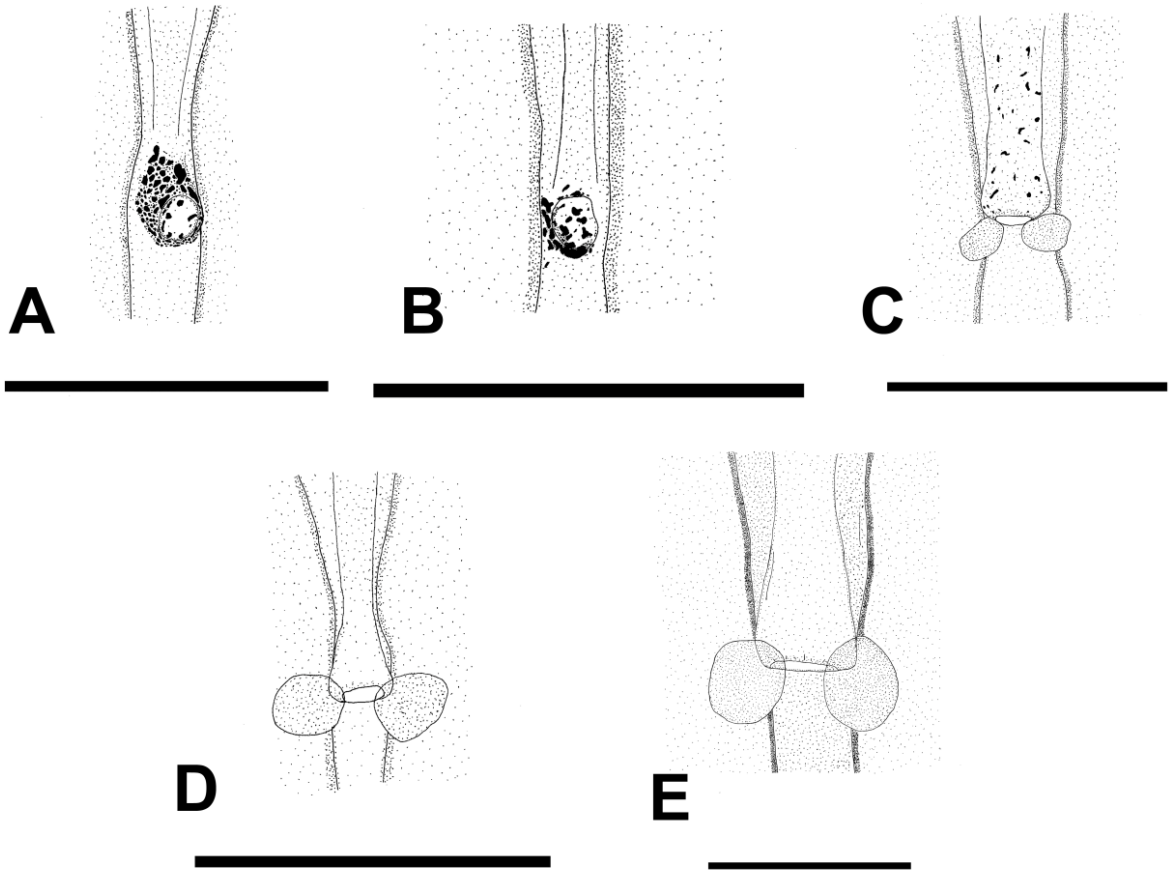

Fig 21. Urogenital papilae. **A:** Male, *Fluviphylax sp.* A; **B:** Male, *Fluviphylax sp.* C; **C:** Female, *Fluviphylax sp.* C; **D:** Female, *Fluviphylax sp.* A; **E:** Male-female, *F. zonatus.*

51. Males, anal fin, black dots: **(0)** absent (Fig. 15B); **(1)** present (Fig. 15 A,C,D) (new character). The presence of black dots in male anal fin is observed in all *Fluviphylax* lineages except in *F. palikur*, *Fluviphylax sp.* E, *Fluviphylax sp.* F and *Fluviphylax sp.* D.

52. Males, anal-fin posterior margin, bright bluish green colouration: **(0)** absent (Fig. 22A-C); **(1)** present (Fig. 22D) (new character). A bright bluish green colouration on male anal fin posterior margin is uniquely seen in *Fluviphylax sp.* A.

53. Males, anal-fin border, bright greenish white blotch: **(0)** absent (Fig. 22 A,B,D) ; **(1)** present (Fig. 22C) (new character). The presence of a bright greenish white blotch in anal fin border occurs only in *Fluviphylax zonatus.*

54. Males, anal-fin first and second rays tip, bright yellow colouration: **(0)** absent (Fig. 22B-D); **(1)** present (Fig. 22A). A bright yellow colouration in male anal fin first and second rays tip is uniquely seen in *Fluviphylax sp.* F and *Fluviphylax sp.* I.

55. Males, anal-fin margin, black zone: **(0)** absent (Fig. 22A,C,D) ; **(1)** present (Fig. 22B) (new character). The presence of a black zone on male anal-fin margin is uniquely seen in *F. obscurus*, *Fluviphylax sp.* D and *Fluviphylax zonatus*.

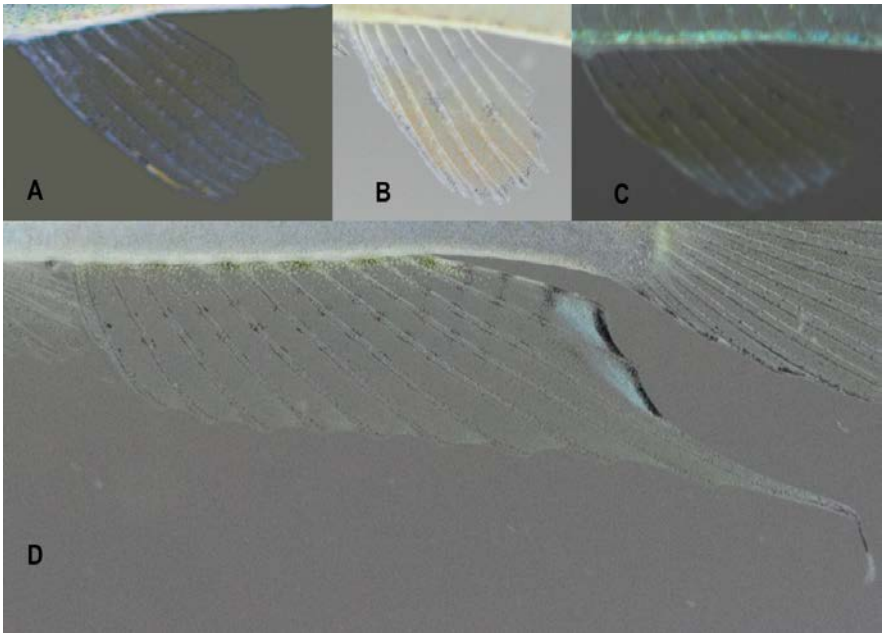

Fig 22. Anal fin colour pattern. **A:** *Fluviphylax sp.* F; **B:** *Fluviphylax obscurus*; **C:** *Fluviphylax zonatus*; **D:** *Fluviphylax sp.* A.

56. Males, caudal-fin margin, black zone: **(0)** absent (Fig. 23A) ; **(1)** present (Fig. 23B-D) (new character). The presence of a black zone in the male caudal fin margin occurs in *F. obscurus*, *F. zonatus*, *F, pygmaeus*, *F. simplex*, *Fluviphylax*

sp. B, *Fluviphylax sp.* C, *Fluviphylax sp.* D and *Fluviphylax sp.* I. The absence of a black zone in the male caudal fin margin occurs in *F. palikur*, *Fluviphylax sp.* A, *Fluviphylax sp.* E, *Fluviphylax sp.* F, *Fluviphylax sp.* G and *Fluviphylax sp.* H.

57. Males, caudal-fin, black dots: **(0)** absent (Fig. 23 A,B); **(1)** present (Fig. 23C,D) (new character). The presence of black dots in the male caudal fin is uniquely seen in *Fluviphylax pygmaeus*, *F. simplex*, *F. zonatus*, *Fluviphylax sp.* A, *Fluviphylax sp.* B, *Fluviphylax sp.* C, *Fluviphylax sp.* G and *Fluvipylax sp.* I.

58. Males, caudal-fin, three to four black bars : **(0)** absent (Fig. 23A,B,D); **(1)** present (Fig. 23C) (new character). The presence of three to four black bars in caudal fin is uniquely seen in *Fluviphylax zonatus*, *F. pygmaeus*, *Fluviphylax sp.* I and *Fluviphylax sp.* A

59. Males, caudal-fin ventral portion, bright greenish white blotch: **(0)** absent (Fig. 23A,B,D) ; **(1)** present (Fig. 23C) (new character). A bright greenish white blotch over caudal fin ventral portion is only seen in *Fluviphylax zonatus*.

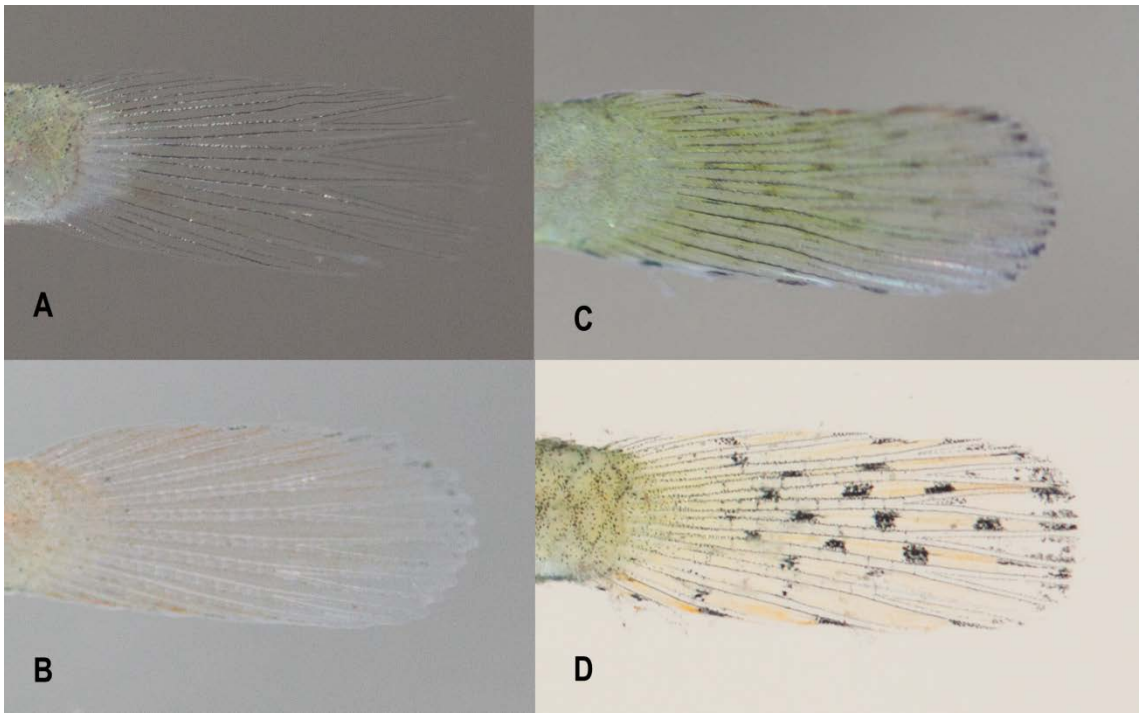

Fig 23. Caudal-fin colour pattern. **A:** *Fluviphylax sp.* E; **B:** *Fluviphylax obscurus*; **C:** *Fluviphylax zonatus*; **D:** *Fluviphylax sp.* B.

60. Males, pectoral-fin posterior region, orange colouration: **(0)** absent (Fig. 20A); **(1)** present (Fig. 20B). The pectoral fin is hyaline in all lineages except in *Fluviphylax sp.* B and *Fluviphylax sp.* C that have an orange colouration on the posterior region of the pectoral fin.

61. Males, pelvic-fin, dark black dots: **(0)** absent (Fig. 13A,B,D,E); **(1)** present (Fig. 13C) (new character). The presence of dark black dots on male pelvic fin is only observed in *Fluviphylax pygmaeus*.

62. Males, pelvic-fin tip, bright yellow colouration: **(0)** absent; **(1)** present (Fig. 43,48) (new character). A bright yellow colouration in male pelvic fin tip is uniquely seen in *Fluviphylax* sp. F and *Fluviphylax sp.* I.

63. Males, pelvic-fin tip, black: **(0)** absent (Fig. 13A,C-E); **(1)** present (Fig. 13B) (new character). A black pelvic fin tip occurs only in *Fluviphylax obscurus* and *Fluviphylax sp.* D.

- **Diagnoses for PAA *Fluviphylax* lineages**

***Fluviphylax pygmaeus* (Myers & Carvalho, 1955)**

Examined material. Brazil: Estado do Amazonas: MNRJ 14160 (original catalog number, 4126), 7 (13.1 - 15.0mm SL) (syntypes); Lake of Borba, Madeira river basin, Município de Borba; A. Parko, 1943. -UFRJ 9120, 92 (6 C&S) (7.8-12.7mm SL); UFRJ 9157, 25; Igarapé Jatuarãna, Madeira River basin, Município de Borba, 04º24'16.2"S 59º32'47.6"W; F. P. Ottoni, P. Amorim & P. Bragança, 06 Nov 2012. -UFRJ 9119, 8 (9.9-14.0mm SL); Balneário do Lira, about 4km following the road from Borba to Mapiá Grande river, Madeira River basin, Município de Borba, 04º25'28.5"S 59º32'47.6"W; F. P. Ottoni, P. Amorim & P. Bragança, 06 Nov 2012. -UFRJ 9201, 27 (8.2-12.6mm SL); UFRJ 9200, 7; UFRJ 9247, 5 (C&S); Igarapé Puxurizal, about 10km following the road from Borba to Mapiá Grande river, Madeira River basin, Município de Borba, 04º28'26.6"S 59º35'18.2"W; F. P. Ottoni, P. Amorim & P. Bragança, 06 Nov 2012. -MZUSP 7122, 4 (9.9 - 11.0mm SL); Igarapé on the left bank of Canumã river; EPA, 28 Nov 1967. Brazil: Estado de Rondônia: MZUSP 29373, 56 (8.4 – 14.1mm SL); Paracaúba Lake, close to the mouth of Machado river, Madeira river basin; M. Goulding, 06 Sep 1980.

Diagnosis. *Fluviphylax pygmaeus* is distinguished from all other congeners in having 3-4 dark black dots on males pelvic fin (vs. absence) and by having the third pelvic fin ray filamentous, surpassing the base of the anal fin base (vs. not filamentous). It is similar to *Fluviphylax sp.* B, *Fluviphylax sp.* H and *Fluviphylax sp.* C and distinguished from the remaining congeners in having an elongate and pointed anal-fin reaching vertically posterior to dorsal-fin base (vs. short and rounded anal fin) and by the elongate pelvic-fin reaching the base of the fifth anal fin ray (vs. short, reaching the

base of the third anal fin ray). It is distinguished from all other congeners but *F. simplex*, *Fluviphylax sp.* F and *Fluviphylax sp.* I by the absence of rostral cartilage (vs. presence). Other character states not unique but useful to identify *F. pygmaeus* are: mesethmoid rounded (vs. subtriangular), second and fourth pharyngobranchials tooth plates rudimentary (vs. well developed), anterodorsal process of opercle long (vs. short); four developed branchiostegal rays (vs. five), posttemporal ventral process short (vs. long) and preorbital canal and lower section of preopercular canal opened (vs. closed).

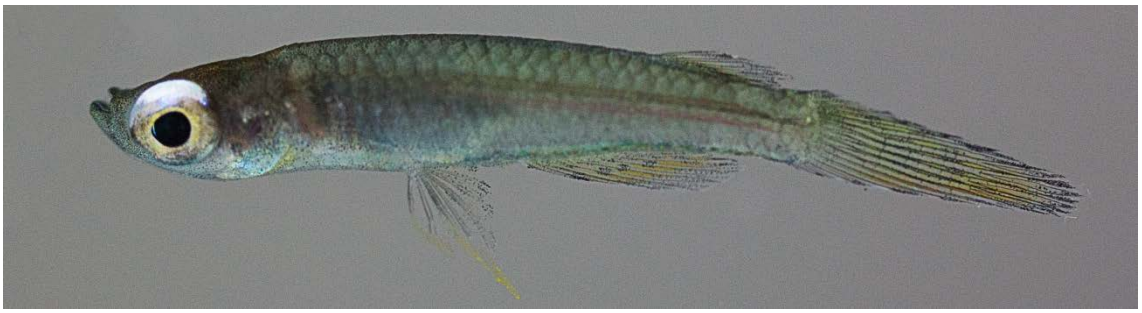

Fig 24. *Fluviphylax pygmaeus*, UFRJ 9120, male; Brazil: Amazonas: Borba.

Fig 25. *Fluviphylax pygmaeus*, UFRJ 9120, female; Brazil: Amazonas: Borba

Distribution and habitat. *Fluviphylax pygmaeus* is known only from the Madeira river basin. Despite the great sediments inflow in the Madeira river basin, in the localities close to Borba, *F. pygmaeus* was collected in high transparency shallow (30-70cm) black water streams in densely vegetation areas.

Remarks. Lucinda & Lucena (2012) reported that all the previous authors (Roberts, 1970; Costa, 1996; Eschemeyer & Fricke, 2011) when referring to the *Fluviphylax pygmaeus* type series do not mention all the information present on the original labels and handwritten notes, forgetting to report that the type locality is in a lagoon at Borba and not only Borba. However, Costa (1996) infact, did already mention this information when listing the examined material of *F. pygmaeus*. Furthermore both Roberts (1970) and Costa (1996) provided information about the type series, making clear that the species was first described without designation of holotype.

One specimen from UFRJ 9120 and two from UFRJ 9247 were parasited by metacercaria, the encysted maturing stage of a trematode. The counter and stained material from the Paracaúba Lake, close to the mouth of Machado river (MZUSP 29373, 3 of 63) were not found. Costa (1996) examined this material and found a slightly different vertebra range count 26-27 vs. 27-29 found in the present study for topotypes, and a different shape of the mesethemoid (subtriangular vs. rounded). The examined populations near Borba are approximately 540km far from the Machado river population.

***Fluviphylax simplex* Costa, 1996**

Material examined. Brazil: Estado do Amazonas: MZUSP 49209, 1 (14.3mm SL) (Holotype); Igarapé of José-Açu Lake, Município de Parintins; EPA, 11-12 Dec 1967. - MZUSP 7817, 114 (9.6 – 14.2mm SL) (Paratypes); Igarapé of José-Açu Lake, Município de Parintins; EPA, 11-12 Dec 1967. MZUSP 5829, 25 (9.1 - 15.5mm SL) (paratypes); Saracá Lake, Município de Silves; EPA, 17-18 Mar 1967. -UFRJ 5373, 360 (5.7-13.4mm SL); UFRJ 5374, 11 (C&S); Máximo Lake margin, Amazon basin, Município de Parintins; C. A. de Figueiredo & C. Codeço, 14 Sep 1996. - UFRJ 9824, 21; José-Açu lake border, close to Bom Socorro community, Amazonas basin, Município de Parintins; C. A. de Figueiredo & C. Codeço. -UFRJ 8307, 3 (13.9-15.1mm SL); Igarapé do Ubim, Amanã Lake drainage, Município de Maraã; H. Lazzaroto, E. Caramashi & F. Oliveira, 20 Aug 2011. - UFRJ 8881, 10 (9.4-15.0mm SL); UFRJ 9248, 3; Igarapé do Baré, Amanã Lake drainage, Município de Maraã, 02°15'59.4"S 64°40'46.0"W; H. Lazzaroto, 22 Aug 2011. - UFRJ 8342, 20; Igarapé do Kalafate, Amanã Lake drainage, Município de Maraã; H. Lazzaroto, E. Caramashi & F. Oliveira, 23 Aug 2011. - UFRJ 9160, 43 (4 C&S) (7.0-10.5mm SL); UFRJ 9209, 16; Igarapé Tarumã-Mirim near Livramento comunity, Lower Negro river basin, Município de Manaus, 03°01'24.3"S 60°10'40.0"W; F. P. Ottoni, P. Amorim & P. Bragança, 08 Nov 2012. - UFRJ 9217, 70 (5 C&S) (7.8-13.0mm SL); UFRJ 9085, 19; Island in Uaicurapá river, Amazon basin, Município de Parintins, 02°45'50.3"S 56°46'32.2"W; F. P. Ottoni, P. Amorim & P. Bragança, 04 Nov 2012. - MZUSP 51763, 50 (9.8 – 13.74mm SL); Igarapé of José Açu Lake, Município de Parintins; EPA, 11-12 Dec 1967. - MZUSP 49057, 62 (7.7 – 11.6mm SL); Miuá Lake, above Codajás, Município de Codajás; EPA, 25 Sep 1968. -MZUSP 5752, 28 (9.9 – 17.1mm SL); Mararauaçu Lake, Município de Urucará; EPA, 15 Mar 1967. -MZUSP 49191, 12 (12.0 – 14.1mm

SL); Janauacá Lake, Solimões river-AM; Alpha Helix Amazon Expedition, 7-25 Jan 1977. -MZUSP 53656, 4 (11.9 – 12.0mm SL); Puraquequara Lake, left bankof Amazonas river, Município de Manaus; M. de Pinna & H. Mesquita, 11 Nov 1996. Brazil: Estado do Pará: MZUSP 9463, 3, (11.7-12.9mm SL); Cuminá-Miri river, Município de Cajuassú; EPA, 26 Jan 1968. - MZUSP 7949, 43 (7.4 – 13.4mm SL); Igarapé in Janari river, above Terra Santa, Município de Terra Santa; EPA 14 Dec 1967.

Diagnosis. *Fluviphylax simplex* is distinguished from all of its congeners by having a bright blue reticulate pattern on males dorsum. It is also diagnosed by a combination of characters: subtriangular mesethemoid (vs. rounded), rostral cartilage absent (vs. present), males pelvic-fin short, reaching between the first to third anal-fin ray (vs. elongated reaching base of the fifth anal fin ray), posttemporal ventral process short (vs. elongated), second and fourth pharyngobranchial tooth plates rudimentary (vs. well developed), retroarticular rectangular (vs. sharp and pointed), five branchisotegal rays (vs. four), free neuromasts placed in shallow grooves (vs. over body surface)

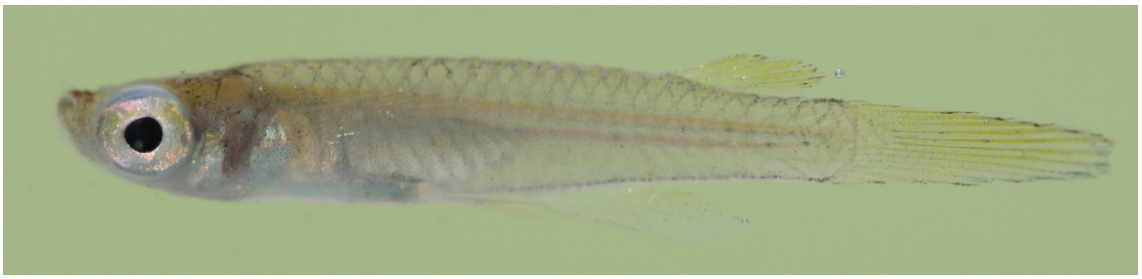

Fig 26. *Fluviphylax simplex*, UFRJ 9217, male; Brazil: Amazonas: Uaicurapá river near Parintins.

Distribution and habitat. *Fluviphylax simplex* has the greatest distribution among its congeners, occurring along the Amazonas and Solimões river, between Amanã lake and Parintins. In Igarapé Tarumã Mirim and Uaicurapá river *F. simplex* was found forming small shoals of about 5-10 specimens.

Remarks. The material from Igarapé Tarumã Mirim UFRJ 9160 and UFRJ 9209 contains both specimens from *F. simplex* and *F. zonatus*. The syntopy was confirmed by both morphological and molecular analysis.

***Fluviphylax obscurus* Costa, 1996**

Material examined. Brazil: Estado do Amazonas: MZUSP 49207, 1 (17.3mm SL) (holotype); Pool in island, Negro River Basin, Município de Barcelos; M. Goulding, 29 Feb 1980. -MZUSP 29374, 7 (12.0 – 13.3mm SL) (paratypes); Pool in island, Negro River Basin, Município de Barcelos; M. Goulding, 29 Feb 1980. -MZUSP 29372, 36 (4 C&S) (9.4 – 14.1mm SL) (paratypes); Central pool in Buiu-Açu island, Negro River basin, near Urubaxi river; M. Goulding, 06 Feb 1980. -MZUSP 29370, 14 (7.5 – 14.8mm SL) (paratypes); Negro river just below Daraá river; M. Goulding, 17 Feb 1980. -UFRJ 9124 , 23 (10.3-13.1mm SL); UFRJ 9246, 5 (C&S); UFRJ 9199, 6; Beach in island of the Mariuá archipelago in Negro river basin, near Barcelos, Município de Barcelos, 0°50'34.7"S 62°58'52.3"W; F. P. Ottoni, P. Amorim & P. Bragança, 18 Nov 2012. -UFRJ 9125, 7 (2 C&S), (10.4-13.0mm SL); Beach in island of the Mariuá archipelago in Negro river basin, near Barcelos, Município de Barcelos, 0°56'14.9"S 62°56'21.5"W; F. P. Ottoni, P. Amorim & P. Bragança, 18 Nov 2012. -UFRJ 9154, 79 (7.3-12.5mm SL); UFRJ 9212, 9; Igarapé in front of São João island, near Campina Community, in Negro river basin, Município de Santa Isabel do Rio Negro, 0°30'21.1"S 64°58'45.4"W; F. P. Ottoni, P. Amorim & P. Bragança, 15 Nov 2012. -UFRJ 9122, 24 (7.3-11.3mm SL); Igarapé tributary to Daraã river, Negro river basin, Município de Santa Isabel do Rio Negro, 0°26'24.1"S 64°45'35.4"W; F. P. Ottoni, P. Amorim & P. Bragança, 15 Nov 2012. -MZUSP 31041, 2 (13.3 – 14.7mm SL); Barcelos, Negro River basin, Município de

Barcelos; M. Goulding, 29 Feb 1980. -MZUSP 62141, 12 (9.9 - 16.8mm SL); Poll in island, Negro River basin, Município de Paricatuba; EPA, 14 Nov 1972. -MZUSP 62228, 4 (11.6 – 13.0mm SL); Poll in island, Negro River basin, Município de Paricatuba; EPA, 14 Nov 1972. -MZUSP 110056, 2 (12.4 – 15.6mm SL); First Igarapé on the left bank of the Negro River just above Santa Isabel do Rio Negro, 00°24’40’’S 65°01’13’’W; Toledo-Piza; Oyakawa, Mattox, Marinho & Santana, 07 Feb 2011. -MZUSP 109894, 51 (5 C&S) (7.6-11.4mm SL); Beach near the confluence between Marauiá river drainage and Jaradi river close to Negro river main channel, Negro river basin, Município de Santa Isabel do Rio Negro, 00°23'33"S 65°12'18"W; Toledo-Piza et. al., 8 Feb 2011.

Diagnosis. *Fluviphylax obscurus* is distinguished from all other congeners except *Fluviphylax* sp. D in having the male pelvic-fin tip black (vs. hyaline). It differs from *Fluviphylax* sp. D in having a preorbital canal closed (vs. opened); preopercle canal lower section closed (vs. opened); anal-fin pigmented (vs. hyaline); mesethmoid large (vs. small). Other character states not unique but useful to identify *F. obscurus* are: posttemporal ventral process long (vs. short), rounded anal-fin (vs. triangular or rectangular), second and fourth pharyngobranchial tooth plates rudimentary (vs. well developed), rostral cartilage present (vs. absent), opercle anterodorsal process short (vs. long), mesethmoid rounded (vs. subtriangular).

Fig 27. *Fluviphylax obscurus*, UFRJ 9124, male; Brazil: Amazonas: Mariuá archipelago near Barcelos.

Fig 28. *Fluviphylax obscurus*, UFRJ 9124, female; Brazil: Amazonas: Mariuá archipelago near Barcelos.

Distribution and habitat. *Fluviphylax obscurus* is known from the upper and middle Negro river basin, including many islands and beaches along Negro river main channel, between Mariuá archipelago near Barcelos and the Marauiá river close to Santa Isabel do Rio Negro.

Remarks. Costa (1996) reported an opened preorbital canal and lower section of preopercular canal for *F. obscurus*. With the examination of recently collected material and reexamination of the type series it was possible to observe that these canals are closed, although some specimens are not well preserved, with many missing scales and consequently opened canals. The original specific ephitet proposed by Costa (1996) were *obscurum* but it was then changed to *obscurus* by Eschmeyer & Fricke (2013).

***Fluviphylax zonatus* Costa, 1996**

Examined material. Brazil: Estado do Amazonas: MZUSP 49207, 1 (15.4mm SL) (holotype); Anavilhanas archipelago, Negro river basin; M. Goulding, 21 Nov 1979. - MZUSP 29367, 132 (3 C&S) (9.4 - 15.3mm SL) (paratypes); Anavilhanas archipelago, Negro river basin; M. Goulding, 21 Nov 1979. -MZUSP 6223, 38 (3 C&S) (8.7 - 15.8mm SL) (paratypes); Igarapé Jaraqui, Negro river left bank tributary, Negro river basin; EPA, Apr 1967. UFRJ 7954, 18 (15.2-17.1mm SL); Beach in Igarapé da

Freguesia a tributary of the left bank of Negro river, about 8km from Novo Airão, Negro river basin, Município de Novo Airão, 2°39'32.8"S 60°59'46.9"W; P. Bragança & P. Amorim, 28 Jan 2011. -UFRJ 7952, 19 (12.8-16.9mm SL); UFRJ 8874, 4 (C&S); UFRJ 7953, 6; Beach in Igarapé da Freguesia a tributary of the left bank of Negro river, about 3km from Novo Airão, Negro river basin, Município de Novo Airão, 2°37'47.9"S 60°58'37.0"W; P. Bragança & P. Amorim, 28 Jan 2011. -UFRJ 7956, 6 (11.6-13.0mm SL); Igarapé do Tarumã-açu, Prainha beach, Negro river basin, Município de Manaus, 2°58'43.3"S 60°06'14.5"W; P. Bragança & P. Amorim, 1 Feb 2011. -UFRJ 7958, 20 (9.8-18.0mm SL); UFRJ 7959, 5; Igarapé do Tarumã-açu, near the condominium Sol Nascente in the city of Manaus, Negro river basin, Município de Manaus, 2°59'06.9"S 60°06'12.1"W; P. Bragança & P. Amorim, 1 Feb 2011. - UFRJ 9209, 16; Igarapé Tarumã-Mirim near Livramento community, lower Negro river basin, Município de Manaus, 03°01'24.3"S 60°10'40.0"W; F. P. Ottoni, P. Amorim & P. Bragança, 08 Nov 2012. - UFRJ 7960, 14 (14.4-15.6mm SL); UFRJ 8873, 5; Igarapé behind Lua beach close to Manaus, Negro river basin, Município de Manaus, 3°01'47.6"S 60°08'23.3"W; P. Bragança & P. Amorim, 1 Feb 2011. -UFRJ 8880, 1 (11.8mm SL); Beach on the left bank of Papagaio river in front of Japim Lake, Unini river drainage, Negro river basin, Município de Novo Airão, 2°3'18.5"S 62°46'53.71"W; H. Lazzaroto, 18 Sep 2010. -UFRJ (9.3-11.1mm SL); 2, Beach with a Caruaçuzeiro tree on the left margin of Arara river, Unini river drainage, Negro river basin, Município de Novo Airão, 1°43'25.78"S 63°35'2.98"W; H. Lazzaroto, 6 Sep 2010. -UFRJ 8878, 8 (9.5-14.4mm SL); UFRJ 9642, 2 (C&S); Beach on the left margin of Preto river, Unini drainage, Negro river basin, Município de Novo Airão, 1°45'55.31"S 63°52'39.2"W; H. Lazzaroto, 10 Sep 2010. -MZUSP 29368, 22 (7.4 – 15.7mm SL); Anavilhanas archipelago, Negro river basin; M. Goulding, Jun 1980. -MZUSP 29369, 69 (6.0 – 15.0mm SL); Anavilhanas archipelago,

Negro river basin; M. Goulding, Feb 1980. -MZUSP 74414, 1 (15.5mm SL), Igarapé Sirinau, a left bank tributary of Cuieiras river, about 25km from its mouth, Negro river basin; Alpha Helix Amazon Expedition, 30 Jan 1977. -MZUSP 63834, 1 (15.1mm SL); Cuieiras river and its tributaries, Negro river basin; Alpha Helix Amazon Expedition, Jan 1977. -MZUSP 6172, 42(6.2 - 14.2mm SL); Negro river above Manaus, Negro river basin; EPA, 22-25 Apr 1967. -MZUSP 103109, 1 (17.0mm SL); Tributary of Preto da Eva river in Encanto da Mata, 2°38'25.8''S 59°44'5.7''W; Excursão MZUSP/USP, 07 Jul 2004. -MZUSP 88810, 1 (16.2mm SL); Igarapé Sucurijú in Preto da Eva river next to the propriety Sítio Bom Jesus in the Km 13 on the road Francisca Mendes, 2°45'15.8''S 59°37'29.6''W; O.T.Oyakawa et. al., 04 Jul 2003. -MZUSP 86948, 2(9.9 – 11.4mm SL); Igarapé Sucurijú in Preto da Eva river; M. de Pinna; L. Souza & L. Rapp Py-Daniel, 14 Agu 2004. -MZUSP 88826, 13 (11.6 – 18.1mm SL); Igarapé tributary to Preto da Eva river, 2°44'35''S 59°40'7.8''W; Excursão MZUSP/USP, 06 Jul 2003. -MZUSP 88729, 105 (6.6 – 16.6mm SL); Igapó at Pousada Paraíso near Igarapé do Tauari, Preto da Eva river, 2°47'25.2''S 59°38'10.8''W; Excursão MZUSP/USP, 05 Jul 2003. -MZUSP 88927, 2(11.9 – 13.4mm SL); Igarapé Barroso, on the bridge in Francisca Mendes road, Preto da Eva river, 2°44'30.8''S 59°38'41.8''W; O.T. Oyakawa et. al. 05 Jul 2003. MZUSP 59956, 124 (8.7 – 15.1mm SL); Igarapé in Cantagalo, Negro river basin; EPA, 24 Jan 1972. -MZUSP 59159, 12 (10.6 – 12.3mm SL); Várzea lake in Cantagalo, Negro river basin; EPA, 28 Jan1972. -MZUSP 77946, 82(10.8 – 12.8mm SL); Igarapé associated with a lake in Cantagalo, Negro river basin; EPA, 25 Jan1972. Brazil: Estado de Roraima: UFRJ 8914, 11 (6.0-14.0mm SL); Igarapé about 10km following the road BR-431 from Jundiá to Macucuau river a tributary of Jauaperi river drainage, Negro river basin, Município de Rorainópolis, 0°10'21.6"S 60°46'23.5"W; E. Henschel, F. P. Ottoni & P. Bragança, 15 Sep 2012. -UFRJ 8916, 28

(7.9-13.9mm SL); Igarapé about 35km following the road BR-431 from Jundiá to Macucuau river a tributary of Jauaperi river drainage, Negro river basin, Município de Rorainópolis, 0°13'44.5"S 60°59'30.4"W; E. Henschel, F. P. Ottoni & P. Bragança, 15 Sep 2012. -UFRJ 8917, 22 (10.1-13.5mm SL); UFRJ 9000, 5; Igarapé about 23km south of Rorainópolis following the road BR-174, Jauaperi river drainage, Negro river basin, Município de Rorainópolis, 0°43'54.7"N 60°27'27.4"W; E. Henschel, F. P. Ottoni & P. Bragança, 14 Sep 2012. -UFRJ 8915, 5 (11.6-13.3mm SL); UFRJ 9641, 3 (C&S); River about 45km following the road BR-431 from Jundiá, Jauaperi river drainage, Negro river basin, 0°13'54.5"N 61°03'52.5"W; E. Henschel, F. P. Ottoni & P. Bragança, 15 Sep 2012. -UFRJ 8966, 19 (8.7-12.4mm SL); Várzea of Igarapé Caleffi, about 84km following the road BR-174 from Caracaraí to Rorainópolis, Anauá river drainage, Branco river basin, 01°23'31.0"N 60°38'39.1"W; E. Henschel, F. P. Ottoni & P. Bragança, 18 Sep 2012.

Diagnosis. *Fluviphylax zonatus* is distinguished from all other congeners by the presence of six or more dark bars over the flank of males (vs. two to five); bright greenish white blotch on males caudal fin ventral portion and on anal fin border (vs. absence). *Fluviphylax zonatus* is similar to *Fluviphylax sp.* E and *Fluviphylax sp.* G and distinguished from all other congeners by having a well developed second and fourth pharyngobranchial tooth plates (vs. rudimentary). It is similar to *F. pygmaeus* and *Fluviphylax sp.* A and differs from all other congeners by having three to four black bars on males caudal fin (vs. absence). Other character states not unique but useful to identify *F. zonatus* are: rostral cartilage present (vs. absence); ventral process of posttemporal elongated (vs. short).

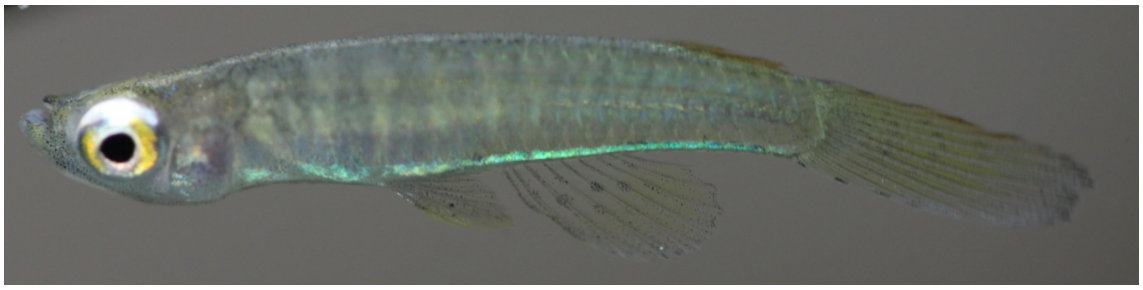

Fig 29. *Fluviphylax zonatus*, UFRJ 7958, male; Brazil: Amazonas: Tarumã Açu near Manaus.

Fig 30. *Fluviphylax zonatus*, UFRJ 7958, female; Brazil: Amazonas: Tarumã Açu near Manaus.

Distribution and habitat. *Fluviphylax zonatus* is known from the Lower Negro river main channel and its tributaries, including the Cuieiras, Jaraqui, Sucurijú, Unini and Jauaperí river drainages, as well as in the Amazonas tributary Preto da Eva river. It was also reported from the Branco river basin in Anauá river drainage, itself a tributary of the Negro river basin. In the Anavilhanas archipelago and localities close to Manaus, it was collected in shallow (50-70cm), black water sandy bottom beaches. In the Jauaperi and Anauá river drainages, it was collected in shallow (30-70cm), lentic, high transparency black-water streams in densely vegetation areas. In the Anauá river *F. zonatus* was syntopic with *Fluviphylax sp.* E herein diagnosed.

Remarks. Costa (1996) designated *F. zonatus* holotype under the catalog number MZUSP 49207 the same number already designated for *F. obscurus* holotype, as observed by Lucinda (2003). The correct catalog number is MZUSP 49208 according to

MZUSP collection database. With the examination of recently collected material and reexamination of the type series it was possible to observe that the preorbital and lower section of preopercular canal are polymorphic, with some specimens presenting closed canals and other opened canals.

Fluviphylax palikur Costa & Le Bail, 1999

Examined Material. Brazil: Estado do Amapá: NRM 28302, 2 (13.6-13.9mm SL) (paratypes); Igarapé at aldeia Cunene, Oiapoque river basin, Juminán, Município de Oiapoque, 04°01’08’’N 051°37’06’’W; S. Kullander & F. Fang, 28 Mar 1994. -UFRJ 8824, 22 (6.9-11,8mm SL); UFRJ 8877, 5 (C&S); UFRJ 8825, 6; Igarapé on the right bank of Oiapoque river, Município de Oiapoque, 03°59’28,5”N 51°41’40,0”W; P. Bragança & E. Henschel, 29 Jul 2012. -UFRJ 8828, 3 (9.1-9.8mm SL); Igarapé on the right bank of Oiapoque river after crossing Vila de Taparabu, município de Oiapoque, 04° 3'8.70"N 51°38'1.23"W; P. Bragança & E. Henschel, 31 Jul 2012.

Diagnosis. *Fluviphylax palikur* is distinguished from all other congeners except *Fluviphylax sp* A by the presence of the anterior process of the fifth ceratobranchial slightly curved laterally (vs. folded laterally); third pharyngobranchial and fifth ceratobranchial teeth with an adjacent lobe (vs. conical); anguloarticular anterior process truncate (vs. pointed); anguloarticular ventral process present (vs. absent); male anal fin rectangular shaped (vs. round or triangular); 12-15 anal-fin rays (vs. 7-10); posterior region of the dorsal fin with a yellow and black blotch in males (vs. absent); male caudal peduncle with yellowish white bars (vs. absent). It is distinguished from *Fluviphylax sp* A by having a rudimentary notch on the autopalatine (vs. well developed); preopercular canal opened (vs. closed); males anal and caudal fins not

filamentous (vs. filamentous); males anal and caudal fins not pigmented (vs. pigmented). Other character states not unique but useful to identify *F. palikur* are: robust dentary (vs. thin) and well developed fourth and second pharyngobranchial tooth plates (vs. rudimentary).

Fig 31. *Fluviphylax palikur*, UFRJ 8824, male; Brazil: Amapá: Oiapoque river.

Fig 32. *Fluviphylax palikur*, UFRJ 8828, female; Brazil: Amapá: Oiapoque river.

Distribution and habitat. *Fluviphylax palikur* is known only from its type locality in the Oiapoque river basin that drains the Guiana Shield. *Fluviphylax palikur* was found near the marginal vegetation and associated with floating meadows in deep channels in a flooded savanna region characterized by the presence of the buriti palm *Mauritia flexuosa*.

Remarks. During the present study the holotype MZUSP 52941 and the paratypes UFRJ 4616; UFRJ 4617 were not localized and they are probably lost.

***Fluviphylax sp.* A**

Examined Material. Brazil: Estado do Amapá: UFRJ 8012, 7 (12.8-19.5mm SL); Igarapé do Davi in the road BR-156, Município de Amapá, 1°55'39.2"S 50°51'52.3"W; P. Bragança & P. Amorim, 16 Jan 2011. -UFRJ 8007, 38 (10.0-17.7mm SL); Stream about 5km before Anauerapucu (Vila Nova) river following the road AP-030 towards Laranjal do Jari, Município de Santana, 0°09'26.3"N 51°31'48.0"W; P. Bragança & P. Amorim, 9 Jan 2011. - UFRJ 7998, 53 (7.6-15.9mm SL); River with flooded margin nexto to the road BR-156 following to Mazagão, Município de Mazagão, 0°31'02.0"N 51°37'23.7"W; P. Bragança & P. Amorim, 9 Jan 2011. - UFRJ 8029, 7 (10.9-14.7mm SL); Balneário do Tomé, Matapi river, next to a bridge, Município de Macapá, 0°13'42.6"N 51°10'08.3"W; P. Bragança & P. amorim, 9 Jan 2011. - UFRJ 7924, 17 (14.0-18.4mm SL); UFRJ 7933, 2 (C&S); Vila Nova (Anauerapucu) river, close to Vila Nova, Município de Santana, 0°08'23.0"N 51°32'14.0"W; C. de Luca & F. Schunek, 7 Feb 2010. -UFRJ 8018, 21 (10.3-14.1mm SL); River and flooded area about 3km from Matapi river, following in the road AP-030 towards Laranjal do Jari, Município de Macapá, 0°13'39.3"S 51°11'26.0"W; P. Bragança & P. Amorim, 9 Jan 2011. -UFRJ 8000, 26 (13.3-17.9mm SL); UFRJ 8001, 7; Igarapé do Henrique, following the road BR-156 between Taratarugalzinho and Calçoene, Município de Pracuúba, 1°45'55.0"N 50°50'44.2"W; P. Bragança & P. Amorim, 16 Jan 2011. -UFRJ 8862, 12 (10.3-16.5mm SL); UFRJ 8863, 6; Macará-Pacu river, road BR-156, between Macapá and Laranjal do Jari, Município de Mazagão, 0°10'59.6"S 51°44'10.8"W; P. Bragança & E. Henschel, 24 Jul 2012. -UFRJ 8822, 48 (8.4-17.9mm SL) Igarapé do Bispo, tributary of Anauerapucu river in the road BR-156, between Macapá and Laranjal do Jari, Município de Mazagão, 0°05'10.7"N 51°37'57.8"W; P. Bragança & E. Henschel, 24 Jul 2012. -IEPA 1827, 256; UFRJ 8876,

7(C&S); Pool near tower 6 of AMCEL cellulose company area, Araguari river drainage, Município de Ferreira Gomes, 0° 50'52.28"N 51°04'42.7"W; C. Gama & D. Halboth, 2 Jun 2002. - IEPA 2757, 7; IEPA 2756, 7; Cachoeira do Henrique, Município de Tartarugalzinho, 01° 45'56.5"N 50°52'41.0"W; F. Costa, C. Gama & D. Halboth, 24 Jul 2002. -IEPA 2599, 3; Córrego Limão on the road BR-156, Município de Macapá, 0°9'59.36"N 51°31'16.11"W; J.da Silva, L. Cohim & J. de Souza, 12 Jan 2008. -MNRJ 21095, 15 (11.9-18.4mm SL); MNRJ 31415, 1 (14.4mm SL); Aporema river, a tributary of the left bank of Araguari river, in the farm Fazenda Modelo do Aporema, Município de Tartarugalzinho; G. Nunan, D. Moraes & W. Bandeira, Apr 1981. -MZUSP 102045, 31 (8.2-19.2mm SL); Igarapé Arapiranga, a right bank tributary of Jari river drainage, Porto do Figueira downstream Cachoeira do Santo Antônio, 00°48’04’’S 052°27’20’’W; Moreira, Loboda & Soares, 25 Mar 2008. Brazil: Estado do Pará: UFRJ 7961, 56 (7.7-17.3mm SL); UFRJ 9643, 5 (C&S); Bridge over Ubá river, tributary to Moju river, in the road PA-252, Município de Mojú, 2º06'09.9"S 48º46'54.7"W; P. Bragança & P. Amorim, 19 Jan 2011. -UFRJ 7964, 51 (7.5-19.0mm SL); UFRJ 8875, 5 (C&S); UFRJ 7965, 7; Acará river close to the ferry in the road PA-256, Município de Tailândia, 2º24'53.9"S 48º41'22.5"W; P. Bragança & P. Amorim, 19 Jan 2011. - MCP 22059, 19 (14.1-18.6mm SL); Acará river, ferry on the road between Tomé-Açu and Moju, about 71km from Tomé-Açu, Município de Tailândia, 2º24'54.0"S 48º41'27.0"W; R. Reis, J. P. Silva, E. Pereira & J. Montoya, 18 Jul 1998. -MCP 22056, 9 (3C&S) (13.4-17.1mm SL); Igarapé on the road between Tomé-Açu and Moju, about 69km from Tomé-Açu, Aracá river drainage, Amazon river basin, Município de Tomé-Açu; 2º24'29.0"S 48º40'14.0"W; R. Reis, J. P. Silva, E. Pereira & J. Montoya, 18 Jul 1998. - UFRJ 9823, 17; Igarapé on PA-256, between Tomé Açu and the road PA-475, about 68km from Tomé-Açu, close to the

Acará river ferry, Município de Tomé-Açu, 2°24'31.7"S 48°40'11.8"W; P. Bragança & E. Henschel, 6 Jul 2013.

Diagnosis. *Fluviphylax sp.* A is distinguished from all other congeners except *F. palikur* by the third pharyngobranchial and fifth ceratobranchial teeth with an adjacent lobe (vs. absent); male caudal peduncle with yellowish white bars (vs. absent); anguloarticular anterior process truncate (vs. pointed); presence of the anguloarticular ventral process (vs. absence); male anal fin rectangular shaped (vs. round or triangular); 12-15 anal fin rays (vs. 7-10); male dorsal fin posterior region with a yellow and black blotch (vs. absence). It is distinguished from *F. palikur* by having autopalatine notch well developed (vs. rudimentary); preopercular canal lower section closed (vs. opened); males anal and caudal fins filamentous (vs. not filamentous); males anal and caudal fins pigmented (vs. not pigmented). Other character states not unique but useful to identify *Fluviphylax sp.* A are: robust dentary (vs. thin); developed fourth and second pharyngobranchial tooth plates (vs. rudimentary) and sexual dimorphism in urogenital papilae morphology present (vs. absent).

Fig 33. *Fluviphylax sp* A, UFRJ 9823 male; Brazil: Pará: Acará river.

Fig 34. *Fluviphylax sp.* A, (not preserved), male; Brazil: Amapá: Cajari river.

Distribution and habitat. *Fluviphylax sp.* A is known from eastern Amazon, both north and south to Amazon river channel, including the Araguari and Jari river basins, draining the Guiana Shield, and many small coastal rivers along Amapá state coast. *Fluviphylax sp.* A was usually found in shallow (10-60cm), high transparency streams, directly exposed to sunlight in savanna areas, characterized by the presence of the buriti palm *Mauritia flexuosa*. It was often found in lentic streams, but sometimes near the bank of lotic streams, where the environmental conditions resembled those of lentic streams. In localities south to the Amazon river main channel, it was found in the Mojú and Acará river drainages, in shallow areas, 30-60cm deep, near the margin of both clear and black high transparency water streams. Both northern and southern populations are often sympatric with the rivulid *Melanorivulus schuncki* Costa & De Luca 2011, and species of the the cichlid genus *Laetacara*.

***Fluviphylax sp.* B**

Material examined. Brazil: Estado do Amazonas: UFRJ 9275, male, (13.2 mm SL); Igarapé near Monte Cristo community a tributary of Vaupés river drainage, Upper

Negro river basin, Município de São Gabriel da Cachoeira, 0°5'23.39"N 67°22'4.73"W, altitude 95m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 12 Nov 2012. -UFRJ 9126, 15 (9.3 − 13.4mm SL); UFRJ 9245, 4 (c&s) (8.0 − 11.5mm SL); UFRJ 9202, 5; same locality; UFRJ 9121, 59 (7.2-13.3mm SL); UFRJ 9276, 6 (c&s) (8.3-12.5mm SL); UFRJ 9210, 11; Lake on Igarapé do Tiburiari near Trovão community a tributary of Vaupés river drainage, Upper Negro river basin, Município de São Gabriel da Cachoeira, 0° 4'26.8"N 67°24'30.8"W, altitude 78m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 12 Nov 2012. - MZUSP 109617, 4 (1C&S) (10.8-13.7 mm SL); Rocky bank on left margin of Neuixi river drainage close to its confluence with Negro river, Município de Santa Isabel do Rio Negro, 0° 21'45.0"S 65°04'13.0"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 Aiuanã river drainage, Middle Negro river basin, Município de Santa Isabel do Rio Negro, 0° 33'05.0"S 64°55'9.0"W; Toledo-Piza et. al., 9 Feb 2011.- MZUSP 29376, 140 (4 C&S) (5.0-13.9 mm SL); beach in a flooded area in Urubaxi river drainage, close to its confluence with Negro river, Upper Negro river basin, Município de Santa Isabel do Rio Negro, 0° 31'0.0"S 64°50'0.0"W; M. Goulding, 7 Feb 1980.

Diagnosis. *Fluviphylax sp.* B is distinguished from all other congeners in having a sharp ventral process on the opercle (vs. absent), second pelvic-fin ray filamentous (vs. not filamentous), absence of mesethemoid (vs. presence), a narrow subopercle (vs. wide), a narrow interopercle (vs. wide) and by the presence of an orange bright blotch on the preorbital region (vs. absent). It is similar to *Fluviphylax sp.* C and distinguished from the remaining congeners in having a sharp and long retroarticular (vs. rectangular), an orange colouration on the posterior region of the pectoral fin (vs. hyaline) and by having

the head free neuromasts placed over the body (vs. in shallow grooves). Other characters not unique but useful to identify *Fluviphylax sp.* B are: rudimentary second and fourth pharyngobrachial tooth plates (vs. well developed); pelvic fin elongated (vs. short); anal fin long and pointed, reaching vertical posterior to dorsal-fin base (vs. short and rounded or rectangular), posttemporal ventral process short (vs. long) and rostral cartilage present (absent).

Fig 35. *Fluviphylax sp* B, UFRJ 9275, males; Brazil: Amazonas: Monte Cristo in Vaupés river drainage.

Fig 36. *Fluviphylax sp* B, UFRJ 9121, female; Brazil: Amazonas: Tiburiari in Vaupés river drainage.

Distribution and habitat. Known from four main river drainages in the upper Negro river, Vaupés, Urubaxi, Neuixi and Aiuanã river drainages. Habitat data are available only from two localities in the Vaupés river drainage. *Fluviphylax sp* B was collected close to the deadwood in a shallow (30-50cm), lentic, high transparency black-water stream in a dense vegetation area and in Tiburiari lake, an open vegetation area connected with the Vaupés river through a straight channel. In the small stream, they were found in small shoals of about 3-5 individuals swimming near the surface whereas in the Tiburiari lake they formed larger shoals of about 20 individuals. Simpatrically were found the Scoloplacid *Scoloplax dolicholophia* Schaefer, Weitzman & Britski, 1989.

Remarks. In a single lot MZUSP 29376 from the Urubaxi river were found both *Fluviphylax sp.* B and *F. obscurus* and due to the small size the identification of each specimen was difficult and sometimes it was not possible. In one specimen from UFRJ 9245 were found some metacercaria, the encysted maturing stage of a trematode, on the base of the unpaired fins.

***Fluviphylax sp.* C**

Material examined. Brazil: Estado do Amazonas: UFRJ 9080, male (12.8 mm SL); Igarapé Mauaú near Romão community, Aracá river drainage, middle Negro river basin, Município de Barcelos, 0°20'31"S 62°56'30"W, altitude 30m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 18 Nov 2012. - UFRJ 9081, 94 (7.3 − 13.5mm SL); UFRJ 9082, 6 (c&s) (10.2 − 12.4mm SL); UFRJ 9194, 5; same locality. Brazil: Estado de Roraima: 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 Jufari river

drainage, Middle Negro river basin, Município de Caracaraí, 1°04'01"S 62°07'40"W; O. Oyakawa et. al., 28 Aug 2011.

Diagnosis. *Fluviphylax sp.* C is distinguished from all other congeners in having a sexual dimorphic pattern on cephalic latero sensory system, in which males have whole opened sensory canals and females have the anterior section of supraorbital, postorbital and upper section of preopercular canals closed, and by a pointed dorsal fin. It is similar to *Fluviphylax sp.* B and distinguished from all other congeners in having a long and sharp retroarticular (vs. rectangular) and an orange colouration on the posterior region of the pectoral fin (vs. hyaline). Other character states not unique but useful to identify *Fluviphylax sp.* C are: presence of 2-4 black bars on the anterior portion of the flank in preserved male (vs. absent); elongate and pointed anal-fin reaching vertical posterior to dorsal-fin base (vs. short and rounded, tip not surpassing dorsal fin base posterior region); male pelvic-fin reaching base of fifth anal fin ray (vs. reaching base of third anal fin ray); posttemporal ventral process short (vs. long) and sexual dimorphism in urogenital papilae morphology present (vs. absent).

Fig 37. *Fluviphylax sp.* C, UFRJ 9081, male; Brazil: Amazonas: Aracá river drainage.

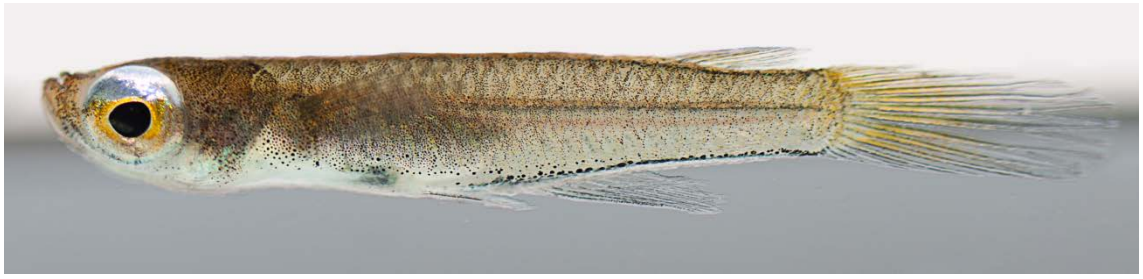

Fig 38. *Fluviphylax sp* C, UFRJ 9081, female; Brazil: Amazonas: Aracá river drainage

Distribution and habitat. Known from two localities in the middle Negro river basin, Igarapé Mauaú in Aracá river drainage and Igarapé do Campo in Jufari river drainage. In Igarapé Mauaú *Fluviphylax sp.* C was collected in a shallow (30 -70 cm), lentic, 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. Simpatrically were found the Hemiramphidae *Hyporhamphus brederi* (Fernández-Yépez, 1948).

***Fluviphylax sp.* D**

Material examined. Brazil: Estado do Amazonas: UFRJ 9391, 1 (10.5 mm SL); UFRJ 9215, 16 (6.5 − 10.7 mm SL); UFRJ 9392, 5 (c&s) (8.1 − 10.3 mm SL); Igarapé on Curicuriari river, middle Negro river basin, Município de São Gabriel da Cachoeira, 0°12'36"S 66°47'59"W, altitude 60m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 11 Nov 2012. - UFRJ 9216, 23 (7.1 − 10.7 mm SL); UFRJ 9150, 7; Igarapé on Curicuriari river, middle Negro river basin, Município de São Gabriel da Cachoeira, 0°13'35"S 66°48'10"W, altitude 59m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 11 Nov 2012.

Diagnosis. *Fluviphylax sp.* D is distinguished from all other congeners by the red colouration present in all fins and over head and by the small mesethmoid (vs. large). It is similar to *F. obscurus* and distinguished from all other congeners in having the male pelvic-fin tip black (vs. hyaline). It differs from *F. obscurus* in having a preorbital canal opened (vs. closed); preopercle canal lower section opened (vs. closed); anal-fin hyaline (vs. pigmented). Other character states not unique but useful to identify *Fluvphylax sp.* D are: posttemporal ventral process long (vs. short) and second and fourth pharyngobranchial tooth plates rudimentary (vs. well developed).

Fig 39. *Fluviphyax sp* D, UFRJ 9391, male; Brazil: Amazonas: Curicuriari river drainage.

Fig 40. *Fluviphyax sp* D, UFRJ 9391, female; Brazil: Amazonas: Curicuriari river drainage.

Distribution and habitat. Known only from the Curicuriari river drainage, upper Negro river basin. *Fluviphylax sp.* D was collected in shallow (70-100 cm), lentic, high transparency black-water streams, with leaf litter on the bottom near the margin. It was found forming small shoals of about four specimens, swimming near the surface

Remarks. All individuals from (UFRJ 9392) presented metacercaria, the encysted maturing stage of a trematode.

***Fluviphylax sp.* E**

Material examined. Brazil: Estado de Roraima: UFRJ 8918, male (12.8 mm SL); Igarapé Água Boa on the Perimetral Norte road (BR-210), about 16km from road BR-174 to Missão Catrimani, Ajarani river drainage, Branco river basin, Município de Caracaraí, 01°57'03.8"N 61°14'42.7"W, altitude 38m; P. H. N. Bragança, E. Henschel and F. P. Ottoni, 17 Sep 2012. -UFRJ 8918, 33 (7.3 − 13.5mm SL); UFRJ 8919, 7 (c&s) (10.2 − 12.4mm SL); UFRJ 9002, 7; same locality. - UFRJ 8966, 19; Flooded margin area of Igarapé Caleffi, about 84km from Caracaraí to Rorainópolis on road BR-174, Anauá river drainage, Branco river basin, Município de Caracaraí, 01°23'31.0"N 60°38'39.1"W; P. H. N. Bragança, E. Henschel and F. P. Ottoni, 18 Sep 2012.

Diagnosis. *Fluviphylax sp.* E is distinguished from all other congeners by having bright cooper blotches on the anterior portion of the flank. It is similar to *F. zonatus* and *Fluviphylax sp.* G and differs from all other congeners by having a well developed second and fourth pharyngobranchial tooth plates (vs. rudimentary). It is distinguished from all congeners except *Fluviphylax sp.* D *and Fluviphylax sp.* F in having all fins hyaline (vs. pigmented) and it is similar to *F. zonatus*, *Fluviphylax sp* C, *F. pygmaeus*, *F. obscurus* and *Fluviphylax sp* G by having dark bars on the flank in preserved males (vs. absent). Other character states not unique but useful to identify *Fluviphylax sp* E are: 5-9 teeth on the second pharyngobrancial; posttemporal ventral process elongated (vs. short or absent) and rostral cartilage present (vs. absent)

Fig 41. *Fluviphylax sp.* E, UFRJ 8918, male; Brazil: Roraima: Ajarani river drainage.

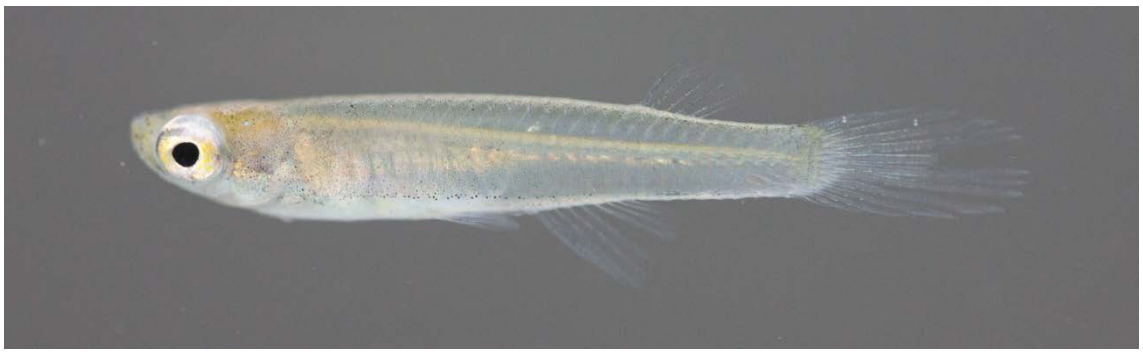

Fig 42. *Fluviphylax sp.* E, UFRJ 8918, female; Brazil: Roraima: Ajarani river drainage.

Distribution and habitat. Known from two main river drainages in the middle Branco river basin, the Ajarani and the Anauá river drainages. In the Ajarani river *Fluviphylax sp.* E was collected in muddy lentic water near the stream margin associated with aquatic vegetation, forming small shoals of about 5 individuals swimming near the surface. In the Anauá river drainage, the new species was collected in shallow (50-70cm) areas on the flooded margin of Igarapé Caleffi, a lentic, hight transparency black water stream. Sintopically in the Anauá river drainage were found the congener *Fluviphylax zonatus*.

***Fluviphylax sp.* F**

Material Examined. Brazil: Estado do Amazonas: UFRJ 9395, male (12.6 mm SL); Island in a stream tributary to Daraã river drainage, Negro river basin, Município de Santa Isabel do Rio Negro, 00°27'17.3"S 64°46'01.4"W, altitude 44m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 15 Nov 2012. - UFRJ 9094, 38 (7.6−11.4mm SL); UFRJ 9396, 6 (C&S) (8.6-12.7mm SL), same locality. - UFRJ 9213, 4 (10.4-11.5mm SL); UFRJ 9192, 2; Tibarrá river in the end of the road, Negro river basin, Município de Santa Isabel do Rio Negro, 00°24'46.8"S 64°56'57.3"W, altitude 69m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 14 Nov 2012.- UFRJ 9128, 43 (8.2−11.5mm SL); UFRJ 9397, 7 (C&S) (8.7-11.3mm SL); Beach on Tibarrá river, Negro river basin, Município de Santa Isabel do Rio Negro, 00°26'23.7"S 64°57'02.1"W, altitude 57m; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 15 Nov 2012.

Diagnosis. *Fluviphylax sp.* F is distinguished from all other congeners in having a preopercle with a rounded flange over opercle. It is distinguished from all other congeners except *Fluviphylax sp.* E and *Fluviphylax sp.* D by having all fins hyaline (vs. pigmented). Other characters not unique but useful to identify *Fluviphylax sp.* F are: posttemporal ventral process short (vs. long), mesethemoid subtriangular (vs. rounded); rostral cartilage absent (vs. present), retroarticular rectangular (vs. long and sharp), pre-orbital and lower section of preopercular canal opened (vs. closed), second and fourth pharyngobranchial tooth plates rudimentary (vs. well developed), anterodorsal process of opercle short (vs. long or absent), pelvic fin short (vs. long), pelvic fin tip bright yellow colouration present (vs. absent), first and second anal fin rays bright yellow colouration present (vs. absent).

Fig 43. *Fluviphyax sp.* F, UFRJ 9213, male; Brazil: Amazonas: Tibarrá river drainage.

Fig 44. *Fluviphyax sp.* F, UFRJ 9213, female; Brazil: Amazonas: Tibarrá river drainage.

Distribution and habitat. Known from the Tibarrá and Daraã river drainages, upper Negro river basin. *Fluviphylax sp.* F was collected in a shallow (20 -120 cm), lentic, high transparency black-water stream. It was found forming small shoals of about 10 individuals swimming near the surface. Simpatrically were found the small Corydoradinae *Aspidoras pauciradiatus* (Weitzman & Nijssen, 1970).

Remarks. Three specimens from UFRJ 9397 and one individual from UFRJ 9396 were parasited by some metacercaria, the encysted maturing stage of trematodes.

***Fluviphylax sp.* G**

Material examined. Brazil: Estado do Amazonas: UFRJ 9389, male (12.5 mm SL); Igarapé do Cajarazinho, tributary of Caurés river on Balaio community, middle Negro river basin, Município de Barcelos, 01°06'17.2"S 62°58'42.3"W; P. H. N. Bragança, P. F. Amorim and F. P. Ottoni, 17 Nov 2012. -UFRJ 9190, 64 (7.0 − 14.2mm SL); UFRJ 9390, 6 (c&s) (8.7 − 13.0 mm SL); UFRJ 9188, 5; same locality.

Diagnosis. *Fluviphylax sp.* G is distinguished from all congeners except *F. zonatus* and *Fluviphylax sp.* E by having the second and fourth pharyngobranchial tooth plates well developed (vs. rudimentary). Other characters not unique but useful to identify *Fluviphylax sp.* G are: rostral cartilage present (vs. absent); ventral process of posttemporal elongated (vs. short); 2-5 black bars over flank (vs. more than five or absent) and male anal fin black dots present (vs. absent).

Fig 45. *Fluviphylax sp.* G, UFRJ 9389, male; Brazil: Amazonas: Caurés river drainage.

Fig 46. *Fluviphylax sp.* G, UFRJ 9389, female; Brazil: Amazonas: Caurés river drainage.

Distribution and habitat. Known only from Igarapé Cajarazinho, Caurés river drainage, middle Negro river basin. *Fluviphylax sp.* G was collected in a shallow (20 - 80 cm), lentic, high transparency clear-water stream, with sandy and leaf litter bottom near stream margin. It was found associated with aquatic vegetation, forming small shoals of about 10-15 individuals, swimming near the surface. Simpatrically were found the Cichlidae *Laetacara fulvipinis* Staeck & Schindler, 2007 and the Rivulidae *Anablepsoides ornatus* (Garman, 1895).

***Fluviphylax sp.* H**

Material examined. Venezuela: Estado Bolívar: MHNLS 12798, 11 (2 C&S) (8.5-12.7 mm SL); Laguna Brava in Caurá river drainage, Orinoco basin, 07° 34'22.0"N 65°11'28.0"W; C. Vispo et. al., 21 Feb 1998.

Diagnosis. *Fluviphylax sp.* H is distinguished from all other congeners in having an indentation on lachrymal dorsal lobe. It is similar to *F. pygmaeus*, *Fluviphylax sp.* B and *Fluviphylax sp.* C and distinguished from the remaining congeners in having an

elongate and pointed anal fin reaching vertical posterior to dorsal-fin base (vs. short and rounded not reaching vertical posterior to dorsal-fin base) and by having an elongated pelvic fin reaching the base of the fifth anal-fin ray (vs. short pelvic fin, reaching between the base of the first and third anal-fin ray). Other character states not unique but useful to identify *Fluviphylax sp.* H are: pre-orbital canal closed (vs. opened), preopercle canal lower section closed (vs. opened), retroarticular rectangular (vs. sharp and pointed), posttemporal ventral process long (vs. short), mesethemoid rounded (vs. subtriangular), rostral cartilage present (vs. absent) and second and fourth pharyngobranchial tooth plates rudimentary (vs. well developed).

Fig 47. *Fluviphylax sp.* H, MNHLS 12798, male; Venezuela, Caurá river.

Distribution and habitat. Known only from one locality in Laguna Brava in the Caurá river drainage, Orinoco basin. The Caurá river drainage is a black water stream similar to those streams from Negro river basin and some main Orinoco drainages like the Caroní, the Ventuari and the upper Orinoco tributaries.

***Fluviphylax sp.* I**

Examined material. Brazil: Estado do Pará: UFRJ 9733,28 (9.0-12.5 mm SL); UFRJ 9734, 5(C&S); UFRJ 9639, 6; Igapó in the Floresta Encantada, Lagoa Verde, Alter do Chão, Município de Santarém, lower Tapajós river drainage, Amazon basin, 02°31'24.5"S 54°55'32.2"W; P. H. N. Bragança and E. Henschel, 10 Jul 2013. - UFRJ 9389, 64 (7.0 − 14.2mm SL); UFRJ 9390, 6 (c&s) (8.7 − 13.0 mm SL); collected in the same locality. - MZUSP 63836, 5 (13.9 – 14.5mm SL); Jacaré Lake, Trombetas river drainage, Município de Oriximiná; Expedition of Dep. de Zoologia of MPEG, 03 Oct 1965. -MZUSP 8458, 1 (12.5mm SL); Jacundá Lake in Alter do Chão, Município de Santarém; EPA, 23 Dec 1967. - MZUSP 21967, 1 (11.6mm SL); Island in front of Monte Cristo, Tapajós river drainage; EPA,12-14 Dec 1970. -MZUSP 93224, 9 (9.0 – 12.6mm SL); Igarapé Juá, about 7km from Santarém in the road to the Airport, Município de Santarém, 02°26’00’’S 54°46’52’’W; L.M. Souza & J.L. Birindelli 13 Nov 2006. - MZUSP 15599, 31(3 C&S) (7.5 – 14.5mm SL); Headwaters of Serrinha, Jacaré Lake, Trombetas biologic reserve, Município de Oriximiná; R.M.C Castro, 20 Jul 1979. -MZUSP 42820, 9 (9.1 – 15.1mm SL); Igarapé Jacaré, left bank of Tapajós river close to Boim, Município de Boim; EPA, 27 Oct 1970. - UFRJ 5372, 19 (7.3-12.4mm SL); UFRJ 9640, 7 (C&S); Igarapé Pauxis, tributary of Pauxis Lake, Amazon basin, Município de Óbidos; C. A. de Figueiredo & C. Codeço, 08 Sep 1996. - MZUSP 8243, 1 (12.3mm SL); Jacupá Lake, Amazon basin, Município de Oriximiná; EPA, 17 Dec 1967.

Diagnosis. *Fluviphylax sp.* I is characterized by a unique combination of characters: second and fourth pharyngobranchial tooth plates rudimentary (vs. well developed); rostral cartilage absent (vs. present); ventral process of posttemporal short (vs. elongated); 2-5 black bars over flank (vs. more than five or absence); males caudal fin

dark black dots present (vs. absent); four branchiostegal rays (vs. five branchiostegal rays); pelvic-fin short (vs. long).

Fig 48. *Fluviphylax sp* I, UFRJ 9733, male; Brazil: Pará: Alter do Chão.

Fig 49. *Fluviphylax sp* I, UFRJ 9733, female; Brazil: Pará: Alter do Chão.

Distribution and habitat. Known from the lower Tapajós and Trombetas river drainages. In the lower Tapajós river, *Fluviphylax sp*. I was collected in shallow areas (20-70cm) near the border of an Igapó forest, and in deeper areas (2-4m) of the flooded forest, over small trees canopies inside the forest.

Wiens and Penkrot (2002) haplotype tree delimitation

The application of the tree-based species delimitation method approached by maximum parsimony (MP) and maximum likelihood (ML) phylogenetic methods, resulted in similar but not identical topologies and delimited lineages. The molecular data comprised 50 specimens from 23 localities embracing all the putative lineages recognized in PAA but *Fluviphylax sp* H from Orinoco basin. The analysis comprised 676 base pairs, 430 characters were constant and 232 parsimony informative. The maximum parsimony analysis resulted in three most parsimonious trees, and a strict consensus tree was performed. In both analysis was possible to recognize the same six species groups herein called: *Fluviphylax palikur*, *F. zonatus*, *F. obscurus*, *F. simplex*, *F. pygmaeus* and *Fluvihylax sp.* B species group. Information about the species groups distribution are also provided.

The MP analysis recognized thirteen lineages, ten of these corresponds to the species delimited through the PAA method: *F. palikur*, *F. obscurus*, *F. simplex*, *F. pygmaeus*, *Fluviphylax sp.* A, *Fluviphylax sp.* B, *Fluviphylax sp.* D, *Fluviphylax sp.* G, *Fluviphylax sp.* F and *Fluviphylax sp.* I. The ML also recognized thirteen lineages but not necessarily the same delimited in the MP. Among the lineages recognized in the ML, seven corresponds to the species delimited through the PAA method: *F. palikur*, *F. pygmaeus*, *F. obscurus*, *Fluviphylax sp.* B, *Fluviphylax sp.* D, *Fluviphylax sp.* G and *Fluviphylax sp* I.

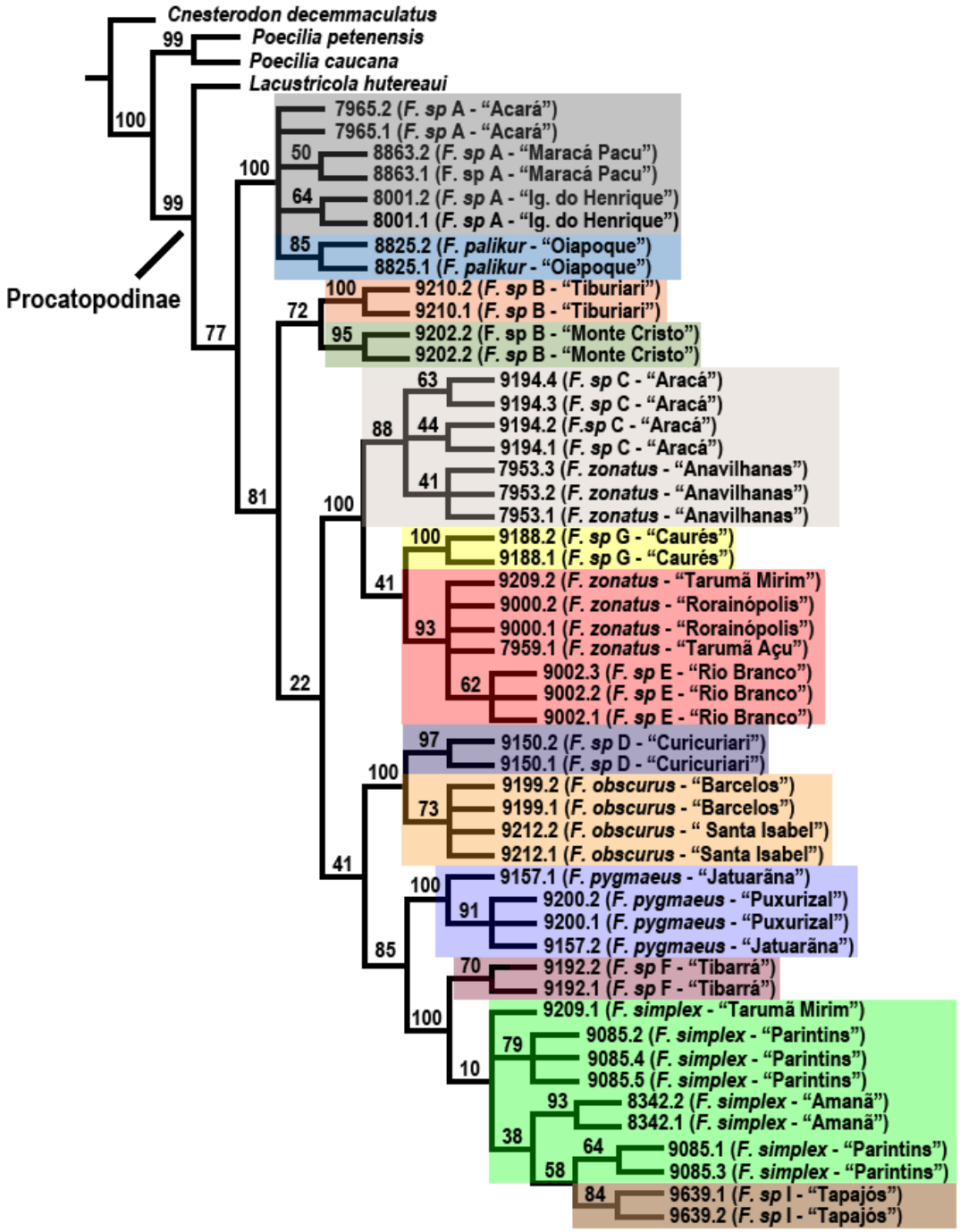

Fig 50. Maximum parsimony haplotype tree of the genus *Fluviphylax*. Terminal names are designated according to PAA result and are preceded by the UFRJ catalog number and are followed by the population collection site identification. Numbers over the branches are bootstrap values.

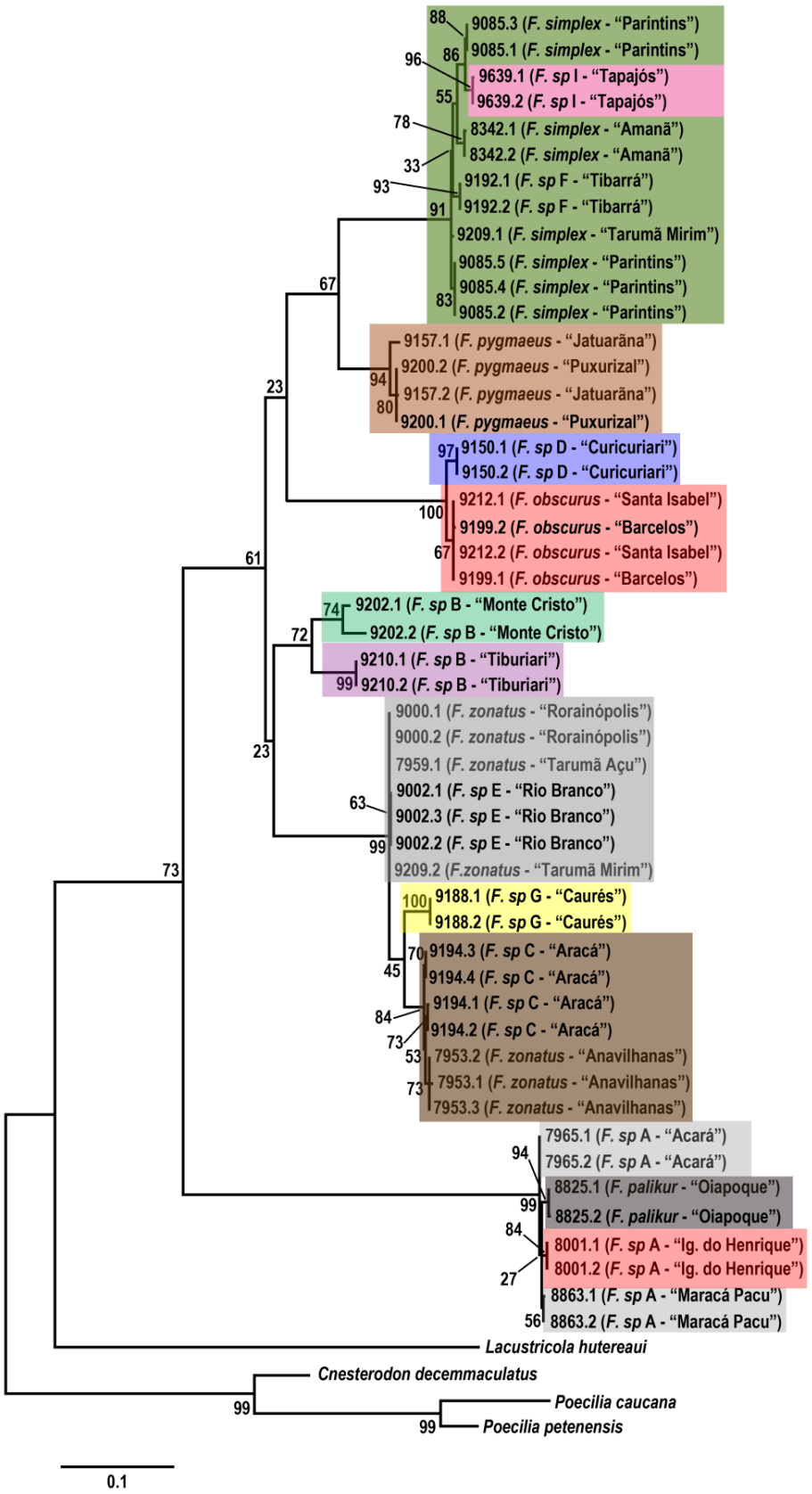

Fig 51. Maximum likelihood haplotype tree of the genus *Fluviphylax*. Terminal names are designated according to PAA result and are preceded by the UFRJ catalog number and are followed by the population collection site identification. Numbers over and above the branches are bootstrap values.

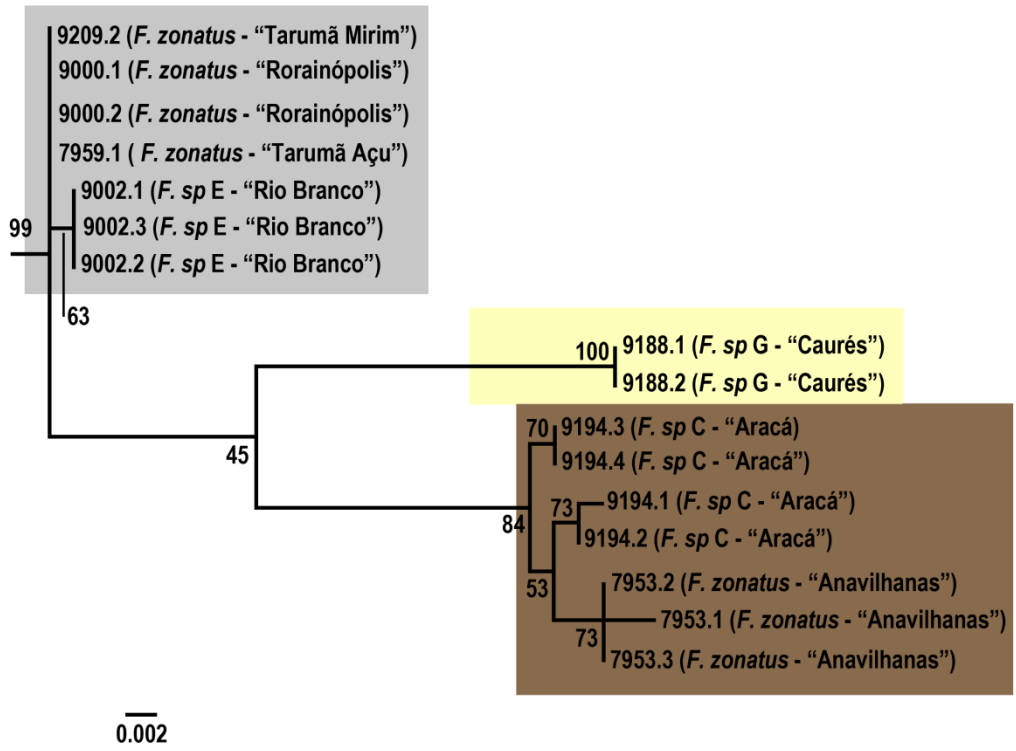

Fig 52. Topology of the *Fluviphylax zonatus* species group from maximum likelihood analysis. Numbers over and above the branches are bootstrap values.

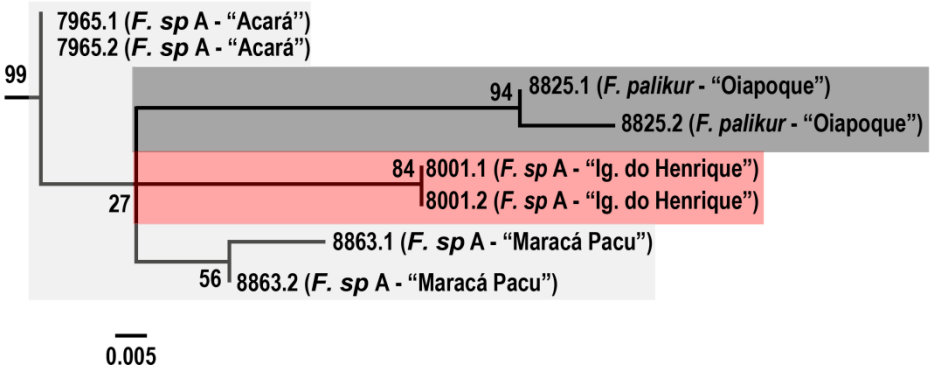

Fig 53. Topology of the *Fluviphylax palikur* species group from maximum likelihood analysis. Numbers over and above the branches are bootstrap values.

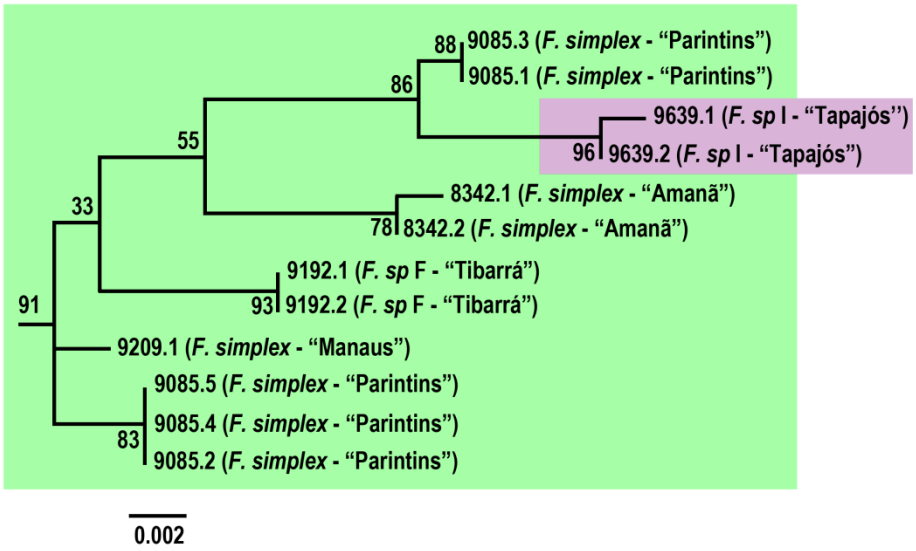

Fig 54. Topology of the *Fluviphylax simplex* species group from maximum likelihood analysis. Numbers over and above the branches are bootstrap values.

Fluviphylax palikur species group

The *F. palikur* species group is a well supported clade recovered with a 100% and 99% bootstrap values in the MP and ML analysis respectively. In the MP the *F. palikur* species group consists of two distinct lineages: the already known *Fluviphylax palikur* and *Fluviphylax sp.* A. In this analyses only *F. palikur* haplotypes constitute a well supported clade with 85% bootstrap support value being considered an exclusive lineage and the other haplotypes are not recognized as independent lineages because of the low bootstrap values, being recognized as belonging to the same non exclusive species *Fluviphylax sp.* A. In the ML the species *F. palikur* was also recovered with a 94% bootstrap value. Other two lineages were recognized in the ML: the well supported exclusive clade comprising the haplotypes of the *Fluviphylax sp.* A from "Ig. do Henrique" with a 84% bootstrap value and the non exclusive lineage that includes the remaining *Fluviphylax sp.* A haplotypes from "Maracá Pacu" and "Acará".

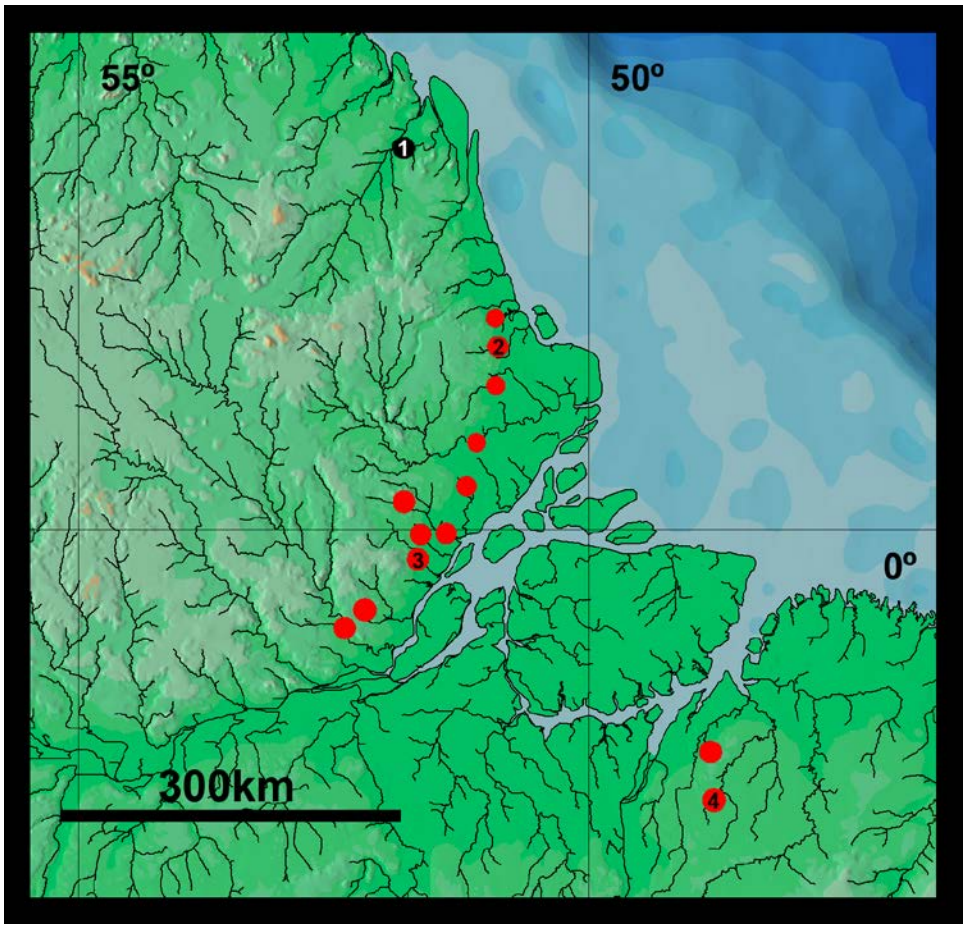

Fig 55. Distribution of *Fluviphylax palikur* species group. Red dots represents *Fluviphylax sp.* A localities and black dot *Fluviphylax palikur* locality. The numbered dots refers to population included in the haplotype analysis (1= "Oiapoque"; 2= "Ig. do Henrique"; 3= "Maracá-Pacú"; 4= "Acará"). One dot may represent more than one sampled location.

Fluviphylax sp. B species group

The *Fluviphylax sp.* B species group represents a new lineage in the genus supported by a moderate bootstrap value in both MP and ML analysis. In the MP the sampled populations, "Tiburiari" and "Monte Cristo", were recognized as two distinct exclusive lineages with high bootstrap values respectively. The ML recognized also the

two sampled populations as distinct exclusive lineages, "Tiburiari" with a 99% bootstrap support value and "Monte Cristo" with a 74% bootstrap value support.

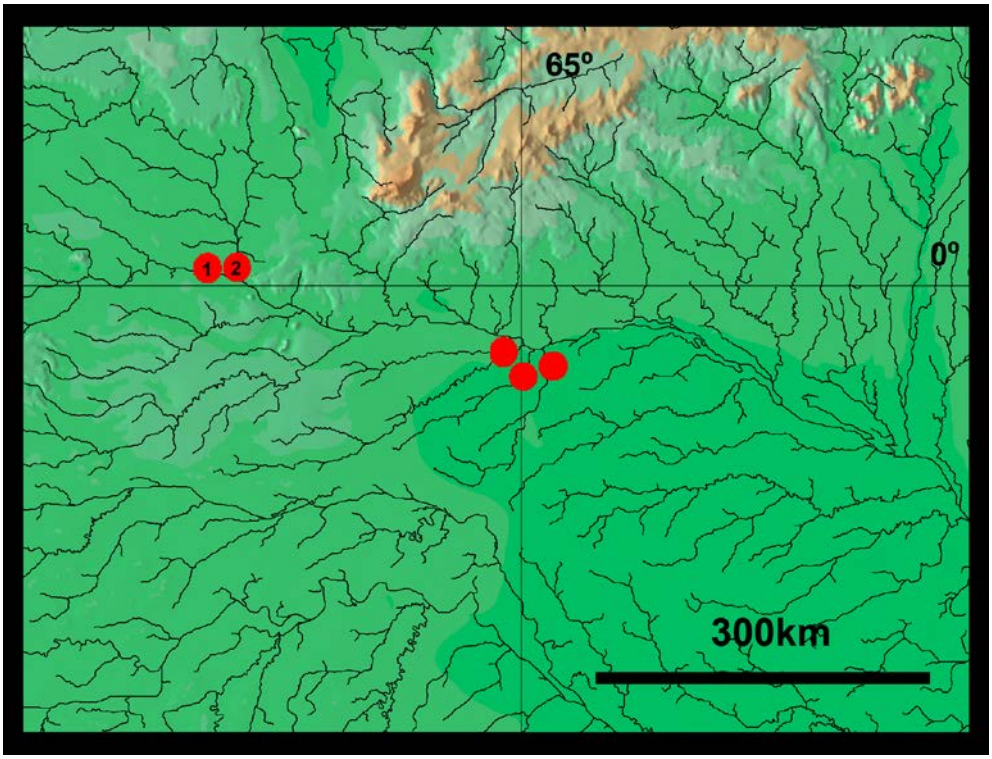

Fig 56. Distribution of *Fluviphylax sp.* B. Red dots represents *Fluviphylax sp.* B localities. The numbered dots refers to population included in the haplotype analysis (1= "Tiburiari"; 2= "Monte Cristo").

Fluviphylax obscurus species group

The *F. obscurus* species group is a well suported clade recovered by a 100% bootstrap value in both MP and ML. Two exclusive lineages were recognized in the MP: *F. obscurus* and *Fluviphylax sp.* D. Individuals of *F. obscurus* from "Barcelos" and "Santa Isabel" clustered in a 73% bootstrap value branch whereas the *Fluviphylax sp.* D individuals from "Curicuriari" have formed a 97% bootstrap value lineage. In the ML analysis the same exclusive lineages were found, the *Fluviphylax sp.* D with a 97% bootstrap value support and *F. obscurus* with a 67% bootstrap value branch.

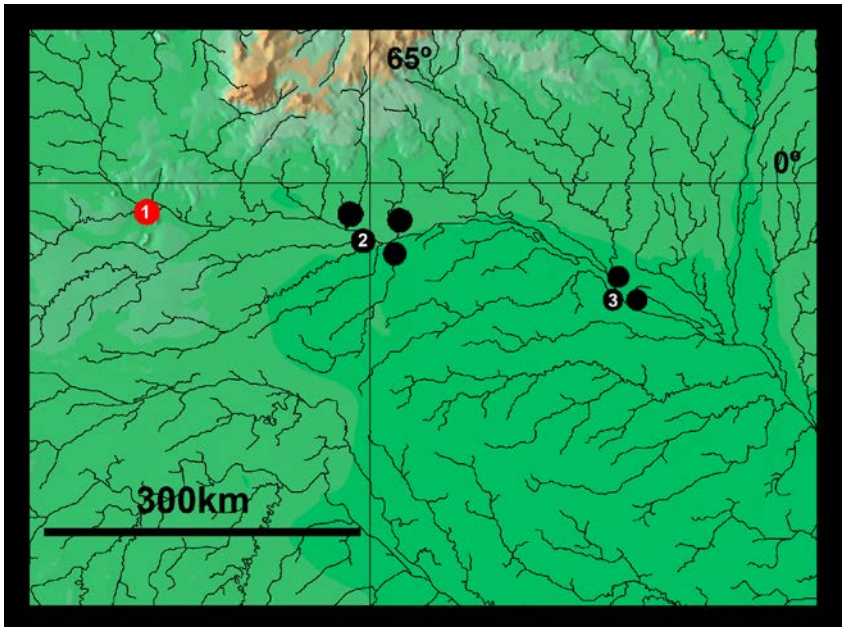

Fig 57. Distribution of *Fluviphylax obscurus* species group. Red dots represents *Fluviphylax sp.* D localities and black dots represents *Fluviphylax obscurus* localities. The numbered dots refers to population included in the haplotype analysis (1= "Curicuriari"; 2= "Santa Isabel"; 3= "Barcelos"). One dot may represent more than one sampled location.

Fluviphylax pygmaeus species group

The *Fluviphylax pygmaeus* species group is a well supported clade recovered by a 100% bootstrap value in the MP and by a 94% bootstrap value support in the ML analysis. In both, only one exclusive lineage was recognized: *F. pygmaeus*. The failing of one of the individuals from "Jatuarãna" locality to cluster with the other specimen from the same locality and the clustering of this with the other two individuals from "Puxurizal" is evidence of gene flow among populations.

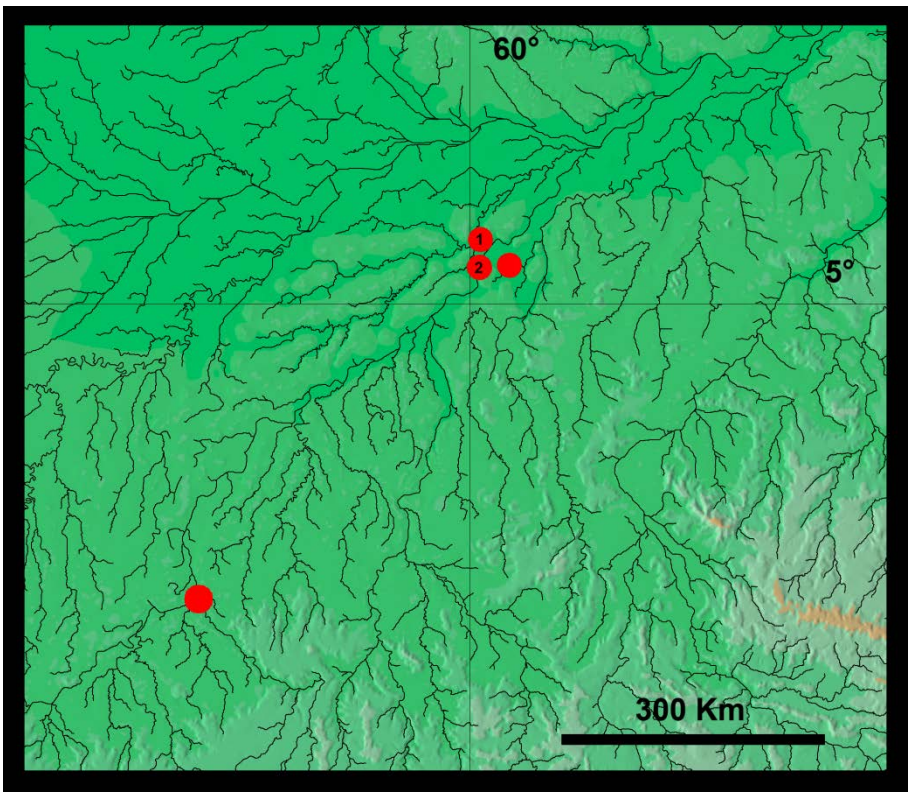

Fig 58. Distribution of *Fluviphylax pygmaeus*. Red dots represents *Fluviphylax pygmaeus* localities. The numbered dots refers to population included in the haplotype analysis (1= "Jatuarãna"; 2= "Puxurizal). One dot may represent more than one sampled location.

Fluviphylax simplex species group

The *F. simplex* species group is a well supported clade recovered with a 100% and 91% bootstrap values in the MP and ML analysis respectively. In the MP analysis were recognized three distinct lineages among the *F. simplex* species group: *F. simplex*, *Fluviphylax sp.* F and *Fluviphylax sp* I. The *Fluviphylax sp.* F represents a basal exclusive lineage supported by a 70% bootstrap value. The *F. simplex* lineage is a non exclusive species grouping individuals from "Tarumã Mirim", "Amanã" and "Parintins". The *Fluviphylax sp.* I is herein considered a valid exclusive species with a 84%

bootstrap value support. The ML analysis recognized only two of the three lineages recovered by the MP: *Fluviphylax sp.* I and *Fluviphylax simplex*. The haplotypes of *Fluviphylax sp.* I clustered in a high supported clade with 96% bootstrap support being considered an exclusive species. The haplotypes of the focal species *Fluviphylax sp.* F clustered within the haplotypes of *F. simplex* being recognized as member of the same lineage. Despite the high bootstrap values present in some internal groups of *F. simplex* in both MP and ML they are considered members of the same non exclusive lineage. This result is due to the splitting of *F. simplex* haplotypes from "Parintins" in two groups showing that some gene flow must be occurring between populations.

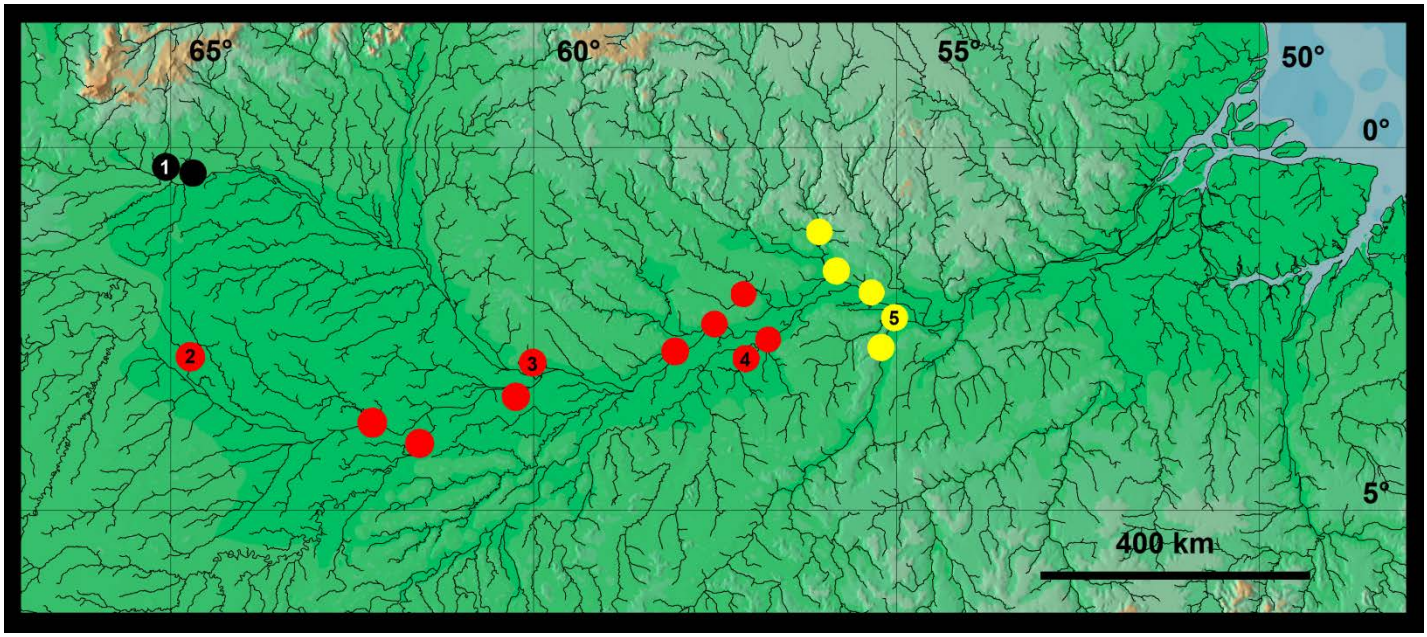

Fig 59. Distribution of *Fluviphylax simplex* species group. Red dots represents *Fluviphylax simplex* localities, black dots represents *Fluviphylax sp.* F and yellow dots represents *Fluviphylax sp.* I. The numbered dots refers to population included in the haplotype analysis (1= "Tibarrá"; 2= "Amanã"; 3= "Tarumã Mirim"; 4= "Parintins"; 5= "Tapajós"). One dot may represent more than one sampled location.

***Fluviphylax zonatus* species group**

The *F. zonatus* species group is a well supported clade with a 100% bootstrap value support in the MP and 99% value support in the ML. The two analysis recognized the same three distinct lineages: (1) *Fluviphylax sp.* G; (2) the lineage containing *Fluviphylax sp.* E from "Rio Branco" and *F. zonatus* from "Tarumã Açu", "Tarumã Mirim" and "Rorainópolis"; (3) the lineage grouping *Fluviphylax sp*. C and *F. zonatus* from "Anavilhanas". The *Fluviphylax sp.* G lineage is a well supported clade recovered by a 100% bootstrap value in both MP and ML. The group containing *F. zonatus* from "Anavilhanas" and *Fluviphylax sp.* C was recognized in both analysis with a 88% bootstrap value support in the MP and a 84% value support in the ML. The lineage containing *Fluviphylax sp.* E from "Rio Branco" and *F. zonatus* from "Tarumã Açu", "Tarumã Mirim" and "Rorainópolis" was recognized in the MP by the high bootstrap value of 93% whereas in the ML despite the low bootstrap value it was recognized due to the high support of the other lineages.

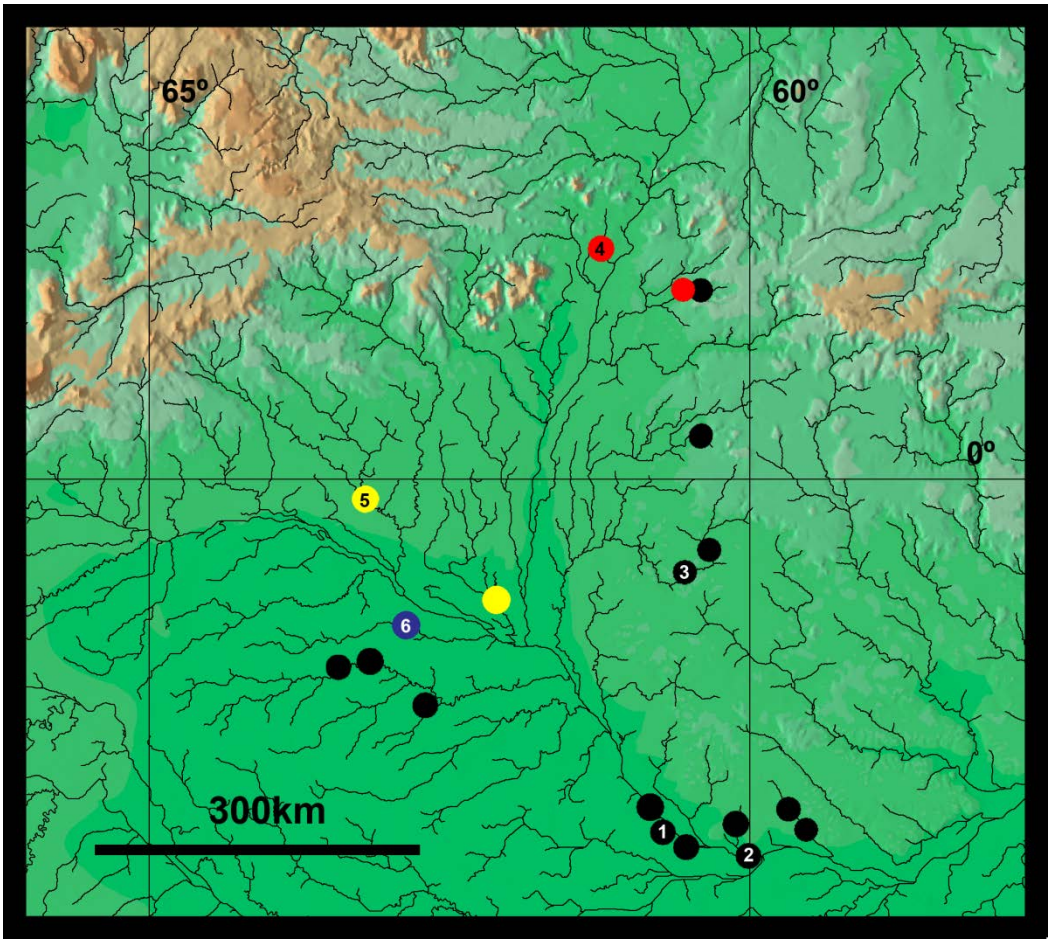

Fig 60. Distribution of *Fluviphylax zonatus* species group. Red dots represents *Fluviphylax sp.* E localities, black dots represents *Fluviphylax zonatus*, yellow dots represents *Fluviphylax sp.* C and the blue dot represents *Fluviphylax sp.* G. The numbered dots refers to population included in the haplotype analysis (1= "Anavilhanas"; 2a-b= "Tarumã Mirim" and "Tarumã Açu"; 3= "Rorainópolis"; 4= "Rio Branco"; 5= "Aracá"; 6= Caurés). One dot may represent more than one sampled location.

Discussion

Species boundaries

This study confirms the importance in integrating distinct methods and independent characters in the species delimitation of the Procatopodinae genus *Fluviphylax*. The application of different approaches turns the delimitation of the species boundaries more rigorous and it is amenable to result in congruencies and discrepancy among the results. The application of two species delimitation methods found both concordance and discrepancies in the delimited species boundaries among *Fluviphylax*.

The population aggregation analysis identified fourteen putative lineages based on the presence of an exclusive combination of morphological characters. Those putative lineages are approached as the focal species of the haplotype trees delimitation method. The focal species are posteriorly confirmed or not according to the topology of the haplotype tree. The distinct results are discussed together but for a organization matter, the identified species groups will be approached separately.

The analysis of the *Fluviphylax palikur* species group encompass two focal species delimited through PAA: *F. palikur* restricted to the Oiapoque river basin and *Fluviphylax sp.* A distributed in the Jari and Amapari river drainages and over Amapá and Pará costal drainages. The MP haplotype tree analysis delimited the same focal species proposed by the PAA, the well supported exclusive species *F. palikur* and the non exclusive species *Fluviphylax sp.* A. However the ML haplotype tree analysis delimited two well supported exclusive species: *F. palikur* and *Fluviphylax sp.* A from "Ig. do Henrique" and the non exclusive species *Fluviphylax sp* A. In view of this divergence in the delimitation of species it is possible to recognize only the two focal

species: *F. palikur* and *Fluviphylax sp.* A. *Fluviphylax palikur* was recovered by all three approaches and *Fluviphylax sp.* A was recognized as a unique non exclusive lineage recovered by PAA and MP analysis.

A complete congruence was observed in the delimitation of the species boundaries of *F. obscurus* species group. The two focal species proposed by PAA, *F. obscurus* and *Fluviphylax sp.* D, were recovered by both the MP and ML haplotype trees. The "Barcelos" and "Santa Isabel" haplotypes of *F. obscurus* clustered as well as the "Curicuriari" haplotypes. The haplotype analysis of the *Fluviphylax pygmaeus* group confirms only one focal species as proposed by the PAA. The failing of one of the individuals from "Jatuarãna" to cluster with the other specimen from the same locality and the clustering of one specimen from "Jatuarãna" with the other two individuals from "Puxurizal" is regarded as possible gene flow among populations.

The *Fluviphylax sp.* B species group, in both MP and ML analysis, was considered as a distinct group in the genus not clustering with any of the previously known *Fluviphylax* groups. This result is also observed when morphological characters are analyzed. The focal species *Fluviphylax sp.* B is diagnosed by the possession of a series of unique character states. Although the group have been recovered by both methods, the haplotype trees supported two exclusive lineages, "Tiburiari" and "Monte Cristo" with high bootstrap values. Rather than recognizing the focal species *Fluviphylax sp.* B as two distinct lineages, only one lineage is recognized because both "Tiburiari" and "Monte Cristo" populations are not morphologically distinguishable, sharing the same character state combination.

The analysis of the *Fluviphylax simplex* species group revealed a distinct species estimation in the ML analysis in contrast with a unique and congruent result from both

MP haplotype tree and PAA method. The PAA delimited three focal species: *F. simplex*, *Fluviphylax sp.* I and *Fluviphylax sp.* F, all of them recovered through MP haplotype tree. The focal species *Fluviphylax sp.* F was considered an exclusive lineage and sister to the remaining focal species from *F. simplex* species group. The focal species *F. simplex* was considered a non exclusive lineage with haplotypes from "Parintins" failing to cluster and originating two well supported branches. This topology suggests that the splitting of *F. simplex* from "Parintins" in two groups is due to gene flow between populations or to a retained ancestral polymorphism. The focal species *Fluviphylax sp.* I have been recovered by all approaches, being considered an exclusive lineage. The ML haplotype tree does not consider the focal species *Fluviphylax sp.* F from "Tibarrá" as an independent lineage but just as another population of *F. simplex*. Because of the congruent result observed in MP and PAA analysis, the three focal species are considered valid species.

The analyses of the *Fluviphylax zonatus* species group provided conflicting evidence of species limits between the approached methods. The PAA identified four focal species, three of them, *F. zonatus*, *Fluviphylax sp.* G and *Fluviphylax sp.* E are probably close relatives by all sharing many morphological and osteological character states, and one focal species, *Fluviphylax sp.* C, not sharing those character states. *Fluviphylax sp.* C differs morphologically from all other species of the *F. zonatus* group by having a sexual dimorphic pattern on cephalic latero sensory system, males with whole opened sensory canals and females with the anterior section of supraorbital, postorbital and upper section of preopercular canals closed, a pointed dorsal fin, a long and sharp retroarticular, an orange colouration on pectoral fin posterior region, an elongate and pointed anal-fin reaching vertically posterior to dorsal-fin base, males pelvic-fin reaching base of fifth anal fin ray, posttemporal ventral process short and by

the urogenital papilae presenting a sexual dimorphic pattern. These morphological characters suggest that *Fluviphylax sp.* C is not a member of the *F. zonatus* group as shown by both MP and ML.

The MP and ML haplotype trees supported three exclusive lineages, not corresponding with the PAA putative species except for the focal species *Fluviphylax sp.* G. The *Fluviphylax sp.* G haplotypes clustered in a well supported branch in both molecular analyses and were morphologically distinct presenting an exclusive character combination. However, *Fluviphylax sp.* G cannot be recognized as a valid lineage due to the resulting analysis topology.

The focal species *F. zonatus* was not confirmed as a single exclusive lineage because the haplotypes from "Anavilhanas" clustered with *Fluviphylax sp.* C from "Aracá", whereas the haplotypes from "Tarumã Mirim", "Tarumã Açu" and "Rorainópolis" clustered with *Fluviphylax sp.* E from "Rio Branco". Despite the haplotypes of *F. zonatus* from "Anavilhanas" and the other *F. zonatus* haplotypes not interdigitate showing some concordance in geographical data, they are indistinguishable morphologically. Because of this although the *F. zonatus* species group have morphologically distinct lineages identified through PAA and some well supported clades (e.g *Fluviphylax sp.* G), no decision should be made without further analysis.

Taxonomic accounts

A new classification for the Poeciliidae was proposed by Costa (1996): the tribes Aplocheilichthyini and Fluviphylacini were grouped in the same subfamily, Aplocheilichthyinae, based on the sharing of six synapomorphies: derived shape of parasphenoid, lachrymal and first hypobranchial, absence of parietals, males larger than

females and accessory caudal cartilage enlarged. In the present study all examined material presented those synapomorphies. Costa (1996) also established the phylogenetic relationships between *Fluviphylax* species and provided a new diagnosis for the genus. The species *Fluviphylax pygmaeus* and *F. simplex* were considered as sister taxa on the basis of a reduced ventral process of posttemporal and the absence of the rostral cartilage, and *F. zonatus* as closely related to *F. pygmaeus* and *F. simplex* by all sharing a sharp and elongated dorsal process of cleithurm, whereas *F. obscurus* was considered as the sister group of all other species. The haplotypes analysis also suggests a close relationship between *F. pygmaeus* and *F. simplex*. However, differently from Costa (1996), the absence of a sharp and elongate dorsal process of cleithrum in *F. obscurus* was not observed. Costa (1996) also listed thirteen synapomorphies for the genus until then diagnosed only by the eye extremely large and by miniaturization: vomer absent, dorsal process of maxilla greatly reduced, interarcual cartilage absent, fourth ceratobranchial teeth absent, anterior process of fifth ceratobranchial short and folded laterally, interhyal absent, basihyal cartilage enlarged, post-temporal scythe-shaped, anterior process of opercle with a distinct narrow process, caudal-fin rays 17-20, anal-fin rays 7-10, cephalic sensory system reduced and colour pattern consisting of melanophores concentrated on dorsal and ventral midlines of body.

Costa & Le Bail (1999) described *Fluviphylax palikur*, the smallest known species in the order Cyprinodontiformes. The new species was diagnosed by the possession of: 13-14 anal-fin rays, 27 scales on longitudinal series, 29-30 vertebrae, origin of dorsal fin at vertical through base of penultimate anal-fin ray, long and anteriorly directed anterior process of the fifth ceratobranchial, unconstricted medial portion of the fourth ceratobranchial, unreduced second pharyngobranchial and by the presence of a black spot preceeded by a yellow blotch on the posterior border of male

dorsal fin. Some of those characters were considered plesiomorphic and a sister-group relationship between *Fluviphylax palikur* and a group comprising the remaining species of the genus was established.

In addition to the necessary modifications after the description of *F. palikur,* the present study identified other characters not in accordance with the synapomorphies originally proposed by Costa (1996). The species from the *F. palikur* species group have a long and anteriorly directed anterior process of fifth ceratobranchial, a straight posstemporal bone and have between 12-15 anal-fin rays. The presence of teeth on the fourth ceratobranchial was here recorded for some individuals of *Fluviphylax sp.* A and *F. simplex*. The absence of the anterior process of opercle was also observed in *Fluviphylax sp.* B, *Fluviphylax sp*. C and *Fluviphylax sp.* D and it was recorded as polymorphic for *Fluviphylax sp.* A. The miniaturization itself is not considered as a diagnostic characteristic, since it is regarded as a process directly linked to the other recorded paedomorphic features. The genus is herein diagnosed by: eye extremely large, vomer absent, dorsal process of the maxilla greatly reduced, interarcual cartilage absent, interhyal absent, basihyal cartilage enlarged, caudal-fin rays 17-20, cephalic sensory system reduced and colour pattern consisting of melanophores concentrated on the dorsal and ventral midlines of body.

Geographical distribution

The diversified Amazonian ichthyofauna is thought to be the product of long term geological processes that changed river courses, watershed limits and land contours. Among the main geological and historical implications over the Amazon basin are the formation of the North and Northeast Andes, the rise of many structural

arches and the occurrence of marine introgressions (Hoorn, 1993; Hoorn et al.,1995; Lundberg et al, 1998). However many of this are recent geological events and the oldest geological patterns, right after the Africa-South America break-up, are still unknown, despite the fossil evidence indicate that many modern groups already existed since the final break-up between South America and Africa during late Cretaceous.

The direct ancestors of the miniaturized genus *Fluviphylax* are thought to have occurred in the Amazon region since the break-up between South America and Africa. This vicariant continental scale process is the best explanation to the present distribution of the African related genus in South America dating its origin back to the late cretaceous period.

Despite having a long history in South America practically none biogeographic pattern could be established by the current distribution of the genus. Biogeography analysis assumes a broad study and comparative information on the geographical distributions of different taxa, identifying consistent patterns of distribution and mapping geomorphological events. However the information on the distribution of the analyzed taxa is critical for conservation and further biogeography analysis.

Conclusion

The present study provides new information about the diversity and distribution of *Fluviphylax* in the Amazon and Orinoco river basins. A high diversity have been reported for the Negro river basin showing that its diversity has been underestimated , and it is probably one of the Poeciliidae family more species rich genus. As a result of the morphological study, informative characters were described and a new diagnosis

provided for the genus. *Fluviphylax* is diagnosed by: eye extremely large, vomer absent, dorsal process of the maxilla greatly reduced, interarcual cartilage absent, interhyal absent, basihyal cartilage enlarged, caudal-fin rays 17-20, cephalic sensory system reduced and colour pattern consisting of melanophores concentrated on the dorsal and ventral midlines of body.

A multidisciplinary approach on the species delimitation of the miniature genus revealed a previously unknown diversity. The application of distinct species delimitation methods led to both consistent and inconsistent results. Among the fourteen species delimited through PAA analyses, six were confirmed by both haplotype trees: *F. palikur*, *F. obscurus*, *F. pygmaeus*, *Fluviphylax sp.* B, *Fluviphylax sp.* D and *Fluviphylax sp.* I. The resulting topology of the *Fluviphylax zonatus* species group turns the recognition of *Fluviphylax sp.* G a precipitated decision as well as the recognition of any of the species lineages belonging to this group. The molecular and morphological approaches over the *Fluviphylax zonatus* species group led to conflicting conclusions about the species boundaries because the haplotype trees delimited lineages that do not correspond to the PAA focal species.

The application of the tree-based species demilitation method approached by MP and ML phylogenetic methods, resulted in similar but not identical topologies and delimited lineages. The MP analysis supported more three species not recognized by the ML, but congruent with PAA: *Fluviphylax simplex*, *Fluviphylax sp.* A and *Fluviphylax sp.* F.

The present study identified as valid, ten of the fourteen lineages proposed by the PAA: *F. palikur*, *F. obscurus*, *F. pygmaeus*, *Fluviphylax sp.* B, *Fluviphylax sp.* D and *Fluviphylax sp.* I, supported by all species delimitation methods; *Fluviphylax*

simplex, *Fluviphylax sp.* A and *Fluviphylax sp.* F, supported by PAA and MP haplotype tree analysis; and *Fluviphylax sp.* H analyzed only by the PAA method.

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Appendix I

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Fluviphylax pygmaeus	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	1	0	0	1	0
Fluviphylax simplex	1	1	1	0	0	1	0	0	0	0	0	0	1	0/1	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0/1	0	1	0
Fluviphylax obscurus	1	1	0	0	0	0/1	0	0	0	0	0	1	1	0	0	1	1	0	0	1	0	0	0	0	1/2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluviphylax zonatus	1	1	0	0	0	1	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0/1	0	0	0/1	0
Fluviphylax palikur	1	1	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0	1	1	1	0	1	0	0	0	0	0	0	0	2	0	1	0	0	0	1	0
Fluviphylax sp. A	1	1	1	1	0	1	0/1	1	1	1	1	0	0/1	0	0	1	1	0	1	1	1	1	0	1	2	2	1	0	0	0	0	2	1	1	0	0	0	0	0
Fluviphylax sp. B	0	-	-	0	0	0/1	0	0	0	0	0	0	0	-	1	0	0	0	0	1	0	0	1	0	1	1	0	1	1	0	0	1	0	0	1	1	0	1	1
Fluviphylax sp. C	1	1	0	0	0	0/1	0	0	0	0	0	0	0	-	0	1	1	0	0	1	0	0	1	0	1	1	0	1	0	0	1	1	0	0	1	?	1	1	1
Fluviphylax sp. D	1	0	0	0	0	1	0	0	0	0	0	1	0/1	0	0	1	1	0	0	1	0	0	0	0	1	0/1	0	0	0	0	0	0	0	0	1	0	0	1	0
Fluviphylax sp. E	1	1	0	0	0	1	0	1	0	1	0	1	1	0/1	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0
Fluviphylax sp. F	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Fluviphylax sp. G	1	1	0	0	0	1	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0/1	0
Fluviphylax sp. H	1	1	0	0	1	1	0	0	0	0	0	1	0	-	0	1	1	0	0	1	0	0	0	0	1	0/1	0	1	0	0	0	1	0	0	0	0	0	0/1	0
Fluviphylax sp. I	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	1	0

	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	**63**
Fluviphylax pygmaeus	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	1	0	0	1	0	0
Fluviphylax simplex	0	0	0	-	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
Fluviphylax obscurus	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1
Fluviphylax zonatus	0	0	1	1	0	0	0	0	0	1	0	1	0	1	0	**1**	1	1	1	1	0	0	0	0
Fluviphylax palikur	0	0	0	-	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluviphylax sp. A	0	0	0	-	1	1	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0
Fluviphylax sp. B	0	0	0	-	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0
Fluviphylax sp. C	0	0	1	0	0	1	0	0	0/1	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0
Fluviphylax sp. D	0	0	?	-	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1
Fluviphylax sp. E	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluviphylax sp. F	0	0	0	-	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Fluviphylax sp. G	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Fluviphylax sp. H	?	?	?	?	0	0	?	?	0	0	0	1	?	?	?	0	0	0	0	?	?	0	?	0
Fluviphylax sp. I	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	1	0