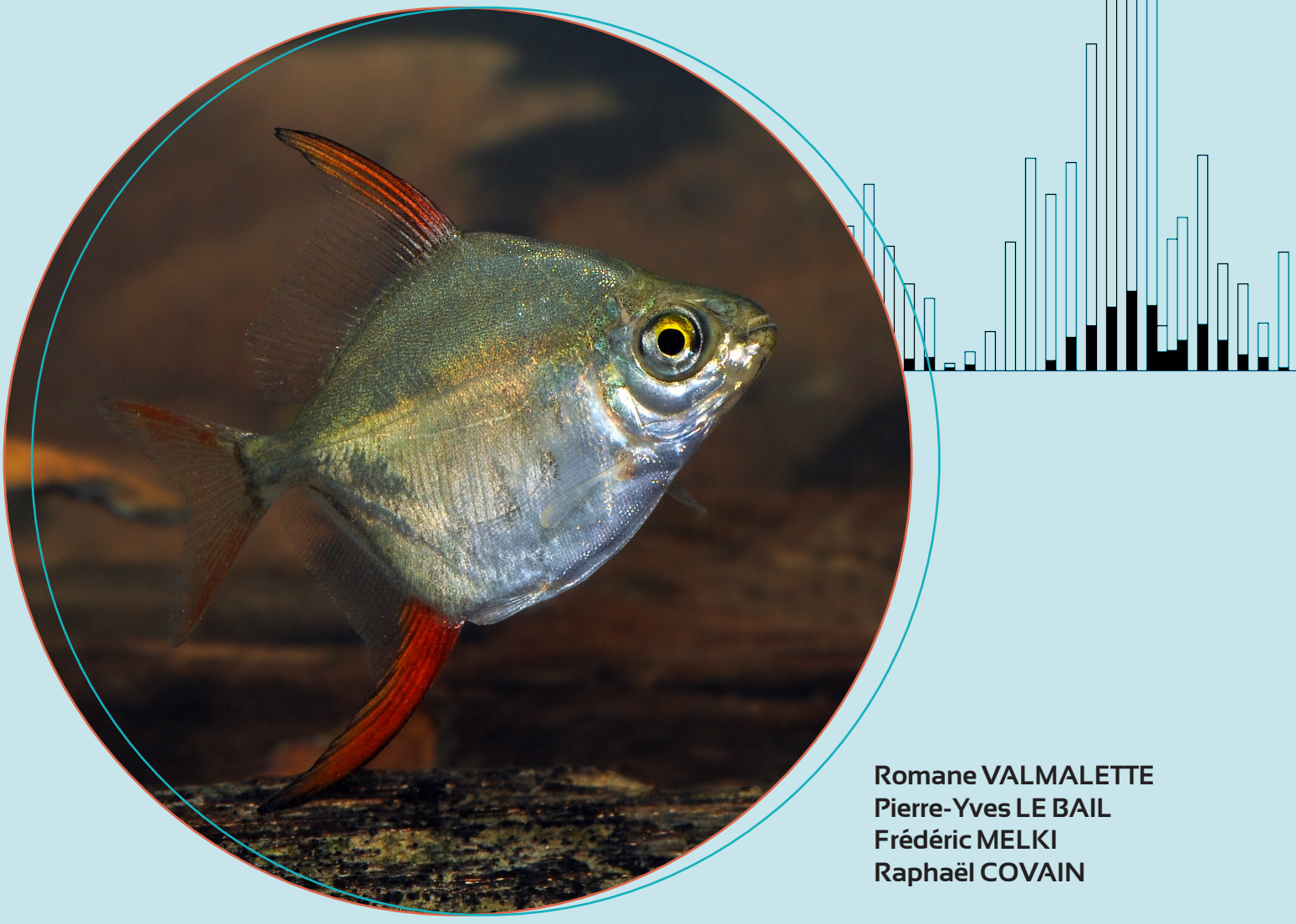
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n°4 Décembre 2025

# Assessing freshwater fish diversity in French Guiana using DNA barcoding



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## Assessing freshwater fish diversity in French Guiana using DNA barcoding

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*Krobia* aff. *guianensis* sp1, crique Canceler, French Guiana. © Frédéric Melki

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Approuague river,
French Guiana.
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# ABSTRACT

The ichthyofauna of French Guiana is among the best documented of South America. The sampling campaigns conducted these recent years in French Guiana and subsequent analyses using DNA barcoding have improved our understanding of intraspecific and interspecific relationships and refined our taxonomical knowledge. Until recently, taxonomic identification was essentially based on morphology with reference to a holotype. But this method can be limited due to the increasing difficulty to determine specific boundaries. Consequently, DNA barcoding quickly became a fundamental tool to assess the diversity of the freshwater ichthyofauna. This method consists in analyzing standardized mitochondrial genetic sequences and comparing them with a reference database in order to identify at the species level. In addition, differences between genetic sequences reflect the level of relationship between specimens. Through illustrated examples, this article shows how genetic analyses have led to enhance the known ichthyodiversity of French Guiana. The DNA barcoding permitted to: 1- validate the taxonomy based on morphological descriptions, 2- reveal putative wrong assignments at specific and generic level and 3- evaluate specific morphological descriptors made after taxonomical corrections based on genetic markers. The checklist of Le Bail *et al.* (2012) was then updated with the data obtained by new sampling campaigns in French Guiana. Despite a satisfying global sampling effort (79%) in French Guiana, the Iracoubo, Approuague and Oyapock rivers still represent fewer than 50% of sampling effort compared to literature. Efforts should focus in these areas to complete the Guianese Barcoding Of Life database.

**KEYWORDS:**
GUIANA SHIELD, ICHTHYOFAUNA, RIVERS, CRYPTIC DIVERSITY, COI GENE, GENETIC ANALYSES.

# RÉSUMÉ

L'ichtyofaune de Guyane française est l'une des mieux documentées d'Amérique du Sud. Les campagnes d'échantillonnage menées ces dernières années et les analyses ultérieures utilisant les codes-barres ADN ont amélioré notre compréhension des relations intra- et interspécifiques et affiné nos connaissances taxonomiques. Jusqu'à récemment, l'identification taxonomique était essentiellement basée sur la morphologie avec référence à un holotype. Toutefois cette approche peut s'avérer limitée en raison de la difficulté croissante à définir les limites spécifiques. Par conséquent, les codes-barres ADN sont rapidement devenus un outil essentiel pour évaluer la diversité de l'ichtyofaune d'eau douce. Cette méthode consiste à analyser des séquences génétiques standardisées d'ADN mitochondrial et à les comparer à une base de données de référence pour identifier les espèces. De plus, les différences entre séquences génétiques reflètent le niveau de parenté entre individus. À l'aide d'exemples illustrés, cet article montre comment les analyses génétiques ont permis d'améliorer la connaissance de l'ichtyodiversité de Guyane française. Les codes-barres ADN ont permis : 1- de valider la taxonomie basée sur les descriptions morphologiques, 2- de révéler des erreurs possibles d'assignation au niveau spécifique et générique et 3- d'évaluer les descripteurs morphologiques spécifiques après corrections taxonomiques basées sur des marqueurs génétiques. La checklist de Le Bail *et al.* (2012) a ensuite été mise à jour avec les données actualisées issues des nouvelles campagnes d'échantillonnage en Guyane française. Malgré un effort global d'échantillonnage satisfaisant (79 %), les fleuves Iracoubo, Approuague et Oyapock représentent encore moins de 50 % de l'effort d'échantillonnage. Les futures campagnes d'échantillonnage devraient se concentrer sur ces régions afin de compléter la base de données du Barcoding Of Life des Guyanes.

# CONTEXT

The diversity of an ecosystem is defined by its species richness and their respective relative abundance. Identifying the entire fauna of an area allows us to associate ecosystem characteristics with species assemblages. An ecosystem is an area defined by a biotope (habitat) and biocenoses (fauna and flora). These two compartments work in a complementary way with habitat characteristics (geology, climate, abiotic feature, etc.) impacting the type of population living in a given place, and biocenoses modifying their respective habitat. Moreover, the faunistic composition of an ecosystem provides information on its global health (Teresa and Casatti, 2017). This information is therefore of great interest to decision makers and managers of natural areas such as those of French Guiana (Parc amazonien de Guyane, Protected areas).

The Guianas encompass three countries: Guyana (former British Guiana), Suriname (former Dutch Guiana), and French Guiana (French oversea department), and part of Amapà (Brazil). The river system of the Guianas is composed of 17 large rivers flowing South to North, organized in parallel and perpendicular to the Atlantic Ocean coastline. The Guianas river system is independent of the Amazon and Orinoco basins. Moreover, the geographic features of Guianese basins strongly influenced the spatial distribution of freshwater fish communities given that their dispersal abilities are highly constrained by physiological adaptations (Lemopoulos and Covain, 2019). Accordingly, fish communities may disperse within a basin or in adjacent basin, depending on salinity and hypoxia tolerance. It would be for these reasons that Guianese freshwaters reveal such a high rate of endemism estimated at around 30% (Albert *et al.*, 2011; Covain and Fish-Muller, 2012; Lemopoulos and Covain, 2019).

The fish richness of Guianese freshwaters varies between geographical areas and river's size (de Merona *et al.*, 2012; Le Bail *et al.*, 2012; Mol, 2012; Lemopoulos and Covain, 2019). Thanks to intensive sampling campaigns, Guianese freshwater fauna is one of the best documented of South America, especially in French Guiana. French Guiana is composed of three biogeographic zones: the western zone comprising Maroni and Mana Rivers, the central zone containing Iracoubo, Sinnamary and Kourou Rivers and the eastern zone including Comté-Orapu, Approuague and Oyapock Rivers (Lemopoulos and Covain, 2019). In 2012, Le Bail *et al.* listed 416 species, including 367 strictly freshwater species. These authors have used conventional approaches consisting in collecting and identifying species based on morphology (Planquette *et al.*, 1996; Keith *et al.*, 2000; Le Bail *et al.*, 2000).

Unfortunately, this approach is time-consuming, requires the expertise of specialists, and is subject to mistakes when type material and original morphologic descriptions are old and vague. Therefore, interpretations may be biased by the low visibility of certain

morphologic differences in some species. Accordingly, Le Bail *et al.* (2012) suspected the presence of hidden cryptic diversity, which was later confirmed by Papa *et al.* (2021).

Today, with the genetic development and the new molecular technologies, DNA barcoding can be used in routine to precisely identify global diversity (Hebert *et al.*, 2003). In animals, DNA barcoding is based on the amplification and the sequencing of a highly variable region of the mitogenome. Usually, the 5' region of the mitochondrial gene coding for the first subunit of the cytochrome *c oxydase* (COI), a protein involved in mitochondrial respiration, is used. DNA barcoding assumes that the intraspecific genetic variation is very low compared to the interspecific genetic variation. The determination of the specific threshold of intraspecific variation allows identifying cryptic diversity, new species, but also retracing the biogeographical history of species and communities. However, the construction of a reference database is a prerequisite to apply this technique at large scale. Such a database is currently in development. Since 2010, the collaborative project launched Gui-BOL (Guianese Barcoding Of Life – Barcoding Guianese Fish) involving about ten researchers from international institutes (MHNG, University of Sao Paulo, University of Oregon, INRAE of Rennes, Smithsonian Institution of Washington, University of Toulouse, Biotope Inc., Hydreco, and University of Suriname) aims to build a genetic database for the entire freshwater fauna of the Guianas.

By relying on the specific sampling campaigns of 1998, 1999, 2000, 2003, 2006, 2007, 2010, 2012, 2014, 2015, and 2017, the objectives of this work are 1) to estimate the sampling effort made for barcoding approaches in French Guiana with regard to existing knowledge, 2) to illustrate with selected examples what the barcoding approach has been able to bring to the knowledge of Guianese ichthyodiversity, in particular concerning cryptic species and the actual distribution of each taxon and 3) to update the reference list of French Guiana freshwater fish fauna regarding species distribution.



*Hypostomus gymnorhynchus*, Chutes Voltaire, French Guiana. © Frédéric Melki

# METHODOLOGY

## SAMPLING TECHNIQUES

Researchers have focused their sampling efforts in the three biogeographical areas of French Guiana's eight principal rivers: the Maroni, Mana, Iracoubo, Sinnamary, Kourou, Comté-Orapu, Approuague and Oyapock rivers. For each station, different fishing technics were used: landing net, trammel net with different mesh, cast-net, angling, trident, etc. All sampled individuals were measured, identified with the morphologic species criteria and assigned to a collection number (*i.e.* voucher). From each specimen, a piece of tissue (fin or muscle) was collected and stored in order to perform DNA barcoding analysis. Each tissue sample was transferred in 80% ethanol, and then preserved at -20°C until analysis. The corresponding specimen was fixed in 4% formaldehyde, and, for conservation and safety reasons, transferred in 75% ethanol. Samples are deposited in official collections either in the Natural History Museum of Geneva (MHNG), or in the National Natural History Museum of Paris (MNHN). Preliminary morphological identifications were performed using reference literature about Guianese fauna (Planquette *et al.*, 1996; Keith *et al.*, 2000; Le Bail *et al.*, 2000; Mol, 2012) and following the checklist of Le Bail *et al.* (2012).

## DNA BARCODING ANALYSES

DNA barcoding involves amplifying the target region of the mitochondrial gene encoding the first subunit of cytochrome c oxidase (mtCOI), also known as the barcode region, and comparing the resulting sequences with a reference database to identify species. Manipulations in laboratory were made for each tissue sample according to the detailed protocol described by Covain *et al.* (2012). The standard region 5' mtCOI region was amplified by PCR (Polymerase Chain Reaction) amplification method, and was sequenced using Sanger's method (Sanger and Coulson, 1975). The obtained genetic sequences were integrated in Gui-BOL database with all metadata related to each sample (identification number, GPS coordinates, collector name, sampling station name). Sequences were edited on BioEdit software (7.0.1 version) (Hall, 1999), and were aligned using an online automatic aligner: clustal omega (Multiple Sequence Alignment, EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/clustalo/; accessed the 02.05.2018)), to determine the homology between sites. Resulting alignment was imported in R 3.4.4. software and submitted to preliminary analyses using the Sequinr 3.4-5 (Charif and Lobry, 2007) and ape 5.1 (Paradis *et al.*, 2004) packages. The Kimura 2 parameter was applied to compute genetic divergences between sequences, assuming a differential rate between transitions and transversions (K2P in ape package or K80 in sequinr package) (Kimura, 1980). A barcoding tree was secondarily built using the K2P distances matrix with the Neighbor

Joining (NJ; Saitou and Nei, 1987) algorithm. The benefit of this algorithm is that branch lengths are preserved, and that we can visualize divergence rates between species or monophyletic groups (Covain *et al.*, 2012). Specific boundaries were evaluated using the threshold values of genetic divergence between species, computed by spider package 1.5.0. (Species Identity and Evolution) in R. The threshold highlights cryptic diversity and even speciation process. This threshold is represented by the barcoding gap bounded by genetic distance of inter and intraspecific diversity (Meyer and Pauley, 2005).



*Pimelodella* gr. *cristata*, Crique Grégoire. DNA Barcoding prove that the interspecific diversity for *Pimelodella* species of French Guiana is underestimated. © Frédéric Melki

# RESULTS AND DISCUSSION

## SAMPLING STATE

Since the start of the sampling campaigns to establish an inventory of the freshwater Guianese fauna, 3989 sequences have been deposited in Gui-BOL, corresponding to 3989 sampled specimens. Of these, 1005 sequences were newly generated in 2017 with the support of the Biotope Foundation for Biodiversity.

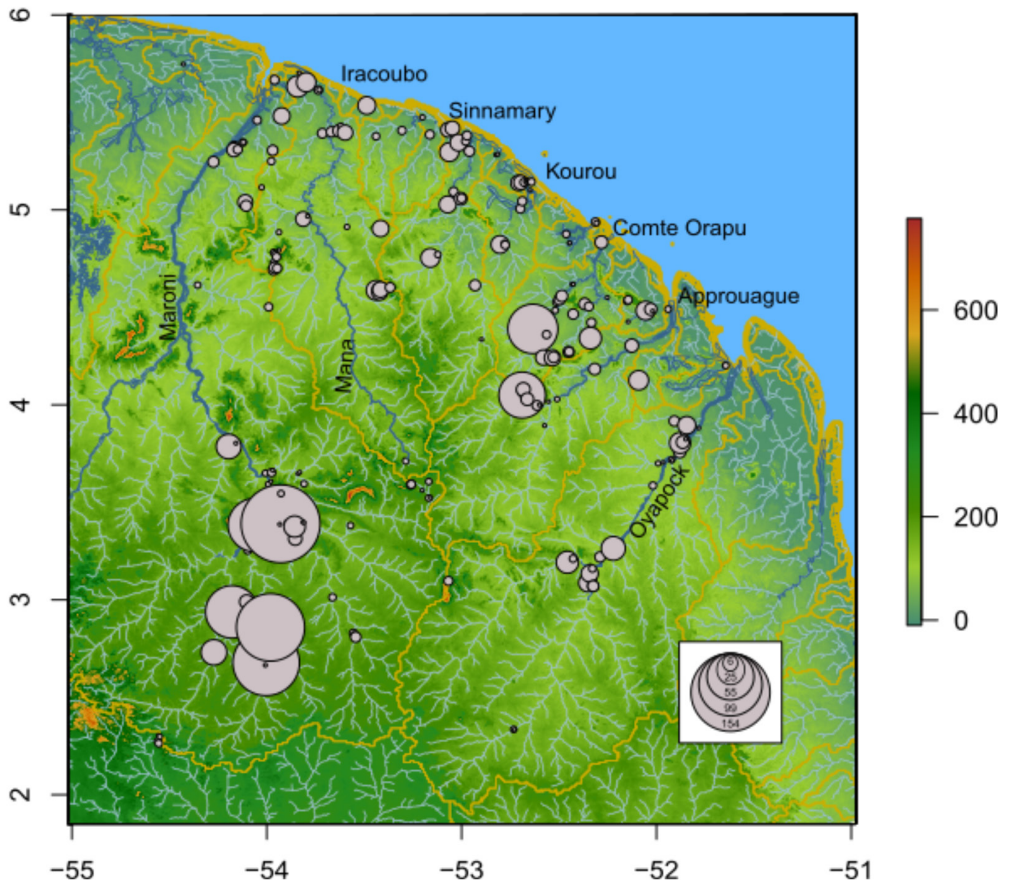



Fig. 1: **Geographic distribution of samples of DNA barcode sequences in French Guiana (until 2017). The axes represent decimals coordinates of the region, longitudinal in x-axes, latitudinal in y-axes. The color scale represents the elevation in meters above mean sea level. The bubbles represent the number of sequences by sampling station.**

Sequencing efforts across the eight French Guiana river basins (figure 1) covered 243 sampling stations, with a number of fish sequences obtained ranging from 1 to 159 per station. At present, sampling in French Guiana is focused along the coastline, in the upper Maroni and in the Comté-Orapu. In contrast, the lower Maroni, the upper Mana and

the upper Oyapock remain under-sampled. Globally, the West of French Guiana appears better sampled than the eastern area.

Table 1

**Sampling recovery percentages and the number of families collected across Guianese basins, estimated in relation to the number of families reported in the literature. Yellow cells indicate current sampling, while grey cells indicate a sampling rate below 50%.**

| | Maroni | Mana | Iracoubo | Sinnamary | Kourou | Comté-Orapu | Approuague | Oyapock | French Guiana |
|---|---|---|---|---|---|---|---|---|---|
| Sampled families number | 35 | 29 | 8 | 21 | 21 | 33 | 20 | 26 | 48 |
| Expected families number | 54 | 37 | 30 | 42 | 36 | 44 | 48 | 48 | 62 |
| Recovery percentage (%) | 64,8 | 78,4 | 26,7 | 50,0 | 58,3 | 75,0 | 41,7 | 54,2 | 77,4 |

Moreover, a pattern is observed in family groups. Indeed, 48 different families were sampled in French Guiana, with disparity between basins. For example, 35 families were present in Maroni River, whereas only 8 families were present in Iracoubo River (table 1). In total, the recovery percentage of sampled families stands around 77%. Mana (78%) and Comté-Orapu Rivers (75%) are better sampled basin regarding the literature. In terms of number of sampled species, the *Characidae*, *Cichlidae,* and *Loricariidae* families are the most represented. These families are the most diversified in terms of the number of genera and species in French Guiana basins.

Table 2

**Sampling recovery percentages and number of sampled species depending on Guianese basins. Estimation given with the number of species described in literature. The yellow cases represent the current sampling; the grey cases represent sampling rate below 50 %.**

| | Maroni | Mana | Iracoubo | Sinnamary | Kourou | Comté-Orapu | Approuague | Oyapock | French Guiana |
|---|---|---|---|---|---|---|---|---|---|
| Sampled species number | 171 | 100 | 14 | 78 | 50 | 100 | 81 | 92 | 330 |
| Expected species number | 279 | 176 | 78 | 156 | 108 | 160 | 188 | 204 | 416 |
| Recovery percentage (%) | 61.3 | 56.8 | 17.9 | 50.0 | 46.3 | 62.5 | 43.1 | 45.1 | 79.3 |

The sampling effort in French Guiana appeared satisfactory, although it still requires improvement (table 2). In total, 330 species have been sampled and sequenced, and represent a robust database for future study. In total, the recovery percentage of species sampling stands around 80%. The Maroni (61%) and Comté-Orapu rivers (62%) are better sampled: 171 species are sampled in Maroni River and 100 species are sampled in Comté-Orapu River. Finally, sequence analysis revealed 113 new occurrences (figure 2)

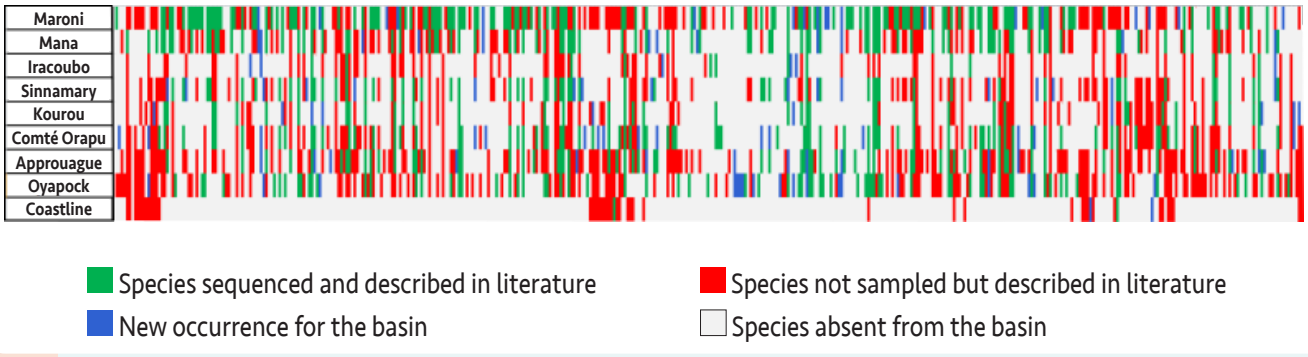



Fig. 2: **Heatmap distribution of sequences for 416 freshwater and estuarine fish species from the different basin of French Guiana (each column represents one morphological species).**

## FRENCH GUIANA'S ICHTHYODIVERSITY

DNA barcodes analyses reveal intraspecific and interspecific relationships and can correct identifications based on morphological descriptions. A low genetic distance between individuals is usually the sign of intraspecific relationships. On the contrary, a high genetic distance corresponds to interspecific relationships. The barcoding hypothesis is that the intraspecific distances are significantly lower than interspecific distances. According to Ward *et al.* (2009), the threshold value between interspecific and intraspecific genetic distances is usually around 1-2%.

Based on the barcoding approach our results showed that species richness of freshwater fishes in French Guiana was underestimated (see figure 2). Through following examples, we showed how genetic analyses improved our vision and comprehension of the taxonomy.

### Confirmation of taxonomy based on morphological descriptions

The genus *Hypostomus* belongs to the Locariidae family and Hypostominae subfamily. These catfishes are present in all Guianese basins. In total, 21 species of *Hypostomus* inhabit Guianese freshwaters (Weber *et al.*, 2012). This genus is characterized by a large, depressed body covered by bony plates, by lacking highly eversible cheek plates, and by never having hypertrophied cheek odontodes, even in nuptial males. *Hypostomus* presents a mean level of intraspecific variation in morphology and in color patterns. Its color



*Hypostomus gymnorhynchus*,
Approuague River © Frédéric Melki



*Hypostomus nematopterus*,
Oyapock drainage, Yaloupi Creek © Frédéric Melki

generally varied between tan to black with or without dark spots. *Hypostomus* species live in freshwater or brackish water (floodplain, shallow or deep rivers, estuarine area). They are benthic catfishes living on the substrate. *Hypostomus* species have a suckermouth placed in ventral position with teeth able to scrape the substrate. *H. plecostomus* can grow up to 40 cm TL.

In French Guiana, four species of *Hypostomus* are morphologically described: *H. nematopterus*, *H. gymnorhynchus*, *H. plecostomus* and *H. watwata*. 83 specimens distributed in eight basins (Sinnamary, Mana, Maroni, Kourou, Approuague, Iracoubo, Comté-Orapu, and Oyapock) have been sequenced.

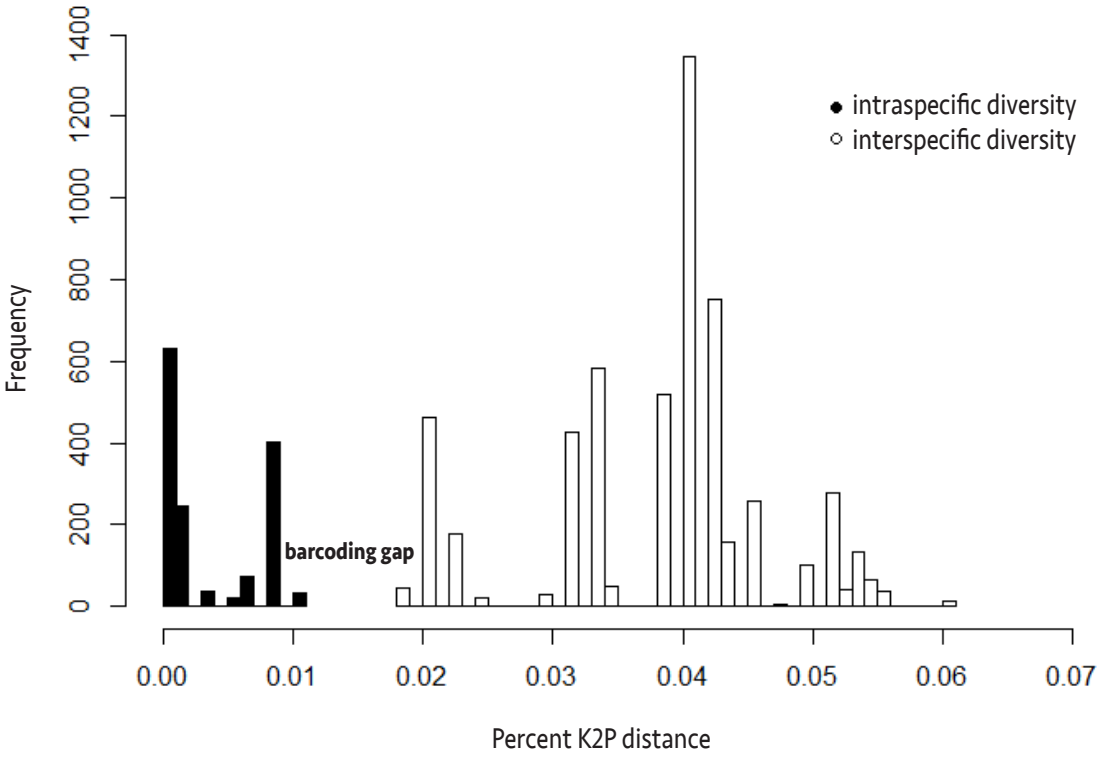



Fig. 3: **Intraspecific (black) and interspecific (white) diversity histograms of *Hypostomus* species in French Guiana. The x-axis represents K2P distances; y-axis represents the frequencies of all pairwise comparisons.**

The figure 3 represents the DNA barcodes gap analysis. The black and white histograms are clearly separated with a barcoding gap comprised between 1 and 2% of genetic distances. Intraspecific distance is below 1% whereas interspecific distance varies between 2 and 6%. This suggests that the four species of *Hypostomus* are well distinct and probably distinguishable from one another. Although a low interspecific distance and barcoding gap of 2% were observed for *Hypostomus* species, this value does not hold across all fish genera (Papa *et al.*, 2021; de Queiroz *et al.*, 2020). The figure 4 represents the relationships between the different *Hypostomus* species. The genetic tree is consistent with the morphological criteria used in classical taxonomy. We observe that the *Hypostomus* species are clearly differentiated from one another, and that their morphological descriptions are consistent with the genetic data.

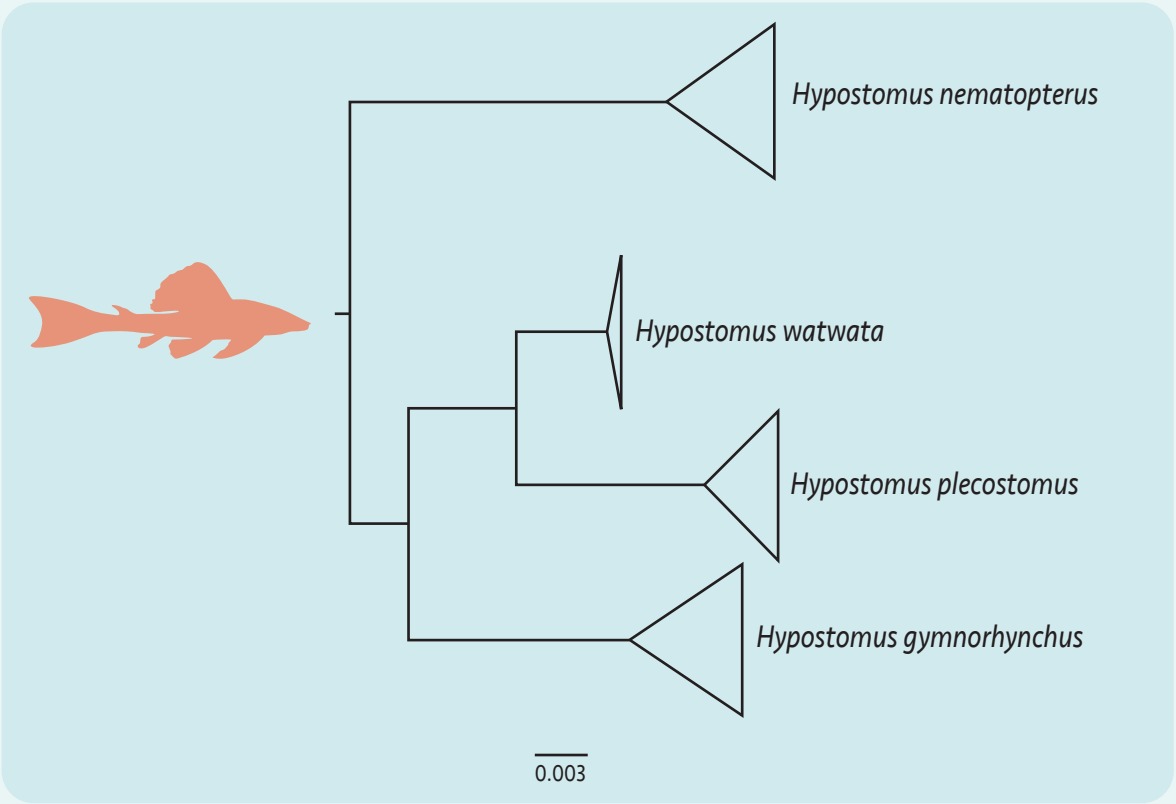



Fig. 4: **Genetic tree based on barcoding analysis. The tree was computed on pairwise K2P distances between COI sequences of the four species of *Hyspotomus* of French Guiana. The scale represents the genetic divergence rate between species computed with K2P method. Triangles: height proportional to the number of sequences in each lineage; depth proportional to intraspecific distances.**

This example shows how the genetic analyses confirm the taxonomy based on morphological descriptors.

## Correction of the taxonomy based on morphological descriptions

These three examples demonstrate how DNA barcoding can uncover discrepancies in morphologically based taxonomy.

### • An underestimation of specific diversity

The genus *Krobia* belongs to the Cichlidae family, and is present in all Guinanese basins. *Krobia* is characterized by a moderately elongated body with ovoïd lateral profile. The shape of the head is slightly convex with a short snout. *Krobia* genus is defined by several morphological criteria: narrowly scaled or naked dorsal and anal fins, spiny fins, uniserial predorsal squamation, a dark lateral interupted band extending from the head to dorsal-fin base end, and a dark spot located on caudal fin in dorsal position (Kullander and Nijssen, 1989, Bragança *et al.*, 2014). Specimen's size varies between 9 and 15 cm and females are usually smaller than males. *Krobia* species live in freshwater or brackish water at shallow, slow flow depth (rivers, marshlands, small creeks). They swim in mid-depth or live close to the substrate in the lower water column, and have an omnivorous diet (detritivore, insectivorous, verminvorous, Roussel ***et al.***, 2021).



*Krobia* aff. *guianensis* sp1, Kaw © P.-Y. Le Bail



*Krobia itanyi*, Mana River © P.-Y. Le Bail



*Krobia* cf. *itanyi* (*K.* aff. *guianensis* sp2), Oyapock River © P.-Y. Le Bail



*Krobia guianensis*, Approuague River © Frédéric Melki

According to Le Bail *et al.* (2012), three species of *Krobia* are found in French Guiana, *K. itanyi* and two undescribed species (*K.* aff *guianensis* sp1, *K.* aff. *guianensis* sp2) (see

photos above). 50 specimens distributed in eight basins (Sinnamary, Mana, Maroni, Kourou, Approuague, Iracoubo, Comté-Orapu, and Oyapock) have been sequenced.

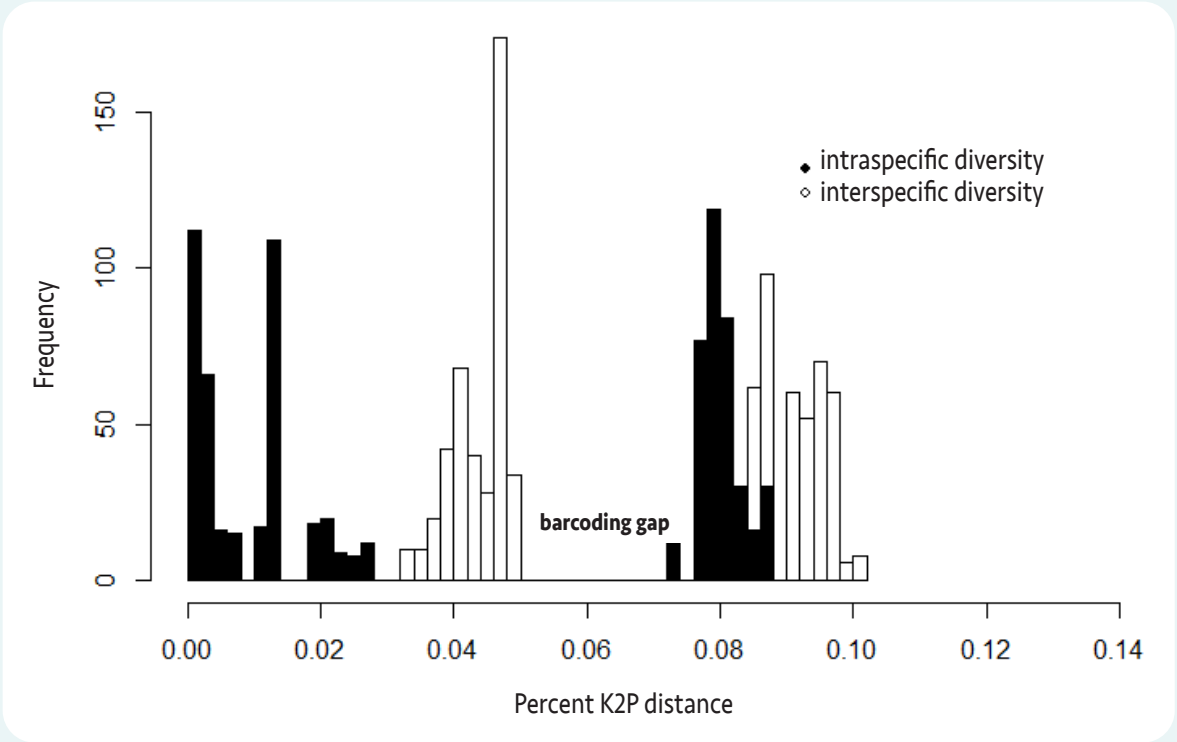



Fig. 5: **Intraspecific (black) and interspecific (white) diversity histograms of *Krobia* species in French Guiana. The x-axis represents K2P distances; y-axis represents the frequencies of all pairwise comparisons.**

The figure 5 represents the DNA barcodes gap analysis. Contrary to *Hypostomus*, intraspecific and interspecific diversity represented by black and white histograms strongly overlap in *Krobias*. Genetic analyses show that some species display an intraspecific variation of up to 8% with a disjoint distribution of frequencies. These observations do not fit the barcoding hypothesis. Moreover, the barcoding gap is located around 6% of genetic distance. This demonstrates that *Krobia* species are not well morphologically identified, and that they comprise more than 3 taxa. Interspecific diversity in *Krobia* is underestimated.

The genetic analysis and the NJ barcode tree (figure 6) confirm the validity of *K. itanyi* and *K.* aff. *guianensis* sp1. The latter is genetically distinct from all other species and can be considered a new species of *Krobia*. *Krobia* aff. *guianensis* sp1 is characterized by red eyes coloration. Conversely, the NJ barcode tree shows that *K.* aff. *guianensis* sp2 consisted in two distinct species. One distributed in Oyapock Basin corresponded to a close representative of *K. itanyi*, and the second distributed in Maroni and Approuague rivers corresponded to *K. guianensis* from Suriname. *Krobia guianensis* differs from *K. itanyi* by a more continuous lateral band extending from the end of the dorsal-fin base (Kullander and Nijssen, 1989; Bragança *et al.*, 2014). Specimens from Oyapock River must be considered as *K.* cf. *itanyi*. They share with the nominal species, while differing from other congeneric species, the conspicuous red and blue markings between the lower

orbital rim and the maxilla. It is not yet clear whether *K.* cf. *itanyi* represents a distinct species, as the observed genetic divergences with the nominal species may be related to the geographical distances between the sampled populations.

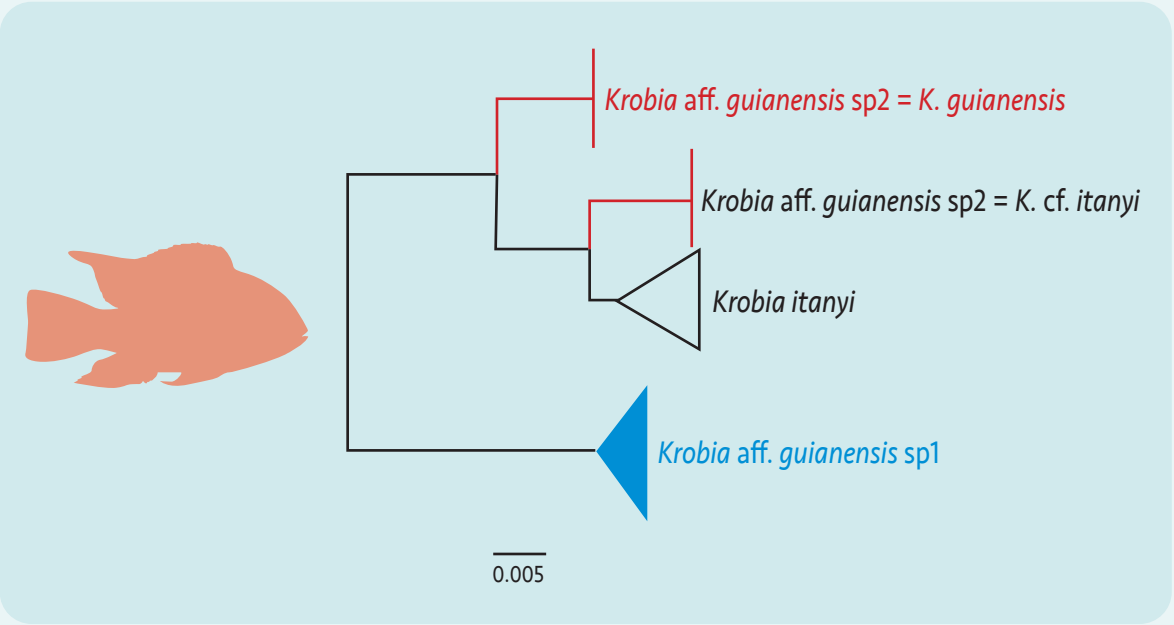



Fig. 6: **Genetic tree based on barcoding analysis. The tree was computed on pairwise K2P distances between COI sequences of the four species of *Krobia* of French Guiana. The scale represents the genetic divergence rate between species computed with K2P method. Triangles: height proportional to the number of sequences in each lineage; depth proportional to intraspecific distances.**

Through this example, an underestimation of specific diversity was highlighted. The genetic analyses have led to correct *Krobia* species taxonomy and to enhance morphological descriptions for this genus.

### • A species in a wrong genus

The herbivore piranhas group *Myleinae* (*Myleus, Myloplus, Mylesinus, Tometes*...) is a herbivorous subfamilly of the *Serrasalmidae*, and is present in all Guianese basins. *Myleinae* is characterized by a massive, laterally compressed body with an ovoid shape. They measure between 16 and 56 cm TL. They have a triangular anal fin (bilobed in male) and short pectoral fins. Their lateral line is concave relative to dorsal profile. *Myleinae* live in rivers with slowly to strongly flowing waters, in floodplains, and flooded forests. They live in groups and swim in mid-depth. The genera of the *Serrasalmidae* are classified partly according to the morphology and arrangement of teeth: *Myleinae* have two rows of teeth on the premaxilla, one row of teeth on dentary and symphyseal teeth (Andrade *et al.*, 2019).

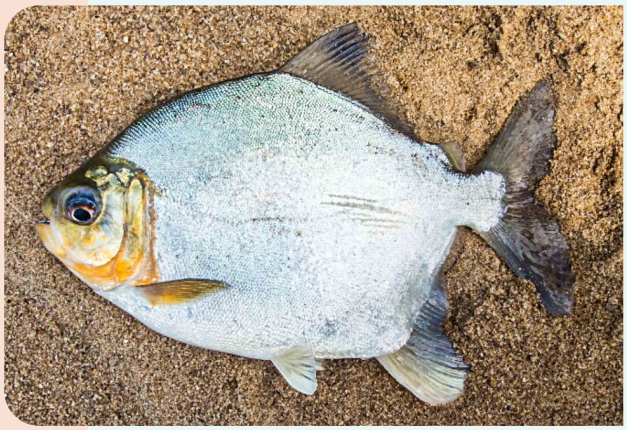
There are eight species of *Myleinae* in French Guiana: *Acnodon oligacanthus*, *Myloplus rubripinnis*, *M. tumukumak*, *M. ternetzi*, *M. rhomboidalis*, *M. planquettei*, *Tometes lebaili and T. trilobatus*. The morphological criterion which differentiates *Myloplus* from *Tometes* is the presence of a space between the two rows of teeth of the premaxillaria in *Myloplus* which does not exist in *Tometes*.



*Acnodon oligacanthus*, Tampoc, © P.-Y. Le Bail



*Myloplus planquettei*, Tampoc, @ M. Nevoux



*Myloplus rhomboidalis*, Approuague, © P.-Y. Le Bail



*Myloplus rubripinnis*, Tampoc, © P.-Y. Le Bail



*Myloplus ternetzi*, Sinnamary, © P.-Y. Le Bail



*Myloplus tumukumak*, Oyapock,© P.-Y. Le Bail



*Tometes lebaili, Marouini*, © P.-Y. Le Bail



*Tometes trilobatus*, Oyapock, © P.-Y. Le Bail

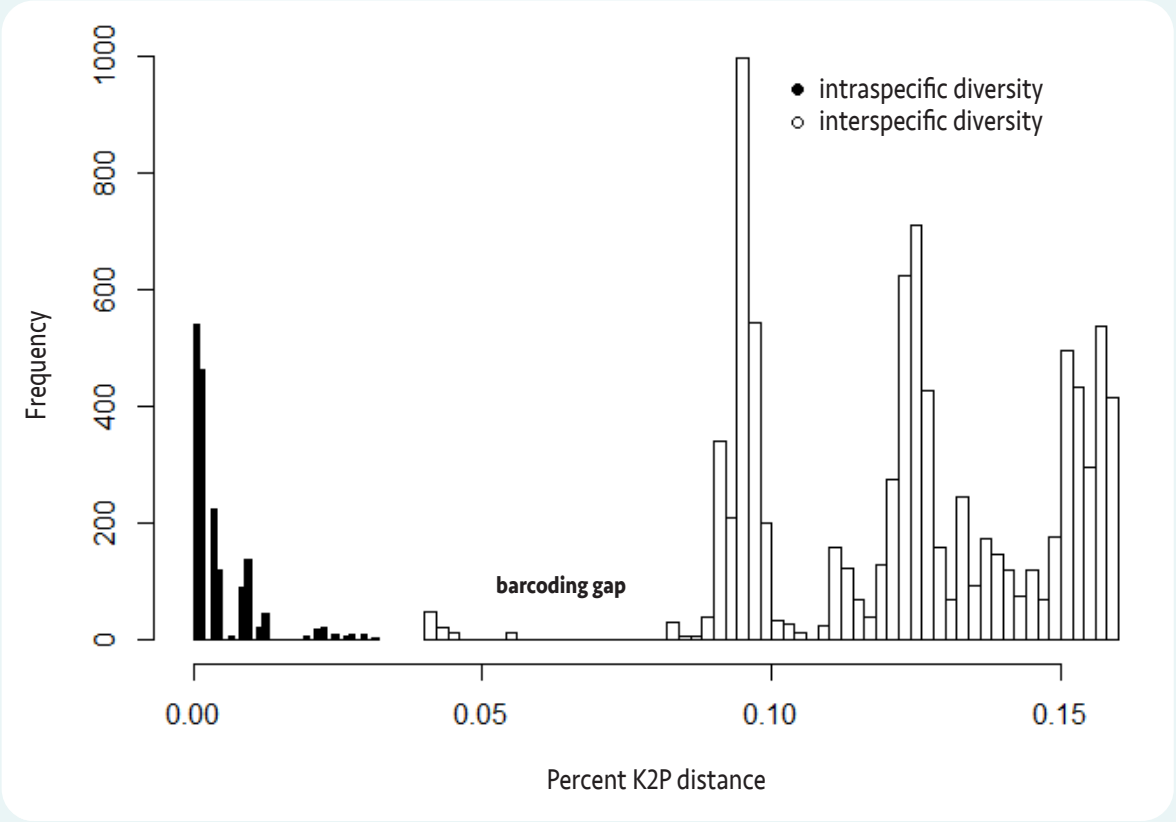



Fig. 7: **Intraspecific (black) and interspecific (white) diversity histograms of *Myloplus* and *Tometes* species in French Guiana. The x-axis represents K2P distances; y-axis represents the frequencies of all pairwise comparisons.**

The interspecific and intraspecific diversity represented in figure 7 by histograms are clearly separated from a barcoding gap comprised between 4 and 9%. Intraspecific distance is below 4% and interspecific distance varies between 8 and 16%. In the histogram, 5 entities clearly appear. These results prove that *Myloplus* and *Tometes* species are well distinct and morphologically distinguishable from one another.

However, the NJ barcode tree, computed using K2P distances between sequenced specimens of *Myloplus* and *Tometes* (figure 8), reveals that two species belonging to *Tometes* genus (*i.e. T. lebaili* and *T. trilobatus*) are genetically close to *Myloplus planquettei*, rendering both *Tometes* and *Myloplus* paraphyletic. Corroborating our results, Ota *et al.* (2020) also observed *Tometes* species nested among *Myloplus* species.

This implies that the morphological criteria used to distinguish the different genera of the Myleinae do not support their respective monophyly, and that a revision of these genera is necessary. Moreover, Kollmann *et al.* (2020) resurrected *Paramyloplus* and *Prosomyleus* to accommodate *Myloplus ternetzi* and *M. rhomboidalis* respectively, and transferred *Myloplus planquettei* to *Myleus*. Accordingly and following the results of Kollmann *et al.* (2020), *Myleinae* of French Guiana are *Acnodon oligacanthus*, *Myloplus rubripinnis*, *M. tumukumak*, *Paramyloplus ternetzi*, *Prosomyleus rhomboidalis*, *Myleus planquettei*, *Tometes lebaili* and *T. trilobatus*.

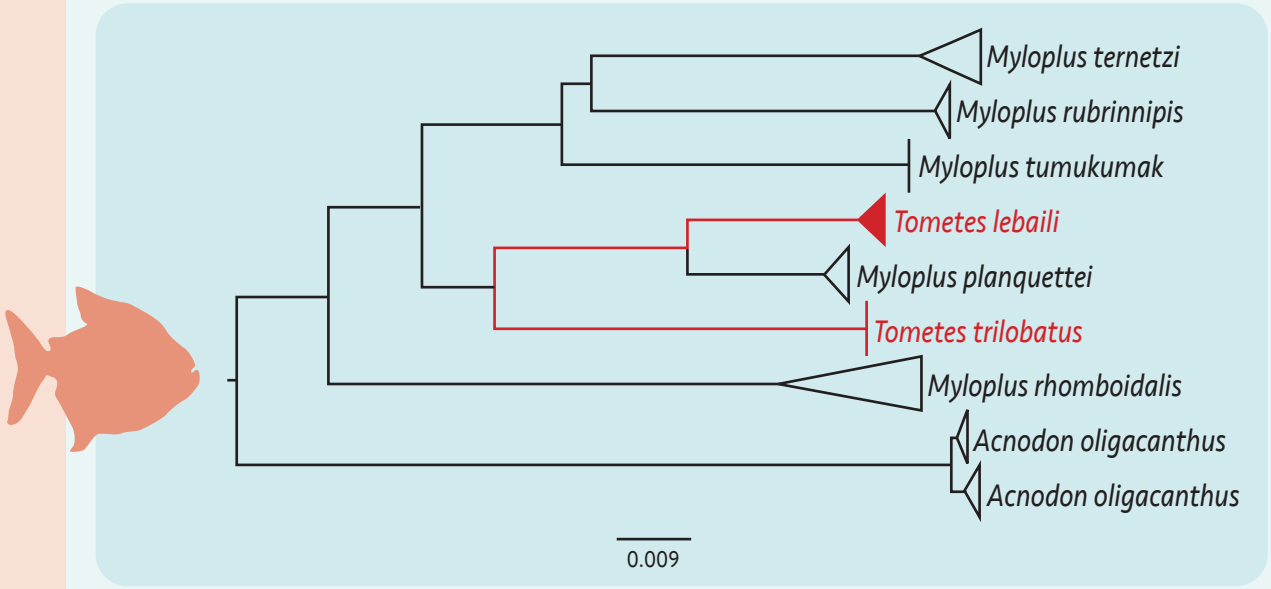



Fig. 8: **Genetic tree based on barcoding analysis. The tree was computed on pairwise K2P distances between COI sequences for the eight species of Myleinae of French Guiana. The scale represents the genetic divergence rate between species computed with K2P method. Triangles: height proportional to the number of sequences in each lineage; depth proportional to intraspecific distances.**

### • A cryptic diversity

*Pimelodella* is the most diverse genus of the Heptapteridae family which contains 24 genera (*e.g. Chasmocranus*, *Heptapterus*, *Imparfinis*, *Rhamdia*...). Eighty species were currently identified in South America (Slobodian and Pastana, 2017) and this genus is present in all Guianese basins. *Pimelodella* species are characterized by a moderated elongated body, which reaches 12 to 30 cm. Their color pattern is brownish tan to yellow with, in general, a dark lateral line from operculum to caudal-fin insertion. They have long dorsal and adipose fins and their caudal fin is strongly forked. These catfishes present three pairs of barbels: one pair of very long maxillary barbels and two pairs of mandibular barbels, shorter than the maxillary ones. These species live in calm areas, creek or slow flowing river, in school of up to ten or more individuals which swim close to the bottom. According to Le Bail *et al.* (2012), six species of *Pimelodella* are distributed in French Guiana: *P. geryi*, *P. leptosoma*, *P. cristata*, *P. megalops*, *P. macturki* and *P. procera*.

Excluding *Pimelodella macturki* which was not present in our sampling of French Guiana, the DNA barcoding gap analysis (figure 9) performed on all other species showed that some species displayed intraspecific K2P distances of up to 11% while other species had interspecific genetic distances of only 1.5%. The expected barcoding gap was not clear because of overlapping inter and intra specific variations, thus not conforming to the barcoding hypothesis. Indeed, we can define two barcoding gaps: one at 2% and a second between 6 and 8% of genetic K2P distances. This result demonstrates that there are two levels of intraspecific diversity in *Pimelodella* species of French Guiana which supports the cryptic diversity hypothesis. The interspecific diversity for *Pimelodella* species is clearly underestimated.



*Pimelodella* aff. *cristata*, Oyapock River, Lanou Creek, © F. Melki



*Pimelodella* cf. *cristata*, Ako Fall in Oyapock River, © F. Melki



*Pimelodella geryi*, Mitaraka Mountains, Maroni River, © F. Melki



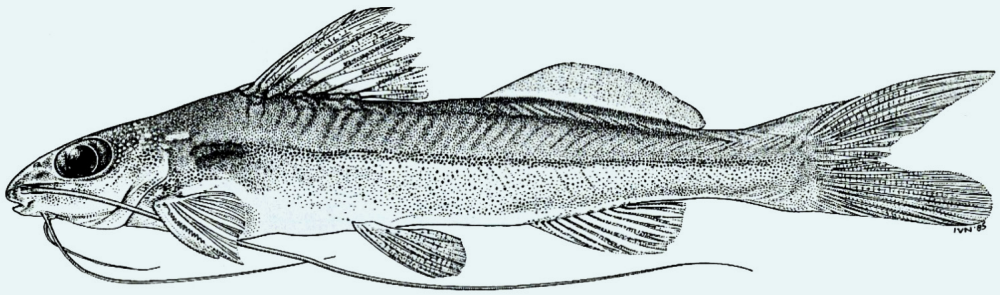
*Pimelodella mackturki*, Mana river, © P.-Y. Le Bail



*Pimelodella procera*, Mitaraka Mountains, Maroni River, © F. Melki



*Pimelodella leptosoma*, Waki Creek in Maroni, © F. Melki



*Pimelodella megalops*, Approuague (from Mees 1986)

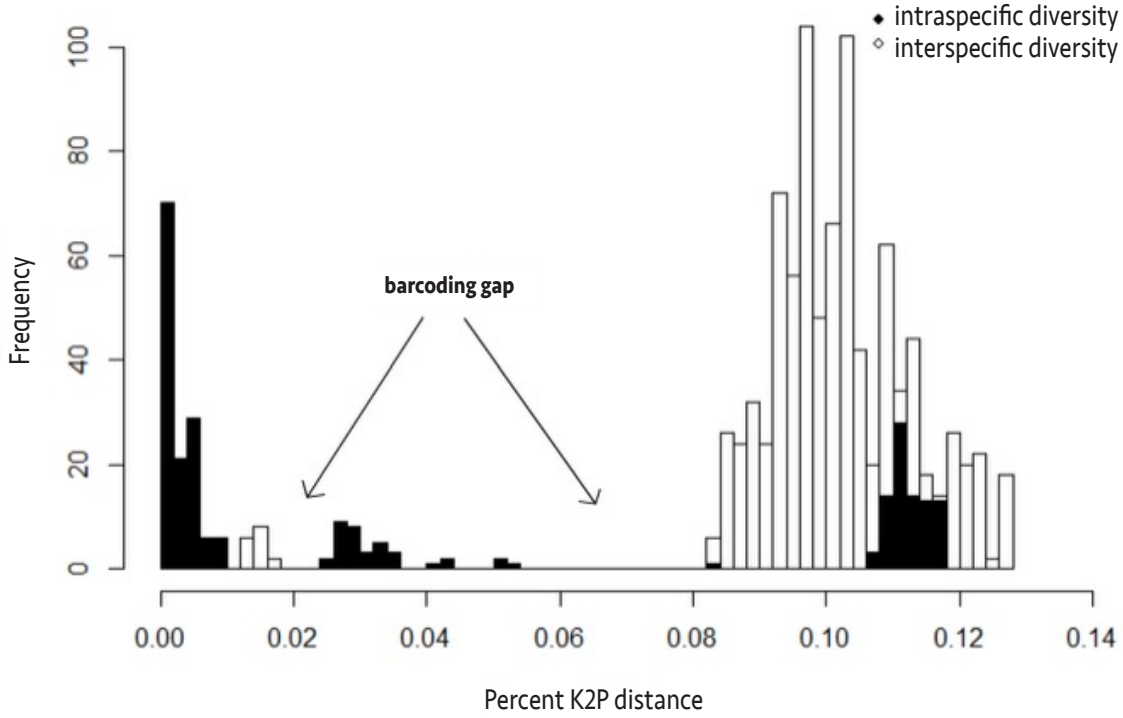



Fig. 9: **Intraspecific (black) and interspecific (white) diversity histograms of *Pimelodella* species in French Guiana. The x-axis represents K2P distances; y-axis represents the frequencies of all pairwise comparisons.**

Furthermore, the figure 10, computed using the K2P metrics between sequences of sampled specimen of *Pimelodella* species, showed that *P. cristata*, and *P. geryi* represented species complexes. For example, *P. cristata* appears three times in different clusters of the genetic tree, with an overlap between geographic distributions and distinct sampled specimen groups. The observed genetic distances between tree branches and the high percentage of intraspecific variation indicate that *P. cristata* is a species complex with cryptic diversity. The actual diversity of *Pimelodella* is underestimated. According to the present genetic analyses *P. cristata* contains at least three species. In a recent work, Papa *et al.* (2021) also attest for cryptic diversity among *P. cristata* and *P. geryi* in Maroni Basin.

Barcoding analyses have led to reevaluate the specific diversity ichthyofauna of French Guiana, especially for: *Cyphocharax spilurus, Gasteropelecus sternicla, Bryconops affinis, Bryconops melanurus, Hyphessobrycon roseus, Moenkhausia collettii, Bario oligolepis, Phenacogaster wayana, Poptella brevispina, Acestrorhynchus microlepis, Erythrinus erythrinus, Nannostomus bifasciatus, Helogenes marmoratus, Ituglanis amazonicus, Pimelodella cristata, P. geryi, Hypopomus artedi, Gymnotus anguillaris, Eigenmania virescens, Symbranchus marmoratus, Krobia* aff. *guianensis.*

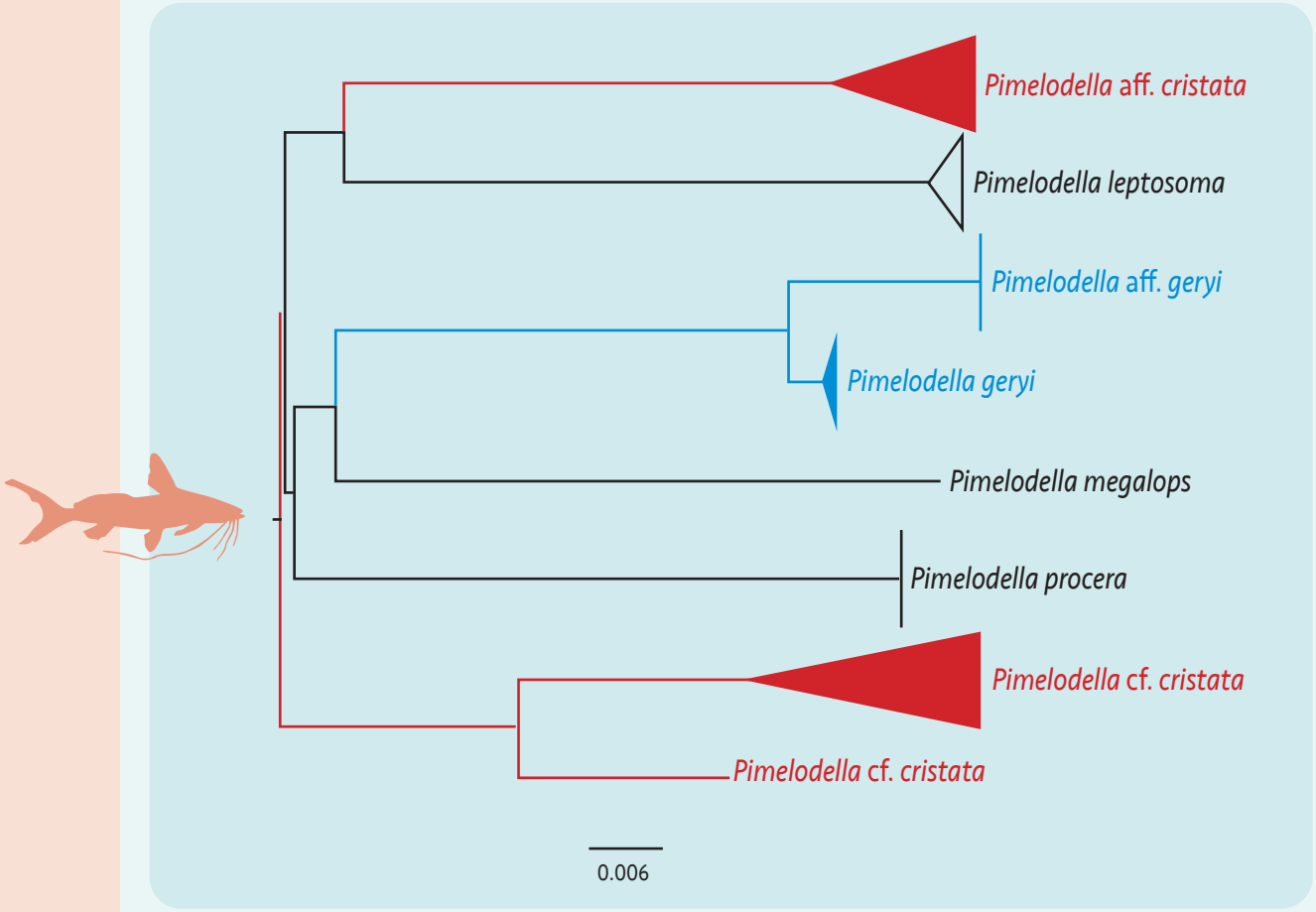



Fig. 10: **Genetic tree based on barcoding analysis. The tree was computed on pairwise K2P distances between COI sequences of the eight species of French Guiana. The scale represents the genetic divergence rate between species computed with K2P method. Triangles: height proportional to the number of sequences in each lineage; depth proportional to intraspecific distances.**

## BARCODING ANALYSIS LIMITS

The genus *Ancistrus* belongs to the Hypostominae subfamily of the Loricariidae or armored catfishes. This family is characterized by a body covered by bony plates (plates bearing odontodes), and above all by a suckermouth in ventral position able to scrape the substrate. Like *Hypostomus* members, *Ancistrus* species are present in all Guianese basins. *Ancistrus* are small species (between 12 and 15 cm) characterized by a stocky appearance and a large head. Eversible odontodes are characteristic, gathered in tuft, claw like, and forwardly oriented. Color pattern is variable in *Ancistrus* depending on species and ecotypes. *Ancistrus* specimens are generally tan to dark brown or black with spots, dots, marbling, or reticulations. Sexual dimorphism is often strongly pronounced with larger males bearing fleshy extensions giving them the name of bristle or bushy noses. *Ancistrus* species feed on the substrate, scraping algae, larvae and microorganisms.



*Ancistrus* aff. *hoplogenys*, Sinnamary River,
© F. Melki



*Ancistrus* aff. *temminckii*, Oyapock River,
© F. Melki



*Ancistrus* cf. *leucostictus*, Comté River,
© F. Melki



*Ancistrus temminckii*, Maroni River,
© F. Melki

In French Guiana six species of *Ancistrus* are morphologically identified: *Ancistrus* sp., *A.* cf. *leucostictus, A.* aff. *hoplogenys* (two forms), *A.* aff. *temminckii*, and *A. temminckii*. 138 specimens distributed in the eight basins (Maroni, Mana, Iracoubo, Sinnamary, Kourou, Comté-Orapu, Approuague, and Oyapock) have been sequenced.

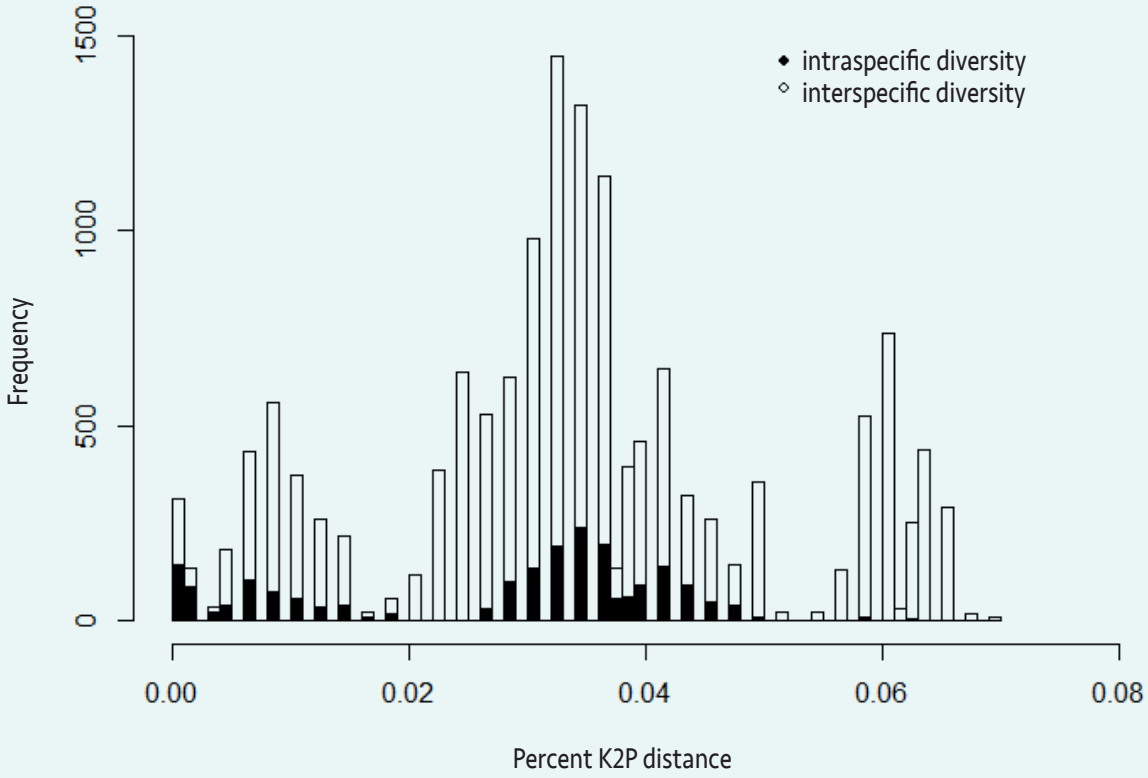



Fig. 11: **Intraspecific (black) and interspecific (white) diversity histograms of *Ancistrus* species in French Guiana. The x-axis represents K2P distances; y-axis represents the frequencies of all pairwise comparisons.**

The figure 11 represents the DNA barcodes gap analysis. Contrasting with previous examples, the black and white histograms are not separated by any barcoding gap. Intra- and interspecific histograms largely overlap implying that genetic boundaries in *Ancistrus* are not clearly established.

The figure 12 confirms the results of the DNA analysis. The NJ barcoding tree shows that *Ancistrus* species, with few exceptions, cannot be identified using DNA barcodes. Indeed, *Ancistrus* cf. *leucostictus* appears eight times in different lineages, *Ancistrus* aff. *hoplogenys* four times and *Ancistrus* aff. *temminckii* four times as well. The observed genetic distances between branches for these three species and the high percentage of intraspecific variation imply that either *Ancistrus* species are not correctly identified and that their actual diversity is greater than expected, or that these species hybridize regularly with important mitochondrial introgression in the different lineages.

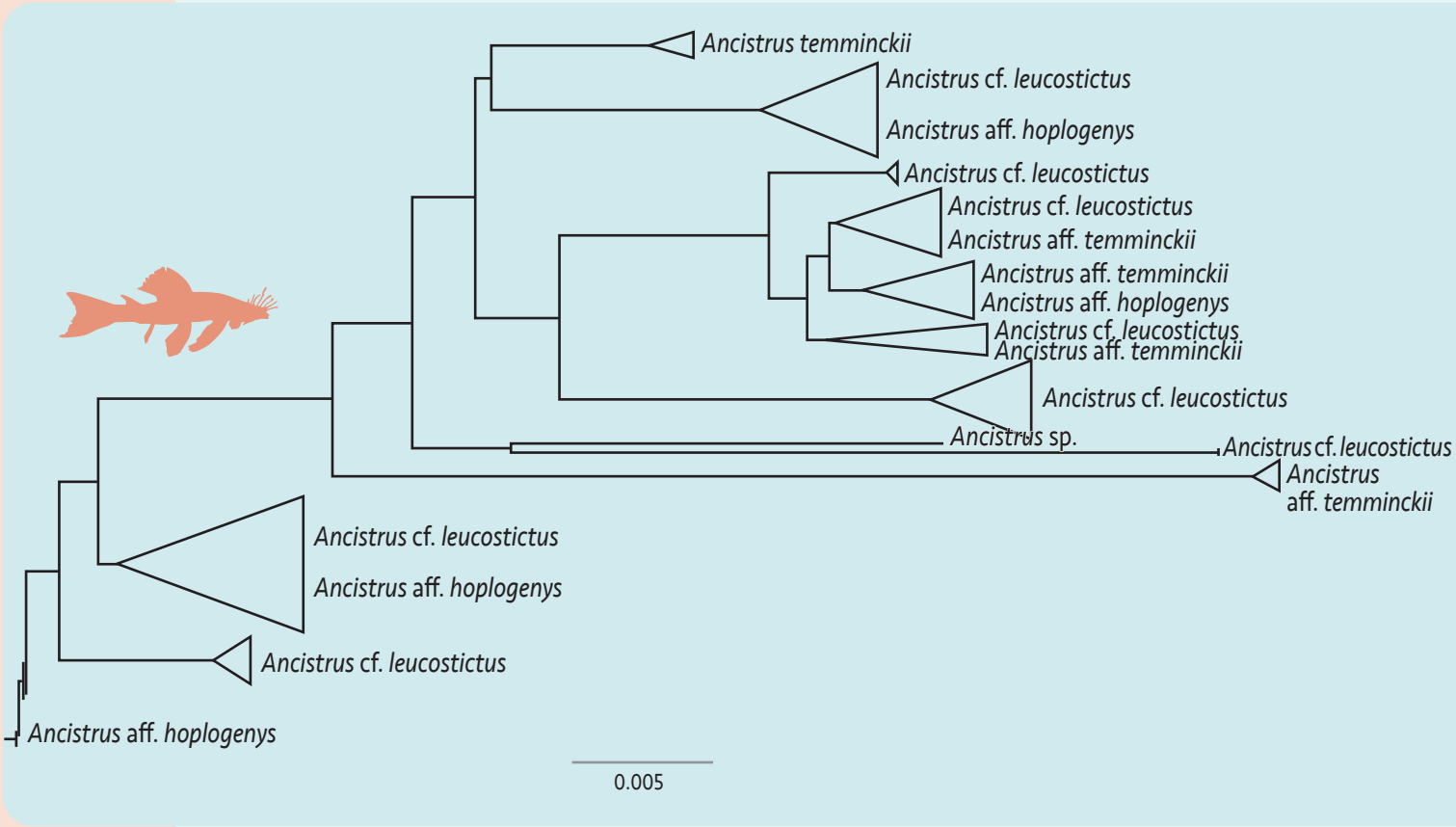



Fig. 12: **Genetic tree based on barcoding analysis. The tree was computed on pairwise K2P distances between COI sequences of the six species of French Guiana. The scale represents the genetic divergence rate between species computed with K2P method. Triangles: height proportional to the number of sequences in each lineage; depth proportional to intraspecific distances.**

The analysis of the genus *Ancistrus* demonstrates that the barcode approach works poorly with several possible identifications for a given morphological species. To correctly identify each species, alternative nuclear markers must be developed to circumvent the hybridization effect between the different species.

# CONCLUSION

The morphological approach is usually used to describe a species. Today, the improvements of new molecular technologies have allowed enhancing taxonomical descriptions. The DNA barcoding analyses permit to compare the morphological species and individual genetic variations and help to precisely determine the species boundaries to better grasp actual freshwater fish diversity. Since 1998, many sampling campaigns were conducted in French Guiana for freshwater fish sequencing. In 2017, the sampling recovery rate was around 79% for all French Guiana basins: Sinnamary, Mana, Maroni, Kourou, Approuague, Comté-Orapu, Iracoubo and Oyapock. Even though the sampling effort was satisfying, additional sampling campaign have to be conducted to 1) completely cover the French Guiana basins to sample all morphologically described species, 2) collect specimens of given species from different basins to possibly detect cryptic diversity. Moreover, *in situ* and *in vivo* photography must be systematically realized to associate coloration patterns of sampled species to specimens maintained in museum collection. The discovery of new freshwater Guianese fish species was confirmed using DNA barcoding analyses. However, these news species have to be described in order to provide the legal status necessary to take them into account in biodiversity protection management plans, and to correctly describe actual fish diversity.

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*Ancistrus* cf. *leucostictus* © Frédéric Melki