Diversity of the Ancistrini (Siluriformes: Loricariidae) from the Guianas: the *Panaque* group, a molecular appraisal with descriptions of new species

by

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ABSTRACT. - DNA barcoding represents a reliable and powerful way to discriminate and identify species using a standardized region of the mt COI gene. However, a correct identification requires two factors: differentiation and assignment. When one component is lacking, the barcode approach usually fails. To circumvent such problem, we developed a dual approach using a nuclear marker as complementary identifier. A first step consisted in characterizing the first intron of the F-RTN4 gene. This intron was found to be the longest, the most divergent and the most variable of the different introns constituting F-RTN4, making it a candidate of choice. This dual approach was applied to a group of closely related armoured catfishes constituting the *Panaque* group within the Guianas. Three groups were found: *Pseudacanthicus*, *Hemiancistrus*, and *Peckoltia-Panaqolus*, and four new species were highlighted. Within the latter group, *Panaqolus koko* n. sp. displayed a pattern of mitochondrial introgression with *Peckoltia otali* n. sp., while *Peckoltia capitulata* n. sp. and *Peckoltia simulata* n. sp. revealed cryptic species of *Peckoltia oligospila*. *Hemiancistrus* appeared significantly distinct from *Peckoltia*. Its type species is redescribed and a neotype is designated to clarify its taxonomic status considering the loss of the holotype.

RÉSUMÉ. - Diversité des Ancistrini (Siluriformes : Loricariidae) des Guyanes : le groupe *Panaque*, une évaluation moléculaire avec descriptions de nouvelles espèces.

Le code barre ADN représente un moyen fiable et puissant de discriminer et d'identifier les espèces en utilisant une région standardisée du gène mitochondrial COI. Une identification correcte requiert toutefois deux critères : différentiation et assignation. Lorsqu'une composante manque, l'approche code barre échoue fréquemment. Afin de circonvenir à un tel problème, nous avons développé une double approche faisant appel à un marqueur nucléaire en tant qu'identifiant complémentaire. Une première étape consista à caractériser le premier intron du gène F-RTN4. Cet intron s'est révélé le plus long, le plus divergent et le plus variable des différents introns constituant F-RTN4, en faisant un candidat de choix. Cette double approche a été appliquée à un groupe de poissons-chats cuirassés étroitement apparentés constituant le groupe *Panaque* dans les Guyanes. Trois groupes ont été trouvés : *Pseudacanthicus*, *Hemiancistrus* et *Peckoltia-Panaqolus*, et quatre espèces nouvelles ont été mises en évidence. Dans le dernier groupe, *Panaqolus koko* sp. n. montre un pattern d'introgression mitochondriale avec *Peckoltia otali* sp. n., alors que *Peckoltia capitulata* sp. n. et *Peckoltia simulata* sp. n. se révèlent espèces cryptiques de *Peckoltia oligospila*. *Hemiancistrus* apparaît significativement distinct de *Peckoltia*. Son espèce type est redécrite et, basé sur la perte de l'holotype, un néotype est désigné afin de clarifier son statut taxonomique.

Key words. - DNA barcode - COI gene - Intron - *Hemiancistrus* - *Peckoltia* - *Panaqolus* - Cryptic species.

Historical methods for identifying, naming and classifying fishes rely essentially on external morphology (Ward *et al*., 2009). Nevertheless, this approach has often proven its limitation, particularly in the detection of cryptic species (see Hillis *et al*., 1996; e.g., Emberton *et al*., 1995; Fisch-Muller *et al*., 2001). Modern techniques, including gene sequencing, appeared as complementary and relevant methods to reveal this hidden diversity (e.g., Hebert *et al*., 2004a; Miura *et al*., 2005; Ellis *et al*., 2006; Lara *et al*., 2010). In this context, the establishment of a standard DNA sequence devoted to the identification of species was a necessary prerequisite. This was the main goal of the Barcoding Of Life Initiative (BOLI) which established the use of a mitochondrial 648-bp 5' target region of the cytochrome *c* oxidase I (COI) gene (Hebert *et al*., 2003). The COI gene encodes part of a large enzymatic complex of the mitochondrial respiratory chain. The sequence, due to the degenerate nature of the genetic code, possesses high mutational rates in third and first posi-

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tions of codons, despite relative conservation in amino acids (Ward and Holmes, 2007). These high mutational rates therefore allow the rapid accumulation of mutations between sequences that forms the conceptual basis of the barcode system. The differences accumulated are expected to be low within species due to the constant transmission of mitochondria, and high among species due to the absence of mitochondrial exchanges. The COI barcode system has already been efficiently used in quantifying and qualifying fish diversity (Ward *et al*., 2005; Hubert *et al*., 2008; Ward *et al*., 2009; Valdez-Moreno *et al*., 2009; Lara *et al*., 2010), and successfully highlighted cryptic species (e.g., Ward *et al*., 2008a; Ward *et al*., 2008b; Lara *et al*., 2010). However, this method has not been without controversy, essentially because it relies on a mitochondrial gene. Particularly, doubts were voiced concerning the ability of the COI gene to discriminate recently radiated species (Moritz and Cicero, 2004; Hickerson *et al*., 2006). Another major concern with the use of a mitochondrial marker is the lack of sensitivity to detect hybridization and mitochondrial introgression (Ward *et al*., 2009). To circumvent this last issue, it is often recommended that comparisons be made with a nuclear marker to detect conflicting signals (Hebert *et al*., 2003; Ward and Holmes, 2007; Ward *et al*., 2009). Different proposals have been made mostly relying on the variable regions of the nuclear ribosomal genes (e.g., Sonnenberg *et al*., 2007; Raupach *et al*., 2010). Nevertheless, no widely accepted standard nuclear marker has presently been developed as a complementary barcode in animals. A possible explanation for this gap may rely on the different natures of both genomes. Moreover, it is well accepted that the coding sequence of nuclear genes evolve much more slowly than mitochondrial ones (Page and Holmes, 1998), what may lead to the absence of the necessary barcoding gap (Meyer and Pauley, 2005) allowing the discrimination of species. In this case, the use of non-coding regions with more relaxed evolutionary constraints such as introns may provide a solution.

The selection of appropriate introns as candidate markers for barcoding purposes can benefit from the following theoretical considerations. A recent investigation of the evolution of the exon-intron structure conducted by Zhu *et al*. (2009) revealed three main evolutionary patterns recovered in all eukaryotic genomes analysed. First, an ordinal reduction of length and divergence in both exon and intron; second, a covariation of GC content and divergence between exons and flanking introns; and three, a decrease of average exon or intron length, GC content and divergence with the increasing number of exons in a gene. Moreover, they noted a strong complicated correlation between the GC content and the length of the introns and exons. To explain these significant trends, the authors hypothesised that these patterns were caused by factors common to either exons or introns or to both (e.g., splicing elements). They noted that the monotonic reduction of length, GC content and divergence as the ordinal variation or as a function of the total number of introns or exons, may reveal the factors that shaped this pattern, since this ordinal trend may reflect a time-orderly evolution. Zhu *et al*. (2009) thus proposed the timely-ordered model for the evolution of the intron-exon structure. This model stipulates that if the number of introns or exons follows an increasing trend, then the first exon and intron are older than the next ones. These older introns had more time to be inserted by regulatory or transposable elements and became accordingly longer. Moreover, the inserted sequences in introns have generally a lower GC content; and the later occurring introns cut the coding sequences into shorter ones except for the first and last exons which are required by splicing-related factors; the subsequent recruited exons, have a higher possibility of coming from intron sequences and therefore have a lower GC content. The first intron of eukaryotic nuclear genes therefore appeared as a possible candidate for identification purpose as that region is supposed to have accumulated enough mutations through time compared with its flanking exons, or subsequent introns.

In the present study, we used a classical barcode approach to investigate species diversity in a group of closely related catfishes belonging to the Loricariidae. The family Loricariidae is the world's largest catfish family including 716 valid species (Ferraris, 2007), without considering the numerous species still awaiting for a formal description, neither the undiscovered nor cryptic ones [300 undescribed species estimated in Reis *et al*. (2003)]. Loricariids are mainly characterized by their body encased in rows of bony dermal plates, and by the possession of a ventral sucker mouth. They feed by scraping the substrate to eat algae, detritus, and invertebrates. Their highly specialized morphology makes the Loricariidae one of the best characterized family among Siluriformes, recognized as a natural group since the earliest classifications for the order (de Pinna, 1998). Their exceptional diversity, usually allied to parental care and to low fecundity, are conditions that were compared to those observed for the cichlid species flocks in the East African rift lakes (Schaefer and Stewart, 1993). Genera sharing the presence of hypertrophied and movable cheek odontodes were placed in the subfamily Ancistrinae Kner, 1853 (Isbrücker, 1980; Fisch-Muller, 2003). Based on a phylogenetic analysis of the Loricariidae using osteological characters, Armbruster (2004) considered the Ancistrinae as one of five tribes of the Hypostominae. The Ancistrini represent the most diversified tribe including about the third of all loricariid species distributed in 26 genera (Ferraris, 2007). It occurs through all main Neotropical drainages, from Panama to Chile on the Western side of the Andes, and to Argentina on the eastern side. The highest generic diversity is mainly represented by rheophilic species distributed in rivers flowing the Brazilian and Guiana Shields. The present work is restricted to a

recently defined group of the Ancistrini, the *Panaque* clade (Armbruster, 2008). In an updated osteological analysis Armbruster (2008) found three groups within the Ancistrini, one composed of a single undescribed taxon, the two others comprising numerous genera and named *Panaque* and *Ancistrus* clades. The *Panaque* clade included *Acanthicus*, *Baryancistrus*, *Hemiancistrus*, *Hypancistrus*, *Leporacanthicus*, *Megalancistrus*, *Panaque*, *Peckoltia*, *Pseudacanthicus*, *Spectracanthicus*, and an undescribed genus. In that analysis, which did not include the type species of *Hemiancistrus* (*H. medians*), corroborating previous studies, *Panaque* (including *Panaqolus*) was found most closely related to *Peckoltia* (Schaefer, 1986; Schaefer and Stewart, 1993; Armbruster, 2004) and to *Scobinancistrus* (Armbruster, 2004: 59). *Scobinancistrus* was also placed in synonymy of *Panaque* (Armbruster, 2004: Table I). The hypothesis of close relationship between *Pekoltia* and *Panaque* was however not supported by the analysis of 12S and 16S mitochondrial rRNA genes (Montoya-Burgos *et al*., 1998), and several studies provided evidence that the genus *Hemiancistrus* forms a polyphyletic assemblage (Montoya-Burgos *et al*., 2002; Armbruster, 2008) and is in need of a revision. In a recent checklist of the Siluriformes, Ferraris (2007) considered *Panaqolus* and *Scobinancistrus* as valid genera.

Within the Guianas (comprising French Guiana, Suriname and Guyana), nine species of the *Panaque* group, placed in four genera, were reported (Le Bail *et al*., 2000; Ferraris 2007; Vari *et al*., 2009): *Hemiancistrus medians* (Kner, 1854), type species of *Hemiancistrus*, described from a single specimen without statement of locality; a species found in the upper Maroni River that was assigned to *Panaque* cf. *dentex* (Günther, 1868) (now *Panaqolus*); *Peckotia braueri* (Eigenmann, 1912) known from the Amazonian Takutu and Branco River basins, and a species assigned to *Hemiancistrus* aff. *braueri* (now *Peckoltia*) found in the Maroni River basin, with a distinct form mentioned for the Oyapock River; *Peckoltia cavatica* Armbruster & Werneke, 2005 endemic to the Rupununi River in Guyana; and *Peckoltia sabaji* Armbruster, 2003 from Essequibo, Branco, Negro, and Orinoco rivers drainages; three *Pseudacanthicus* species, *P. fordii* (Günther, 1868), known from type material from Suriname, *P. serratus* (Valenciennes, 1840) from Suriname and French Guiana, and *P. leopardus* (Fowler, 1914) from the Rupununi River basin. Two additional Surinamese species that are essentially known from their respective holotypes were never, to our knowledge, collected again in Suriname. Described as *Chaetostomus megacephalus* by Günther (1868) and *C. macrops* by Lütken (1874), they were both placed in *Hemiancistrus* (Fisch-Muller, 2003; Ferraris, 2007) and in *Pseudancistrus* (Armbruster, 2004, Vari *et al*., 2009), a genus that is included in Armbruster's *Ancistrus* group. Eigenmann (1912) provided a complementary description of *H. megacephalus* from material collected in the Essequibo River basin. However, based on the examination of the holotype and one of the specimens identified by Eigenmann, *H. megacephalus sensu* Eigenmann may well prove to be distinct from the species. The assignation of species to genera such as *Hemiancistrus* and *Peckoltia* remains a problem. Both taxa are poorly defined despite a recent attempt to revise *Peckoltia* (Armbruster, 2008), and their taxonomic history has for long been intimately linked (Miranda Ribeiro, 1912; Isbrücker, 1980; Cardoso and Lucinda, 2003; Armbruster, 2003, 2004, 2008), species being regularly moved from one genus to the other. In this work, we followed the taxonomy provided by Ferraris (2007), except for *H. macrops* and *H. megacephalus* that still deserve further investigations.

Recent field work in the Guianas resulted in a representative sampling of the *Panaque* group for molecular analyses, including unidentified and tentatively identified forms. Based on a dual barcode evaluation to prevent species misassignment, the systematics of the Guianese representatives of the *Panaque* group are revised here, and the new taxa highlighted are described. The methods used in the present work are primarily addressed for discriminating and identifying species, and have only limited phylogenetic resolution (Moritz and Cicero, 2004).

MATERIAL AND METHODS

DNA barcodes

For an assessment of the diversity of the Guianese Ancistrini constituting the *Panaque* group, the standard COI barcode region was amplified. A total of 15 specimens (Tab. I) representing all available species and populations was submitted to molecular analyses. Among the fifteen, nine represented strictly Guianese lineages and two were downloaded from GenBank to provide comparative material for a correct assignment of the taxa. In addition, because of the close resemblance of the Oyapock form of *Peckoltia* aff. *braueri* with the lower Amazonian *Peckoltia oligospila* (Günther 1864), three specimens representing two populations of *P. oligospila* were added to the data set. Due to the confusing taxonomy of the group and the close relatedness of its representatives, a fragment of the Fish Reticulon-4 (F-RTN4) nuclear gene was also amplified to detect potential conflicting signals. Tissue samples were housed in MHNG and ANSP, and preserved in 80% ethanol and stored at -20°C. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the instructions of the manufacturer. The PCR amplifications were carried out using the Taq PCR Core Kit (Qiagen), using the Fish-F1 and Fish-R1 primers (Ward *et al*., 2005). The amplifications and sequencing processes were performed as in Covain *et al*. (2012) for the COI gene, and as in Chiachio *et al*. (2008) for the F-RTN4 gene.

Table I. - Taxa list, specimen and sequence data for the 15 Ancistrini of the *Panaque* group analyzed for COI and F-RTN4 genes. Institutional acronyms follow Fricke and Eschmeyer (2010). [1] according to the exporter. [2] specimen voucher ZSM 32728, no locality stated (Cramer *et al*., 2007).

Species	Catalog number	Field number	Locality
Hemiancistrus medians [H. med.]	MHNG 2664.078	GF00-084	French Guiana, Marouini River
Hemiancistrus medians [H. med.]	MHNG 2717.005	SU08-173	Surinam, Tapanahony River
Panaqolus changae [Pn. chan.]	ANSP 181097	P6218	Peru, aquarium trade, Itaya River[1]
Panaqolus sp. L204 [Pn. L204]	MHNG 2710.093	PE08-900	Peru, aquarium trade, San Alexandro River[1]
Panaqolus koko [Pn. Mar.]	MNHN 2011-0013	GF00-115	French Guiana, Marouini River
Peckoltia capitulata [Pc. Appr.]	MNHN 2011-0011	MUS 331	French Guiana, Approuague River
Peckoltia simulata [Pc. Oya.]	MHNG 2681.058	GF06-120	French Guiana, Oyapock River
Peckoltia simulata [Pc. Oya.]	MHNG 2681.058	GF06-119	French Guiana, Oyapock River
Peckoltia cavatica [Pc. cava.]	MHNG 2651.020	GY04-030	Guyana, Rupununi River
Peckoltia otali [Pc. Mar.]	ANSP 187118	SUR07-05	Surinam, Litani River
Peckoltia sabaji [Pc. saba.]	MHNG 2651.016	GY04-029	Guyana, Rupununi River
Peckoltia oligospila [Pc. olig.]	MHNG 2602.017	BR98-154	Brazil, Guamá River
Peckoltia oligospila [Pc. olig.]	MHNG 2602.017	BR98-155	Brazil, Guamá River
Peckoltia oligospila [Pc. olig.]	MHNG 2601.078	BR98-076	Brazil, Mãe do Rio River
Pseudacanthicus leopardus [Ps. leop.]	MHNG 2651.024	GY04-025	Guyana, Rupununi River

	COI					RTN4				
Species	GenBank No.	GC content	GC1	GC2	GC3	GenBank No.	GC content 1	Length 1	GC content 2	Length 2
Hemiancistrus medians [H. med.]	JF746998	0.46	0.55	0.43	0.39	JF747011	0.33	694	0.41	197
Hemiancistrus medians [H. med.]	JF746999	0.46	0.55	0.43	0.39	JF747012	0.33	694	0.41	197
Panaqolus changae [Pn. chan.]	EU359435	0.42	0.52	0.43	0.31	JF747023	0.32	694	0.41	196
Panaqolus sp. L204 [Pn. L204]	EU359436[2]	0.43	0.54	0.44	0.32	JF747024	0.32	694	0.41	196
Panaqolus koko [Pn. Mar.]	JF747003	0.42	0.53	0.43	0.30	JF747016	0.32	694	0.41	196
Peckoltia capitulata [Pc. Appr.]	JF747000	0.41	0.52	0.43	0.28	JF747013	0.32	694	0.4	196
Peckoltia simulata [Pc. Oya.]	JF747001	0.43	0.54	0.44	0.32	JF747014	0.32	694	0.41	196
Peckoltia simulata [Pc. Oya.]	JF747002	0.43	0.54	0.44	0.32	JF747015	0.31	694	0.41	196
Peckoltia cavatica [Pc. cava.]	JF747004	0.42	0.52	0.43	0.30	JF747017	0.32	694	0.41	190
Peckoltia otali [Pc. Mar.]	JF747005	0.41	0.52	0.43	0.27	JF747018	0.32	694	0.4	196
Peckoltia sabaji [Pc. saba.]	JF747006	0.42	0.53	0.43	0.29	JF747019	0.32	694	0.41	196
Peckoltia oligospila [Pc. olig.]	JF747007	0.42	0.53	0.43	0.31	JF747020	0.32	694	0.41	196
Peckoltia oligospila [Pc. olig.]	JF747008	0.42	0.53	0.43	0.31	JF747021	0.31	694	0.41	196
Peckoltia oligospila [Pc. olig.]	JF747009	0.42	0.53	0.43	0.31	JF747022	0.31	694	0.41	196
Pseudacanthicus leopardus [Ps. leop.]	JF746997	0.42	0.55	0.43	0.28	JF747010	0.33	694	0.41	196

Sequences were deposited in GenBank, and accession numbers are provided in table I.

The DNA sequences were edited and assembled using BioEdit 7.0.1 (Hall, 1999). Prior to the alignment, all sequences were confronted to GenBank database using the blastn 2.2.24 algorithm (Altschul *et al*., 1997) to confirm the identity of the amplified genes. Additionally, F-RTN4 fragments were queried against the genome of *Danio rerio* in Ensembl database (http://www.ensembl.org/index.html) to identify the ordinal position and intervals of the amplified introns of the F-RTN4 gene. The sequences were secondarily manually aligned since the coding COI gene aligned unambiguously in a single block, and very few indels were present in the F-RTN4 introns. The GC content and base composition were computed using the seqinr 2.0-9 package (Charif and Lobry, 2007) in R 2.10.1 (R Development Core Team, 2009), and usual tests for homogeneity of nucleotide frequencies and substitution saturation (Xia *et al*., 2003) were performed using Dambe 4.5.56 (Xia and Xie, 2001).

To evaluate the ability of the intronic regions of F-RTN4 to discriminate and assign the different species to the correct taxa, and accordingly confirm or detect conflicting

signals with COI barcodes, different types of analyses were performed. These analyses were also used to verify that the selected region fitted the timely-ordered model suggesting evolutionary constraints acting on this region. The length of each intron was measured in number of bases and submitted to the upper tail Wilcoxon signed-rank test to assess significant differences in length between introns according to their ordinal position. The alignments of introns were secondarily converted into distances matrices using the Kimura 2 Parameters (K2P) metrics (Kimura, 1980) as implemented in ape 2.5 (Paradis *et al.,* 2004; Paradis, 2006) in R, to evaluate sequence divergence, and submitted to the upper tail Wilcoxon signed-rank test to detect significant differences in variation between the different introns according to their ordinal position. The Spearman's rank correlation coefficient was also computed to assess the type of association recorded between GC contents and length of introns. Due to the low taxonomic level, too few or even no variation was observed in our data to compute this last statistic. To enlarge the range of variation of introns and allow the computation of the coefficient of correlation, all F-RTN4 sequences deposited in GenBank from previous studies were downloaded (Chiachio *et al*., 2008; Cardoso and Montoya-Burgos, 2009).

Subsequently, Shannon's information theoretic entropy (Shannon, 1948) was computed for both markers, COI and selected intron of F-RTN4, to measure the diversity of bases and hence bases' conservation in the alignments using the bio3d 1.0-6 package (Grant *et al*., 2006) in R. To detect potential conflicting phylogenetic signals, both alignments were submitted to the Incongruence Length Difference (ILD) test (Farris *et al*., 1994) as implemented in PAUP* 4.0b10 (Swofford, 1998), and after conversion of both alignments into distances matrices using the K2P metrics to the Mantel test (Mantel, 1967) using the ade4 1.4-14 package (Dray and Dufour, 2007) in R. The ILD test was conducted using a heuristic search with 100 replicates, TBR branch swapping, and random addition of taxa, and the Mantel test was performed using 9,999 random permutations of both matrices. The pattern of selection pressure acting on mt COI gene and the selected intron of F-RTN4 was assessed using a global estimation of $\omega = d_N/d_S$ for coding regions and ζ for non-coding regions to detect differences in the selective forces acting on silent *versus* replacement changes (Pybus and Shapiro, 2010). The parameter ζ (Wong and Nielsen, 2004) assuming that neutral (i.e., synonymous) nucleotide substitution rate is constant in both the coding and non-coding regions of the same gene, represents the nucleotide substitution rate in the non-coding region, normalized by the synonymous nucleotide substitution rate in the coding region. Therefore, the interpretation of ζ becomes identical to that of ω. The computation of ω was performed with HyPhy 2.0 (Kosakovsky Pond *et al*., 2005) following the methodology proposed by Kosakovsky Pond *et al*. (2009). The parameter ζ was estimated using a batch file developed for HyPhy 2.0 by O. Fedrigo (http://www.duke.edu/~ofedrigo/Olivier_Fedrigo/HyPhyScripts.html). Assuming the timely-ordered model, the computation of ζ for the selected intron of F-RTN4 was performed using synonymous changes of a flanking exon as neutral proxy. Both estimates require a topology, which was obtained from a different study currently in progress and is not presented here.

Finally Neighbour Joining (NJ) trees (Saitou and Nei, 1987) were reconstructed based on the K2P distances matrices to provide a cluster ordination of the species. The NJ algorithm has the advantage over other agglomerative partitioning methods to translate distances into branch lengths. To estimate robustness of the groupings, a nonparametric bootstrap analysis (Efron, 1979) was performed following Felsenstein's (1985) methodology using 9,999 pseudoreplicates. In addition, levelplot graphs allowing a graphical representation of both distance matrices were computed using the lattice 0.18-3 and colorRamps 2.3 packages (Sarkar, 2010; Keitt, 2009) in R.

Taxonomy

Material belonging to the new species described here is deposited in the Muséum national d'Histoire naturelle, Paris (MNHN), the Muséum d'histoire naturelle, Geneva (MHNG), and the Academy of Natural Sciences, Philadelphia (ANSP). Comparative material includes primary type-specimens of *Hemiancistrus macrops* (Lütken, 1874), *Hemiancistrus megacephalus* (Günther, 1868), *Peckoltia braueri* (Eigenmann, 19129, *Peckoltia oligospila* (Günther, 1864), *Panaqolus dentex* (Günther, 1868), *Pseudacanthicus leopardus* (Fowler, 1914) *Pseudacanthicus serratus* (Valenciennes, 1840) and *Pseudacanthicus spinosus* (Castelnau, 1855), and twelve specimens collected in the Maroni/Marowijne River basin that we assigned to *Hemiancistrus medians* (Kner, 1854) (see COMPARATIVE MATERIAL). Institutional acronyms follow Fricke and Eschmeyer (2010).

Measurements and counts indicated in descriptions are based on all specimens listed, except those less than 30 mm SL and one individual with end of caudal peduncle and fin missing (ANSP187118, estimated size 64.5 mm SL). Specimens were measured with a digital calliper to the nearest 0.1 mm. Measurements follow Fisch-Muller *et al*. (2001), and are expressed as percents of standard length (SL) except for subunits of the head, which are expressed as percents of head length (HL). Counts follow Schaefer and Stewart (1993), excluding the marginal caudal plates, and with the addition of the counts of plates along the dorsal-fin base, plates between the anal and the caudal fins, and hypertrophied cheek odontodes.

RESULTS

DNA barcodes analysis of Guianese Ancistrini, *Panaque* group

The obtained sequences reached a total length of 652 bp for the COI gene and 1,797 to 1,813 bp for the F-RTN4. Comparisons made against the GenBank database using blastn 2.2.24 produced high similarity scores ranging between 1,169 and 767 for the 100 first Blast Hits indicating homology between our sequences and the COI sequences deposited in the database. The E-value was null for all comparisons which indicated that the obtained scores were not due to chance. For F-RTN4 sequences, similarity scores ranged between 2,892 and 255 with E-values ranging between 0 and 7 x 10^{-64}. These results also indicated that the amplified segments were homologues of the F-RTN4 gene with high probability. Comparison made between our F-RTN4 fragments and the *Danio rerio* genome in Ensembl located the *D. rerio* homologue gene rtn4rl2a-001 on chromosome 1, region 37,951,786-37,976,785. The amplified fragments comprised partial exons 1 (positions 1 to 5) and 3 (pos. 1,173: 1,823), and complete introns 1 (pos. 6: 746) and 2 (pos. 974: 1,172), and exon 2 (pos. 747: 973) (Fig. 1).

The sequence alignment of the 15 COI barcodes reached a total length of 652 positions. No insertions, deletions, or stop codons were observed in any sequence. The base composition was: A = 0.251, T = 0.322, G = 0.172, and C = 0.255. The χ^2 test of heterogeneity of nucleotide frequencies among OTUs failed to reject the null hypothesis ($\chi^2 = 5.3$, p-value = 1) implying that the data set is not at base-composition equilibrium. A slight tendency toward AT enrichment was present in the data since the GC content per sequence (Tab. I) was always below 0.5 (mean = 0.43 ± 0.014). In first codon position (GC1) the GC content reached a mean value of 0.53 ± 0.01, *versus* 0.43 ± 0.004 in second position (GC2), and 0.31 ± 0.035 in third position (GC3). The maximum in GC content was thus observed in first position, with a mean value above 0.5, whereas a minimum was reached in third position with a significant enrichment in AT bases (0.69). The test on the Index of substitution saturation (Iss) resulted in Iss = 0.0869 significantly smaller than Iss.c assuming both a symmetrical (Iss.c_{sym} = 0.73) and an asymmetrical (Iss.c_{asym} = 0.5368) topology (p-value < 0.0001), implying little substitution saturation in the data.

The alignment of the first intron of F-RTN4 reached a total length of 694 bases. Insertions and deletions consisted in two deletions of one base, and one insertion of two bases in the sequence of *Pseudacanthicus leopardus*. The base composition was: A = 0.309, T = 0.371, G = 0.154, and C = 0.166. The χ^2 test of heterogeneity of nucleotide frequencies among OTUs failed to reject the null hypothesis ($\chi^2 = 2.38$, p-value = 1) implying that the first intron of F-RTN4 is not at base composition equilibrium. A significant trend toward AT enrichment was present in the data since the GC content per sequence (Tab. II) was always below 0.5 (mean = 0.32±0.007). The test on the Index of substitution saturation (Iss) resulted in Iss = 0.0482 significantly smaller than Iss.c assuming both a symmetrical (Iss.c_{sym} = 0.734) and an asymmetrical (Iss.c_{asym} = 0.5419) topology (p-value < 0.0001), implying little saturation in the data.

Comparisons between intron 1 and intron 2 of F-RTN4 (Tab. I) revealed significant difference in length between both introns, intron 1 being the longest (Wilcoxon test: V = 120, p-value = 0.0002), as well as significant differen-

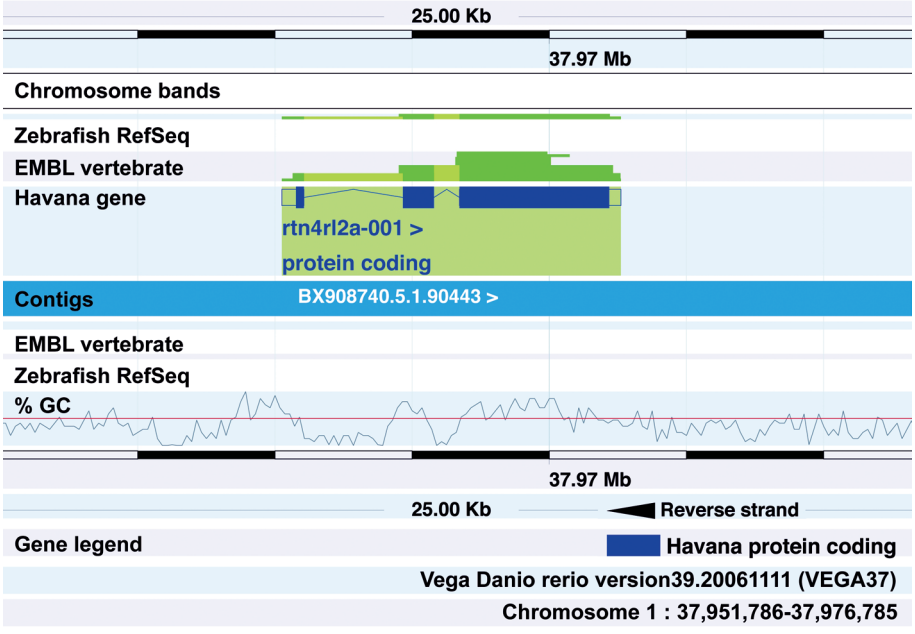

Figure 1. - Localization and main characteristics of the F-RTN4 gene homologue in *Danio rerio*.

Table II. - Morphometric and meristic data for the type series of *Peckoltia otali*, *Peckoltia capitulata*, *Peckoltia simulata* and *Panaqolus koko*. H: holotype. N: number of specimens measured. P: paratype. SD: standard deviation. Computed statistics include holotype.

	Peckoltia otali					*Peckoltia capitulata*				*Peckoltia simulata*					*Panaqolus koko*				
	H	N	Range	Mean	SD	H	P	Mean	SD	H	N	Range	Mean	SD	H	N	Range	Mean	SD
Standard length (mm)	76.5	23	39.7-76.5	62.1	8.4	75.9	59.5	68.4		83.4	3	80.4-83.4	82.3	1.6	90.1	6	62.7-90.1	77.6	11.1
Percents of standard length																			
Total length	133.1	20	130.2-140.4	133.9	3.0	–	–	–	–	136.8	2	132.4-136.8	134.6	3.1	132.1	6	126.2-132.4	130.9	2.4
Predorsal length	44.3	23	42.7-47.7	45.0	1.1	40.6	40.5	40.6	0.1	42.8	3	40.5-42.8	41.8	1.2	40.2	6	38.9-42.2	40.4	1.4
Head depth at supraoccipital	24.4	23	22.5-25.7	23.8	1.0	20.4	18.4	19.4	1.4	20.4	3	19.8-20.4	20.0	0.3	19.5	6	18.7-21.5	19.9	1.0
Cleithral width	35.2	23	33.8-37.1	35.2	0.9	30.5	30.2	30.4	0.2	30.7	3	29.5-30.7	30.2	0.6	31.5	6	30.6-32.1	31.4	0.5
Head length	36.9	23	35.4-40.1	37.2	1.2	33.6	33.4	33.5	0.1	34.4	3	33.7-34.4	34.0	0.4	33.8	6	32.9-35.1	33.9	0.7
Dorsal spine length	31.2	21	27.5-34.3	30.0	1.5	27.9	27.7	27.8	0.1	28.8	3	28.4-31.1	29.4	1.4	28.9	6	28.6-30.7	29.5	0.8
Dorsal–fin base length	26.8	23	25.1-28.2	26.6	0.9	25.8	26.0	25.9	0.1	26.4	3	26.1-26.4	26.3	0.1	28.7	6	27.6-29.0	28.4	0.6
Interdorsal distance	16.3	23	13.0-17.4	15.2	1.0	17.6	18.1	17.8	0.3	16.3	3	16.0-16.5	16.3	0.2	16.7	6	16.4-18.4	17.1	0.7
Pectoral spine length	33.3	23	30.0-34.3	32.2	1.2	30.7	30.6	30.6	0.1	31.2	3	29.4-32.2	30.9	1.4	29.8	6	29.8-31.3	30.3	0.5
Pelvic spine length	29.0	23	24.8-29.6	27.3	1.1	25.9	25.0	25.5	0.6	27.2	3	24.5-27.2	26.2	1.5	26.4	6	25.9-27.1	26.6	0.5
Thoracic length	22.4	23	22.1-26.2	23.9	1.0	21.5	24.3	22.9	2.0	22.8	3	20.4-22.8	21.4	1.3	23.7	6	21.6-26.7	23.7	1.6
Abdominal length	24.1	23	20.9-24.6	23.3	0.9	22.8	22.2	22.5	0.5	24.1	3	23.5-24.3	23.9	0.4	24.4	6	22.2-24.4	23.7	0.8
Caudal peduncle length	29.4	23	26.5-29.8	28.2	1.0	32.2	30.9	31.1	1.5	29.6	3	29.6-30.5	30.2	0.5	29.0	6	27.2-29.1	28.1	0.9
Caudal peduncle depth	12.9	23	12.4-13.8	13.1	0.4	11.3	11.7	11.5	0.3	10.2	3	10.2-10.5	10.3	0.1	13.1	6	12.0-13.4	12.9	0.5
Adipose spine length	11.1	23	8.4-12.1	9.8	0.8	10.5	10.6	10.5	0.9	10.4	3	9.0-11.8	10.4	1.4	7.3	6	5.9-7.5	6.9	0.6
Anal fin length	19.2	22	14.4-19.2	17.0	1.3	14.1	13.9	14.0	0.1	15.9	3	15.3-15.9	15.7	0.3	15.9	6	14.4-16.3	15.3	0.7
Upper caudal spine length	27.1	18	25.7-31.6	28.5	1.6	–	–	–	–	33.6	3	31.5-33.6	32.4	1.1	19.5	6	19.5-26.3	24.2	2.4
Lower caudal spine length	32.2	20	28.2-37.5	32.4	2.3	–	–	–	–	23.3	2	23.3-25.8	24.5	1.8	31.0	6	27.1-33.3	30.3	2.1
Body width at dorsal–fin origin	29.5	23	28.2-32.4	30.5	1.1	26.5	26.0	26.3	0.4	27.0	3	25.6-27.0	26.4	0.7	28.5	6	26.3-29.5	27.9	1.1
Percents of head length																			
Supracleithral width	79.8	23	76.1-84.4	80.0	2.4	77.5	80.4	78.9	2.1	74.2	3	71.1-74.3	73.2	1.8	77.2	6	75.5-80.7	77.6	1.8
Snout length	58.2	23	52.2-59.3	56.7	1.9	52.4	59.1	55.7	4.7	61.3	3	57.9-61.3	59.5	1.7	60.8	6	56.1-60.8	58.1	1.9
Interorbital width	32.3	23	29.8-34.4	32.6	1.2	34.5	34.4	34.5	0.1	33.8	3	33.8-37.1	35.5	1.7	33.2	6	29.4-33.2	31.6	1.5
Plated internostril distance	11.7	23	10.6-14.8	12.3	1.1	12.9	13.8	13.4	0.6	13.2	3	11.8-13.2	12.3	0.8	10.8	6	8.3-10.9	9.9	1.0
Orbital diameter	19.9	23	19.9-23.6	21.6	1.0	19.4	20.9	20.1	1.0	18.8	3	17.5-20.6	19.0	1.5	18.9	6	18.9-20.8	19.8	0.8
Dentary tooth cup length	13.8	23	11.0-14.6	13.3	0.8	11.4	12.1	11.7	0.5	10.1	3	10.1-11.0	10.6	0.5	10.3	6	9.5-11.2	10.3	0.6
Premaxillary tooth cup length	13.8	23	11.0-15.0	13.4	0.9	12.2	12.1	12.1	0.1	10.1	3	10.1-11.1	10.6	0.5	8.1	6	6.4-8.4	7.7	0.7
Interbranchial distance	53.6	23	48.2-56.4	53.1	2.2	51.0	51.8	51.4	0.6	51.2	3	49.6-52.2	51.1	1.3	51.6	6	51.2-54.6	52.9	1.4
Counts																			
Lateral plates	26	23	24-26	25	1	25	25	25	0	25	3	25-26	26	1	25	6	25-26	25	1
Plates along dorsal–fin base	8	23	7-8	8	1	8	8	8	0	8	3	8	8	0	8	6	8-9	8	1
Dorsal to adipose plates	6	23	5-7	6	1	6	6	6	0	5	3	5-6	6	1	7	6	6-7	7	1
Anal to caudal plates	12	23	11-12	12	1	11	12	12	1	9	3	8-9	8	1	8	6	7-8	7	1
Dentary teeth	–/23	20	17-28	25	3	14/14	–	–	–	19/20	3	19-29	25	6	4/5	7	4-6	5	1
Premaxillary teeth	24/24	22	17-33	25	4	15/17	–	–	–	17/16	3	17-22	20	3	4/4	8	4-6	5	1
Hypertrophied cheek odontodes	25/22	23	10-25	19	4	26/29	19/17	23	5	24/25	3	24-25	25	1	29/30	9	10-29	22	7

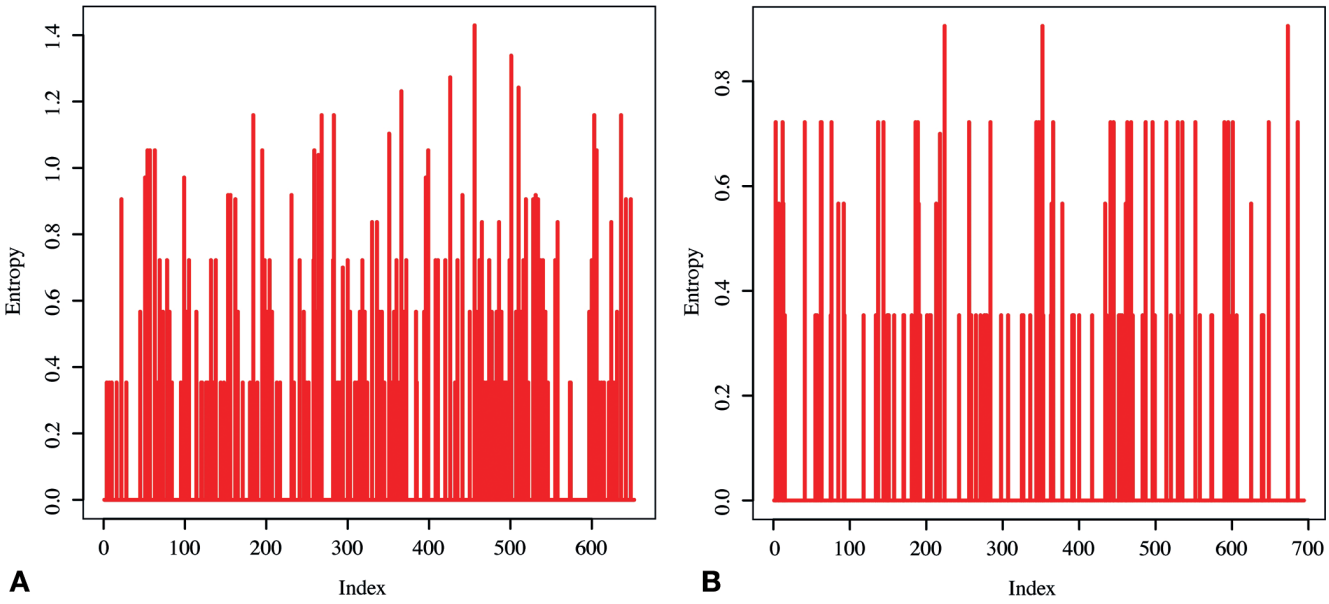

Figure 2. - Entropy plots of each position in alignments for 15 Ancistrini of the *Panaque* group. **A**: COI gene (652 bp). **B**: F-RTN4 intron 1 gene (694 bp).

ces in K2P divergences (V = 4216, p-value < 0.0001), intron 1 being the most divergent. A significant negative correlation between length and GC content was also recorded for both introns (ϱ_1 = -0.65, p-value < 0.0001 for intron 1 and ϱ_2 = -0.44, p-value < 0.0001 for intron 2). In addition, comparisons between intron 1 and the 3' flanking exon 2 revealed a significant positive correlation between their respective K2P divergences (ϱ = 0.75, p-value < 0.0001) but no significant correlation between their respective GC content (ϱ = 0.20, p-value = 0.4849). Since intron 1 showed patterns meeting the general patterns observed in the evolution of intronic regions, subsequent analyses were performed with this marker.

The pattern of base diversity provided by the Entropy plots (Fig. 2) of the COI gene and F-RTN4 intron 1 showed a regular pattern of substitutions all along both sequence alignments even though the intronic region displayed less variation. This pattern implies that the information was regularly distributed along sequences and is not restricted to a particular region of the alignment.

No conflicting phylogenetic signal was detected between COI and F-RTN4 intron 1 as the ILD test failed to reject the null hypothesis of congruence between data partitions (ILD: $p(X > X_{obs}) = 1$) and that K2P distances matrices were highly correlated (r = 0.97, p-value = 0.0001). In the COI alignment, the rate of synonymous substitution d_S was much higher than the rate of non-synonymous substitutions d_N leading to a very small value of ω = 0.0459 implying strong negative (= purifying) selection acting on this marker. For the first intron of F-RTN4, the parameter ζ computed using the 3' flanking exon 2 (length = 225 bp; ω = 0.388) as neutral proxy was very high (ζ = 4.79) implying positive selection acting on this marker. The likelihood ratio tests used in the Wong-Nielsen test confirmed this hypothesis in significantly rejecting the null hypothesis of neutral or negative selection (p-value = 0.0039).

The NJ tree reconstruction computed with the K2P distance matrix of COI sequences grouped the different species within three strongly supported clusters (100% bootstrap) corresponding to *Pseudacanthicus leopardus*, *Hemiancistrus medians*, and a mix of *Peckoltia-Panaqolus* representatives (Fig. 3A). The first diverging species corresponded to *P. leopardus* which possessed the deepest genetic divergences to other representatives of the *Panaque* group with K2P corrected distances ranging between 0.122 and 0.146. The second diverging group gathered the two barcoded populations of *H. medians*. The within species variation recorded was null between the specimen from Marouini River in French Guiana and the specimen from Tapanahony River in Suriname, whereas between species variation ranged from 0.094 to 0.119. The *Peckoltia-Panaqolus* group was split into two poorly supported groups (< 50% bootstrap), one comprising *Pn. changae* and *Pn.* sp. L204 in a sister position to *Pc.* sp. Approuague and the two specimens of *Pc.* aff. *braueri* Oyapock, and the second comprising the three specimens of *Pc. oligospila* in a sister position to *Pn.* cf. *dentex* plus *Pc. cavatica*, *Pc.* aff. *braueri* Maroni, and *Pc. sabaji*. Within the first group, *Pn. changae* formed the sister species of *Pn.* sp. L204 with low statistical support (64% bootstrap), the two species diverging by 0.038 K2P distances. The sister group of *Panaqolus* grouped the three species from Eastern French Guiana with only the two specimens of *Pc.*

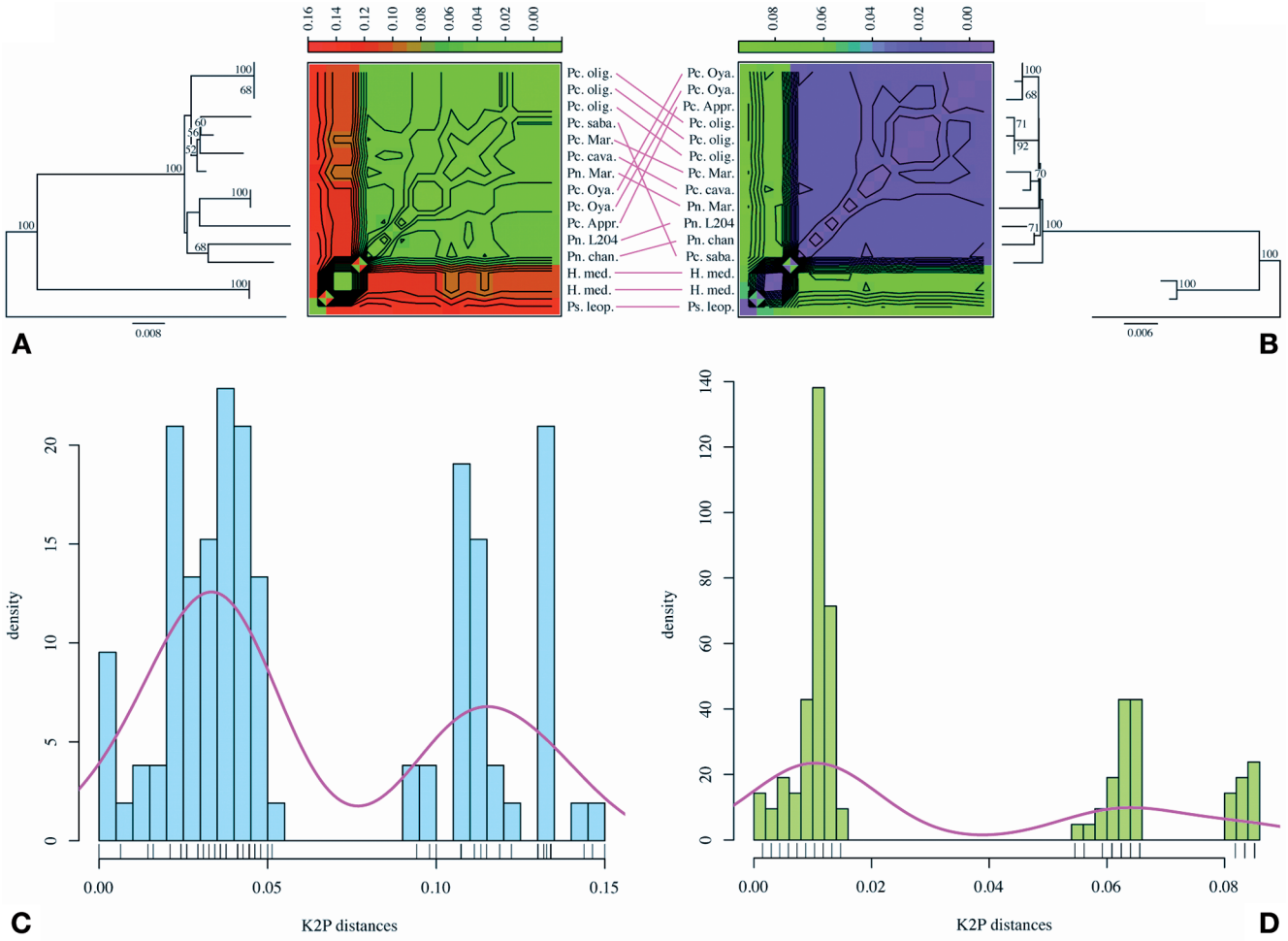

Figure 3. - Analysis of COI and F-RTN4 intron 1 genes of 15 Ancistrini of the *Panaque* group. **A:** NJ tree reconstructed from the K2P distances matrix computed on 652 bases of the mitochondrial COI gene facing the levelplot representation of the ordinated K2P matrix with scale indicating the levels of variation in K2P distances; numbers above branches indicate bootstrap support using 9,999 pseudoreplicates; scale indicates K2P distances; tips labelled as in table I. **B**: NJ tree reconstructed from the K2P distances matrix computed on 694 bases of the F-RTN4 gene intron 1 facing the levelplot representation of the ordinated K2P matrix with scale indicating the levels of variation in K2P distances; numbers above branches indicate bootstrap support using 9,999 pseudoreplicates; scale indicates K2P distances; tips labelled as in Table 1. **C**: Histogram of variation of the K2P distances matrix of COI gene using 105 pairwise comparisons; scale indicates the frequencies of pairwise comparisons in a definite range. **D**: Histogram of variation of the K2P distances matrix of F-RTN4 intron 1 gene using 105 pairwise comparisons; scale indicates the frequencies of pairwise comparisons in a definite range.

aff. *braueri* Oyapock displaying significant support (100% bootstrap). The K2P divergence between the two specimens from Oyapock was null whereas a divergence of 0.036 was recorded between *Pc*. aff. *braueri* Oyapock and *Pc*. sp. Approuague implying divergence of between species level. The divergence between *Panaqolus* representatives and their sister *Peckoltia* species ranged between 0.044 and 0.051. Within the second group, the only strongly supported grouping comprised the different populations of *Pc*. *oligospila* from the Capim River drainage (100% bootstrap). The within species variation recorded was null whereas divergence to other sister species ranged between 0.029 and 0.031. The first diverging species in the sister group of *Pc*. *oligospila* was *Pn*. cf. *dentex* in a position weakly supported (52% bootstrap). *Panaqolus* cf. *dentex* displayed small divergence with its sister species with K2P distances ranging between 0.006 (*Pc*. aff. *braueri*) and 0.016 (*Pc*. *sabaji*). The sister group of *Pn*. cf. *dentex* was also poorly supported (56% bootstrap) and recovered *Pc*. *cavatica* in a sister position to *Pc*. aff. *braueri* and *Pc*. *sabaji*, this last grouping being also weakly supported (60% bootstrap). *Peckoltia cavatica* diverged from *Pc*. aff. *braueri* by a K2P distance of 0.014 and from *Pc*. *sabaji* by a distance of 0.024. These latter diverged by K2P distances of 0.016.

Due to the lack of resolution and the poor generic assignment obtained within the *Peckoltia-Panaqolus* group using the COI K2P matrix (i.e., mixing of species belonging to different genera), a new NJ tree ordination was computed using

the K2P matrix of the F-RTN4 gene intron 1 (Fig. 3B). The topological results were highly congruent with the previous analysis and the three highly supported main clusters (100% bootstrap) corresponding to *P. leopardus*, *H. medians* and *Peckoltia-Panaqolus* were recovered. Within the *Peckoltia-Panaqolus* group, deeper relationships were not supported (50% < bootstrap). *Peckoltia sabaji* was the first diverging species and connected at base of the group. The second diverging group comprised *Pn. changae* and *Pn.* sp. L204. The sister relationship between these two species was moderately supported (71% bootstrap). The third diverging species was *Pn.* cf. *dentex* in a sister position to all remaining *Peckoltia*. The last group of *Peckoltia* was split into two groups, one strongly supported (100% bootstrap) comprising *Pc.* aff. *braueri* Oyapock and *Pc.* sp. Approuague, and a second comprising the remaining species. Within this last group, the three specimens of *Pc. oligospila* were highly supported (92% bootstrap) and formed the sister group of *Pc.* aff. *braueri* Maroni and *Pc. cavatica*. The sister relationship between the two latter species was well supported (70% bootstrap).

Using these two species ordinations, both matrices were reordinated and levelplots reconstructed (Fig. 3A). Even though three levels of variation were present in the COI matrix corresponding to within species (between populations), between species, and between genera levels, the pairwise distances followed a bimodal distribution (Fig. 3C). The within-species level (light green) displayed indeed no variation (K2P = 0). Following the distribution of pairwise distances, the between species level (green to khaki) ranged from 0.006 to 0.051 (mean = 0.033 ± 0.01), and the between genera level (red) from 0.094 to 0.146 (mean = 0.117 ± 0.013). Assuming the current taxonomy, the between species range of variation became 0.014 to 0.04 (mean = 0.031 ± 0.008), and the between genera 0.006 to 0.146 (mean = 0.083 ± 0.043). The smallest between genera K2P distance was recorded between *Pn.* cf. *dentex* and *Pc.* aff. *braueri* (d_{K2P} = 0.006). The mitochondrial signature of *Pn.* cf. *dentex* was thus very similar to that of *Pc.* aff. *braueri*, and smaller divergences between *Pn.* cf. *dentex* and other *Peckoltia* representatives were indeed observed (0.014 to 0.032) compared to divergences observed between *Panaqolus* and *Peckoltia* (0.029 to 0.051). Comparison made to the levelplot representing the F-RTN4 intron 1 K2P matrix (Fig. 3B), revealed three levels of variation corresponding to within species, between species, and between genera levels. The global rate of variation of F-RTN4 intron 1 was half of that of COI. The within species level (pink) ranged from 0.0014 to 0.004 (mean = 0.0024 ± 0.0011), the between species level (purple) from 0.0043 to 0.014 (mean = 0.014 ± 0.0022), and the between genera level (green) from 0.0546 to 0.0851 (mean = 0.069 ± 0.01). Two maxima were observed within the between genera level (Fig. 3D), one located at 0.062 ± 0.003, and a second at 0.084 ± 0.001. Using F-RTN4 intron 1, *Pn.* cf. *dentex* displayed variations to *Peckoltia* representatives (range between 0.0103 and 0.0132) comparable to that observed with the two other species of *Panaqolus* (d_{K2P} = 0.0147) whereas within *Peckoltia* variations ranged between 0.003 and 0.0118.

Taxonomic implications

Based on these results, *Hemiancistrus* is valid, but only represented by the type species *H. medians* within the Guianas. Species here placed in *Peckoltia* but considered in *Hemiancistrus* either previously (*P. braueri*) or presently by some authors (*P. sabaji*) do not cluster with *H. medians* but belong to the *Peckoltia-Panaqolus* group. With a COI K2P distance of 11% between *Hemiancistrus* and the *Peckoltia-Panaqolus* group (*versus* an intra-group K2P distance ranging from 0 to 3.8%), *Hemiancistrus* clearly appears very divergent from both *Peckoltia* and *Panaqolus*. It has a similarly high degree of divergence with *Pseudacanthicus* (d_{K2P} = 0.13). The identity of *H. medians* is clarified below accordingly, and the species is redescribed. The twelve populations included in the *Peckoltia-Panaqolus* group represent nine distinct species according to the genetic and morphological divergences. Four new species are recognized for the Guianas, three *Peckoltia* and one species that we assigned to *Panaqolus* for the time being, and are described here.

Identity of *Hemiancistrus medians*

Hemiancistrus medians is the type species of *Hemiancistrus* as originally designated by Bleeker (1862:2). The name of *Ancistrus medians* was made available by Kner (1854: 256; 6 of separate) with an unusual diagnosis placed in the general introduction of his main group named "Loricaten" or "Goniodonten". Kner mentioned that the royal Museum from Stuttgart possessed a wrongly named *barbatus* hypostomid, that was absent from "Hof-Naturalien-Cabinet" (Vienna) before proceeding with the description of this specimen (holotype). Although the description by Kner did not mention its origin, the historical catalogue of the Museum of Stuttgart's collection shows that the only material available to Kner was a specimen registered under the number SMNS 186. Confirming its typical status, it was first registered as *Hypostoma barbatum* Cuv., as mentioned by Kner. This identification was then changed to *Chaetostomus medians* Kner, thus probably only later than the complementary description of the species by Günther (1864: 242) who placed it in the genus *Chaetostomus* [=*Chaetostoma*]. The catalogue indicates that it is one dried specimen, locality "Surinam", collected by "Kappler", and received Feb. 1849. More ancistrine specimens were obtained later from the same collector, including: two alcohol specimens registered as *Chaetostoma medians* Kner (SMNS 791; received 1860), and one dried

specimen originally identified as *Chaetostomus serratus* (SMNS 1729; received 1870). August Kappler was a German researcher and entrepreneur in Suriname. He founded the settlement of Albina on the Marowijne (Surinamese) or Maroni (French) River, where he lived for several years, and according to our knowledge he collected his materials in the vicinity of Albina (R. Fricke, pers. comm.).

The holotype SMNS 186 was searched for, without success, in 1991 by Ronald Fricke, Curator of Fishes in the Staatliches Museum für Naturkunde, who concluded that it had to be considered lost (see Isbrücker, 1992). In the same publication, Isbrücker invalidly restricted the type-locality on the base of the specimens SMNS 791 ("Rivière Marouini", Maroni system, French Guiana, mentioning that the area was Surinamese during Kappler's time and not French). He provided illustrations based on more recently collected specimens. His view of *H. medians* is the same as that of previous authors, in particular Günther (1864) and Regan (1904) who provided complementary descriptions of the species based on two specimens also collected in Suriname by Kappler, but sent to the British Museum.

Recently, Ronald Fricke found a dried specimen with label indicating SMNS 186, *Pseudacanthicus serratus*, 1 ex, Surinam, Kappler (type-written) and also "Holotype of *Chaetostomus medians* Kner, 1854" (hand-written). It has the inventory number 186 written in ink on its lower side. The specimen, considered as putative holotype, was photographed by N. Khardina and is illustrated on the All Catfish Species Inventory Image Base (Morris, Yager and Sabaj Pérez, 2006; images available at http://acsi.acnatsci.org/base/image_show_wrapper.html?target=589063, accessed on 2 Feb. 2011).

Because the typical status of the specimen labelled SMNS 186 has only recently been claimed and, if confirmed, renders the identity of the species different from current usage, and because the original description is crucial but in German language from the mid-nineteenth century, we repeat it here followed by an English translation. It is described in these terms: "Er ist ein *Ancistrus* von gedrungener Gestalt mit wenig strahliger Rückenflosse, gekielten und grobzähnigen Rumpfschildern, einem Bündel sehr langer Haken von Form wie bei *Anc. mystacinus m.* und den folgenden Arten, mit kurzem Kopfe, breiter Schnauze, grossen Augen, sehr langen, bis hinter die Anale reichenden Bauchflossen und sehr stachliger Pectorale; Rumpf und Flossen sind mit grossen, dunklen Flecken besetzt, die Bauchseite ist dicht und klein beschildert. Schon das letzte Merkmal allein unterscheidet ihn als eine von allen mir bekannten verschiedene Art, indem ich keinen *brachypteren Ancistrus* mit beschildertem Bauche kenne, welcher dagegen allen *macropteren Lictoren* eigen ist. Da somit diese Art das vermittelnde Glied zwischen beiden Gruppen darstellt, so dürfte die Benennung *Anc. medians* vielleicht nicht unpassend erscheinen." A literal translation of this description is: "It is an *Ancistrus* of stocky stature with dorsal fin having few rays [Kner's *Brachypteri* subgroup], keeled and rough-toothed trunk plates, a tuft of very long hooks whose form is like in *Anc. mystacinus m.* and the following species [*A. pictus*, *A. brachyurus*, and *A. scaphirhynchus*, species at present ranged in *Lasiancistrus* and in *Dekeyseria*], with a short head, broad snout, large eyes, very long pelvic fins, which reach behind the anal, and a very pointed pectoral; trunk and fins covered by large dark spots, the ventral side is densely and finely plated. The last character alone already distinguishes it as a different species from all the ones I know, because I do not know any *brachypteren Ancistrus* [defined by Kner as having dorsal fin with few rays, meaning 7 to 9 considering the species included in this sub-group, and belly usually naked] with a plated belly, which on the other hand is particular for all *macropteren Lictoren* [defined by Kner as having dorsal fin with more rays, meaning 11 to 13 considering the species included in this sub-group, and belly constantly plated]. As this species therefore represents an intermediate link between the two groups, perhaps the name *Anc. medians* doesn't seem inappropriate". On page 281, the author briefly placed *Ancistrus medians* according to his systematic position, between *Brachypteri* and *Macropteri Ancistrus*, together with another species that he listed as *Hyp.* (*Anc.*) *itacua*, based on ZMB specimens that would later become the type material of *Hemiancistrus braueri* Eigenmann, 1912, now included in *Peckoltia*.

The specimen SMNS 186 indicated as putative holotype of *H. medians* is a representative of a species of *Pseudacanthicus*. Based on Kner's original description of species, there are several reasons to reject it as the holotype of *H. medians*. It does not agree to the description in the following characters: stocky structure, or stout body (SMNS 186 not much elevated, and elongated head and body); rough-toothed trunk plates (plates with particularly long and strong spines); a tuft of very long hooks (jugal hooks not very long); broad snout (elongated and more or less pointed, as in all *Pseudacanthicus*); trunk and fins covered by large dark spots (specimen at present uniformly coloured; one cannot exclude that it was spotted at time of description, however both Surinamese species *Pseudacanthicus serratus* (Valenciennes, 1840) and *P. fordii* (Günther, 1868) are white spotted, and no other known species of *Pseudacanthicus* has large dark spots on the trunk and fins; small dark spots are present in the Amazonian *P. spinosus* (Castelnau, 1855), and irregular dark spots and vermiculations in *P. leopardus* (Fowler, 1914) from the Rupununi River in Guyana); ventral side densely and finely plated (specimen has no more abdominal skin at all, showing skeleton; *Pseudacanthicus* species generally have no plates on the abdomen; very small plates with odontodes are sometimes present, but restricted to some areas, and generally widely separated from one another; often only odontodes

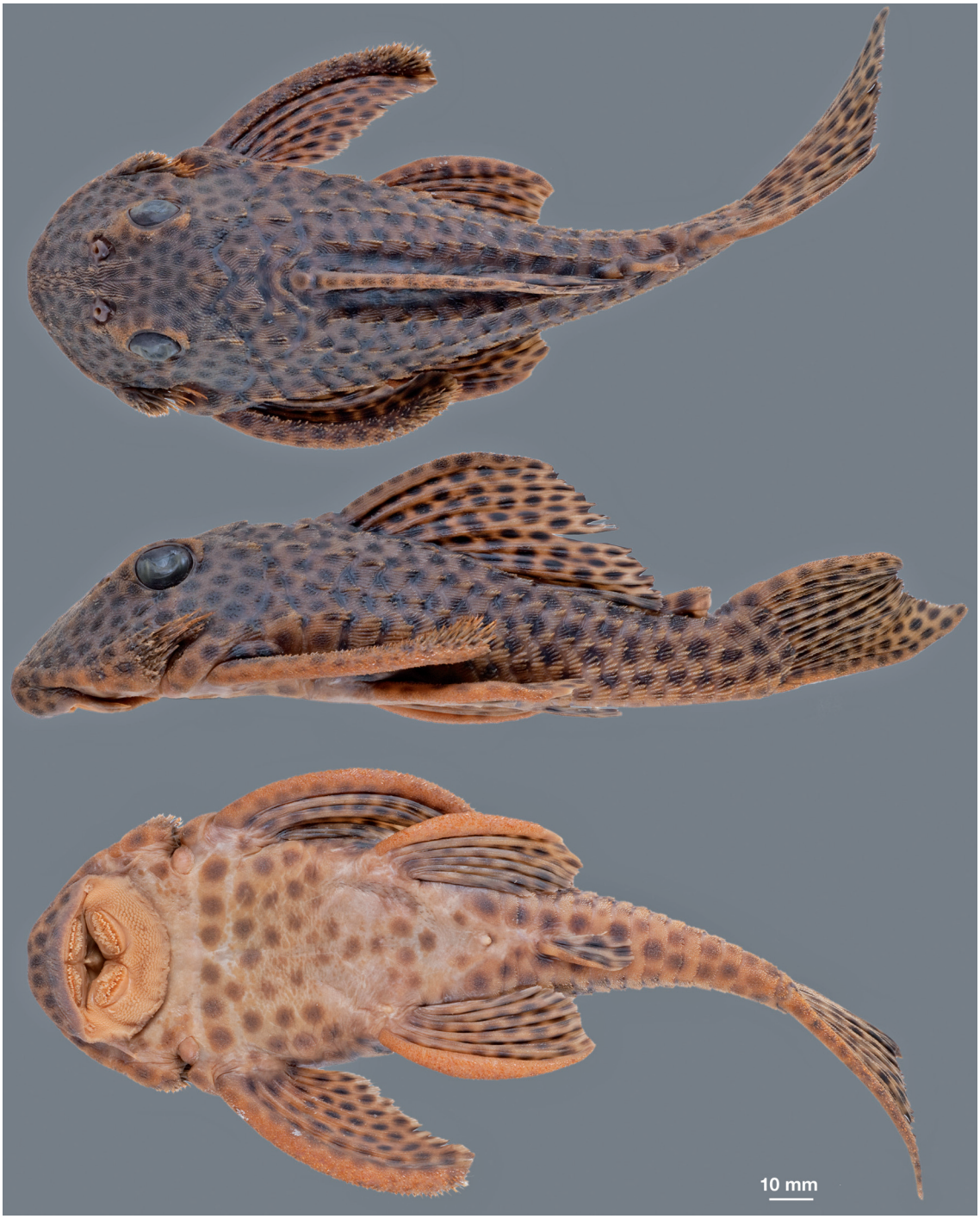

Figure 4. - Neotype of *Ancistrus medians* Kner, 1854, SMNS 26503 (ex-MHNG 2675.094), 164.1 mm SL.

are visible). In addition, as just mentioned, the specimen has no skin on the abdomen. It appears very unlikely that skin was removed from a dried specimen subsequent to Kner's description, especially for a holotype. We conclude that the specimen is not the holotype, and that it is not SMNS 186. It was very likely labelled as such subsequently, having been confused with SMNS 1729: one dried specimen received from Kappler in 1870 and originally registered as *Chaetostomus serratus*, now *Pseudacanthicus*. The SMNS does not claim to have another specimen listed as number 1729 in their collection.

Considering the previous efforts by Isbrücker and Fricke

Figure 5. - Juvenile specimens of **A**: *Hemiancistrus medians*, MNHN 2002-0854, 28.8 mm SL; and **B**: *Peckotia otali* n. sp., MNHN 1988-1851, paratype, 26.5 mm SL.

to find the type specimen, combined with the observation that the recently discovered putative type was incorrect, we believe that the holotype of *Hemiancistrus medians* is really missing in SMNS collection.

Hemiancistrus medians as recognized until recently, and redescribed and illustrated by previous authors (Günther, 1864; Regan, 1904; Isbrücker, 1992) agrees with Kner's original description of the species. In order to clarify the taxonomic status and fix the type locality of *Hemiancistrus medians*, and in order to preserve the stability of nomenclature, the designation of a neotype is needed. We thus designate here the following specimen as the neotype of *Ancistrus medians* Kner, 1854: SMNS 26503 (ex MHNG 2675.094), 164.1 mm SL mm SL, French Guiana, Maroni River basin, Grand Inini River, Saut "S", 3°36'19"N-53°48'25"W, P.Y. Le Bail *et al*., 1 Oct. 1997. The specimen is illustrated in figure 4.

As described by Kner, *H. medians* has a stocky structure, body being stout, deep and wide. Trunk plates are keeled and rough-toothed, with odontodes horizontally aligned on lateral plate series, odontodes of the central line on each plate longer than others. The snout is broad and rounded. The eye is large (23.3% HL for neotype; 18.6-26.9, mean 23.9 ± 2.4 for 12 specimens of 61.9-196.5 mm SL), dorsal margin of the orbit forming a crest. Jugal odontodes are strong and hooked, longest largely behind posterior margin of orbit in large specimens. Their numbers vary from 20 in a small specimen (61.9 mm SL) up to 60 in a large one (196.5 mm SL) (neotype: 49/53). The mouth is broad. The tooth row cup is medium sized (dentary: 16.9% HL, 15.1-19.8, mean 17.1 ± 1.3; premaxillary 16.9% HL, 15.5-19.6, mean 16.9 ± 1.1), bearing strong teeth with two elongated cusps very similar in size and shape. The number of teeth is variable, slightly higher on dentary (32/25; 14-41, mean 24.1 ± 6.6) than on premaxillary (21/24; 12-35, mean 20.6 ± 6.2). Dorsal fin is high, with a long spine (30.6% SL; 28.3-37.2, mean 32.7 ± 2.7) and seven branched rays. Pectoral-fin spine is long (35.6% SL; 35.0-37.5, mean 36.1 ± 0.7) and strong, its distal part bearing dorsally elongated odontodes. Pelvic fin is very long (29.0% SL; 27.2-31.0, mean 29.4 ± 1.2), but in examined specimens reaching only to behind middle of anal fin, not passing anal fin as described for the holotype. Body and fins are covered by numerous

Figure 6. - Colouration in life. **A**: *Hemiancistrus medians*, MHNG 2717.005, Suriname, Tapanahony River, Kumaru Konde Sula (R. Covain). **B**: *Peckoltia otali* n. sp., French Guiana, Tampoc River (P.Y. Le Bail). **C**: Holotype of *Peckotia capitulata* n. sp., MHNG 2723.086 (F. Naneix). **D**: Holotype of *Peckoltia simulata* n. sp., MNHN 2011-0012 (R. Covain).

large, dark brown, roundish spots. Spots are less numerous and comparatively larger in juveniles (Fig. 5A). They appear black on a yellowish background in living specimens (Fig. 6A). Ventrally, spots are generally missing behind the lip and in the area surrounding the pelvic-fin origin (incl. neotype), and often more broadly. The ventral covering of the species is extremely variable. It may be densely and finely plated as described by Kner, although not covered completely in any of the examined specimens. The neotype has the abdomen largely covered with very small plates that are mostly not contiguous. Platelets are contiguous and form a dense granular cover in restricted areas under the cleithrum and on sides of the abdomen. Most other specimens, even of large size, show a less densely covered abdomen. Small individuals and some large ones have even the abdomen almost plateless: few platelets, sometimes small granular areas, are present close to the pectoral-fin origin, under the cleithrum, and/or on side of the abdomen, none in central part of abdomen. The high variability of this character may explain the difference shown by several conspecific specimens with Kner's description of the holotype, as was already highlighted by Günther (1864: 242) who nevertheless "had no doubt that our specimens are identical with *Ancistrus medians* of Kner".

Hemiancistrus medians was mostly found in the main channel of rivers within the upper Maroni/Marowijne basin in French Guiana and Suriname (Fig. 7). The species was collected in fast flowing waters in the immediate vicinity of waterfalls or rapids. In all places, the substrate was mainly boulders and stones, with gravels in the shallows, sand in the deeper, still water areas, and mud and decayed organic litter in the deepest holes. Exposed wet rocks were covered by the Podostemaceae *Mourera fluviatilis* (Fig. 8). *Hemiancistrus medians* was collected with the Hypostominae *Hypostomus gymnorhynchus*, *Ancistrus* cf. *leucostictus*, *Ancistrus temminckii*, *Guyanancistrus brevispinis*, *Lithoxus planquettei*, *Panaqolus koko* n. sp., *Peckoltia otali* n. sp., *Pseudancistrus barbatus*, and the Loricariinae *Cteniloricaria platystoma, Harttia guianensis, Metaloricaria paucidens*, and an unidentified Hypoptopomatinae (n. gen. aff. *Parotocinclus*).

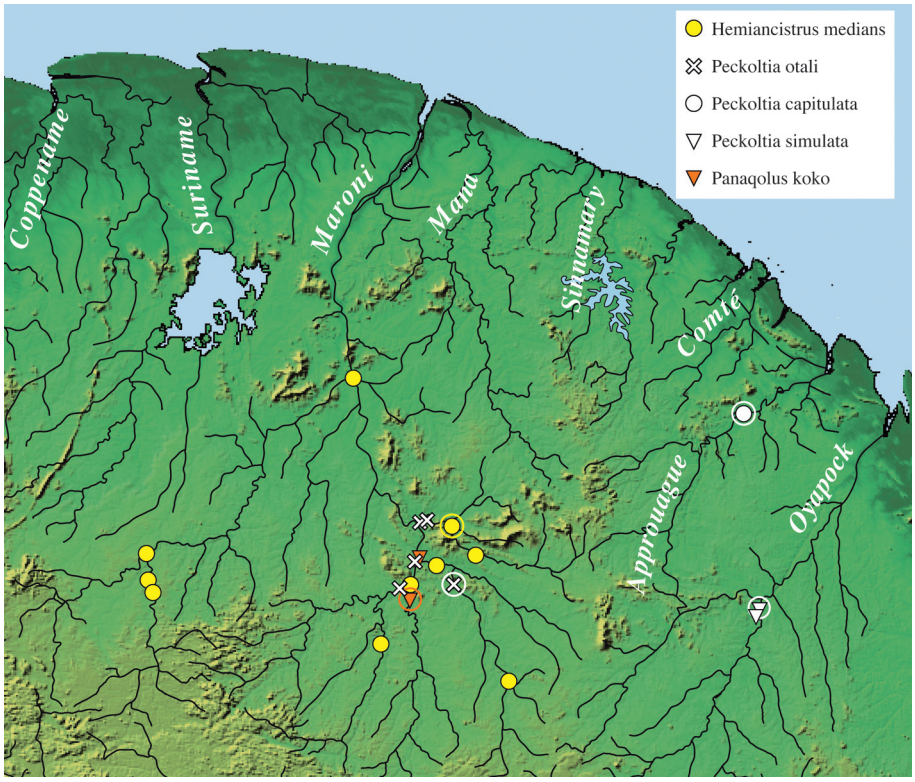

Figure 7. - Geographic distribution of *Hemiancistrus medians*, *Peckotia otali*, *Peckotia capitulata*, *Peckotia simulata*, and *Panaqolus koko*. Circled symbols refer to type localities. One symbol may overlap distinct localities.

Figure 8. - Saut Pierkuru, Tampoc River, Maroni basin ((S. Fisch-Muller).

DESCRIPTIONS OF NEW SPECIES

Peckoltia otali Fisch-Muller and Covain, new species
(Fig. 5B, 7, 9)

Hemiancistrus aff. *braueri* Eigenmann, 1912: Le Bail *et al.*, 2000: 232 (description), figs p. 233 (living specimen; map of distribution).

Holotype. - MNHN 2011-0005, ex-MHNG 2723.082, 76.5 mm SL, French Guiana: Tampoc River in Saut Tampoc, tributary of Lawa River, Maroni basin, 3°19'27''N-53°50'12''W, P.Y. Le Bail, P. Keith, P. Gaucher and C. Richard-Hansen, 17 Nov. 1998.

Paratypes. - All from Maroni River basin. French Guiana: MHNG 2723.082, 6, incl. 1 cleared & stained, 50.6-68.8 mm SL; MNHN 2011-0006, 5, ex-MHNG 2723.082, 57.8-66.4 mm SL; MNHN 2011-0007, 1, ex-MHNG 2723.082, 68.3 mm SL, same data as holotype. MHNG 2723.083, 1, 52.9 mm SL; MNHN 2011-0008, 1, ex-MHNG 2723.083, 59.8 mm SL, Tampoc River, Elahé, same collectors, 21 Nov. 1998. MNHN 1988-1851, 3, 26.5-49.3 mm SL, Litani River, Saut Tetombé, upstream of Pilima, 3°14'54''N-54°09'28''W, M. Jégu, 4 Oct. 1998. MNHN 2002-0848, 1, 74.4 mm SL, Marouini River, 2 h of boat from Antecume Pata, Wayana Amerindian ichthyocide fishing, Y. Fermon, R. Commergnat and R. Ksas, 18 Dec. 2001. MHNG 2723.084, 1, 20.4 mm SL; MNHN 2011-0009, 2, ex-MHNG 2723.084, 19.2-63.7 mm SL, Grand Inini River, downstream Saut Batardeau, 3°29'23''N-53°42'50''W, P.Y. Le Bail, P. Keith and M. Jégu, 28 Sept. 1997. MHNG 2723.085, 1, 69.0 mm SL; MNHN 2011-0010, 1, ex-MHNG 2723.085, 65.0 mm SL; ZMA 119.859, 2, 60.3-63.9 mm SL, Maroni River, Saut Singatetei, 4°23'N-54°26'W, P.Y. Le Bail, 9 Jul. 1983. Suriname: Sipaliwini: ANSP 187118, 1, SUR07-05, tag 7023, estimated 64.5 mm SL, end of caudal peduncle and fin missing, Litani River at mouth and confluence with Marowijne/Maroni River, just upstream from settlement of Konya Kondre, 03°17'24''N-54°04'38''W, J. Lundberg, M. Sabaj, P. Willink, J. Mol *et al.*, 21 Apr. 2007.

Diagnosis

Peckoltia otali is distinguished from all congeneric spe-

Figure 9. - Holotype of *Peckotia otali* n. sp., MNHN 2011-0005, 76.5 mm SL.

cies by a unique colour pattern of adults, and from Guianese species by its specific barcode sequence (JF747005). It shows numerous blackish-brown spots of irregular size and shape, distributed on head and on entire body except naked ventral areas, resulting in a mottled aspect of dorsum, while spots are aligned to form transverse bands on fins, at least on caudal fin. Juvenile specimens present large transversal blackish bands, or dorsal saddles, on the body that are similar to those

characteristic of several *Peckoltia* including the type species *P. vittata*. Brown spots on posterior part of the body are also observed in *Peckoltia oligospila*, *P. sabaji*, *P. capitulata* and *P. simulata*, but in these species spots are rounded, comparatively larger and regularly spaced, and they usually do not form bands on fins. *Peckoltia otali* is further distinguished from these species by a deeper body (22.5-25.7% SL, mean 23.8, *versus* less than 23.4 at occiput; 12.4-13.8% SL, mean 13.1, *versus* less than 11.7 at caudal peduncle) and a wider body (33.8-37.1% SL, mean 35.2, *versus* less than 32.7 at cleithrum). It is distinguished from *P. bachi* that is also mottled, by having the eye high on the head (*versus* low) and a much narrower interorbital (29.8-34.4% HL, mean 32.6, *versus* 57.9-59.9, mean 58.8).

Description

Morphometric and meristic data given in table II. Small-sized species (largest specimen observed 76.5 mm SL, holotype, breeding male). Body stout, deep and wide. Dorsal profile gently convex from snout tip to dorsal-fin origin, then sloped ventrally to procurrent caudal-fin rays, and rising straight to caudal fin. Ventral profile flat to caudal fin.

Snout rounded anteriorly, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit, supraoccipital with very slight rounded crest. Eye moderately large. Dorsal margin straight flattened from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle roughly ovoid in cross section, slightly flattened ventrally, and more compressed posteriorly.

Lips covered with short, wide papillae. Buccal papilla generally small, sometimes absent. Lower lip wide, not reaching pectoral girdle, upper lip much narrower. Maxillary barbel reaching posteriorly one-third to two-third of distance to gill opening, sometimes bifurcated. Teeth bicuspid, lateral lobe up to one third smaller than medial lobe.

Head and body plated. Tip of snout naked. Two rows of plates and curved nuchal plate between triangular supraoccipital process and dorsal fin. Five series of lateral plates extending to caudal fin. Abdominal region naked in juveniles, and largely naked in adults. Patches of platelets usually restricted to regions close to pectoral-fin base, pectoral girdle, and, by largest specimens, also anteriorly to anal pore. First anal-fin pterygiophore exposed to form a small platelike structure.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. In breeding males (including holotype), odontodes on plates of posterodorsal part of body and on adipose fin enlarged to confer hirsute appearance. Odontodes on posterior third of pectoral-fin spine generally enlarged but more in mature males. Opercle supporting odontodes in juveniles but not in most of large specimens (more than 60 mm SL). Posterodorsal margin of opercle covered by one or two plates. Hypertrophied cheek odontodes straight with tips curved, the longest reaching posterior margin of cleithrum in large specimens. Cheek plates evertible to approximately 90º from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip reaching adipose fin or the plate before. Dorsal-fin spinelet V-shaped, dorsal-fin spine locking mechanism functional. Adipose fin roughly triangular, preceded by single median unpaired plate, short and raised. Adipose spine straight or slightly curved. Pectoral-spine tip reaching one-fourth to one-third of pelvic spine, somewhat longer and more robust in large males. Anal fin with weak spine of approximately same length of first branched ray. Caudal fin slightly concave, ventral lobe longer than dorsal lobe. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14, i.

Colouration

In life (Fig. 6B), base colour yellow-orange, except whitish abdominal region without plates. Base colour tan in alcohol, head darker tan. Sometimes a hardly distinct lighter band between both eyes, and faint dorsal saddles on body. In adults, numerous small dark blackish-brown spots of irregular size and shape distributed on head and entire body except naked abdominal regions; spotting pattern resulting in an irregularly mottled aspect (Fig. 9). Spots may form few irregular transverse bands on posterior part of body of medium-sized specimens. Juveniles show contrasted pattern of colouration with five transversal blackish bands, or dorsal saddles, along body (Fig. 5B). Dark spots present on all fins, centred on fin rays and often combined to form transverse bands, more generally on caudal fin, and especially in smaller specimens.

Distribution and habitat

Peckoltia otali was collected from several localities in the upper Maroni River basin (Fig. 7). It lives in same biotopes as the loricariids *Guyanancistrus brevispinis*, *Hemiancistrus medians*, *Lithoxus planquettei*, *Panaqolus koko* n. sp., *Pseudancistrus barbatus*, *Hypostomus gymnorhynchus*, and *Cteniloricaria platystoma*. In rapids, it is mostly found in sunny and shallow clear water, swiftly flowing currents, with medium-sized rocks substrate. It is a discreet species due to its colouration that resembles its natural environment.

Etymology

Named *otali*, a Wayana Amerindian name meaning secret, in reference to the colouration of the species, similar to its biotope, making it difficult to observe. Wayana Amerindians live on the sides of the Upper Maroni River basin where the new species was found. A noun in apposition.

***Peckoltia capitulata* Fisch-Muller and Covain, new species** (Fig. 7, 10)

Holotype. - MHNG 2723.086, 75.9 mm SL, French Guiana: Approuague River, rapids of Saut Athanase, 4°11'12"N-52°20'03"W, F. Naneix, 24-27 Feb. 2004.

Paratype. - MNHN 2011-0011, ex-MHNG 2723.086, MUS 331, 1, 59.5 mm SL, same origin as holotype.

Diagnosis

Peckoltia capitulata is characterized by its specific barcode sequence (JF747000), distinguishing it from Guianese

Figure 10. - Holotype of *Peckotia capitulata* n. sp., MHNG 2723.086, 75.9 mm SL.

species, and by a spotted pattern of colouration of posterior part of body, distinguishing it from all congeners except *Peckoltia oligospila*, *P. bachi*, *P. sabaji*, *P. simulata*, and *P. otali*. In contrast to these five species, no spot is present on the head of *Peckoltia capitulata*. It is additionally distinguished from the spotted species as well as from most other *Peckoltia* species by a shorter head (length 33.4-33.6% SL *versus* more than 33.7).

Peckoltia capitulata is also easily separated from both *P. bachi* and *P. otali* by rounded spotting (*versus* mottling); from *P. bachi* by a much narrower interorbital (34.4-34.5% HL, mean 34.5, *versus* 57.9-59.9, mean 58.8); from *P. otali* by several measurements including those listed in diagnosis of the latter; from *P. sabaji* by smaller spots on caudal peduncle and less slender body. It is further distinguished from *P. oligospila* by lower occipital depth (18.4-20.4% SL, mean 19.4, *versus* 21.1-23.4, mean 21.9), smaller cleithral width (30.3-30.5% SL, mean 30.4, *versus* 30.9-32.8, mean 32.1) and shorter orbital diameter (6.5-7.0% SL, mean 6.8, *versus* 6.9-8.1, mean 7.4); from *P. oligospila* and *P. simulata* by a shorter dorsal-fin spine (27.7-27.9% SL, mean 27.8, *versus* more than 28.4) and higher caudal peduncle (11.3-11.7% SL, mean 11.5, *versus* less than 10.6); and from *P. simulata* by tooth shape and length of hypertrophied cheek odontodes (detailed in diagnosis of the latter species).

Description

Morphometric and meristic data given in table II. Small to medium-sized species (largest specimen examined 75.9 mm SL, no breeding male). Body moderately stout. Dorsal profile gently convex from snout tip to supraoccipital process, then straight to dorsal-fin origin, sloped ventrally to procurrent caudal-fin rays, and rising straight to caudal fin. Ventral profile flat to caudal fin.

Snout slightly pointed (holotype) to rounded (paratype) anteriorly, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit, supraoccipital with very slight rounded crest. Eye moderately large. Dorsal margin straight flattened from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle roughly ovoid in cross section, slightly flattened ventrally, and more compressed posteriorly.

Lips covered with short, wide papillae. Buccal papilla small. Lower lip wide, far from reaching pectoral girdle, upper lip much narrower. Maxillary barbel reaching posteriorly halfway the distance to gill opening. Teeth bicuspid, lateral lobe up to one-half smaller than medial lobe.

Head and body plated. Tip of snout naked. Two rows of plates and curved nuchal plate between triangular supraoccipital process and dorsal fin. Five series of lateral plates extending to caudal fin. Abdomen naked. Few patches of platelets below pectoral girdle. First anal-fin pterygiophore exposed to form a small platelike structure.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. Odontodes on plates of posterodorsal part of body and on adipose fin slightly enlarged. Odontodes on posterior third of pectoral-fin spine enlarged in holotype. Opercle supporting few odontodes. Posterodorsal margin of opercle covered with one or two plates. Hypertrophied cheek odontodes straight with tips curved, not reaching posterior margin of cleithrum. Cheek plates evertible to approximately 90º from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip not reaching preadipose plate. Dorsal-fin spine locking mechanism functional. Adipose fin preceded by single median unpaired plate, short and raised. Adipose spine thin and very slightly curved. Pectoral-spine tip reaching about one-fourth (paratype, left spine; right fin cut close to origin) to quite half (holotype) of pelvic spine, somewhat longer and more robust in large males. Anal fin with weak spine slightly shorter than first branched ray. Caudal fin apparently concave, damaged in both specimens. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14, i.

Colouration

Base colour light tan in life (Fig. 6C), somewhat darker in alcohol (Fig. 10). Head with darker areas, and without spot. Three or four faint dorsal saddles on body. Body and fins brown spotted. Spots very small and numerous at dorsal-fin origin level, but becoming rapidly larger posteriorly, about the size of the pupil before end of dorsal-fin base, and less numerous on caudal peduncle. Spots few in number, darker, larger and more rounded on dorsal and caudal fins. No spot on ventral face, abdomen with diffuse pigmentation.

Distribution and habitat

Peckoltia capitulata was collected with a cast net at a single place of the Approuague River in swift current of Saut Athanase (Fig. 7). Numerous specimens of *Guyanancistrus brevispinis*, *Hypostomus gymnorhynchus*, and the Loricariinae *Harttia guianensis*, *Rineloricaria platyura*, and *Loricaria* sp., were also found. Water at Saut Athanase is slightly acidic (pH 5-6.4), soft (20-22 $\mu S.cm^{-1}$), and relatively warm (27-30°C). At time of collection, the river was highly turbid as a result of illegal gold mining activities.

Etymology

The specific epithet *capitulata* is Latin and means having a small head.

***Peckoltia simulata* Fisch-Muller and Covain, new species**
(Fig. 7, 11)

Hemiancistrus aff. *braueri* forme Oyapock: Le Bail *et al.*, 2000: 232.

Holotype. - MNHN 2011-0012, ex-MHNG 2681.032, GF06-062, 83.4 mm SL, French Guiana, Crique Moulou Koulou, small tributary of the Oyapock River, 3°06'05"N-52°20'34''W, R. Covain, S. Fisch-Muller, P.Y. Le Bail and J.I. Montoya-Burgos, 4 Nov. 2006.

Paratypes. - MHNG 2681.058, GF06-119 & 120, 2, 80.4-83.0 mm SL), French Guiana, Crique Fifine, left side tributary of the Oyapock River, 3°04'44''N-52°20'34"W, R. Covain, S. Fisch-Muller, P.Y. Le Bail and J.I. Montoya-Burgos, 5 Nov. 2006.

Diagnosis

Peckoltia simulata is characterized by its specific barcode sequences (JF747001- JF747002), distinguishing it from Guianese species, and by a spotted pattern of colouration of body including posterior part, distinguishing it from all congeners except *Peckoltia oligospila*, *P. bachi*, *P. sabaji*, *P. otali* and *P. capitulata*. It is distinguished from the latter by teeth shape, with both lobes similar, long (unless if worn), lateral lobe being only very slightly smaller than medial lobe (*versus* distinctly smaller). Longer hypertrophied cheek odontodes, longest one passing posterior end of cleithrum (*versus* not reaching) additionally separate *P. simulata* from spotted species. In addition to several measurements, it is further separated from *P. bachi* and *P. otali* by rounded spotting (*versus* mottling), from *P. sabaji* by smaller spots on caudal peduncle, and from *P. capitulata* by presence of spots on head (*versus* absence). It can be further distinguished from *P. oligospila* by having a smaller body depth (19.8-20.4% SL *versus* 21.1-23.4), narrower body (29.5-30.7% SL *versus* 30.9-32.8) and shorter orbital diameter (5.9-7.0% SL *versus* 6.9-8.1).

Description

Morphometric and meristic data given in table II. Fairly medium sized species (largest specimen examined 83.4 mm SL, no breeding male). Body moderately stout. Dorsal profile gently convex from snout tip to supraoccipital process, then straight to dorsal-fin origin, sloped ventrally to procurrent caudal-fin rays, and rising straight to caudal fin. Ventral profile flat to caudal fin.

Snout slightly pointed, low median ridge in front of nostrils, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit supraoccipital with distinctly elevated crest. Eye moderately large. Dorsal margin straight flattened from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle roughly ovoid in cross section, slightly flattened ventrally, and more compressed posteriorly.

Lips covered with short, wide papillae. Buccal papilla small. Lower lip wide, far from reaching pectoral girdle, upper lip much narrower. Maxillary barbel reaching posteriorly half the distance to gill opening or slightly more. Teeth bicuspid, both lobes very similar, lateral lobe only slightly smaller than medial lobe.

Head and body plated. Tip of snout naked. Two rows of plates and curved nuchal plate between pointed tip of supraoccipital process and dorsal fin. Five series of lateral plates extending to caudal fin. Abdominal region largely naked. Patches of platelets restricted to regions close to pectoral girdle, pectoral-fin base, and between pelvic fins posteriorly to anal pore. Some large specimens more largely plated. First anal-fin pterygiophore exposed to form a small platelike structure.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. Odontodes on plates of posterodorsal part of body and on adipose fin slightly enlarged. Odontodes on tip of pectoral-fin spine generally enlarged, longest in males. Opercle supporting few odontodes. Posterodorsal margin of opercle covered with one or two plates. Hypertrophied cheek odontodes straight with tips curved, reaching first plate of mid-ventral lateral series. Cheek plates evertible to approximately 90º from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip reaching preadipose plate. Dorsal-fin spine locking mechanism functional. Adipose fin preceded by single median unpaired plate, short and raised. Adipose spine straight or slightly curved. Pectoral-spine tip reaching past middle of pelvic spine. Anal fin with weak spine slightly shorter than first branched ray. Caudal fin forked, ventral lobe longer than dorsal lobe. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14, i.

Colouration

Base colour brownish orange-coloured in life (Fig. 6D), tan in alcohol, lighter on lower part of caudal peduncle and ventrally, abdomen whitish (Fig. 11). Faint dorsal saddles. Dark rounded spots on head, body and fins. Spots small to medium-sized (smaller or equal to pupil) on head, larger (less than eye) posteriorly. Spots rather irregularly distributed on head as well as on body, where they often superimpose. Similar spots on ventral surface, rarer on naked areas. Spots more contrasted, rounded and spaced on fins.

Distribution and habitat

Peckoltia simulata was collected in two small forest creek tributaries of the Oyapock River in the vicinity of Camopi (Fig. 7), with cast net and dip net on sandy and

Figure 11. - Holotype of *Peckotia simulata* n. sp., MNHN 2011-0012, 83.4 mm SL.

Figure 12. - Crique Moulou Koulou, Oyapock River tributary (S. Fisch-Muller).

gravelled bottom with rocks, woods and leaves (Fig. 12). One specimen was hidden in a hollow piece of wood oriented against the current. The new species was collected with representatives of *Ancistrus* cf. *leucostictus*, *A.* aff. *temminckii*, *Guyanancistrus longispinis*, *Farlowella reticulata*, *Rineloricaria stewarti*, and *Otocinclus mariae*. Water parameters were: temperature 25.0-25.7°C, pH 6.1-6.2, and conductivity 13-14 μS.cm^{-1}.

Etymology

Named *simulata*, a Latin adjective meaning counterfeit, in reference to its similarity with *Peckoltia oligospila*.

***Panaqolus koko* Fisch-Muller and Covain, new species** (Figs 7, 13, 14)

Panaque cf. *dentex* (Günther, 1868): Le Bail *et al.*, 2000: 248 (description), figs p. 249 (living specimen).

Holotype. - MNHN 2011-0013, ex-MHNG 2675.096, GF00-115, 90.1 mm SL, male, French Guiana, Marouini River, vicinity of Antecume Pata, Wayana Amerindian fisherman ("Nivrée 2000" mission),19 Oct. 2000.

Paratypes. - All from Maroni River basin. French Guiana: MHNG 2723.088, 1, 73.5 mm SL; MNHN 2011-0014, 1, ex-MHNG 2723.088, 79.9 mm SL, French Guiana, Lawa River, Elahé, Le Bail, P. Keith, P. Gaucher and C. Richard-Hansen, 21 Nov. 1998. MHNG 2723.089, 1, ex-MNHN 2002-0851, 89.8 mm SL; MNHN 2002-0851, 2, 61.2-69.4 mm SL, Marouini River, 2 h of boat from Antecume Pata, Wayana Amerindian ichthyocide fishing, Y. Fermon, R. Commergnat and R. Ksas, 18 Dec. 2001.

Diagnosis

Panaqolus koko is diagnosed by its large and almost spoon-shaped teeth characteristic of *Panaqolus* but bifid instead of most generally unicuspid in congeneric species, and is characterized by its specific barcode sequence (JF747003). It is additionally distinguished from all *Panaqolus* except *P. dentex* and *P. nocturnus* by a uniformly blackish-brown colouration, *versus* banded pattern of colouration (*P. purusiensis*, *P. gnomus*, *P. maccus*, and *P. changae*) or spotted pattern of colouration (*P. albomaculatus*). The dark pigment on membrane and branched rays of all fins distinguishes *P. koko* from *P. dentex*, as well as a smaller interorbital width (29.4-33.2% HL, mean 31.6, *versus* 38.7), a shorter pectoral spine (29.8-31.3% HL, mean 30.3, *versus* 34.8) and a greater caudal peduncle depth (12.0-13.4% SL, mean 12.9, *versus* 10.8). The large eye distinguishes it easily from *P. nocturnus* (orbit length 18.8-20.8% HL *versus* 13.7-15.9).

Description

Morphometric and meristic data given in table II. Body moderately deep, head and body depressed. Dorsal profile gently convex from snout tip to dorsal-fin origin, straight and posteroventrally slanted to adipose-fin origin, slightly concave up to first procurrent caudal-fin rays, then rising straight to upper caudal-fin ray. Ventral profile flat to caudal fin. Ventral margin of caudal peduncle rounded.

Snout tapering anteriorly to a largely blunted point, slight rounded ridge from antero-lateral corner of nostril to end of dorsal margin of orbit, tip of supraoccipital pointed and slightly elevated. Eye large. Dorsal margin straight from base of first branched dorsal-fin ray to base of adipose fin between light ridges formed with lateral plates of dorsal series. First lateral plates of mid-ventral series forming slight lateral ridge. Caudal peduncle ovoid in cross section, slightly flattened ventrally.

Oral disk circular to diamond shape, lips covered with short and wide papillae. No buccal papilla. Maxillary barbel larger than one-half orbital diameter. Premaxillary and dentary teeth few (4-6), strong and thick, close to spoon-shape but bicuspid, major cusp large, moderately elongated, lateral cusp short, triangular to rounded (Fig. 14). Teeth slightly larger on dentary, those in middle of tooth row slightly larger than those on either end.

Head and body plated. Snout with a very small naked area near tip. Snout covered by numerous small platelets, with discreet naked interspaces. Posterodorsal margin of opercle covered by two or three plates. A single plate on midline between supraoccipital process and curved nuchal plate preceding dorsal fin. Two plates between ventral supraoccipital and dorsal peterotic-supracleithrum margins. Five series of lateral plates extending to caudal fin. Branchial and abdominal region generally naked, without plates except for area adjacent to branchial opening, rarely on larger area below pectoral girdle. Area dorsal to pelvic-fin base below ventral margin of lateral plates with 0-4 small plates (1 and 2 on each side in holotype), area otherwise naked. First anal-fin pterygiophore covered by skin, except for one specimen (73.5 mm SL) that exhibits small plate-like structure.

Figure 13. - Holotype of *Panaqolus koko* n. sp., MNHN 2011-0013, 90.1 mm SL.

Head and body plates covered by odontodes of relatively uniform size and distribution. Odontodes on lateral series of plates not arranged in distinct longitudinal rows and not forming keels on sides. Odontodes on plates of posterodorsal part of body and on adipose fin slightly enlarged. Odontodes on posterior third of pectoral-fin spine generally enlarged, longest in males. Hypertrophied cheek odontodes straight with tips curved, the longest reaching posterior margin of cleithrum in large specimens. Cheek plates evertible to approximately 90º from head.

Dorsal-fin origin slightly anterior to pelvic-fin origin; when adpressed, dorsal-fin tip reaching one or two plates before adipose fin. Dorsal-fin spinelet V-shaped, dorsal-fin spine locking mechanism functional. Adipose fin roughly triangular, preceded by single median unpaired plate, short and raised. Adipose spine slightly curved. Pectoral-spine tip reaching one fourth to one-third of pelvic spine, somewhat longer and more robust in large males. Anal fin with weak spine, approximately same length of first branched ray or shorter. Caudal fin slightly concave, ventral lobe longer than dorsal lobe. Fin-ray formulae: dorsal II,7; pectoral I,6; pelvic i,5; anal i,4; caudal i,14,i. Dorsal procurrent caudal rays: 4-5 (mean 4; holotype 4). Ventral procurrent caudal rays: 3-5 (mean 4; holotype 5).

Figure 14. - Dentition of *Panaqolus koko* n. sp., MHNG 2723.089, paratype, 69.4 mm SL.

Colouration

Body and fins uniformly blackish-brown, dark brown ventrally (Fig. 13). In life (see Fig. in Le Bail *et al*., 2000: 233), dorsum nearly black with some diffuse lighter areas, that probably correspond to the three light brown saddles or blotches described by Schaefer and Stewart (1993) for *P. dentex* and *P. nocturnus*. These light areas probably reflect a stress pattern, as commonly observed in the Loricariidae.

Distribution and habitat

Despite several fish collections, *Panaqolus koko* is only known by a few specimens collected in three stations in the surroundings of Antecume Pata in the upper Maroni River basin (Fig. 7). It was collected in main river channel on a stony substrate at two meters depth. It was further caught with ichthyocide by Wayana Amerindians together with the hypostomins *Hemiancistrus medians*, *Peckoltia otali* and *Pseudancistrus barbatus*, and with the loricariins *Harttia guianensis, Loricaria cataphracta* and *Rineloricaria stewarti*.

Etymology

Named *koko*, a Wayana Amerindian name meaning night, in reference to the dark colouration of the species, and in allusion to the similarly coloured and named *Panaqolus nocturnus*. A noun in apposition.

DISCUSSION

This assessment of the diversity of the Ancistrini constituting the *Panaque* group within the three countries of the Guianas unambiguously reveals the presence of four new species in French Guiana and Suriname. The barcode approach appears as a relevant tool to characterize such diversity, and in revealing hidden diversity, or complex evolutionary patterns. However, despite an effective differentiation between sequences, and accordingly between species, a correct assignment to congeneric species is missing in the present case. Ward (2009) indeed demonstrated that the "10-fold" rule (Hebert *et al*., 2004b) classically used as threshold to distinguish different species (i.e., ten times the mean intraspecific variation for the group under study) was correct, but rather conservative especially considering cryptic speciation. Ward (2009) refined the "10-fold" rule and proposed that specimens with divergences greater than 2% were likely to be different species with a probability greater than 0.95. This threshold applies to most of our results since interspecific variations were generally greater than 2.1%. Nevertheless between species divergences of 1.4% and 1.6% were observed between *Pc. otali* and *Pc. cavatica* on one hand, and between *Pc. otali* and *Pc. sabaji* on the other hand. Moreover, at the between genera level, *Pn. koko* displayed a divergence of 0.6% with *Pc. otali* (and accordingly divergences of 1.4% and 1.6% with *Pc. cavatica* and *Pc. sabaji*, respectively). Considering Ward's criterion, this very small divergence recorded between *Pn. koko* and *Pc. otali* would lead to consider the former as a distinct population of the latter, whereas both belong to distinct genera. Thus, if one considers that a correct identification necessitates first differentiation and second, a correct assignment (e.g., to congenerics), then the COI marker was not sufficient in the present case to distinguish *Panaqolus koko* from *Peckoltia otali*. To circumvent this issue, we used a nuclear marker to detect potential conflicting signals.

The first step consisted thus in identifying, selecting and characterizing the first intron of the F-RTN4 gene to verify that it formed a candidate of choice for a correct assignment of the species. A preliminary assessment consisted thereby in evaluating that its pattern of evolution fitted the timely-ordered model proposed by Zhu *et al*. (2009). Since this intron is larger, presumably because it is older, it accumulates more variations due to the more numerous indels and mutations, the quantity of information accumulated along the sequences is expected to be more important than in other regions of the gene. Our results corroborate in great part the pattern of intronic evolution observed by Zhu *et al*. (2009), and particularly the first point (ordinal reduction of length and divergence in both exon and intron). Comparisons made between introns 1 and 2 confirmed indeed that intron 1 is significantly longer (in mean 3.5 times longer) and more variable (about two times) than intron 2. Concerning the second point (co-variation of GC content and divergence between exons and flanking introns), a strong and significant correlation is also observed between divergence of intron

1 and its 3' flanking exon. However the co-variation in GC content between these two entities appeared independent and contrasts with Zhu *et al*. (2009) study. This absence of co-variation is very probably due to the lack of variation in GC content in both intron 1 and exon 2, and to the small size of our data set and close relatedness of the species that constitutes it. Larger sampling and higher taxonomic levels would probably correct for this potential artefact. A strong negative correlation between both introns of F-RTN4 and their respective GC content was also observed in our data and those obtained from GenBank. The pattern of co-variation between GC content and intron length appears complex, and if no universal pattern of co-variation between intron length and GC content is observable across taxa, a significant correlation of both parameters seems always present. Significant negative correlation was indeed observed in primate genomes (Gazave *et al*., 2007) whereas a significant positive correlation was observed in fruit flies (Haddril *et al*., 2005). The third point was not estimated since it implies comparisons to multiple genes. The within gene characteristics of the selected intron meet generally the global pattern of evolution of intronic regions and can fit accordingly the timely-ordered model.

A second step consisted in evaluating the quality of the signal of the first intron of the F-RTN4 by making between-genes comparisons to the mt COI gene, since a good candidate for barcoding process is expected to contain a significant amount of variation for discrimination and identification purposes as well as a significant amount of phylogenetic signal for a correct assignment of the species (e.g., to the correct genus). The maximum parsimony and distances based tests of congruence of both molecular markers did not detect any conflicting phylogenetic signal between COI and the first intron of the F-RTN4 genes, revealing that a significant common signal was present in both data sets. This result is reinforced by the significance of the tests of substitution saturation that highlighted little saturation in both markers, making each of them good candidates for phylogenetic reconstructions.

Our F-RTN4 fragment was also expected to share the main qualities of the COI gene as characterized in Ward and Holmes (2007). The Shannon's information theoretic entropy plots computed for both markers revealed that each of them was highly variable, especially the COI gene. The information was distributed all along sequences providing enough variation for identification and discrimination purposes. This pattern of variation is essentially due to the degenerate nature of the genetic code that allows numerous substitutions in positions 1 and above all 3 of codons for COI. In F-RTN4, the three maxima observed corresponded to the three insertion-deletions of the *Pseudacanthicus leopardus* sequence. Moreover, important variations were also regularly distributed along sequences. A close examination of the alignment reveals that the substitutions occurring in those positions were not obtained at random but display variations that were lineage dependent. The observed mutations are indeed preserved through lineages implying inheritance from common ancestors. The lack of variation of the first intronic region of F-RTN4 is intriguing compared to that of the COI gene. Non-coding regions are indeed expected to be highly variable, or at least more variable than coding regions due to presumably less evolutionary constraints acting on them. Different studies demonstrated that the first intron of genes tends to be the most conserved of all introns (e.g., Keightley and Gaffney, 2003; Chamary and Hurst, 2004; Gaffney and Keightley, 2006; Vinogradov, 2006), implying that they are more selectively constrained. Bradnam and Korf (2008) demonstrated that early introns (e.g., the first intron) were in average significantly longer than subsequent ones, and hypothesized that this increase in length was probably due to an increase in the presence of functional elements that may be involved in controlling gene expression. This hypothesis is not necessarily in contradiction with the timely-ordered model that stipulates that first introns had more time to be inserted. These inserted elements could effectively be new regulatory elements responsible for new gene functions (e.g., due to alternative splicing). The Wong and Nielsen test (2004) conducted on the first intron of F-RTN4 using its 3' flanking exon as neutral proxy confirmed that this intron was under strong positive selection. However, if the 3' flanking exon appears as the best choice as neutral proxy, assuming the observed co-variation in divergence between intron and flanking exons as stipulated by Zhu *et al*. (2009), its short length could have biased the test. Indeed, a second test using exon 3 of F-RTN4 as neutral proxy (length = 651 bp, $\omega = 0.034499$) resulted in a neutral evolution of the first intronic sequences ($\zeta = 1$; p-value = 0.9999).

Several attempts have been made to develop alternative nuclear markers, and the use of the variable regions of the nuclear ribosomal genes has been proposed (Sonnenberg *et al*., 2007; Raupach *et al*., 2010). Nuclear ribosomal genes are usually considered highly conserved and are classically used to resolve deep phylogenetic relationships (e.g., Le *et al*., 1993; Zardoya and Meyer, 1996). The nuclear ribosomal genes consist of a succession of conserved and variable regions. Among the latter, the D1-D2 LSU (28S) region was proposed by Sonnenberg *et al*. (2007), and the D3 (28S) and V4-V7 (18S) by Raupach *et al*. (2010) as supplement for barcoding purpose. However, these regions remain highly conserved (at least in vertebrates), and the observed mutations remain scarce. For example, using accession numbers provided by Sonnenberg *et al*. (2007) for four European cyprinids belonging to four distinct genera (EF417161: *Alburnoides bipunctatus*; EF417162: *Alburnus alburnus*; EF417165: *Leuciscus cephalus*; EF417167: *Rutilus rutilus*), we obtained a mean K2P divergence of 0.003 ± 0.001

between these four genera for an alignment of 1,052bp of the D1-D2 LSU. By comparison, using four closely related species of the same genus, *Pc. oligospila*, *Pc. otali*, *Pc. simulata*, and *Pc. capitulata*, we obtained a mean divergence of 0.009 ± 0.003 for an alignment of 692 bp of the first intron of F-RTN4, i.e., three times more variable for a fragment one third shorter. These data are not directly comparable since they imply different taxa in the comparison of both makers. However, the fact of obtaining a between genera divergence three times smaller than a between species divergences sustains the hypothesis of the intronic regions to be more variable, and consequently reinforces the relevance of such markers in barcoding comparisons. The first intron of genes thus appears as good candidate for barcoding purposes. Despite complex evolutionary mechanisms (for a review see Roy and Gilbert, 2006), it possesses sufficient variability for a correct identification and discrimination of the different species; it contains a significant amount of phylogenetic signal for a correct assignment of species to their respective taxa (genus, family, order…); it is functional and evolutionary constrained so that mutational pattern is not obtained at random, but rather preserved through lineages preserving thus the quality of the phylogenetic signal (e.g., limitation of multiple substitutions saturation). One of the main concern using intronic regions, especially early introns, relies however on the occurrence, origin and size of inserted elements responsible for size polymorphism. At higher taxonomic level (e.g., familial rank) these multiple insertions can reach several hundred bases making detection of homology difficult, if not impossible when inserted elements are not homologue. A possible solution to overcome this problem may consist in the selection of coding regions. In this frame, Montoya-Burgos *et al.* (2010) developed recently the Inter-Specific Selective Hybridization (ISSH) method to enrich cDNA libraries in fast evolving genes in non-model organisms. This method could therefore allow the detection of exonic regions with high mutational rates providing good nuclear markers for species identification.

The dual approach used in the present study was particularly useful in the resolution of species level taxonomy of genera such as *Peckoltia*. *Peckoltia* species were indeed said to show no morphometric or meristic differences and no obvious difference in morphology, the only difference between species being the colour pattern (Armbruster, 2008: 51). Adults of all three new Guianese species of *Peckoltia* show the presence of dark spots on posterior part of body instead of dark saddles that are present in most of congeneric species. *Peckoltia otali* is clearly distinguished from the five known dorsally dark-spotted species (*Pc. oligospila, Pc. bachi*, *Pc. sabaji*, *Pc. simulata,* and *Pc. capitulata*) by additional colouration characteristics, and by morphometry. On the contrary *Pc. simulata* and *Pc. capitulata* represent cryptic species, both being very similar in colour pattern and morphologically close to *Pc. oligospila*. Nevertheless the divergence recorded between these two sister species (d_{K2P} = 0.036) and between each of them and *Pc. oligospila* (d_{K2P} = 0.048 for *Pc. capitulata*, and d_{K2P} = 0.036 for *Pc. simulata*) coupled with the topological results that never connected them within or in sister position to *Pc. oligospila* clearly demonstrate that *Pc. simulata* and *Pc. capitulata* represent distinct taxa. At the generic level, the barcode approach also unambiguously shows that none of those *Peckoltia* species, like those previously included in *Hemiancistrus* and in *Peckoltia* (e.g., *Pc. braueri*, *Pc. sabaji*), can be assigned to *Hemiancistrus* due to the very high genetic divergence recorded between representatives of both genera.

Conversely, an unexpected result of the DNA barcode analysis was obtained considering *Panaqolus koko*. The COI sequence of this species was indeed highly similar to that of *Pc. otali* whereas these two taxa represent undoubtedly distinct species, and even distinct genera. Only five silent transitions where recorded between sequences (positions 372, 385, 396, 426, and 624) leading to a between genera K2P distance of 0.6%. *Panaqolus koko* also showed smaller divergence with representatives of *Peckoltia* based on the COI gene (K2P distance ranging between 0.6% and 3.3%) whereas to other *Panaqolus* it displayed variations ranging between 2.6% and 3.1%. These intriguing results coupled to the fact that *Pn. koko* and *Pc. otali* are sympatric and endemic to the Maroni/Marowijne basin, and that both frequent the same biotopes, suggest that *Pn. koko* and *Pc. otali* may hybridise. *Panaqolus koko* shares indeed the mitochondrial signature of *Pc. otali* implying mitochondrial introgressive hybridization. This hypothesis was reinforced by the dual approach used herein. The F-RTN4 NJ tree placed indeed *Pn. koko* in a sister position to all *Peckoltia* representatives, except *Pc. sabaji*, and *Pc. otali* in a sister position of *Pc. cavatica*. *Panaqolus koko* does not group with other *Panaqolus* species, even though this topological result is not supported by bootstrap value.

Panaqolus was described by Isbrücker and Schraml (in Isbrücker *et al.*, 2001) based on a group of small species previously included in *Panaque* but defined as the *Panaque dentex* group by Schaefer and Stewart (1993). *Panaque* and *Panaqolus* are Ancistrini diagnosed by acutely angled rows of robust spoon-shaped teeth. *Panaqolus* is notably distinguished from *Panaque* by the absence of a posterior orbital notch and of a ventrolateral keel on caudal peduncle (Schaefer and Stewart 1993). These characters are shared by the new species *Pn. koko*, but the latter has morphological differences with congeneric species that have to be underlined. Teeth in *Pn. koko* approach spoon shape, but some may be more elongated, approaching the condition observed in *Scobinancistrus*. In addition they are always bicuspid, with a lateral cusp smaller but not absent or minute. Schaefer and Stewart (1993) highlighted a large polymorphism in

shape and number of teeth for the Venezuelan *Pn. maccus*, and tentatively included in that species two specimens from the Guiana shield drainage having bicuspid teeth similar to those of the new species. In addition to dentition, head and body shape, *Pn. koko* appears quite different from other *Panaqolus* species. It is notably more elongated and narrower, with a smaller interorbital distance and a larger eye. However direct comparison of morphometric data with some previously described species is made difficult when not impossible because the data provided in recent literature (Schaefer and Stewart, 1993; Chockley and Armbruster, 2002) do not correspond to standard measurements. Waiting for further evidence for its placement into a distinct genus if needed, we prefer to take a conservative position in placing the new species within *Panaqolus*.

The diversity of the *Panaque* group revealed in this study exceeds what was previously recorded for the Guianas. Here, four new species are added to the seven valid taxa. Among the latter, two were described very recently (*Pc. cavatica* and *Pc. sabaji*), one seems to have never been collected again (*Ps. fordii*), and one is confirmed from the Maroni drainage by only few specimens (*Ps. serratus*). Despite several decades of sampling throughout the Guianas countries, species constituting the *Panaque* group remain scarce and poorly known, as attested by literature and poor representation in collections (MNHN, RMNH and ZMA collections examined by SFM and RC). Apart from *Hemiancistrus medians*, that was collected in several places in the Maroni river basin, other members were sporadically collected. It appears that no specimen of the *Panaque* group was collected in rivers from Central Suriname (see Mol *et al.*, 2012). Within the Essequibo drainage, the area of distribution of *Peckoltia cavatica*, described from the Upper Rupununi River close to Massara in Guyana, is here extended to Siparuni River, a left tributary of the Rupununi. *Peckoltia cavatica* was also found again close to its type locality in sympatry of *Pc. sabaji*. Excluding the representatives of *Peckoltia* within the Essequibo drainage, each species of each genus is allopatric. The Essequibo region is indeed still under the strong influence of the Amazon drainage (see de Souza *et al.*, 2012) and consequently exhibits the highest diversity within *Peckoltia* representatives (*Pc. braueri*, *Pc. cavatica*, and *Pc. sabaji*). From East to West, *Peckoltia simulata* is found in the Oyapock River, *Pc. capitulata* in the Approuague River, and *Pc. otali* in the Maroni/Marowijne River drainages. This latter basin exhibits also the highest diversity of genera of the *Panaque* group, including *Hemiancistrus medians*, *Pseudacanthicus serratus*, *Panaqolus koko* and *Peckoltia otali*. It makes the Maroni River the richest strictly coastal drainage of the Guianas for this group of species. Even if it corroborates other studies (e.g., Covain *et al.*, 2012), additional comparisons to other groups have to be conducted to confirm this observation.

COMPARATIVE MATERIAL

Hemiancistrus medians: all from Maroni River basin: Neotype, SMNS 26503, ex-MHNG 2675.094, Grand Inini River, Saut S; MNHN 2011-0015, ex-MHNG 2675.094, 1, same locality; MHNG 2593.085, 1, GenBank number AJ318368, Grand Inini River, creek upstream Saut S; MHNG 2593.86, 1, Grand Inini River, dead end branch of Saut S; MHNG 2717.005, 1, Suriname, Tapanahony River, Kumaru Konde Sula; MNHN 1998-1905, 2, Grand Inini River; MNHN 1998-16, 1, Litani River, Saut Tetombé; MNHN 1998-1616, 1, Marouini River, vicinity of Antecume Pata; MHNG 2675.095, 1, MHNG 2675.096, 2, inc.1 c&s, MNHN 2000-5740, 1, MNHN 2000-5752, 3, Litani River upstream of Antecume pata; MCP 38715, 1, ex-MHNG 2664.078, MHNG 2664.078, 4, MNHN 2011-0016, 3, ex-MHNG 2664.078, Marouini River, vicinity of Antecume Pata; MNHN 2002-0854, 3, Marouini River, 2 h of boat from Antecume Pata; ZMA 119.868, 6, French Guiana, Maroni River, Saut Singatetei just N of confluence with Tapanahony River; IRD Cayenne, 1, Tampoc River, Kayodé; ZMA 115.301, 1, French Guiana, Maroni basin, Marouini River downstream of Epoia.

Panaqolus changae: ANSP 181097, 1, P6218, Peru, vicinity of Iquitos, Itaya River, Amazonas basin. ***Panaqolus dentex:*** Holotype, BMNH 1867.6.13.37, Peru, Xeberos, upper Aipena River system, Huallaga basin. ***Panaqolus* sp. L204**: MHNG 2710.093, 1, PE08-900, Peru, aquarium trade, San Alexandro River, tributary of Aguaytia, Ucayali basin.

Peckoltia bachi: Holotype, BMNH 1897.12.1.61, Brazil, Jurua River. Paratypes of *P. ucayalensis*, ANSP 68652-68653, 2, Peru, Ucayali River, Contamana; MEPN unnum., 1, Ecuador, Condor Yacu; MHNG 2358.059, 1, Peru, Ucayali River, Pucallpa; MHNG 2721.054, 5, Peru, aquarium trade. ***Peckoltia braueri:*** Holotype, ZMB 3174, paratype, ZMB 3174, Guyana [? Takutu River, Negro River basin]; MHNG 2624.091, 2, aquarium trade, export Boa Vista. ***Peckoltia cavatica:*** CSBD uncat., 3, ex-MHNG 2651.020; MHNG 2651.020, 2, GY04-030, Guyana, Rupununi River, Pregogo; MHNG 2651.044, 1, Guyana, Siparuni River, tributary of middle Essequibo, Iwokrama Forest. ***Peckoltia oligospila***: all from Brazil: Holotype, BMNH 1849.11.8, Brazil, Capin (=Capim) River, tributary of Guamá River, lower Amazon basin; MHNG 2546.097, 8 inc. 1 c&s; MHNG 2552.007, 4, Guamá River at Ourem; MHNG 2550.027, 1, Guamá River 20 km downstream of Ourem; MHNG 2601.078, 1, BR98 076, Mãe do Rio River, tributary of Guamá River; MHNG 2602.006, 1, Guamá River; MHNG 2602.017, 6, BR 98 154-155, Guamá River near Ourem. ***Peckoltia sabaji***: CSBD uncat., 1, ex-MHNG 2651.016; MHNG 2651.016, 1, GY04-029, Guyana, Rupununi River, Pregogo.

Pseudacanthicus fordii: Syntype, BMNH 1866.8.14.150, Suriname. ***Pseudacanthicus leopardus***: Holotype, ANSP 39345, Guyana, Rupununi River; CSBD uncat., 3, ex-MHNG 2651.024; MHNG 2651.024, 3, Guyana, Rupununi River, Pregogo;

MHNG 2588.050, 2; MHNG 2624.096, 3; MHNG 2677.047, 3, aquarium trade (Negro River basin, probably Demini River). ***Pseudacanthicus serratus***: Holotype, RMNH 3125, Suriname; MHNG 1223.014, 1, dried, French Guiana, Mana River, Saut Sabbat; RMNH 6915, 1, Suriname; RMNH uncat., 1, French Guiana, Maroni River basin, Litani River; ZMA 106.331, 2, Suriname, Suriname River, rapid 1 km. South of Botopasi village; ZMA 106.523, 2, Suriname, Marowijne River, ca. 3 km. N of Albina. ***Pseudacanthicus spinosus***: Holotype, MNHN A.9577, Amazon River.

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