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Genomic resources for the monitoring and management of *Tometes trilobatus*, *Hoplias aimara* and *Myloplus rhomboidalis*, three exploited freshwater fish species in French Guiana

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Abstract – The Neotropical region, hosts a quarter of all freshwater fish species, while providing important food resources for local human populations. The management of neotropical freshwater ecosystems is thus of primary importance for both biodiversity conservation and local human sustainability. Recent technological advances in the field of genomics offer new tools for managers and practitioners to monitor entire fish assemblages using environmental DNA (eDNA) metabarcoding, or to detect specific species or populations using targeted eDNA. The availability of species genomics information is thus crucial to implement eDNA monitoring methods. Nevertheless, specific primers allowing species-centred approaches are lacking for most species. In French Guiana, only 18 mitochondrial genomes of freshwater fishes have been published out of more than 400 species known from French Guiana. In this study, we provide genomic resources for *Myloplus rhomboidalis* (locally called Koumarou), *Hoplias aimara* (Aimara) and *Tometes trilobatus* (Pakou), three exploited fish species in French Guiana. We provide complete mitochondrial genomes and tools for the detection of the three fish species by developing a targeted species approach using digital PCR (dPCR) for each species.

Keywords: Digital PCR / mitochondrial genome / environmental DNA / Neotropical / species detection

1 Introduction

One-quarter of the global freshwater fish diversity is found in the Neotropical region (Lévêque *et al.*, 2008). Almost 2200 freshwater fish species are known from the Guiana Shield compared to less than 500 in Europe (Lemopoulos and Covain, 2019; Costa *et al.*, 2021). Nevertheless, the Guiana Shield biodiversity is severely threatened by gold mining which induces deforestation, soil degradation, and a drastic decline in water quality (Cantera *et al.*, 2022, 2023a, 2023b; Timsina *et al.*, 2022). This is particularly worrisome as aquatic ecosystems provide pivotal ecosystem services to local human communities. For those human populations, protein intake largely relies upon river fish. The management of Guianese

freshwater ecosystems is thus of primary importance for both biodiversity conservation and local human sustainability.

Recent technological advances in the field of genomics offer new tools for managers and practitioners to monitor entire fish assemblages using environmental DNA (eDNA) metabarcoding or to detect specific species or populations using targeted eDNA (Taberlet *et al.*, 2018; Condachou *et al.*, 2024). Nevertheless, the implementation of eDNA techniques in management and conservation plans relies on the availability of species genomics information. Indeed, building genomic reference databases is crucial for analysing eDNA samples and requires the sequencing of voucher specimens (Marques *et al.*, 2021). Information about these voucher specimens must be properly referenced and the authenticity of each sequence must be checked by phylogenomic analysis (Botero-Castro *et al.*, 2016). Until now due to the ease of access to the mitochondrial genome in comparison to the nuclear genome, most studies used mitochondrial information

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to build DNA reference databases. Using environmental DNA also implies designing universal or specific primers that can be optimized with such genomic species information. While many universal primers have been designed and evaluated for teleostean fishes (Valentini *et al.*, 2016; Miya, Gotoh and Sado, 2020), specific primers allowing species-centered approaches are lacking for most species. For instance, complete genome sequences are available for no more than 2.4% of the 15 521 threatened species listed by the International Union for Conservation of Nature (IUCN) (Hogg *et al.*, 2022). This situation also applies to freshwater fishes of French Guiana, with only 18 published mitochondrial genomes out of more than 400 species occurring in French Guiana (Sato *et al.*, 2018).

The goal of the study was to provide mitochondrial genomic resources for the study of *Myloplus rhomboidalis* (locally called Koumarou), *Hoplias aimara* (Aimara) and *Tometes trilobatus* (Pakou). Those three large species are among the most researched species by local communities (Longin *et al.*, 2021) and possibly suffer from overexploitation as population trends are decreasing (Allard *et al.*, 2017). Moreover, *T. trilobatus* is strictly endemic to the Oyapock River (Le Bail *et al.*, 2012) and is considered as Near Threatened by the IUCN (Allard *et al.*, 2017). *H. aimara*, although widespread in French Guiana, locally suffers from overfishing for local subsistence and recreational angling, leading to the establishment of local regulations to lower fishing pressure (DEAL, 2017).

In this paper, we used a shotgun sequencing approach to obtain the complete mitochondrial genomes of the species, using multiple specimens from each species to take into account intra-specific variability. We then used these genomic resources to develop and test new digital PCR (dPCR) assays for each species, that could be used in future eDNA studies for species detection and monitoring of local populations.

2 Materials and methods

2.1 Target species

To provide genomic resources for designing *Myloplus rhomboidalis*, *Hoplias aimara*, and *Tometes trilobatus* target species assays, we sequenced the whole mitochondrial genomes of individuals of these three species ($N=9$) and individuals from closely related species ($N=29$) (Tab. 1). No specimen from closely related species was sequenced because *T. lebaili* is the only species from the *Tometes* genera in the Oyapock drainage basin.

2.2 Mitogenome sequencing and phylogenetic analysis

We used a genome-skimming approach as recently performed for other fish species (Murienne *et al.*, 2016; Ory *et al.*, 2019) which allows us to retrieve the high-copy fraction of the genome (*e.g.*, organelle) using shallow shotgun sequencing. Total genomic DNA was extracted from muscle tissue using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. The quality and quantity of extracted genomic DNA was

evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a PicoGreen double-stranded DNA quantitation assay kit (Life Technologies, Carlsbad, CA, USA). Library construction was performed using the Illumina TruSeq Nano DNA Sample Prep Kit following the supplier's instructions (Illumina Inc., San Diego, CA, USA). After shearing by ultrasonication with a Covaris M220 (Covaris Inc., Woburn, MA, USA), purified fragments were A-tailed and ligated to sequencing-indexed adapters. Fragments with an insert size of around 450 bp were selected with Agencourt Ampure XP beads (Beckman Coulter, Inc.), and enriched with 8 cycles of PCR before library quantification and validation. The pool of libraries was then hybridized on one lane of HiSeq 3000 or NovaSeq6000 flow cell using the Illumina TruSeq PE Cluster Kit v.3, and paired-end reads of 150 nucleotides were collected using the Illumina TruSeq SBS Kit v.3 (200 cycles). Sequence data were stored on the NG6 platform (Mariette *et al.*, 2012). Quality filtering was performed by the Consensus Assessment of Sequence and Variation (CASAVA) pipeline.

The complete mitochondrial genomes were assembled *de novo* using NOVOplasty (Dierckxsens *et al.*, 2017) and annotated using MitoAnnotator (Iwasaki *et al.*, 2013). Phylogenetic analyses were performed separately for Serrasalmidae and Erythrinidae. Ribosomal genes were aligned using MAFFT v7 (Katoh and Standley, 2013) and the Iterative refinement method with local pairwise alignment information, with subsequent trimming using trimal 1.4 with the automated1 option. Coding genes were aligned using TranslatorX (Abascal, Zardoya and Telford, 2010) to consider the amino-acid sequence. Individual genes were concatenated using FasnconcatG (Kück and Meusemann, 2010; Kück and Longo, 2014). We used a midpoint rooting approach. A Maximum Likelihood phylogenetic analysis was performed on all thirteen protein-coding genes and rRNA using RAxML-ng (Kozlov *et al.*, 2019) and a GTR+G model was applied for each gene. ML tree search was based on ten random and ten parsimony starting trees. Nodal support was estimated using Transfer Bootstrap Expectation (Lemoine *et al.*, 2018) using 1000 replicates and an automated stopping procedure.

2.3 Development of dPCR probes and primer for the three species of interest

We followed the framework put forward by the DNAqua-Net consortium and the MIQE guidelines to validate our targeted eDNA assays (Huggett, 2020; Thalinger *et al.*, 2021).

2.3.1 Primer design and in silico validation

To implement our dPCR assay, we developed specific primers and probes for the three species of interest (*M. rhomboidalis*, *T. trilobatus* and *H. aimara*). Primers and probes were designed using primer3 software implemented in Geneious (Untergasser *et al.*, 2007).

For the *H. aimara*, these assays targeted a 112 bp region of the *nad5* gene, using the specific forward primer 5'-AATCGCAACATCCTTTACTG -3', the specific reverse primer 5'-TACTGCAGGGGTATTTTCAT -3' and the specific probe Cy5-AACCCACGATTCCTGCCCC-

Table 1. Species, voucher, locality information, collection date, GenBank accession number and coverage (average, minimum and maximum) of the analyzed specimens. Vouchers annotated as ‘**’ are stored in the CRBE biological collection and vouchers annotated as ‘**’ are stored in the MNHG. Coordinates of each collection site are indicated in Table S1.

Family	Species	Voucher	River basin, site	Country	Collection date	Accession number	Coverage
Erythrinidae	<i>Hoplerythrinus unitaeniatus</i>	GEN5094*	Oyapock, Crique Marie	French Guiana	2012-10-09	PP754834	124 Min: 61 Max: 656
–	<i>Erythrinus erythrinus</i>	MITA-15-0012*	Maroni, Mitiraka	–	2015-03-13	PP721227	48 Min: 25 Max: 100
–	<i>Hoplias aimara</i>	HYD15-1069*	Comte, Roche Fendé	–	2015	PP754828	90 Min: 34 Max: 130
–	<i>H. aimara</i>	GEN2797*	Approuague, Crique Laboue	–	2011-11-10	PP754837	43 Min: 18 Max: 118
–	<i>H. aimara</i>	GEN4296*	Sinnamary, Crique Saul	–	2012-10-18	PP754835	150 Min: 90 Max: 288
–	<i>H. aimara</i>	GEN3082*	Oyapock, Crique Trois Sauts Aval	–	2012-10-22	PP754836	36 Min: 13 Max: 69
–	<i>H. intermedius</i>	GEN2521*	NA	NA	NA	NC_033860	NA
–	<i>H. malabaricus</i>	GEN1380*	Maroni, Crique Grande Waki	French Guiana	2012-08-16	PP754838	157 Min: 74 Max: 283
–	<i>H. malabaricus</i>	GEN5218*	Mana, Crique Korossibo	–	2011-10-28	PP754839	17 Min: 4 Max: 33
–	<i>H. malabaricus</i>	GEN5218*	Counamama, Crique Petit	–	2012-10-24	PP754833	554 Min: 266 Max: 863
–	<i>H. malabaricus</i>	GEN5538*	NA	NA	NA	NC_057531	NA
–	<i>H. malabaricus</i>	GEN5538*	NA	NA	NA	AP011992	NA
–	<i>H. malabaricus</i>	GEN5538*	Mana, Crique Petit Laussat Aval	French Guiana	2012-08-29	PP754832	37 Min: 17 Max: 131
Serrasalimidae	<i>Colossoma macropomum</i>		NA	NA	NA	KPI88830	NA
–	<i>Piaractus brachipomus</i>		NA	NA	NA	NC_025315	NA
–	<i>Piaractus</i> sp.		NA	NA	NA	KM435146	NA
–	<i>P. mesopotamicus</i>		NA	NA	NA	NC_024940	NA
–	<i>Metynnix hypsauchen</i>		NA	NA	NA	NC_039113	NA
–	<i>M. lippincottianus</i>	Kaw-METlip-1*	Kaw, Amont Kaw	French Guiana	2018-05-23	PP754827	242 Min: 167 Max: 828
–	<i>Pristobrycon striolatus</i>	FI-15-086*	Maroni	–	2015	PP754846	136 Min: 85 Max: 196
–	<i>Pygopristis denticulata</i>	SUJM-102**	Mapana Creek	Suriname	28-10-2007	PP754825	188 Min: 65

Table 1. (continued).

Family	Species	Voucher	River basin, site	Country	Collection date	Accession number	Coverage
–	<i>Pygocentrus nattereri</i>	FL-15-397*	NA	NA	NA	NC_015840	Max: 256 NA
–	<i>Serrasalmus rhombeus</i>		Maroni, Aval Apatou	French Guiana	2015	PP754842	84 Min: 17
–	<i>S. eigenmanni</i>	FI-15-053*	Maroni	–	2015	PP754848	Max: 126 219 Min: 102
–	<i>S. eigenmanni</i>	18-G-OYA-52*	Oyapock, Manoa Itou	–	2018-10-10	PP754849	Max: 305 502 Min: 329
–	<i>Acnodon oligacanthus</i>	FI-15-088*	Maroni	–	2015	PP754845	Max: 735 208 Min: 161
–	<i>Myloplus rhomboidalis</i>	FL-15-266*	Approuague, Athanasé	–	2015	PP754843	Max: 271 87 Min: 40
–	<i>M. rhomboidalis</i>	FL-15-148*	Maroni	–	2015	PP754844	Max: 124 178 Min: 45
–	<i>M. rhomboidalis</i>	HYD15-0221*	Mana, Angouleme	–	2015	PP754829	Max: 323 111 Min: 60
–	<i>M. rhomboidalis</i>	FL-16-231*	Sinnamary	–	2016	PP754841	Max: 157 115 Min: 70
–	<i>M. rubripinnis</i>	FL-15-071*	NA	NA		NC_039115	Max: 151 NA
–	<i>M. rubripinnis</i>		Maroni	French Guiana	2015	PP754847	188 Min: 100
–	<i>M. ternetzi</i>	GEN163*	Maroni, Crique Nouvelle France	–	2011-10-04	GEN163	Max: 255 353 Min: 233
–	<i>Myleus</i> sp.		NA	NA		NC_015751	Max: 553 NA
–	<i>M. cf. schomburgkii</i>		NA	NA		NC_039114	NA
–	<i>Tometes trilobatus</i>	OYA-Chi-12-12*	Oyapock, Saut Chien	French Guiana	2012-11-15	PP754826	48 Min: 25
–	<i>T. lebaili</i>	GFSU14-141**	Maroni Saut Wayo	–	2014-09-23	PP754830	Max: 100 53 Min: 12
–	<i>Myloplus planquettei</i>	GF15-129**	Maroni Langa Sulu	–	2015-11-24	PP754831	Max: 76 187 Min: 38
							Max: 245

QXL670. For *M. rhomboidalis*, a 125 bp fragment of the *nad4* gene was targeted, using the specific forward primer 5'-CGGTGTTACCTCTGAC-3', the specific reverse primer 5'-GGTCAAGTATGACTATCATTCG-3', and the specific probe ROX-CCGTAGCCGGTTCAATGGTCTAGCTGC-QXL 610. For the *T. trilobatus*, a 127 bp fragment of the *cox1* gene was targeted, using the specific forward primer 5'-CCAGCCATTTACAAATATCAA-3', the specific reverse primer 5'-TGAGGTTTCGATCTGTAAGG-3' and the specific probe TAMRA-CTCTCTGCCTGTTCTGGCTGCCGA-BHQ2.

The specificity of the *H. aimara* primer and probes were tested *in silico* against all complete mitogenomes of the Erythrinidae family ($N=13$). The dPCR design of *T. trilobatus* and *M. rhomboidalis* was tested against all complete mitogenomes of the Serrasalmidae family ($N=24$). Then, the specificity of each primer and probes were checked against the NCBI database using blast (Altschul *et al.*, 1990).

2.3.2 In vitro validation of dPCR

To test *in vitro* the specificity of each dPCR design, DNA was extracted from individuals of each species and their close relatives. Extraction was performed from muscle tissue using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA), following a protocol adapted from the manufacturer's instructions. To test *in vitro* specificity of *M. rhomboidalis*, DNA was extracted from three *Myloplus* individuals: *M. planquettei* collected in French Guiana (Maroni river basin); *M. rubripinnis* (FL-15-071) sampled in French Guiana (Maroni river basin) and *M. ternetzi* (GEN163) sampled in French Guiana (Maroni river basin). Concerning *T. trilobatus* this specificity was tested with *T. lebailli* (GFSU14-141) collected in French Guiana (Maroni river basin). The specificity of *H. aimara* design was tested against four individuals of *H. aimara* collected in different French Guiana river basins (HYD15-1069, GEN4296, GEN3082, GEN2797). This design was also tested against other Erythrinidae species: *H. malabaricus* (GEN1380, GEN5538, GEN2521), *Hoplerythrinus unitaeniatus* (GEN5094) and *Erythrinus erythrinus* (MITA-15-0012).

2.3.3 dPCR assays

For each of the three *in vitro* specificity test, dPCR reaction mixtures were prepared in a pre-plate as follows. For Nanoplate 26K reactions (Qiagen, Cat.No/ID:250002), 10 μ L of 4X QIAcuity Probe PCR kit (Qiagen, Cat.No/ID:250101), 1 μ L of the 20X sets of primers and probes (*M. rhomboidalis*, *H. aimara* and *T. trilobatus*), 18 μ L of eDNA and RNase-free water were combined to reach a final reaction volume of 40 μ L.

Reaction mixtures were transferred into a QIAcuity Nanoplate and loaded onto the QIAcuity Eight instrument to perform dPCR. The amplification step was performed following this cycling protocol: 2 min at 95°C for enzyme activation, 15 s at 95°C for denaturation, and 30 s at 58°C for annealing/extension during 40 cycles. Then an imaging step was completed by reading the following channels: orange (excitation 543–565 nm; emission 580–606 nm) for *T. trilobatus*, crimson (excitation: 590–640 nm; emission 654–692)

for *H. aimara* and red (excitation 570–596 nm; emission 611–653 nm) for *M. rhomboidalis*. Data were analyzed using the QIAcuity Software Suite V1.2 and expressed as copies/ μ L of reaction volume (40 μ L final). Negative controls were displayed on each assay.

3 Results

3.1 Mitogenomes organisation

The mitochondrial genomes (Tab. 1) show the typical gene arrangement for vertebrates. As an example, we hereafter provide information about the mitogenome of *Hoplias aimara* (specimen GEN4296). The mean insert size at the library preparation step was 517 base pairs. After sequencing on one lane of an Illumina NovaSeq6000 along with other libraries, we obtained 4 142 388 paired reads. The circular mitochondrial genome was assembled based on 15 692 mitochondrial reads representing 0.21% of the total reads. All protein-coding genes started with an ATG codon except for the cytochrome c oxidase subunit I (*cox1*) genes which started with the GTG codon. Six TAA stop codons and one TAG stop codon were identified. Six incomplete stop codons were found (*nad2*, *cox2*, *nad3*, *nad4*, *nad6*, *cob*) adjacent to transfer RNAs encoded on the same strand.

3.2 Phylogeny of Erythrinidae

After concatenating the individual markers, our final supermatrix contained 14 047 sites with 2429 patterns distributed over 15 partitions. Our analysis stopped after 50 bootstrap replicates and yielded a Maximum Likelihood tree with a LogLikelihood of -47 792. The monophyly of genus *Hoplias* was highly supported with 100% bootstrap frequency [BF] (Fig. 1). *H. intermedius* was the sister group to *H. malabaricus* species (100% BF) and *H. aimara* sister to those two species. When several specimens from the same species were included, they all formed distinct monophyletic groups.

3.3 Phylogeny of Serrasalmidae

After concatenating the individual markers, our final supermatrix contained 14 102 sites with 4333 patterns distributed over 15 partitions. Our analysis stopped after 100 bootstrap replicates and yielded a Maximum Likelihood tree with a LogLikelihood of -80 952. Three distinct phylogenetic groups are depicted in Figure 2. They correspond to the subfamily Colossomatinae, and the Serrasalmiinae tribes Myleini and Serrasalmiini (Fig. 2). Genera *Myloplus* and *Tometes* were not monophyletic. *Acnodon oligacanthus* was sister to *Myloplus rhomboidalis* (96% BF).

3.4 Specificity testing

Testing the *H. aimara* design against all mitogenomes from the Erythrinidae family revealed the specificity of the design with a high level of mismatch between *H. aimara* *nad5* primers and probes regions and other Erythrinidae species (Fig. 1). One mismatch is present within the *nad5* region of the different

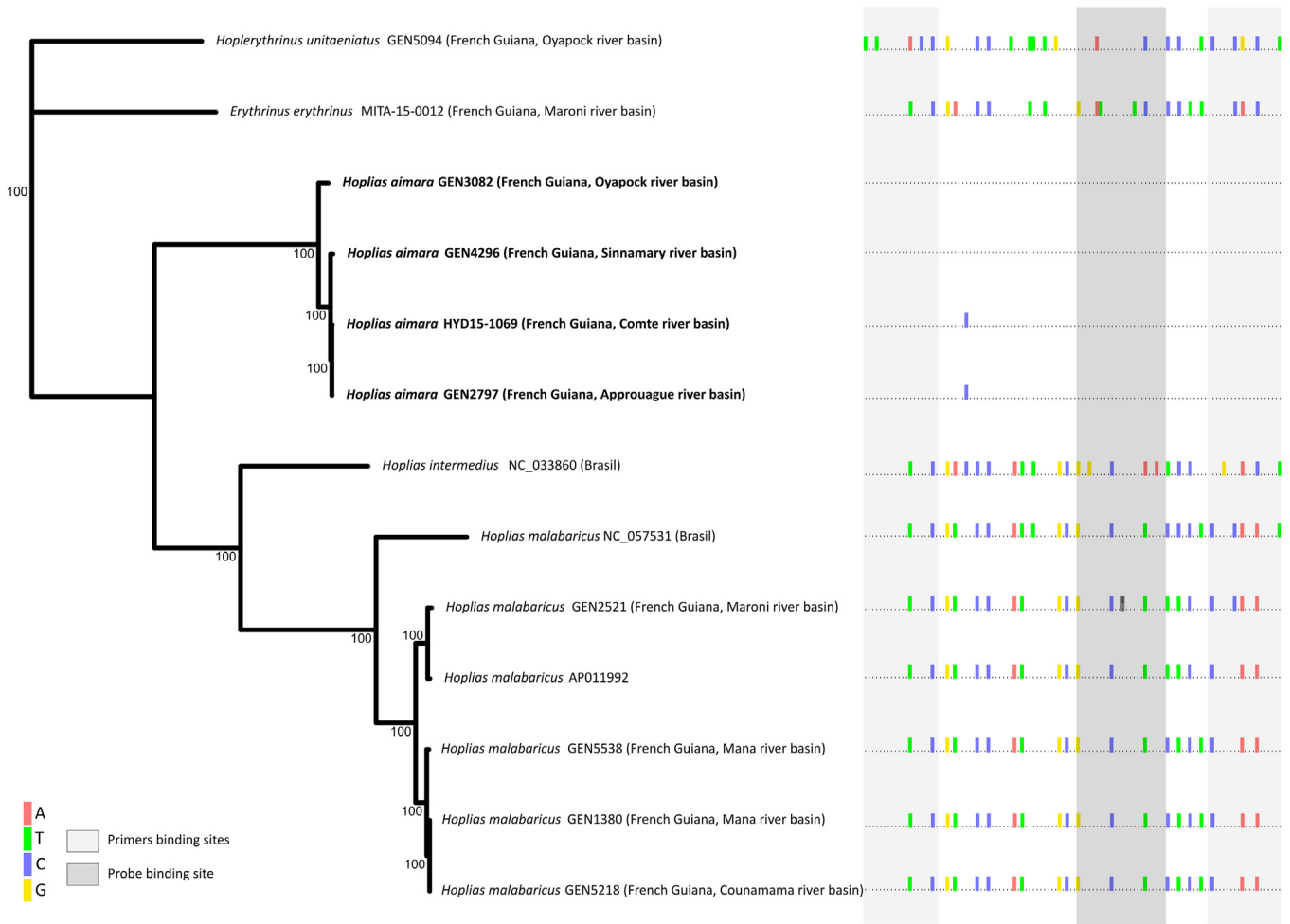


Fig. 1. Maximum likelihood phylogeny of the Erythrinidae family inferred from complete mitochondrial genomes using RAxML-NG. Bootstrap support frequencies are indicated on nodes. Amplified nad5 fragments of the *H. aimara* target assay are represented on the right. Highlighted nucleotides correspond to disagreement from the reference *H. aimara* sequence (GEN4296).

H. aimara species but not in the primer or probe binding site thus not affecting the design specificity. The dPCR test with *H. aimara* tissue showed DNA amplification of each of the 4 individuals of *H. aimara* tissue and no DNA amplification of any *H. malabaricus*, *H. unitaeniatus* or *E. erythrinus* tissue.

The *in silico* specificity testing of *T. trilobatus* design revealed no co-amplification of any species belonging to the Serrasalminidae family (Fig. 3). The *in vitro* test for *T. trilobatus* revealed the specificity of the design as no amplification of closely related species (*T. lebaili*) occurred.

4 Discussion

This study provides new genomic resources for three commercially and culturally valuable fish species in French Guiana. Complete mitogenomes have been newly analysed for 16 species (26 specimens: 10 Erythrinidae and 16 Serrasalminidae). Phylogenomic analysis of the Serrasalminidae family showed a topology similar to the one obtained from the analysis of 2708 UCE (Ultra Conserved Elements) loci (Mateussi *et al.*, 2020). The Colossomatinae subfamily is sister-group to the Serrasalminae subfamily which is

subdivided into two distinct tribes (Myleini and Serrasalmini). Our phylogenomic analysis also supports previous findings on the non-monophyly of genus *Myloplus* and *Tometes* (Mateussi *et al.*, 2020). The phylogenetic position of genus *Acnodon* has long been uncertain in the literature (Orti *et al.*, 2008; Mateussi *et al.*, 2020; Kolmann *et al.*, 2021). Based on our complete mitogenome analysis, the position of this genus in the phylogeny was consistent with the one proposed by (Mateussi *et al.*, 2020) thus confirming genus *Acnodon* as a sister group to *Myloplus rhomboidalis*. It is interesting to notice that all those results are congruent between our study using complete mitochondrial genomes and previous studies based on nuclear UCEs (Mateussi *et al.*, 2020), highlighting the robustness of both findings.

The phylogenomic analysis of the Erythrinidae family provides a first overview of the phylogenetic position of French Guiana species of Erythrinidae. The genus *Hoplias* was found to be monophyletic, with *H. intermedius* sister to *H. malabaricus*. The French Guiana specimens of *Hoplias malabaricus* present a high degree of divergence (92.7% identity) with the specimen sampled in Brazil (NC_057531). The mitogenome of AP011992 is nested well within the French

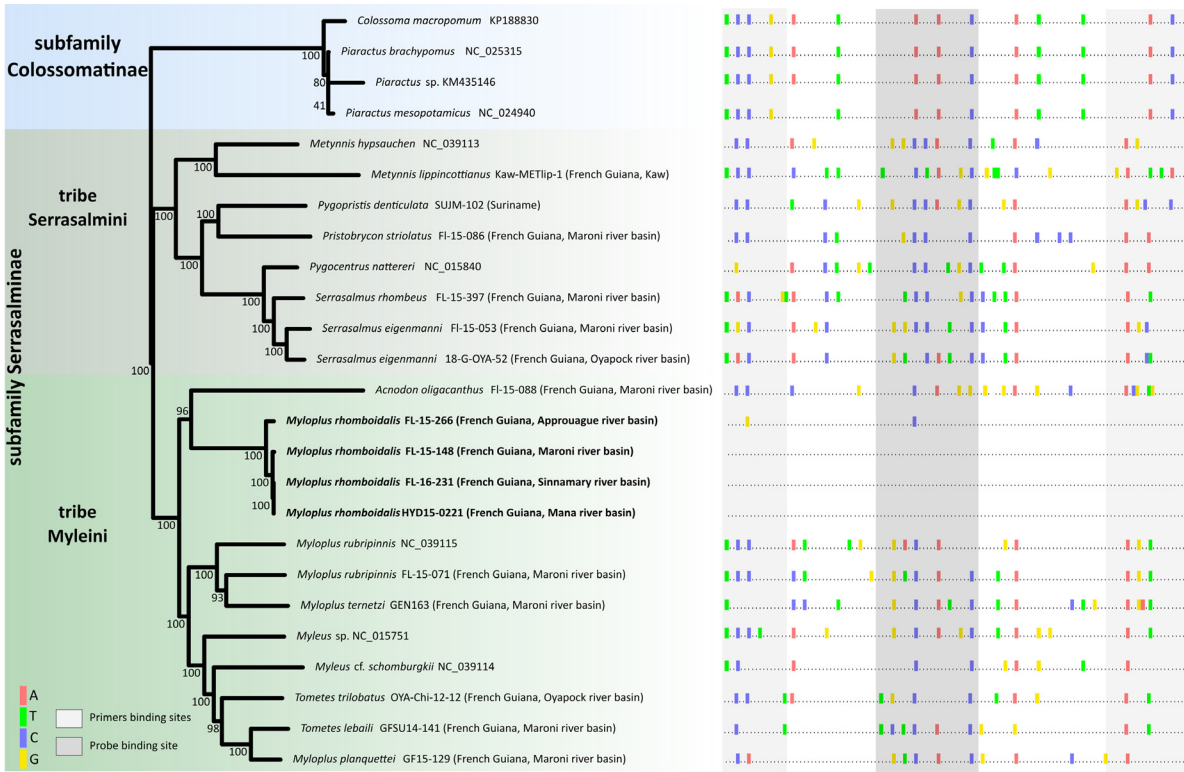


Fig. 2. Maximum likelihood phylogeny of the Serrasalminae subfamily inferred from complete mitochondrial genomes using RAxML-NG. Bootstrap support frequencies are indicated on nodes. Amplified *nad4* fragments of the *M. rhomboidalis* target assay are represented on the right. Highlighted nucleotides correspond to disagreement from the reference *M. rhomboidalis* sequence (FL-15-148).

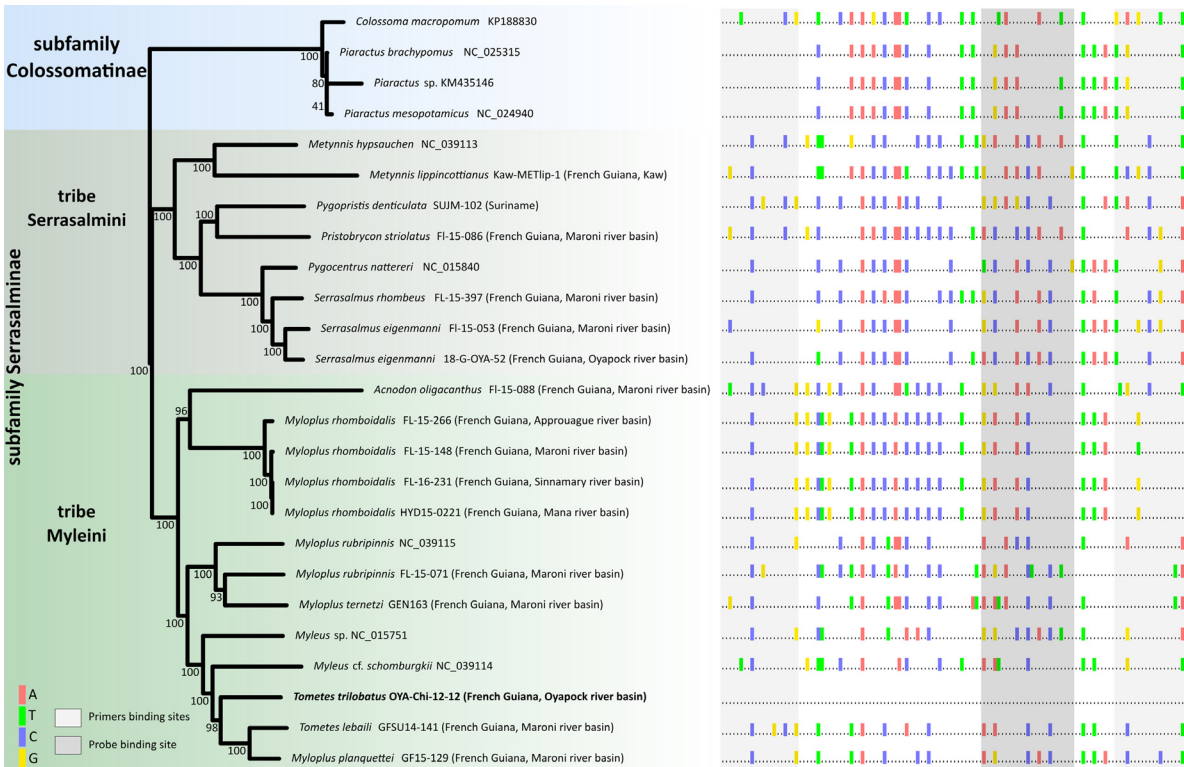


Fig. 3. Maximum likelihood phylogeny of the Serrasalminae subfamily inferred from complete mitochondrial genomes using RAxML-NG. Bootstrap support frequencies are indicated on nodes. Amplified *cox1* fragments of the *T. trilobatus* target assay are represented on the right. Highlighted nucleotides correspond to disagreement from the reference *T. trilobatus* sequence (OYA-Chi-12-12).

Guiana lineage but no information about its origin was available.

Information provided by the complete mitogenome enabled the design of specific primers and probes. This study thus provides ready-to-use sets of probes and primers specific for three species of high interest in French Guiana. Alignments of the target region of each species showed a high species specificity of each assay while taking into account population-level variability. The *M. rhomboidalis* design provided in this study might be less efficient for populations originating from the Approuague River. Indeed, even a few mismatches (*i.e.*, two) can alter the amplification efficiency of the set of primers and probe.

Targeted approaches are widely used corresponding to 68% of eDNA-based-fish studies (Yao *et al.*, 2022). Yet, only 19% of those studies target species of economic interest (Yao *et al.*, 2022). The three targeted species in this study are of high commercial and cultural value, providing food resources for several local communities. The development of such eDNA monitoring approaches should allow managers to perform a non-invasive follow-up of these fish populations. Indeed, dPCR procedures offer a fast and low-cost tool to evaluate species presence on large spatial scales (Condachou *et al.*, 2024). Moreover, targeted approaches such as dPCR are promising tools for investigating fish abundance. In controlled environments, several studies found positive relationships between eDNA concentration and fish biomass (Takahara *et al.*, 2012; Klymus *et al.*, 2015; Benoit *et al.*, 2023). Fernandez *et al.* (2023), showed a positive correlation between eels biomass estimated with electrofishing and eDNA concentration in Spanish Rivers. In freshwater of southern Australia, Rourke *et al.* (2024) also found a relationship between golden perch eDNA concentration and relative biomass estimated with electrofishing but not with relative abundance. Indeed, in natural environments, various biotic and abiotic factors affect the fate (production, degradation, transport) of eDNA in waters and thus alter the eDNA/biomass relationship (Yates, Fraser and Derry, 2019). Future studies should therefore explore the complex links between eDNA abundance in the water and targeted species biomass and abundance.

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Conflicts of interest

Laetitia Pigeyre is a research scientist at a private company specializing in the detection of environmental DNA by digital PCR.

Data availability statement

Genbank accession number for sequences will be available at the time of the publication. Vouchers are conserved and available in the biological collection of the CRBE (Toulouse 3 University) and at the Natural Museum of Geneva.

Author contribution statement

CC, JM, and SB conceived the ideas and design methodology; JM, SB, RV and RC, collected the data; LP and YC conducted the laboratory work; CC, JM and SB analyzed the data; CC, JM and SB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Supplementary material

Table S1. Site coordinates.

The Supplementary Material is available at <https://www.kmae-journal.org/10.1051/kmae/2024011/olm>.

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