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SHORT REPORT

Phylogenomics of the critically endangered *Harttiella* (Siluriformes, Loricariidae) based on complete mitochondrial genomes

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Abstract

The genus *Harttiella*, belonging to the armoured catfish family Loricariidae subfamily Loricariinae (Siluriformes), comprises species with narrow geographical ranges, predominantly endemic to river basins in French Guiana and Suriname. Despite recent taxonomic advancements, including the description of new species, *Harttiella* faces conservation challenges due to its rarity, limited dispersal capacities, and habitat fragmentation. To elucidate the evolutionary history and aid in conservation efforts, we sequenced complete mitochondrial genomes for all known *Harttiella* species. Our analysis revealed distinctive molecular features, including unique stop codon usage and positioning, potentially serving as molecular synapomorphies for the genus. Phylogenetic reconstructions supported previous findings and highlighted the complex evolutionary relationships within the genus. Furthermore, our study provides foundational genomic resources for developing targeted environmental DNA approaches to monitor and conserve *Harttiella* populations effectively. These findings contribute to a comprehensive understanding of *Harttiella* evolution and inform conservation strategies aimed at preserving this unique group of freshwater fish.

Keywords Conservation · Endangered species · Mitogenomic · Neotropics

Introduction

The genus *Harttiella* belongs to the armoured catfish family Loricariidae subfamily Loricariinae (Siluriformes). Detailed molecular phylogenetic analyses (Covain et al. 2016) recently clarified the relationship of 31 different genera of the Loricariinae (vs. Hypoptopomatinae, Hypostominae, Lithogeninae, Rhinelepinae), with *Harttiella*, along with two other genera, *Harttia* and *Cteniloricaria*, belonging to the Harttiini tribe (vs. Loricariini). The genus *Harttiella* was established in 1971 (Boeseman 1971) based on specimens from Suriname originally described as *Harttia crassicauda* (Boeseman 1953). More recently, six new species were described, all endemic to French Guiana: *Harttiella intermedia*, *Harttiella janmoli*, *Harttiella longicauda*, *Harttiella lucifer*, *Harttiella parva* and *Harttiella pilosa* (Covain et al. 2012). Additionally, two potentially new species (*Harttiella* n. sp. aff. *lucifer* and *Harttiella* n. sp. Makwali) have been collected but await formal description.

Harttiella species typically exhibit very limited geographical ranges, with the exception of *H. longicauda*, which is distributed across four river drainage basins. Most species are endemic to a single river basin, occupying only a few localities, predominantly found in small perennial forest streams at elevations ranging from 120 to 800 meters. These species exhibit a preference for specific sandy and rocky bottom microhabitats associated with steep-sloped streams and waterfalls, which are spatially constrained within mountainous regions of French Guiana and Suriname. Due to their rarity, low fecundity, and limited dispersal capacities, the seven formally described species in French Guiana and Suriname are considered threatened by the IUCN (Allard et al. 2017; Ballen 2023a, b, c, d, e, f, g), with four species classified as Critically Endangered, two species as Endangered, and one as Vulnerable.

Our objective is to sequence the complete mitochondrial genomes of all *Harttiella* species. This initiative aims to establish a robust phylogenetic framework for the

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Harttiini tribe, supporting the development of a National Action Plan for the conservation of *Harttiella* species in French Guiana. Given that the type species of the genus, *H. crassicauda*, is the sole species known outside of French Guiana, determining its phylogenetic position is crucial for understanding the regional biogeography of the group. Moreover, our phylogenetic framework will enable the testing of hypotheses concerning the mechanisms driving local biodiversity. Specifically, we seek to investigate whether the presence of two species in Mont Galbao (*H. lucifer* and *Harttiella* n. sp. Makwali) results from a dispersal event between river basin heads or from the isolation of an ancestral population of *H. lucifer* in the Makwali stream, which is part of the Maroni drainage headwaters housing several *H. lucifer* populations. Additionally, the mitochondrial genomes generated in this study will serve as foundational resources for future genetic investigations, facilitating the design and testing of specific primers for targeted studies, including environmental genomic approaches focused on detecting and monitoring *Harttiella* populations.

Materials and methods

Taxonomic sampling. The complete list of *Harttiella* species is provided hereafter (see Table 1 for details and Fig. 1 for a map of the sampled localities). It contains seven nominal species and two putative undescribed species still awaiting formal description. We also included information about their conservation status according to the national and international Red List evaluation of the IUCN (Allard et al. 2017; Ballen 2023a, b, c, d, e, f, g).

Our dataset includes for the first time all the described species of *Harttiella* and one of the two putative species pending formal taxonomic description. We also included in our dataset representatives of the two other genera present in the Harttiini tribe, namely *Harttia fowleri* and *Cteniloricaria platystoma*. Our dataset thus includes all the genera present in the tribe Harttiini.

DNA extraction and sequencing. To provide genomic resources for designing species-specific assays, we sequenced the complete mitochondrial genomes of all known *Harttiella* species (except *Harttiella* n. sp. aff. *lucifer*) as well as their closest allies, *Harttia* and *Cteniloricaria*. We

Table 1 List of the species and specimens included in this study

Species	Endemic Region	Known Localities	IUCN Status	Specimens Included
Harttiella crassicauda	Suriname	Nassau Mountains, Paramaka Creek (Maroni River tributary)	Endangered	MUS221:MHNG2674.051 – Paramaka Creek, Nassau Mountains, Suriname (4.820278, -54.605556)
Harttiella intermedia	French Guiana	Trinite Mountains (Sinnamary basin headwaters)	Critically Endangered	MUS651:MHNG2713.087 PARATYPE – Crique Grand Leblond, Trinite Mountains (4.60972, -53.35917)
Harttiella janmoli	French Guiana	Kotika Mountains (Maroni River basin)	Critically Endangered	Ech1:MHNG2695.059 – Montagne Kotika (3.95444, -54.18056)
Harttiella longicauda	French Guiana	Mana, Sinnamary, Approuague, Comte/Orapu river basins	Vulnerable	HYD15-077 – Bois Bande, Comte basin (4.25404, -52.5296); GEN5336 – Crique Georges, Sinnamary basin (5.09911, -53.05293)
Harttiella lucifer	French Guiana	Mana and Maroni basins (11 sites total)	Endangered	GEN3760 – Crique Montagne, Mana basin (4.712, -53.94666); NFV2301 – Crique Nouvelle France, Maroni basin (3.631971, -53.165539)
Harttiella parva	French Guiana	Atachi Bakka Mountains (Maroni basin)	Critically Endangered	MUS612:MHNG2723.093 (PARATYPE) – Atachi Bakka Mountains (3.55, -53.9167)
Harttiella pilosa	French Guiana	Comte/Orapu basin (2 localities)	Critically Endangered	PYLB17-042 – Crique Grillon, Orapu River (4.28018, -52.4519)
Harttiella n. sp. Makwali	French Guiana	Mont Galbao (2 sites)	Not Evaluated	Gal-08-1 – Crique Makwali near Mont Galbao, Maroni basin (3.60066, -53.29842)
Harttiella n. sp. aff. *lucifer*	French Guiana	Mont Itoupe (Oyapock drainage basin; 2 sites)	Not Evaluated	Not included

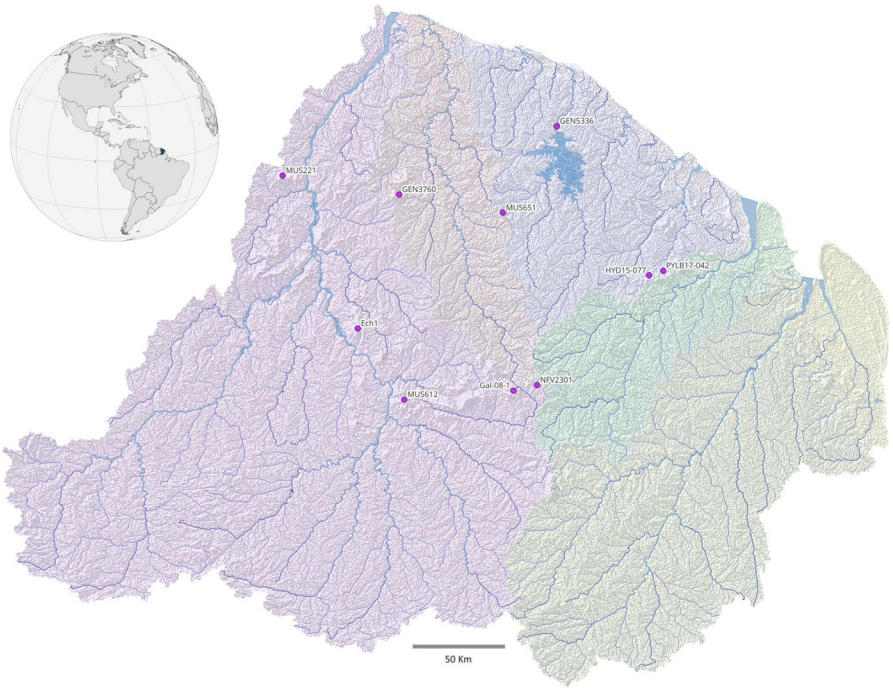

Fig. 1 Map presenting the localities of the samples included in this study. Major river basins are highlighted in color (from West to East): *purple* Maroni, *orange* Mana, *blue* central, *green* Approuague and *yellow* Oyapock. *Harttiella crassicauda* (MUS221), *H. intermedia* (MUS651), *H. janmoli* (Ech1), *H. longicauda* (HYD15-077, GEN5336), *H. lucifer* (NFV2301, GEN3760), *H. parva* (MUS612), *H. pilosa* (PYLB17-042), *Harttiella* n. sp. Makwali (Gal-08-1)

used a genome-skimming approach, as recently performed for other fish species (Murienne et al. 2016; Ory et al. 2019; Condachou et al. 2024b), 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 a protocol adapted from the manufacturer's instructions. The quality and quantity of extracted genomic DNA were 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 Bioruptor pico (Diagenode S.A., Seraing, Belgium), 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 Illumina HiSeq3000 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).

Mitogenomes analyses. The complete mitochondrial genomes were assembled *de novo* using NOVOplasty (Dierckxsens et al. 2017), recursively using the closest relative as a seed. Assembled mitogenomes were annotated using MitoAnnotator (Iwasaki et al. 2013). 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 (Capella-Gutiérrez et al. 2009) with the automated1 option. Coding genes were aligned using TranslatorX (Abascal et al. 2010) to consider the amino-acid sequence. Individual genes were concatenated using FasconcatG (Kück and Meusemann 2010; Kück and Longo 2014). We used *Harttia fowleri* and *Cteniloricaria platystoma*, two members of the Harttiini tribe as outgroups. A Maximum Likelihood phylogenetic analysis was performed on all thirteen protein-coding genes and two 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 an automated stopping procedure.

As an example of the use of those genomic resources, we here provide a depiction of the region targeted by primers and probes specifically targeting *H. lucifer* and *Harttiella* n. sp. Makwali species. The details of the primer design, *in silico* and *in vitro* testing can be found in (Condachou et al. 2024a).

Data quality. To check the quality of the assembly, reads were mapped in Geneious R9 (Kearse et al. 2012) using the low-sensitivity option. We also checked for potential contamination or mislabeling issues by extracting the COI gene

and using the BOLD identification engine to verify that the taxonomic assignation matched our initial identification.

Results

The mean insert size at the library preparation step was around 550 base pairs. After sequencing one lane of an Illumina HiSeq3000 or NovaSeq6000 along with other libraries (96 libraries per lane), we obtained between four million and 16 million paired reads per sample. The mitochondrial reads were represented by less than 1% of the total reads. After remapping the reads in Geneious, the mean coverage ranged from 20X for Ech1 (the paratype of *Harttiella janmoli* preserved in formaline) to 280X for Gal08-01 (*Harttiella* n. sp. Makwali). The mitochondrial genomes (Fig. 2) show the typical gene arrangement for vertebrates (see Satoh et al. 2016, for a review of the structure of mitochondrial genomes in fishes). Among the nine types of start codon present in fish (Satoh et al. 2016), ATG was used predominantly. GTG was used exclusively for cox1 and in eight cases for atp8. Of the four types of complete stop codons, only TAA and TAG were observed in Harttiini. Incomplete stop codons T- was found exclusively in nad2, cox2, cox3, nad3 and nad4L.

After concatenating the individual markers, our final supermatrix contained 14,056 sites with 2,167 patterns distributed over 15 partitions. Our analysis stopped after 1,000 bootstrap replicates and yielded a Maximum Likelihood tree (Fig. 3) with a LogLikelihood of -44,581. The monophyly of *Harttiella* was highly supported with 100% bootstrap frequency [BF]. *Harttiella lucifer* was the sister to the remaining *Harttiella* species (100% BF). *Harttiella intermedia* was nested within the two specimens of *H. longicauda*. *Harttiella pilosa*, *H. crassicauda*, *H. janmoli* and *Harttiella* n. sp. Makwali formed a monophyletic group supported by high bootstrap frequency (100%). The primers and probes targeting the species *H. lucifer* and *Harttiella* n. sp. Makwali (Fig. 4) show that all the species not targeted have mismatches in both primers and probes, highlighting the specificities of the developed essays. The datasets generated and analysed during the current study are available on GenBank (https://www.ncbi.nlm.nih.gov/) under accession numbers PP747111-PP747121.

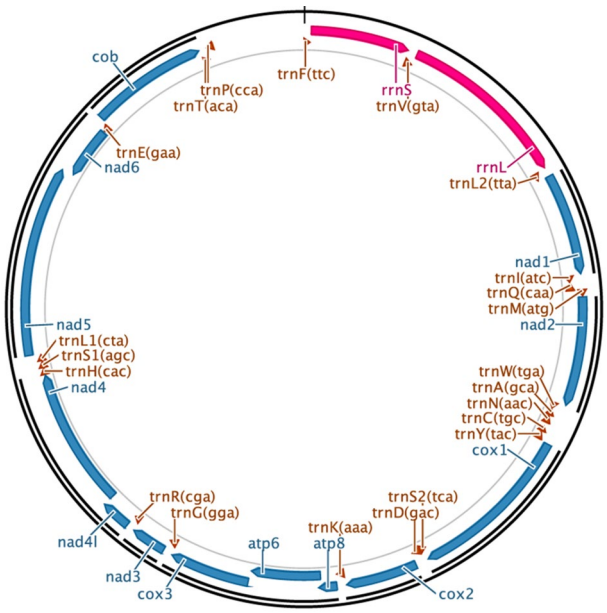

Fig. 2 Structure of the 16,472 bp long mitochondrial genome of *Harttiella lucifer* (NFV2301). Ribosomal genes (*pink*), coding genes (*blue*) and transfer RNAs (*brown*) are depicted with arrows representing their position on the L or H strand

Discussion

The complete mitochondrial genomes acquired for the tribe Harttiini exhibit the typical genome structure observed in vertebrates, a pattern largely shared among fish, although exceptions have been noted (Satoh et al. 2016). While the genome structure and codon usage appear highly conserved, our analysis revealed two molecular features that could serve as molecular synapomorphies for the genus *Harttiella*. Firstly, in the cox1 gene, *Harttiella* species utilize the TAG stop codon, whereas outgroup species employ TAA. Additionally, the stop codon in *Harttiella* is positioned eleven amino acids downstream compared to outgroup species. Similarly, in the atp8 gene, the stop codon in *Harttiella* occurs seven amino acids downstream compared to outgroups.

Our phylogenetic tree broadly confirms the topology found using a combination of mitochondrial (12S and 16S) and nuclear (f-rtn4r) markers (Covain et al. 2016). *Harttiella lucifer* is a sister to the remaining species. The latter split into two groups, one comprising *H. longicauda* and *H. intermedia*, and a second group with the remaining species. *Harttiella intermedia* presents a phylogenetic position nested with the two specimens of *H. longicauda*. This result resonates with previous findings based on cox1 (Covain et al. 2012) or based on a combination of mitochondrial and nuclear markers (Covain et al. 2016). This is particularly troubling as morphometric analyses (Covain et al. 2012) showed that the two species are perfectly distinct. As already concluded, *H. intermedia* may have split from *H. longicauda* through a recent vicariant event (Covain et al. 2016) but alternative explanations such as morphological plasticity or introgression could be plausible.

On a local scale, our phylogenetic framework allows investigation of the potential mechanisms responsible for

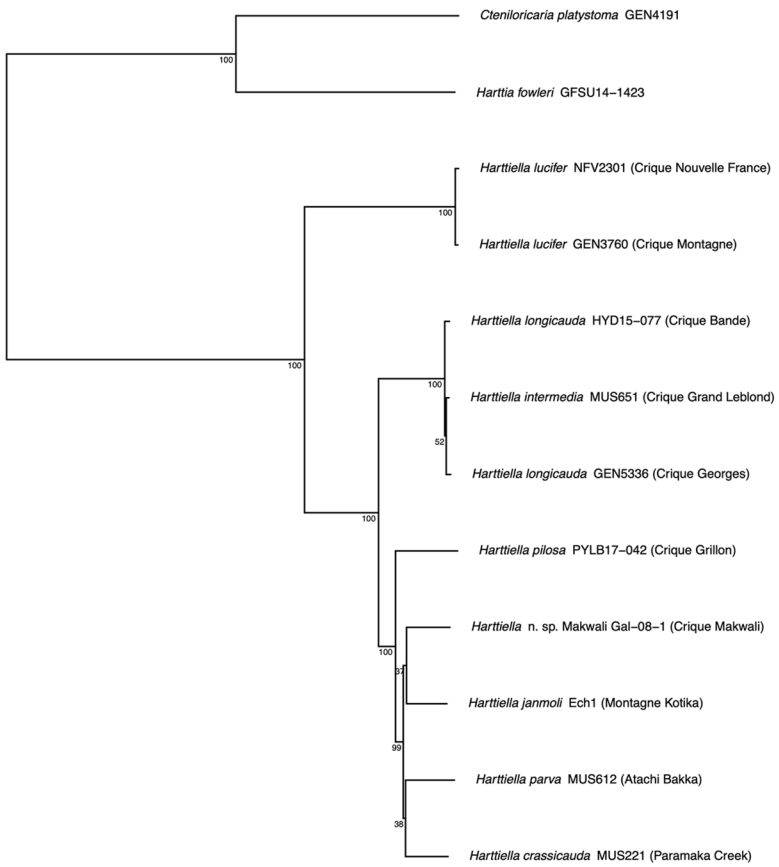

Fig. 3 Maximum Likelihood phylogeny of the Harttiini tribe inferred from complete mitochondrial genomes using RAxML-NG. Bootstrap support frequencies are indicated on nodes

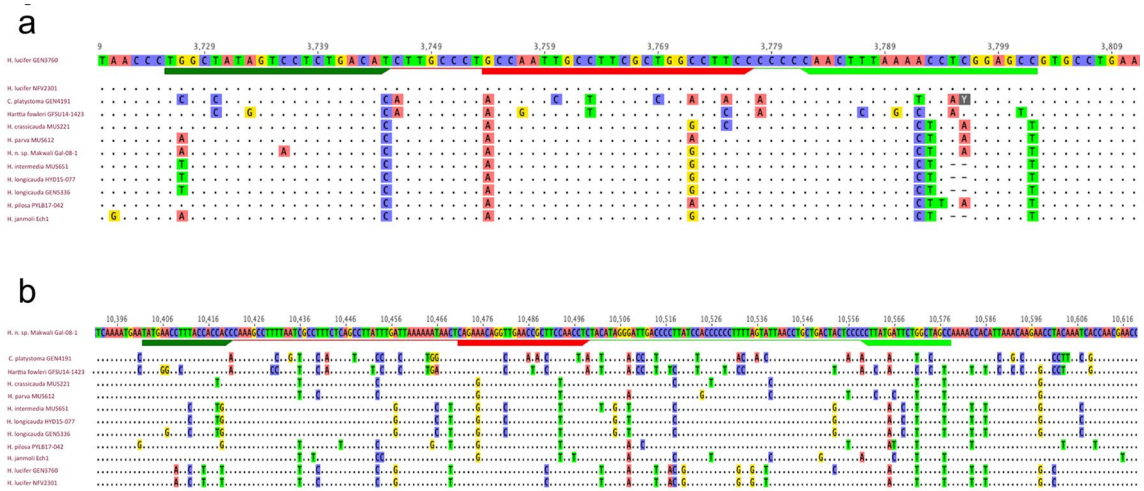

Fig. 4 Alignment depicting the position of the primers and probes designed for the specific detection of *Harttiella lucifer* (panel a) and *Harttiella* n. sp. Makwali (panel b) (see Condachou et al. 2024a for further details). Forward primer in *dark green*, reverse primer in *light green* and probe in *red*

the buildup of local biodiversity. In particular, we wanted to test whether the two species present in Mont Galbao (*H. lucifer* and *Harttiella* n. sp. Makwali) could be linked to a speciation event as the two species are geographically close in the same river drainage. Surprisingly, the two species are not phylogenetically closely related, with *Harttiella* n. sp.

Makwali sister to *H. janmoli* and *H. lucifer* sister to all the remaining species of the genus.

The genus *Harttiella* is currently under particular scrutiny and subject to a "National Action Plan". One "action" corresponds to the development of innovative prospection methods to obtain a better understanding of the distribution of the species and possibly monitor the known populations. In this context, environmental DNA appears as a promising approach, either targeting multiple species through metabarcoding or using species-specific approaches such as digital PCR (Condachou et al. 2024a). The complete mitochondrial genomes analysed in the present study provide meaningful genomic resources to develop specific primers and probes for further environmental DNA studies.

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Declarations

The authors declare that they have no conflict of interest. This work follows the Nagoya protocol within the Convention on Biological Diversity. Access and Benefit Sharing permit ABSCH-IRCC-FR-245902-1 covering all the species under consideration in this study was obtained from the French Ministry of Environment.

Competing interests The authors declare no competing interests.

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