

GENOME SEQUENCING

Whole-Genome Sequencing of two *Listrura* and five *Microcambeva* Species (Trichomycteridae, Siluriformes), Rare and Threatened catfishes from the Atlantic Forest

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Biodiversity Genomes

Listrura and *Microcambeva* are two genera of small, rare and elusive catfishes of the Trichomycteridae family, endemic to the Atlantic Forest coastal drainages in Brazil. *Listrura* comprises 13 species with slender bodies, absent pelvic fins, and a distinct caudal-fin and caudal-peduncle morphology, while *Microcambeva* includes eight species with translucent bodies, typically buried in sandy substrates with exposed nasal barbels. The two genera inhabit fragile environments that are undergoing accelerated degradation, with *Listrura camposae* and *L. nematopteryx* classified as "Critically Endangered" (CR), and *L. picinguabae* as "Near Threatened" (NT). *Microcambeva* species face similar threats, with *M. bendego* proposed as CR and *M. draco* as "Endangered" (EN). Over the past two decades, collection efforts and taxonomic descriptions have expanded the diversity of these two genera, with descriptions of 11 species of *Listrura* and seven of *Microcambeva* species in that period. Recently, Costa & Katz (2021) further subdivided each genus into three subgenera based on pectoral-fin morphology and molecular phylogenetic results. To address gaps in genetic data for those taxa, we herein present whole-genome sequences for three *Listrura* species — *L. nematopteryx*, *L. tetraradiata*, and *L. boticario* — and five *Microcambeva* species — *M. barbata*, *M. watu*, *M. jucuensis*, *M. bendego*, and *M. ribeirae*. These genomic data are critical for future studies on the evolutionary history and systematics of these unique catfishes, contributing to foundational knowledge of their biodiversity.

Introduction

The Trichomycteridae genera *Listrura* de Pinna 1988 and *Microcambeva* Costa and Bockmann 1994 currently include 13 and eight species respectively, of diminutive and elusive catfishes. *Listrura* species have a distinctive morphology, characterized by a slender and elongate body, nearly eel-like in some species, with a unique caudal-fin morphology with a prominent membrane on the caudal peduncle supported by numerous procurent rays, and entirely lacking pelvic fins and girdle (Fig. 1a and 1b) (de Pinna 1988; Villa-Verde et al. 2013, 2022). *Microcambeva* species are characterized by a translucent, miniaturized morphology (standard length not exceeding 50mm) (Fig. 1c to 1g). They are distinguished by relatively subtle morphological characters (Costa and Bockmann 1994; Sarmiento-Soares et al. 2019).

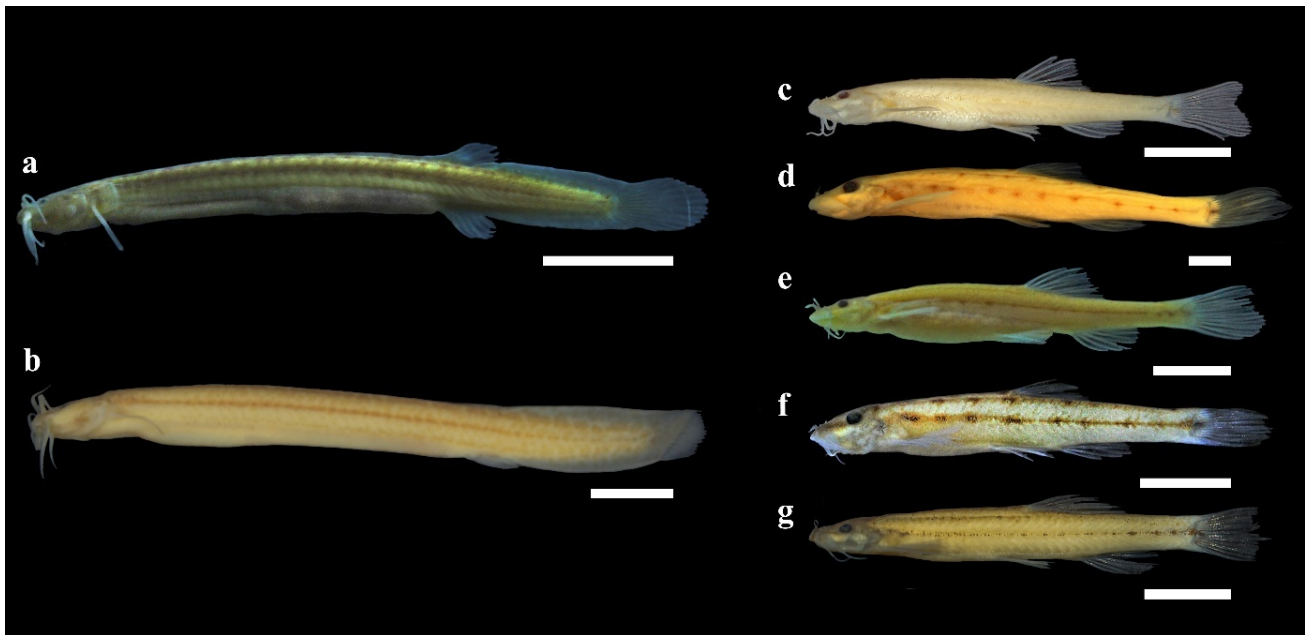


Figure 1. Specimens of *Listrura* and *Microcambeva*. (a) *Listrura nematopteryx*, (b) *L. boticario*, (c) *Microcambeva barbata*, (d) *M. ribeirae*, (e) *M. jucuiensis*, (f) *M. bendego* and (g) *M. watu*. Scale bar: 5.0mm

Both *Listrura* and *Microcambeva* are endemic to coastal drainages of the Atlantic Forest biome in Brazil (Fig. 2). Those catfish species are found in habitats that are relatively unusual for most freshwater fish. *Listrura* species are typically found in shallow marginal water bodies with dense leaf litter and/or sandy substrates where they hide, often separated from the main river channel (Nico and de Pinna 1996; Villa-Verde et al. 2022). Those habitats are found in coastal drainages draining the Serra do Mar and Serra Geral Mountain ranges, with the exception of *Listrura depinnai* Villa-Verde, Ferrer & Malabarba which occurs in the Pampa Biome (Villa-Verde, Ferrer, and Malabarba 2014; Medeiros et al. 2024). *Microcambeva* species are found in shallow sandy riverbanks in narrow streams in coastal drainages of the eastern margin of the Brazilian Shield, where they are often buried, with only their nasal barbels exposed (Costa and Bockmann 1994; Sarmento-Soares et al. 2019; Medeiros, Sarmento-Soares, and Lima 2021).

For a considerable time, records of specimens of these two genera were scarce, with the majority of them comprising a few specimens from the type series. The scarcity of *Listrura* and *Microcambeva* specimens in museum collections can be attributed to a number of factors, including their diminutive size, small population size, secretive and cryptic habits, and the lack of collecting efforts focused on the environment favored by those species (Sarmento-Soares et al. 2019; Villa-Verde et al. 2022). Knowledge on the biodiversity of those genera has undergone considerable progress over the past two decades. During that period, the majority of the currently known species of both genera have been described, 11 of *Listrura* and seven of *Microcambeva* (Medeiros et al. 2020; Medeiros, Sarmento-Soares, and Lima 2021; Medeiros et al. 2024). Additionally, the first proposals of phylogenetic relationships

among their species have been published in the past four years (Costa, Henschel, and Katz 2020; Costa and Katz 2021; Medeiros et al. 2024). Due to the particular fragility of their habitats and the paucity of available records, species of both genera are frequently included in Brazilian lists of threatened species. In the latest published list (2024), two species of *Listrura*, *L. camposae* and *L. nematopteryx*, were classified as ‘Critically Endangered’ (CR), while *L. picinguabae* was listed as ‘Near Threatened’ (NT). *Listrura depinnai* and *L. boticario* were classified as ‘Least Concern’ (LC), whereas *L. costai* and *L. tettraradiata* were categorized as ‘Data Deficient’ (DD). Remaining species were not evaluated (ICMBio 2024). Regarding *Microcambeva* species, Medeiros et al. (2020) suggested that *M. bendego* should be classified as CR. Officially, *M. draco* was classified as ‘Endangered’ (EN), and *M. watu* as NT, and both *M. ribeirae* and *M. filamentosa* as LC. Remaining species were classified as DD.

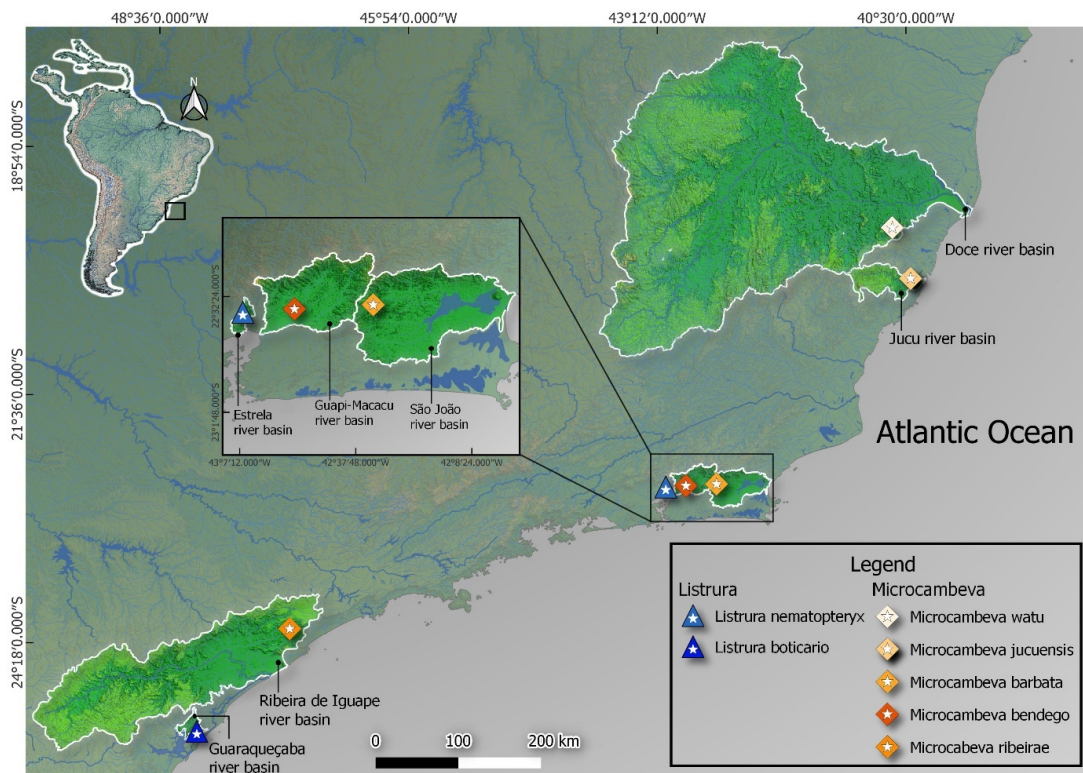


Figure 2. Sampling localities for the species sequenced in the present study.

Here, we present the draft version of the whole-genome sequencing efforts for two species of *Listrura* – *L. nematopteryx*, and *L. boticario* – and five of *Microcambeva* – *M. barbata*, *M. watu*, *M. jucuensis*, *M. bendego*, and *M. ribeirae* –, including the type-species of each genus, filling a key knowledge gap about the genetic diversity within those elusive and endangered genera. Such data will serve as a foundation for more in depth studies of evolutionary and systematics in those of related taxa and unique catfish species.

Methods

Tissues samples from one specimen per species were used, obtained from Museu Nacional, Rio de Janeiro (MNRJ), Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), Coleção Ictiológica da Universidade Federal do Rio Grande do Norte, Natal (CIUFRN) and Coleção Ictiológica da Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS). *Listrura nematopteryx* (Fig. 1a) was collected from a small stream near the Imbariê region, municipality of Duque de Caxias, Rio de Janeiro (22°39'09.5"S 43°13'04.0"W) (Fig. 2), close to its type locality, which has since been destroyed by urban growth. *Listrura boticario* (Fig. 1b) was collected from the Figueira River, in the municipality of Guaraqueçaba, Paraná (25°10'43.0"S 48°18'42.0"W) (Fig. 2). *Microcambeva barbata* (Fig. 1c) was collected in the Aldeia Velha river, municipality of Cassimiro de Abreu, Rio de Janeiro (22°29'53.0"S 42°16'03.0"W) (Fig. 2). *Microcambeva ribeirae* (Fig. 1d) was collected in the Rio Preto, Sete Barra municipality, São Paulo (24°11'33.0"S 47°53'25.0"W) (Fig. 2). *Microcambeva jucuiensis* (Fig. 1e), was collected in the Jucu river, in the municipality of Viana, in Espírito Santo (20°22'52.98"S 40°30'14.98"W) (Fig. 2). *Microcambeva bendego* (Fig. 1f) was collected in the type locality, in the Guapiaçu river, in the Guapimirim municipality, in the Rio de Janeiro (22°35'33"S, 42°53'20"W) (Fig. 2). *Microcambeva watu* (Fig. 1g) was collected in the type locality in a small stream near the road between São João de Petropolis and Vila Vinte e Cinco de Julho, in Santa Teresa municipality, Espírito Santo (19°47'2.00"S 40°38'52.00"W) (Fig. 2).

DNA was extracted using the Qiagen DNeasy Genomic Extraction Kit following standard protocol. A paired-end sequencing library was then prepared with the Illumina TruSeq kit, adhering to the manufacturer's instructions. Sequencing was carried out on an Illumina Hi-Seq platform, using a paired-end 2 × 150 bp format. The resulting FASTQ files were processed to remove adapter/primer sequences, as well as low-quality regions, using Trimmomatic v0.33 (Bolger, Lohse, and Usadel 2014). The cleaned sequences were assembled with SPAdes v2.5 (Bankevich et al. 2012), followed by a final assembly step using Zanfona (Kieras, O'Neill, and Pirro 2021).

Genome assembly statistics

Genome assembly completeness was analyzed with BUSCO v5 (Simão et al. 2015) and CEGMA 2.4 (Parra, Bradnam, and Korf 2007) using the gVolante server (Nishimura, Hara, and Kuraku 2017, <https://gvolante.riken.jp>).

Results and Data Availability

All raw read data and assembled genomes are available in the GenBank database (Table 1). The quality assessments conducted using BUSCO and CEGMA indicated that the genome assemblies range from average to good.

However, a significant proportion of the genomes appear to be fragmented ([Table 1](#)). Further assembly steps are needed to improve the continuity of the assemblies presented here.

Table 1. Genome assembly statistics for (a) *Listrura nematopteryx*, (b) *L. boticario*, (c) *Microcambeva barbata*, (d) *M. ribeirae*, (e) *M. jucensis*, (f) *M. bendego* and (g) *M. watu*.

(a) <i>Listrura nematopteryx</i>	
Voucher number	MNRJ37022
Raw Data	SRR30722138
Assembled Genomes	JBIDEM000000000
No. of scaffolds	613,746
No. of base pairs	657,742,134
N50	4,506
L50	24,783
GC content (%)	39.77
Average genome coverage	60x
BUSCO scores	Vertebrata: C:50.2% (S:49.7%, D:0.5%); F:31.5%; M:18.3%; N:3354 Actinopterygii: C:48.6% (S:47.8, D:0.8%); F:12.8%; M:38.6%; N:3640
CEGMA scores	CVG: C: 45.06%; C+F: 84.98%; M: 15.02%; N:233 CEG C: 68.55%; C+F: 92.74%; M: 7.26%; N: 248
(b) <i>Listrura boticario</i>	
Voucher number	MNRJ 39068
Raw Data	SRR30360593
Assembled Genome	JBIEOJ000000000
No. of scaffolds	653,027
No. of base pairs	692,340,185
N50	8,583
L50	12,179
GC content (%)	40.53
Average genome coverage	60x
BUSCO scores	Vertebrata: C:61.5% (S:60.6%, D:0.9%); F:26.2%; M:12.3%; N:3354 Actinopterygii: C:59.46% (S:58.6, D:0.9%); F:12.3%; M:28.2%; N:3640
CEGMA scores	CVG: C: 54.94%; C+F: 89.90%; M: 10.30%; N:233 CEG C: 75.81%; C+F: 97.58%; M: 2.42%; N: 248
(c) <i>Microcambeva barbata</i>	
Voucher number	MNRJ 47108
Raw Data	SRR26167089
Assembled Genome	JAYDZF000000000
No. of scaffolds	476,640
No. of base pairs	763,273,019
N50	17,098,239
L50	17
GC content (%)	40.15%
Average genome coverage	60x
BUSCO scores	Vertebrata: C:83.3% (S:82.7%, D:0.6%); F:10.6%; M:6.1%; N:3354 Actinopterygii:

	C:81.3% (S:80.2%, D:1.1%); F:6.0%; M:12.7%; N:3640
CEGMA scores	CVG: C: 78.54.0%; C+F: 95.71%; M: 4.29%; N:233 CEG C: 88.71%; C+F: 99.19%; M: 0.81%; N: 248
(d) <i>Microcambeva ribeirae</i>	
Voucher number	MZUSP116750
Raw Data	SRR26070011
Assembled Genome	JAYECN000000000
No. of scaffolds	1,281,735
No. of base pairs	1,872,330,592
N50	29,304
L50	9,919
GC content (%)	40.15%
Average genome coverage	60x
BUSCO scores	Vertebrata: C:72.4% (S:69.2%, D:3.2%); F:20.4%; M:7.2%; N:3354 Actinopterygii: C:52.5% (S:48.6%, D:3.9%); F:11.0%; M:36.5%; N:3640
CEGMA scores	CVG: C: 71.67%; C+F: 97.42%; M: 2.58%; N:233 CEG C: 74.19%; C+F: 97.58%; M: 2.42%; N: 248
Average genome coverage	60x
BUSCO scores	Vertebrata: C:80.4% (S:79.6%, D:0.8%); F:11.3%; M:8.3%; N:3354 Actinopterygii: C:79.5% (S:78.6%, D:0.9%); F:7.1%; M:13.4%; N:3640
CEGMA scores	CVG: C: 74.25%; C+F: 94.42%; M: 5.58%; N:233 CEG C: 87.90%; C+F: 97.58%; M: 2.82%; N: 248
(e) <i>Microcambeva jucuensis</i>	
Voucher number	UFRGS 15080
Raw Data	SRR30360568
Assembled Genome	JBIMOW000000000
No. of scaffolds	2,150,831
No. of base pairs	2,014,421,424
N50	4309
L50	87,483
GC content (%)	39.62
Average genome coverage	60x
BUSCO scores	Vertebrata: C:79.8% (S:53.7%, D:26.1%); F:15.6%; M:4.6%; N:3354 Actinopterygii: C:80.5% (S:53.9%, D:26.6%); F:9.3%; M:10.2%; N:3640
CEGMA scores	CVG: C: 79.83%; C+F: 97.85%; M: 5.12%; N:233 CEG C: 91.13%; C+F: 99.60%; M: 0.40%; N: 248
(f) <i>Microcambeva bendego</i>	
Voucher number	TIUFRN 5548
Raw Data	SRR26167281
Assembled Genome	JAYDZG000000000
No. of scaffolds	856,387
No. of base pairs	927,108,182
N50	12,713,763
L50	20

GC content (%)	42.03
Average genome coverage	60x
BUSCO scores	Vertebrata: C:80.4% (S:79.6%, D:0.8%); F:11.3%; M:8.3%; N:3354 Actinopterygii: C:79.5% (S:78.6%, D:0.9%); F:7.1%; M:13.4%; N:3640
CEGMA scores	CVG: C: 74.25%; C+F: 94.42%; M: 5.58%; N:233 CEG C: 87.90%; C+F: 97.58%; M: 2.82%; N: 248
(g) <i>Microcambeva watu</i>	
No. of scaffolds	1,524,213
No. of base pairs	1,426,256,390
N50	2,761
L50	85,325
GC content (%)	39.38
Average genome coverage	60
BUSCO scores	Vertebrata: C:72.4% (S:60.7%, D:11.7%); F:20.2%; M:7.4%; N:3354 Actinopterygii: C:71% (S:58%, D:13%); F:11.2%; M:17.8%; N:3640
CEGMA scores	CVG: C: 69.53%; C+F: 93.56%; M: 6.44%; N:233 CEG: C: 84.68%; C+F: 99.19%; M: 0.81%; N: 248

Complete BUSCOs or CEGs (C); Complete and single-copy BUSCOs (S); Complete and duplicated BUSCOs (D); Fragmented BUSCOs or partial CEGs (F); Missing BUSCOs or CEGs (M); Total BUSCOs or CEGs queried (N). CEGMA scores are shown for vertebrate ortholog database (CVG) and eukaryotic ortholog database (CEG).

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