

TABLE S1 | Accession numbers and specimen information for the 12S rRNA reference sequences. LGC refers to Laboratório de Genética da Conservação, Pontifícia Universidade Católica de Minas Gerais; MBML-PEIXES refers to Museu de Biologia Mello Leitão, Fish collection, Instituto Nacional da Mata Atlântica.

Species	Gene	Specimen / Lot Voucher	Accession LGC12Sdb	Genbank Accession Number	Sequence
<i>Trichogenes claviger</i>	12S	MBML-PEIXES 14206	LGC_DC8125	PX556613	TACGAAAAGACCCAAGTTGACAGCCACGGCGTAAAGGGTGGTTAAGACACAA- ACAAAATAAAGCCAAAGACCTCCTAGGCCGTCATACGCATTCCGGAGGCAC- GAAACCCAAACACGAAAGTAGCTTTACTTCAATATCTGACCCACGAAAGCTA- AGAAACAACTGGGATTAGATACCCCACTATGCTTAGCCTTAAACCTAGATG- GCACATTACAAAAACATCCGCCAGGGTACTACGAGCATAGCTTAAAAACCAA- AGGACTTGGCGGTGCCTCAGATCCCCTAGAGGAGCCTGTTCTAGAACCATA- ACCCCGTTAAACCTCACCACCTCTTGTCTTCCCGCCTATATACCGCCGTCGT- CAGCTTACCCTGTGAAGGATAATAGTAAGCAAATGGGCAAGCCCAAAAC- GTCAGGTCGAGGTGTAGCGTACGAGGTGAAAGAAATGGGCTACATTTTCTA- ACACAGAATATTACGAAAGGCGCCTTGAAACCTGGCGCCCAAAGGTGGATT- TAGCAGTAAAAAGCAAATAGAGCGTTCTTTTGAATTAGGCTCTGAGGCGCG- CACACACCGCCGTCACCTCTCCCTCCTTACCCTATCAACTTCCATTCTAATA- ACTTAACCTATAAATGCGGGGAG
<i>Trichogenes claviger</i>	12S	MBML-PEIXES 14206	LGC_DC8126	PX556614	CCCAGTTGACAGCCACGGCGTAAAGGGTGGTTAAGACACAAACAAAATA- AAGCCAAAGACCTCCTAGGCCGTCATACGCATTCCGGAGGCACGAAACCCA- AACACGAAAAGTAGCTTTACTTCAATATCTGACCCACGAAAGCTAAGAAAAC- AACTGGGATTAGATACCCCACTATGCTTAGCCTTAAACCTAGATGGCACATTA- CAAAAACATCCGCCAGGGTACTACGAGCATAGCTTAAACCCAAAGGACTT- GGCGGTGCCTCAGATCCCCCTAGAGGAGCCTGTTCTAGAACCATAACCCCG- GTTAAACCTCACCACCTCTTGTCTTCCCGCCTATATACCGCCGTCGTAGCT- TACCCTGTGAAGGATAATAGTAAGCAAATGGGCAAGCCCAAAACGTCAG- GTCAGGTTGAGGTGTAGCGTACGAGGTGAAAGAAATGGGCTACATTTCTAACA- CAGATATTACGAAAGGCGCCTTGAAACCTGGCGCCCAAAGGTGGATTAGCAG- TAAAAAGCAAATAGAGCGTTCTTTTGAATTAGGCTCTGAGGCGCGCACAC- CGCCGTCACCTCTCCCTCCTTACCCTATCAACTTCCATTCTAATAACTTAAC- TATAAATGCGGGGAGGCAAGT
<i>Trichogenes claviger</i>	12S	MBML-PEIXES 14206	LGC_DC8127	PX556615	TACGAAAAGACCCAAGTTGACAGCCACGGCGTAAAGGGTGGTTAAGACACAA- ACAAAATAAAGCCAAAGACCTCCTAGGCCGTCATACGCATTCCGGAGGCAC- GAAACCCAAACACGAAAGTAGCTTTACTTCAATATCTGACCCACGAAAGCTA- AGAAACAACTGGGATTAGATACCCCACTATGCTTAGCCTTAAACCTAGATG- GCACATTACAAAAACATCCGCCAGGGTACTACGAGCATAGCTTAAAAACCAA- AGGACTTGGCGGTGCCTCAGATCCCCTAGAGGAGCCTGTTCTAGAACCATA- ACCCCGTTAAACCTCACCACCTCTTGTCTTCCCGCCTATATACCGCCGTCGT- CAGCTTACCCTGTGAAGGATAATAGTAAGCAAATGGGCAAGCCCAAAAC- GTCAGGTCGAGGTGTAGCGTACGAGGTGAAAGAAATGGGCTACATTTTCTA- ACACAGAATATTACGAAAGGCGCCTTGAAACCTGGCGCCCAAAGGTGGATT- TAGCAGTAAAAAGCAAATAGAGCGTTCTTTTGAATTAGGCTCTGAGGCGCG- CACACACCGCCGTCACCTCTCCCTCCTTACTCTATCAACTTCCATTCTAATA- ACTTAACCTATAAATGCGGGGAG
<i>Trichogenes claviger</i>	12S	MBML-PEIXES 14206	LGC_DC8128	PX556616	GAAAGACCCAAGTTGACAGCCACGGCGTAAAGGGTGGTTAAGACACAAACA- AAAATAAAGCCAAAGACCTCCTAGGCCGTCATACGCATTCCGGAGGCACGA- AACCCAAACACGAAAGTAGCTTTACTTCAATATCTGACCCACGAAAGCTAA- GAAACAACTGGGATTAGATACCCCACTATGCTTAGCCTTAAACCTAGATGG- CACATTACAAAAACATCCGCCAGGGTACTACGAGCATAGCTTAAAAACCAA- GGACTTGGCGGTGCCTCAGATCCCCTAGAGGAGCCTGTTCTAGAACCATA- ACCCCGTTAAACCTCACCACCTCTTGTCTTCCCGCCTATATACCGCCGTCGT- CAGCTTACCCTGTGAAGGATAATAGTAAGCAAATGGGCAAGCCCAAAAC- GTCAGGTCGAGGTGTAGCGTACGAGGTGAAAGAAATGGGCTACATTTTCTA- ACACAGAATATTACGAAAGGCGCCTTGAAACCTGGCGCCCAAAGGTGGATT- TAGCAGTAAAAAGCAAATAGAGCGTTCTTTTGAATTAGGCTCTGAGGCGCG- CACACACCGCCGTCACCTCTCCCTCCTTACCCTATCAACTTCCATTCTAATA- ACTTAAC

TABLE S2 | Filter membranes ('Replica') for eDNA extraction, DNA library construction and sequencing metabarcoding; the concentrations (ng/μL) and absorbance ratios (A260/280) were estimated via a Nanodrop 2000.

N	Sampling Site	Replicate	DNA concentration [ng/μL]	Absorbance A260/280
1	P1	1A	24.8	1.46
2		1B	3.4	1.73
3		1C	15.8	1.37
4	P2	2A	4.5	1.66
5		2B	8.8	1.33
6		2C	9.3	1.81
7	P3	3A	6.7	1.6
8		3B	3	1.61
9		3C	10.4	1.65
10	P4	4A	3.5	1.93
11		4B	3.1	1.84
12		4C	4.7	1.54
13	P5	5A	10.3	1.69
14		5B	7.5	1.45
15		5C	4.8	1.7
16	P6	6A	3.7	1.66
17		6B	3.5	1.51
18		6C	3.8	1.14
19	P7	7A	3.8	1.68
20		7B	4.6	1.55
21		7C	8.1	1.36
22	P8	8A	21.1	1.42
23		8B	3	1.68
24		8C	2.8	1.06
25	P9	9A	3.3	1.17
26		9B	3	0.92
37		9C	2.6	1.18
28	P10	10A	3.1	1.32
29		10B	5	1.61
30		10C	4.1	1.35
31	Control sample 1		2.9	1.41
32	Control sample 2		2.7	1.54

TABLE S3 | Sequence counts for each sample along the bioinformatic process, from raw reads to total non-chimeric merged sequences, used for ASV determination.

Identifiers		Site information						
Sample	Field ID	Sampling site	Latitude	Longitude	Local	Sub-basin 2	Sub-basin 1	Basin
EM126_RBS1	RBS1	P1	-20.475639	-41.063917	Tributary of ribeirão Braço Sul	Ribeirão Braço Sul	Rio Caxixe	Itapemirim
EM126_MK2	MK2	P2	-20.512722	-41.032611	Córrego Picada Comprida	Ribeirão Braço Sul	Rio Caxixe	Itapemirim
EM126_MK3	MK3	P3	-20.495500	-41.040056	Tributary of córrego Picada Comprida	Ribeirão Braço Sul	Rio Caxixe	Itapemirim
EM126_MK4	MK4	P4	-20.499444	-41.037889	Tributary of córrego Picada Comprida	Ribeirão Braço Sul	Rio Caxixe	Itapemirim
EM126_MK5	MK5	P5	-20.523444	-41.018111	Tributary of ribeirão Caetés	Ribeirão Caetés	Rio Fruteiras	Itapemirim
EM126_MK6	MK6	P6	-20.527389	-41.016472	Tributary of córrego do Ouro	Córrego do Ouro	Rio Fruteiras	Itapemirim
EM126_MK7	MK7	P7	-20.530611	-41.012278	Tributary of córrego do Ouro	Córrego do Ouro	Rio Fruteiras	Itapemirim
EM126_RBS8	RBS8	P8	-20.477083	-41.071583	Tributary of ribeirão Braço Sul	Ribeirão Braço Sul	Rio Caxixe	Itapemirim
EM126_SPA9	SPA9	P9	-20.436750	-41.023167	Tributary of rio Jucu Braço Norte	-	Rio Jucu Braço Norte	Jucu
EM126_PPA1	PPA1	P10	-20.453028	-40.995472	Tributary of rio Fruteiras	-	Rio Fruteiras	Itapemirim

TABLE S3 | (Continued)

Identifiers		Sequence counts							
Sample	Field ID	Raw reads (pairs)	Paired reads	Primer detected reads	Repaired reads	Denoised (R1)	Denoised (R2)	Merged	Non-chimeric Merged
EM126_RBS1	RBS1	63911	63910	58437	58355	57646	53589	28251	27842
EM126_MK2	MK2	83731	83730	60950	60592	60401	60046	47292	46986
EM126_MK3	MK3	43882	43877	16851	16411	16137	16063	8189	8189
EM126_MK4	MK4	54538	54533	43663	43501	43073	42657	27980	27933
EM126_MK5	MK5	34461	34460	11844	11497	11274	11197	2972	2972
EM126_MK6	MK6	69814	69788	28335	27710	27313	27118	9583	9583
EM126_MK7	MK7	61281	61280	25629	25110	24835	24621	19693	19639
EM126_RBS8	RBS8	91499	91475	73948	73657	72320	72130	55692	55345
EM126_SPA9	SPA9	76691	76690	60577	60354	60064	59817	8524	8487
EM126_PPA1	PPA1	65220	65218	35123	34650	34466	34270	11831	11785

Neotropical Ichthyology

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Official Journal of the Sociedade Brasileira de Ictiologia

HOW TO CITE THIS ARTICLE

- Silva-Novelli JP, Hilário HO, Carvalho DC, Martins-Pinheiro RF, Sarmento-Soares LM. Detection of the critically endangered catfish *Trichogenes claviger* (Siluriformes: Trichomycteridae) using environmental DNA. Neotrop Ichthyol. 2026; 24(2):e260013. <https://doi.org/10.1590/1982-0923-2025-0013>