



Complete mitochondrial genome of four *Scleromystax barbatus* (Siluriformes: Callichthyidae) populations

Correspondence:
Roger Henrique Dalcin
roger_dalcin@hotmail.com

Roger Henrique Dalcin¹, Luz Elena De La Ossa-Guerra²,
 Roberto Ferreira Artoni³ and Vinícius Abilhoa⁴

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The complete mitogenome of four *Scleromystax barbatus* populations distributed along the species natural range was reconstructed, comprising the first study with this genus and the second with Corydoradinae specimens sampled from their natural environment and deposited in permanent collections. The mitogenome of *S. barbatus* is a circular molecule of 16,694 base pairs (bp) comprising 37 genes, 22 of which are tRNA, two are rRNA, 13 are protein-coding genes and one control region (D-loop). An 18-nucleotide insertion sequence was found between the ATPase subunit 6 and COIII genes. Most genes are encoded on the heavy strand, while the ND6 and eight tRNAs are found on the light strand. Phylogenetic analyses using other available Callichthyidae mitogenomes confirmed the monophyly of Callichthyinae and Corydoradinae and indicate that *S. barbatus* populations form a separate and more closely related branch of *Corydoras nattereri* + *Corydoras paleatus*.

Keywords: Markers, Mitochondrial DNA, Molecular identification, Phylogenetic analysis.



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- 1 Programa de Pós-Graduação em Zoologia, Universidade Federal do Paraná, Campus Centro Politécnico, Av. Cel. Francisco H. dos Santos, 100, 81530-000 Curitiba, PR, Brazil. roger_dalcin@hotmail.com (corresponding author).
- 2 Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular, Universidade Federal de São Carlos, Rodovia Washington Luis, 13565-905 São Carlos, SP, Brazil. 03ledg@gmail.com.
- 3 Programa de Pós-Graduação em Biologia Evolutiva, Universidade Estadual de Ponta Grossa, Av. Carlos Cavalcanti, 4748, 84030-900 Ponta Grossa, PR, Brazil. rfartoni@gmail.com.
- 4 Museu de História Natural Capão da Imbuia, MAPCF/PMC, Rua Prof. Nivaldo Braga, 1369, 82810-080 Curitiba, PR, Brazil. vinicius.abilhoa@gmail.com.

O mitogenoma completo de quatro populações de *Scleromystax barbatus* coletadas ao longo da área de distribuição natural foi reconstruído, compreendendo o primeiro estudo com este gênero e o segundo com espécimes de Corydoradinae amostrados em seu ambiente natural e depositados em coleções permanentes. O mitogenoma de *S. barbatus* é uma molécula circular de 16.694 pares de bases (pb) compreendendo 37 genes, dos quais 22 são tRNA, dois são rRNA, 13 são genes codificadores de proteínas e uma região controle (D-loop). Uma sequência de inserção de 18 nucleotídeos foi encontrada entre a subunidade 6 da ATPase e os genes COIII. A maioria dos genes é codificada na fita pesada, enquanto o ND6 e oito tRNAs são encontrados na fita leve. Análises filogenéticas usando outros mitogenomas de Callichthyidae disponíveis confirmaram a monofilia de Callichthyinae e Corydoradinae e indicam que as populações de *S. barbatus* formam um ramo separado e mais intimamente relacionado a *Corydoras nattereri* + *Corydoras paleatus*.

Palavras-chave: Análises filogenéticas, DNA Mitocondrial, Identificação molecular, Marcadores.

INTRODUCTION

Corydoradinae is a subfamily of Callichthyidae, one of the largest monophyletic family of catfish widely distributed in the Neotropical region (Reis, 2003, 1998). Currently, this subfamily is composed of three genera and 207 valid species (Britto, 2003; Tencatt *et al.*, 2022a; Fricke *et al.*, 2023). Nevertheless, despite its richness, the main studies concerning Corydoradinae are focused on the description of new species, especially in *Corydoras* Lacepède, 1803 (Rocha *et al.*, 2022; Tencatt *et al.*, 2022b). Some attempts, however, have been performed to resolve phylogenetic relationships between groups (Britto, 2003; Shimabukuro-Dias *et al.*, 2004; Alexandrou *et al.*, 2011; Marburger *et al.*, 2018).

Among Corydoradinae members, *Scleromystax* Günther, 1864 exhibits the lowest diversity and is restricted to Atlantic Rainforest coastal rivers (Britto, 2003; Britto *et al.*, 2016). This genus was revalidated by Britto (2003), including three species previously belonging to *Corydoras*. Subsequently, two *Scleromystax* species were described (Britto, Reis, 2005; Britto *et al.*, 2016), and one species previously included in *Aspidoras* Ihering, 1907 has been recently reclassified to *Scleromystax* (Tencatt *et al.*, 2022a), bringing the total number of known species to six for the genus.

Scleromystax barbatus (Quoy & Gaimard, 1824) is the type species of the genus and exhibits the widest distribution among *Scleromystax* representatives, extending to the coastal rivers of the Atlantic Rainforest, from the Paraíba do Sul River, in the State of Rio de Janeiro, to Itapocu River, in the State of Santa Catarina (Reis, 2003; Menezes *et al.*, 2007). The species presents an accentuated sexual dimorphism. Adult males present odontoids in the lateral region of the head, spines on the dorsal fin, and elongated pectoral fins (Britto, 2003). It also inhabits shallow environments with weak to moderate currents and substrates consisting of clay or sandy bottoms, moving in small shoals or alone in clear stream and river waters. Its diet is omnivorous, and its food is obtained

by exploring the bottom in search of small invertebrates, plant fragments and algae (Aranha *et al.*, 1998; Gonçalves, Braga, 2013). Molecular genetic variation within *S. barbatus* populations of southern Brazil indicate that this species is an interesting model for phylogeographic studies (Tschá *et al.*, 2017).

The rapid evolution of mitochondrial DNA and its small size make this an important tool in evolutionary genetics, molecular ecology, species identification and conservation biology (Harrison, 1989; Ma *et al.*, 2012; Zhang *et al.*, 2022). Complete mitochondrial genome sequences are becoming an increasingly important apparatus in the study of fish phylogenies due to advances in sequencing techniques and biological data analyses (Betancur-R *et al.*, 2017; Rocha-Reis *et al.*, 2020). With regard to Corydoradinae, however, studies are scarce and concentrate only on *Corydoras* (Moreira *et al.*, 2016; Liu *et al.*, 2019b; Sun *et al.*, 2022). In this context, the present study aims to sequence, assemble, and annotate the complete mitogenome of four *S. barbatus* populations, providing an important addition to future phylogenetic Corydoradinae studies as the first mitogenome from a *Scleromystax* member.

MATERIAL AND METHODS

Specimen samplings. Individuals from four *Scleromystax barbatus* populations were collected by electrofishing and trawling along their known distribution between 2019 and 2021 (Tab. 1). In the field, individuals were anesthetized and sacrificed. Tissue samples (pieces of muscle and pectoral fin) were removed from each individual and preserved in 100% alcohol. The specimens were then fixed in 4% formalin and transferred to 70% alcohol in the laboratory. All specimens were deposited in the ichthyological collection at the Museu de Historia Natural Capão da Imbuia, Curitiba (MHNCI).

Sequencing and mitogenome assembly. Genomic DNA was extracted using the saline method and the purity was checked on a 1% agarose gel and on NanoVue. The extracted DNA was sequenced using the DNBSeg sequencing platform (BGI Genomics), which generated 150pb paired-end sequences. The quality of the clean data was verified using FASTQC. We then used the GetOrganelle v. 1.7.6.1 (Jin *et al.*, 2020) for the *de novo* assembly of the mitogenome, using *Corydoras* Genbank sequences as a reference (Tab. 2). We checked genome circularity using the Bandage v. 0.9.0 software (Wick *et al.*, 2015). Annotation was performed using the MitoAnnotator tool (<http://mitofish.aori.utokyo.ac.jp/annotation/input.html>), included in the Mitofish Database v. 3.73 (Iwasaki *et al.*, 2013).

Phylogenetic analysis. Phylogenetic analyses were performed using 13 PCGs (protein-coding genes), including four mitogenomes from *S. barbatus* and some related Callichthyidae species obtained at Mitofish and Genbank (Tab. 2). All species were aligned using the MAFFT v. 7.017 software (Kato *et al.*, 2002). The tree was constructed using Mr. Bayes v. 3.2 (Ronquist, Huelsenbeck, 2003).

TABLE 1 | Code used in the analysis of *Scleromystax barbatus* populations, locality, river, and coordinates where the collections were carried out.

Code	Locality	River	Latitude/Longitude	Voucher
AE	Itariri, SP	Aerado	24°17'51.31"S 47°08'47.83"W	MHNCI 12734
AR	Angra dos Reis, RJ	Ariró	22°54'7.33"S 44°20'46.56"W	MHNCI 12735
PE	Barra do Sul, SC	Pernambuco	26°23'19.60"S 48°40'55.94"W	MHNCI 12736
PS	Teresópolis, RJ	Vargem Grande	22°24'24.55"S 42°52'27.34"W	MHNCI 12737

TABLE 2 | Species used from Genbank and the present study and their respective accession and source numbers.

Species	Accession number	Author
<i>Scleromystax barbatus</i> (AE)	OQ507208	This study
<i>Scleromystax barbatus</i> (AR)	OQ507209	This study
<i>Scleromystax barbatus</i> (PE)	OQ507210	This study
<i>Scleromystax barbatus</i> (PS)	OQ507211	This study
<i>Corydoras aeneus</i> (Gill, 1858)	MZ571336	Sun <i>et al.</i> (2022)
<i>Corydoras arcuatus</i> Elwin, 1938	NC_049096	Liu <i>et al.</i> (2019d)
<i>Corydoras duplicareus</i> Sands, 1995	NC_049095	Liu <i>et al.</i> (2019c)
<i>Corydoras nattereri</i> Steindachner, 1876	KT239009	Moreira <i>et al.</i> (2016)
<i>Corydoras paleatus</i> (Jenyns, 1842)	MZ571337	Sun <i>et al.</i> (2022)
<i>Corydoras panda</i> Nijssen & Isbrücker, 1971	NC_049097	Liu <i>et al.</i> (2019a)
<i>Corydoras rabauti</i> LaMonte, 1941	NC_004698	Saitoh <i>et al.</i> (2003)
<i>Corydoras sterbai</i> Knaack, 1962	NC_048967	Liu <i>et al.</i> (2019b)
<i>Corydoras trilineatus</i> Cope, 1872	NC_049098	Chen <i>et al.</i> (2020)
<i>Hoplosternum littorale</i> (Hancock, 1828)	KX087170	Parente <i>et al.</i> (2018)

RESULTS

The mitochondrial genome of the four *Scleromystax barbatus* populations is a circular molecule 16,694 base pairs (bp) in length. It consists of 37 genes, 22 of which are tRNA, two are rRNA, 13 are protein-coding genes, and one is a control region (D-loop). An 18-nucleotide insertion sequence was found between the ATPase subunit 6 and COIII genes. Most of the genes are encoded on the heavy strand, while ND6 and eight tRNAs are found on the light strand. All protein-coding genes use ATG start codons, except for COI, which uses GTG. Seven protein-coding genes end with the complete stop codon, five of which end with TAA (ATP8, ATP6, ND4L, ND5 and ND6), one with TAG (ND1) and one with AGG (COI), while the remaining protein-coding genes are terminated with incomplete stop codons (ND2, COII, COIII., ND3, ND4, Cyt b). Fig. 1 and Tab. 3 depict the mitogenome annotation of the AR population, which is the closest to the *S. barbatus* type-locality (Reis *et al.*, 2003). A summary of the other populations is reported in Fig. S1 and Tab. S2. The nucleotide composition is summarized in Tab. 4.

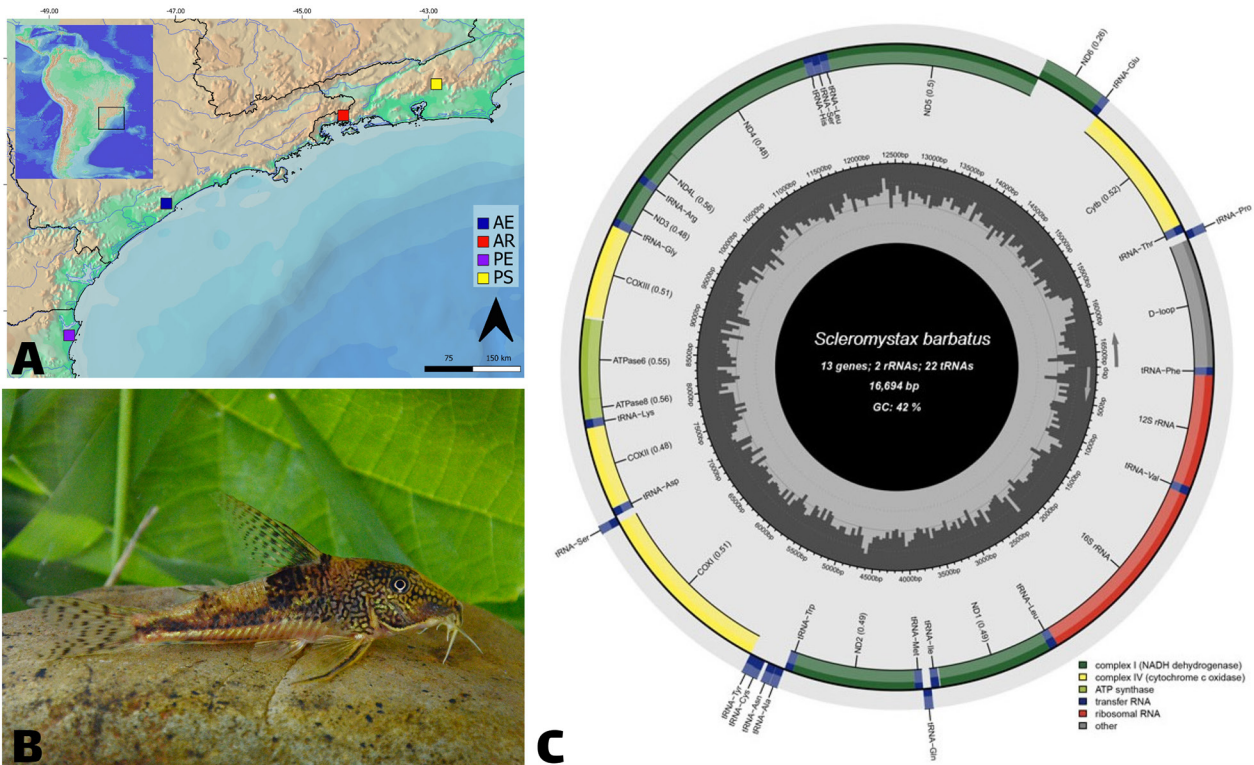


FIGURE 1 | **A.** Geographic location of the *Scleromystax barbatus* populations in coastal Atlantic Rainforest rivers. **B.** Male of *S. barbatus*. Photo by Caio Feltrin. **C.** Complete mitochondrial genome of *S. barbatus* from the AR population. Population codes in Tab. 1.

TABLE 3 | Annotation of the complete mitochondrial genome of a *Scleromystax barbatus* individual belonging to the Ariró River (AR) population.

Gene	Strand	Position		Length(bp)	Codon	
		Start	End		Start	Stop
tRNA-Phe	H	1	68	68		
12S rRNA	H	69	1016	948		
tRNA-Val	H	1017	1088	72		
16S rRNA	H	1089	2756	1668		
tRNA-Leu ^a	H	2757	2831	75		
ND1	H	2832	3803	972	ATG	TAG
tRNA-Ile	H	3812	3883	72		
tRNA-Gln	L	3882	3952	71		
tRNA-Met	H	3952	4021	70		
ND2	H	4022	5066	1045	ATG	T--
tRNA-Trp	H	5067	5137	71		
tRNA-Ala	L	5139	5207	69		
tRNA-Asn	L	5209	5281	73		
tRNA-Cys	L	5312	5378	67		
tRNA-Tyr	L	5378	5447	70		
COI	H	5449	7008	1560	GTG	AGG
tRNA-Ser ^b	L	6996	7066	71		
tRNA-Asp	H	7071	7140	70		
COII ^c	H	7145	7835	691	ATG	T--
tRNA-Lys	H	7836	7909	74		
ATPase 8	H	7911	8078	168	ATG	TAA
ATPase 6 ^c	H	8069	8752	684	ATG	TAA
COIII ^c	H	8771	9554	784	ATG	T--
tRNA-Gly	H	9555	9626	72		
ND3 ^c	H	9627	9975	349	ATG	T--
tRNA-Arg	H	9976	10045	70		
ND4L	H	10046	10342	297	ATG	TAA
ND4 ^c	H	10336	11716	1381	ATG	T--
tRNA-His	H	11717	11786	70		
tRNA-Ser ^d	H	11787	11853	67		
tRNA-Leu ^e	H	11855	11927	73		
ND5	H	11928	13754	1827	ATG	TAA
ND6	L	13751	14266	516	ATG	TAA
tRNA-Glu	L	14267	14335	69		
Cyt b	H	14338	15475	1138	ATG	T--
tRNA-Thr	H	15476	15547	72		
tRNA-Pro	L	15546	15615	70		
D-loop	H	15616	16694	1079		

TABLE 4 | Nucleotide composition of the four *Scleromystax barbatus* populations used in this study. %T (thymine percentage), %C (cytosine percentage), %A (adenine percentage), %G (guanine percentage), %GC content (guanine and cytosine percentages). Population codes in Tab. 1.

Population	Genome size (bp)	%T	%C	%A	%G	%GC
AE	16,651	26.5	26.4	32.0	15.2	41.6
AR	16,694	26.4	26.4	31.2	15.1	41.6
PE	16,614	26.5	26.4	31.9	15.2	41.6
PS	16,693	26.4	26.4	32.0	15.2	41.6

Phylogenetic analyses using the mitochondrial genome of Callichthyidae species indicated that the Corydoradinae subfamily formed a well-separated Callichthyinae branch (Fig. 2). In addition, the validity and taxonomic identification of *S. barbatus* was also strengthened.

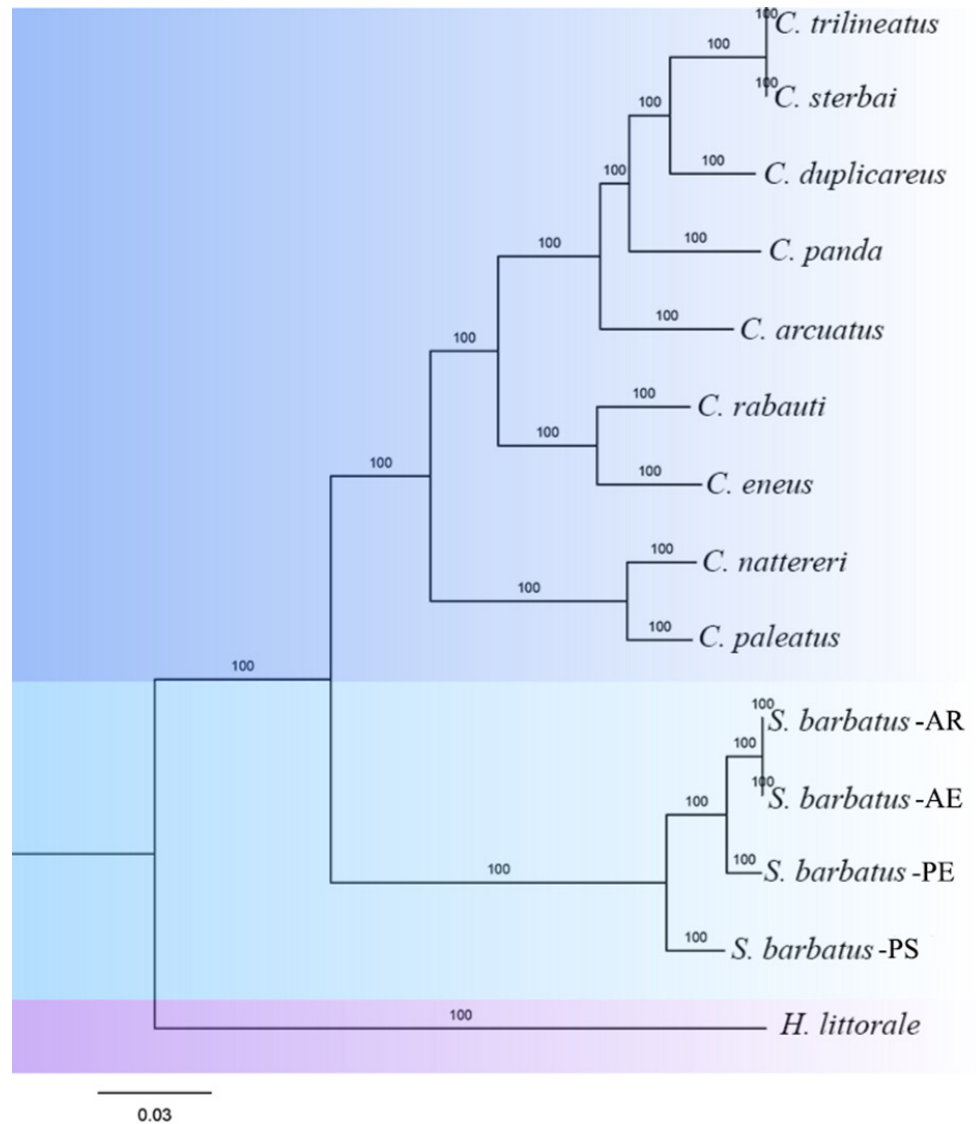


FIGURE 2 | Phylogenetic analysis of 13 Corydoradinae and *Hoplosternum littorale* (Callichthyinae member) species as the outgroup based on the nucleotide sequences of 13 PCGs from the mitochondrial genome. Bootstrap values are shown next to nodes and the scale bar shows 0.03 changes. Population codes in Tab. 1.

DISCUSSION

Phylogenetic analyses confirm the monophyly of the Callichthyidae subfamilies and among the Corydoradinae members whose mitogenome information is available. The *Scleromystax barbatus* populations are closer to the clade formed by *Corydoras nattereri* + *Corydoras paleatus*, similar to the findings reported by phylogenies with molecular (Shimabukuro-Dias *et al.*, 2004; Alexandrou *et al.*, 2011; Marburger *et al.*, 2018) and morphological (Britto, 2003) markers.

The order and arrangement of the mitochondrial genomes of the evaluated *S. barbatus* populations were identical to each other and similar to other Siluriformes members (Saitoh *et al.*, 2003; Moreira *et al.*, 2016; Rocha-Reis *et al.*, 2020; Zhang *et al.*, 2022) with the ND6 gene and eight tRNAs found on the light strand. The size of the mitogenomes ranged from 16,614 to 16,694 bp, and the nucleotide composition was 41.6% G+C for all populations, very close to the percentages found in other Corydoradinae members (Moreira *et al.*, 2016; Liu *et al.*, 2019a,b; Chen *et al.*, 2020; Xu *et al.*, 2020; Zhang *et al.*, 2022).

The four *S. barbatus* populations presented an 18-nucleotide sequence insertion between the ATPase subunit-6 and COIII genes, also found in other Callichthyidae members, with similar values noted for Corydoradinae members (Saitoh *et al.*, 2003; Moreira *et al.*, 2016) and higher values for Callichthyinae members (Parente *et al.*, 2018), suggesting that this insertion may be a synapomorphy among Callichthyidae subfamilies.

This is the first report on the mitogenome of *S. barbatus* populations and the second study on Corydoradinae species using individuals sampled from their natural environment and deposited in permanent collections. In this context, this study fills an existing gap for the Neotropical region, furthering information on a genus restricted to coastal Atlantic Rainforest streams and may be useful for future studies on Callichthyidae population genetics and evolution.

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AUTHORS' CONTRIBUTION

Roger Henrique Dalcin: Conceptualization, Methodology, Writing–original draft.

Luz Elena De La Ossa–Guerra: Conceptualization, Methodology, Writing–original draft.

Roberto Ferreira Artoni: Conceptualization, Resources, Writing–review and editing.

Vinícius Abilhoa: Conceptualization, Writing–review and editing.

Neotropical Ichthyology



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ETHICAL STATEMENT

Experiments were approved by the Ethical Committee for Animal Use from the Biological Sciences Section of the Universidade Federal do Paraná (CEU/BIO–UFPR), process 23075, 066050/2022–03 number 1489.

COMPETING INTERESTS

The authors declare no competing interests.

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