GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Genetic diversity of *Onychostoma gerlachi* in Kon Tum province (Vietnam) based on mitochondrial COI sequences

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GSC Biological and Pharmaceutical Sciences, 2025, 31(01), 061-068

Publication history: Received on 22 February 2025; revised on 05 April 2025; accepted on 07 April 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.31.1.0144

Abstract

In this study, Onychostoma gerlachi specimens were collected from three districts of Kon Tum Province—Đăk Glei, Kon Plong, and Kon Rẫy—to determine the mitochondrial COI sequence region for genetic diversity analyses. The analysis of COI fragment in 15 inviduals *O. gerlachi* revealed high nucleotide and haplotype diversity in each district. Nucleotide differences among samples ranged from 10 to 55 nucleotides. Phylogenetic tree analysis and genetic distance revealed significant genetic divergence among the three *O. gerlachi* populations from Đăk Glei, Kon Plong, and Kon Rẫy. These findings suggest long-term reproductive isolation and adaptation to distinct selective pressures in each population of *O. gerlachi*.

Keywords: *Onychostoma gerlachi*; genetic diversity; COI gene; mtDNA; Vietnam

1. Introduction

Onychostoma gerlachi Peters, 1881, belonging to the genus *Onychostoma* (Cypriniformes, Cyprinidae), is a freshwater fish species found in Kon Tum Province, Vietnam. Additionally, *O. gerlachi* has been reported in several freshwater regions of southern China [1]. The genus *Onychostoma* comprises 19 species, mainly distributed across the Yellow River, Yangtze River, Pearl River, and Red River systems [1]. Due to its high economic value and popularity among local communities, several species in this genus have been listed as endangered fish [2]. In Vietnam, *O. gerlachi* is one of five rare and endemic fish species [3]. However, due to its economic significance and extensive exploitation by local communities [3], *O. gerlachi* has been classified as "Near Threatened" in Vietnam's Red Data Book and is legally protected under Decree 26/2019/NĐ-CP (According to the Vietnamese government). Fishing of this species is prohibited annually from April to August, coinciding with its peak spawning period from June to July [3]. According to the IUCN Red List, *O. gerlachi* was categorized as Near Threatened (NT) in 2007 due to an estimated 30% population decline in the Mekong and Chao Phraya River basin. Therefore, further studies on its genetic diversity and conservation status are crucial.

In genetic diversity studies, particularly in fish research, certain mitochondrial genome regions are commonly used as molecular markers. The mitochondrial genome exhibits relatively low conservation across species while maintaining a sufficiently high mutation rate to assess intraspecific genetic diversity [4-6]. Among these, the Cytochrome Oxidase Subunit 1 (COI) gene has been widely accepted as a species-level DNA barcode and has been standardized for various animal groups [7]. This genetic marker has been extensively applied in assessing fish genetic diversity [8-12]. Previous studies on the mitochondrial genome of *O. gerlachi* have provided a foundation for further investigations into the species' genetic diversity [1, 2]. Therefore, in this study, we utilized the COI gene sequence to investigate the genetic diversity of *O. gerlachi* populations collected from Kon Tum Province, Vietnam.

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2. Material and methods

2.1. Sample Collection

Fish samples were collected from three districts of Kon Tum Province: Đăk Glei, Kon Plong, and Kon Rẫy. Sampling was conducted using two methods: direct net fishing and purchasing from local fisheries. After collection, fish samples were rinsed, preserved on ice, and transported to the laboratory for further analyses. Sampling locations are shown in Figure 1.

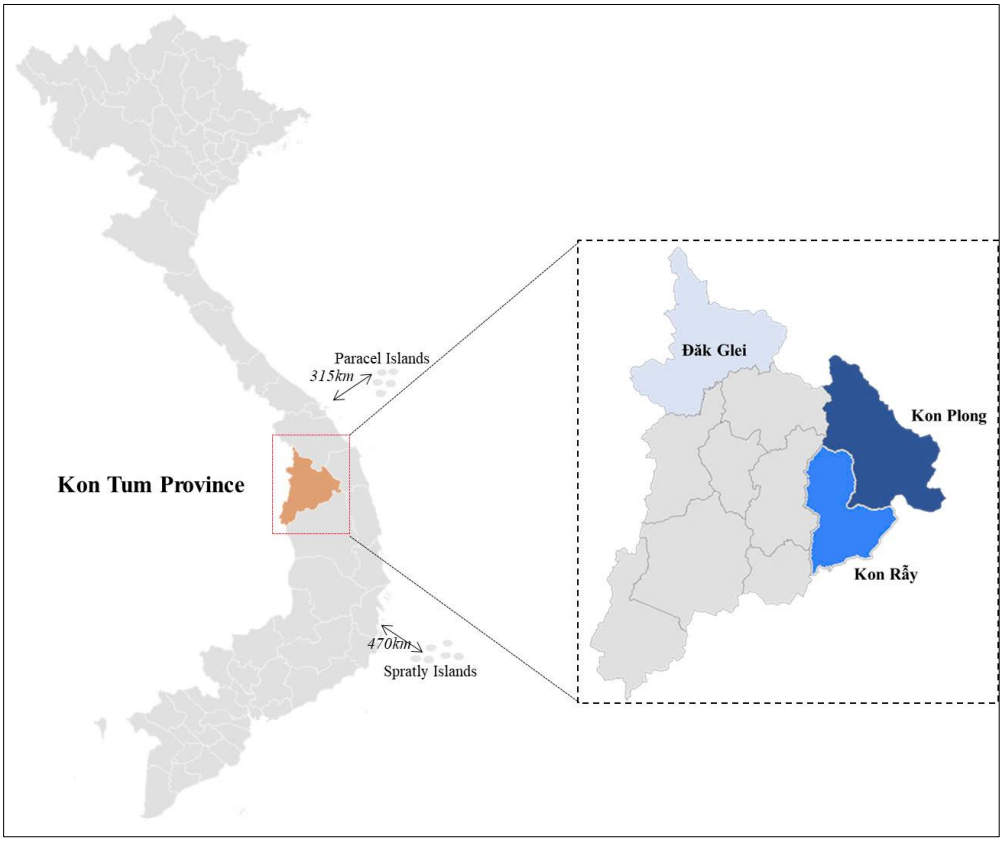

Figure 1 Sampling regions

2.2. DNA Extraction

A small tissue sample from *O. gerlachi* was incubated with proteinase K at 72°C for cell lysis. DNA extraction was performed using the TopPURE GENOMIC DNA EXTRACTION kit (HI-112) following the manufacturer's instructions. The extracted DNA was stained with GelRed and subjected to electrophoresis on a 1% agarose gel in 1X TAE (Tris-acetate-EDTA) buffer.

2.3. DNA Amplification

A 655-bp fragment of the COI gene was amplified using two primers: FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') [13]. PCR reactions were performed using Prime Taq Premix, which contains DNA polymerase, buffer, and free nucleotides. The PCR mixture consisted of 12.5 µL Prime Taq Premix (2X), 0.5 µL forward primer, 0.5 µL reverse primer, 0.5 µL DNA template, and sterile distilled water to a final volume of 25 µL. After an initial denaturation step at 94°C for 5 min, amplification was performed over 35 cycles (denaturation at 94°C for 15 s, annealing at 54°C for 15 s, extension at 72°C for 10 s), followed by a final extension at 72°C for 5 min. The PCR products were stained with GelRed and analyzed via electrophoresis on a 1% agarose gel in 1X TAE buffer.

2.4. DNA Sequencing

PCR products were sequenced by DNAsequencing (Vietnam) using the Sanger method [14]. The output chromatograms were analyzed using FinchTV 1.4.0, and manual editing was performed to resolve inconsistencies between forward and reverse sequences. All sequences were aligned with the reference COI sequence (GenBank accession number: KJ994644.1) and trimmed using MEGA12 software [15].

2.5. Genetic diversity analysis

Haplotype analysis of the fish samples was conducted using DnaSP version 6 [16]. A phylogenetic tree was constructed using the maximum likelihood method (bootstrap value = 1000) in MEGA12 [15] , based on the best-fitting nucleotide substitution model (K2+I), with *Cyprinus carpio* as the outgroup (GenBank accession number: MT805055.1). A haplotype network was generated using PopArt version 1.7 [17] based on the minimum spanning network method. Genetic diversity measures (nucleotide diversity, haplotype diversity, and average nucleotide difference), AMOVA, and genetic distances were analyzed using Arlequin version 3.5.2.2 [18].

3. Results

A total of 15 COI gene sequences from *O. gerlachi* were successfully sequenced and encoded as shown in Table 1. Analysis revealed that these sequences were 583 bp in length. Nucleotide composition analysis determined the relative proportions as follows: C: 27.97%, T: 28.00%, A: 24.49%, and G: 19.54%. Haplotype analysis identified a total of 14 haplotypes, as presented in Table 2. Notably, most haplotypes were distinct from one another, except for two samples collected from Đăk Glei (C2 and C6), which shared the same haplotype.

Maximum likelihood (ML) phylogenetic analysis revealed a clear clustering pattern corresponding to the three sampling locations—Đăk Glei, Kon Plong, and Kon Rẫy—with a bootstrap value of 100% (Figure 2). Notably, samples from Kon Rẫy formed a distinct clade, whereas those from Đăk Glei and Kon Plong clustered separately, despite their considerable geographic distance (Figure 1). These findings indicate genetic differentiation in the COI region of *O. gerlachi* among the three sampled populations. Haplotype network analysis further confirmed the genetic divergence among these three groups. The substantial genetic differences between haplotypes resulted in distinct separation within the network (Figure 3).

Table 1 Sample name of 15 individuals of *O. gerlachi* in Đăk Glei, Kon Plong, and Kon Rẫy

Location	Sample order	Sample code name
Đăk Glei	KL.C.01	C1
	KL.C.02	C2
	KL.C.03	C3
	KL.C.06	C6
	KL.C.08	C8
Kon Plong	KP.R.02	R2
	KP.R.03	R3
	KP.R.05	R5
	KP.R.07	R7
	KP.R.09	R9
Kon Rẫy	KR.Tre.01	T1
	KR.Tre.02	T2
	KR.Tre.06	T6
	KR.Koi.04	K4
	KR.Koi.10	K10

Table 2 Haplotype analysis in COI gene of 15 individuals *O. gerlachi*.

Haplotype	Sequence name	Count
Hap_1	C1	1
Hap_2	C2, C6	2
Hap_3	C3	1
Hap_4	C8	1
Hap_5	R2	1
Hap_6	R3	1
Hap_7	R5	1
Hap_8	R7	1
Hap_9	T2	1
Hap_10	K10	1
Hap_11	T1	1
Hap_12	R9	1
Hap_13	T6	1
Hap_14	K4	1

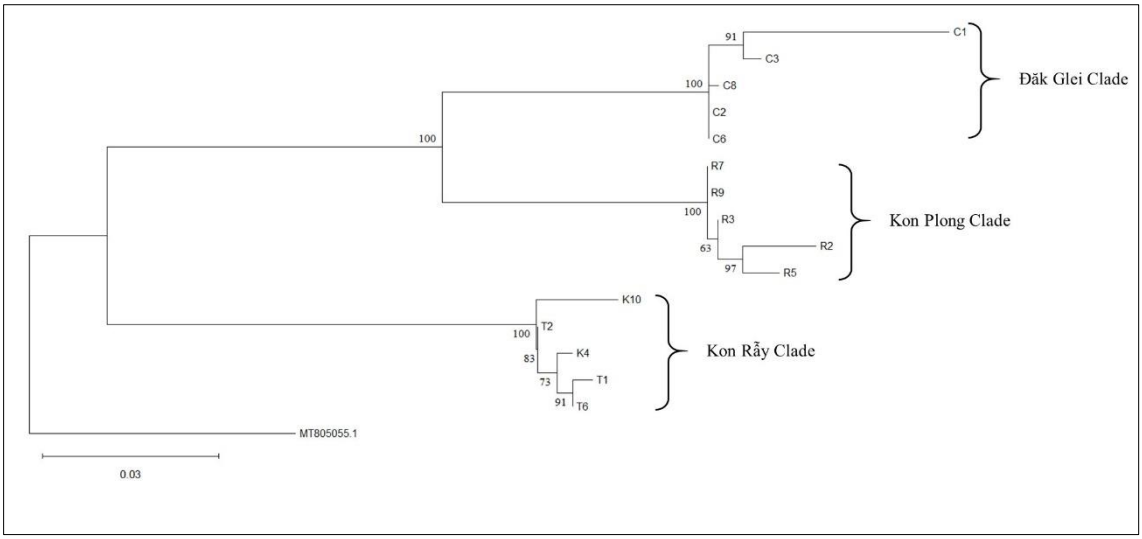

Figure 2 The ML tree base on COI gene of *O. gerlachi* in Kon Tum Province, Vietnam

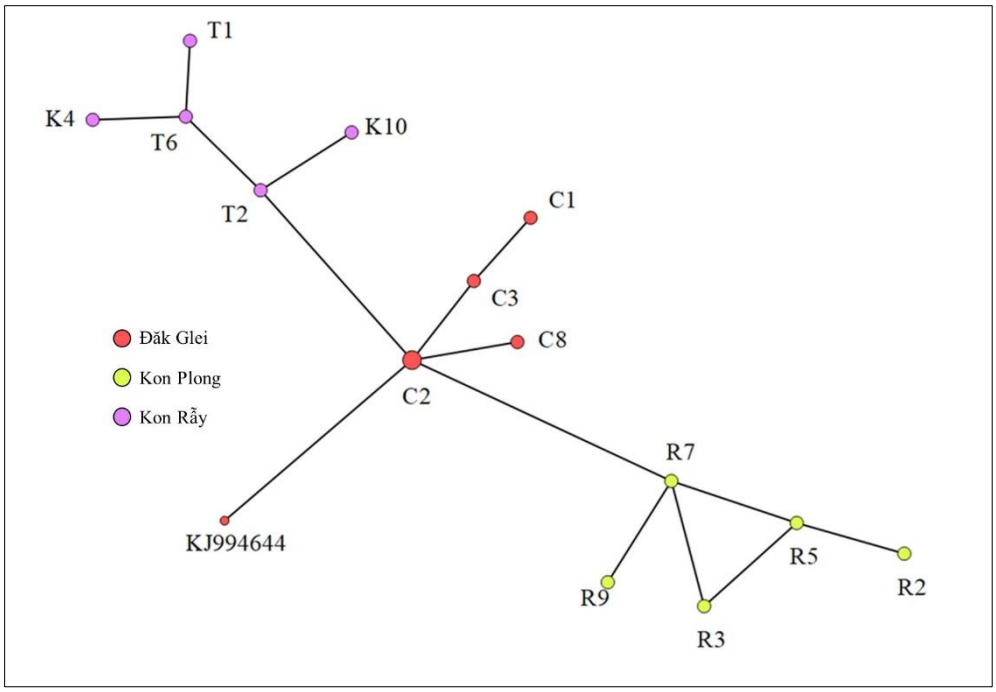

Figure 3 The haplotype network analysis of *O. gerlachi* in COI gene

Genetic diversity indices revealed that the *O. gerlachi* samples exhibited exceptionally high haplotype and nucleotide diversity, with values of 0.9905±0.0281 and 0.094977±0.048802, respectively (Table 3). Nucleotide variation analysis identified 133 polymorphic sites within the COI region of *O. gerlachi*, with an average nucleotide difference of 55.371429±25.375472 nucleotides per individual. Pairwise nucleotide differences among the 14 haplotypes of *O. gerlachi* are shown in Figure 4, where haplotypes from Kon Rẫy exhibited the highest nucleotide divergence, followed by those from Kon Plong (Figure 4). Genetic diversity analysis for each population indicated similarly high levels of genetic variation across all three sampling locations: Đăk Glei, Kon Plong, and Kon Rẫy (Table 3). Intra-population nucleotide differences among haplotypes were also analyzed (Figure 5). The results showed substantial genetic variation within each population. In Đăk Glei, individual C1 exhibited the highest nucleotide divergence (Figure 5A). In Kon Plong, R5 and R7 displayed relatively higher nucleotide differences compared to other individuals in the population (Figure 5B). Kon Rẫy exhibited the most pronounced genetic differentiation, with individual T10 standing out due to its exceptionally high pairwise nucleotide divergence (Figure 5C).

Table 3 Genetic diversity statistics in COI of *O. gerlachi.*

	Haplotype diversity	**Nucleotide diversity**	**Pairwise differences**	**Polymorphic sites**
Đăk Glei	0.9000±0.1610	0.017728±0.011423	10.300000±5.677276	23
Kon Plong	1.0000±0.1265	0.013058±0.008587	7.600000±4.275169	16
Kon Rẫy	1.0000±0.1265	0.013058±0.008587	7.600000±4.275169	16
Total	0.9905±0.0281	0.094977±0.048802	55.371429±25.375472	133

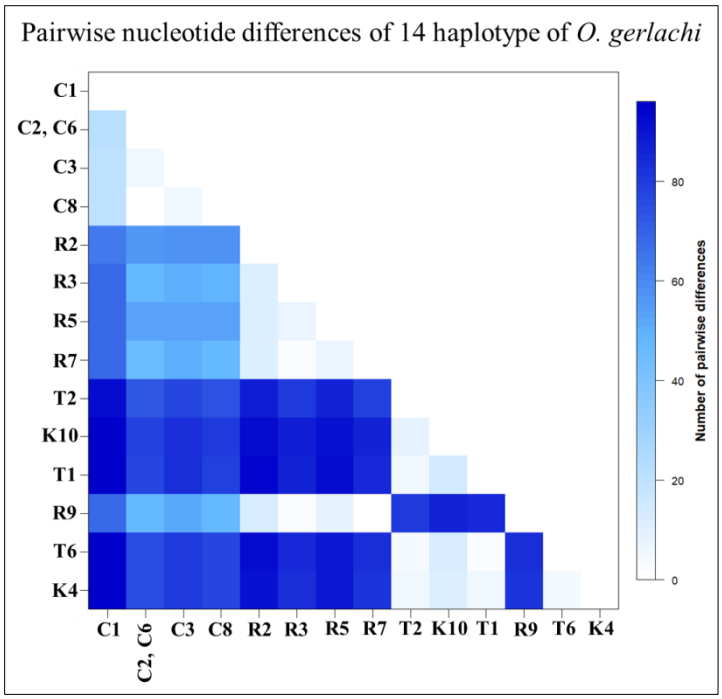

Figure 4 Nucleotide pairwise differences in COI gene of *O. gerlachi* haplotypes

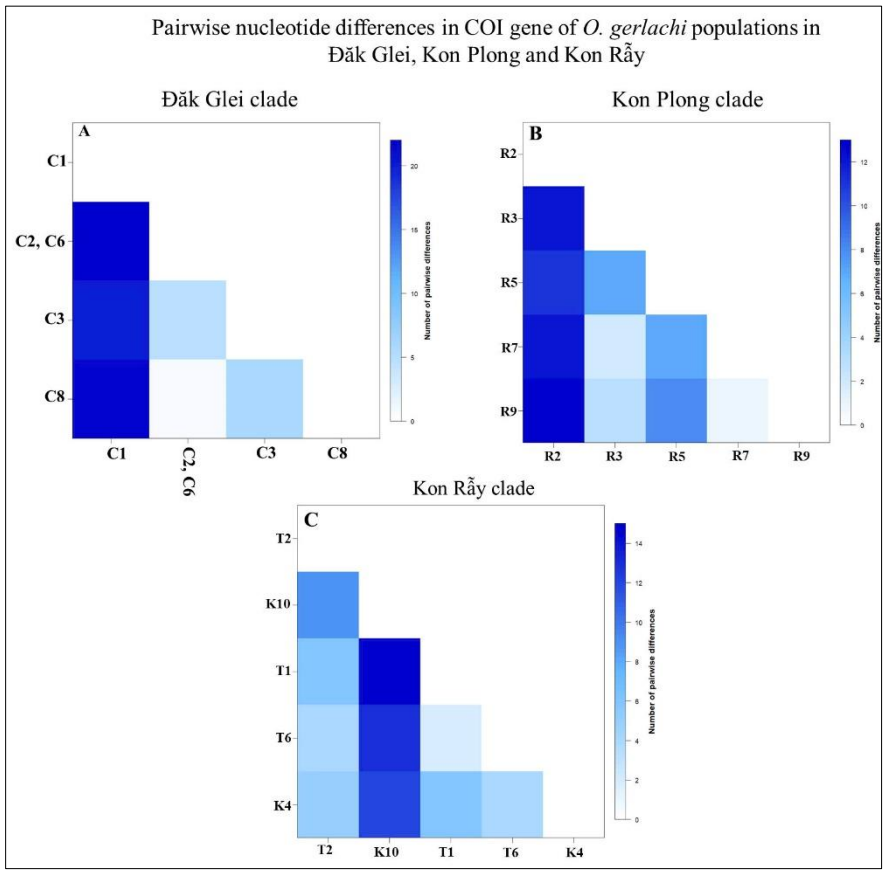

Figure 5 Nucleotide pairwise differences in COI gene of *O. gerlachi* population in Đăk Glei, Kon Plong, and Kon Rẫy (A. *O. gerlachi* in Đăk Glei population; B. *O. gerlachi* in Kon Plong population; C. *O. gerlachi* in Kon Rẫy population)

We conducted an assessment of genetic differentiation among the *O. gerlachi* samples collected from the three regions: Đăk Glei, Kon Plong, and Kon Rẫy. The results indicated that the F_{ST} values between these regions were greater than 0.5 (Table 4). This finding was further supported by the *p*-values from the F_{ST} analysis, all of which were below 0.05 (Table 5). Therefore, it can be concluded that there is substantial genetic differentiation in the COI region among the *O. gerlachi* populations from the three districts.

Table 4 Population pairwise genetic distance ($\boldsymbol{F_{ST}}$) analysis by Arlequin

Population pairwise genetic distance ($\boldsymbol{F_{ST}}$)			
	Đăk Glei	**Kon Plong**	**Kon Rẫy**
Đăk Glei	0.00000	-	-
Kon Plong	0.83560	0.00000	-
Kon Rẫy	0.89005	0.91216	0.00000

Table 5 The $\boldsymbol{p}$-values of $\boldsymbol{F_{ST}}$

***p*-values of $\boldsymbol{F_{ST}}$ analysis**			
	Đăk Glei	**Kon Plong**	**Kon Rẫy**
Đăk Glei	-	-	-
Kon Plong	0.00901±0.0091	-	-
Kon Rẫy	0.00000±0.0000	0.01802±0.0121	0.00000

4. Discussion

Our analysis revealed significant genetic differentiation among *O. gerlachi* populations across the surveyed regions. This raises concerns about the genetic divergence of *O. gerlachi* within our study. Based on geographic analysis, river mouths, streams, small tributaries, and interviews with local communities, we identified physical barriers that restrict water flow between small channels. As a result, reproductive isolation has likely occurred among the three populations in Đăk Glei, Kon Plong, and Kon Rẫy.

Furthermore, fishing pressure has significantly contributed to genetic variation among these populations. Over time, the accumulation of nucleotide substitutions has led to substantial genetic divergence. DNA in mitochondria is highly conserved, the observed variation of 10 to 55 nucleotide differences in the COI gene suggests that these *O. gerlachi* populations have been genetically isolated for an extended period. This finding provides valuable insights into the pronounced genetic differentiation of *O. gerlachi* populations in Đăk Glei, Kon Plong, and Kon Rẫy. Therefore, further comprehensive studies, including samples from additional geographic regions, are necessary to achieve a broader understanding of their genetic diversity.

5. Conclusion

The COI gene region was successfully sequenced from 15 *O. gerlachi* samples collected from three districts in Kon Tum: Đăk Glei, Kon Plong, and Kon Rẫy. Haplotype and nucleotide diversity were confirmed in the COI region of *O. gerlachi* across the surveyed areas. Genetic analysis demonstrated significant genetic differentiation among the three studied populations based on the COI gene region. The flow fragmentation and fishing pressure have intensified selective pressures on each *O. gerlachi* population**.**

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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