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Research Paper

Identification of Tuna (*Thunnus* sp.) in Sashimi Products from Restaurants in Manado City Using DNA Barcoding

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ABSTRACT

Tuna sashimi is a high-value fishery product widely consumed in Manado City; however, morphological verification of the tuna species used in such products is practically unfeasible. This study aimed to identify tuna species in sashimi products sold at restaurants in Manado City using DNA barcoding based on the cytochrome c oxidase subunit I (COI) gene. Sashimi samples were collected from four restaurants under actual operational conditions. The research procedure included tissue preparation, DNA extraction, amplification of the COI gene with universal primers, agarose gel electrophoresis, and Sanger sequencing. Sequences were assembled and edited using Geneious Prime v2025.2.2, then compared against the GenBank database via BLAST for species identification. All four samples (VL1, VL2, VL3, and VL4) produced single DNA bands of 709 bp. BLAST analysis showed 100% in sequence similarity with *Thunnus albacares*, belong to yellowfin tuna group. Multiple Sequence Alignment revealed one synonymous transversion in sample VL4. Pairwise sequence similarity among samples ranged from 99.85% to 100%, and the Kimura 2-Parameter (K2P) genetic distance was 0.000-0.0015. Phylogenetic analysis using the Neighbor-Joining method (1000 bootstrap replicates) confirmed the clustering of all samples with reference sequences of *T. albacares*. These results demonstrate the effectiveness of COI-based DNA barcoding for tuna species authentication in sashimi products and provide a scientific basis for seafood product traceability in Manado City.

Keywords: COI; DNA barcoding; molecular identification; tuna sashimi; *Thunnus albacares*

INTRODUCTION

Tuna (*Thunnus* sp.) is the most economically valuable marine fishery commodity in the global market, with total world tuna and tuna-like species catches reaching 8.3 million tonnes in 2022.[1] Species such as yellowfin tuna (*T. albacares*), bigeye tuna (*T. obesus*), and skipjack (*Katsuwonus pelamis*) dominate catches in Indonesian waters, with yellowfin and bigeye being the primary species supplying the sashimi market in Southeast Asia.[2] Differences in economic value among tuna species have fueled the practice of mislabeling, whereby incorrect labels are applied to tuna products. Studies have reported that more than 47% of tuna products sold in Los Angeles restaurants were mislabeled, supported by a global meta-analysis documenting an aggregate mislabeling rate of approximately 39%.[3],[4],[5] Sashimi thinly sliced raw fish or seafood served without cooking from the Japanese culinary tradition is increasingly popular in Manado City. Unlike the premium Japanese market, which associates sashimi exclusively with bluefin tuna (*T. thynnus*) due to its high fat content and distinctive texture, in the Southeast Asian market including Indonesia, sashimi products are not limited to bluefin species. The species commonly used as sashimi in this region are *T. albacares* (yellowfin) and *T. obesus* (bigeye), owing to their higher availability in the tropical Indo-Pacific waters and more affordable prices compared to bluefin.[6],[7] Therefore, sashimi criteria in Manado City are more commonly based on freshness quality, bright meat color, good texture, and food safety standards for raw consumption, rather than on a specific species type.[6]

Because sashimi preparation removes all morphological markers, conventional species identification cannot be performed at the point of consumption, creating conditions vulnerable to mislabeling and species substitution.[8] In Indonesia, DNA barcoding-based studies have also detected label discrepancies in processed tuna products, affirming the need for molecular verification in domestic markets.[9] Although Manado City has a high fish consumption culture and tuna sashimi is increasingly popular, no research has yet applied DNA barcoding to verify the species identity of tuna sashimi sold locally.[10]

DNA barcoding using the mitochondrial cytochrome c oxidase subunit I (COI) gene has emerged as the standard for seafood product authentication.[11],[12],[13] The COI gene exists in high copy numbers per cell, exhibits low intraspecific variation yet sufficient interspecific divergence for species-level identification, and can be amplified even from degraded DNA in processed food samples.[14] The universal primers developed by Ivanova *et al.* (2007) generate a 709 bp diagnostic barcode fragment that is effective in differentiating most tuna species.[15] A study on COI sequence variation showed that 11 of 13 *Thunnus* species could be distinguished, proving its utility in regional markets.[16]

This study aimed to identify tuna species in sashimi products from restaurants in Manado City using COI-based DNA barcoding, to characterize intraspecific genetic variation among samples through Multiple Sequence Alignment and genetic distance analysis, and to reconstruct phylogenetic relationships to validate species-level placement. The research findings provide baseline species authenticity data, contributing to consumer protection and supporting the traceability of the local seafood supply chain.

MATERIALS & METHODS

Sample Collection

This exploratory study was conducted from September to November 2025 at the Advanced Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University. Tuna sashimi samples were collected from four different restaurants in Manado City under actual operational conditions consistent with consumer purchasing practices, including one nationally scaled outlet. Each sample (±50 g) was stored in a sealed sterile container, transported to the laboratory within 15 minutes, and immediately processed. Sashimi tissue was cut into ±50 g portions using a sterile knife, placed into Falcon tubes containing 95% alcohol with identity codes VL1–VL4, and stored at -20°C until DNA extraction.

Equipment Sterilization and Tissue Sample Preparation

Prior to testing, glassware and micropipettes were cleaned, wrapped in aluminum foil, and sterilized using an autoclave at 121°C for 15 minutes. The working area was sterilized using 70% alcohol and UV irradiation to prevent DNA contamination. Subsequently, ±25 mg of muscle tissue was cut from each sample using a sterile knife and placed in 1.5 mL microtubes for the DNA extraction process.

DNA Extraction

DNA extraction was performed using the Tissue Genomic DNA Mini Kit (Geneaid) according to the manufacturer's protocol with minor modifications. Tissue was lysed using GT buffer and Proteinase K at 60°C for 30 minutes, followed by the addition of GBT buffer and a second incubation at 60°C for 20 minutes. DNA was bound to a silica column via centrifugation, washed with cold absolute ethanol to remove contaminants, and eluted with elution buffer. Purified DNA was stored at -20°C until PCR amplification.

PCR Amplification and Agarose Gel Electrophoresis

The COI gene fragment (±709 bp) was amplified using the universal primers FF2D (forward) and FR1D (reverse).[15] Each PCR reaction with a total volume of 40 µL consisted of: 20 µL MyTaq HS Red Mix (Bioline), 1.5 µL of each primer, 15 µL sterile Milli-Q water, and 2 µL DNA template. Thermocycler conditions (T-Personal, Biometra): initial denaturation at 94°C for 2 minutes; 35 cycles of 94°C for 30 seconds, annealing at 52°C for 40 seconds, extension at 72°C for 1 minute; final extension at 72°C for 10 minutes. PCR products were run on a 0.8% agarose gel in 0.5× TBE buffer at 100 V for 30 minutes, stained with ethidium bromide, and visualized under UV light. Successfully amplified amplicons were sent to First Base (Malaysia) for Sanger sequencing using primers FF2D and FR1D.

Sequence Analysis and Species Identification

Forward and reverse chromatograms were assembled and trimmed using the MUSCLE algorithm in Geneious Prime v2025.2.2. Consensus sequences in FASTA format were compared against the NCBI GenBank database using BLASTn. Species identification was declared valid at sequence similarity ≥98%. Multiple Sequence Alignment (MSA) of all four samples was performed with the MUSCLE algorithm to detect nucleotide polymorphisms and characterize substitution types. Codons involved were analyzed using the vertebrate mitochondrial genetic code. Pairwise sequence similarity and genetic distance (Kimura 2-Parameter/K2P model) were calculated in Geneious Prime. Phylogenetic reconstruction used the Neighbor-Joining method.[17] with 1000 bootstrap replicates. Reference sequences of *T. obesus*, *T. alalunga*, *T. thynnus*, *T. maccoyii*, *T. tonggol*, and *Katsuwonus pelamis* (outgroup) were downloaded from GenBank for comparative analysis. The inclusion of various *Thunnus* species was intended to display the taxonomic position of the samples, validate the barcode gap between intraspecific variation of *T. albacares* and interspecific genetic distance, and reinforce the validity of species identification based on clustering in the phylogenetic tree.

RESULT

PCR Amplification

COI gene amplification was successfully performed on all four tuna sashimi samples (VL1–VL4) obtained from several different outlets in Manado City, including one nationally scaled outlet. Agarose gel electrophoresis (0.8%) showed a clear single DNA band at ±709 bp (Figure 1), consistent with the fish barcode fragment standard.[18] No non-specific amplification or primer-dimers were detected, indicating high-quality DNA template and optimal PCR conditions. These results demonstrate that the primers used were capable of specifically amplifying the target gene region, consistent with reported consensus COI barcode sequences from various fish in the Taiwan Strait having an average length of 709 bp.[18] All PCR products were deemed suitable for use in the sequencing process.

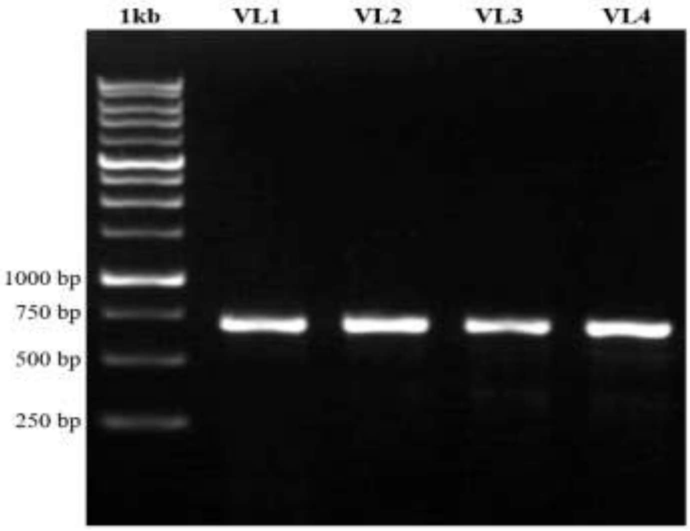

Figure 1. Agarose gel electrophoresis (0.8%) of COI gene PCR products from tuna sashimi samples (VL1–VL4).

Multiple Sequence Alignment and Genetic Variation

All four samples produced high-quality Sanger sequencing chromatograms with clear and distinct nucleotide peaks, as verified in Geneious Prime v2025.2.2. Consensus sequences were ±709 bp in length. MSA using the MUSCLE algorithm showed that VL1, VL2, and VL3 had identical COI sequences, while VL4 differed at one nucleotide at alignment position 343: a transversion of thymine (T) to adenine (A) (Figure 2). Codon analysis confirmed that this substitution changed codon CCT to CCA, both of which encode the amino acid proline. This mutation is synonymous (silent) with no effect on the COI amino acid sequence, as synonymous substitutions do not alter protein structure.[14]

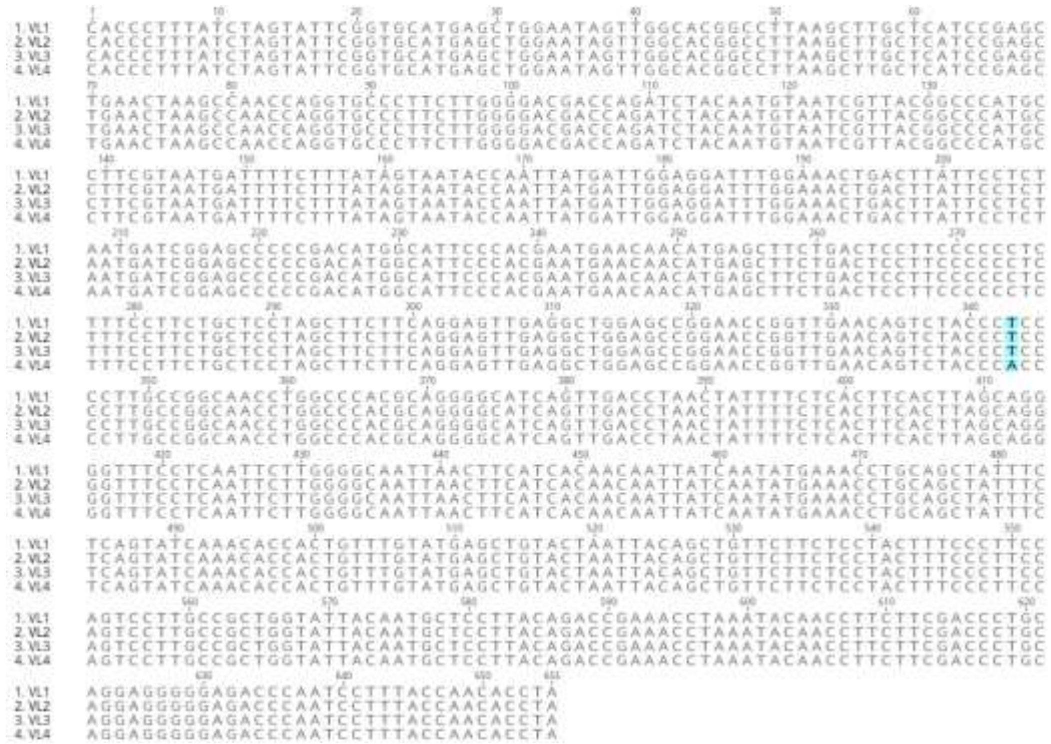

Figure 2. Multiple Sequence Alignment (MUSCLE algorithm) of COI sequences from samples VL1–VL4.

Pairwise sequence similarity among VL1-VL4 ranged from 99.85% to 100% (Table 1), and the K2P genetic distance was 0.000-0.0015 (Table 2), confirming that all variation remains within intraspecific bounds. In contrast, the genetic distance between the study samples and *Katsuwonus pelamis* reached ±0.11, demonstrating a clear barcode gap between intraspecific and interspecific divergence.

Table 1. Pairwise sequence similarity (%) of the COI gene from tuna sashimi samples VL1–VL4

Sample	VL1	VL2	VL3	VL4
VL1	100.00%	100.00%	100.00%	99.85%
VL2	100.00%	100.00%	100.00%	99.85%
VL3	100.00%	100.00%	100.00%	99.85%
VL4	99.85%	99.85%	99.85%	100.00%

Table 2. Kimura 2-Parameter (K2P) genetic distance among COI sequences of tuna sashimi samples VL1–VL4

Sample	VL1	VL2	VL3	VL4
VL1	0.0000	0.0000	0.0000	0.0015
VL2	0.0000	0.0000	0.0000	0.0015
VL3	0.0000	0.0000	0.0000	0.0015
VL4	0.0015	0.0015	0.0015	0.0000

BLAST Species Identification

BLAST analysis of all four COI sequences against the NCBI GenBank database identified *Thunnus albacares* as the top match for all samples, with 100% query coverage and 100% sequence similarity. All top accession numbers corresponded exclusively to *T. albacares* sequences from various geographic regions (Indonesia, the Philippines, India, and the Dongsha Islands), indicating consistent identification across the GenBank reference collection. Detailed BLAST results for each sample are presented in Tables 3-6.

Table 3. BLAST results for specimen VL1

No.	Scientific Name	Query Cover (%)	% Similarity	Accession
1	*Thunnus albacares*	100	100	OM743312.1
2	*Thunnus albacares*	100	100	PV242005.1
3	*Thunnus albacares*	100	100	KF528374.1
4	*Thunnus albacares*	100	100	NC_014061.1
5	*Thunnus albacares*	100	100	KP259550.1
6	*Thunnus albacares*	100	100	JN086153.1
7	*Thunnus albacares*	100	100	MH638704.1
8	*Thunnus albacares*	100	100	MK567346.1
9	*Thunnus albacares*	100	100	MN508955.1
10	*Thunnus albacares*	100	100	KY984984.1

Table 4. BLAST results for specimen VL2

No.	Scientific Name	Query Cover (%)	% Similarity	Accession
1	*Thunnus albacares*	100	100	OM743312.1
2	*Thunnus albacares*	100	100	NC_014061.1
3	*Thunnus albacares*	100	100	KF528374.1
4	*Thunnus albacares*	100	100	KP259550.1
5	*Thunnus albacares*	100	100	JN086153.1
6	*Thunnus albacares*	100	100	MH638704.1
7	*Thunnus albacares*	100	100	PV242005.1
8	*Thunnus albacares*	100	100	MN508955.1
9	*Thunnus albacares*	100	100	KY984984.1
10	*Thunnus albacares*	100	100	KX781891.1

Table 5. BLAST results for specimen VL3

No.	Scientific Name	Query Cover (%)	% Similarity	Accession
1	*Thunnus albacares*	100	100	OM743312.1
2	*Thunnus albacares*	100	100	NC_014061.1
3	*Thunnus albacares*	100	100	KF528374.1
4	*Thunnus albacares*	100	100	KP259550.1
5	*Thunnus albacares*	100	100	JN086153.1
6	*Thunnus albacares*	100	100	MH638704.1
7	*Thunnus albacares*	100	100	PV242005.1
8	*Thunnus albacares*	100	100	MN508955.1
9	*Thunnus albacares*	100	100	KY984984.1
10	*Thunnus albacares*	100	100	OU607616.1

Table 6. BLAST results for specimen VL4

No.	Scientific Name	Query Cover (%)	% Similarity	Accession
1	*Thunnus albacares*	100	100	DQ107652.1
2	*Thunnus albacares*	100	100	DQ885058.1
3	*Thunnus albacares*	100	100	KP975855.1
4	*Thunnus albacares*	100	100	MK567344.1
5	*Thunnus albacares*	100	100	MH638705.1
6	*Thunnus albacares*	100	100	KP975851.1
7	*Thunnus albacares*	100	100	DQ107648.1
8	*Thunnus albacares*	100	100	KP975853.1
9	*Thunnus albacares*	100	100	OM743312.1
10	*Thunnus albacares*	100	100	KP975859.1

Phylogenetic Analysis

The Neighbor-Joining phylogenetic tree reconstructed from COI sequences clustered all four samples (VL1-VL4) within a single strongly supported clade together with several *T. albacares* reference sequences from GenBank (Figure 3). Samples VL1, VL2, and VL3 formed an identical sub-cluster, while VL4 was positioned slightly apart within the same *T. albacares* clade, consistent with its variant harboring a single-nucleotide transversion. All other *Thunnus* species (*T. obesus*, *T. alalunga*, *T. thynnus*, *T. maccoyii*, *T. tonggol*) formed separate bootstrap-supported clades, and *K. pelamis* (outgroup) was clearly separated from the *Thunnus* cluster, validating the tree topology and confirming the presence of a barcode gap.

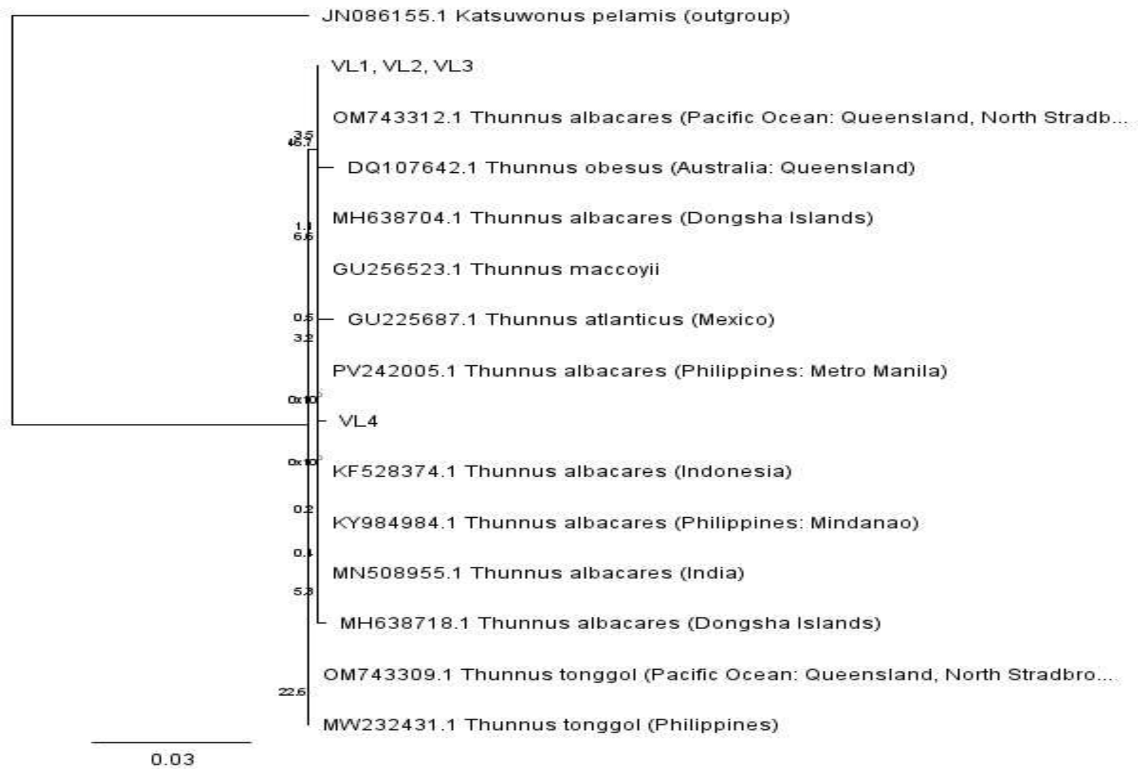

Figure 3. Neighbor-Joining phylogenetic tree based on COI sequences of samples VL1-VL4 and reference sequences of Thunnus sp. and Katsuwonus pelamis (outgroup) from GenBank.

DISCUSSION

All four tuna sashimi samples from restaurants in Manado City were identified as *Thunnus albacares* (yellowfin tuna) through COI-based DNA barcoding, with 100% BLAST similarity and strong phylogenetic support. These results are consistent with the regional fisheries context: yellowfin tuna dominates commercial catches in the Indo-Pacific and is the primary species supplying the sashimi market at Southeast Asian restaurants.[2],[6] The absence of species substitution across all four samples differs from the 30-47% mislabeling rates reported in sashimi and sushi studies from the United States, Europe, and parts of Asia,[3],[4] indicating that, at least for this sample set, the sashimi supply chain in Manado is consistent with product claims.

The single synonymous transversion (T→A at position 343) detected in VL4 represents a silent codon mutation (CCT→CCA, both proline) that falls well within the intraspecific variation range of *T. albacares*. The pairwise K2P genetic distances among all samples (0.000-0.0015) are far below the 2% intraspecific threshold applied in fish DNA barcoding,[14] and the ≈0.11 distance separating the samples from *K. pelamis* affirms a clear barcode gap. This variation likely reflects population- or geographic-level polymorphism, as similar synonymous substitutions in COI have been documented in *T. albacares* from various ocean basins.[19] Minor variation may also be influenced by chromatogram signal quality at certain base positions, although the high overall similarity among samples ensures that such variation does not significantly affect species identification.

The high-quality PCR products and chromatograms obtained from fresh sashimi tissue reinforce the well-established utility of COI barcoding for raw or minimally processed seafood, where DNA degradation is minimal.[20] The FF2D/FR1D universal fish barcoding primer succeeded on all samples, consistent with their design as universal fish barcoding primers.[15] The 709 bp amplicon size, the standard barcode length for vertebrates, provides sufficient sequence length for convincing BLAST matching and phylogenetic resolution among *Thunnus* species.[16]

Phylogenetic placement further reinforces species identity: all study samples clustered within the *T. albacares* clade with ≥95% bootstrap support, clearly separated from congeners including *T. obesus* and *T. thynnus*. This separation validates the discriminatory power of the COI barcode for species commonly found in Indonesia's tuna trade.[16] This study reinforces previous recommendations that DNA barcoding be integrated into routine seafood quality control and regulatory traceability programs in Indonesia, particularly for premium raw products such as sashimi, which carry high economic incentives for species substitution.

This study has limitations that should be acknowledged. The sample size from four restaurants does not allow broad generalization about the prevalence of mislabeling in Manado City. Future research should expand sampling to more vendors, a wider variety of products, and seasonal variation. The addition of alternative markers such as cytochrome b could improve resolution for closely related *Thunnus* species that are difficult to distinguish by COI alone.

CONCLUSION

COI-based DNA barcoding successfully identified all four tuna sashimi samples from restaurants in Manado City as *Thunnus albacares* (yellowfin tuna), with 100% BLAST query coverage and sequence similarity, and strong phylogenetic support. One synonymous transversion in sample VL4 represents normal intraspecific variation with no taxonomic implications. No evidence of species substitution or mislabeling was found in this sample set. These results demonstrate that DNA barcoding is a reliable and accurate method for species authentication in sashimi products, providing a molecular basis for seafood traceability and consumer protection within the tuna supply chain in Manado City

Declaration by Authors

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