



## Molecular Characterization of *Anisakis typica* (Nematoda: Anisakidae) from the Mediterranean Sea Coasts of Turkey: First Mitochondrial *rnrS* Sequence Data

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**Abstract:** There is no study about the molecular characterization of the mitochondrial *rnrS* gene of *Anisakis typica* from the Turkish Mediterranean coasts. Thus, molecular characterization of the *rnrS* gene of *A. typica* from Turkish waters is aimed for the first time in the present study. Ascaridoid marine nematodes were collected and morphologically identified by light microscope. Identified *Anisakis* type I larvae were randomly selected and molecularly analysed by Restriction Fragment Length Polymorphism (RFLP) for the internal transcribed spacer (ITS) region. *Anisakis typica* larvae identified by PCR-RFLP analyses were amplified and sequenced with both directions for the *rnrS* gene. The *rnrS* sequence of *A. typica* from the Mediterranean coasts of Turkey (MT395672) showed 99.80% identity (100% query coverage) with *rnrS* gene of *A. typica* (JX500052) from the Caribbean Sea, Atlantic Ocean. Between *A. typica* from the Mediterranean Sea (MT395672) isolate and the Caribbean Sea isolate differed by only one nucleotide (C–T, at alignment position 437). Pairwise genetic distance between the *rnrS* sequence of *A. typica* herein and other *A. typica* from the Caribbean Sea was 0.002. Consequently, we determined the mitochondrial *rnrS* data of *A. typica* from the Mediterranean Sea in the present study for the first time. The valid genetic data (MT395672) reported here can be used to molecular identification of *Anisakis* species from the Mediterranean Sea and worldwide.

**Keywords:** *Anisakis typica*, Mediterranean Sea, molecular characterization, small subunit of rRNA (*rnrS*).

## Türkiye'nin Akdeniz Kıyılarından *Anisakis typica*'nın (Nematoda: Anisakidae) Moleküler Karakterizasyonu: İlk Mitokondriyal *rnrS* Dizi Verileri

**Öz:** Türkiye Akdeniz kıyılarındaki *Anisakis typica*'nın mitokondriyal *rnrS* geninin moleküler karakterizasyonu hakkında bir çalışma bulunmamaktadır. Bu nedenle mevcut araştırmada Türk sularından izole edilen *A. typica*'nın *rnrS* geninin moleküler karakterizasyonu amaçlanmıştır. Ascaridoid deniz nematodları toplandı ve morfolojik olarak ışık mikroskobu ile teşhis edildi. Tanımlanan *Anisakis* tip I larvaları rastgele seçildi ve Sınırlayıcı Enzim Parça Uzunluk Çeşitliliği (RFLP) yöntemi ile internal transcribed spacer (ITS) bölgesi yönünden moleküler olarak analiz edildi. RFLP analizleri ile *Anisakis typica* olarak teşhis edilen larvaların *rnrS* geni çoğaltıldı ve iki yönlü DNA dizi analizleri yapıldı. Türkiye'nin Akdeniz kıyılarından elde edilen *A. typica*'nın *rnrS* geni (MT395672) Atlantik Okyanusu Karayip Denizinden elde edilen *A. typica*'nın *rnrS* geni (JX500052) ile %99,80 oranında (%100 sorgu kapsamı) benzerlik gösterdi. Akdeniz'den *A. typica* (MT395672) izolatu ile Karayip Denizi izolatu arasında sadece bir nükleotit (C – T, hizalama pozisyonu 437) farklıydı. Buradaki *A. typica*'nın *rnrS* dizisi ile Karayip Denizi'nden diğer *A. typica* arasındaki ikili genetik mesafe 0.002'dir. Sonuç olarak bu çalışmada ilk kez Akdeniz'den *A. typica*'nın mitokondriyal *rnrS* verilerini belirledik. Burada bildirilen geçerli genetik veri (MT395672), *Anisakis* türlerinin Akdeniz'den ve dünyadan moleküler tanımlanması için kullanılabilir.

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**Anahtar kelimeler:** Akdeniz, *Anisakis typica*, küçük alt ünite rRNA (*rnrS*), moleküler karakterizasyon.

## INTRODUCTION

Adult nematodes of *Anisakis* Dujardin, 1845 are mainly found in the gastrointestinal canal of marine mammals. Different species of marine mammals and fish and squids serve as definite and intermediate or paratenic hosts, respectively. Until now based on molecular genetic markers such as nuclear and mitochondrial DNA, nine distinct *Anisakis* species have been reported in the world. Among the mitochondrial markers, the cytochrome oxidase I (*cox1*), II (*cox2*) and the small subunit of rRNA (*rrnS*) genes are often prepared for molecular characterizations of *Anisakis* species. The specimen of *A. typica* has been widely distributed in definitive and intermediate or paratenic hosts at marine waters between 35–40°N to 36°S as geographic coordinates (Mattiucci & Nasseti 2008, Mattiucci et al., 2018).

Both mitochondrial *cox2* and nuclear ITS data of *Anisakis* species from marine fish were previously reported from coasts of Turkey (Pekmezci et al., 2014). Moreover, molecular characterization of *rrnS* loci of only *Hysterothylacium aduncum* and *Contraecaecum overstreeti* among ascaridoids nematodes of marine fish was made in the Turkish waters (Pekmezci & Yardimci, 2019; Pekmezci, 2019). Up to date, there is no study about the molecular characterization of the mitochondrial *rrnS* gene of *A. typica* from the Turkish Mediterranean coasts. Therefore, the molecular characterization of the *rrnS* gene of *A. typica* from Turkish waters is aimed for the first time in the current study.

## MATERIAL AND METHOD

**Morphological examinations, PCR and RFLP analysis:** Nematodes were collected from *Merluccius merluccius* in the Mediterranean coasts of Turkey. These were individually cut into three parts. The anterior and posterior parts were used for morphological identifications. The middle parts were used for DNA extractions using commercial kits. Nematodes were morphologically identified by light microscope according to Berland, (1961) and Petter and Maillard, (1988). Morphologically identified representative specimens were randomly selected and genetically analysed. The ITS regions of nuclear DNA were amplified using NC5/NC2 primers (Zhu et al., 1998). The ITS regions were then digested with *HhaI* and *HinfI* enzymes using RFLP analysis to identify the species (D’Amelio et al., 2000). All PCR amplicons and RFLP patterns were electrophoresed on 2% gel and visualized under UV illumination. The *rrnS* gene of the mitochondrial DNA of the selected *Anisakis* species was amplified with MH3/MH4.5 primer pairs (D’Amelio et al., 2007).

**DNA Sequencing and Genetic analysis:** Selected three individuals were sequenced both directions with MH3/MH4.5 primer pairs using Sanger methods for the *rrnS* gene. We checked the sequence quality, assembled and then trimmed to remove primers in Geneious R11 (Kearse et al., 2012). The assembled sequence was blasted in GenBank database to examine the nucleotide similarity (Altschul et al., 1990). Obtained *rrnS* data from Genbank were aligned by ClustalW in MEGA X multiple sequence alignments (Kumar et al., 2018) and adjusted manually. Pairwise distances were estimated using the K2P model in MEGA X (Kumar et al., 2018).

## RESULTS

Ascaridoid nematodes were morphologically identified as third stage of *Anisakis* type I larvae. Some specimens of *Anisakis* larvae were classified as *A. typica* by RFLP analyses with *HhaI* and *HinfI* enzymes as previously described by D’Amelio et al., (2000). The amplified *rrnS* gene of *A. typica* was produced ~500 bp in the PCR analyses. After DNA sequencing of *rrnS* gene and trimmed to primers, the 494 bp length products were obtained in the present study. There were no intraspecific nucleotide differences detected within *rrnS* gene of three representatives. Therefore, the *rrnS* sequence of one representative was submitted to GenBank was given the accession number: MT395672. Nucleotide difference in the *rrnS* sequences between *A. typica* from the Mediterranean Sea (MT395672) and the Caribbean Sea differed by one nucleotide (C–T, at alignment position 437) (Figure 1). Pairwise genetic distance between the *rrnS* sequence of *A. typica* herein and other *A. typica* from the Caribbean Sea was 0.002.

MT395672	1	TATAA	60
JX500052	17	.....	76
MT395672	61	ttagggtga	120
JX500052	77	.....	136
MT395672	121	GCCAGATTAGTACCTGGTTAGATAAAATTTAAAGAGCAGGAGTAAAGTTGATTATTAAC	180
JX500052	137	.....	196
MT395672	181	TGTAAGAAATATTGGCAGGTTTTAAATTAATCTTTGGAGGTTGAGTAGTAATTGAGAACCC	240
JX500052	197	.....	256
MT395672	241	TCATTAACAACCTTTTTATTGGGCGCATGTATGATCGTTTATTTTATCTCTTAAGGATTGT	300
JX500052	257	.....	316
MT395672	301	AATTTTAATTTTTGGTTGTAATAATAGATAGATATCTGGCTTATGAAAAAGATTTAATTT	360
JX500052	317	.....	376
MT395672	361	GACCTACAATAAATAATTTTGTGGATGCCCGGCTTGTGGCCCGGCTGAAATTTGTAAGAAG	420
JX500052	377	.....	436
MT395672	421	ACAGTAAACCTCTTTTCATGGGGGGTTGAAGTTTATTTAAAAACGGTACAAATCATCCAT	480
JX500052	437	.....T.....	496
MT395672	481	CAATTGCCATAGG	494
JX500052	497	.....	510

**Figure 1.** Nucleotide difference in the *rrnS* sequences between *A. typica* from the Mediterranean Sea (MT395672) and the Caribbean Sea (JX500052) isolates.

## DISCUSSION

This study represents first data on the molecular characterization of the *rrnS* gene region of *A. typica* isolated from the Mediterranean Sea. The sequence analysis of the *rrnS* loci of *A. typica* was firstly characterized from the Caribbean Sea, Atlantic Ocean by Mattiucci et al., (2014) and previously submitted as accession number JX500052 in GenBank database. A new valid *rrnS* sequence of *A. typica* (accession number MT395672) in the present study was the second record in the GenBank database. Our *rrnS* sequence of *A. typica* from the Mediterranean coasts of Turkey (MT395672) showed 99.80% identity (100% query coverage) with *rrnS* gene of *A. typica* (JX500052) from the Caribbean Sea, Atlantic Ocean (Mattiucci et al., 2014). Moreover, a search in GenBank database showed that *rrnS* sequence of *A. typica* in the present study were ranged from 88.91% to 91.11% identical to *rrnS* sequences of *A. physeteris* (JX500055), *A. brevispiculata* (JX500056), *A. paggiae* (JX500057), *A. simplex* x *A. pegreffii* (AB831878), *A. nascettii* (JX500054), *A. ziphidarum* (JX500053), *A. pegreffii* (LC222461) and *A. simplex* (AY994157). These results showed that mitochondrial *rrnS* gene could be used effectively in the genetic distinction of *Anisakis* species. Pairwise genetic distance for mitochondrial *rrnS* loci showed a very low-level variation among the Mediterranean Sea isolate of *A. typica* (MT395672) herein and the Caribbean Sea isolate of *A. typica* previously found (JX500052) (p distance=0.002). We considered the very low level of nucleotide differences (0.2%) between those isolates of *A. typica* as intraspecific nucleotide differences because of the different geographical locations.

## CONCLUSION

We determined the mitochondrial *rrnS* data of *A. typica* from the Mediterranean Sea in the present study for the first time. Moreover, the new valid *rrnS* sequence (accession number MT395672) was the second record in the GenBank and this unique data can be also used to molecular identification of *Anisakis* species from the Mediterranean Sea and worldwide.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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