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

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Geliş/Received: 17.05.2020
Kabul/Accepted: 15.06.2020

Abstract: There is no study about the molecular characterization of the mitochondrial rrnS gene of Anisakis typica from the Turkish Mediterranean coasts. Thus, molecular characterization of the rrnS gene of A. typica from Turkish waters is aimed for the first time in the present study. Ascaroidid 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 rrnS gene. The rrnS sequence of A. typica from the Mediterranean coasts of Turkey (MT395672) showed 99.89% identity (100% query coverage) with rrnS 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 rrnS sequence of A. typica here and other A. typica from the Caribbean Sea was 0.002. Consequently, we determined the mitochondrial rrnS 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 (rrnS).

Türkiye’nin Akdeniz Kıyılarından Anisakis typica’nın (Nematoda: Anisakidae) Moleküler Karakterizasyonu: İlk Mitokondriyal rrnS Dizi Verileri

Öz: Türkiye Akdeniz kıyılarından Anisakis typica’nın mitokondriyal rrnS geninin moleküler karakterizasyonu hakkında bir çalışma bulunmamaktadır. Bu nedenle mevcut araştırımda Türk sularından izole edilen A. typica’nın rrnS geninin moleküler karakterizasyonu amaçlanmıştır. Ascaroidid deniz nematodları toplandı ve morfolojik olarak ışık mikroskobu ile teşhis edildi. Tanımlanan Anisakis tip I larvaları rastgele seçilip ve Sınırlayıcı Enzim Parçaları Uzunluğunu Çeşitlettiği (RFLP) yöntemle ile internal transcribed spacer (ITS) bölgesi yönünden moleküler olarak analiz edildi. RFLP analizleri ile Anisakis typica larvaların teşhis edilen larvaların rrnS geni çoğaltıldı ve iki yönlü DNA dizi analizleri yapıldı. Türkiye’nin Akdeniz kıyılarından elde edilen A. typica’nın rrnS geni (MT395672) Atlantik Okyanusu Karayip Denizi'nden elde edilen A. typica’nın rrnS geni (JX500052) ile %99,80 oranında (%100 sorgu kapsamlı) benzerlik gösterdi. Anideniz’den A. typica (MT395672) izolatı ile Karayip Denizi izolatı arasında sadece bir nükleotit (C–T, hierarchy pozisyonu 437) farklıydı. Buradaki A. typica’nın rrnS dizisi ile Karayip Denizi’nden diğer A. typica arısmakları ikili genetik mesafe 0.002'dir. Sonuç olarak bu çalışmada ilk kez Akdeniz’den A. typica’nın mitokondriyal rrnS verilerini belirledik. Burada bildirilen geçerli genetik veri (MT395672), Anisakis türlerinin Akdeniz’den ve dünyadaki moleküler karakterizasyonu için kullanılabilir.

Anahtar kelimeler: Akdeniz, Anisakis typica, küçük alt ünite rRNA (rrnS), 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 & Nasetti 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 *Contracaecum overstreeti* among ascaridoids nematodes of marine fish was made in the Turkish waters (Pekmezci & Yardımcı, 2019; Pekmezci, 2019). Up to date, there is no study about the Turkish waters (Pekmezci & Yardımcı, 2019; Pekmezci et al., 2014). Moreover, *A. typica* from the Mediterranean coasts of Turkey. These specimens of *A. typica* from 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 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.

**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.

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 rrsS gene region of A. typica isolated from the Mediterranean Sea. The sequence analysis of the rrsS 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 rrsS sequence of A. typica (accession number MT395672) in the present study was the second record in the GenBank database. Our rrsS sequence of A. typica from the Mediterranean coasts of Turkey (MT395672) showed 99.80% identity (100% query coverage) with A. typica from the Caribbean Sea, Atlantic Ocean by Mattiucci et al., (2014) and previously submitted as accession number MT395672) in the present study was the second record in GenBank database. Moreover, a search in GenBank database showed that rrsS sequence of A. typica in the present study were ranged from 88.91% to 91.11% identical to rrsS sequences of A. physeteris (JX500055), A. breviplicata (JX500056), A. paggiae (JX500057), A. simplex x A. pegreffii (AB831878), A. nascettii (JX500054), A. ciphiolarum (JX500053), A. pegreffii (LC222461) and A. simplex (AY994157). These results showed that mitochondrial rrsS gene could be used effectively in the genetic distinction of Anisakis species. Pairwise genetic distance for mitochondrial rrsS 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 rrsS data of A. typica from the Mediterranean Sea in the present study for the first time. Moreover, the new valid rrsS 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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