

Short Communication / Kısa Bilimsel Çalışma

Pathology and molecular identification of *Anisakis pegreffii* (Nematoda: Anisakidae) infection in the John Dory, *Zeus faber* (Linnaeus, 1758) caught in Mediterranean Sea

Banu YARDIMCI, G. Zafer PEKMEZCİ, E. Emek ONUK

Department of Diseases of Aquatic Animals, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, Kurupelit, Samsun, TURKEY.

Summary: One specimen of John Dory, *Zeus faber* (Linnaeus, 1758), was caught from commercial sites at the costs of Gulf of Antalya at Mediterranean Sea, Turkey. *Zeus faber* was dissected carefully. After examination Anisakidae larvae were recovered from in the abdominal cavity, liver and muscles. A total of eight larvae were found in the abdominal cavity and seven larvae encapsulated on surface of liver at necropsy. In the macroscopic examination, liver was seen to pale and surface of the capsula was haemorrhagic with subcapsular cysts including larvae in 0.3 cm diameter. In the histopathologic examination severe degeneration and hepatic necrosis was identified. Just below the liver capsula, *Anisakis* larvae were seen covered with fibrous capsula. The larvae morphologically identified as *Anisakis* larvae Type I were subjected to further molecular characterization by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to identify the species. All *Anisakis* larvae were identified at species level by PCR-RFLP as belonging to *A. pegreffii*. Within the present study, for the first time, we report on the molecular identification of *A. pegreffii* larvae from John Dory, *Z. faber*, and we describe their pathological significance in a paratenic or intermediate hosts in Turkish waters.

Keywords: *Anisakis pegreffii*, pathology, PCR-RFLP analysis, *Zeus faber*.

Akdeniz’de yakalanan Dülger balığındaki *Anisakis pegreffii* (Nematoda: Anisakidae) enfeksiyonunun moleküler identifikasyonu ve patolojisi

Özet: Çalışmanın materyalini Akdeniz’de Antalya körfezi avlanma sahasında yakalanan bir dülger balığı *Zeus faber* (Linnaeus, 1758) oluşturdu. *Zeus faber* dikkatli bir şekilde diseke edildi ve karın boşluğu, karaciğer ve kaslar Anisakidae ailesindeki nematod larvaları yönünden incelendi. Makroskopik olarak karın boşluğunda sekiz larva ile karaciğer yüzeyinde kapsüle olmuş 7 larva bulundu. Makroskobik incelemede, solgun görünümde olan karaciğer yüzeyinde kanamalara ve kapsül altında 0,3 cm çapında kistlere rastlandı. Histopatolojik incelemede, hepatositlerde şiddetli dejenerasyon ve nekroz tanımlandı. Karaciğer kapsülü altında fibröz kapsülle çevrili *Anisakis* larvaları gözlemlendi. Morfolojik olarak tanımlanan *Anisakis* Tip I larvalar tür teşhisi için PZR-RFLP ile moleküler olarak incelendi. Tüm *Anisakis* larvalar PZR-RFLP ile *Anisakis pegreffii* olarak teşhis edildi. Bu çalışma ile Türkiye sularında avlanan dülger balığında *Anisakis pegreffii*’nin moleküler identifikasyonu ve paratenik ya da ara konakdaki patolojik bulguları ilk kez tanımlandı.

Anahtar sözcükler: *Anisakis pegreffii*, patoloji, PZR-RFLP, *Zeus faber*.

The John Dory, *Zeus faber* (Linnaeus, 1758) is widely distributed in the Aegean Sea, Mediterranean Sea, western coastal waters of Europe, the English Channel, North Sea, and the coastal waters of South Africa, Australia and New Zealand. It inhabits waters of southern Japan, the East and South China Seas, and the Pacific, Indian and Atlantic Oceans (7). *Anisakis pegreffii* is the most frequently isolated in many fish species from Mediterranean waters and appears to be responsible for many cases of human anisakiasis (9). In Turkey, *A. simplex* and *A. pegreffii* were morphologically

identified different fish species (1,16); while *A. pegreffii* was molecularly detected in *Trachurus trachurus* (17). Moreover, *A. simplex* was morphologically found in *Z. faber* from the coastal waters of Gökçeda (1). Nematodes of genus *Anisakis* parasitize a wide range of marine hosts with marine mammals, especially cetaceans, serving as definitive hosts, while fish, squid and other invertebrates serve as paratenic or intermediate hosts. In fish, third-stage larvae of the genus *Anisakis* encapsulates on the surfaces of the visceral organs, mainly in the mesenteries and liver. Recently, molecular

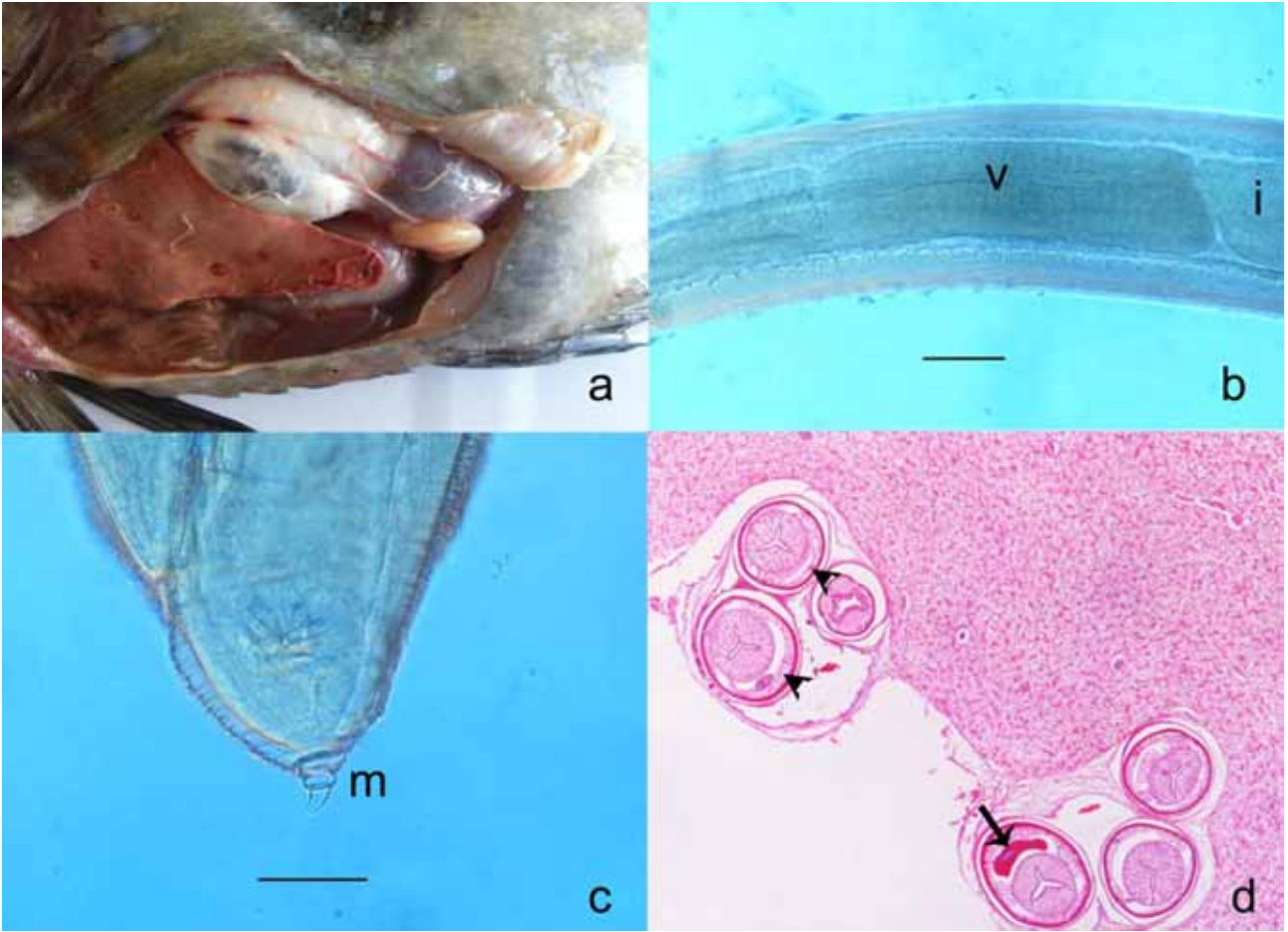


Figure 1. a. Macroscopic appearance of visceral organs and parasites. b. *Anisakis* Tip I larvae, ventriculus (v)-intestine (i) junction, scale 150 μ . c. Caudal end and mucron (m), scale 50 μ . d. Transverse sections of the *Anisakis* larvae below the liver capsula. Polymyarian muscle cells, separated into four quadrants by the chords were visible (arrow heads) and excretory cells were banana-shaped (arrow), HE x400.

Şekil.1. a. Parazitlerin ve iç organların makroskopik görünümü. b. *Anisakis* Tip I larva, ventriculus (v)-intestine (i) birleşim yeri, ölçü 150 μ . c. Arka uç ve mukron (m), ölçü 50 μ . d. Karaciğer kapsülü altındaki *Anisakis* larvalarının transversal kesiti. Polymyarian kas hücreleri (ok başları) ve muz şeklinde salgı hücreleri (ok), HE x400.

techniques, sequencing and restriction fragment length polymorphism (RFLP) analyses of the ribosomal DNA (rDNA) internal transcribed spacer region (ITS region), have been proven to be particularly useful for the accurate identification of ascaridoid nematodes at the species level for eggs, larvae, and adults (4,18).

Most reports about the pathogenic effect of larval *Anisakis* in fish are correlated with the effects of the larvae to the liver of the host. The most probable larval damage is mainly caused by compression of the tissues. Sometimes larval damage is more deleterious due to more deeply penetration to the liver. Some authors reported reduction in liver size in several fish species after heavy infections of larval *Anisakis* (3,8,14). Liver atrophy of hake in superficial but heavy infections of *Anisakis* was reported by Remotti (14) but according to Guiart (6) this liver damage was caused by toxins secreted by the worm. In contrast, no evidence of degenerative liver changes or general inflammatory reaction was found in the same host by Brian (3) who

concluded that atrophy of the liver was due to mechanical compression.

In this paper, for the first time, we report on the molecular identification of *A. pegreffii* larvae from John Dory, *Z. faber* (Linnaeus, 1758), and we describe their pathological significance in a paratenic or intermediate hosts in Turkish waters.

Parasite detection and pathological examination: One specimen of *Z. faber* was caught from commercial sites at the costs of Gulf of Antalya at Mediterranean Sea, Turkey. *Z. faber* was dissected carefully and examined for larvae of Anisakidae nematodes in the abdominal cavity, liver and muscles. Firstly, total length of larvae found in abdominal cavity was measured with a microscope. Then, a small portion of the mid-body of larvae was excised and stored in 70% ethanol at -20 °C for molecular study. The remainder of the larvae was cleared in lactophenol for morphological examination and measured with a microscope. Morphological identification was conducted according to Berland (2).

The larvae morphologically identified as belonging to the genus *Anisakis* were subjected to further molecular characterization by RFLP to identify the species. Encapsulated nematodes were found on the liver tissue was fixed in 10% neutral formaldehyde solution for pathological examination. Tissue samples were routinely taken processed and embedded in paraffin. Tissue sections 4–6 µ in width were stained with haematoxyline-eosin (HE) and examined under light microscope (Nikon Eclipse 80i).

PCR-RFLP analysis: A total of 15 *Anisakis* larvae Type I, at third-stage, recovered from the *Z. faber* were examined. Genomic DNA was extracted from larvae in abdominal cavity and paraffin-embedded encapsulated larvae on the liver, using the DNA purification kit (DNA DNeasy tissue kit, Qiagen) according to manufacturer's instructions. PCR amplification of internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) region of parasites was carried out according to Zhu et al (18) with NC5 (forward 5'-GTAGGTGAACCTGCGGAAGG ATCATT-3') and NC2 (reverse 5'-TTAGTTTCTTCCT CCGCT-3') primers. The restriction endonucleases, *HhaI* and *HinfI*, were used in RFLP analyses of ITS rDNA for the identification of the members of *Anisakis* genus according to the genetic markers defined by D'Amelio et al. (4).

Morphological and molecular identification of *Anisakis* larvae: A total of eight larvae were macroscopically found in the abdominal cavity and seven larvae encapsulated on surface of liver at necropsy

(Figure 1.a). Parasites were identified morphologically as *Anisakis* Type I larvae in abdominal cavity. The identification was based on the presence of a long ventriculus with an oblique ventricular-intestinal junction, without a ventricular appendage and intestinal caecum and a rounded tail possessing a mucron (Figure 1.b,c). Our morphological measurements were similar to literatures (11,16) except small differences (Table 1).

In the macroscopic examination, liver was seen to pale and surface of the capsula was haemorrhagic with subcapsular cysts including larvae in 0.3 cm diameter. In the histopathologic examination severe degeneration and hepatic necrosis was identified. Just below the liver capsula, *Anisakis* larvae were seen covered with fibrous capsula. Larvae had a thin cuticle. Polymyarian muscle cells, separated into four quadrants by the chords were visible. Excretory cells were banana-shaped (Figure 1.d). The nematode was identified as a larva belonging to the genus *Anisakis*.

All *Anisakis* larvae were identified at species level by RFLP as belonging to *A. pegreffii*. All larvae amplifications of the ITS rDNA region produced a fragment of approximately 1000 bp. Three clear bands of 330, 280 and 240 bp for *A. pegreffii* were observed in RFLP patterns digested with *HinfI* (Figure 2), whilst digestion with *HhaI* produced two fragments of 550 and 430 bp in *A. pegreffii* (data not shown).

In the present study, for the first time, we report on the molecular identification of *A. pegreffii* larvae from John Dory, *Z. faber*, and we describe their pathological

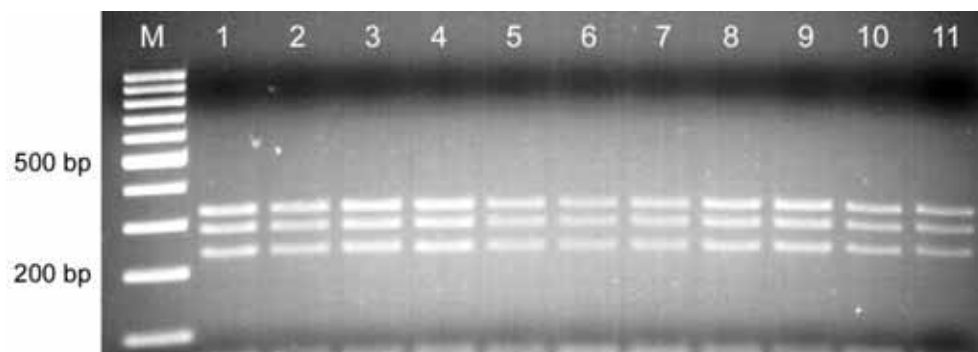


Figure 2. RFLP analysis with *HinfI* of the ITS PCR products amplified from the *Anisakis* type I larvae. Lane 1-11: *A. pegreffii*. The 100-bp DNA ladder marker was used to estimate the size of the bands (lane M).

Şekil 2. *Anisakis* tip I larvalardan çoğaltılan ITS PCR ürününün *HinfI* ile RFLP analizi. 1-11: *A. pegreffii*. Bantların büyüklüğünü değerlendirmek için 100-bp DNA marker (M) kullanıldı.

Table 1. Comparative measurements (mm) of some morphological characters in third-stage larvae of *Anisakis pegreffii*. Tablo 1. Üçüncü dönem *Anisakis pegreffii* larvalarının bazı morfolojik karakterlerinin karşılaştırmalı ölçüleri (mm).

Morphological characters	Larvae of <i>Anisakis pegreffii</i>		
	Present study	Quiazon et al., 2008	Tepe and Oguz 2013
Total body length	12.80-24.65	11.10-26.78	12.05-17.64
Maximum body width	0.40-0.51	0.38-0.60	0.30-0.54
Length of oesophagus	0.98-1.87	1.04-2.11	0.69-1.78
Ventriculus length	0.53-0.74	0.50-0.78	0.50-0.81
Tail length	0.08-0.11	0.05-0.12	0.07-0.21
Mucron length	0.01-0.02	0.02-0.03	-

significance in a paratenic or intermediate hosts in Turkish waters.

Capsule formation around the *Anisakis* larvae is a feature common in the parasitic infection in the other species of fish (5,13). It was observed that, the visceral structure and the liver lesions of this larval form was found to be similar with Anisakiosis cases in the other species (10,15). Different studies have demonstrated that the ITS region (ITS1, the 5.8S and the ITS2) of nuclear rDNA provide useful genetic markers for the accurate identification of sibling species and morphospecies within Anisakid species (4,12,18). In this present study, the identification of the larval nematodes recovered from the examined John Dory, *Z. faber* (Linnaeus, 1758), as belonging to the species *A. pegreffii* was very well supported to PCR-RFLP technique by D' Amelio et al. (4); Pontes et al. (12). *A. pegreffii* is the dominant species of *Anisakis* in the Mediterranean Sea, being widespread in all the fish species. Actually, this species is presently the most important anisakid larvae in different pelagic and demersal fish from Mediterranean waters (9). In the Mediterranean Sea, its definitive hosts include dolphins, such as *Tursiops truncatus*, *Stenella coeruleoalba*, and as intermediate/paratenic hosts, 18 fish species and 1 squid species have been reported by Mattiucci and Nascetti (9). On the other hand, the absence of other species of the genus *Anisakis*, for instance, *A. simplex* s.s., which is a species mainly distributed in the North Atlantic Sea, seems so far to suggest that these individuals of John Dory, *Z. faber*, were from a Mediterranean population. Indeed, *Anisakis* spp. have been proved to be used as a good biological tags in suggesting the possible origin and migratory routes of their intermediate/paratenic hosts (9).

Acknowledgement

This study was presented in The 12th Symposium Prospects for The 3rd Millennium Agriculture 26th-28th of September 2013, Cluj-Napoca, Romania.

References

1. Akmırza A (2013): *Gökçeada kıyı sularındaki balıkların parazitik nematodları*. T Parazitol Derg, **37**, 199-202.
2. Berland B (1961): *Nematodes from some Norwegian marine fishes*. Sarsia, **2**, 1-50.
3. Brian L (1958): *Ricerche sul ciclo biologico e l'ecologia di Ascaris capsularia Rud.* Atti dell'Accademia ligure di scienze e lettere, **14**, 249-263.
4. D'Amelio S, Mathiopoulos KD, Santos CP, Pugachev ON, Webb SC, Picanco M, Paggi L (2000): *Genetic markers in ribosomal DNA for the identification of members of the genus Anisakis (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism*. Int J Parasitol, **30**, 223-226.
5. Elarifi AE (1982): *The histopathology of larval anisakid nematode infections in the liver of whiting, Merlangius merlangus (L.), with some observations on blood leucocytes of the fish*. J Fish Dis, **5**, 411-419.
6. Guiart J (1938): *Étude parasitologique et épidémiologique de quelques poissons de mer*. Bulletin de l'Institut océanographique de Monaco, No. 755, 15 pp.
7. Janssen GM (1979): *The occurrence of Zeus faber (Linnaeus, 1758) in the coastal waters of the Netherlands (Pisces, Zeiformes)*. Bull Zool Mus Univ Amsterdam, **6**, 153-158.
8. Kahl W (1938): *Nematoden in Seefischen. 11. Erhebungen über den Befall von Seefischen mit Larven von Anacanthocheilus rotundatus (Rudolphi) und die durch diese Larven hervorgerufenen Reaktionen des Wirtsgewebes*. Zeitschrift für Parasitenkunde, **10**, 513-534.
9. Mattiucci S, Nascetti G (2008): *Advances and trends in the molecular systematics of Anisakis nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes*. Adv Parasitol, **66**, 47-148.
10. Mattiucci S, Paoletti M, Borriani F, Palumbo M, Palmieri RM, Gomes V, Casati A, Nascetti G (2011): *First molecular identification of the zoonotic parasite Anisakis pegreffii (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal anisakiasis in Italy*. BMC Infect Dis, **31**, 11-82.
11. Quiazon KMA, Yoshinaga T, Ogawa K, Yukami R (2008): *Morphological differences between larvae and in vitro cultured adults of Anisakis simplex (sensu stricto) and Anisakis pegreffii (Nematoda: Anisakidae)*. Parasitol Int, **57**, 483-489.
12. Pontes T, D'Amelio S, Costa G, Paggi L (2005): *Molecular characterization of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species*. J Parasitol, **91**, 1430-1434.
13. Prusevich TO (1964): *On the formation of capsules around larvae of Anisakis sp. in the tissues of the shorthorn sculpin Myoxocephalus scorpius*. Trudy Murmanskogo Biologicheskogo Instituta, **5**, 265-273. (In Russian)
14. Remotti E (1933): *Ancora sull' Ascaris capsularia Rud.* Bollettino dei Musei e laboratori di zoologia e di anatomia comparata della R. Università di Genova, **68**, 1-15.
15. Smith, JW, Wootten R (1978): *Anisakis and anisakiasis*. Adv Parasitol, **16**, 93-163.
16. Tepe Y, Oguz MC (2013): *Nematode and acanthocephalan parasites of marine fish of the eastern Black Sea coasts of Turkey*. Turk J Zool, **37**, 753-760.
17. Utuk AE, Pişkin FÇ, Balkaya İ (2012): *Molecular detection of Anisakis pegreffii in horse mackerels (Trachurus trachurus) sold for human consumption in Erzurum province of Turkey*. Kafkas Univ Vet Fak Derg, **18**, 303-307.
18. Zhu XQ, Gasser, RB, Podolska M, Chilton N (1998): *Characterization of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences*. Int J Parasitol, **28**, 1911-1921.

Geliş tarihi: 22.11.2013 / Kabul tarihi: 20.03.2014

Address for correspondence:

Yrd. Doç. Dr. Banu Yardımcı
Ondokuz Mayıs Üniversitesi
Veteriner Fakültesi
Su Ürünleri Hastalıkları AD.
Kurupelit, Samsun
e-mail: byardimci@omu.edu.tr