
Avrupa Danaburnu *Gryllotalpa gryllotalpa* (Orthoptera: Gryllotalpidae) ile İlişkili Parazitik Bir Nematod Olan *Oscheius myriophila* (Nematoda: Rhabditida)'nin İzolasyonu ve Karakterizasyonu

Research Article

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**ABSTRACT**

A nematode strain was isolated from a population of European mole cricket, *Gryllotalpa gryllotalpa* L. (Orthoptera: Gryllotalpidae), collected from the Black Sea Region of Turkey. Based on morphometrical and molecular (ITS partial sequence) properties, it was identified as *Oscheius myriophila*. This species of dauer juveniles resembled with *Rhabditis myriophila* (Poinar, 1986), however, it differs in having larger body length (571.3-693.9) and distance from the head to the nerve ring (100-116.8), and smaller tail length (53.4-76.8) and width at anus (10.4-13.8). This stage is the third-stage juvenile enclosed in a second-stage cuticle that surrounds the nematode like a sheath. The sequences of the ITS region of rDNA confirmed this identification. The species is recorded for the first time from *G. gryllotalpa*.

**Key Words**

Oscheius myriophila, Gryllotalpa gryllotalpa, parasitic nematode.

**ÖZ**

Bu çalışmada, Türkiye'de Karadeniz Bölgesi'nden toplanan Avrupa danaburnu (*Gryllotalpa gryllotalpa* L., Orthoptera: Gryllotalpidae) populasyonundan bir nematod suşu izole edildi. Suş, morfometrik ve moleküler (ITS kısmi sekansı) özelliklerine göre *Oscheius myriophila* olarak tanımlandı. Dauer juvenillerin bu türleri *Rhabditis myriophila* (Poinar, 1986)'ya benzemekle birlikte, *O. myriophila* sahip olduğu; vücut uzunluğu (571.3-693.9) ve baştan sinir halkasına olan uzunluk (100-116.8), ve küçük kuyruk uzunluğu (53.4-76.8) ve anüs genişliği (10.4-13.8) özellikleriyle farklıdır. Bu evre, nematodu bir kılıf gibi kuşatan ikinci evre kütkül içinde bulunan üçüncü evre juvenildir. rDNA’nın ITS bölgesinin sraları bu tanımlamayı doğruladı. Bu tür *G. gryllotalpa*’da ilk kez kaydedildi.

**Anahtar Kelimeler**

Oscheius myriophila, Gryllotalpa gryllotalpa, parazitik nematod.

**Article History:** Received: Sep 3, 2016; Revised: Nov 20, 2016; Accepted: Jan 20, 2017; Available Online: Apr 1, 2017.

**DOI:** 10.15671/HJBC.2017.152

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INTRODUCTION

The European mole cricket, Gryllotalpa gryllotalpa (Linnaeus 1758), is one of the most serious insect pests in turf and field crops in both Turkey and in all over the world [1,2]. They are burrowing insects and feed on a variety of organisms in the soil. These insects do not attack plants directly, but by tunneling, extended surface tunnels, cause significant damage to grass and crops of gardens, as they chop off any roots encountered when digging [3].

Nematode-arthropod associations are plentiful and range from beneficial to antagonistic [4, 5]. These associations have been divided into four categories: phoretic (nematodes are transported by an insect), necromenic (nematodes obtain nutrition from insect cadavers), facultative parasitism, and obligate parasitism [6]. Insect parasitism evolves in this sequence, with parasites evolving from non-parasitic insect associates [6]. Nematodes also interact with bacteria in at least three ways such as trophism (nematodes eat bacteria), parasitism (pathogens cause nematode diseases if not resisted), and mutualism (nematodes and bacteria cooperate).


The aim of the present work was to provide a morphometric and molecular characterization of the nematode, which detected first time in the population of Gryllotalpa gryllotalpa from Turkey during a survey and to contribute to the knowledge of the species.

MATERIALS and METHODS

Nematodes were isolated from G. gryllotalpa adults and nymphs during a survey conducted in 2011 in the Eastern Black Sea Region of Turkey. Collected nymphs and adults stages of G. gryllotalpa were individually placed in plastic boxes (17×11×7 cm) including sterile soil and perforated covers to permit airflow during transit to the laboratory. Subsequently, cadavers were placed in modified White traps to allow nematode emergence according to procedures described by Kaya & Stock [20]. Harvested nematodes (Gg1) were washed three times by sedimentation in distilled water.

For morphometric analysis of isolate Gg1, 20 IJs were randomly selected from G. gryllotalpa cadaver. Infective juveniles were collected for one week after they first appeared from cadavers [21]. The IJs were killed and fixed by hot 4% formalin (60°C) for 2 minutes and kept in this solution for 12 h at room temperature. Fixed nematodes were transferred to anhydrous glycerin and mounted on slides using cover-glass supports to avoid flattening them. Morphological observations were made following the taxonomic criteria by Homnick et al. [22]. Measurements were taken using a Zeiss AxioCam ERC 5s equipped with differential interference contrast optics.

Molecular characterization of the new isolate Gg1 was done by analysis of 18S internal transcribed spacer (ITS) ribosomal DNA sequences. DNA was extracted from IJs using a modified method published by Joyce et al [23].

ITS region of rDNA of nematode was amplified by PCR in a 50 µl reaction mix containing: 5 µL of the DNA suspension, 5 µL of 10X PCR buffer, 2 µL of MgCl₂ (25 mM), 1 µL of dNTP mixture (10 mM each dNTP), 1.5 U of Taq DNA polymerase, 1 µl of the forward primer TW81: 5’-GTTTCCGTAG-GTGAACCTGC-3’, and 1 µl of the reverse primer AB28: 5’-ATATGCTTAAGTTCAGCGGGT-3’ and ddH₂O to final volume [23]. Subsequently, 5 µl of the product was loaded on 1% agarose gel, and a target fragment was purified using a Qiagen Gel Purification Kit (Qiagen Ltd, The Netherlands).
The purified PCR product was cloned into pGEM-T easy vector and transferred to DH10β high efficiency competent cells (Promega, Netherlands), according to the manufacturer’s instructions. After selection of transformed colonies, plasmid isolation was performed and digested by restriction enzymes to confirm whether the gene was successfully cloned into the vector or not. Plasmid DNA samples that had the right clone were sequenced (Macrogen, Korea). The obtained sequence of Oscheius isolate was compared with sequences of the Oscheius species available in GenBank (NCBI). The DNA sequences were edited by BioEdit [24] with sequences of related species and new isolates available in GenBank. The evolutionary relationship of the isolates with 4 species of Oscheius and 2 species of Rhabditis were evaluated [25]. Phylogenetic analyses (Maximum Parsimony analyses) of sequence data were done using MEGA [26].

RESULTS

The morphometrical examination of dauer juveniles of nematode isolate Gg1 matched with the original descriptions of the respective species (Table 1). This species of dauer juveniles resembled with Rhabditis myriophila [16], however, it differs in having larger body length (571.3-693.9) and distance from the head to the nerve ring (100-116.8), and smaller tail length (53.4-76.8) and width at anus (10.4-13.8) (Figure 1).

The full sequence length of the ITS1-5.8S-ITS2 region including the partial sequence of 18S and 28S rRNA genes of the isolate of O. myriophila Gg1 was 1148 bp. The BLAST search indicated a 99% similarity among the O. myriophila Gg1 isolate sequence and isolate from USA (AY602176). Multiple sequence alignments of the ITS rDNA region of Oscheius and Rhabditis species are presented.
in Figure 2. The evolutionary relationship of the isolate and closely related other species were evaluated. Sequence and phylogenetic analysis of 1148 bp segment of ITS rDNA by MEGA revealed a high degree of homology to genus of *Oscheius* and *Rhabditis* (Figure 3).

**DISCUSSION**

A new nematode was first isolated from European mole crickets and Turkey. Based on the taxonomical characteristics; the nematode isolate was identified as *Oscheius myriophila*.

According to morphologic data, dauer juvenile of *O. myriophila* Gg1 isolate is similar to the *Rhabditis myriophila* [16], however, differs in having larger body length (L= 571.3-693.9 µm) and larger distance from anterior end to nerve ring (NR = 100-116.8 µm).

The new isolate (dauer juvenile) also closely resembles *O. pheropsophi* [27] but differs in body length (L= 571.3-693.9 µm vs. L= 491-643 µm in *O. pheropsophi*); shorter distance from anterior end to base excretory pore (EP= 97.8–118.8 µm; EP = 124-148 µm in *O. pheropsophi*). New isolate is larger in body length (571.3-693.9 µm) and distance from anterior end base of basal bulb (128.8-139.8 µm) than *O. pheropsophi*, *O. colombiana*, *O. amsactae* and *R. myriophila* (Table 1).

The genus *Rhabditis* [28] includes several nematode species are associated with soil invertebrates. Several of species of the genus *Oscheius* were recorded from cadaver/soil like *Rhabditis* (*O.)* *tipulae* re-described by Sudhaus [29] associated with leather jackets larva of *Tipula paludosa* (Diptera: Tipulidae), *R. (O.)* *myriophila* [16]; *Rhabditis* (*O.)* *columbiana* [30] associated with burrower bug, *Cyrtomenus bergi* (Hemiptera: Cydnidae). *R. caulleryi* [14] and *R. myriophila* [16] also cultured from millipedes [17], *R. (O.)* *necromena* [17] associated with millipede *Oncocladosoma castaneum* (Diplopoda: Paradoxosomatidae), *R. (O.)* *pheropsophi* [27] associated with bombardier beetle, *Pheropsophus aequinoctialis* L. are shown to be of economic importance as a biological con-

| Table 1. Morphometrics data of *Oscheius myriophila* Gg1. All measurements are in µm and in the form: mean±SD (range). |
|---|---|---|---|---|---|
| Isolates | *O. pheropsophi*<sup>a</sup> | *O. colombiana*<sup>b</sup> | *O. amsactae*<sup>c</sup> | *R. myriophila*<sup>d</sup> | *O. myriophila* Gg1 |
| Species | | | | | |
| n | 16 | 25 | 10 | 6 | 20 |
| L | 568±50 (491-643) | 505±32 (439-535) | 335-409 | 564 (504-611) | 630.2±31.5 (571.3-693.9) |
| W | 24±2.5 (22-31) | 23±3 (19-28) | 14.2-16.5 | 23 (19-26) | 25.4±2.7 (21-30.4) |
| EP | 136±7 (124-148) | 96±11 (82-116) | 68.7-82.0 | 107 (97-114) | 108±6.7 (97.8-118.8) |
| NR | 90±9 (79-108) | 89±13.5 (73-113) | 59.2-69.5 | 89 (83-96) | 110.5±4.9 (100-116.8) |
| ES | 130±6 (120-141) | 118±12 (98-139) | 90.8-97.9 | 129 (126-136) | 134.6±3.2 (128.8-139.8) |
| ABW | 15±2 (11-19) | 13±3.5 (9.5-20) | 7.9-11.1 | 15 (14-16) | 12.3±1 (10.4-13.8) |
| T | 88±13 (64-106) | 56±6 (48-66) | 48.9-60.4 | 78 (75-80) | 82±6.2 (72.2-92.2) |
| a | 23±2 (19-27) | 22.5±3.5 (17-27) | 21.8-26.5 | NA | 25±2.2 (20.5-28.5) |
| b | 4±0.4 (3.7-5.2) | 4.3±0.6 (3.2-5.4) | 3.5-4.3 | NA | 4.7±0.2 (4.3-5.2) |
| c | 6.6±1 (5.2-8.6) | 9.2±1.3 (6.6-11.1) | 6.6-7.4 | NA | 9.3±0.9 (8.3-11.6) |

NA: not available; n: number of specimens; L: total body length, EP: distance from anterior end to base excretory pore, NR: distance from anterior end to nerve ring, ES: distance from anterior end base of basal bulb, T: tail length, ABW: anal body width, W: maximum body width, a: L/W, b: L/ES, c: L/T. [27], [30], [33], [16].
Figure 2. Multiple sequence alignment of the ITS rDNA region of Oscheius and Rhabditis species. Code Ggl corresponds to the isolate of Oscheius myriophila. Codes AF083019 and EU196022 refer to Oscheius insectivora and Oscheius guentheri strains, respectively. Code UB1588 corresponds to the Rhabditis myriophila strain. Sequence alignments were performed using the ClustalW-algorithm.

Figure 3. Phylogenetic relationships of the Oscheius and Rhabditis species based on analysis of ITS rDNA regions neighbor joining method. Number on branches more than 70% indicates the percentage of 1000 bootstrap replicates.
trol agent. While R. (O.) maqbooli [18] and R. (O.) shamimi [19] were recovered from soil and R. (O.) guentheri [7] was isolated from decaying rice plants. R. (O.) amsactae was also recovered from cadaver of red-hairy caterpillar. We described a new isolate, Oscheius myriophila Ggl, as an associate of the European mole cricket, Gryllotalpa gryllotalpa.

Some Oscheius species such as O. carlanon-sis [31], O. siddiqii, O. niazi [32] and O. amsactae [33] were currently reported as entomopatho-genic nematode. Furthermore O. gingeri was also trapped by baiting with G. mellonella from the soil like entomopathogenic nematode. All of which increase the importance of our isolate. Considering that the findings of this study will contribute significantly to integrated pest management of mole crickets and under soil pests such as Agrotis segetum, Agriotes lineatus and Melolontha melolontha [34-37]. Future work will indicates the potential role of this nematode in natural regulation of mole crickets.

References

7. W. Sudhaus, D.J. Hooper, Rhabditis (Oscheius) guen-theri sp.n., an unusual species with reduced posterior ovary, with observations on the Dolichura and Insectivora groups (Nematoda: Rhabditidae), Nematologica, 40 (1994) 508-533.
8. A.F. Schneider, Monographie der nematoden, Berlin (1866).
10. G. Osche, Systematik und phylegenie der gattung Rh- abditis (Nematoda), Zoologische Jahrabuchen (Systematik), 81 (1952) 175-312.
11. A.B.O. Lam, J.M.C. Webster, Morphology and biology of Panagrolaimus tipulae n. sp. (Panagrolaimidae) and Rhabditis (Rhabditella) from leather jacket larvae, Tipula paludosa (Diptera; Tipulidae), Nematology, 17 (1971) 201-212.