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Original article (Özgün makale)

Morphological and Morphometric Identification of *Steinernema abbasi* (Nematoda: Steinernematidae) from Düzce, Türkiye

Taylan ÇAKMAK[1*]

Düzce İlinde Saptanan *Steinernema abbasi* (Nematoda: Steinernematidae)'nin Morfolojik ve Morfometrik Tanısı

Öz: Nematodların biyolojik mücadele etmeni olarak kullanılma potansiyeli son yıllarda artış göstermiştir. Entomopathogenik nematodlar (EPN) (*Steinernema* and *Heterorhabditis*), tarla ve bahçe tarımında zararlı sorununun çözümünde başarılı olmuşlardır. Biyolojik mücadele pragramlarında zararlıların ve doğal düşmanların doğru tanısı ile bu doğal düşmanların etkiliğinin belirlenmesi kritik öneme sahiptir. Bu çalışmada amaç, Düzce'den fındık bahçelerinden toplanan entomopatojenik nematodların tanısının yapılmasıdır. Morfolojik ve morfometrik karakterlere bağlı olarak, nematodların tanısı için metod sunulmuş ve bilinmeyen entomopatojenik nematod, ırk1, *Steinernema abbasi* olarak tanımlanmıştır.

Anahtar Kelimeler: Biyolojik Mücadele, entomopatojenik nematode, fındık, nematod tanısı, toprak faunası, Türkiye

Abstract: Interest in the use of nematodes as biological pest control agents has grown exponentially over the past two decades. Entomopathogenic nematodes (EPNs) (*Steinernema* and *Heterorhabditis*) have been particularly successful in managing pest problems in agriculture and horticulture. One of the most important requirements in biological control programmes is the accurate identification of pests and any beneficial organisms with biocontrol potential. The aim of this study was to identify an unknown entomopathogenic nematode that was collected from a hazelnut orchard in Düzce, Türkiye. Our study reports methods for the identification of *Steinernema abbasi*, based on morphological and morphometric characterization. The unknown entomopathogenic nematode, Strain 1, was identified as *S. abbasi.*

[1] Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, Düzce, Türkiye
[*] Sorumlu yazar (Corresponding author): taylancakmak@duzce.edu.tr
ORCID ID (Yazar sırasıyla): 0000-0003-4151-5724
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Introduction

Insect pests are a major problem in agriculture, causing significant reductions in crop yield and quality. In horticulture, the negative impact of certain insects on the quality of fruit and ornamental plants is a major concern. As a result, various strategies have been employed to control these insects, both locally and systemically, such as the use of various agrochemicals. While most of these chemicals suppress the targeted insects, their use has been found to have a major impact on the environment (Nguyen & Hunt, 2007). There have also been reports of bioconcentration, bioaccumulation and biomagnification of these toxic materials. Therefore, the use of environmentally friendly biological control strategies is desirable.

One group of biological agents that can be used as biocontrol agents for insect pests are the entomopathogenic nematodes (EPNs) (Johnigk & Ehlers, 1999). The two most commonly used genera are *Steinernema* and *Heterorhabditis*. Steinernema species (Nematoda: Rhabditida) are produced in large-scale liquid culture for the biological control of insect pests (Ehlers 2001). These soil-dwelling nematodes have a specific third instar juvenile adapted to long-term survival, which is called the "dauer juvenile" (DJ). They carry cells of specific enteric bacterial symbionts of the genus *Xenorhabdus* in a pouch in the anterior part of the intestine (Ciche et al. 2006). DJs actively search for insects, enter the insect body through orifices or less sclerotic parts of the cuticle and release the bacteria. Approximately two days after invasion, the insects die of septicemia (Dowds & Peters 2002). The aim of this study was to identify an unknown EPN isolated from a hazelnut orchard in Düzce Province, Turkiye, using morphometrics and morphological analysis.

Materials and Methods

Collection and examination of nematodes

Samples were collected from a hazelnut orchard (40°56'40.1"N 31°15'04.8"E) in Düzce Province in the western Black Sea region of Turkiye. A 100 g soil sample from each sampling site was placed in a glass container with three last instar larvae of the wax moth, *Galleria mellonella* (L.), and covered with a lid. Samples were then stored at room temperature. After 10 days, dead larvae were collected and transferred to White traps to collect the emerging IJs. Five juveniles were collected from the test tube containing the unknown EPN, placed on a glass slide, and examined under a light microscope to identify the genus/genera to which the nematode(s) belonged, based on certain morphological characteristics, such as the type of cuticle (striated or smooth) and the presence or absence of horns. Twenty

G. mellonella larvae were placed in each of two different Petri dishes lined with slightly moistened double-layer filter paper. Infective juveniles (IJs) from the tube were used to inoculate the larvae at 100 IJs/larva. The Petri dishes were then covered and sealed with parafilm to prevent contamination. The Petri dishes was then stored at a temperature of around 15°C and left for five days. The *G. mellonella* larvae died, which is typical of infection by *Steinernema* spp. The larval carcasses were placed in a white trap to obtain the IJs (Kaya & Stock, 1997). The *G. mellonella* carcasses were dissected on day 5 to obtain first generation males. The males were transferred from the dissected cadaver to a staining block containing water and then immediately transferred to permanent fixation for further morphometric analysis.

Nematode preparation

The males were fixed and mounted which observations and all measurements were made on males using a calibrated light microscope with a drawing tube. Thirty (30) males were measured. The measurement of the infective IJs was done by making temporary slides followed by immediate measurement. Twenty-five (25) juveniles were measured. The following morphometrics were used to identify the nematode: length (L), maximum body diameter (MBD), excretory pore (EP), nerve ring (NR), esophagus (ES), tail length (T), hyaline (H), anal body diameter (ABD), spicule length (SL) and gubernaculum length.

Other calculated parameters were:

a= L/MBD c'= T/ABD H%= H/T*100
b= L/ES D%= EP/ES*100 GS%= GL/SL*100
c= L/T E%= EP/T*100 SW%= SL/ABD*100

Results and Discussion

Identification

Although some overlap in morphometric values was common for other species in the *Steinernema bicornutum* group (IJs with "horn like" structure in the head region) and *S. carpocapsae*, the infective juveniles and first generation males of the unknown EPN showed the greatest similarity to *Steinernema abbasi*, based on morphometric and morphological comparison only (Elawad et al. 1997).

Morphometrics and morphology of the infective juvenile

The average morphometric values of the unknown IJs showed some overlap with *Steinernema abbasi*, *S. bicornutum*, *S. ceratophorum* and *S. carpocapsae*, so that most of the values were interchangeable with the standard morphometric values used to describe the third instar juveniles of these four species. The calculated ratios a, b, c of the unknown nematode appear to have similar values to the

standard average ratios of *S. abbasi* and *S. carpocapsae* consistently (Table 1). However, there were inconsistencies with *S. bicornutum* and *S. ceratophorum*. Two horn-like structures were also observed on the IJs of the unknown strain. Nevertheless, the group comparison is based on the "bicornutum group". Other species of this group that were beyond the range of measurements were eliminated to facilitate comparison.

Comparison of the unknown nematode, based on the length of IJs, also showed that the unknown nematode (535 µm) was closest to *S. abbasi* (541 µm) and *S. carpocapsae* (558 µm). However, morphologically there is no horn-like structure in *S. carpocapsae*. Therefore, *S. carpocapsae* was discarded as a possibility. Furthermore, the measurements of the unknown IJ were almost outside the range of measurements of *S. bicornutum* and *S. ceratophorum*.

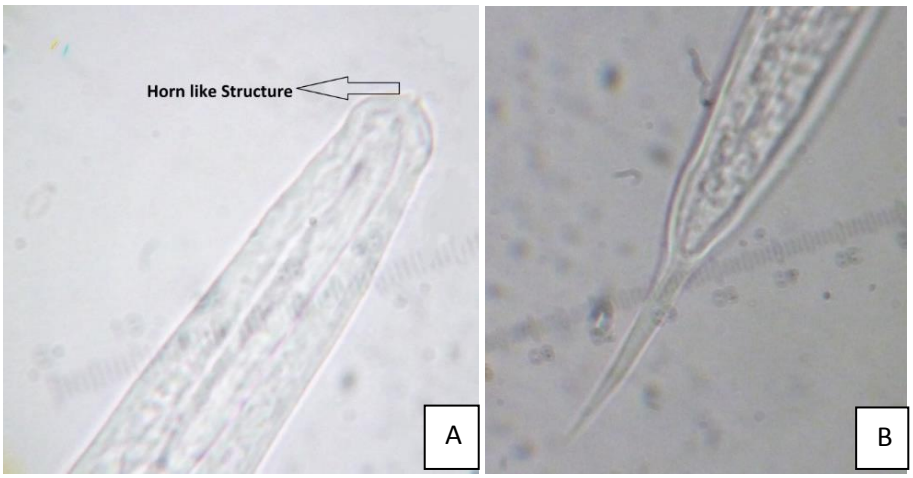

Figure 1. A. Anterior end of infective juvenile showing the horn-like structure. B. Posterior end of infective juvenile

Infective juveniles of *S. abbasi* are characterised by horn-like structures; thin, elongated body; sheath (J2 cuticle) present but sometimes lost; excretory pore always weak; near posterior end of metacorpus. Distance from anterior end to excretory pore always greater than body width at the same level. Oesophagus with cylindrical procorpus and slightly swollen metacorpus. Nerve ring just above metacorpus. Tail gradually tapering, dorsally curved at the tip, with a slight ventral depression that closely matches the unknown nematode.

This comparison of the unknown nematode with the above morphometric features of the infective juveniles shows that the nematode shares most of the morphometric characteristics of *S. abbasi*. Although the morphometric values within the "bicornutum group" don't show much overlap with other species, except *S. abbasi*, in order to make the identification more accurate, the characteristics of the first generation of males were diagnosed to give additional information (Fig. 1).

Table 1. Morphometric measurements of infective juveniles of Strain 1 and comparison with the closest species

Species/ Character	Strain 1	*S. abbasi*	*S. bicornutum*	*S. ceratophorum*	*S. carpocapsae*
n	25	15	20	45	55
L	535,2±21,7	541 ± 24	769,5±52,3	706 ± 62	558
	(480-565)	(496-579)	(648-873)	(591–800)	(438-650)
a	22,5±2,5	18 ± 0.91	26,5±1,5	25.9 ± 1.1	21
	(17-26)	(17-20)	(23-29)	(23.7–27.9)	(19-24)
b	5,9±0,3	6 ± 0.32	6±0,3		4,4
	(5,2-6.7)	(5.5-6.6)	(5,6-6,9)		(4.0-4.8)
c	11±0,9	9.8 ± 0.83	10,7±0,66	10.6 ± 0.9	10
	(9,93-14)	(8.1-10.8)	(9,7-12)	(8.8–12.9)	(9.1-11.2)
Body diam.	23,7±2,5	29 ± 1	29,5±1,6	27 ± 3	25
	(20-29)	(27-30)	(25-32,5)	(23–34)	(20-30)
EP	45±3,5	48 ± 1.5	60,6±3,3	55±5	38
	(39-52)	(46-51)	(53,5-65)	(47–70)	(30-60)
NR	70±2,5	68 ± 2.4	92±3,5	92 ±6	85
	(64-75)	(64-72)	(87,5-100)	(79 -103)	(76-99)
ES	91±4,8	89 ± 1.8	123,9±6	123 ± 7	120
	(80-105)	(85-92)	(112,5-135)	(108–144)	(103-190)
T	48,5±3,3	56 ± 3.2	72±4,97	66 ± 5	53
	(40-54)	(52-61)	(62,5-77,5)	(56–74)	(46-61)
ABD	12,9±0,9	29±1		15 ± 2	
	(12-15)	(27-30)		(9–18)	
D%	49,6±4,7	53 ± 0.02	50±3	44.9 ± 3.1	26
	(41-60)	(51-58)	(40-60)	(40.0–55.8)	(23-28)
E%	93±8,2	86 ± 0.05	80±6	84.2 ± 6.0	60
	(81-113)	(79-94)		(73.8–96.4)	(54-66)
Two "Horns" on Head	+	+	+	+	-
Reference		Elawad Ahmad & Reid (1997)	Tallosi & Ehlers (1995)	Jian et al. (1997)	Poinar (1967)

* Measurements are in µm and in the form: mean±SD (range).

Justification based on the morphometrics and morphology of first generation males

The length of the first generation males of the unknown nematode (1269 µm) showed closeness to *S. abbasi*, *S. bicornutum* and *S. ceratophorum*. In addition to the measurements, the absence of mucrons in first generation males was also used in the diagnosis (Table 2). Maximum body diameter, excretory pore (EP), nerve ring (NR), eusophagus length (ES), spicule length (SL), spicule width (SW), gubernaculum length (GL) and gubernaculum width (GW) of the unknown nematode had values similar to *S. abbasi*. In addition, the observation of caudal and precloacal papillae also helped supportour reasoning. We also observed one of the diagnostic features of *S. abbasi*, a single large midventral precloacal papilla. The tail was also short, conoid and relatively similar to the drawings of *S. abbasi*.

Furthermore, comparison of excretory pore (EP) and spicule length (SP) showed similarities with *S. carpocapsae*, *S. bicornutum* and *S. ceratophorum*. The presence of mucrons showed a clear difference between *S. carpocapsae* and the unknown nematode. In additionfurther comparisons made by looking at characteristic features of *S. abbasi* helped to eliminate other species, e.g., shape of spicule and gubernaculum, golden dark yellow coloured spicules and absence of terminal mucrons. Comparison using both morphological and morphometric measurements showed great similarities between the unknown species and *S. abbasi*. Therefore, it is possible to state that the unknown nematode most closely approximated *S. abbasi* in terms of 1st generation males (Fig. 2).

Figure 2. *Unknown nematode* A. General overview of 1st generation male, B. Excretory pore, C. Tail region - spicule and gubernaculum shape

Table 2. Morphometric measurements of 1st generation males of Strain 1 of an unknown nematode and a comparison with the closest species

Species/ Character	Strain 1	*S. abbasi*	*S. bicornutum*	*S. ceratophorum*	*S. carpocapsae*
n	20	15	20	35	25
L	1269±116,7	1252 ± 189	1352±149	1358±134	1450
	1080-1498	(999-1534)	(945-1539)	(1136-1694)	(1090-1710)
Body diam.	85,76±5,7	87 ± 6,7	108±11	146±21	102
	76-98	(82-98)	(80-127)	(104-185)	(77-131)
EP	74,32±5,2	80 ± 7,8	82±8	85±11	61
	(66-82)	(68-89)	(67,5-97,5)	(50-104)	(47-74)
NR	97,6±14,2	103.20 ± 6,48	123±8	123±14	110
	(68-124)	(99-123)	(107-137)	(90-147)	(93-124)
ES	134,56±20	133 ± 6	156±7	165±10	155
	(100-166)	(121-144)	(137-167)	(149-190)	(136-167)
Testis reflection	176,48±28	274 ± 33		393±94	563
	(110-220)	(234-319)		(163-574)	(400-808)
T	17,52±2,5	26 ± 3	31,5±2,5	30±4	30
	(14-22)	(20-31)	(25-35)	(23-38)	(23.4-39)
ABD	28,12±4	43 ± 4,90		52±5	42.6
	(22-38)	(37-55)		(45-70)	(32.5-54.6)
SL	67±4,8	65 ± 5,70	65±4	71±7	64.6
	(60-78)	(57-74)	(52,5-70)	(54-90)	(58.5-71.5)
Spicule Width	13,36±4,8	12 ± 1.30		11	11.Oca
	(10-18)	(10-14)		(9-16)	(9.1-13)
GL	47,72±6	45 ± 4.30	47,9±3,5	40±4	47
	(39-62)	(33-50)	(37,5-50)	(25-45)	(39-56)
Gubernaculum Width	6,56±0,89	7 ± 0.10		7±1	5,2
	(5-8)	(6-8,5)		(5-9)	(3.9-6.5)
D%	56±9	60 ± 5	50±3	51,4±7,2	
	(42,5-74)	(51-68)	(50-60)	(32.8-64.8)	
E%	424,2±77,3		260±24		
	(300-571)		(220-310)		

Table 2 continued

SW%	238,2±37,4	156±22		140±20	
	(173-327)	(107-187)		(10-20)	
GS%	71,2±10,5	70±0,07		60±10	
	(56-93)	(58-85)		(40-80)	
Mucron	Absent	Absent	Absent	Absent	Present
Reference		Elawad Ahmad & Reid (1997)	Tallosi & Ehlers (1995)	Jian et al. (1997)	Poinar, 1967.

* Measurements are in µm and in the form: mean±SD (range).

Conclusion

Previous work using morphological and morphometric measurements has shown the high intraspecific variation of *Steinernema* species (Tabassum & Shahina, 2004). Our study combined and compared different identification methods for *Steinernema* spp. based on morphological and morphometric characterization. However, as some identifications cannot be justified by the current methods, further studies need to be conducted with different methods.

Based on the morphometric values and morphological analysis of the unknown nematode in the current study, namely strain 1, *S. bicornutum*, *S. ceratophorum*, *S. carpocapsae* and *S. abbasi* had values that most often matched those of the unknown nematode. However, it had more values in common with *S. abbasi* which justified the unknown nematode being identified as *S. abbasi*.

Although identification based on morphometrics can be helpful in giving a good idea of the possible identification of the nematode species in question, errors due to inconsistencies in morphometric values are not uncommon due to obscuration of certain anatomorphological structures or poor fixation of the specimen. Therefore, molecular analysis is required in addition to morphological and morphometric characteristics for a more holistic and accurate and identification.

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