

Plant-parasitic Nematodes from Afghanistan with Discussion on the Taxonomic Status of *Merlinius neohexagrammus* Ivanova, 1978 (Nematoda: Dolichodoridae)

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ABSTRACT

During a survey of plant-parasitic nematodes in Bamyan and Mazar Sharif provinces of Afghanistan, several species of plant-parasitic nematodes belonging to infraorder Tylenchomorpha were detected and identified. *Filenchus facultativus*; *Filenchus discrepans*; *Filenchus elegantulus*; *Boleodorus volutus*; *Neopsilenchus magnidens*; *Coslenchus costatus*; *Ditylenchus myceliophagus*; *Tylenchorhynchus clarus*; *Paratylenchus microdorus* and *Merlinius neohexagrammus* are reported here for the first time from Afghanistan. Detailed examination of *M. neohexagrammus* using SEM microscopy confirmed this species in the genus *Merlinius* by having a labial disc not marked and with four longitudinal striations on the lip region, amphid apertures located at the lateral edge of labial disc and lateral field at mid-body areolated.

Keywords: Afghanistan, First report Morphology, *Merlinius*, *Nagelus*, Morphology, SEM.

INTRODUCTION

Currently there are few documented studies on plant-parasitic nematode fauna of Afghanistan. Faizyar (1971) reported *Paratrichodorus christiei* (Allen, 1957) Siddiqi, 1974 from Nangarhar province. Khan and Khan (1977) reported several genera of Tylenchida including *Basiria* Siddiqi, 1959; *Helicotylenchus* Steiner, 1945; *Psilenchus* de Man, 1921; *Rotylenchus* Filipjev, 1936; *Tylenchus* Bastian, 1865; and *Tylenchorhynchus* Cobb, 1913 from the rhizosphere of pistachio (*Pistachia vera* L.) in Hirat and also described *Filenchus afghanicus* (Khan and Khan, 1978) Siddiqi, 1986, *Filenchus sheri* (Khan and Khan, 1978) Siddiqi, 1986, and *Filenchus quartus* (Szczygieł, 1969) Siddiqi, 1986. Khan (1982) described *Basiria shahidi* from rhizosphere of pistachio in Hirat. Khan and Khan (1985) described *Hoplolaimus jalalabadiensis* (Khan and Khan, 1985) Ebsary,

1991 from the roots of fig in Jalalabad. Shahina and Maqbool (1993) described *Criconemoides afghanicus* from poplar host plant. Roivainen *et al.* (1988) gave a list including 88 genera and species of plant-parasitic and free-living nematodes from Afghanistan. In a survey of plant-parasitic nematodes carried out during May-June 2009 in the Bamyan and Mazari sharif provinces of Afghanistan, several species of infraorder Tylenchomorpha, including *Merlinius neohexagrammus* Ivanova, 1978 were indentified. *Merlinius neohexagrammus* was described about 33 years ago by Ivanova, 1978 in Tajikistan. Since then, little information on the morphology of this species has been added and no SEM studies have been made. *M. neohexagrammus* has also been put in different genera such as *Merlinius*, *Geocenamus* and *Nagelus* (Powers *et al.*, 1983; Brzeski, 1991; Siddiqi, 2000, respectively). The objective of this work was to identify plant-parasitic nematodes of two main provinces of Afghanistan and also the

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morphological and morphometrical characterization of a population of *M. neohexagrammus* from Afghanistan. The later aim was supported by SEM studies, and the taxonomic status of this species is also discussed.

MATERIALS AND METHODS

During May and June 2009, fifty-five soil samples were collected from Bamyan and Mazari sharif provinces, Afghanistan. The nematodes were extracted by centrifugal flotation technique, killed and fixed by FGA (formalin, glycerin and acetic acid) and transferred to glycerin according to the De Grisse method (1969). Permanent slides were made and studied using a light microscope (Nikon E600). For scanning electron microscopy (SEM), nematodes were prepared according to Wergin's method (1981) and were observed with a Scanning Electron Microscope (Cam Scan MW 2300).

RESULTS

In total, twenty-six species of plant parasitic nematodes of infraorder Tylenchomorpha were found (see Table 1). Most of the identified species belonged to the family Tylenchidae. Ten species from seven genera including *Filenchus facultativus*, *Filenchus discrepans*, *Filenchus elegantulus*, *Boleodorus volutus*, *Neopsilenchus magnidens*, *Coslenchus costatus*, *Ditylenchus myceliophagus*, *Tylenchorhynchus clarus*, *Paratylenchus microdorus* and *Merlinius neohexagrammus*, are reported here for the first time.

Merlinius neohexagrammus Ivanova, 1978

(Figures 1-3)

Measurements: See Table 2

Description

Female

Body C-shape after fixation. Cuticular annulation distinct, 1.8-2.5 μm wide in mid-body. Lateral fields one-fourth of corresponding body width, areolated at mid-body, two incisures originate from base of the stylet knobs, increasing to four at the level of median bulb, becoming six at about 8-10 annuli anterior to deirid. Lip region slightly offset, 5.5-7.0 μm high and 9-11 μm wide with six annuli, cephalic framework well developed. In en face view by SEM, there is a labial disc and four longitudinal striations on the lip region. Amphid apertures located at lateral edge of labial disc. Stylet well developed, knobs rounded with anterior surface sloping posteriorly. Orifice of dorsal esophageal gland 2-3 μm posterior to stylet knobs. Median bulb oval in dimension of 30.0 \times 14.5 μm , with prominent valvular apparatus in center. Isthmus 36.5 μm long, encircled by nerve ring at about its middle. Hemizonid 1-2 annuli long, situated 2-3 annuli anterior to the excretory pore. Deirids conspicuous, located in four-incisure region of lateral fields and at the level of the isthmus, and in distance of 132-138 μm from anterior end. Basal bulb elongate, cylindrical, 43.0 \times 16.5 μm size, cardia rounded and smaller than base of esophageal bulb. Ovaries outstretched, oocytes usually in one row. Spermatheca axial, sperms rounded, opening of vulval slit with single epiptygma, and vagina 12 μm long. Rectum not overlapped by intestine. Tail elongate, conoid, slightly arcuate ventrally, with 26-35 annuli, and terminus smooth, hyaline region 4-7 μm long. Phasmids generally anterior to middle of tail.

Table 1. List of Tylenchina species found and frequency in Afghanistan.

Species	Frequency ^a	Host	Locality
<i>Filenchus facultativus</i>	7.9	Turfgrass	Mazari Sharif
<i>Filenchus cylindricaudus</i>	5.6	Turfgrass	Mazari Sharif
<i>Filenchus discrepans</i>	9.0	Potato	Bamyan
<i>Filenchus elegantulus</i>	11.2	Turfgrass	Mazari Sharif
<i>Filenchus hamatus</i>	8.4	Apple tree	Bamyan
<i>Basiria graminophila</i>	1.4	Almond tree	Bamyan
<i>Boleodorus thylactus</i>	35.7	Clover	Mazari Sharif
<i>Boleodorus volutus</i>	12.2	Potato, tomato	Bamyan
<i>Irantylenchus vicinus</i>	23.6	Almond tree , potato, wheat	Mazari sharif, Bamyan
<i>Psilenchus hilarulus</i>	17.1	Clover	Mazari Sharif
<i>Psilenchus hilarus</i>	19.0	Almond tree	Balkh (Mazari Sharif)
<i>Neopsilenchus magnidens</i>	13.8	Astragalus spp	Bamyan
<i>Coslenchus costatus</i>	10.0	Potato	Bamyan
<i>Ditylenchus myceliophagus</i>	39.1	Clover , wheat	Mazari sharif, Balkh (Mazari Sharif)
<i>Pratylenchus neglectus</i>	55.6	Clover	Mazari Sharif
<i>Pratylenchus thornei</i>	22.0	Potato, tomato	Bamyan
<i>Tylenchorhynchus brassica</i>	3.4	Almond tree	Balkh (Mazari Sharif)
<i>Tylenchorhynchus clarus</i>	6.2	Almond tree	Mazari Sharif
<i>Merlinius brevidens</i>	47.8	Clover , wheat	Mazari Sharif , Bamyan
<i>Merlinius microdorus</i>	33.0	potato	Bamyan
<i>Scutylenchus rugosus</i>	78.8	-	Bamyan, Balkh (Mazari Sharif)
<i>Merlinius neohexagrammus</i>	47.6	Almond tree	Mazari Sharif
<i>Helicotylenchus digonicus</i>	37.0	Potato, tomato	Bamyan
<i>Helicotylenchus pseudorobustus</i>	69.9		
		Almond tree	Mazari Sharif
<i>Helicotylenchus vulgaris</i>	45.0	potato	Bamyan
<i>Paratylenchus microdorus</i>	3.6	Turfgrass	Mazari Sharif

^a Percentage of samples in which a nematode species was found.

Male

Males are abundant and with general morphology similar to females. Testis single, and outstretched. Spicule and gubernaculum ventrally arcuate. Bursa large with coarsely crenate margins, enveloping tail terminus.

Locality

Collected from the rhizosphere of almond trees (*Prunus amygdalus* L.) in Mazari Sharif Province, Afghanistan (GPS coordinates: N 36° 35.5' 62" - E 067° 06.8' 63").

Remarks

M. neohexagrammus is most closely related to *M. affinis* (Allen, 1955) Siddiqi, 1970 and *M. grandis* (Allen, 1955) Siddiqi, 1970 by having well developed cephalic framework, stylet length, and cylindrical to conical tail with smooth terminus. It differs from *M. affinis* in the number of lip region annuli (6 vs 8), offset lip region vs continuous, and lateral field at mid-body areolated vs not areolated. It also differs from *M. grandis* by having an offset lip region vs continuous, number of tail annuli (30 vs 43) and lateral field at mid-body areolated vs not areolated. It can be

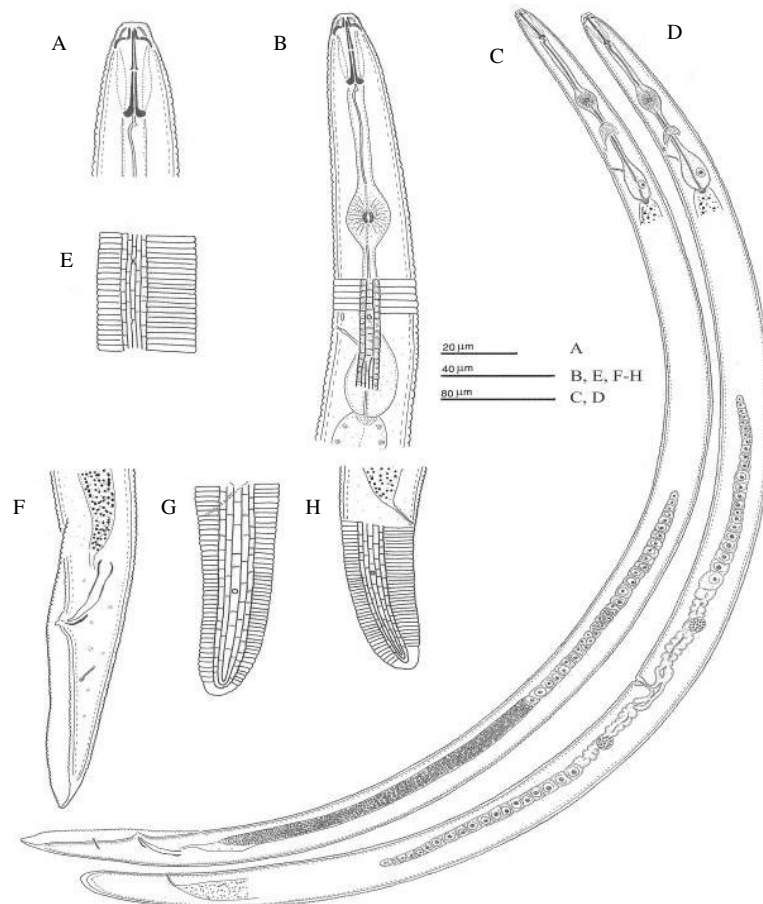


Figure 1. *Merlinius neohexagrammus*. Female: (A) Anterior body region; (B) Pharyngeal region; (D) Entire body; (E) Lateral field at mid body, (G, H) Tail. Male (C and F): (C) Entire body, (F) Tail.

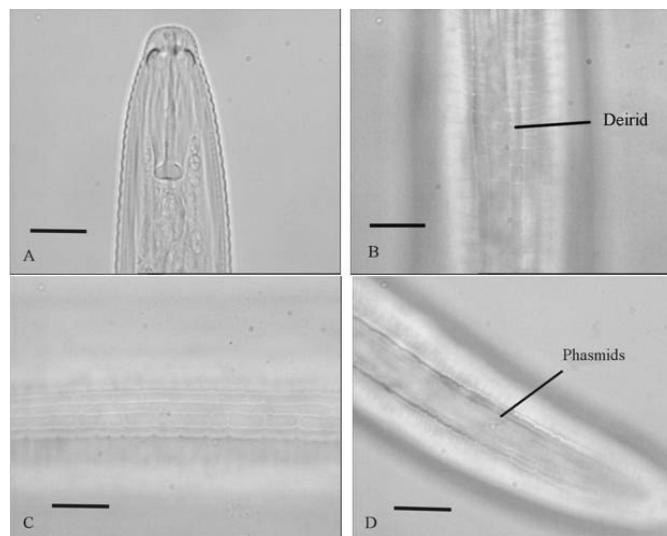


Figure 2. *Merlinius neohexagrammus*. Female (A-D): (A) Anterior body region; (B) Deirid region; (C) Areolation on the lateral fields, (D) Phasmid region. Scale bars: 10 μm.

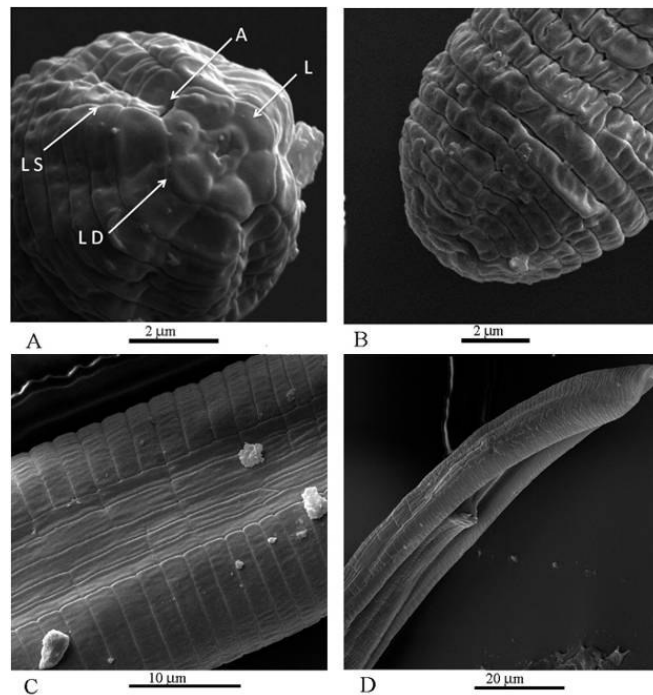


Figure 3. *Merlinius neohexagrammus*. Female (A-C): (A and B) Head region; (C) Areolation on the lateral fields at mid body, (D) Male tail. (A: Amphid; L: Labials; LD: Labial disc, LS: Longitudinal striations).

Table 2. Morphometrics of *Merlinius neohexagrammus* collected from Afghanistan and its comparison with type population (all measurements in micrometers).

Population characters	Afghanistan		Ivanova, 1978	
	Females	Males	Female	Male
n	9	8	10	5
L	1119±36.8 (1075-1191)	1054±95 (870-1177)	1000-1200	720-900
a	34.1±2.3 (31.5-36.9)	36.3±2.2 (32.2-39.9)	18.5-22	30-35
b	5.9±0.3 (5.3-6.3)	5.7±0.5 (4.7-6.5)	5.1-7.1	5.1-5.7
c	19.7±2.1 (15.9-22.3)	13.5±2.2 (8.6-15.7)	18.2-22	12-16
c'	2.4±0.3 (2-2.8)	3.7±0.5 (3-4.5)	1.8-2.3	-
V or T	55.2±1.7 (53.2-58.1)	44.6±2.8 (40.9-49.9)	51-56	-
Stylet	26.5±1.7 (25-29)	25.8±0.8 (25-27)	24-26	23-26
m	48.5±2.3 (44.8-52)	48.8±2 (46.2-52)	-	-
Median bulb	89.9±5.4 (80-97)	89.5±2.9 (85-93)	-	-
MB	47.6±1.6 (44.7-50.3)	48.8±1.3 (47.5-50.8)	46-52	47-50.5
Excretory pore	143±8.6 (132-158)	137±12 (110-147)	-	-
Esophagus length	189±11.2 (170-202)	183±4.9 (178-190)	-	-
Lip region to vulva	618±27 (584-665)	-	-	-
Body width	33±2.6 (30-37)	29±1.7 (27-31.5)	-	-
Anal body width	23.9±1.6 (22-27)	21.1±0.8 (20-22)	-	-
Vulva to anus	443±27.8 (416-491)	-	-	-
Tail	57.4±7 (49-71)	79±11 (63-101)	-	-
Phasmid from anus	20±1.8 (17-22)	-	-	-
Phasמידs/Tail	35.2±4.1 (29.6-40.8)	-	-	-
Spicules	-	29.1±1.0 (28-30)	-	27.5-30
Gubernaculum	-	9.6±1.3 (8-11)	-	8.5-9.5



separated from *M. hexagrammus* (Sturhan, 1966) Siddiqi, 1970, *M. alpinus* (Allen, 1955) Siddiqi, 1970 and *M. macrodens* (Allen, 1955) Siddiqi, 1970 by having shorter stylet length and presence of areolation on the lateral fields vs absence.

DISCUSSION

The taxonomy of *M. neohexagrammus* is not constant because different investigators place this species under different genera, such as Powers *et al.* (1983) and Fortuner and Luc (1987) who placed this species in *Merlinius*, Brzeski (1991) moved it to *Geocenamus* and Siddiqi, 2000 transferred it to *Nagelus*.

Powers *et al.* (1983) characterized *Nagelus* by four characters: somewhat broadly oval, laterally elongated face, with amphid apertures well within the continuous boundary of the first annulus; no longitudinal striations interrupting the lip annuli; deirid surrounded by six longitudinal incisures and an irregularly tapering tail with a long hyaline region. Therefore, they proposed to move *N. affinis* (Allen, 1955) Siddiqi, 1979; *N. alpines* (Allen, 1955) Siddiqi, 1979; *N. conicus* (Allen, 1955) Siddiqi, 1979; *N. grandis* (Allen, 1955) Siddiqi, 1979; *N. hexagrammus* (Sturhan, 1966) Siddiqi, 1970; *N. kirjanovae* (Doucet, 1978) Doucet, 1980; *N. lineatus* (Allen, 1955) Siddiqi, 1979; *N. macrodens* (Allen, 1955) Siddiqi, 1979 and *N. superbus* (Allen, 1955) Siddiqi, 1979 to *Merlinius* and *Merlinius obscures* (Allen, 1955) Siddiqi, 1979 to *Nagelus*.

Fortuner and Luc (1987) agreed with characterization of *Nagelus* referred by Powers *et al.*, (1983) and they included *M. neohexagrammus* in the species list of the genus *Merlinius*. Brzeski (1991) synonymized the genus *Merlinius* and some species *Nagelus* with *Geocenamus*. He defined variability of some characters and overlaps in some morphological definitions and values considered to differentiate these genera. As a result, the author mentioned *M.*

neohexagrammus in his species list of *Geocenamus*.

Siddiqi (2000) differentiated *Nagelus* from *Geocenamus* and *Merlinius* on the base of face patterns. En face view observations of *Nagelus* revealed that there is not a labial disc and longitudinal striations on the lip region while the labial disc annuli is interrupted by longitudinal grooves in the two other genera. He separated *Geocenamus* mainly by having well marked, rounded labial disc as compared to *Merlinius* whose labial disc is rectangular to oval and not prominent. He also characterized *Nagelus* by a robust, 20-40 μm long stylet, distinct deirids located in six-incisure region of lateral fields, elongate-conoid to subcylindrical female tail, with a distinct hyaline region. Finally, he transferred *neohexagrammus* to the genus *Nagelus*, in spite of labial disc, striations on lip annuli and deirids located in the four-incisure region.

Our light microscopy and SEM observations on the population of *M. neohexagrammus* from Afghanistan revealed that it has a distinct labial disc grooved by four longitudinal striations on the lip region and deirids in the four line zone of lateral fields. Also, the SEM images of the lip region and deirids zone showed more similarities with *Merlinius* than *Nagelus*. Based on our observations, we agree with Powers *et al.* (1983) and Fortuner and Luc (1987) that this species should be transferred to *Merlinius*.

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REFERENCES

1. Brzeski, M. W. 1991. Taxonomy of *Geocenamus* Thorne and Malek, 1968. (Nematoda: Belonolaimidae). *Nematologica.*, **37**: 125-173.
2. De Grisse, A. T. 1969. Redescription ou Modifications de Quelques Techniques Utilisées dans L'étude des Nématodes Phytoparasitaires. *Mededelingen Rijksfakulteit Landbouw Wetenschappen Gent.*, **34**: 351-369.
3. De Ley, P. and Blaxter, M. 2004. A New System for Nematoda: Combining Morphological Characters with Molecular Trees, and Translating Clades into Ranks and Taxa. *Nematol. Monogr, Persp.*, **2**: 633-653.
4. Faizyar, A. 1971. List of Plant Pests and Diseases of Economic Importance in Afghanistan. FAO Reg. Off. Near East, Cairo, UAR, 32 PP.
5. Fortuner, R. and Luc, M. 1987. A Reappraisal of Tylenchina (Nemata). 6. The Family Belonolaimidae Whitehead, 1960. *Revue de Nematologie.*, **10**: 183-202.
6. Ivanova, T.S. 1978. Ectoparasitic Nematodes of Subfamily Tylenchorhynchinae Eliava, 1964 from Tadzhikistan. *Izvestija Akademii Nauk Tadzhikskoj SSR, Otdelenije biologicheskikh Nauk.*, **4**: 35-46. (in Russian)
7. Kapoor, M. 1983. Taxonomic Studies on Nematodes of Some Medicinal and Aromatic Plants of North India. PhD. Thesis. *Helminth. Abstr., Ser. No. 1511*, 177 PP.
8. Khan, M. L. and Khan, S. H. 1977. A New and a Known Species of Tylenchus Bastian, 1865 (Nematoda: Tylenchinae) from Afghanistan. *All-India Helminth. Symp.*, Date? Year? Sriangar, India, 29 PP.
9. Khan, M. L. 1982. Species of *Basiria* Siddiqi, 1959 Associated with Fruit Trees in India (Nematode: Psilenchinae). *Indian J. Nematol.*, **12**: 99-106.
10. Khan, M. L. and Khan, S. H. 1978. Two New and a Known Species of Tylenchus Bastian (Nematoda: Tylenchinae) from Afghanistan. *Nematol. Medit.*, **6**: 213-221.
11. Khan, M. L. and Khan, S. H. 1985. Three New species of Hoplolaiminae (Hoplolaimidae: Nematoda) with New Report of *Scutellonema unum* Sher, 1963 from Tunisia. *Indian J. Nematol.*, **14**: 115-120.
12. Powers, T. O., Baldwin, J. G. and Bell, A. H. 1983. Taxonomic Limits of the Genus *Nagelus* (Thorne and Malek, 1968) Siddiqi, 1979 with Description of *Nagelus borealis* n. sp. from Alaska. *J. Nematol.*, **15**: 582-593.
13. Roivainen, O. H., Siddiqi, M. R., Ahadi, Q. A. and Noori, M. A. 1988. A Survey of Plant Parasitic and Other Nematodes Associated with Field Crops and Fruit Trees in Afghanistan. *FAO Plant Prot. Bull.*, **36**: 61-67.
14. Shahina F. and Maghbool M. A. 1993. *Pakricronemoides anastomoides* (Maghbool and Shahina) gen. n., *Criconemoides afghanicus* sp. n. and *Macroposthonia curvata* Alpine sub sp. n. (Nematoda: Criconematidae). *Afro-Asian J. Nematol.*, **3**: 188-195.
15. Siddiqi M. R. 1979. Taxonomy of the Plant Nematode Subfamily Merliniinae Siddiqi, 1970, with Descriptions of *Merlinius processus* n. sp. *M. loofi* n. sp. and *Amplimerlinius globigerus* n. sp. from Europe. *Syst. Parasitol.*, **1**: 43-59.
16. Siddiqi, M. R. 2000. *Tylenchida Parasites of Plants and Insects*. 2nd Edition, Commonwealth Institute of Parasitology, Slough, UK, PP. xvii and 833.
17. Wergin, W. P. 1981. Scanning Electron Microscopic Techniques and Application for Use in Nematology. In: "*Plant Parasitic Nematodes*", (Eds.): Zuckerman, B. M. and Rohde R. A.. Academic Press, New York, **III**: 175-204.



نماتودهای انگل گیاهی از افغانستان با بحث روی موقعیت تاکسونومی *Merlinius neohexagrammus* (Nematoda: Dolichodoridae)

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چکیده

در طی مطالعه نماتودهای انگل گیاهی از استان های بامیان و مزار شریف از کشور افغانستان، چندین گونه از نماتودهای انگل گیاهی متعلق به بالا راسته Tylenchomorpha جمع آوری و شناسایی شدند. از بین گونه های شناسایی شده گونه های *Filenchus facultativus*، *Filenchus*، *Neopsilenchus*، *Boleodorus volutus*، *Filenchus elegantulus discrepans*، *Ditylenchus myceliophagus*، *Coslenchus costatus magnidens*، *Merlinius* و *Paratylenchus microdorus*، *Tylenchorhynchus clarus* و *Merlinius neohexagrammus* برای اولین بار از کشور افغانستان گزارش می شوند. بررسی مجدد روی گونه *Merlinius neohexagrammus* و با استفاده از میکروسکوپ الکترونی روبشی (SEM) تایید کرد که این گونه، دارای یک دیسک لب غیر برجسته و چهار شیار طولی روی سر، منافذ آمفید در لبه خارجی دیسک لب و همچنین باند جانبی دارای تریانات می باشد. در نتیجه با این مشخصات، گونه ی مورد بحث باید در جنس *Merlinius* قرار گیرد.