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SOME NEW RECORDS OF *HELICOTYLENCHUS* (STEINER, 1945) SPECIES FROM PAKISTAN (NEMATODA: HOPLOLAIMIDAE)

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ABSTRACT

The detailed morphological and taxonomical studies revealed the presence of number of plant parasitic nematodes from grass (*Cynodon dactylon* L.) at different areas of University of Karachi. The genus *Helicotylenchus* was encountered with highest occurrence among other plant parasitic genera. In the present investigations, two known spiral nematode species viz. *Helicotylenchus abuharazi* Zeidan and Geraert, 1990 and *Helicotylenchus wajihi* Sultan, 1981 associated with grass were found as new records and were studied for their taxonomic descriptions. Brief redescription along with their measurements and illustrations are provided herein. These species were reported for the first time from Pakistan.

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INTRODUCTION

Species of the genus *Helicotylenchus* Steiner, 1945 comprise one of the most common and consistent component of hoplolaimid attacking agricultural crops under diverse climatic and edaphic conditions. The genus *Helicotylenchus* Steiner, 1945 contains more than 200 species worldwide with *H. dihystera* as its type species (Haque and Khan, 2021; Siddiqi, 2000; Uzma et al., 2015). Currently, 35 species of the genus *Helicotylenchus* have been reported from Pakistan by various researchers from different hosts and localities which are given in Table 1 (Shahina et al., 2019; Uzma et al., 2015). Two new records of spiral nematodes viz. *Helicotylenchus abuharazi* Zeidan and Geraert (1990) and *Helicotylenchus wajihi* Sultan (1981) were reported for the first time from Pakistan extracted from the grass of University of Karachi. Both the species are briefly described along with measurements, illustrations and photomicrographs.

MATERIALS AND METHODS

Nematodes were extracted from soil by Cobb's sieving and decanting method (Cobb, 1918) followed by modified Baermann funnel technique (Baermann, 1917). Nematode specimens were killed genteelly and fixed in TAF. Specimens were processed to glycerin by Seinhorst slow method (Seinhorst, 1959) and mounted in glycerin. Measurements and illustrations were made with a camera Lusida attached to a compound microscope. Body dimensions were calculated using de Man's formula (de Man, 1884). Photographs were taken by the help of automatic camera Nikon DS-FI attached with compound microscope using Nomarski's interference contrast system. Identification of nematodes was made by the systematics provided by Siddiqi (2000).

RESULTS AND DISCUSSION

Helicotylenchus abuharazi Zeidan and Geraert, 1990

Description

Female

Body spiral shape after fixing it. Lip region hemispherical with 4-5 distinct annules.

Table 1: List of *Helicotylenchus* species reported from Pakistan.

Nematode species	Reference	Host	Localities
H. abunaamai Siddiqi, 1972	Firoza and Maqbool (1991)	*Citrus* spp., *Lycopersicon esculentum*	Swat
H. arachisi Mulk & Jairajpuri, 1975	Firoza and Maqbool (1991)	*Citrus* spp., *Lycopersicon esculentum*, *Pyrus malus*,	Swat
H. californicus Sher, 1966	Maqbool (1986)	*Citrus* spp. *Vitis vinifera*	Hyderabad, Multan, Sargodha, Sahiwal, Thatta, Chagi, Quetta
H. canadensis Waseem, 1961	Saeed et al. (1986)	*Nicotiana tabacum*	PCSIR, Karachi
H. certus Eroshenko and Nguen Vu Thanh, 1981	Aatika et al. (2017) Zarina and Shahina (2012)	*Capsicum annuum*, *Solanum tuberosum*, *Triticun aestivum*, *Sorghum vulgare*, *Zea mays*	Chak, Hazara, Mazaharabad, Pakistan Pull, Sheikhupura
H. conicephalus Siddiqi, 1972	(Maqbool, 1986)	*Citrus* spp. *Musa paradisiaca*	Gujranwala, Multan, Sargodha, Muzaffargarh, Sahiwal, Thatta Larkana, Nawabshah,
H. crenacauda Sher, 1966	Maqbool, 1986 Khan et al. (2008)	*Mangifera indica* *Prunus persica*	Multan, Mardan, Sahiwal, Chagi , D.I. Khan, Muzaffargarh Peshawar, Quetta
H. digonicus Perry in Perry, Darling & Thorne, 1959	Anwar and Chaudhary (1976)	*Mangifera indica*	Punjab
H. dihystera (Cobb, 1893) Sher, 1961	Kafi (1963)	*Cynodon dactylon*	Karachi
H. discocephalus Firoza & Maqbool, 1993*	Firoza and Maqbool (1993)	*Triticum aestivum*	Azad Kashmir, Muzaffarabad
H. egyptiensis Tarjan, 1964	(Maqbool, 1986)	*Grewia asiatica* *Musa paradisiaca* *Prunus domestica* *Pyrus communis* *Saccharum officinarum* *Triticum aestivum*	Dadu, Khairpur, Rawalpindi, Sukkar, Tharparkar, Sanghar, Sukkar, Thatta, Hazara, Peshawar, Quetta, D.I. Khan, Mardan, Peshawar, Hyderabad, Nawabshah, Sanghar, Thatta, Nawabshah, Sanghar, Thatta
H. erythrinae (Zimmermann, 1904) Golden, 1956	Malik and Yasmeen (1978)	*Oryza sativa*	Gujrat
H. exallus Sher, 1966	(Maqbool et al., 1985)	*Cynodon dactylon*	Karachi
H. falcatus Eroshenko & Nguen Vu	Firoza and Maqbool (1991)	*Musa paradisiaca*, *Cocos*	Karachi

Thanh, 1981		*nucifera*	
H. goodi Tikyani, Khera & Bhatnagar, 1969	Khan et al. (1987)	*Phoenix dactylifera*	Turbat
H. gulabi Jain, Siddiqui and Aruna Parihar, 2000	Aatika et al. (2017)	*Capsicum annuum*, *Solanum tuberosum*, *Zea mays*	Islampur, Shamsabad, Umrao Khan
H. handooi Khan, Ghazi & Soomro, 2008*	Khan, Ghazi & Soomro, 2008	*Prunus amygdalus*	Kork, Klat, Khuzdar
H. indicus Siddiqi, 1963	Saeed and Ashrafi (1973)	*Manilkara zapota*	Malir, Karachi
H. jasminii Jain, Siddiqui & Aruna Parihar, 2000	Aatika et al. (2017) Maqbool and Shahina (2001)	*Sorghum vulgare*, *Zea mays*	Burj Jieway Khan, Islampur, Mazaharabad, Noorpur
H. lemoni Firoza & Maqbool, 1996*	Firoza and Maqbool (1996)	*Citrus* spp.	Bahawalpur
H. macronatus Mulk & Jairajpuri, 1975	(Maqbool et al., 1985)	*Saccharum officinarum*	Tandojam
H. martini Sher, 1966	Khan et al. (1992)	*Cocos nucifera*	Karachi
H. meloni Firoza & Maqbool, 1994*	Firoza and Maqbool (1994)	*Cucumis melo*	Mirpursakro
H. microdorus Prasad, Khan & Chawla, 1965	Maqbool et al. (1975)	*Saccharum officinarum*	Sindh
H. microtylus Firoza & Maqbool, 1993*	Firoza and Maqbool (1993)	*Pistachio vera*	Quetta
H. multicinctus (Cobb, 1893) Golden, 1956	Saeed and Ashrafi (1973)	*Musa paradisiaca*	Malir, Karachi
H. obliquus Maqbool & Shahina, 1986*	Maqbool and Shahina (1986)	*Mangifera indica*	Hyderabad
H. oscephalus Anderson, 1979	Firoza and Maqbool (1992)	*Pinus* spp.	Naran
H. platyurus Perry in Perry, Darling & Thorne, 1959	Firoza and Maqbool (1991)	Wheat	Nawabshsh
H. pseudorobustus (Steiner, 1914) Golden, 1956	Maqbool (1986)	*Annona squamosa* *Oryza sativa* *Vitis vinifera*	Pishin, Quetta, Zhob, Gujranwala, Larkana, Muzaffargarh, Sukkar, Nawabshah, Sanghar, Sheikhupura, Chagi, Quetta, Zhob
H. seshadrii Singh & Khera, 1994	Firoza and Maqbool (1991)	*Citrus* spp.	Karachi
H. sidiqii Zarina & Akhter, 2016*	Zarina and Akhter (2016)	*Capsicum annuum*	Malir, Karachi
H. striatus Firoza & Maqbool, 1994*	Firoza and Maqbool (1994)	*Cucumis melo*	Mirpursakro

H. thornei Roman, 1965	Maqbool (1986)	*Citrus* spp. *Mangifera indica*	Faisalabad, Sargodha, Gujranwala, Hyderabad, Karachi, Multan, Sahiwal, Thatta
H. urobelus Anderson, 1978	(Samina and Erum, 2019)	*Abelmoschus esculentus, Capsicum frutescens, Cucumis sativus, Cucurbita moschata, Mentha spp., Phaseolus vulgaris, Prunus persica, Triticum aestivum, Vigna radiata, Zea mays*	Khurrum Agency
H. verecundus Zarina & Maqbool, 1991*	Zarina and Maqbool (1991)	*Pancratium verecundum*	Nursery, University of Karachi
H. willmottae Siddiqi, 1972	Firoza and Maqbool (1992)	*Nicotiana tabacum*	Mardan

Lateral fields having four incisures, inner two incisures, not fused distally. Stylet 21.6-23.2 µm long, bearing anteriorly directed basal knobs. Opening of dorsal esophageal gland 8-13µm, from stylet knobs. Oesophageal junction located at 90-100 µm from head end and oesophageal gland located at 66-108 µm. Excretory pore located above the level of oesophago-intestinal junction at 78-105 µm. Nerve ring encircles isthmus at 77-94 µm behind the median bulb. Hemizonids located 1-2 annules anterior to excretory pore. Female reproductive system didelphic (amphidelphic). Spermatheca non-functional. Phasmid located 0-1 annules anterior to anus. Tail is dorso convex-conoid with a short projection bearing 13-14 annules (Figures 1 A-G; 2 A-F; Table 2).

Male

Not found.

Remarks

Soil samples of grass (*Cynodon dactylon* L.) were collected from Karachi University campus for identification of *Helicotylenchus* genus, the measurement of these specimens are closely related with the original description of Zeidan and Geraert (1990).

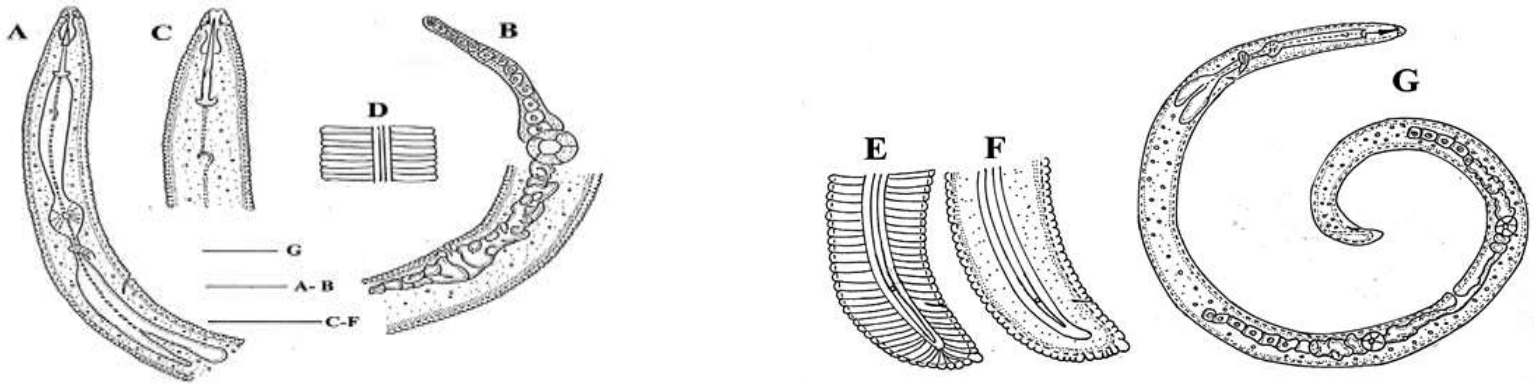

Figure 1 (A-G): *Helicotylenchus abuharazi* Zeidan & Geraert, 1990. Female: A. Oesophageal region; B. Female gonad; C. Anterior region; D. Lateral filed; E, F. Tail regions; G. Whole body.

Table 2: Measurement of *Helicotylenchus abuharazi* (measurements are in µm except L).

Measurements	Females (n=10)	
	Range	Mean ± SD
L (mm)	0.58 – 0.66	0.617±0.025
a	26.5-32.4	29.86±2.18
b	4.7-5.5	5.0±1.9
b’	3.5-4.7	4.0±1.3
c	33.4-58.4	45.27±6.81
c’	0.8-1.3	0.95±0.23
V%	59.8-62.9	62.75±2.06
Stylet	21.6-23.2	22.1±0.50
DGO	8-13	10.6±1.8
Nerve ring	77-94	86.1±5.6
Excretory pore	78-105	88.1±8.7
Hemizonid	1-2	1.5±0.07
Phasmid	0-1	0.5±0.03
Tail length	10-20	13±5.2
Anal body width	12-15	13.4±1.28
Body width	19-23	20.7±1.48
Tail annules	13-14	13.5±0.5

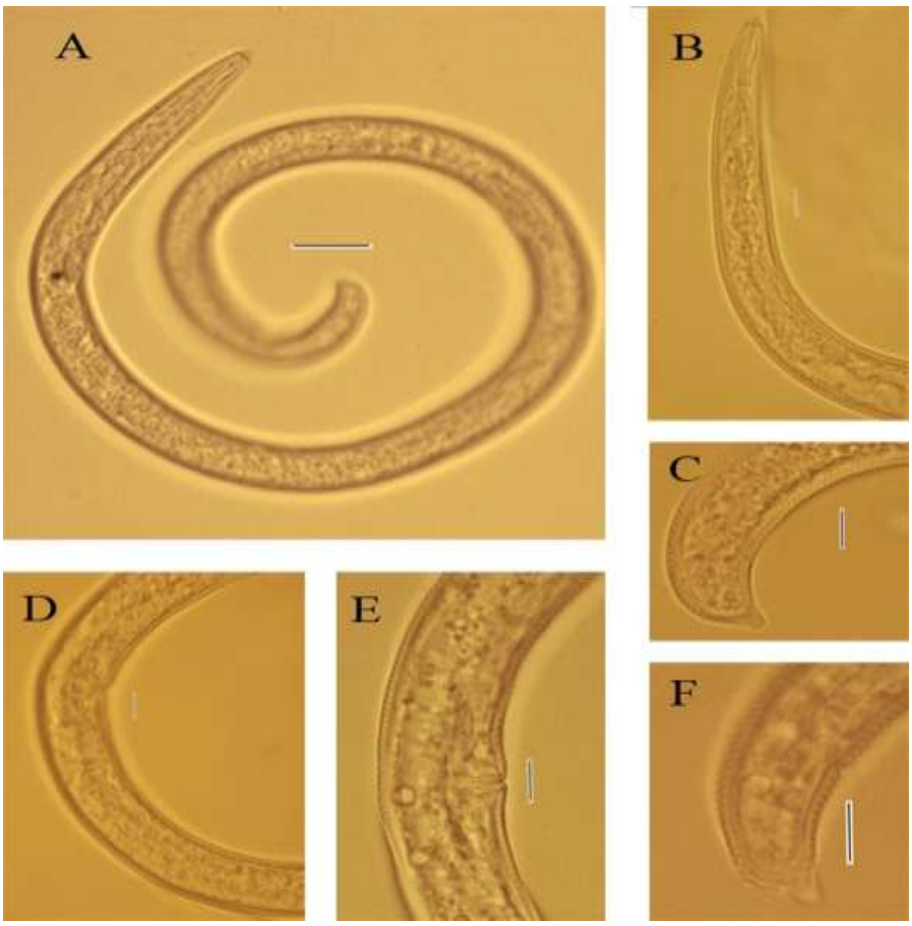

Figure 2 (A-F): *Helicotylenchus abuharazi* Zeidan & Geraert, 1990. Female: A. Whole body; B. Anterior region; C, F. Tail regions; D, E. Vulval region (Scale : A= 40 μm; B-F= 100 μm).

***Helicotylenchus wajihi* Sultan, 1981**

Description

Female

Body loose spiral when relaxed by gentle heat. Lip region hemispherical with 4-6 annules. Lateral fields occupying ¼ of the body width, marked with four incisures in the middle. Stylet 21.6-24 µm long. Oesophageal junction located at 5-6.3 µm from anterior end and oesophageal gland located at 4-5.1 µm. Excretory pore located above the level of oesophago-intestinal junction at 89-90.4 µm. Nerve ring encircles in the middle of isthmus. Female reproductive system didelphic (amphidelphic) with empty spermatheca. Phasmid located 5-7 annules anterior to anus. Tail bearing 8-9 annules and conical in shape (Figure 3 A-F; 4 A-F; Table 3).

Male

Not found.

Remarks

Soil samples of grass (*C. dactylon* L.) were collected from Karachi University campus for identification of *Helicotylenchus* genus, the measurement of these specimens are closely related with the original description of Sultan (1981).

CONCLUSION

In the present investigations, two known spiral nematode species viz. *Helicotylenchus abuharazi* Zeidan and Geraert, 1990 and *Helicotylenchus wajihi* Sultan, 1981 associated with grass were found as new records and were studied for their taxonomic descriptions. These species were reported for the first time from Pakistan.

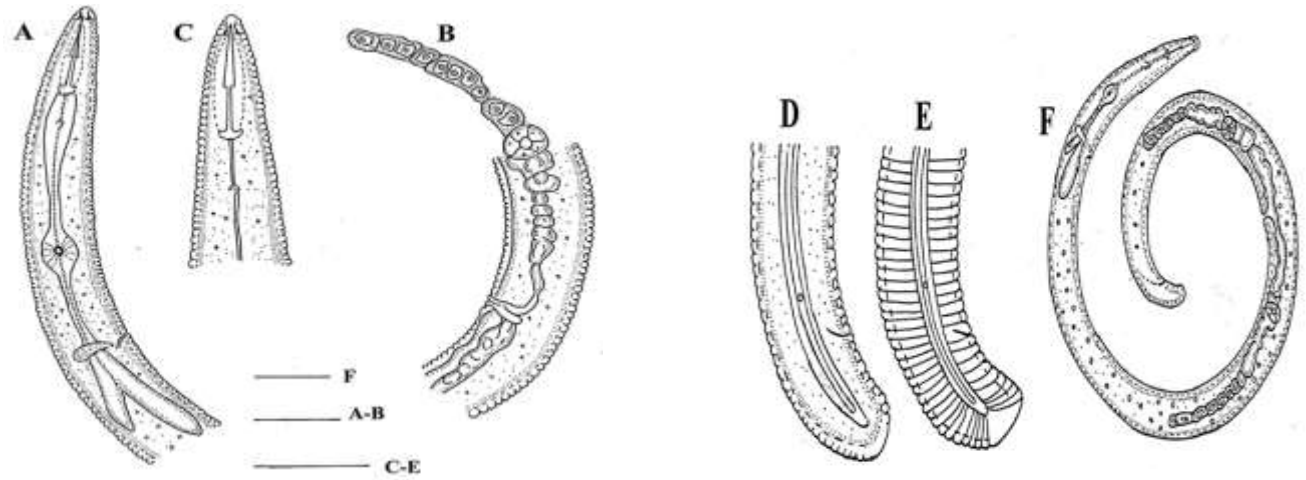

Figure 3 (A-F): *Helicotylenchus wajihi* Sultan, 1981. Female: A. Oesophageal region; B. Female gonad; C. Anterior region; D, E. Tail regions; F. Whole body.

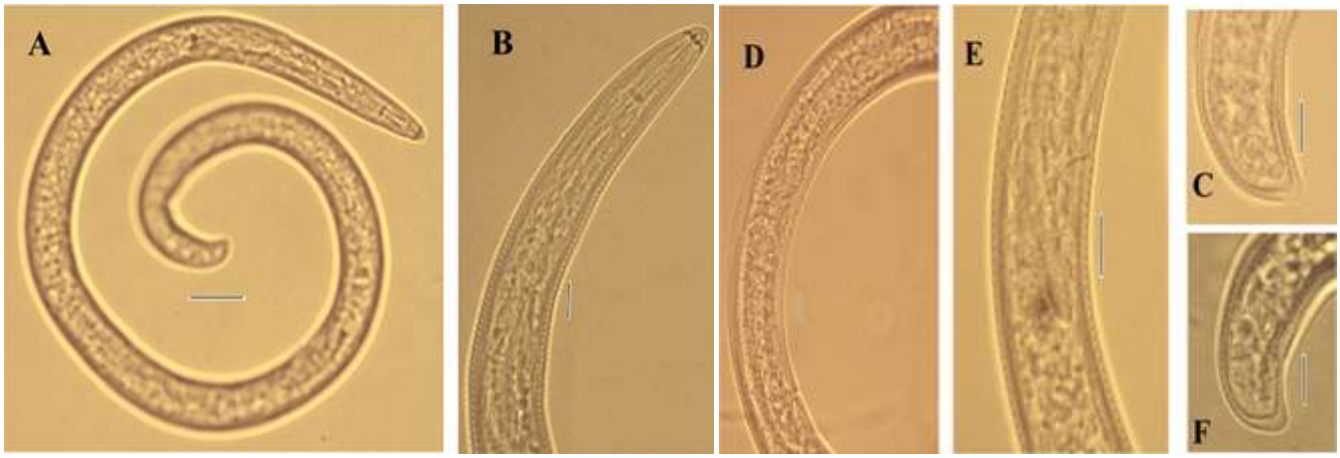

Figure 4 (A-F): *Helicotylenchus wajihi* Sultan, 1981. Female: A .Whole body; B. Anterior region; C, F. Tail region; D. Vulval region; E. Oesophageal region (Scale: A= 40 µm ; B-F= 100 µm).

Table 3: Measurement of *Helicotylenchus wajihi* (measurements are in µm except L).

Measurements	Females (n=7)	
	Range	Mean ± SD
L (mm)	0.50-0.62	0.56±0.019
a	23-29.90	29.1±1.94
b	5.4-6.3	5.65±0.44
b’	4.0-5.1	4.63±0.36
c	35.8-52.3	44±6.58
c’	0.9-1.1	1.0±0.064
V%	60.8-63.9	62.04±1.33
Stylet	21.6-24	22.14±0.97
DGO	5-6.3	5.8±0.44
Nerve ring	70-76	73.2 ± 2.4
Excretory pore	89-90	89.7 ±0.63
Hemizonid	83-87	85±0.62
Phasmid	5-7	6±0.5
Tail length	12-14	13.04±0.84
Anal body width	13-14	13.7±0.34
Body width	20-22	21.0±0.68
Tail annules	8-9	8.4±0.49

AUTHORS’ CONTRIBUTION

Both the authors designed the study, collected nematode samples, identified them, made diagrams, performed measurement of nematodes, wrote and proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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