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RESEARCH ARTICLE

New Records of Three *Pratylenchus* Species from Pakistan

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ABSTRACT

Plant-parasitic nematode of the genus *Pratylenchus* pose a significant threat to global agriculture by seriously reducing yields across numerous crops. During, a recent survey conducted in Karachi, Pakistan, several plant parasitic nematode (PPN) species from root and soil samples. Among these, nematodes from the genus *Pratylenchus* were frequently encountered. The study showed several notable findings: three species of *Pratylenchus acuticaudatus* Braasch & Decker, 1988, *P. ekrami* Bajaj & Bhatti, 1984, and *P. mulchandi* Nandakumar & Khera, 1970 are reported as new records of Pakistan. Additionally, *Pratylenchus wescolagricus* Corbett, 1983 was found in the rhizosphere of coconut (*Cocos nucifera*), representing a new host record for this species in the country.

Keywords: Plant parasitic nematodes, *Pratylenchus* species, taxonomical characterization, Pakistan.

INTRODUCTION

Plant parasitic nematodes (PPN) represent a major group of pathogens affecting crop globally. Among these, root lesion nematode of the genus *Pratylenchus* are considered third greatest economically destructive, following only cyst and root knot nematodes, due to the significant financial losses they cause in agricultural and horticulture system. As bio-trophic parasites, they live in soil and are adaptable to both temperate and tropical climates across the world. The genus, which includes approximately 103 recognized species, is known for its broad host range, capable of parasitizing a large number of plant species (Jatala & Bridge, 1990; Nguyen *et al.*, 2019; Sasser, 1987). Species within the *Pratylenchus* genus are polyphagous, yet each exhibits distinct preferences for specific hosts or groups of plants. The growth of significant profitable crops, such as coffee, bananas, peanuts, beans, wheat, maize, vegetables and a variety of fruits, is known to be

inhibited by their parasitism. For these impacted crops, reported yield losses usually range between 5 and 15% (Sikora & Fernandez, 2005; Castillo & Vovlas, 2007). The diagnosis of lesion nematodes, which mainly target plant roots and other underground structures, is made by removing them from soil or damaged roots and looking at them under a microscope (Donald, 2002). Above-ground symptoms are typically nonspecific signs of root stress, and early root damage frequently goes unnoticed. The plant's foliage may show no symptoms at all from low to moderate infestations. Conversely, high populations, particularly in young plants, can lead to stunting, symptoms of water and nutrient deficiency, and potentially plant dieback.

The subtropical climate of Pakistan exists in the moderate temperate zone which spans from 24-36° North to 61-76° East. The majority of Pakistan exists as an arid region

because it receives limited rainfall except in the southern Himalayan mountainous areas. The agricultural sector utilizes 54.97 million hectares out of Pakistan's total 79.61 million hectares of land (Maqbool & Shahina, 2001; Shahina et al., 2019). Research has identified 27 lesion nematode species which infect 45 different plant species in this region. The research adds three previously unknown root lesion nematode species to Pakistan's record while confirming the presence of one established species in a previously unreported host plant.

MATERIALS AND METHODS

Root and soil sampling: Thirty root and soil samples were randomly collected from multiple sites throughout Karachi and Sindh. Each sample had an approximate weight of 1000g. The research sites consisted of multiple plant nurseries situated in Gulistan-e-Johar Gulshan-e-Iqbal and North Nazimabad as well as University of Karachi gardens and experimental fields. The nematode collection process followed established methods described by Fortuner (1991) and Siddiqui (2000).

Collection and Storage: The plastic bags containing soil and root samples were kept in refrigerator maintained at 4° C to avoid high temperature. The bags received essential identification tags which included the host plant species and collection site and date of collection. The tagging system enabled scientists to identify and document all collected specimens correctly.

Processing: Nematodes were extracted from soil samples with a combination of Cobb's sieving and decanting methods, and modified Baermann funnel (Cobb, 1918; Baermann, 1917). After extraction, nematode specimens were initially relaxed by gentle heat and then fixed in TAF. They were then treated according to Seinhorst's (1959) slow method and mounted permanently in glycerol on glass slides for observation.

Light microscopy: Nematodes were processed for light microscopy as described by Hooper (1986). Micrographs were snapped by a Nikon DS-FI automatic camera fixed to a compound microscope.

Measurements and drawing: Body parts were determined from the formulae proposed by Man (1884). Measurements and drawings were made by the assistance of a compound microscope through a camera Lucida.

Identification: The studied nematode species were morphologically determined to species level.

Table 1. Morphometric of *Pratylenchus* species in μm .

Characters	<i>P. acuticaudatus</i>	<i>P. ekrami</i>	<i>P. mulchandi</i>	<i>P. wescolagricus</i>
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Identification was inferred through morphological studies with the aid of graphical keys and taxonomic indices prepared by Castillo and Vovlas (2007) and Siddiqui (2000).

RESULT AND DISCUSSION

Four species of *Pratylenchus* viz., *P. acuticaudatus* Braasch & Decker, 1988, *P. ekrami* Bajaj & Bhatti, 1984, *P. mulchandi* Nandakumar & Khera, 1970, and *P. wescolagricus* Corbett, 1983 were found during the present study. These species are described and illustrated herein.

Pratylenchus acuticaudatus Braasch & Decker, 1988:

Description: Female: *Pratylenchus acuticaudatus* is characterized by the subsequent morphological features in female. The labial region is low and flattened anteriorly, with a robust framework and not divided into two annuli; the head measures 7-8 μm wide by 2 μm high. Postmortem, the body exhibits a slight ventral curvature and fine annulation, with annuli approximately 1.0 μm apart at mid-body and 1.5 μm apart near the anal region. The stylet is robust and 17 μm long, bearing anterior knobs that are flat to slightly round. Lateral fields are broad, marked by four distinct lines that become faint near the tail tip; phasmids are indistinct. The median bulb is predominantly oval. An 85-110 μm long oesophageal gland extends ventrally over the intestine. The excretory hole is positioned 75-80 μm from the anterior end. A nerve ring encircles the isthmus just anterior to excretory pore, besides a separate hemizonid, 02 (two) annuli in length, is also situated anterior to the hole. The outspread anterior gonad typically contains a sole row of oocytes and reduced, sperm-free spermatheca, which overlaps the intestine. The tail is 25-30 μm long and bears 17-26 annuli (Table 1, Figure 1 & 2).

Male: Not found

Remarks: In a recent survey at the University of Karachi, specimens of *Pratylenchus acuticaudatus* were isolated from soil nearby the roots of damask rose (*Rosa damascena*). This report is first record of this nematode species from Pakistan (Maqbool & Shahina, 2001; Shahina et al., 2019). Morphological character of the specimens matched with the description as given by Braasch & Decker, 1988), only slight difference were noticed in stylet length.

	Mean ± SD (Range)	Mean ± SD (Range)	Mean ± SD (Range)	Mean ± SD (Range)
N	5	5	7	5
L	510±69.7 (430-600)	534.1±63.2 (450-625)	556.66±35.5 (500-600)	479±18 (450-500)
a	26.16±2.43 (23.8-30)	26.3±3.86 (22.5-33)	30±2.04 (27.7-33.3)	31±0.89 (30-32)
b	5±0.2 (4.7-5.3)	5.3±0.67 (4.0-5.9)	4.91±0.31 (4.5-5.4)	5.32±0.29 (5.1-6)
b'	4.44±0.43 (3.9-5)	4.9±0.56 (4.2-6)	4.42±0.33 (4-5)	4.3±0.14 (4.1-4.5)
c	19.5±3.39 (14.3-24)	22±6.41 (15-31)	20±2.38 (17-24)	24±0.81 (23-25)
c'	2.77±0.52 (1.4-2.4)	1.86±0.26 (1.4-2.2)	2.25±0.55 (1.5-3)	2.75±0.18 (2.5-3)
V%	77.2±1.72 (75-80)	79.4±2.05 (77-83)	76±0.70 (75-77)	78.8±0.74 (78-80)
Stylet	17.5±0.40 (17-18)	15.2±0.74 (14-16)	16.5±0.37 (16-17)	17.8±0.74 (17-19)
Median bulb from anterior end	57.2±1.72 (55-60)	-	-	-
Excretory pore	77.4±1.85 (75-80)	81.6±5.46 (74-90)	-	89.5±3.73 (85-96)
Junction of pharyngeal gland	85.75±3.76 (80-90)	97.6±5.00 (90-105)	115±3.68 (110-120)	98.8±0.74 (98-100)
Oesophagus gland	96±8.6 (85-110)	108.4±1.85 (105-110)	120.8±2.48 (118-125)	111.25±7.39 (100-120)
Tail length	27.2±1.72 (25-30)	25±3.68 (20-30)	27.8±2.47 (25-32)	20.5±0.5 (20-21)
Anal body width	9.5±1.11 (08-11)	11.5±1.7 (9-14)	11±0.70 (10-12)	8.5±0.44 (8-9)
Body width	16±1.41 (14-18)	17.2±1.72 (15-20)	17±0.70 (16-18)	15.8±0.74 (15-17)
Post Vulval Uterine Sac	18±1.41 (16-20)	36.5±0.5 (36-37)	17.8±2.31 (15-21)	26±1.41 (24-28)
Tail annules	19.5±2.29 (17-26)	31.8±5.07 (26-40)	17±0.70 (16-18)	18±1.41 (16-20)

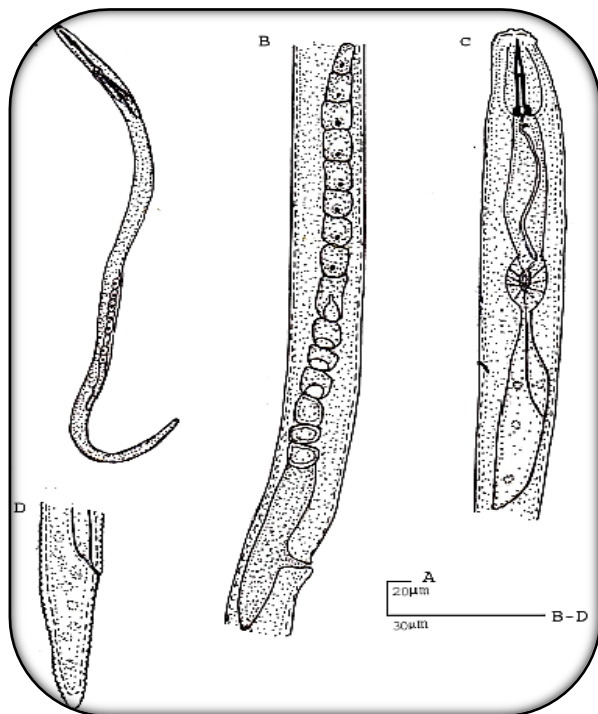


Figure 1. *Pratylenchus acuticaudatus* Braasch & Decker, 1988 (A-D): A= Female whole body, B= Reproductive system C= Pharynx and D= Female tail. Scale A= 20µm, B-

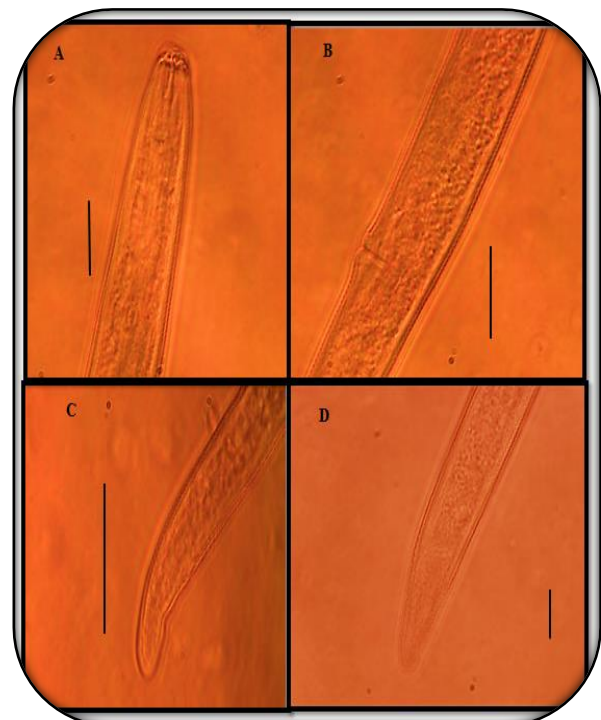


Figure 2. *Pratylenchus acuticaudatus* Braasch & Decker, 1988 (A-D): A= Anterior region, B= Vulval region, C & D= Tail region. Scale A-C= 20µ, D= 10µm. D= 30µm.

***Pratylenchus ekrami* Bajaj & Bhatti, 1984:**

Description: Female: Upon death, the body assumes a relaxed, open C-shape with mild ventral curvature. The gently sclerotized cephalic region aligns with the body's contour, displaying three clear annuli. Beginning just behind the labial area, the lateral field is marked by 02 (two) lines; the external appearances are aerolated, increasing to 04 (four) lines at the metacarpus level and continuing past the phasmid before becoming faint. A well-developed stylet features rounded basal knobs and a conus equal to or slightly shorter than its shaft. A highly muscular, oval median bulb is present. The esophagus overlaps a weakly defined intestinal junction. The hemizonid spans two annuli in width, with the excretory pore situated between these annuli, approximately 86 μ m from the anterior end. A transverse, slit-like vulva is located at 79% of the body length. The anterior genital branch

contains a round, sperm-filled spermatheca and oocytes arranged in a single file. A post-vulval uterine sac measures 36.5 μ m in length. The anal body width is 12 μ m. The tail is elongated and sub-cylindrical, 25 μ m long, with a slightly rounded terminus and 26-40 tail annuli; the phasmid is located anterior to the tail tip (Table 1, Figure 3 & 4).

Male: Not found.

Remarks: Specimens of *Pratylenchus ekrami* were collected from soil around the roots of tomato plants (*Solanum lycopersicum*) in a field area at Karachi University, representing the first recorded occurrence of this nematode species in Pakistan (Maqbool & Shahina, 2001; Shahina et al., 2019). The morphological measurements of the recovered specimens align closely with the original description by Bajaj & Bhatti (1984), with only minor variations observed in esophageal and stylet length.

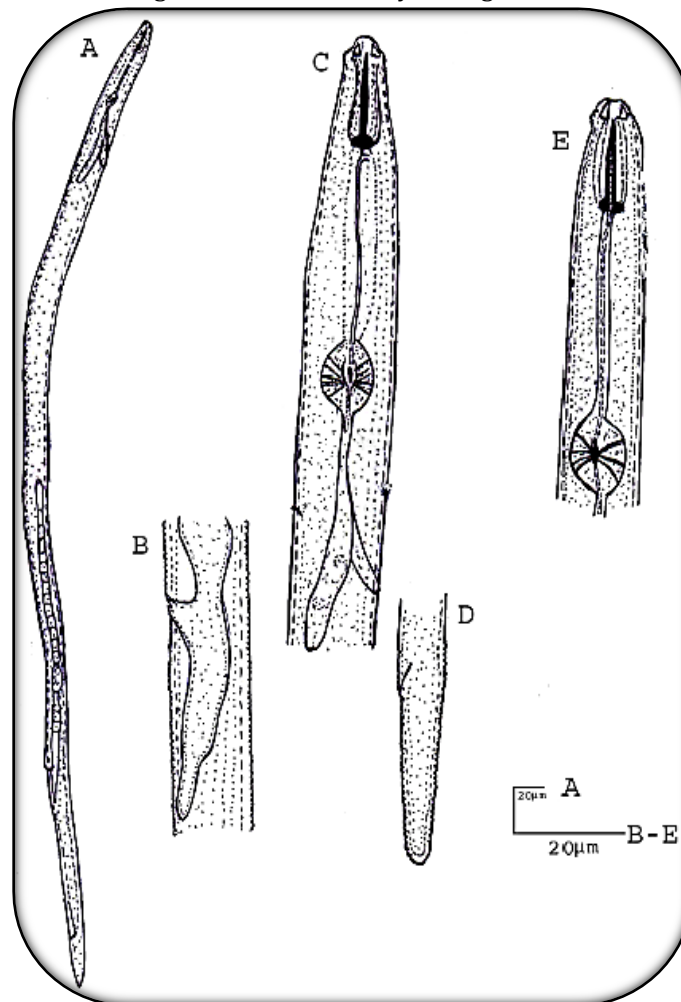


Figure 3. *Pratylenchus ekrami* Bajaj & Bhatti, 1984(A-E), Female: A= whole body= B= Vulva, C= Pharynx, D= Anus and tail length, E= Anterior region. Scale A-E= 20 μ m.

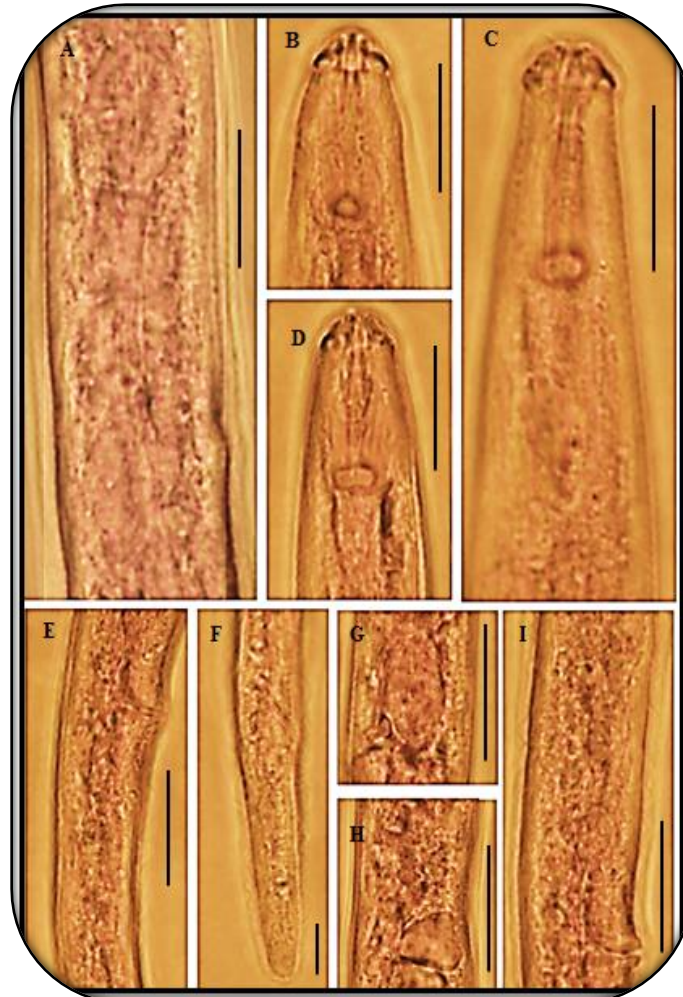


Figure 4. *Pratylenchus ekrami* Bajaj & Bhatti, 1984(A-I): A= Reproductive system, B-D= Anterior region, E= Vulval region, F= Tail region, G-I= Reproductive system. Scale A-I= 20 μ m.

***Pratylenchus mulchandi* Nandakumar & Khera, 1970:**

Description: Female: Upon death, the body assumes a slender, slightly curved (ventrally) posture, relaxing into a distinct C-shape. The head consists of three annuli, the first of which is sometimes deeply indented. The lateral fields, comprising one-third of the body's diameter, are marked by four lines. The outer line appears crenated. These fields originate with two lines at the metacarpus, increasing to four lines near the esophageal-intestinal junction. They widen at mid-body, continue with four lines until just before the anus and then reduce to two lines at the phasmid level before becoming indistinct. The stylet measures 17 μ m, with a robust, slightly shorter conical portion leading to rounded basal knobs that may curve forward. The metacarpus is oval. The excretory pore is positioned opposite the pharyngo-intestinal junction, with the hemizonid located just anterior to it. A transverse, slit-like vulva is present at 76% of the body

length. The single, anterior gonad contains oocytes arranged in one or two rows, and the oval spermatheca is empty. The tail comprises 16 coarsely annulated segments and terminates in a smooth, broadly rounded, truncate tip, often with a pronounced ventral twist. The phasmids are situated posterior to the anus (see Table 1 and Figures 5 & 6).

Male: Not found.

Remarks

Soil samples were taken from the root zones of sugarcane (*Saccharum officinarum*) in agricultural fields near Karachi. The nematode species recovered from these samples represents its first documented occurrence in Pakistan (Maqbool & Shahina, 2001; Shahina et al., 2019). Morphological measurements of this population align with those reported for *Pratylenchus mulchandi* by Nandakumar & Khera (1970), with the exception of body width and tail length.

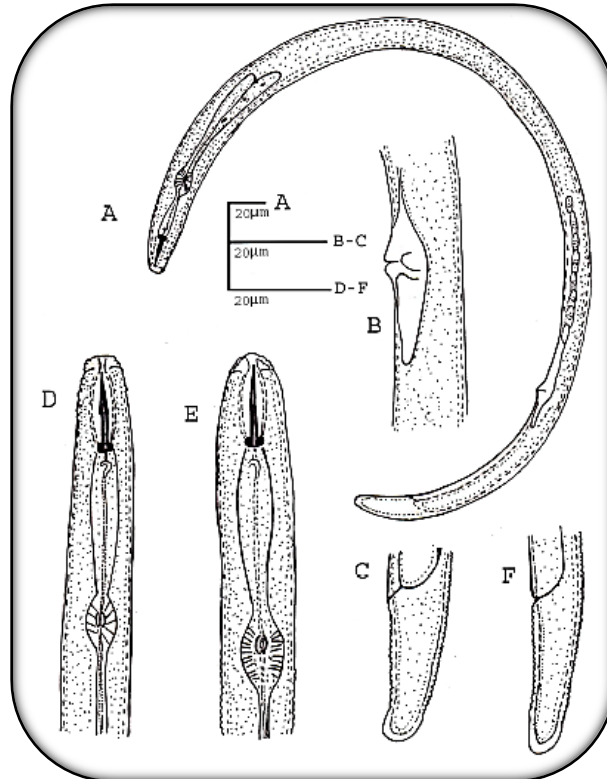


Figure 5. *Pratylenchus mulchandi* Nandakumar & Khera, 1970 (A-F), Female: A= whole body, B= Vulva, C= Anus and tail length, D-E= Anterior region, F= Tail length. Scale A-F= 20μm.

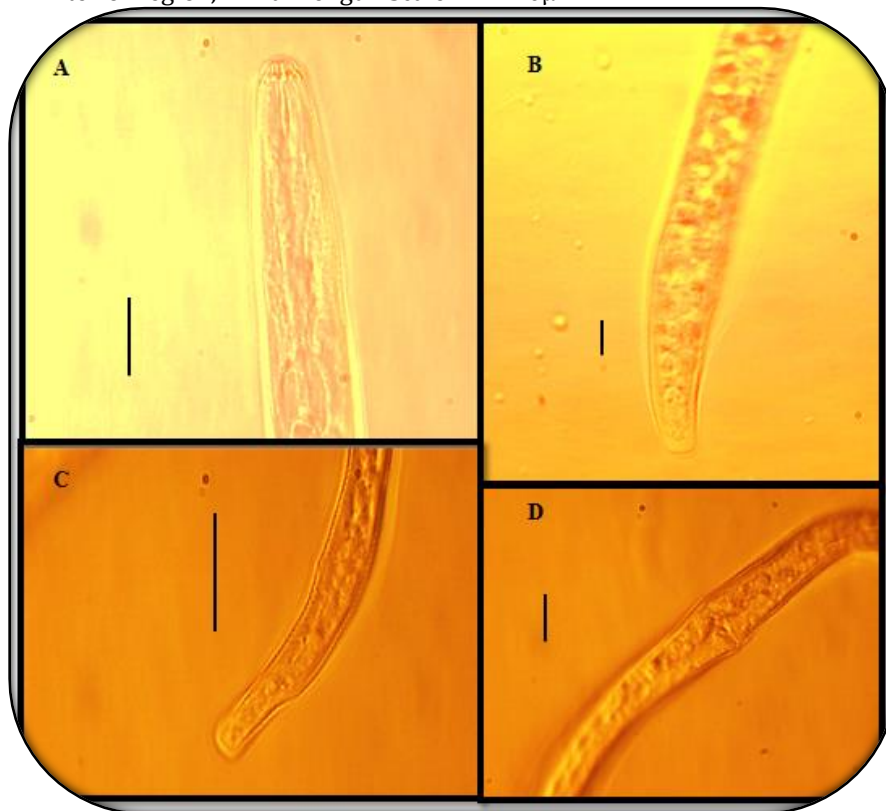


Figure 6. *Pratylenchus mulchandi* Nandakumar & Khera, 1970 (A-D). A= Anterior region, B-C= Tail region, D= Vulval region. Scale A-B= 20μm, C= 40μm, D= 20μm.

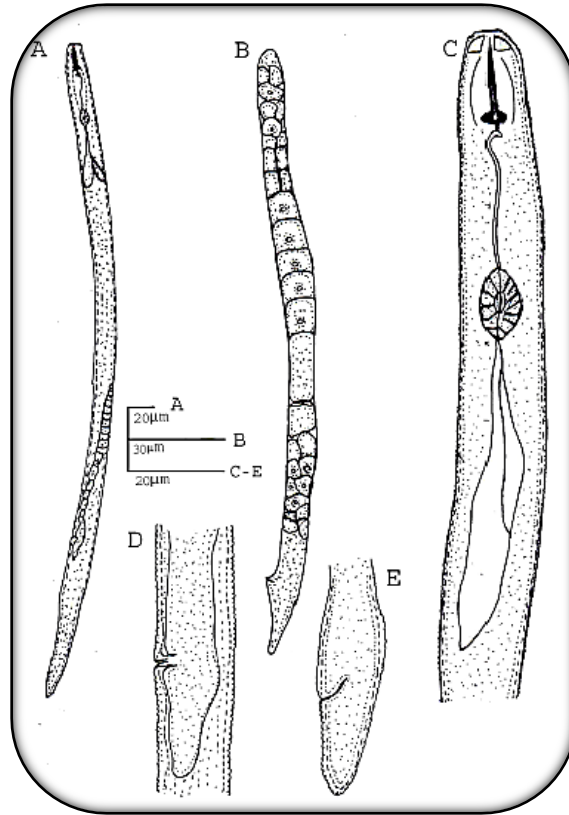


Figure 7. *Pratylenchus wescolagricus* Corbett, 1983 (A-E). A= Female whole body, B= Reproductive system, C= Pharynx, D= Vulva, E= Anus and tail length, Scale A-E= 20µm.

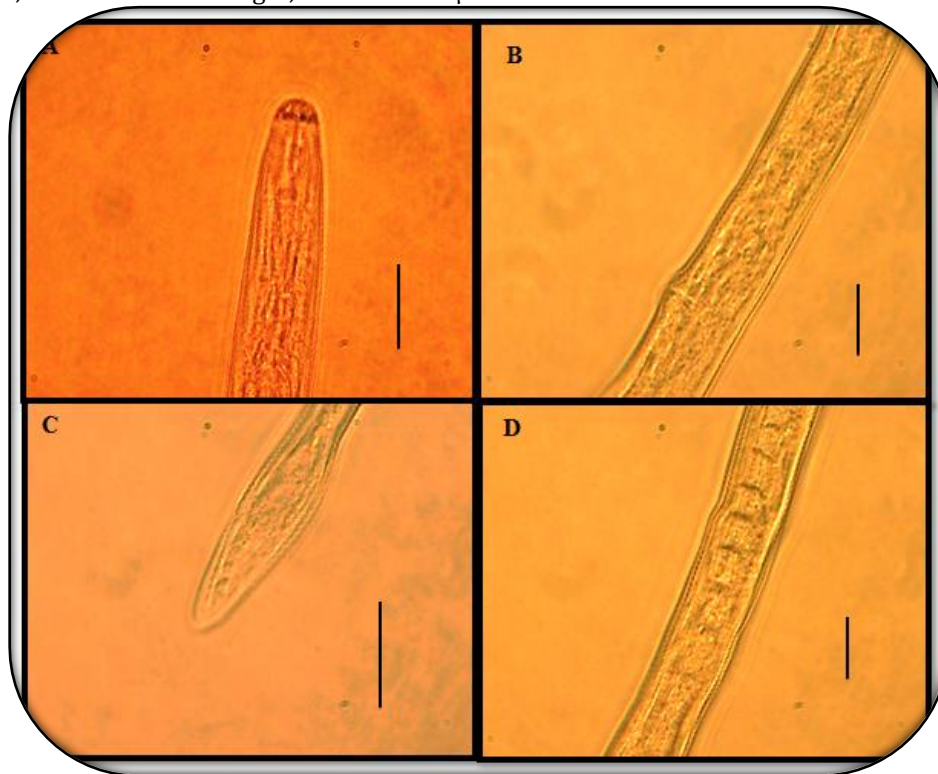


Figure 8. *Pratylenchus wescolagricus* Corbett, 1983(A-D). A= Anterior region, B= Vulval region, C=Tail region, D= Reproductive system. Scale A-D = 20µm.

***Pratylenchus wescolagricus* Corbett, 1983:**

Description: Female: The nematode is characterized by surface striations visible over the body, the worm is straight or slightly bent at rest and it tapers to either end. The sides of the body are intersected by four deeply impressed lines. These lines start as a single line at the stylet base, separate into two near the median bulb and become four posterior to the nerve ring which reach past their level of phasmid at anus. At the anal orifice all four lines are perfectly distinct, one or two of them more or less prolonged nearly to the extremity of the tail. The conoid labial region bears 4 annuli and is heavily sclerotized, extending over 2 body annulation. The stylet is 18µm long and possesses weakly anchor-shaped knobs. The dorsal pharyngeal gland opening is located 2.5µm behind the stylet. The pharynx is typical for the genus, with an isthmus length equal to the body diameter. Within the oesophageal overlap, a distinct dorsal gland nucleus and two sub-ventral pharyngeal gland nuclei are visible. The oesophageal-intestinal junction is situated 12-13% along the overlap from its anterior end, with the total pharyngeal gland overlap measuring 98-100µm. The excretory pore is opposite the oesophageal-intestinal junction, and the hemizonid lies two annuli anterior to it. The vulva is a transverse slit, 5/6 of the body diameter in width. The spermatheca is small and spherical. A post-vulval uterine sac extends 24µm from the body diameter. The tail tip is rounded and smooth, with a clear hyaline area; the phasmid is located anterior to the tail terminus. The tail itself consists of 16-20 annuli (Table 1 and Figures 7 & 8).

Male: Not found.

Remarks

Specimens of *P. wescolagricus* were isolated from the rhizosphere soil of coconut (*Cocos nucifera*) in Karachi, Pakistan, representing the first documented report of this nematode species associated with coconut in the country. Previously in Pakistan, *P. wescolagricus* had only been reported from soil surrounding loquat (*Eriobotrya japonica* L.) trees in Khyber-Pakhtunkhwa province (Sami Ullah & Khanum, 2023). Morphometric measurements of the Karachi specimens align with the original description by Corbett (1983), exhibiting minor variations in body and tail lengths.

Occurrence of *Pratylenchus* spp., especially *P. wescolagricus* in the city of Karachi has reported and highlight their threat to most important commercial plants. The current observation reinforces the necessity

for more detailed investigations about local plant parasitic interactions. In order to protect agricultural productivity, such evidence highlights the need for continual research and monitoring of nematodes to ensure sustainable effective management in the region.

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Namra Inam	: Sampling, extraction and identification of nematodes
Abdul Q. Rajput	: Supervision of research, manuscript writing
Saleem Shahzad	: Review and finalization of manuscript
Saboohi Raza	: Review of manuscript
Erum Iqbal	: Research idea and identification of manuscript
Sumit Nadeem	: Data collection and presentation