New and Known Nematodes Associated with Cotton Plantation in Sindh, Pakistan

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ABSTRACT

Cotton is a major crop of Pakistan after wheat and it occupies the largest area in Pakistan compared to other crops. Cotton is cultivated in Sindh on more than 1 million acres. Two popular varieties of cotton are grown in Sindh. The aim of the present study was to determine the diversity of plant parasitic and soil nematodes associated with cotton plants in Sindh. During 2017–2018 extensive surveys were conducted at the time of cropping and at harvesting and soil root samples were collected from different fields in the cotton producing regions of five districts of Sindh viz., Sanghar, Mirpurkhas, Umerkot, Mityari and Tando Allahyar. The analysis of samples resulted in the identification of one new species of soil nematode viz., Acrobeloides gossypii n. sp., along with three new record species viz., Tylenchorhynchus ewingi Hopper, 1959; T. crassicaudatus Williams, 1960 and Pratylenchus pseudofallax Café-Filho & Huang, 1989. Description, measurements and illustrations of these species are incorporated herein.

INTRODUCTION

Several fiber crops are important agriculture commodities in the subtropics and tropics with cotton being the most important one in term of total production (Starr et al., 2005). Because cotton is grown as cash crop, it is often grown in a monoculture system that favors the development of a nematode community dominated by one or a few parasitic species (Starr et al., 1993). Upland cotton Gossypium hirsutum L. accounts for approximately 90% of the world production. Cotton is one of the four major crops of Pakistan and accounts for 8% of the value-added in agriculture and contributes about 2% to national GPD. The two popular varieties of cotton grown in Sindh are NIAB and newly introduced Bt (genetically modified) variety (www.sbi.gos.pk/sindh.profile). Several nematode species are reported from the cotton, but have not proven to be pathogenic. Meloidogyne incognita, Rotylenchulus reniformis and Hoplolaimus columbus are the most frequently encountered species found associated with cotton in the world and are responsible for the significant yield losses (Starr et al., 2005). These species are also recorded on cotton crop in Pakistan (Zarina and Shahina, 2013). In spite of its importance as a commercial crop, very few investigations have been undertaken to evaluate the role of nematodes as pests of this crop except for few reports of nematode infection. Recently a new species of soil nematode was described from Mubarak Village (Salma et al., 2018)

MATERIALS AND METHODS

Soil samples were collected from the cotton root zone at a depth of 15-30 cm. These soil and root samples were placed in polythene packs at 4-20ºC. The packs were labeled containing basic information about host, locality and date of collection. For separating nematodes from the soil, Cobb’s decanting and sieving method (Cobb, 1918) and modified Baermann’s funnel technique (Baermann, 1917) were used. The nematodes were killed by delicate warming the suspension on a hot plate at 60-70ºC. Killing was additionally done by pouring of high temperature water (80-90ºC) over nematodes in a little amount of water. The killed nematodes were instantly fixed in TAF for 24 h (Courtney et al., 1955). Fixed samples were kept in vials. After 24 h, the samples were washed thrice with distil water, then 2 ml of 1.25% glycerine was added to the cavity and placed it in incubator at 55ºC for 5-6 days. Nematodes were mounted in a little drop of pure glycerin (anhydrous glycerol) in the focal point of a glass-slide and secured with a 19mm cover slip containing three little pieces of paraffin wax. The slide was delicately warmed on a hot plate till the wax was softend and permitted the cover slip to settle down. At the point when the wax was set the cover slip was fixed by zut with a little delicate brush. The permanent slides were labeled containing the
details of host, locality, number of specimens of males and females and mounting date. For light microscopy the settled nematodes were prepared utilizing techniques for Hooper (1986). Photomicrographs of nematodes were made with a programmed camera connected to a compound magnifying lens outfitted with an impedance differentiate framework. Measurements were taken with an ocular micrometer and also by tracing the outline of the structure on a paper with a drawing tube attached to a compound microscope. Measurements were taken from the drawing with a ruler or curvimeter for curved lines. Illustrations were made with the help of a drawing tube attached to the compound microscope. de Man’s (1884) formula was used for measurement of nematodes. Identification of nematodes was made after Siddiqi (2000).

Acrobeloides gossypii n. sp. 
(Figs. 1 and 2)

**Measurements**
See Table 1 for measurement.

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**Fig. 1.** Female of *Acrobeloides gossypii* n. sp.: A, pharyngeal region; B, basal bulb; C, anterior region; D, posterior region; E, vulval region showing PUS; F, reflexed ovary.

**Fig. 2.** Male of *Acrobeloides gossypii* n. sp.: A, whole body; B, pharyngeal region; C, tail region; D, anterior region; E, basal bulb; F, spicules and bifurcated gubernaculum.

**Description**

**Female**

Body cylindrical slightly ventrally curved upon fixation, tapering towards both ends, narrowing just behind vulva. Cuticle annulated, annuls 2-3 µm wide at mid body. Lateral field with five incisures, occupying 8-19% of the mid body diameter. Lip region with three pairs of lips. Three low and rounded labial brobolae. Amphid opening slit like stoma cephaloboid 12-16 µm long. Cheilostom with small rhabdia. Gymnostom and stegostom are narrower than cheilostom and walls appear to be sclerotized. Pharyngeal corpus cylindrical 4.3-4.6 times isthmus length. Basal bulb oval with strongly developed valves. Cardia conoid-truncated, enveloped by intestinal tissue. Excretory pore at 83-95% of total neck length at posterior part of isthmus. Deirid at the level of excretory pore, 84-87% of neck length. Nerve ring surrounding anterior half of isthmus at 82-100% of neck length. Genital system monodelphic, prodelphic ovary posteriorly directed, with double
flexure in its post vulval part. Oviduct long and tubular, spermatheca strongly developed 130-200 \( \mu \text{m} \) long. Post uterine sac (PUS) 0.7-1.4 times the corresponding body diameter long, distinct, vagina extending inwards to one seventh of body diameter. Rectum 0.6-0.9 times anal body width long. Rectal glands present near intestine rectum junction. Anal lips not protruding. Tail conoid to subcylindriod with 19-21 annules ventrally. Terminus smooth obliquely truncated. Phasmid just behind mid tail 25-32 \( \mu \text{m} \) or 53.3-59.5% of tail length.

**Male**

General appearance similar to female but with smaller body. Body curved upon fixation more so in posterior region. Reproductive system cephaloboid, monorchic, testis reflexed ventrally. Tail conoid ventrally curved with five pairs of rounded lips and three high and conical rounded labial probolae. Pharyngeal corpus 4.0-5.2 times isthmus length. Spermatheca 56-100 \( \mu \text{m} \) long, post uterine sac 116-170 \( \mu \text{m} \) long or 1.1-1.7 times the corresponding body diameter long. Female tail conoid, sub-cylindrical with obliquely truncated terminus 42-55 \( \mu \text{m} \); c = 16-29.2; c’ = 1.1-1.9;

**Diagnosis and relationship**

*Acrobeloides gossypii* n. sp., is characterized by a body length of 811-1695 \( \mu \text{m} \) in female and 714-1392 \( \mu \text{m} \) in males. Lateral field with five incisures, lip region with three pairs of rounded lips and three high and conical rounded labial probolae. Pharyngeal corpus 4.0-5.2 times isthmus length. Spermatheca 56-100 long, post uterine sac 116-170 \( \mu \text{m} \) long or 1.1-1.7 times the corresponding body diameter long. Female tail conoid, sub-cylindrical with obliquely truncated terminus 42-55 \( \mu \text{m} \); c = 16-29.2; c’ = 1.1-1.9;

### Table I. Measurements (in \( \mu \text{m} \)) of *Acrobeloides gossypii* n. sp.

<table>
<thead>
<tr>
<th>Morphological characters</th>
<th>Holotype (♀)</th>
<th>Paratypes (15 ♀♀)</th>
<th>Paratypes (15 ♂♂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length (L)</td>
<td>960</td>
<td>117.6±351.6 (811-1695)</td>
<td>939.6 ± 175.9 (714-1392)</td>
</tr>
<tr>
<td>a</td>
<td>12.3</td>
<td>11.3±2.2 (8.1-15.5)</td>
<td>14.4±1.61 (11.1-16.6)</td>
</tr>
<tr>
<td>b</td>
<td>5.5</td>
<td>6.1±1.3 (4.5-8.0)</td>
<td>5.7±0.92 (4.6-7.5)</td>
</tr>
<tr>
<td>c</td>
<td>19.2</td>
<td>22.7±4.9 (16-29.2)</td>
<td>21.7±2.3 (19.5-27.8)</td>
</tr>
<tr>
<td>c’</td>
<td>1.7</td>
<td>1.56±0.18 (1.1-1.9)</td>
<td>1.1 ± 0.15 (0.9-1.3)</td>
</tr>
<tr>
<td>V/T</td>
<td>71.2</td>
<td>71.9±2.1 (70.2-75)</td>
<td>-</td>
</tr>
<tr>
<td>Labial probolae</td>
<td>3</td>
<td>4.00±0.7 (3-5)</td>
<td>3.5±0.5 (3-4)</td>
</tr>
<tr>
<td>Width lip region</td>
<td>14</td>
<td>13.6±1.16 (12-15)</td>
<td>12.2±1.01 (11-14)</td>
</tr>
<tr>
<td>Stoma</td>
<td>12</td>
<td>16.0±3.2 (12-22)</td>
<td>14.5±2.16 (12-15)</td>
</tr>
<tr>
<td>Pharyngeal corpus</td>
<td>130</td>
<td>135±8.1 (125-150)</td>
<td>121±8.37 (110-134)</td>
</tr>
<tr>
<td>Isthmus</td>
<td>28</td>
<td>23.7±6.6 (14-28)</td>
<td>17.3±1.88 (16-20)</td>
</tr>
<tr>
<td>Bulb</td>
<td>30</td>
<td>28.8±1.8 (26-32.5)</td>
<td>26.7±2.0 (24-30)</td>
</tr>
<tr>
<td>Pharyngeal length</td>
<td>178</td>
<td>186.7±20.9 (166-227.5)</td>
<td>165.9±13.8 (150-187.5)</td>
</tr>
<tr>
<td>Nerve ring anterior end</td>
<td>134</td>
<td>127.8±4.4 (125-135.5)</td>
<td>125.2±10.0 (112-134)</td>
</tr>
<tr>
<td>Excretory pore anterior end</td>
<td>148</td>
<td>147.5±8.5 (136-150)</td>
<td>136.8±4.4 (132-144)</td>
</tr>
<tr>
<td>Deirid-anterior end</td>
<td>152</td>
<td>147.4±3.3 (143-152)</td>
<td>-</td>
</tr>
<tr>
<td>Annuli width</td>
<td>3</td>
<td>2.6±0.4 (2-3)</td>
<td>3.5 ± 0.5 (3-4)</td>
</tr>
<tr>
<td>Cuticle thickness</td>
<td>4</td>
<td>6.7 1.05 (5-8)</td>
<td>4.6 ± 0.65 (4-5)</td>
</tr>
<tr>
<td>Body width: neck base</td>
<td>54</td>
<td>73.6±16.1 (54-100)</td>
<td>51.3±3.5 (46-58)</td>
</tr>
<tr>
<td>Body width: midbody</td>
<td>78</td>
<td>114.6±45.5 (52-170)</td>
<td>71.3±13.1 (64-87.5)</td>
</tr>
<tr>
<td>Body width: anus</td>
<td>40</td>
<td>33.9±9.7 (25-50)</td>
<td>39.5±6.21 (32-40)</td>
</tr>
<tr>
<td>Vagina</td>
<td>20</td>
<td>23.7±3.2 (20-27.5)</td>
<td>-</td>
</tr>
<tr>
<td>Spermatheca</td>
<td>56</td>
<td>121.3±47.4 (56-200)</td>
<td>-</td>
</tr>
<tr>
<td>Post uterine sac (PUS)</td>
<td>154</td>
<td>140.5±27.7 (90-170)</td>
<td>-</td>
</tr>
<tr>
<td>Rectum</td>
<td>24</td>
<td>27.0±3.6 (24-30)</td>
<td>-</td>
</tr>
<tr>
<td>Tail</td>
<td>50</td>
<td>51.8±6.92 (42-55)</td>
<td>45.3±3.77 (40-50)</td>
</tr>
<tr>
<td>Vulva-anterior end</td>
<td>620</td>
<td>866.5±253.7 (616-1225)</td>
<td>-</td>
</tr>
<tr>
<td>Vulva-anus</td>
<td>5.8</td>
<td>324.3±64.4 (230-416)</td>
<td>-</td>
</tr>
<tr>
<td>Spicules</td>
<td>48</td>
<td>-</td>
<td>49.7 ± 6.2 (38-57.5)</td>
</tr>
<tr>
<td>Gubernaculum</td>
<td>20</td>
<td>-</td>
<td>27.1±5.2 (24-35)</td>
</tr>
</tbody>
</table>
male tail conoid with minutely rounded terminus 42-57 µm; c = 19-27.8; c’ = 0.9-1.31. Spicules 38-57.5 µm long and gubernaculum 24-35 µm long.

The new species resembles A. bodenheimeri (Steiner, 1936) Thorne, 1937 but differs from it by having more robust body (body width 52-170 µm vs. 33-40 µm); lesser a value (8.1-15.5 vs. 16.6-23.5); more posterior located vulva (70.2-75 vs. 64-69%). Post uterine sac (PUS) 1.1-1.7 vs. 9-1.1 times the corresponding body diameter long.

**Type habitat and locality**

Samples were collected from root zone of cotton (Gossypium hirsutum L.) from Matiari, Sindh, Pakistan.

**Measurements**

**Female** (n=8)

L = 0.65 ± 0.07 (0.54–0.75) mm; a = 31.84 ± 1.94 (29.3-35); b = 4.85 ± 0.45 (4.5-5.5); c = 15 ± 1.23 (13-17); c’ = 2.5 ± 0.37 (2-3); V = 55.8 ± 2.35 (53-59.3); Stylet = 19.2 ± 0.64 (18-20) µm.

**Male** (n=6)

L = 0.56 ± 0.02 (0.54-0.60) mm; a = 32.2 ± 1.88 (30-35); b = 4.58 ± 0.30 (4-5); c = 13.6 ± 0.36 (13-16.5); Stylet = 18.5 ± 0.35 (18-20) µm; spicule = 19.1 ± 0.60 (18-20) µm; gubernaculum = 9.4 ± 0.32 (9-10) µm.

**Remarks**

Specimens of Tylenchorhynchus ewingi Hopper, 1959 collected from soil around the roots of cotton (Gossypium hirsutum L.) at two localities Umerkot and Sanghar, are the first record species from Sindh, Pakistan. Measurements are in close agreement with the description given by Hopper (1959). It causes stunted and retarded growth of the crop plants. Robbins *et al.* (1989) also recorded T. ewingi from cotton rhizosphere from India.

**Tylenchorhynchus crassicaudatus**

*Williams, 1960*

(Fig. 4)

**Measurement**

**Female** (n=10)

L= 0.62 ± 0.11 (0.57-0.70) mm; a= 30.4 ± 1.77 (29-33); b= 5.3 ± 0.29 (5.0-5.7); c= 14.9 ± 0.64 (14-16); c’= 3.3 ± 0.13 (3.1-3.5); V= 55 ± 1.49 (52.5-58); Stylet = 20.2 ± 0.68 (19-21) µm.

**Male** (n=8)

L= 0.65 ± 0.04 (0.58-0.70) mm; a= 29.7 ± 1.15 (27.9-31.5); b= 5.50 ± 0.27 (4.7-5.5); c= 14.5 ± 1.21 (12.8-17); spicule= 21.8 ± 1.01 (20-23) µm; gubernaculum= 11.15 ± 0.68 (10-12) µm; stylet= 19.8 ± 0.57 (19-21) µm.

**Type material**

Holotype (female): Acrobeloides gossypii n. sp. Slide No. 1 deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Pakistan. Paratype females (n= 25), males (n= 25) on slides No. 2-20, are deposited in the above said nematode collection.

**Etymology**

The species name refers to the type host cotton (Gossypium hirsutum L.) from where the samples were collected.
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Fig. 4. *Tylenchorhynchus crassicaudatus*, Williams (1960): A, oesophageal region of female; B, oesophageal region of male; C, lateral field; D, vulval region; E, female tail; F, male tail.

**Remarks**
*Tylenchorhynchus crassicaudatus* Williams, 1960 was reported from soil around the roots of cotton (*Gossypium hirsutum* L.) from Tando Allahyar as a new record from Sindh, Pakistan. Measurements and morphological characters are quite similar to those given by Williams (1960).

**Pratylenchus pseudofallax**
*Café-Filho & Huang, 1989*  
(Fig. 5)

**Measurements**
*Female (n = 10)*  
$L = 0.44 \pm 0.05$ (0.42-0.55) mm; $a = 1.98 \pm 9.3$ (22-30); $b = 3.77 \pm 0.44$ (3.0-4.4); $b' = 6.11 \pm 0.74$ (5.0-7.3); $c = 18.5 \pm 1.91$ (15-21); $c' = 2.2 \pm 0.46$ (1.75-3.0); $V = 78.6 \pm 1.20$ (77.1-80.2); stylet $= 15.34 \pm 0.74$ (14-16.5) µm.

Fig. 5. *Pratylenchus pseudofallax*, Café-Filho and Huang (1989): A, whole body; B, vulval region; C, oesophageal region; D-E, tail region.

**Remarks**
*Pratylenchus pseudofallax* Café-Filho and Huang (1989) was reported as a new record from cotton growing areas of Sindh for the first time from Pakistan. This species was collected from Mirpurkas during the present studies. The measurements of these nematode specimens correspond well with the original description of Café-Filho and Huang (1989).

**CONCLUSION**

One new species of soil nematode *viz.*, *Acrobeloides gossypii* n. sp., along with three new record species *viz.*, *Tylenchorhynchus ewingi*, Hopper (1959); *T. crassicaudatus*, Williams (1960) and *Pratylenchus pseudofallax*, Café-Filho and Huang (1989) identified from this survey. This study will help further for the management of the cotton soil nematodes.
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Statement of conflict of interest
The authors declare no conflict of interest.

REFERENCES


