Research Article

Aphelenchoides bougainvilli n. sp. (Nematoda: Aphelenchoididae) from around *Bougainvillea spectabilis* L. from Pakistan

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Abstract | A population of *Aphelenchoides bougainvilli* n. sp. was retrieved from soil sample collected around roots of paper flower (*Bougainvillea spectabilis* L.), thoroughly described and illustrated from village Sarai Naurang located in southern district Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The population was identified as new species and related with Group 2 of *Aphelenchoides* species, distinguished via length of female's body 428-444 µm (0.42-0.44 mm), cephalic region rounded and offset. Lateral field found with three incisures. Cuticle finely annulated, about 1µm apart. Stylet 10 µm (10±0), delicate with minute basal knobs. Excretory pore located 2 µm anterior to the metacarpus and 44 µm from cephalic end. Vulva anteriad, having slight elevated anterior and posterior lips. Post urine sac (PUS) short (15-20 µm), and covering 16.6-20% of vulva-anus distance. Tail small (24-26.5 µm), conical, about 3 times long to anal diameter and with a small, single mucro (1 µm).

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Introduction

Nematodes and fungi are considered the most rich organisms in soil environment and offer substantial role in ecosystem as well as perform central part in sustaining the stability of food webs and nutrient recycling (Zhang *et al*., 2020).

The genus *Aphelenchoides* (Fischer, 1894) representing species rich group in order Aphelenchida. They are found in soil and also have been associated with lichens, moss and algae from coniferous trees, twigs and bark (on and under) and insect frass from trees. Few species of *Aphelenchoides* are facultatively ecto and endoparasites of plant and are known as foliar and bulb nematodes while most of them leads mycetophagous life style or fungivorous nematodes (Hunt, 1993). Foliar nematodes are generally infectious agent of flowering plants in nurseries, greenhouses, growing crops as well as timber plants. The damages produced by them can be a reason of marketability difficulties in flowerings due to interfere with presence of plant or decreases production of crops (Sanchez Monge *et al*., 2015). *Aphelenchus* and *Aphelenchoides* spp may fed upon fungi and would be a good biological control agents counter to several plant parasitic nematodes and plant pathogenic fungi (Lamondia and Timper, 2016).

Approximately, 200 species of *Aphelenchoides* have been described worldwide (Kim *et al.*, 2018) while, Handoo *et al.* (2020) after conducting a through literature review, they assigned 182 valid nominal species to the genus *Aphelenchoides*. According to the compilation of Pakistani nematode species, the genus *Aphelenchoides* contained 19 species (Shahina *et al.*, 2019). After that, further five species comprising three new and two known species were also added (Salma *et al.*, 2020; Samreen *et al.*, 2020; Salma *et al.*, 2021). In addition, during recent study the population of new species designated as *Aphelenchoides bougainvilli* n. sp. were obtained from soil around paper flower (*Bougainvillea spectabilis* L.) at Sarai Naurang village, district Lakki Marwat, KPK, Pakistan. The objective of the study was: documentation of new aphelenchid nematodes species of their significant biologically importance in agricultural system as thoroughly described in current research article.

Materials and Methods

Host and locality

The soil was obtained around the rhizosphere of paper flower (*Bougainvillea spectabilis* L.) up to 25cm depth using garden tool *i.e.*, hand trowel from village Sarai Naurang (GPS: 32°49'43.0"N 70°46'33.0"E), district Lakki Marwat, KPK, Pakistan.

Processing of soil sample

Nematodes were taken out from soil as per Cobb's sieving and decanting technique (Cobb, 1918), then further run by Baermann's funnel method (Baermann, 1917). Killing process of retrieved nematodes was done by means of boiled water (approximately 80-90°C) and immediately preserved in Tri-ethanol Amine Formaline (TAF) for next one day for hardening effect (Courtney *et al.*, 1955). Specimens cleaned thrice by purified water for exclusion of excess fixative, thereafter 2ml glycerin (1.25%) were added and placed in incubator at 55°C for about 5-6 days for slow dehydration (Seinhorst, 1959). Permanent mounting was ensured in glycerin drop. Cover slip (19mm) was placed over the drop with the provision of wax lumps from four sides, subsequently heated and finally sealed as wax melted (Hooper *et al.*, 2005).

Light microscopy

For taxonomical purpose specimens were examined, morphologically identified and subsequently measured according to the formula of de Man (1884) as (L= whole body length; a= whole body length/width; b'= whole body length/esophagus; c= length of whole body/ tail length; c'= length of tail/ anal diameter; V%= head-vulva distance/ whole body length x 100; G1%= length of anterior ovary/whole body x 100). The illustration was worked out via camera lucida under compound microscope. Photographs were taken through digital camera (Nikon DS-fi-1) fixed with compound microscope (Nikon Eclipse E-400).

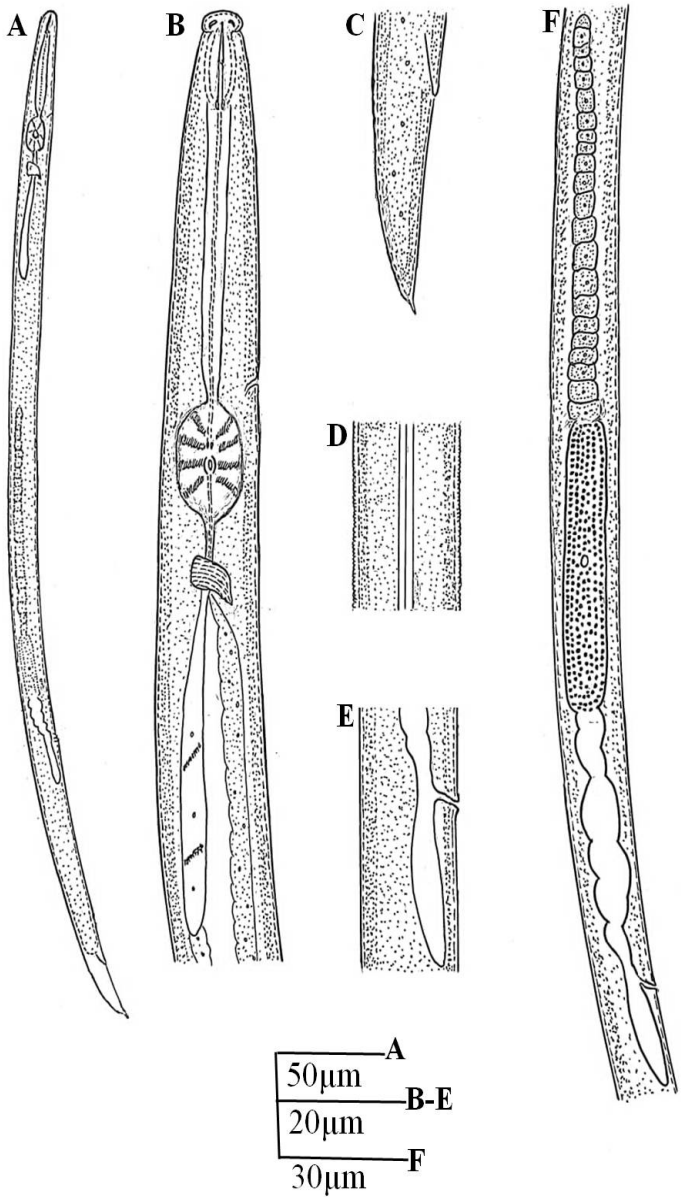

Figure 1: *Aphelenchoides bougainvilli n. sp. Female:* **A)** *Whole body;* **B)** *Pharyngeal region;* **C)** *Tail;* **D)** *Lateral field showing lateral lines;* **E)** *Vulval region and PUS;* **F)** *Female gonad.*

Results and Discussion

Systematics

(Siddiqi, 1980)

Order: Aphelenchida Siddiqi (1980)

Superfamily: Aphelenchoidea (Fuchs, 1937) Thorne, 1949
Family: Aphelenchoididae (Skarbilovich, 1947) Paramonov, 1953
Subfamily: Aphelenchoidinae Skarbilovich, 1947
Genus: *Aphelenchoides* Fischer (1894)

Aphelenchoides bougainvilli n. sp.
(Figure 1 and 2, Table 1)

Table 1: *Morphometric data of Aphelenchoides bougainvillii n. sp. All measurements are in µm in the form of Mean±SD (range) except L (mm).*

Morphological characters	Holotype female	Paratype females (n=15)
L (mm)	0.43	0.43±0.58 (0.42-0.44)
a	30.7	32.81±1.91 (30-35.5)
b'	3.9	4.0±0.19 (3.8-4.4)
c	16.5	15.4±1.00 (14-17)
c'	2.8	2.7±0.22 (2.5-3.1)
V%	71.9	71.4±0.81 (70.6-73)
Lip width	6	5.7±0.21 (5.5-6)
Lip height	2	2.1±0.15 (2-2.4)
G1 %	33.1	36.6±3.64 (32-42)
Stylet	10	10±0 (10)
Conus length	5	5±0 (5)
Procorpus	46	47±1.85 (45-50)
Pharyngeal length	108	107.5±1.87 (105-110)
Distance to the base of median bulb	60	62±2.30 (59-66)
Median bulb length	13	12.8±0.42 (12.5-13.2)
Median bulb width	9	9.2±0.21 (9-9.5)
Median bulb ratio (L/W)	1.4	1.3±0.04 (1.3-1.4)
Width at median bulb	14	14±1.65 (12-16)
Nerve ring	66	68.5±1.68 (66-71)
Excretory pore	44	43.9±0.75 (43-45)
Max. body width	14	15.9±1.35 (14-18)
Width at vulva	13	13.3±0.43 (13-14)
Post uterine sac (PUS)	20	17.1±2.08 (15-20)
Overall ovary length	143	143.9±2.45 (140-148)
Tail	26	25.2±0.84 (24-26.5)
Anal body width	9	9±0.75 (8-10)
Rectum length	7	6.9±0.86 (6-8)
Vulva-anus distance	98	95.1±3.11 (90-99)
Mucro	1	1±0 (1)

Description

Female: Habitus of body usually smaller, slender shaped, tapering at both ends, ventrally concave and dorsally convex upon fixation. Cuticle is finely annulated, approximately 1µm apart with a lateral field with three incisures, consisting about 15% of corresponding body width. Cephalic end rounded, offset from body contour, about 6 µm wide, 2 µm high. Stylet delicate, measured 10 µm (10±0) having minute basal swelling. Conus part of stylet approximately 50% of the entire length. Procorpus cylindrical. Metacarpus oblong, with highly strong valves situated centrally to slightly posteriorly, approximately 9 µm wide and 13 µm in length. Excretory pore positioned at 2 µm anterior to the median bulb, the position is approximately 1/12th of metacarpus length. Isthmus long. Nerve ring placed just posterior to metacarpus and approximately half of the metacarpus length. Hemizonid invisible. Pharynx slender, about three times long to body diameter, overlapping intestine dorsally. Intestine simple.

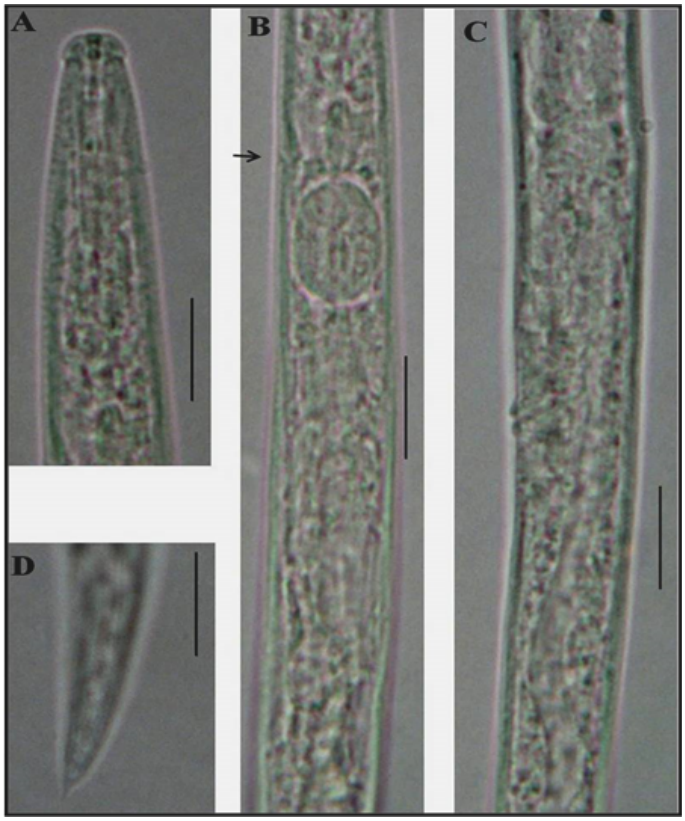

Figure 2: *Aphelenchoides bougainvilli n. sp. Female:* **A)** *Cephalic region;* **B)** *Pharynx showing position of excretory pore as shown by arrow;* **C)** *Vulval region;* **D)** *Tail region (Scale: A-D=100µm).*

Genital system prodelphic and monodelphic, eggs designed in single row. Spermatheca well developed, packed with usually small rounded sperms. Vagina oblique with thick walled. Vulva with slight raised

lips. Rectum is approximately 0.7-0.8 times to anal width. PUS short, usually no sperms, extending about 16.6-20% of vulva to anal distance. Tail small, conical, approximately three times long to anal diameter, with small mucro.

Male: Not found.

Differential diagnosis and relationship: *Aphelenchoides bougainvilli* n. sp. categorized via length of female's body (0.42-0.44mm). Lateral field with three incisures. Cephalic end offset, rounded. Cuticle finely annulated, approx. 1µm apart. Stylet 10 µm (10±0) with minute basal knobs. Excretory pore situated 2 µm anterior to the metacarpus and measured about 44 µm from cephalic region. Tail conoid with small mucro. Based on grouping scheme of *Aphelenchoides* species (Shahina, 1996), newly prescribed species is related to Group II having "one or may be two mucronate structures on tail terminus". As per excretory pore location, the population of new species found nearest to four species belonging to Group II including *Aphelenchoides eradicitus* Eroshenko (1968), *Aphelenchoides parabicaudatus* Shavrov (1967), *Aphelenchoides platycephalus* Eroshenko (1968), *Aphelenchoides submersus* Truskova (1973) and one species of Group I viz., *Aphelenchoides rotundicaudatus* Fang *et al.* (2014).

Aphelenchoides bougainvilli n. sp. differentiated from *Aphelenchoides eradicitus* in larger body length (0.42-0.44 vs 0.30-0.31 mm); greater a and c ratios (a=30-35.5 vs 23.4-23.9; c= 14-17 vs 7.1-8.3); slightly posterior located vulva (70.6-73 vs 64%); excretory pore 2 µm above median bulb against at mid of median bulb and lateral field (3 vs 4). From *Aphelenchoides parabicaudatus,* new species differs by larger body length (0.42-0.44 vs 0.31-0.35 mm); higher a and c ratios (a= 30-35.5 vs 21.4-26; c= 14-17 vs 10.5-12.7); lower c' value (2.5-3.1 vs 3.7); more posterior located vulva (70.6-73 vs 61-64%); stylet length (10 µm (10±0) vs 8 µm) and lateral field (3 vs 4). From *Aphelenchoides platycephalus* in larger body length (0.42-0.44 vs 0.24-0.27 mm); greater a and c ratios (a= 30-35.5 vs 24-27.8; c= 14-17 vs 9.1); posterior located vulva (70.6-73 vs 67.7-69%) and lateral lines (3 vs 4). From *Aphelenchoides submersus,* the new species differs in slightly shorter body length (0.42-0.44 vs 0.49-0.72 mm); lower stylet length (10 vs 13 µm) and in lateral lines (3 vs 4). Further, it also distinguished from *Aphelenchoides rotundicaudatus* for having excretory pore one body diameter anterior to metacarpus. Also new species differs in slightly longer stylet length (10 vs 8-9 µm); from head to excretory pore (43-45 vs 27-33 µm) and in lateral lines (3 vs 4).

The new species also compared with *A. acacia* Samreen *et al.* (2020) and *A. marwataensis* (Salma et al. 2021). New species differ from *A. acacia* in higher c ratio (14-17 vs 9.6-12.3); lower c' ratio (2.5-3.1 vs 4.3-5.7); greater V% (70.6-73 vs 60.3-67.5); smaller stylet (10 vs 11-13 µm); smaller tail (24-26.5 vs 35.2-45 µm) and location of excretory pore (anterior to median bulb vs posterior to median bulb). From *A. marwataensis* new species differ in smaller c' ratio (2.5-3.1 vs 3.4-4.2); greater V% (70.6-73 vs 68.8-70.2); smaller stylet (10 vs 10-12 µm); smaller tail (24-26.5 vs 29.6-34 µm); smaller PUS (15-20 vs 34-38 µm); excretory pore location (anterior to median bulb vs posterior to median bulb) and shape of tail terminus (bifid with ventral mucro vs conoid, arcuate ventral mucro).

Type habitat and locality: Population of new species was retrieved from soil around roots of paper flower (*Bougainvillea spectabilis* L.) from village Sarai Naurang (GPS: 32°49'43.0"N 70°46'33.0"E), district Lakki Marwat, KPK, Pakistan.

Type specimens: Holotype female as well as paratype females forwarded to Nematode Collection Lab at National Nematological Research Centre (NNRC), University of Karachi (UoK), Karachi, Pakistan.

Etymology: *Aphelenchoides bougainvilli* n. sp. allotted its name according to type host paper flower (*Bougainvillea spectabilis* L.).

Conclusions and Recommendations

The genus *Aphelenchoides* with its typically small worm-shaped body, exist in diverse habitat. The newly prescribed species belongs to one of the species rich genus of the order Aphelenchida, isolated from paper flower and thoroughly described in detailed from remote areas such as southern district *i.e.*, Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The close morphological scrutiny of the said new species evidently separated from seven species and categorized in group II of assemblage system of *Aphelenchoides* species. Furthermore, Aphelenchid nematodes has significant biological and agricultural importance in the soil ecosystem hence, it is recommended that

further consideration is to be required to unearth the additional species and to study their biology as well ecology for better agricultural productions and plant health.

Novelty Statement

The current research effort provides existence as well as morphological data about the novel fungivores nematode species that having a great biologically and agricultural importance. This discovery in scientific world will be useful to facilitate future identification of additional species in the order Aphelenchida from unexplored areas of district Lakki Marwat, KPK, Pakistan.

Authors' Contribution

Samreen Khan: designed the study as well as carried out surveys, processed samples followed by measurements, identified species, line drawing, photography and finely drafted the manuscript.
Salma Javed: Supervised and thoroughly reviewed the manuscript.
Nasira Kazi: Helped in identification and revising the manuscript.

Conflict of interest
The authors stated that non-conflict of any interest.

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