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DESCRIPTION OF *CEPHALENCHUS REGIA* N. SP. ASSOCIATED WITH WALNUT (*JUGLANS REGIA*) FROM LAWAT KUNDIYAN, DISTRICT NEELUM, AZAD KASHMIR, PAKISTAN

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ABSTRACT

Lawat Valley is one of the greenest valleys in Neelum District, situated near the bank of the Neelum River in Azad Kashmir, Pakistan. Surveys were conducted from seven different localities in the Lawat area of Neelum district during 2018-2020. A total of 250 samples of soil and roots were collected from areas where various fruits and vegetables were grown at a depth of 10-20 cm. The main objective of the survey was to study pathogenic nematodes and observe the incidence of diseases caused by parasitic nematodes. From Lawat Kundian, six nematode species were identified in apricot trees, and seven were identified in walnut trees, including a newly identified species, *Cephalenchus regia*. *C. regia* n.sp. was found in the soil around the dry fruit of walnut (*Juglans regia*) in Lawat Kundian. It differs from other species in having a longer stylet, pharynx, and tail (22-24; 115-130; 150-200 µm, respectively), as well as an anteriorly situated vulva (57.4 - 62.5%).

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INTRODUCTION

Plant-parasitic nematodes are present in all soils, irrigation water, and even in blowing dust in agricultural regions worldwide. They are one of the most challenging pest problems encountered in our agricultural economy, causing significant crop losses. In early records, Maqbool et al. (1984) recovered and described *Cephalobus sacchari* from sugarcane in Sindh, whereas Maqbool and Ghazala (1986) also described *C. longicaudatus* from the roots of weeds in hilly areas of Lake Saiful Muluk, Naran.

Lawat Valley is one of the greenest valleys in Neelum district, located near the bank of the Neelum River. It is a stunning tourist destination and a captivating place for tourism. The valley features Patlian Lake, Kanali Waterfall, and Nairean/Neriaan Waterfall. Notably, Patlian Lake is the highest glacier lake in Neelum Valley. Research on nematodes in Lawat village, district Neelum, has not been reported. Therefore, the objective of this study was to conduct surveys to isolate, identify, and determine the infectivity of plant-parasitic nematodes on apricot and walnut trees.

MATERIAL AND METHODS

Sample collection and extraction of nematodes

During the current studies for research work, 250 soil samples were collected from the soil around the roots of agrarian site of Lawat Kundian, district Neelum Pakistan. The soil samples were randomly collected which had covered the entire field and brought to lab carefully and were kept at 4°C till further process. Nematode specimens were isolated from the soil sample by Baermann funnel method (1917) and decanting by Cobb's sieving method (1918).

Killing and fixing and identification of nematodes

Nematodes were killed with boiled water (80ºC), and fixed in TAF according to Courtney et al. (1955). Fixed nematodes were washed and transferred in glycerin by following the Seinhorst (1959) method. Permanent slides were made by using glass fiber to avoid flattening of specimens. Identification of nematodes was done through measurements given by de Man (1884) formula with an ocular micrometer under a compound microscope (Nikon E400 light microscope) and identification was based on the systematics given by Siddiqi (2000). Illustrations were drawn by drawing tube attached to the Nickon Eclipse E400 microscope. Photomicrographs were also captured by compound microscope using Nomarski's interference contrast system (Nikon DS-Fi1).

RESULTS AND DISCUSSION

Cephalenchus regia n. sp. (Figure 1, 2, 3 and 4)

Measurements

Females (Paratypes)

(n = 10): L = 646 ± 63.9 (530-700) µm; a =44.3 ± 7.01 (36.6-52.9); b = 5.6 ± 1.70 (4.5-7); c = 3.18 ± 0.44 (3.9-5.5); V = 59.82 ± 3.97 (57.14 – 62.51); Stylet = 23 ± 0.81 (22-24) µm; Pharynx = 120.66 ± 8.13 (115-130) µm; Tail= 172 ± 22.17 (150-200) µm; Anal body width = 8 ± 2.91 (6-9) µm; Maximum body width = 15 ± 1.63 (12-19) µm.

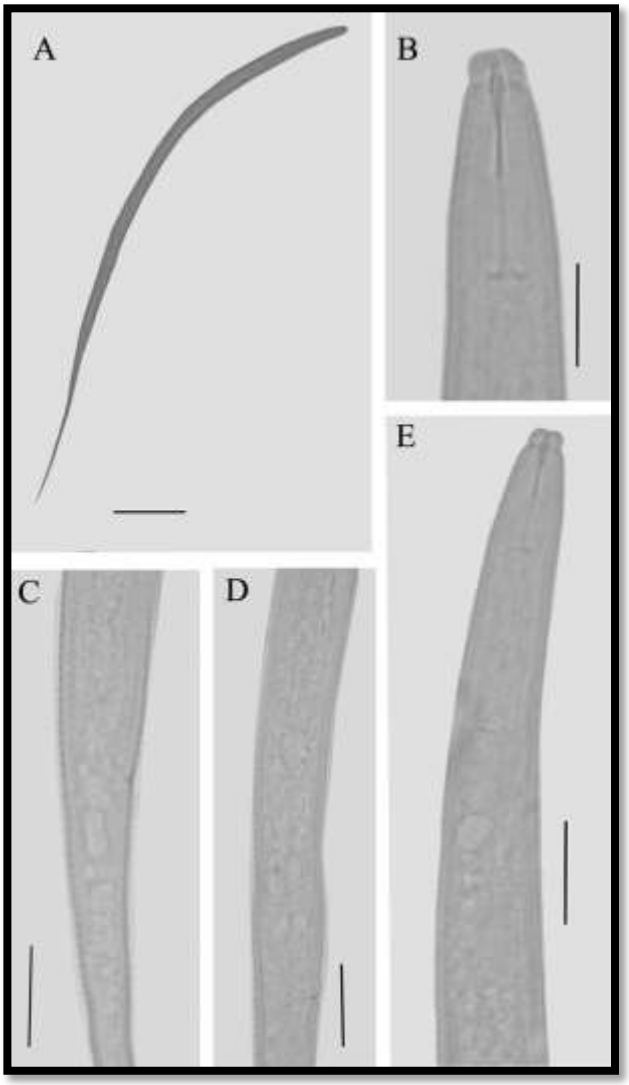

Figure 1. Light microphotograph of *Cephalenchus regia* n.sp., (A-E). Female A. whole body; B. anterior region; C. tail region; D. vulval region; E. pharyngeal region.

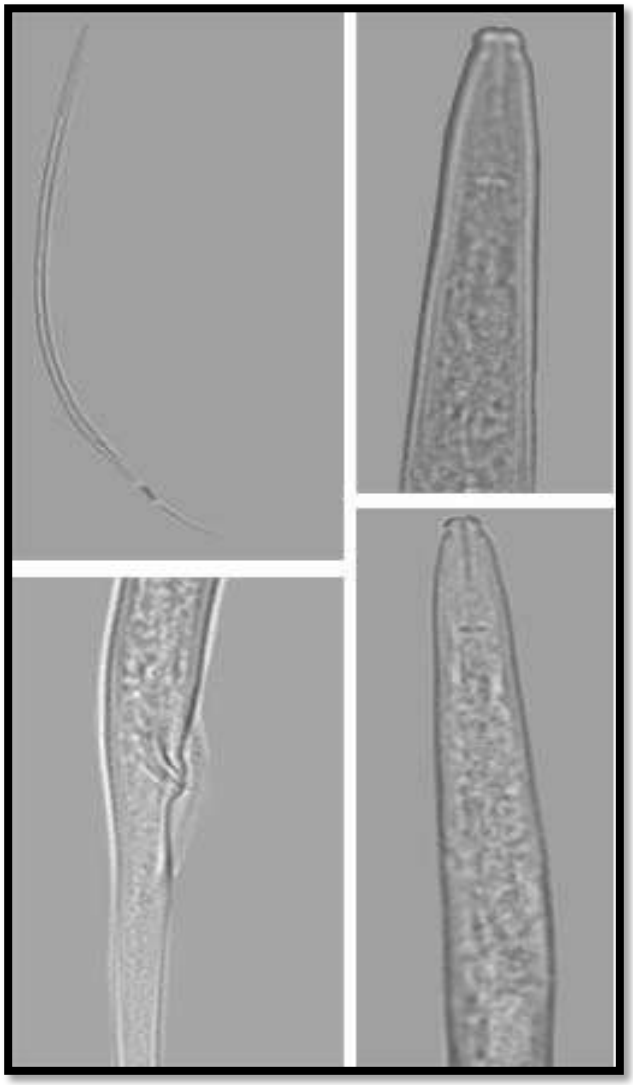

Figure 2. *Cephalenchus regia* n. sp., Light microphotograph of (A-G). Male A. whole body; B. pharyngeal region; D. tail region showing spicule and gubernaculum.

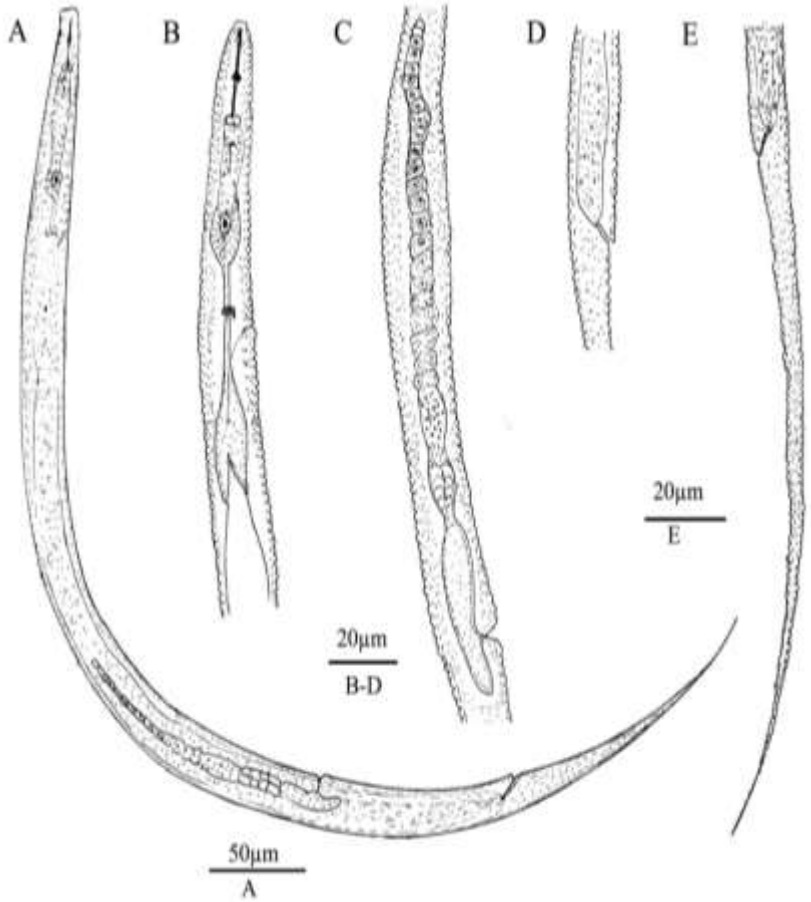

Figure 3. *Cephalenchus regia* n. sp., (A-E). Female B. pharyngeal region; C, vulval region; D. E, tail region.

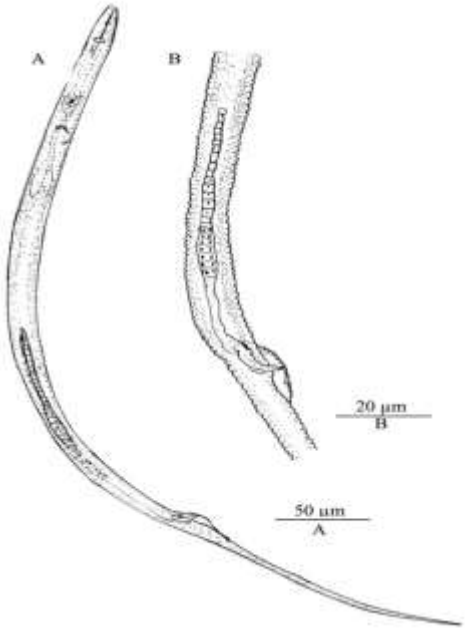

Figure. 4. *Cephalenchus regia* n. sp., (A-B). Male A. whole body; B. tail region showing spicule and gubernaculum.

Holotype

Female

L = 0.530 µm; a = 52.9; b = 5.6; c = 5.5; V = 57.14; Stylet = 23 µm; Pharynx = 130 µm; Tail= 200 µm; Anal body width = 8 µm; Maximum body width = 15 µm.

Male (Paratypes)

(n = 04): L = 530 ±36.5 (490-550) µm; a = 47.28 ± 10.18 (36.6-61); b = 5.90 ± 1.64 (4.58-7); c = 3.9 ± 0.46 (3.5-4.06); Stylet = 21.8 ± 0.89 (22 -24) µm; Pharynx = 108 ± 12.54 (80-120)µm; Tail= 170 ± 21.62 (150-200)µm; Anal body width = 10 ± 1.2 (9-10) µm; Maximum body width = 14 ± 2.19 (10- 15)µm; Spicules= 16± 1.8 (15 -18) µm; gubernaculum = 6 ± 1. 65 (5-8).

Description

Female

Nematode slender curved ventrally upon fixation. Head smooth, low and rounded, width of head 3-7 µm whereas height is between 4-6 µm. Stylet 22-24 µm long with a thick and large basal knob. Cuticle coarsely annulated, annules 1-2. µm wide at mid body. Lateral field with four longitudinal lines, labial region is offset from the body by a depression. Six labial and four cephalic papillae present. DGO about 4-5 µm long. Excretory pore situated at posterior region of isthmus or mid of isthmus and basal bulb 73 -75 µm from anterior end. Nerve ring located 68-70 from anterior end. Pharynx 115-130 µm long and slender procarpus with weak median bulb. Vulva protruding with long monodelphic ovary. Oocytes placed in a single row, spermatheca filled with sperms, post vulval uterine sac about 10-18 µm long. Distance from vulva to anus 80-100 µm. Tail filliform 150-200 µm long or about 6-9 times of the anal body width. Phasmid distinctly prominent 15-20 µm anterior to anus.

Male

Male body similar in general characters to female, body arcuate 530 ±36.5 (490-550) µm long. Head 4.2 ± 1.06 (4-6) µm high whereas 6 ± 1.5 (3-7) µm wide at base. Stylet 21.5 ± 0.89 (22-24) µm long. DGO about 3.4 ± 0.94 (2-4) µm long. For secretion excretory pore placed at the base of isthmus 72.4 ± 4.35 (65-73) µm from anterior end. Nerve ring present 67.6 ± 4.33 (60-70) from anterior end.

Pharynx 108 ± 12.54 (80-120) µm long has a slender procarpus and weak median bulb. Testis outstretched, spicule paired, cephalated, 16 ± 1.8 (15-18) µm long. Gubernaculum 6 ± 1, 65 (5-8) µm long. Tail filiform 170 ± 21.62 (150-200) µm, about 9-10 anal body widths long.

Type host and locality

The specimens of *Cephalenchus regia* n. sp., were isolated from the soil samples of dry fruit walnut *(J. regia)* from Lawat Kundiyan, district Neelum Azad Kashmir, Pakistan.

Type specimens

Holotype and paratype females were deposited in NNRC, nematode collection, at University of Karachi.

Etmology

The species is named after the soil was collected from a dry fruit walnut from Lawat Kundiyan, district Neelum, Azad Kashmir, Pakistan.

Diagnosis and relationship

C. regia n. sp., was collected from the soil around dry fruit walnut Lawat Kundiyan closely resembles to *Cephalobus nemoralis* Mizukubo and Minagawa (1985); *C. longicaudatus* Maqbool and Ghazala (1986) and *C. sacchari* Maqbool et al. (1984). It can be different from *C. nemoralis* by having longer stylet (22-24 vs 16-19) µm; longer pharynx (115-130 vs 85-115) µm; anterior vulva (57.4 – 62.5 vs 63-69)%; longer tail (150-200 vs 104-157) µm. *C. regia* n. sp., can be recognized from *C. longicaudatus* in female by having longer stylet (22-24 vs 16-17) µm; longer tail (150-200 vs 130-161) µm; longer pharynx (115-130 vs 84-92) µm; in male by having smaller body (490-550 vs 520-630) µm and longer stylet (22 -24 vs 16-16.8) . It can also differ from *C. sacchari* on the basis of longer stylet (22-24 vs 17.6-18.4) µm; deirid anteriorly situated (65-70 vs 83-86) µm; spicule large size (15 -18 vs 12-15) µm.

CONCLUSION

In this study, the fauna of plant parasite nematodes in a dry fruit walnut root rhizosphere Lawat Kundiyan, district Neelum Azad Kashmir, Pakistan were investigated and one new species was identified. During this study, 7 nematode species from walnut trees were identified in which *Cephalenchus regia* n. sp., was described and illustrated.

AUTHORS' CONTRIBUTION

JB and TAK designed, formulated and laid out the study, JB collected the samples, isolated and identified the nematodes, TAK helped in the identification of nematodes, provided technical assistance, and supervised the work, JB wrote the manuscript, TAK proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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