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Research Article – Zoology

Some studies on the species of Criconematids (Nematoda: Tylenchida) from Hilly Districts of Nepal

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Abstract

A nematological survey was conducted in three hilly districts – Bhaktapur, Kavre and Dhading surrounding capital city of Nepal ranging the altitudes between 1340m to 1540m. Randomly collected soil samples from vegetable fields processed through Cobb's sieving and decantation method yielded various tylenchid nematodes. Among six species of criconematid nematodes found associated with vegetable crops, four species *viz. Hemicriconemoides mangiferae* Siddiqi, 1961, *Criconemoides brevistylus* Singh and Khera, 1976, *Discocriconemella retroversa* Sauer and Winoto, 1975 and *Hemicycliophora arenaria* Raski, 1958 are described here with their morphometric data along with localities and host plants and illustrated with photomicrographs.

Keywords: Plant nematodes, criconematids, taxonomy, Nepal

Introduction

Criconematid is a group of plant parasitic nematodes belonging to Superfamily Criconematoidea (Taylor, 1936). Criconematid nematodes are distinct from other tylenchid nematodes in having retrose annules, scales, spines or an extra cuticular body sheath, a poorly developed labial region, lips forming a labial disc, often with four submedial lobes, usually elongated spear wherein the length of the conus is not proportional to length of shaft, pro-metacorpus generally amalgamated, with non-overlapping intestine, females with a single anteriorly directed genital branch with no post-uterine extension. Criconematids are obligatory ectoparasites often feeding on woody perennials and other crops which not only suppress root growth forming lesions including necrosis of leaves but also show stunded growth of plants.

In Nepal, agricultural productivity has been seriously affected by plant parasitic nematodes (Amatya and Shrestha, 1968). Analysis of soil samples collected from vegetable crops of three hilly districts- Bhaktapur, Kavre and Dhadhing surrounding the Kathmandu Valley, Nepal yielded several plant parasitic nematodes. Among them, some species of plant parasitic nematodes affecting vegetable crops have been reported (Keshari and Gupta, 2016). In this paper, four species of criconematids are reported here as the known species. However, these species are the first report from these regions of Nepal. Brief descriptions, microphotographs and information on host, locality of these species are provided.

Materials and methods

Soil samples collected during 2012-2014 from three major vegetable producing districts of Nepal–Bhaktapur, Kavre and Dhadhing at altitudes of 1319 m, 1439 m and 1518 m, respectively were screened. Nematodes were isolated by modified by Cobb (1918) sieving and

decantation and modified Baermann's funnel techniques. They were killed and fixed in hot FA (4:1) for 24 hours (Seinhorst, 1962). After fixation, the nematodes were transferred to glycerine–alcohol (5 parts glycerine and 30% alcohol) and kept in a desiccator for slow dehydration. Permanent mounts were made in anhydrous glycerine by using wax ring technique. Measurements, morphological observations and microphotographs were made using an Olympus microscope . Identifications were made using the keys developed by Siddiqi (2000) and Gereart (2010). In the given measurements, figures in parentheses indicate the mean \pm the standard deviation.

Results and discussion

Descriptions and illustrations of *Hemicriconemoides* mangiferae, *Criconemoides* brevistylus, *Discocriconemella* retroversa and *Hemicycliophora* arenaria are provided here.

Hemicriconemoides mangiferae (Fig. 1(A-C) (Siddiqi, 1961)

Description

Females: Body ventrally arcuate upon fixation. Outer cuticular sheath tightly enclosing body. Body annules flat, 3.0–4.5µm apart at midbody. Lip region 6µm high, slightly setoff, with two annules. Oral disc narrow, rectangular, dorso-ventrally oriented with a slit–like oral aperture. Amphidial apertures located behind oral disc and covered by plugs. Labial plate not protruding from first lip annule. Cephalic framework moderately sclerotized. Stylet well developed 71.3µm long. Basal knobs massive, anchor shaped. Dorsal oesophageal gland opening 3.0–4.5µm from stylet base. Nerve ring 73–100µm from anterior end. Excretory pore 85–105µm from anterior end. Hemizonid inconspicuous. Reproductive system monoprodelphic. Oocytes arranged in single row. Vulva an open transverse slit. Vagina anteriorly directed, 15–

18µm long. Uterus muscular. Spermatheca spherical with sperms. Anus pore–like, less than one vulval body width from vulva. Tail conoid, tip smooth.

Male: Not found

Hosts and localities: Soil around the roots of (i) Cucumber (*Cucumis sativus*) Bhaktapur, (ii) tomato (*Lycopersicum esculentum*) from Kavre.

Remarks: The measurements and descriptions of *Hemicriconemoides mangiferae* confirm well with its original description with some variation in body length, number of body annules, length of stylet and length and shape of tail tip. *H. mangiferae* shows similarities in structure of tail shape and vulval shape, anuules between vulva and anus as given by Vovlas *et al.* (1990) and Decraemer and Geraert (1992).

Table 1. Measurements (μm) of female species of *H.* mangiferae.

Characters	H.mangiferae (Siddiqi 1961)
Ν	7
Length	$477-614(53 \pm 4.5)$
А	$17.6-21.6(19.3 \pm 1.3)$
В	$4.4-5.3(4.9\pm0.3)$
С	$15.2-24.1 (19.2 \pm 2.6)$
V	$91.1 - 93.0 \ (92.1 \pm 0.7)$
Stylet	$67.5 - 75.0 (71.3 \pm 2.1)$
Ex. Pore	$150 - 162.4 \ (158.3 \pm 4.6)$
Tail	$24-30(28.5\pm3.0)$
R	148–165
Rst	23–24
Roes	31–37
Rex	30–34
Rv	13–16
Ran	9–12
VL/VB	$1.37 - 1.91 (1.7 \pm 0.17)$
St%L	$12.1 - 15.1(13.2 \pm 0.98)$



Fig. 1. *Hemicriconemoides mangiferae* (A-C); *Criconemoides brevistylus* (D-F); *Discocriconemella retroversa* (G-I); *Hemicycliophora arenaria* (J-L). Magnification=400X.

Description

Females: Body ventrally slightly curved like C shape. Lip region rounded, continuous with body contour, with three rounded annuli, first smaller than second and second smaller than third, all three smaller than first body annulus. Cephalic framework distinct, stretching over first three annuli. Body annules 3.5µm apart at midbody. Lateral field demarcated with six anastomoses. Hemizonid frequently not seen. Stylet well developed with cupped basal knobs. Stylet 56.5–62µm long. Dorsal oesophageal gland opening at 5.0µm from stylet base. Excretory pore at 98–145µm from anterior. Spermatheca indistinct, small, round, without sperm cells. Vagina straight. Vulva open. Dorsal vulval lip straight, not overhanging, at most with two small lobes. Tail tapering to a rounded tip which is slightly projecting dorsally.

Male: Not found.

Host and locality: Soil around the roots of cauliflower (*Brassica oleracea*), Bhaktapur.

Remarks: This specimens comes close to a number of species in the genus with a large number of body annuli with anastomoses in the lateral field and a stylet length of 56.5–62.0µm. The present specimen is closest to *Criconemoides brevistylus* described by Singh and Khera (1976) from West Bengal, India except for lip annuli with slightly larger diameters.

Table 2. Measurements (μm) of female species of *C*. *brevistylus*.

Characters	C. brevistylus Singh and Khera, 1976
Ν	7
Length	$450-614(54 \pm 4.5)$
А	$12.2-15.5 (14.3 \pm 1.6)$
В	$3.8-4.8(4.4 \pm 1.4);$
С	$16-25.5\ (20.2\pm2.2)$
V	89–93 (91 ± 1.4)
Stylet	56.5-62 (58 ± 2.2)
Ex.pore	$98-145~(128\pm2.2)$
Tail	$19-28 (22.5 \pm 2.4)$
R	134–158
Rst	18–23
Roes	30–42
Rex	32–40
Rv	10-13
Ran	7–10
VL/VB	$1.0-1.4 (1.1 \pm 0.6)$
St%L	$10.6-13.5 (11.8 \pm 1.0)$

Discocriconemella retroversa (Fig. 1(G-I) (Sauer and Winoto 1975)

Description

Females: Body posture C shaped after fixation, cylindrical with constant diameter for the most part of the body. Body annuli 3-4 μ m at midbody, few lateral anastomoses. Cephalic disc 7-10 μ m wide with margin not retrorse, separated from the first body annulus by a collar distinctly visible in lateral view. Cephalic framework weakly developed. Stylet strongly developed, conus 81-85 % of stylet length. Basal knobs massive, anteriorly directed anchor-shaped. Oral oesophageal gland opening 7 μ m behind stylet base. Nerve ring 70-74 μ m from anterior end.

Excretory pore at level of or posterior to oesophagus base. Reproductive system monoprodelphic. Gonad extending anteriorly 132-212 μ m, about 45% body length. Ovary outstretched. Spermatheca round, situated 16-17 annuli anterior to the vulva. Uterus muscular. Sphermatheca spherical. Vulva opening on the posterior margin of a ventrally enlarged annulus. Anal opening, distinct, pit-like, on the posterior margin of an annulus. Tail bluntly conoid, terminal annulus.

Male: Not found.

Host and locality: Soil around the roots of brinjal (*Solanum melagena*) from Dhading, Nepal.

Remarks: Discocriconemella retroversa is characterized by a body length of 285–350µm, with 107-117 smooth annuli, rare lateral anastomoses, a long stylet length of 88– 91µm, and a round cephalic disc, with paired dorsal, and submedian projections. *D. retroversa* is similar to *D. pannosa* (Sauer and Winoto, 1975) in having body and stylet length. However, *D. retroversa* is distiguished from *D. hengsungica* (Choi and Geraert, 1975) by its much shorter stylet (88–91 vs 68-80µm).

Table 3. Measurements (μm) of female species of *D. retroversa*

Characters	D. retroversa (Sauer and Winoto 1975)
Ν	6
Length	$285 - 350 (302 \pm 20)$
Α	$6.5 - 9 \ (8.2 \pm 0.9)$
В	$2.8 - 3.8 (3.3 \pm 1.2)$
С	$18-25~(20\pm2)$
V	88–93 (91 ± 0.8)
Stylet	stylet=88–91 (89 \pm 2.4)
Ex.pore	$66-70~(68.2\pm0.8)$
Tail	15–21 (17 ± 1.9)
R	94–105 (101 \pm 1.8)
Rst	$17-20~(18\pm1.1)$
Roes	$23-29(27 \pm 1.5)$
Rex	29-31 (29 ± 2.3
Rv	$11-13(11\pm 3.8)$
Ran	7–8 $(7 \pm 0.5);$
VL/VB	$0.8 - 1.1 (1 \pm 0.1)$
St%L	$13-16(15\pm1.1)$

Hemicycliophora arenaria (Fig. 1J-L) (Raski, 1958)

Description

Females: Body arcuate, tapering towards blunt extremities, with a pronounced ventral constriction justbehind vulva. Sheath annules slightly flatter than body annules, marked with faint longitudinal striae, about 70 in number at midbody. Lateral fields marked by two irregular incisures often enclosing a third incisure forming a zig-zag line, areolated. Female body with 180-206 annules. Cephalic region continuous, with two annules on both sheath and body cuticles ; labial disc not elevated; frame-work moderately sclerotized. Stylet 67-82µm long. Orifice of dorsal oesophageal gland 10µm behind stylet base. Oesophagus with a short amalgamated isthmus crossed by nerve ring. Excretory pore one to five annules behind oesophageal base. Vulva lips modified, projecting one or two annules, not tubular; sheath cuticle forming a short sleeve in this region. Vagina thickwalled, dilated, directed inward and forward. Gonad prodelphic. Spermatheca indistinct. Post-vulval part of body three times vulval body-width long. Tai1 sub-cylindrical with a broadly rounded tip with 19-23 tail annules.

Male: Not found.

Host and locality: Soil around roots of cucumber (*Cucumis sativus*) from Kavre, Nepal.

Remarks: The measurements and descriptions of *Hemicycliophora arenaria* conform well with its original description except in having shorter stylet 67–82µm. It is very closely related to *H. obtusa* (Thorne, 1955) and *H. signata* (Williams, 1978) except in having pointed female tails.

Table 4. Measurements (µm) of female species of *H. arenaria*

Characters	H. arenaria (Raski, 1958)
Ν	6
Length	$0.77 1.12 \ (0.9 \pm 0.4) \ \text{mm}$
Α	$6.5 - 10 \ (8.6 \pm 0.9)$
В	$3.5 - 5.1 (4.2 \pm 1)$
С	$18-25(20\pm 2)$
V	83–90 (88 ± 0.8)
Stylet	67-82 (78 ± 1.2)
Ex.pore	120-132 (126 ± 6.2)
Tail	$15-21(17 \pm 1.9)$
R	$107 - 111 (109 \pm 3.8)$
Rst	$17-20(18 \pm 1.1)$
Roes	23–29 (27 ± 1.5)
Rex	$25 - 30 (29 \pm 1.3)$
Rv	$9-12~(10\pm1.8)$
Ran	$7-8 (7 \pm 0.5)$
VL/VB	$0.8-1.1 (1 \pm 0.1)$
St%L	$11-16(14 \pm 1.1)$

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