

Descriptions of *Kashmira dimorphicauda* gen. n., sp. n. and *Aphelenchoides hypotris* sp. n. (Nematoda: Aphelenchida) from Kashmir Valley, India

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Abstract. *Kashmira dimorphicauda* gen. n., sp. n. and *Aphelenchoides hypotris* sp. n. are described and illustrated from freshwater stream soil in Kashmir Valley, India. *Kashmira* gen. n. is characterized by having dimorphic tails: coinoid-spicate tail in female and subcylindroid with rounded, non-spicate tip with a minute mucro in male. It belongs to the family Seinuridae. *Kashmira dimorphicauda* gen. n., sp. n. has females with 0.317-0.415 (0.365) mm long body, sclerotized labial framework, tubular stylet 13-15 μ m long lacking basal thickenings, vulva located at 77-79 percent, degenerate rectum and anus and dorsally convex-conoid tail with spicate terminus. Male has typical spicules with elongate rod-like apex directed anteriorly, well developed pointed rostrum direct ventrally and ventral limb strongly curved, about half as long as the dorsal limb. *Aphelenchoides hypotris* sp. n. is characterized by its shorter body length under 0.3 mm than most other *Aphelenchoides* spp. (hence the name), stylet 6.5-8.5 μ m long, with minute rounded basal knobs, excretory pore close to oesophageal bulb, potvulval uterine sac 18-35 μ m or 2.0-3.5 vulval body widths long, tail subcylindroid, ventrally curved 17-26 (20) μ m long with a pointed terminal mucro and 8-10 μ m long spicules with indistinct apex and rostrum.

Keywords. Aphelenchida, *Aphelenchoides hypotris* sp. n., description, Kashmir, *Kashmira dimorphicauda* gen. n., sp. n., Seinuridae, taxonomy.

INTRODUCTION

A large number of soil samples was collected and analysed during a survey of nematodes associated with freshwater streams in Kashmir valley, Jammu and Kashmir, India. Study of nematode fauna belonging to different groups was undertaken as part of an ongoing project entitled "Studies on ecology and diversity of plant and soil nematodes of Pir Panjal Range in Jammu and Kashmir" funded by the Ministry of Environment and Forests, Government of India.

During the surveys, the senior author encountered several interesting species belonging to the order Aphelenchida Siddiqi, 1980. Among them was a new species belonging to the family Seinuridae Husain & Khan, 1967 (Baranovskaya, 1981), but it did not fit into any of its existing genera. A new genus, *Kashmira*, is therefore proposed to receive it. It was found associated with the rhizosphere of willow (*Salix tetrasperma* Roxb.) tree near

streams in Sopore and Baramulla districts of Jammu and Kashmir. It is described here as *Kashmira dimorphicauda* gen. n., sp. n. Another new species belonging to the genus *Aphelenchoides* Fischer, 1894 was recovered from soil around roots and trunk bark of *Salix babylonica* L. near a stream in Lolaab village, Kupwara district. It is named and described here as *Aphelenchoides hypotris* because of its minute size of under 0.3 mm.

MATERIAL AND METHODS

The samples were processed using modifications of Cobb's (1918) sieving and decantation and Baermann's (1917) funnel techniques. Extracted nematodes were simultaneously killed and fixed in hot F. A. (4:1) and dehydrated in glycerine – alcohol solution (5 parts glycerine + 95 parts 30% alcohol). The dehydrated nematodes were mounted on glass slides in anhydrous glycerine. All diagrams and morphological observations were made on an

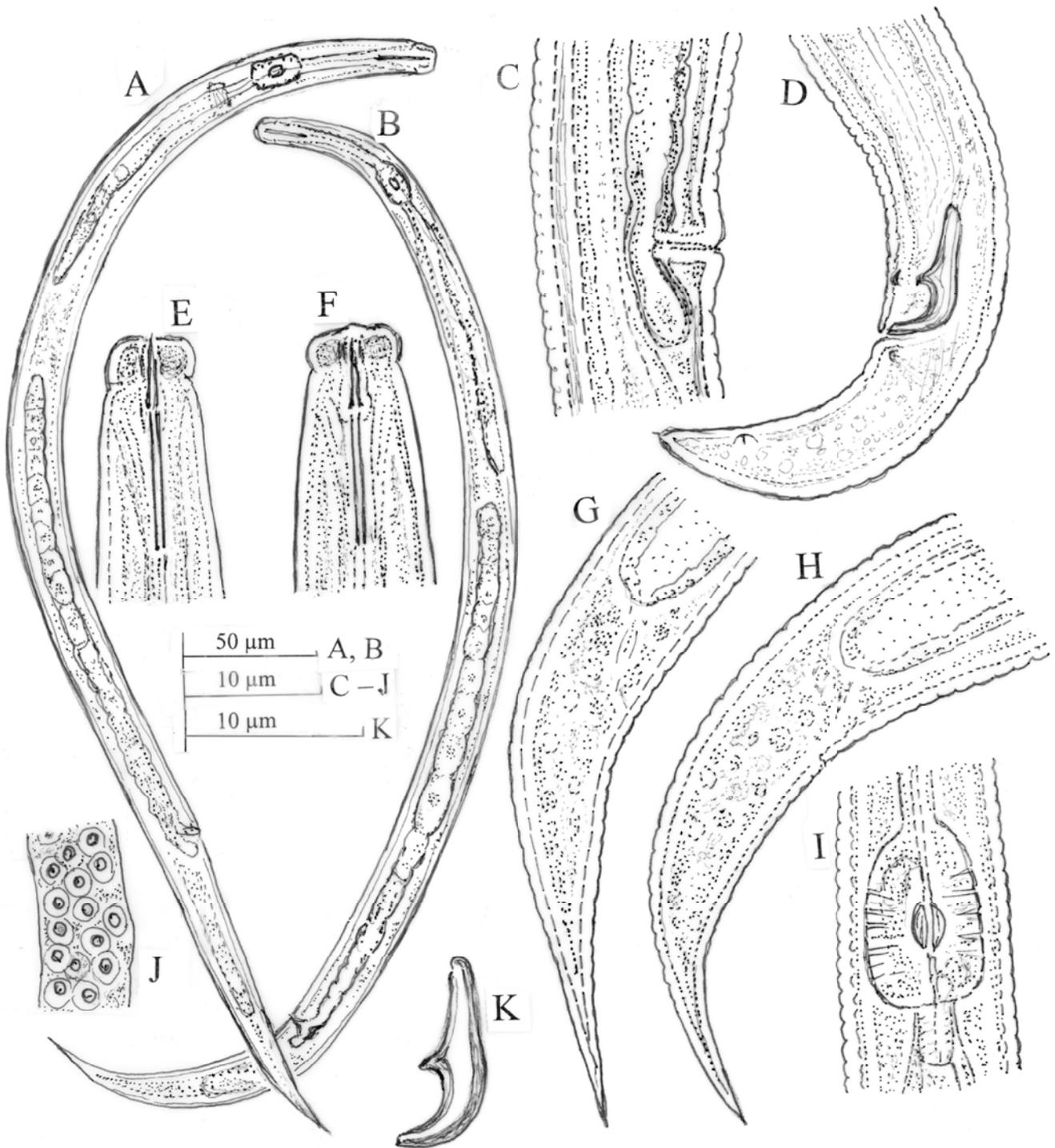


Figure 1. *Kashmira dimorphicauda* gen. n., sp. n. A and B. Entire females. C. Vulval region. D. Male tail, E and F. Stylet region of female and male, respectively. G and H. Posterior ends of female, note degenerated rectum and anus. I. Oesophageal bulb showing valvular apparatus and orifices of dorsal and subventral glands, and isthmus. J. Sperm in gonoduct. K. Spicule.

Olympus BX51 DIC microscope and photographed using an Olympus DP25 digital camera.

DESCRIPTIONS

Genus *Kashmira* gen. n.

Diagnosis: Seinuridae. Body elongate-cylindrical, small sized under one mm. Cuticle thin, finely annulated. Lateral fields present. Cephalic region smooth, high, slightly wider than adjacent body; lips amalgamated, sensilla indistinct. Stylet hollow needle-like, with distinct conus and no basal thickenings or knobs, under three times head width long. Procorpus narrow, cylindrical, apparently non-muscular. Median oesophageal bulb rather oblong, with oval cuticular valvular apparatus at or just behind centre; musculature mostly in middle third of bulb, with orifice of dorsal oesophageal gland located within bulb anterior to valvular apparatus. Isthmus short, cylindrical, giving attachment to intestine and oesophageal glands, which are elongated extending over intestine dorsally; their nuclei arranged in tandem. Nerve ring circum-intestinal a little behind isthmus. Excretory pore distinct, slightly behind nerve ring. Monodelphic-prodelphic, with a short postvulval uterine sac. Vulva transverse, lips similar, slightly raised over body surface in type-species, Vagina short leading inwards. Ovary single with a single row of oocytes. Intestine ends in a blind sac; rectum and anus obscure. Tails dissimilar between sexes. In type species female tail dorsally convex-conoid, with terminal quarter spicate, tip pointed to finely rounded. In male tail is subcylindroid with rounded terminus, usually with a minute terminal mucro. Males common. Spicules typical, with elongate rod-like apex directed anteriorly, well developed pointed rostrum directed ventrally and ventral limb strongly curved, about half as long as the dorsal limb; distance from top of apex to tip of rostrum more than that between the latter to spicule distal end. Three pairs of ventro-sublateral caudal papillae present, one precloacal and two postcloacal. Terminal bursa absent. Phasmids not seen.

Type species: *Kashmira dimorphicauda* gen. n., sp. n.

No other species.

Relationship. In the shape and structure of cephalic region, stylet lacking basal swellings, median oesophageal bulb with musculature concentrated near middle and a simple female and male gonad, *Kashmira* gen. n. is close to *Seinura* Fuchs, 1931 and belongs to the family Seinuridae Husain & Khan, 1967. It can easily be differentiated from its genera by having dissimilar tails between sexes and typical spicules with elongate apex, well-developed rostrum and dorsal limb about double the length of ventral limb.

Tail of the male of *Kashmira* is subcylindroid with rounded terminus provided with a minute mucro, thus appearing like tails in most *Aphelenchoides* species, whereas the female tail is conoid to a spicate terminus resembling tails of some *Seinura* spp. though most of its species have flagelliform or filiform tails. For example, Andr ssy (1958)

described *Aphelenchoides speciosus* on a single male having a spicate tail. This species was considered to belong in the genus *Seinura* Fuchs, 1931, by Baranovskaya (1981) because of the spicate tail character. However, in their revision of the genus *Seinura*, Hechler & Taylor (1965) regarded it as *species inquirenda*. Ebsary (1991) included it in the list of *species inquirendae* as well.

Aphelenchoides speciosus has recently been redescribed on both males and females by Khusainov (2014) because it has short stylet with distinct basal swellings and the oesophageal bulb is rounded and strongly muscular throughout. The tail in this species is not very different from that of *Kashmira dimorphicauda*. However, unlike *A. speciosus*, the male tail of *K. dimorphicauda* shows definite dimorphism terminally being rounded and having a tiny mucro as found in most *Aphelenchoides* species.

Etymology. The generic name is derived from Kashmir Valley where it was found. It is feminine in gender.

Kashmira dimorphicauda gen. n., sp. n.

(Figures 1 & 2)

Measurements

Holotype female: L = 0.355 mm; a = 24; b = 7.1; b' = 2.96; c = 11.5; c' = 3.4; V = 77.7; stylet = 13 μ m.

Paratype females (n=20): L = 0.317-415 (0.365) mm; a = 21-27 (23.7); b = 6.3-7.6 (7.2); b' = 2.8-3.9 (3.5); c = 9-15 (13); c' = 2.8-3.9 (3.2); V = 77-79 (78.6); stylet = 13-15 (14) μ m.

Paratype males (n=10): L = 0.290-317 (0.308) mm; a = 21-25 (22.6); b = 5.5-6.2 (5.8); b' = 3.2-3.8 (3.5); c = 9-14 (12); c' = 2.9-3.8 (3.4); T = 48-55 (51); stylet = 14-16 (15) μ m.

Female: Body slightly arcuate ventrally when relaxed by gentle heat (Fig. 1, A, B). Annules distinct, 1.0-1.3 μ m wide on body Lateral fields about one-sixth as wide as body, with three incisures, reducing to two towards extremities. Lip region prominent, knob-like, slightly wider than adjacent body; framework sclerotized (Fig. 1, E). Stylet hollow needle-like lacking basal thickenings (Fig. 1, E; 2, a, b), 13-15 (14) μ m or 2-2.3 head diameters long; conus about 6 μ m in length; basal thickenings absent. Median oesophageal bulb oval, about 1.4 -1.6 μ m of corresponding body diameter and half as much wide, with a distinct valvular apparatus in centre or just behind it (Fig. 1, I; 2, c). Distance from anterior end to centre of oesophageal bulb 48-52 μ m. Isthmus short 2-4 μ m long. Nerve ring circum-oesophageal, about one corresponding body width behind bulb. Oesophageal glands lobe-like, extending to 55-76 μ m behind the bulb; total oesophageal length from anterior body end to end of oesophageal glands 120-150 μ m. Excretory pore at 56-67 (60) μ m from anterior end, located well behind oesophageal bulb. Reproductive system mono-prodelphic with ovary anteriorly outstretched. Postvulval uterine sac short, 0.4-0.8 vulval body diameter (Fig. 1, C).

Vulva a transverse slit, lips slightly protruding from the body surface. Uterus with proximal muscular and distal

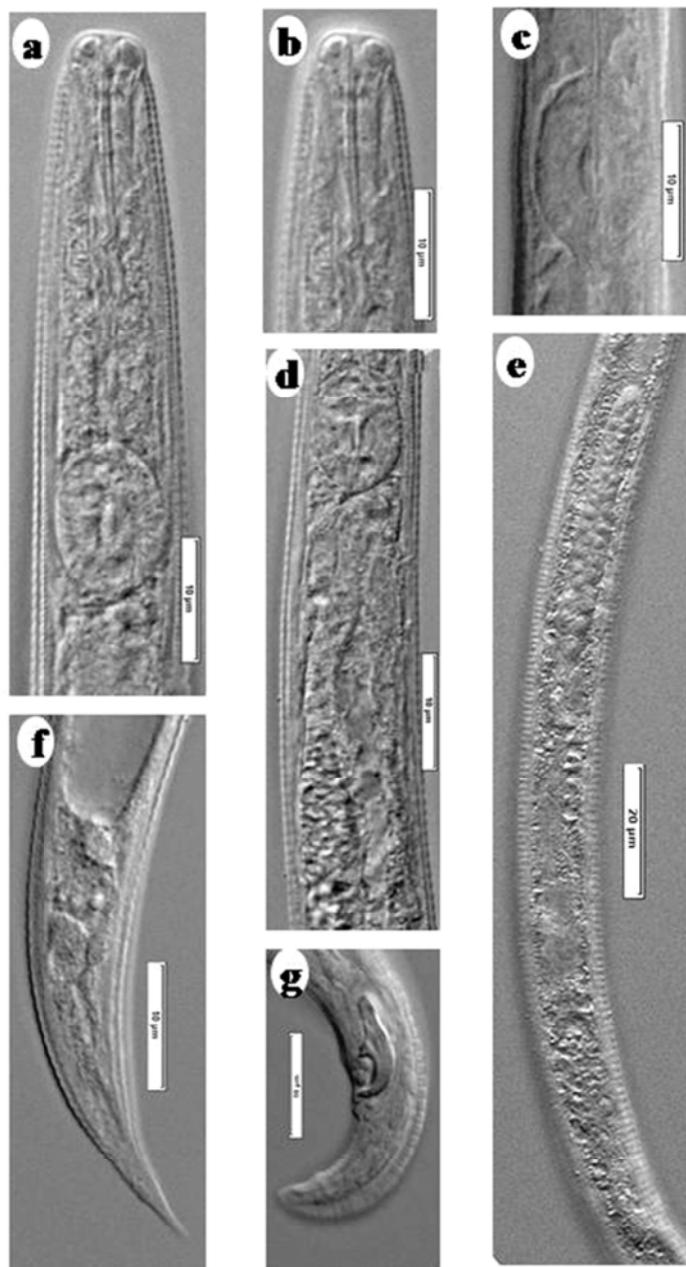


Figure 2. Photomicrographs of *Kashmira dimorphicauda* gen. n., sp. n. (a) Oesophageal region (anterior), (b) anterior end enlarged, (c) Oesophageal bulb, (d) Oesophageal region (posterior), (e) female genital tract, (f) female tail, (g) male tail showing spicule.

glandular parts. Spermatheca oval, often with sperm. Uterine egg $76 \times 16 \mu\text{m}$. Posteriorly, the intestine ends blindly. Rectum and anus degenerate leaving some indication (Fig. 1, G, H), hence tail length and ratios *c* and *c'* are arbitrary. Tail arcuate ventrally, its anterior half subcylindroid posterior tapering to a spicate terminus (Fig. 1, A, B, G, H; 2, f).

Male: Slightly smaller than female. Cephalic framework sclerotized (Fig. 1, F). Stylet and oesophagus as described for female. Testis single, anteriorly outstretched, not reaching gland lobe. *Vas deferens* filled with sperm $2.0\text{-}2.5 \mu\text{m}$ in diameter. Spicules typical for genus, with large cylindroid apex about one third of spicule length and with rounded head; rostrum well developed pointing ventrally; distance from tip of apex to tip of rostrum $7\text{-}8 \mu\text{m}$. Dorsal limb of spicule $14\text{-}15.5 \mu\text{m}$ long, ventral limb $7\text{-}7.5 \mu\text{m}$ in length (Fig. 1, D, K; 2, g). Gubernaculum absent. Tail subcylindroid to a rounded tip bearing a minute pointed mucro (Fig. 1, D; 2, g), ventrally curved, quite different from that of female, $20\text{-}25 \mu\text{m}$ or $2.2\text{-}3.3$ (2.8) anal body diameters long, ventrally curved. There are three pairs of ventro-sublateral genital papillae: a pair anterior to the cloacal aperture opposite the spicule rostrum, another pair a little behind cloacal aperture and a third pair $5\text{-}7 \mu\text{m}$ anterior to the tail tip (Fig. 1, D).

Type habitat and locality: Collected in July 2014 by the first author from soil around roots of willow (*Salix tetrasperma* Roxb.) tree near a stream in Hardushiva village, Baramulla district, Jammu and Kashmir, India. Also collected from willow tree soil in Lolaab, Kupwara district, Jammu and Kashmir, India.

Type specimens: Holotype female and three paratype females and two paratype males are in Nematode Collection of School of Bioscience and Biotechnology, Baba Ghulam Shah Badshah University, Rajouri, Jammu and Kashmir, India. Two paratype females and one paratype male are in Indian National Nematode Collection, IARI, New Delhi; India. Ten paratype females and two paratype males are in The British Nematode Collection; FERA, Sand Hutton, Yorkshire, UK. Fourteen paratype females and four males are with USDA Nematode Collection numbers T-6317p to T-6318p in the USDA Nematode Collection, at Beltsville, MD, USA.

Differential diagnosis and relationship: *Kashmira dimorphicauda* gen. n., sp. n. is characterized by its shorter and thicker body (♀ $L = 0.317\text{-}0.415$ (0.365) mm; $a = 21\text{-}27$), stylet $15\text{-}16 \mu\text{m}$ long; spicules with large apex, prominent ventrally pointing rostrum and dorsal limb almost double the size of ventral limb and male tail tip which is bluntly rounded, not carrying spicate or flagellum-like process. There is a dimorphism in the tail – in female, the tail is elongate pointed with drawn out terminus whereas males have a bluntly rounded terminus with minute terminal mucro.

Kashmira dimorphicauda has spicules similar in shape to those of *Paraseinura musicola* Timm, 1960 and

Rhadinaphelenchus cocophilus (Cobb, 1919) J. B. Goodey, 1960, as illustrated by Brathwaite and Siddiqi (1975). *Paraseinura* Timm, 1960 is characterized by having stylet with double conus, stylet base with minute swellings and filiform tails in both sexes. *Rhadinaphelenchus* has longer and very slender body, stylet with minute basal swellings, elongate-cylindroid female tail and male tail having a terminal bursa. A very interesting point about spicules of *R. cocophilus* was made by Hunt (1993) who stated, "Spicules paired, small; dorsal limb $9\text{-}11 \mu\text{m}$ long with an elongated rounded apex and ending distally before the ventral limb whose distal end appears to recurve to join the dorsal limb so than the entire spicule appears notched distally." We have noted that the distal end of the dorsal limb of *K. dimorphicauda* also recurves and joins with the ventral limb, but the spicule terminus looks rounded. The genus *Rhadinaphelenchus* was synonymized with *Bursaphelenchus* by Baujard (1989), but Hunt (1993) reinstated it as a valid genus.

Kashmira dimorphicauda sp. n. comes close to the species in Group number I of the genus *Seinura* Fuchs, 1831, based on the compendium of morphometric and morphological characters provided by Shahina & Hunt (1995). It comes close to *Seinura tritici* Bajaj & Bhatti, 1982 and *S. nagini* Husain & Khan, 1965. From *S. tritici*, it differs by having female body $317\text{-}415 \mu\text{m}$ long, stylet $13\text{-}16 \mu\text{m}$ long and rounded male tail terminus (versus $L = 570\text{-}700 \mu\text{m}$, stylet $16\text{-}18 \mu\text{m}$ long and male tail is conoid, pointed in *S. tritici*). From *S. nagini* it differs in the shape of head and tail, and posteriorly located vulva (in *S. nagini* head truncate continuous, elongate pointed tail with drawn out terminus in females and conoid in males, and vulva located anteriorly at 60-64%).

Aphelenchoides hypotris sp. n.

(Figures 3 & 4)

Measurements

Holotype female: $L = 0.283$ mm; $a = 32$; $b = 7.2$; $c = 13.8$; $c' = 3$; $V = 68$; stylet = $7.8 \mu\text{m}$.

Paratype females (n=20): $L = 0.256\text{-}0.297$ (0.28) mm; $a = 28\text{-}34$ (30); $b = 5.4\text{-}6.8$ (6); $b' = 3.2\text{-}3.7$ (3.4); $c = 11\text{-}15$ (13); $c' = 2.8\text{-}3.8$ (3.2); $V = 61\text{-}69$ (65); stylet = $6.5\text{-}8.5$ (7.3) μm .

Paratype males (n=10): $L = 0.254\text{-}0.285$ (0.27) mm; $a = 26\text{-}34$ (31); $b = 4.2\text{-}7.0$ (5); $b' = 3.2\text{-}3.4$ (3.3); $c = 10\text{-}15$ (12.7); $c' = 3\text{-}4$ (3.4); $T = 48\text{-}55$ (51); stylet = $6.5\text{-}8.5$ (7.5) μm .

Females: Body small, $256\text{-}297$ long, tapering at both ends, gradually narrowing posterior to the anus with a slight ventral curvature upon fixation; maximum width $8\text{-}11 \mu\text{m}$. Lateral fields about one-fourth as wide as body, with 2 distinct incisures forming a band (Fig. 1, G), reducing in size towards extremities. Cuticle smooth, fine transverse striae present, $0.7 \mu\text{m}$ apart in oesophageal region. Cephalic region rounded, slightly wider than adjacent body. Stylet delicate $6.5\text{-}8.5 \mu\text{m}$ or $1.3\text{-}1.9$ lip region diameters long; conus

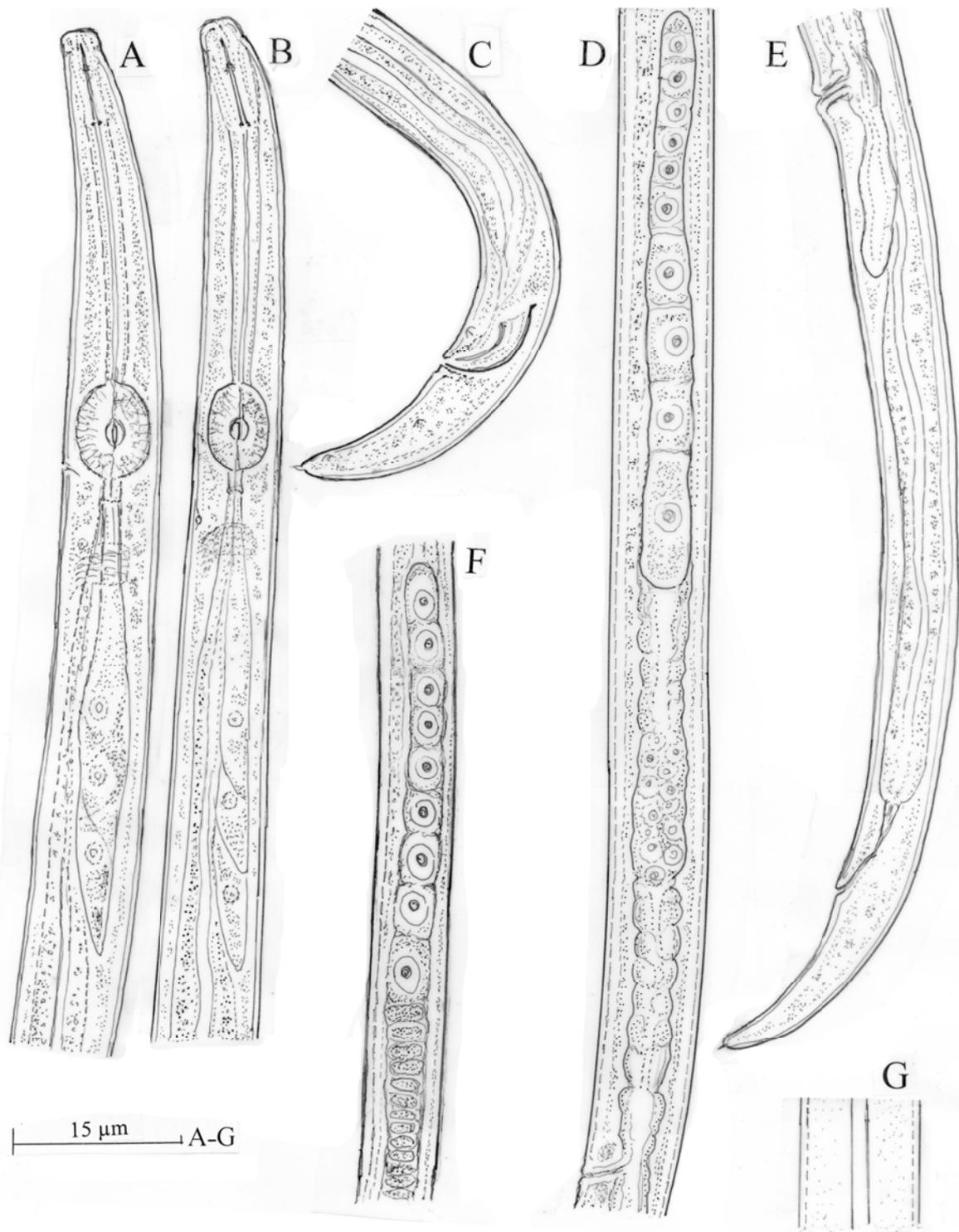


Figure 3. *Aphelenchoides hypotris* sp. n. A and B. Oesophagus of female and male, respectively. C. Male tail end. D. Anterior female gonadal branch. E. Posterior region showing postvulval uterine sac and tail. F. Testis. G. Lateral field at midbody.

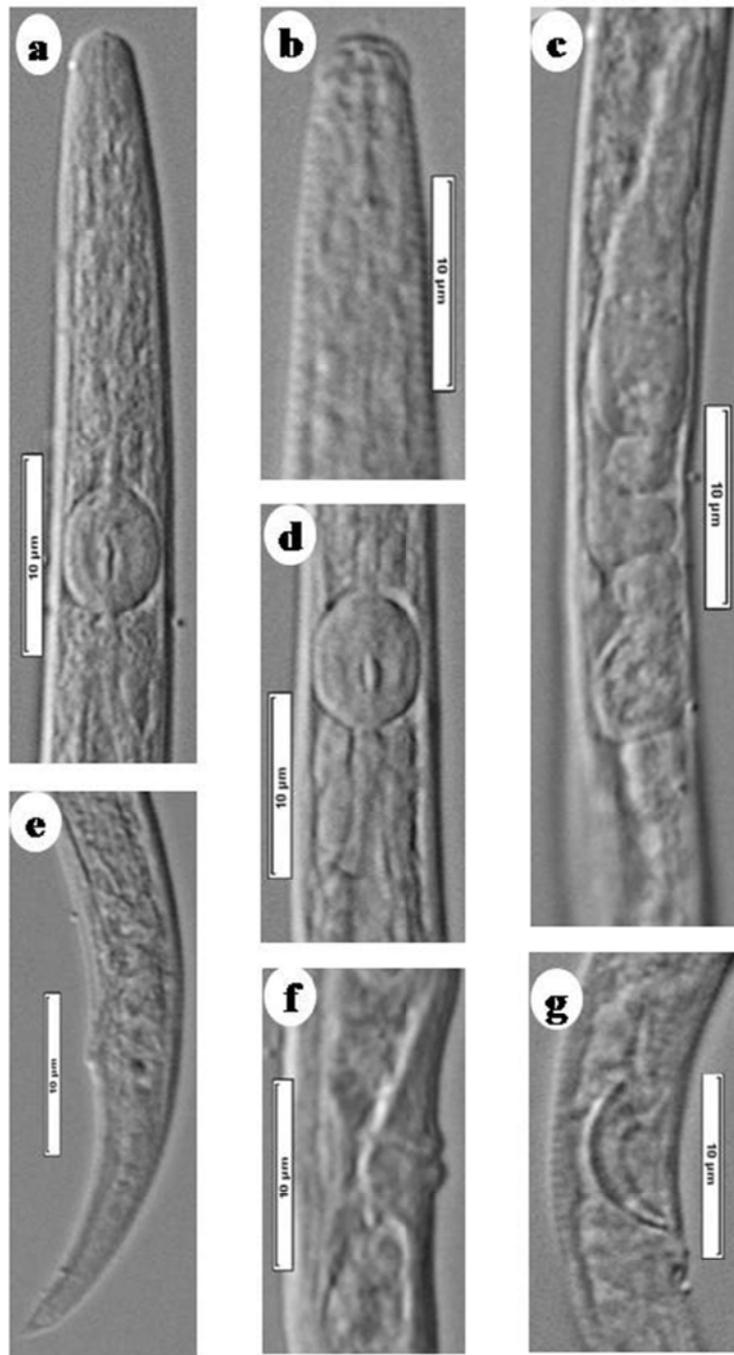


Figure 4. Photomicrographs of *Aphelenchoides hypotris* sp. n. (a) Oesophageal region (anterior), (b) anterior end enlarged, (c) female genital tract, (d) Oesophageal region (posterior), (e) female tail, (f) Vulval region, (g) male tail showing spicule.

2.5-3.0 μm long, about 1/3 of its total length; basal knobs tiny 0.6-0.9 μm across but well separated from each other appearing furcate at base (Fig. 3, A; 4, a, b). Procorpus 39-41 μm long, cylindrical with a slight constriction at median bulb. Oesophageal bulb oval or rarely rounded, 7.5-9.0 (8) μm long, 5-7 μm wide, with a distinct round to oval valvular apparatus in centre or slightly behind it (Fig. 3, A; 4, a, d). Distance from anterior end to base of oesophageal bulb 37-43 μm . Isthmus short 2-4 μm long, joined to intestine and oesophageal glands; latter 38-45 (41) μm long, extending over intestine mostly dorsally. Nerve ring circumoesophageal, 5-10 μm behind base of bulb. Excretory pore rather obscure, located about posterior margins of bulb, 38-42 μm from anterior end.

Reproductive system mono-prodelphic with ovary anteriorly outstretched, with prominent postvulval uterine-sac, anterior reproductive branch lying on the right side and postvulval uterine sac on left side of intestine. Vulva a transverse slit, with slightly protruding lips (Fig. 3, E; 4, f). Vagina straight but slightly directed anteriorly, two-fifths to half as long as vulval body diameter. Uterus with proximal muscular and distal glandular parts. Spermatheca oblong, rarely rounded, with moderately large sperm 1.5-2.0 μm in diameter (Fig. 3, D). Postvulval uterine sac variable in length, 18-35 μm or 2.0-3.5 vulval body diameters long (Fig. 3, E). Rectum 1.5-1.8 anal body diameters long. Anal body diameter 5.0-6.8 (6) μm . Tail elongated, regularly tapering, subcylindroid, slightly arcuate ventrally, 18-27 (23.5) μm long, provided with a pointed about one μm long mucro being axial or ventrally subterminal.

Males: Body straight to slightly arcuate. Lip region hemispherical, slightly wider than adjacent body. Stylet and oesophagus similar to female. Testis single, anteriorly outstretched; spermatocytes large, in a single file (Fig. 3, F); sperm large, packed in vas deferens appearing transversely oval (Fig. 3, F). Spicules arcuate, rose-thorn-shaped, but apex and rostrum poorly developed (Fig. 3, C; 4, g), 8-10 (9.2) μm measured along median line. Bursa absent. Caudal papillae three ventro-sublateral pairs, often difficult to see, a pair seen a little anterior to cloacal aperture, another a little behind cloacal aperture and a third at 2-4 μm anterior to tail tip. Tail subcylindroid, slightly arcuate ventrally, 17-26 (20) μm long, with 1-1.5 μm long pointed mucro at tip (Fig. 3, C; 4, E).

Type habitat and locality: Collected from soil around roots of *Salix babylonica* L. close to a stream near Lolaab village, Kupwara district, Jammu and Kashmir, India.

Type specimens: Holotype female and three paratype females and two paratype males are in Nematode Collection of School of Bioscience and Biotechnology, BGSB University, Rajouri, Jammu and Kashmir, India. Two paratype females and one paratype male are deposited in Indian National Nematode Collection, IARI, New Delhi, India. Five paratype females and two paratype males are in The British Nematode Collection, FERA, Sand Hutton,

Yorkshire, UK. Three paratype females and three males and two juveniles with USDA Nematode Collection number T-6320p are in the USDA Nematode Collection, at Beltsville, MD, USA.

Differential diagnosis and relationship. *Aphelenchoides hypotris* sp. n. is recognized by its small body size ($\text{♀L} = 0.256\text{-}0.297$ (0.28) mm), stylet 6.5-8.5 μm long with basal knobs tiny less than 1 μm across, separated from each other appearing furcate at base, vulva at 61-69 percent of body length, postvulval uterine sac 2.0-3.5 vulval body diameters long, a subcylindroid tail ending in rounded terminus provided with about 1 μm long pointed mucro and 8-10 μm long spicules with poorly developed apex and rostrum.

Aphelenchoides hypotris sp. n. has body size under 0.3 mm, and in this respect, it comes close to *Aphelenchoides editocaputis* Shavrov, 1967, *A. platycephalus* Eroshenko, 1968 and *A. curiolis* Gritzenko, 1971. It differs from *A. editocaputis* in having less offset cephalic region, narrow lateral fields with three incisures and obscure rectum (offset cephalic region, wide lateral fields with four incisures and rectum about two anal body widths long in *A. editocaputis*). *A. platycephalus* has four incisures in lateral field, excretory pore anterior to oesophageal bulb and more tapering tail tip with longer mucro in both females and males. *A. curiolis* has a smaller body 0.2-0.24 mm, excretory pore opposite nerve ring, vulva at 69.8-71 percent and shorter postvulval uterine sac about one vulval body width long.

Aphelenchoides parabicaudatus Shavrov, 1967, *A. orientalis* Eroshenko, 1968 and *A. appendurus* Singh, 1967 have excretory pore at close to or opposite oesophageal bulb and similar spicules. Rashid *et al.* (1986) redescribed *A. parabicaudatus* collected in Bahia and Espirito Santa States of Brazil. It differs from *A. hypotris* in having body over 0.3 mm long, shorter postvulval uterine sac and tail tip slightly bifurcate with a long sharply pointed ventral projection. *A. orientalis* is longer ($\text{♀L} = 0.34\text{-}0.41$ mm, stylet 10.8 μm long, vulva at 69.8-73.4 % and postvulval uterine sac more than half vulva-anus distance in length. *A. appendurus* is considerably larger (0.72-0.88 mm) and has a longer (16.5-17.5 μm) stylet. *A. montanus* Singh, 1967 has a small 0.39-0.4 mm long body, under 8 μm long stylet, but the excretory pore is located behind oesophageal bulb and female tail mucro is flower bud-like with base encircled with two serrated rings and 17-21.7 μm long spicules. *A. helicus* Heyns, 1964 has excretory pore opposite oesophageal bulb but differs in being longer (0.48-0.50 mm) and having round tail tip lacking a mucro.

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purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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