Nematol. medit. (1989), 17: 7-10

Plant Nematodes Laboratory, Faculty of Biology «Kl. Ohridski» University, Sofia 1421, Bulgaria

TWO NEW SPECIES OF *DIPHTHEROPHORA* (DIPHTHEROPHORIDAE, NEMATODA) FROM BULGARIA

by
S. NEDELCHEV and B.M. CHOLEVA

Summary. Two new nematode species of *Diphtherophora*, *D. vasilevi* and *D. malkovi* found in the rhizosphere of raspberry in South Bulgaria are described.

During a survey of the nematode fauna of raspberry plantations in south Bulgaria two underscribed species belonging to the family Diphtherophoridae were found. They possess characteristics of the genus *Diphtherophora*, but also show some differences, and when more species are described it would be possible to re-organize the species of Diphtherophora into new subgenera/genera. The two species are described below.

The nematodes were extracted from soil samples by means of Cobb's wet sieve technique, killed by heat, fixed in T.A.F. and mounted in glycerine. Measurements were made with a disc micrometer.

DIPHTHEROPHORA VASILEVI sp. n. (Fig. 1, A-P)

Measurements: paratype females (n = 25): L = 0.57-0.86 (0.70) mm; a = 20.7-31.8 (26.2); b = 4-6.2 (4.9); c = 27.2-47.7 (36.7); V = 56-65 (59.8); spear = 13-17 (14.5) μm; paratype males (n = 15): L = 0.48-0.78 (0.59) mm; a = 24.4-43.3 (34.3); b = 3.4-4.9 (4.3); c = 22.2-29.8 (25.9); spicule = 16-20 (18.4) μm; spear = 13-15 (14) μm; holotype female: L = 0.72 mm; a = 22; b = 5.2; c = 45; V = 57.8; spear = 14 μm.

Description: female body curved ventrally in «C» shape when fixed, gradually narrowing from vulva to each end. Cuticle thin transparent, two layered, separated from the body, except at head, excretory pore, vulva, anus and tail end; subcuticle about 1 μm thick. Body cavity full of granules. Head continuous with body contour with 6 lip and 10 head papillae. Amphids with wide aperture; sensillar sac connected to the amphid fovea by a short sclerotized tube. Spear typical of the genus. Oesophagus 120-176 μm long, anterior part cylindrical, posterior part elongate-bulboid, ending in a small cardia. Gland nuclei obscured by abundant granules in body cavity in this region. Excretory pore 55-74 μm from anterior end. Nerve ring at mid-oesophagus.Vulva a transverse slit perpendicular to the body axis. Cuticle in front of the vulva partly overhangs it as an epiptygma, but posteriorly it is flush with body surface. Vagina with conspicuous muscles and sclerotized lumen wall widened towards the inner end. Tail conoid-rounded with striae and a pair of lateral pores.

Male similar to female in body shape, also with separated swollen cuticle. Ventromedian neck papilla and excretory pore at 26-37 μm and 61-79 μm from anterior end respectively. A pair of lateral hypodermal pores are situated between the base of the spear and the ventromedian neck papilla. Testis single outstretched anteriorly. The body is widest immediately behind the testis. Spermatozoids oval to extended oval (2-3 μm long). Preanal supplements three: the first 10-15 μm from cloacal aperture; distance between the first and the second 20-44 μm, and between the second and third 25-60 μm. Spicules with a bifurcated well differentiated tip; corpus with bristle-like structures and proximal end widened. Under the effect of the muscles and the opening of the cloaca the spicules show considerable change in their shape. The spicular pouch and muscles from a spicular capsule similar to that of *Trichodorus* (Siddiqi, 1974). The retractor muscles are fixed to the body wall proximally and distally to the ventral and subventral wall of the spicular pouch. Gubernaculum with a well-developed wedge-like part entering between the spicules. Two groove-forming wings are joined laterally to the wedge, where the spicules move. At the joint of each of the wings with the wedge-like part, the tissue is strongly sclerotized forming a longitudinal rib with an extended prox-

imal end. The gubernaculum is connected proximally to the spicular pouch walls. Its distal end is connected with the cuticular sclerotized widening between the gubernaculum and the opening of the cloaca, called the «telamon» (Kirjanova and Krall, 1969; Krall, 1978). The «telamon» is also observed in the juvenile and the female as a widening of the rectal wall from the side of the tail immediately before the anus. Hence it can be concluded that the spicules, the gubernaculum and the «telamon» all originate from the back wall of the rectum. The tail is longer than that of the female.

Differential diagnosis: *Diphtherophora vasilevi* sp. n. can be distinguished from all known Diphtherophora species in having an epiptygma overhanging the vulva and the presence of a pair of lateral cervical pores, a ventromedian neck papilla and three preanal supplements in the male.

Habitat and type locality: raspberry (*Rubus idaeus* L.) rhizosphere, Eastern Rhodopes, South Bulgaria.

Type material: The holotype is deposited in the Plant Nematode Laboratory, Faculty of Biology, Sofia University «Kliment Ohridski»; paratypes are deposited in the Museum of the Central Laboratory of Helminthology, Sofia, Bulgaria.

DIPHTHEROPHORA MALKOVI sp. n. (Fig. 2, A-K)

Measurements: paratype females (n = 25): L = 0.55-0.70 (0.61) mm; a = 12-20 (16); b = 3.8-5.1 (4.3); c = 32-55 (47); V = 56-61 (59); spear = 17-19 (17.4) μm; paratype males (n = 20): L = 0.55-0.82 (0.64) mm; a = 17-27 (22); b = 4-5.1 (4.5); c = 31-46 (37); spear = 17-19 (17.6) μm; spicules = 27-32 (30.5) μm; holotype female: L = 0.70 mm; a = 15.8; b = 4.6; c = 46.5; V = 58; spear = 18 μm.

Description: female body slightly curved from the vulva to posterior end, gradually narrowing towards both ends.

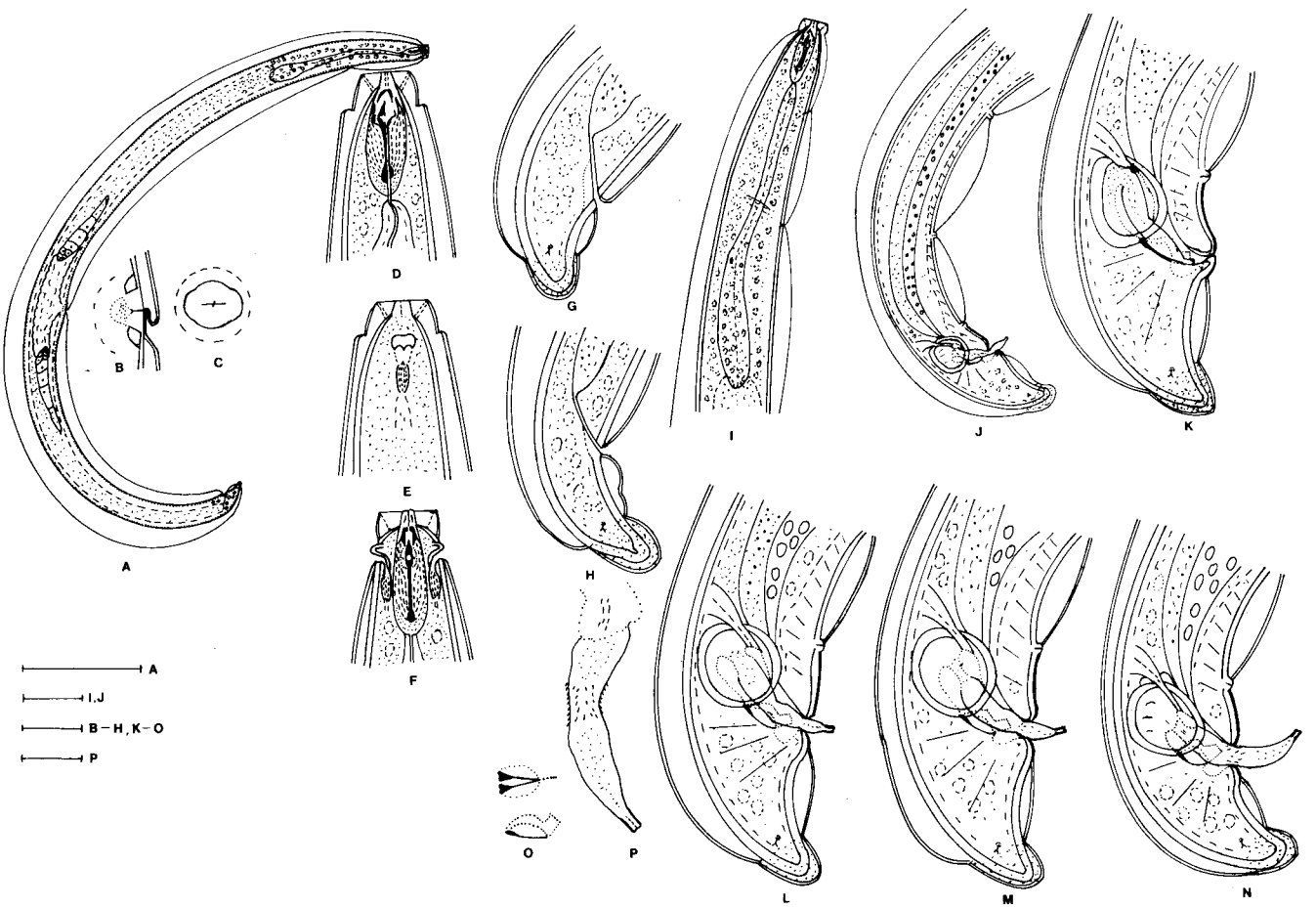

Fig. 1 - *Diphtherophora vasilevi sp. n.*: A) Entire female; B) lateral view of vulva; C) ventral view of vulva; D-E) lateral view of head end of female; F) ventral view of head end of female; G-H) female tails; I) anterior region of male; J) posterior region of male; K,L,M,N) male tails; O) ventral and lateral view of gubernaculum; P) spicule. Scale bars: A – 100 μm; I, J – 20 μm; B-H, K-O – 10 μm; P – 5 μm.

Cuticle thin, transparent, two-layered, separated from the body except at head, amphid, pores and papillar zone; subcuticle about 1 μm thick. Body cavity in oesophageal region full of granules. Head continuous with body contour with 6 lip and 10 head papillae. Sponge-like amphids connected to sensillar sac by means of a short sclerotized tube. Distal end of sensillar sac opposite mid-spear. A pair of lateral pores situated at the spear base and a pair of subdorsal pores located in the terminal half of the tail. Spear typical of the genus. Oesophagus 126-164 μm long. Excretory pore 59-80 μm from anterior end. Nerve ring at mid-oesophagus. Sexual system didelphic with reflexed ovaries; usually one of the sexual tubes is better developed; the vulva is a transverse slit; the vagina has well-developed musculature with a sclerotized ring, its cross-section being triangular. The rectum length: anal diameter ratio is about 1; rectum has thickened back wall, tail sides asymmetrical.

Male body almost straight or with a slightly curved posterior half the greatest diameter being in the maturation zone of the spermatozoids. Two ventromedian papillae at 23-32 μm and 50-68 μm respectively from anterior end. The excretory pore is situated 62-75 μm from anterior end 7-12 μm from the second ventromedian papilla. A pair of lateral pores located close to spear base of 21-24 μm from anterior end. There are two ventromedian supplements: the first 5-8 μm from cloacal aperture and the second 5-10 μm from the first. A pair of subdorsal pores located in the terminal half of the tail. The oesophagus is 126-177 μm long, cylindrical up to the nerve ring, then widening gradually and ending in a small cardia. Intestine usually extending slightly forward, overlapping the oesophagus both laterally and dorsoventrally. Testis single, reaching midbody. Spicules strongly developed, the corpus having bristle-like structures and a bifurcated tip. Spicular capsule and possible mechanism of action identical to the ones described for *D. vasilevi*. Gubernaculum with ribs thickening toward the distal end. «Telamon» present. Membranous cuticle appearing somewhat like a bursa. Tail asymmetrical.

Differential diagnosis: *Diphtherophora malkovi* sp. n. is similar to *D. obesus* but differs in the female having a pair

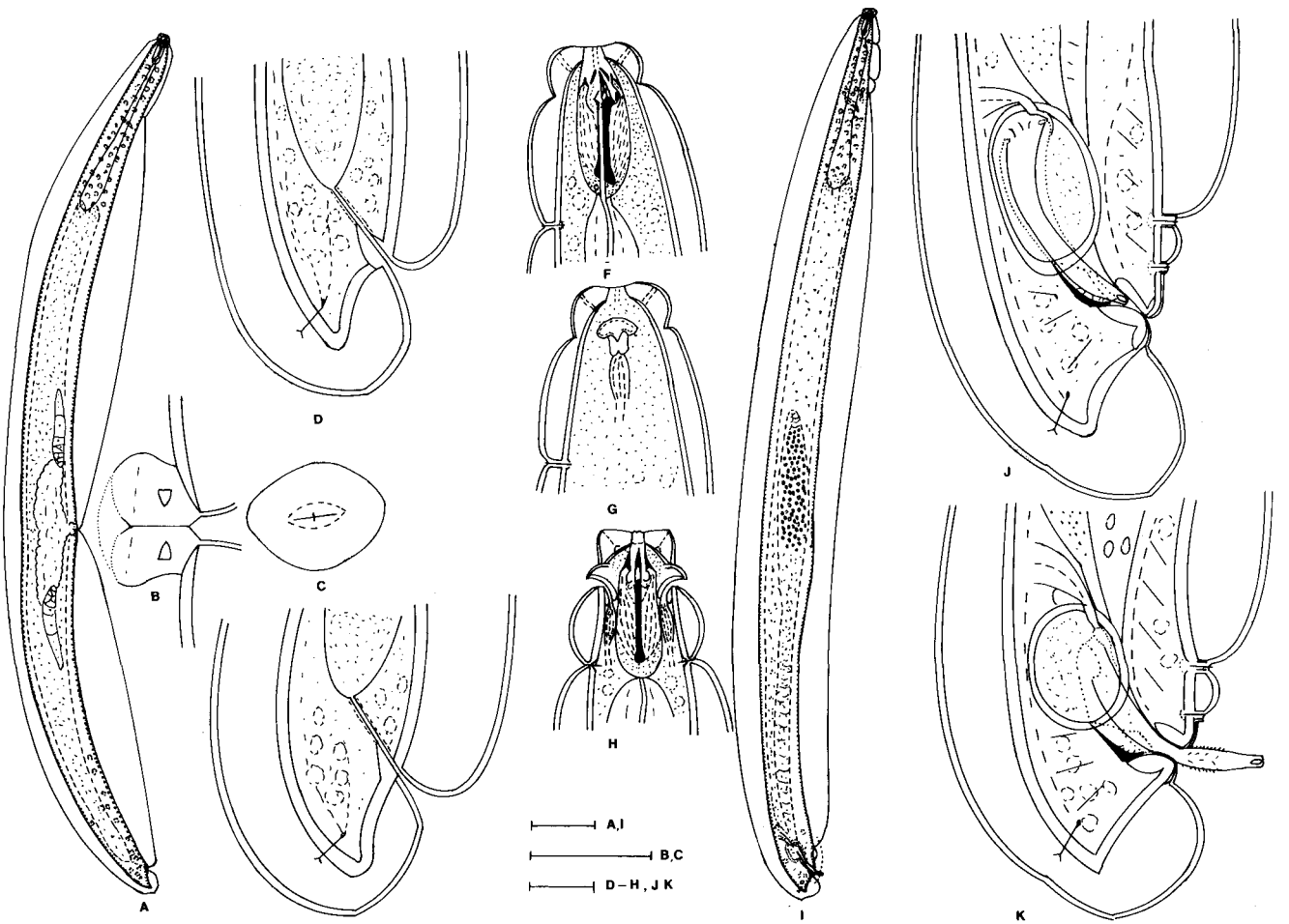

Fig. 2 - *Diphtherophora malkovi sp. n.*: a) Entire female; B) lateral view of vulva; C) ventral view of vulva; D-E) female tails; F-G) lateral view of head end of male; H) ventral view of head end of male; I) entire male; J-K) male tails. Scale bars: A,I – 50 μm; B,C – 10 μm; D-H,J,K – 10 μm.

of lateral pores near spear base and a significantly shorter tail, and two ventromedian neck papillae and two supplements in males.

Habitat and type locality: Soil around raspberry rhizosphere in Pazardjik district, South Bulgaria.

Type material: The holotype is deposited in the Plant Nematode Laboratory, Faculty of Biology, Sofia; paratypes are deposited in the Museum of Central Helminthological Laboratory, Sofia, Bulgaria.

Literature cited

KIRJANOVA E.S. and KRALL E.L., 1969 - Parasitic Nematodes of Plants and their Control. Leningrad, USSR: «Nauka», vol. I, pp. 447 (in Russian) (English translations available from US Dept. Commerce, Nat. Tech. Inst. Serv., Springfield, Virginia 22161, USA).

KRALL E.L., 1978 - Parasiticheskie Kornevie Nematodi. Semeistvo Hoplolaimidae. Leningrad, USSR: «Nauka», pp. 420 (in Russian).

SIDDIQI M.R., 1974 - Systematics of the genus *Trichodorus* Cobb, 1913 (Nematoda: Dorylaimida) with descriptions of three new species. *Nematologica*, *19* (1973): 259-278.

Accepted for publication on 3 December 1988.