Nematode parasites and associates of Dendroctonus spp. and Trypodendron lineatum (Coleoptera: Scolytidae), with a description of Bursaphelenchus varicauda n.sp.

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Abstract: Nematode parasites and associates of four bark beetle species in British Columbia were surveyed. Bursaphelenchus varicauda n.sp., Ektaphelenchus macrostylus, Panagrolaimus dentius, and Cryptaphelenchus latus were found associated with Dendroctonus pseudotsugae. Parasitorhabditis obtusa was found in the gut and Contortylenchus reversus in the hemocoel of both D. pseudotsugae and D. rufipennis. The latter also had hemocoel infections of Sphaerulariopsis dendroetoni, which were not found concomitant with C. reversus infections. Contortylenchus brevicomis occurred in the hemocoel of D. brevicomis. The first report of a tylenchid larva parasitizing Trypodendron lineatum in North America is presented. Bursaphelenchus varicauda n.sp. was obtained from the gallery frass of D. pseudotsugae. It resembles B. corneolus and B. bestiolus but differs from the former species in female tail shape, the position of the excretory pore, spicule shape, and position of the male caudal papillae, and from the latter species in spicule shape and in the length of the esophagus and postuterine sac. Key words: taxonomy, new species, entomogenous nematodes, Douglas-fir beetle, bark beetle.


There have been very few reports of nematode parasites of bark beetles in British Columbia. Reid (14) reported that of the seven nematode species associated with the bark beetle Dendroctonus ponderosae, Sphaerularia hastata was the only endoparasite. Khan (3,4) described S. hastata and S. unguulacauda from the Douglas-fir beetle, Dendroctonus pseudotsugae and both of these species have now been placed in the genus Sphaerulariopsis (12). Ektaphelenchus macrostylus has been described from under the elytra of Douglas-fir beetles (5).

The nematodes described here were obtained from Dendroctonus pseudotsugae, D. brevicomis and D. rufipennis, and from Trypodendron lineatum. These four bark beetle species are economically important pests of the British Columbia forest industry. The impact of nematode parasites on the populations of these beetles is not known. We therefore surveyed these four beetles to determine the prevalence of hemocoel parasites in their populations.

MATERIALS AND METHODS

Bark was stripped from logs or trees infested with bark beetles, placed in large polythene bags for transport to the laboratory, transferred to emergence cages, and the emerged beetles collected twice daily. Frass scraped from the beetle galleries was placed in Baermann funnels for nematode extraction (16).

Beetles were dissected in 0.75% NaCl solution after examination for subelytral nematodes. All nematodes were killed and fixed in hot (50 C) triethanolamine/formalin (T.A.F.) and stored in fresh fixative. Stained preparations were made with 0.01% cotton blue in lactophenol, processed through Baker's solutions, and mounted in glycerine (16).

Drawings and measurements were made under transmitted light using phase contrast microscopy. Measurements follow the de Man formula except for G (length of female gonad expressed as a percentage of body length) and V (distance of vulva from tail end). All values are given as mean ± one standard error.

Some previously described nematode species are presented with more detailed morphometric measurements and, in some instances, with supplementary notes on their biology.

SPECIES DESCRIPTIONS

Bursaphelenchus varicauda n.sp.

Holotype female: L = 0.78 mm; a = 27.9; b = 9.8; c = 19.0; c' = 2.6; V =
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74.4%; \( G_1 = 59.0\% \); tail length = 41 \( \mu \)m; stylet = 15 \( \mu \)m.

**Paratype females** (n = 7): L = 0.79 ± 0.02 (0.71–0.89) mm; a = 30.3 ± 1.2 (26.4–35.8); b = 10.1 ± 0.2 (9.6–10.9); c = 19.5 ± 1.1 (15.4–24.7); \( c' = 2.7 ± 0.1 (2.5–3.2) \); \( V = 76.0 ± 1.1 (72.7–82.4)\% \); \( G_1 = 60.6 ± 1.8 (53.9–69.8)\% \); tail length = 41.7 ± 2.7 (32–50) \( \mu \)m; stylet = 14.9 ± 0.4 (12–17) \( \mu \)m.

Paratype males (n = 6): L = 0.72 ± 0.04 (0.54–0.84) mm; a = 35.7 ± 1.6 (29.7–41.1); b = 8.8 ± 0.3 (7.4–9.6); c = 19.7 ± 1.0 (15.3–23.0); \( c' = 2.2 ± 0.1 (1.8–2.7) \); \( T = 69.3 ± 2.1 (64.7–80.0)\% \); tail length = 36.7 ± 0.9 (33–40) \( \mu \)m; spicule = 15.1 ± 0.6 (13–17) \( \mu \)m; stylet = 16.0 ± 0.1 (15–17) \( \mu \)m.

Female body long and thin, with body inflexion at vulva. Cuticle finely striated. Head rounded, offset with slight distal flattening (Fig. 1A). Stylet long and apherlenchoid with small basal knobs. Esophagus 70–82 \( \mu \)m long in females. Procorpus of esophagus narrow at base of stylet and widening posteriorly, ending in a constriction at the median bulb. Median bulb large and oval with prominent valvular apparatus; anterior duct of esophageal gland opens just anterior to valve apparatus; posterior esophageal gland opening not discernible. Esophageal glands long. Intestine straight; anus 32–50 \( \mu \)m from tail terminus. Excretory pore situated about 10 \( \mu \)m behind median bulb. Nerve ring just posterior to median bulb-intestine junction. Tail of female conical in shape, 32–50 \( \mu \)m long; terminus variable (Fig. 2), most frequently ending in three fingerlike processes (Fig. 1B).

Female gonad monodelphic, prodelphic, occasionally reflexed, beginning at level of esophageal glands. Oviduct and uterus filled with sperm. Walls of uterus thickened. Vagina slopes posteriorly to vulval opening; vulval opening covered by an anterior cuticular flap (Fig. 1F). Postuterine sac long, extending 70–120 \( \mu \)m posteriorly from vulva; postuterine sac thick walled.

Males generally shorter than females and with similar cephalic morphology. Esophagus 72–90 \( \mu \)m long. Tail of male conoid, 33–40 \( \mu \)m long and sharply curved ventrally. Small bursa extending around tail terminus. Three pairs of caudal papillae present (Fig. 1C), the first pair just anterior to the cloaca, the second pair midway between the cloaca and the tail terminus, and the third pair at the level of the anterior margin of the bursa. Testis single, 0.35–0.62 mm long, usually reflexed at the anterior extremity (Fig. 1D). Spicules paired, separate along most of their length but fused at the posterior extremity, with strong ventral incurvation and prominent ventral process at the proximal end (Fig. 1E). Gubernaculum absent.

**Type specimen:** Holotype female (slide No. 271), one paratype female (slide No. 271a), and two paratype males (slide No. 271b) have been deposited with the Canadian National Collection of Nematodes, Ottawa, Ontario.

**Species diagnosis:** B. varicauda resembles B. corneolus Massey, 1966 (9) and B. bestiolus Massey, 1974 (10). It differs from B. corneolus in the shape of the female tail and in the position of the excretory pore, which is more posterior in B. corneolus. Male B. corneolus differ from male B. varicauda in spicule shape and in the position of the caudal papillae, which are all postanal in the former species. Female B. bestiolus differ from female B. varicauda in tail shape and length of the postuterine sac, which is eight to nine body widths long in the former but only two to five body widths long in the latter species. The esophagus of female B. bestiolus is shorter in relation to body length than is that of female B. varicauda. B. bestiolus differs also from B. varicauda in spicule shape.

Host: D. pseudotsugae. Specimens were obtained from under the elytra and in the frass of the beetle.

**Type locality:** Boston Bar, December 1969. This new species has also been collected from Douglas-fir beetles in Hixon, North Bend, Pemberton, Maple Ridge, Whistler Mountain, and Williams Lake from 1970 to 1971.

**Contortylenchus reversus** (Thorne, 1935) Rühm, 1956

**Egg laying females** (n = 18): L = 1.32 ± 0.05 (0.93–1.66) mm; a = 18.3 ± 0.6 (14.9–23.7); \( V = 93.8 ± 0.3 (92.5–95.8)\% \);
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Fig. 2. *Bursaphelenchus varicauda*, n.sp. Intraspecific variations in tail shape of paratype females.

$G_1 = 113.7 \pm 5.3 (93.5-196.8)\%$; $V_t = 80.6 \pm 2.5 (70-100) \mu m$.

**Free-living females** (n = 14): $L = 0.40 \pm 0.01 (0.34-0.43)$ mm; $a = 31.0 \pm 0.6 (28.6-35.8)$; $V = 91.0 \pm 0.2 (89.8-92.4)\%$; $G_1 = 25.3 \pm 1.2 (17.2-32.3)\%$; $V_t = 35.9 \pm 0.6 (30-40) \mu m$; stylet = 14.6 \pm 0.3 (13-17) \mu m.

**Free-living males** (n = 12): $L = 0.40 \pm 0.01 (0.35-0.45)$ mm; $a = 31.7 \pm 0.7 (28.9-35.8)$; $c = 24.3 \pm 0.6 (20.8-28.0)$; $c' = 2.0 \pm 0.1 (1.8-2.2)$; $T = 61.3 \pm 3.7 (40.7-77.3)\%$; tail length = 16.3 \pm 1.0 (15-18) \mu m; stylet = 14.3 \pm 0.6 (11-17) \mu m; spicules = 14.5 \pm 0.4 (12-17) \mu m; gubernaculum = 5.7 \pm 0.1 (5-6) \mu m.

Egg laying females occur only in the hemocoel and were isolated from all host stages except the eggs and first-instar larvae.

A detailed redescription of this species, its life cycle and its physiological effects on its host has been presented in previous publications (17,19,20,21).

**Hosts and localities:** *D. pseudotsugae* from Boston Bar, Hixon, North Bend, Pemberton, Maple Ridge, Whistler Mountain, and Williams Lake between 1969 and 1971. The prevalence of *C. reversus* infections varied from 5\% to 50\% at these sites. *C. reversus* was also found in 5\% of *D. rufipennis* from Prince George and Pemberton in November 1970.

*Ektaphelenchus macrostylus* Khan, 1960.

**Females** (n = 10): $L = 0.78 \pm 0.01 (0.71-0.81)$ mm; $a = 28.4 \pm 1.1 (25.1-36.7)$; $b = 8.4 \pm 0.1 (7.8-9.1)$; $V = 78.0 \pm 0.03 (76.0-79.8)\%$; $G_1 = 44.2 \pm 1.6 (37.1-52.2)\%$; stylet = 13.4 \pm 0.8 (11-19) \mu m.

**Males** (n = 10): $L = 0.69 \pm 0.02 (0.61-0.78)$ mm; $a = 33.5 \pm 1.3 (26.6-41.6)$; $b = 7.6 \pm 0.1 (6.9-8.3)$; $c = 19.6 \pm 0.5 (17.0-22.3)$; $c' = 2.3 \pm 0.1 (2.0-2.6)$; $T = 28.0 \pm 0.7 (24.1-30.6)\%$; stylet = 9.6 \pm 0.4 (7-11) \mu m; spicule = 21.0 \pm 0.3 (20-22) \mu m.

This species was originally described by Khan (5), who presented measurements of "$L_1$", "$a$", "$b$", "$c$", "$V$", and "$T$" for only one male and one female obtained from the Douglas-fir beetle in British Columbia. His "$b$" ratio for the female was 4.0, half that obtained in the present study. Khan also mentioned finding females of this species under the elytra and males in the bark. We found only ensheathed *L_3* larvae under the elytra; males and females occurred in only the gallery frass. The life cycle occurs almost entirely in the galleries, and the *L_3* larvae attach to the adult beetles prior to host emergence for transfer to another habitat.

**Host and localities:** *D. pseudotsugae* from Boston Bar, Hixon, North Bend, Pemberton, Maple Ridge, Whistler Mountain, and Williams Lake between 1969 and 1971.


**Females** (n = 10): $L = 0.49 \pm 0.02 (0.42-0.57)$ mm; $a = 15.5 \pm 0.5 (13.1-18.0)$; $b = 4.6 \pm 0.2 (4.0-5.4)$; $c = 11.1 \pm 0.5$
(10.1–13.3); \( c' = 2.5 \pm 0.1 \) (2.0–2.8); \( V = 61.7 \pm 0.6 \) (58.5–64.9)%; \( G_1 = 69.5 \pm 2.5 \) (59.6–82.2)%; tail length = 43.8 ± 1.2 (40–50) \( \mu \text{m} \).

**Males** (\( n = 10 \)):

- \( L = 0.44 \pm 0.01 \) (0.39–0.49) \( \text{mm} \);
- \( a = 17.9 \pm 0.8 \) (14.3–22.8);
- \( b = 4.5 \pm 0.1 \) (3.9–5.1);
- \( c = 10.6 \pm 0.3 \) (9.2–12.5);
- \( c' = 2.3 \pm 0.1 \) (1.8–2.7);
- \( T = 66.9 \pm 1.9 \) (59.5–76.3)%;
- tail length = 41.8 ± 1.6 (35–49) \( \mu \text{m} \);
- spicule = 22.2 ± 0.6 (20–25) \( \mu \text{m} \);
- gubernaculum = 12.4 ± 0.3 (11–14) \( \mu \text{m} \).

**P. dentatus** was first described by Thorne (22) from *D. ponderosae* in Utah. Originally placed in the genus *Panagrodontus*, it was later transferred to the genus *Panagrolaimus*, with which the former genus has been synonymized (15). Thorne presented measurements in the Cobb formula [or one male and one female specimen. His specimens were slightly longer than ours. The full length of the reflexed female gonad was not given (22) and, therefore, a full comparison with our specimens is not possible. Thorne, like ourselves, found the ensheathed larvae only under the elytra of the beetle and the adult nematodes in the galleries.

**Host and localities:** *D. pseudotsugae* from Boston Bar, Hixon, North Bend, Pemberton, Maple Ridge, Whistler Mountain, and Williams Lake between 1969 and 1971.

**Cryptaphelenchus latus**

(Thorne, 1935) Rühm 1956

**Females** (\( n = 12 \)):

- \( L = 0.31 \pm 0.01 \) (0.26–0.38) \( \text{mm} \);
- \( a = 20.0 \pm 0.4 \) (18.1–22.7);
- \( b = 7.5 \pm 0.2 \) (6.5–8.6);
- \( V = 78.5 \pm 0.3 \) (76.8–80.3)%;
-\( G_1 = 48.7 \pm 2.0 \) (37.2–63.5)%;
- stylet = 8.9 ± 0.3 (8–10) \( \mu \text{m} \).

**Males** (\( n = 7 \)):

- \( L = 0.26 \pm 0.01 \) (0.23–0.29) \( \text{mm} \);
- \( a = 20.6 \pm 0.7 \) (19.9–21.4);
- \( b = 6.6 \pm 0.1 \) (6.1–7.0);
- \( c = 10.7 \pm 0.7 \) (8.9–11.8);
- \( c' = 2.1 \pm 0.1 \) (1.9–2.4);
- \( T = 39.3 \pm 1.3 \) (35.1–44.3)%;
- tail length = 24.3 ± 1.0 (21–29) \( \mu \text{m} \);
- stylet = 9.1 ± 0.4 (8–11) \( \mu \text{m} \);
- spicules = 15.9 ± 0.4 (14–17) \( \mu \text{m} \);
- gubernaculum = 9.7 ± 0.4 (8–11) \( \mu \text{m} \).

Adult nematodes were recovered from gallery frass. *C. latus* was originally described from *D. ponderosae* (22) and named *Aphelenchoides latus* but was transferred to the genus *Cryptaphelenchus* by Rühm (15). Thorne's original description presented the Cobb formula for one male and one female specimen. Thorne's male was larger than ours, and his male and female had "a" ratios one-half that of ours. Thorne's specimens also had slightly longer esophagi in proportion to their body length than do our specimens, and although the lengths of the tails in the two descriptions are similar, the base of the tails of Thorne's specimens were wider. Differences between Thorne's morphometric measurements and ours are probably due to his small sample size.

**Host and localities:** *D. pseudotsugae* from Boston Bar, Hixon, North Bend, Pemberton, Maple Ridge, Whistler Mountain, and Williams Lake between 1969 and 1971.

**Parasitorhabditis obtusa**

(Fuchs, 1915) Dougherty, 1953.

**Females** (\( n = 10 \)):

- \( L = 0.70 \pm 0.04 \) (0.54–0.88) \( \text{mm} \);
- \( a = 19.7 \pm 0.5 \) (18.1–22.7);
- \( b = 4.2 \pm 0.4 \) (3.4–4.6);
- \( c = 32.3 \pm 1.0 \) (26.8–38.5);
- \( c' = 1.1 \pm 0.4 \) (0.9–1.2);
- \( V = 93.6 \pm 0.3 \) (92.7–95.4)%;
- \( G_1 = 73.2 \pm 2.2 \) (61.1–82.2)%;
- tail length = 21.8 ± 1.0 (18–27) \( \mu \text{m} \).

**Males** (\( n = 13 \)):

- \( L = 0.72 \pm 0.03 \) (0.54–0.91) \( \text{mm} \);
- \( a = 20.8 \pm 0.8 \) (16.1–24.5);
- \( b = 4.5 \pm 0.3 \) (4.0–6.1);
- \( c = 28.3 \pm 1.8 \) (23.0–39.6);
- \( c' = 1.2 \pm 0.1 \) (0.9–1.4);
- \( T = 78.2 \pm 1.3 \) (71.8–85.5)%;
- tail length = 25.7 ± 0.9 (22–30) \( \mu \text{m} \);
- spicule = 45.1 ± 0.9 (38–55) \( \mu \text{m} \);
- gubernaculum = 23.3 ± 0.6 (20–28) \( \mu \text{m} \).

Larval stages of the nematode are found living in the lumen of the midgut and occasionally in the hindgut of adult beetles. The nematodes move constantly, working against the flow of food material in the gut. Adult nematodes are found in the frass of the galleries.

Fuchs (2) first described *P. obtusa* from *Ips typographus* and placed it in the genus *Rhabditis*. It was later transferred to the genus *Parasitorhabditis* of which it is now the type species (1). Thorne (22) described *P. obtusa* from *D. ponderosae*. His measurements showed females with tail lengths one-half those of our specimens and consequently "c" ratios twice those of ours. Nickle (11) stated that larval parasitorhabditids reduced the epithelial layer of the ventriculus of *Ips paraconfusus*. However, as *P. obtusa* does not attach to the mucosa but is free in the lumen, it is
difficult to envisage the mechanism leading to such an effect, unless the nematodes are numerous enough to cause gut occlusion. The mode of re-infection of beetles by these nematodes is unknown, but it is probably either oral during feeding or anal during the quiescent overwintering period of adult beetles.


Females (*n* = 16): L = 3.61 ± 0.14 (2.82-4.82) mm; w = 86.9 ± 2.69 (70-100) μm; a = 41.8 ± 1.6 (32.8-60.0); V = 95.9 ± 0.4 (94.7-97.0)%; G = 93.5 ± 0.6 (88.5-96.5); Vt = 147.2 ± 5.0 (110-180) μm.

Eggs, second- and third-stage larvae, and the mature females were found in the host hemocoel.

*C. brevicomi* has been redescribed in detail and its life cycle elucidated (6,18). Some detrimental effects of this parasite on its host have also been reported (7).

Host and locality: D. brevicomis from Lytton collected in December 1969 and 1970. Prevalence of *C. brevicomi* was 32% and 17%, respectively, at these collection dates.


Mature gravid females, eggs, and at least three larval stages were obtained from the hemocoel of *D. rufipennis*. The eggs (80 × 40 μm) are deposited in the hemocoel after initial cleavage. At least one molt occurs within the egg. No detailed study was undertaken of the postembryonic development of *S. dendroctoni* but fourth-stage female larvae, identified by their vaginal primordia, were observed in the hemocoel.

Early in development, after the females become sexually mature, the uterus, ovary, and oviduct are extruded through the vulva and enlarge to more than 100 times their original size, dwarfing the body of the female nematode to which they are still attached. The size of these protruded reproductive organs may reach a length of 1.6 mm and a width of 0.25 mm. The rounded projections on the surface of the everted uterus contain enlarged nuclei of the uterine cells. The female body at this stage is approximately 0.4 mm long and 30 μm wide.

The original description of this nematode from *D. rufipennis* in Colorado and northwestern Montana included two larval stages (8). Nevertheless, fourth-stage larvae of *S. dendroctoni* were observed in the host hemocoel in the present study making it possible that all four larval stages occur in the hemocoel. Massey found males together with the females in the beetle hemocoel. This report merits further examination because in other Sphaerulariidae the male is free-living and does not enter the host.

Egg production of female beetles parasitized by *S. dendroctoni* is known to be reduced (8). Poinar and Hess (13) in their study on the related nematode *Sphaerularia bombi* observed that nutrients were absorbed by the nematode through the walls of the extruded uterus.

Host and localities: *D. rufipennis* collected from Pemberton and Prince George in November 1970. Prevalence of infection at these sites was 45% and 14%, respectively.

*Trypoderdon lineatum* There have been no previous reports on the occurrence of nematodes associated with *Trypoderdon lineatum* in North America. However, larval nematodes were obtained from the hemocoel of only four (three females and one male) of 64 beetles collected in Maple Ridge. Between 20 and 100 larvae were found in the hemocoel of individual beetles. They were all at the same stage of development.

Larva 0.37 mm long and 12 μm wide. Head rounded with prominent cephalic frame. Tail pointed, approximately 45 μm long. Stylet short, 5 μm in length, with prominent basal knobs. Procorpus of esophagus straight, ending in narrow, elliptically-shaped median bulb 5 μm wide; valvular apparatus not well developed. Esophageal glands overlap intestine to a distance of
20 μm behind the median bulb. Nerve ring just posterior to median bulb. Intestine straight and undifferentiated. Genital primordium two-thirds of way down body, consisting of three cells, the central cell being larger than the proximal and distal ones.

The structure of the genital primordium indicates that this is the first-stage larva. No other larval stages were found. The nematode did not have any obvious deleterious effect on the beetle.

LITERATURE CITED


