

Rhabditis (Crustorhabditis) stasileonovi (Belogurov) from beach hoppers (Talitridae; Amphipoda) from the Pacific Coast of North America

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Summary – Material collected from intersegmental spaces beneath the dorsal plates (pereonites) of beach hoppers from the Pacific Coast of North America is herein referred to *Rhabditis (Crustorhabditis) stasileonovi* (Belogurov, 1977) Sudhaus & Nimrich, 1989 (Rhabditida; Rhabditidae). This species, hitherto known only on the basis of female anatomy, was described from beach wrack in the Commander Islands by Belogurov (1977) who proposed a new genus, *Marispelodera*, for it. Sudhaus and Nimrich (1989) reported the species from beach wrack on Vancouver Island but referred the species to the subgenus *Rhabditis (Crustorhabditis)*. Females are exceptional in that the tail is extremely short and rounded. Males described herein agree in all essential respects to the subgenus *Rhabditis (Crustorhabditis)* and therefore corroborate Sudhaus and Nimrich's (1989) interpretation. Adult and larval worms were collected from intersegmental spaces of *Megalorchestia californiana*, *M. benedicti* and *Traskorchestia traskiana* from beaches near Bamfield, B. C., Canada. Larvae only were collected from the above three hosts and *Megalorchestia corniculata* from Santa Barbara, California, USA, but larvae were cultured to adulthood on agar plates. The material from the two localities differed in minor morphological details and in life history: in Canada, larvae, males and ovoviviparous females occurred on hosts, whereas in California, only larvae occurred on hosts, and females cultured from these larvae were oviparous.

Résumé – *Rhabditis (Crustorhabditis) stasileonovi* (Belogurov) provenant de talitres (Talitridae; Amphipoda) de la côte pacifique d'Amérique du Nord – Des spécimens récoltés sur les espaces intersegmentaires situés entre les plaques dorsales (péréonites) de talitres provenant de la côte pacifique d'Amérique du Nord sont rapportés ici à *Rhabditis (Crustorhabditis) stasileonovi* (Belogurov, 1977) Sudhaus & Nimrich, 1989 (Rhabditida; Rhabditidae). Cette espèce, connue jusqu'ici par les seules données de l'anatomie des femelles, a été décrite, à partir de spécimens provenant de laisses des plages des îles Commander, par Belogurov (1977) qui propose de la classer dans un nouveau genre, *Marispelodera*, créé pour elle. Sudhaus et Nimrich (1989) signalent cette même espèce dans des laisses provenant de plages de l'île de Vancouver, mais la rapportent au sous-genre *Rhabditis (Crustorhabditis)*. Les femelles apparaissent exceptionnelles par leur queue extrêmement courte et arrondie, les mâles, décrits ici, sont conformes pour l'essentiel au sous-genre *Rhabditis (Crustorhabditis)* et permettent donc de confirmer l'opinion de Sudhaus et Nimrich (1989). Des adultes et des formes larvaires ont été collectées sur les espaces intersegmentaires de *Megalorchestia californiana*, *M. benedicti* et *Traskorchestia traskiana* provenant de plages de la région de Bamfield, B. C., Canada. Seules des larves ont été collectées sur ces trois hôtes, et sur *M. corniculata* provenant de Santa Barbara, CA, USA, mais ces larves ont pu être élevées jusqu'au stade adulte sur agar. Le matériel provenant de ces deux localités diffèrent par des caractères morphologiques mineurs et par leur cycle biologique: au Canada, les larves, les mâles et les femelles ovovivipares sont tous présents sur l'hôte, tandis qu'en Californie, seules les larves sont présentes sur l'hôte et les femelles provenant de leur élevage sont ovipares.

Key-words : Amphipoda, phoresy, Rhabditida, *Rhabditis (Crustorhabditis)*, Talitridae, nematodes.

Chitwood *et al.* (1970) reported what they interpreted as a new species of *Parasitorhabditis* from dorsal pockets between segments of three species of amphipods (*Megalorchestia californiana*, *M. corniculata* and *Traskorchestia traskiana*) from Californian and Washington coasts. They did not describe the material however, and its assignment to the genus *Parasitorhabditis* remains questionable especially since other members of the genus occur in wood-boring insects. We found adult and larval worms under the pereonites (dorsal plates) of amphipods from sandy beaches on the West Coast of Vancouver island and from the vicinity of Santa Barbara, California. This material is indistinguishable from larvae

described by Chitwood *et al.* (1970), but distinct species may have identical larval morphology.

Our material agrees with adult female worms from beach wrack described as *Rhabditis (Crustorhabditis) stasileonovi* (Belogurov, 1977) Sudhaus & Nimrich, 1989. Belogurov (1977) described his material from the Commander Islands in the Bering Sea, but more recently, Sudhaus and Nimrich (1989) reported females of the same species from beach wrack on Vancouver Island. The species is still known based only on female anatomy. We are not absolutely certain of our designation of this material as *Rhabditis (Crustorhabditis) stasileonovi*, but this seems the most prudent taxonomic move. In

light of this uncertainty we offer a full description of our material.

Materials and methods

Beach hoppers were identified using keys in Bousfield (1982) and Smith and Carlton (1975). Three species of amphipods (Crustacea; Talitridae) – *Megalorchestia californiana*, *M. benedicti*, and *Traskorchestia traskiana* – were collected from beach wrack from various beaches near Bamfield, British Columbia from July 24 through August 4, 1994. In addition, the same species and a fourth, *M. corniculata*, were collected from similar ecotypes on beaches near Santa Barbara, California October 8 through October 10, 1994.

Hoppers were placed individually in vials containing 70% ethanol immediately upon capture. Vials were agitated manually for about 1 min to release nematodes from beneath the pereonites. Vial contents were emptied and nematodes were counted with aid of a dissecting microscope. In addition, some amphipods were dissected in water to ascertain the exact site occupied by nematodes and to provide material for culture.

Nematodes collected by dissection from hoppers were transferred to plates containing nutrient agar (Fisher Scientific Bio Cert Nutrient Agar). Plates were shipped to Vancouver for further study where nematodes were replated onto plates containing a lawn of *Escherichia coli* on 0.7% agar prepared as follows: 12.5 mg peptone, 3 mg NaCl, and 27 mg agar were added to 1 l water and autoclaved; to this was added 1 ml each of cholesterol (5 mg/ml in ethanol), 1 M MgSO₄ and 1 M CaCl₂, and 100 ml of 1 M KH₂PO₄ at pH 6.0.

Rhabditis (Crustorhabditis) stasileonovi (Belogurov, 1977) Sudhaus & Nimrich, 1989

Figs 1 & 2

MEASUREMENTS (British Columbia specimens)

Male (n = 10): L = 1184 (1075-1333) μm; Max. diam = 48 μm (near midbody); Buccal capsule = 21 (15-23) μm; Pharynx = 195 (183-202) μm; corpus 104 (99-116) μm; isthmus and bulb = 91 (84-103) μm; Max. diam. bulb = 26 (23-29) μm; Nerve ring = 165 (159-170) μm from ant. end; Excretory pore = 194 (183-202) μm from ant. end; Blind end testis = 518 (459-553) μm; Posterior flexure = 347 (284-383) μm from ant. extrem.; Spicules = 63 (60-66) μm; Gubernaculum = 33 (29-36) μm; Tail = 30 (26-35) μm. a = 24.7 (21.8-27.9); b = 6.1 (5.4-6.7); c = 39.3 (34.7-44.6).

Female (n = 8): L = 1274 (1076-1530) μm, Max. diam. = 52 (47-67) μm (near midbody); Buccal capsule = 21 (18-25) μm; Pharynx = 199 (182-210) μm; Corpus 108 (101-113) μm; Isthmus and bulb = 90 (81-98) μm; Max. diam. bulb = 28 (24-31) μm; Nerve ring = 168 (159-179) μm from ant. end; Excretory pore = 200 (183-209) μm from ant. end; Blind end

ovary = 614 (529-799) μm; Posterior flexure = 333 (263-422) μm from ant. extrem.; Vulva = 1195 (1006-1447) μm from ant. end; Tail = 22 (17-28) μm; a = 24.4 (20.6-28.4); b = 6.4 (5.8-7.5); c = 58.6 (44.5-74.5); V = 93.7 (92.8-94.6).

DESCRIPTION (British Columbia specimens, Fig. 1)

General: Small slender worms, 1-1.5 mm long, with maximum width near midbody. Oral opening surrounded by six small round well separated lips, each with sharply pointed anteriorly directed projection. Amphidial pore slightly posterior to projection on lateral lip. Body with faint striations beginning just posterior to lips and extending to near level of anus. Buccal cavity tubular surrounded by cuticular capsule beginning about halfway down lips and extending to anterior end of pharynx. Metastomal teeth sharply pointed in form of single sharply pointed denticle. Pharynx with corpus, isthmus and bulb. Corpus with slight swelling at base. Deirids present near level of swelling at base of corpus. Nerve ring surrounding isthmus and excretory pore near level of anterior end of bulb. Two prominent gland cells extending posteriorly at level of pharyngo-intestinal junction. Lateral alae absent.

Male: Testis leading anteriorly before flexing posteriorly and leading on right ventral side through seminal vesicle to ejaculatory duct. Two lateral anteriorly directed diverticula, present, leaving ejaculatory duct about 150 μm anterior to anus. Spicules fused for about 2/3 of their length. Gubernaculum a simple plate like thickening of dorsal wall of cloaca. Tail subconical. Bursal membrane with faint ridging on surface and delicately fluted outer edge. Caudal papillae consisting of single unpaired sessile papilla on anterior anal lip and ten paired pedunculate papillae associated with caudal bursa. Anterior two pairs caudal papillae grouped and pre-cloacal; next five pairs grouped and last three pairs grouped. Third, seventh and ninth pair of caudal papillae subdorsal, remaining pairs subventral in position.

Female: Monodelphic. Ovoviviparous. Ovary leading anteriorly along right dorsal side before flexing posteriorly and emptying into oviduct. Oviduct extending posteriorly along ventral side, and near level of midbody, leading through muscular sphincter to uterus. Uterus of gravid females containing 1-50 well-developed larvae, leading posteriorly to short vagina and vulva. Tail evenly rounded.

HOSTS AND LOCALITY

Adult and larval worms were isolated from beach hoppers from sandy and rocky beaches near Bamfield, B. C., Canada. Adults probably also inhabit beach wrack. Worms described here came from *Megalorchestia californiana*. Adult and larval worms were found under the pereonites. Worms were also recovered from *M. benedicti* and *Traskorchestia traskiana*. In 67 specimens

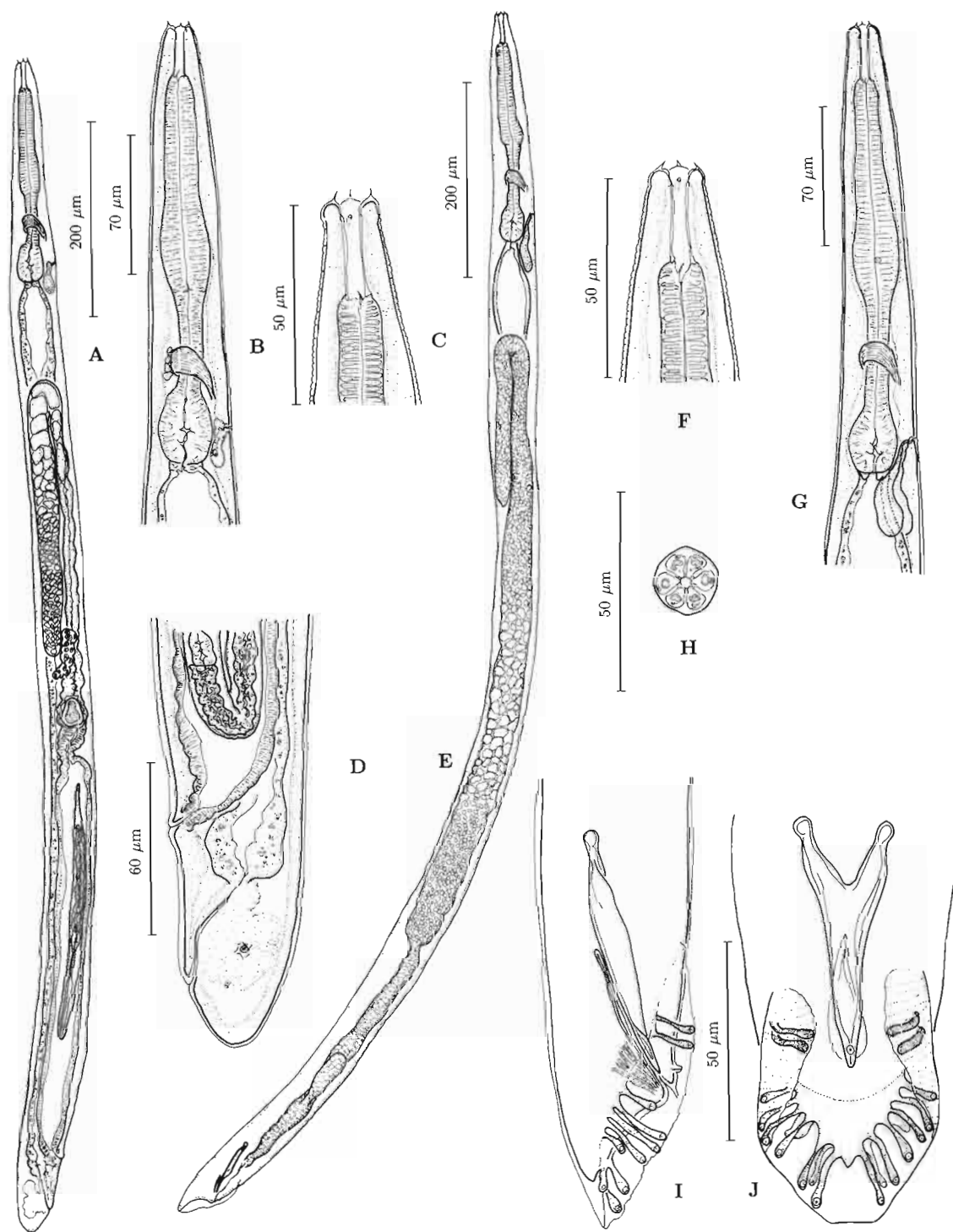


Fig. 1. *Rhabditis (Crustorhabditis) stasileonovi* (Belogurov), from British Columbia. A: Female, entire, lateral view; B: Female, pharyngeal region, lateral view; C: Female, anterior extremity, lateral view; D: Female, caudal extremity, lateral view; E: Male, entire, lateral view; F: Male, anterior extremity, lateral view; G: Male, pharyngeal region, lateral view, showing deirid; H: Male, apical view; I, J: Male, caudal extremity in lateral and ventral views, respectively.

of *Megalorchestia californiana* examined, mean intensity was 36.1 ± 6.43 (1-242) and prevalence was 86.6 %.

ADDITIONAL MATERIAL (Fig. 2)

Small unsheathed larvae (presumably late third-stage) were found under the pereonites *Megalorchestia corniculata*, *M. californiana*, *M. benedicti* and *Traskorchestia traskiana* collected from beach wrack near Santa Barbara, California, USA. Mean intensity of larvae was 1031.7 ± 152.1 (27-4150) and prevalence was 100 % in a sample of 37 specimens of *Megalorchestia corniculata* examined. Adults were never found on amphipods in this locality. However, some larvae were transferred from *Megalorchestia corniculata* to agar plates containing 0.7 % agar, and from such cultures we acquired adult worms described below.

Phoretic larvae (n = 10)

a = 29.7 (28.1-31.4); b = 5.4 (5.3-5.4); c = 5.9 (5.7-6.1). Slender worms 812 (737-848) μm long. Oral opening surrounded by two lateral and four submedian shallow lips. Buccal capsule tubular = 21 (17-24) μm , beginning 2-3 μm posterior to lip apex. Pharynx = 149 (141-159) μm ; Corpus = 81 (77-89) μm ; Isthmus and bulb = 68 (64-75) μm ; Max. diam. bulb. = 16 (15-17) μm ; Nerve ring = 133 (124-148), and Excret. pore = 159 (147-171) μm , from ant. end. Lateral alae consisting of two shallow sublateral ridges on each side, beginning near level of pharyngeal bulb and extending to approximately 100 μm posterior to anus. Genital primordium = 81 (50-106) μm long, its anterior end at 328 (290-343) μm from ant. body end. Tail attenuate = 137 (124-148) μm long.

Adults from culture

Male (n = 6): L = 1044 (834-1160) μm ; Max. diam. = 59 (53-66) μm (near midbody); Buccal capsule = 20 (17-24) μm ; Pharynx = 163 (152-179) μm ; Corpus = 89 (75-103) μm ; Isthmus and bulb = 74 (51-90) μm ; Max. diam. bulb = 28 (25-30) μm ; Nerve ring = 124 (115-138) from ant. end. Excret. pore = 150 (130-164) from ant. end; Ant. flexure = 268 (243-315), and blind end of testis = 488 (461-575) μm from ant. extrem.; Spicules fused for about 2/3 of their length = 66 (61-72) μm ; Gubernaculum = 31 (22-36); Tail = 29 (22-35) μm ; a = 17.9 (15.6-20.7); b = 6.4 (5.4-7.4); c = 37.3 (29.4-52.7).

Female (n = 2): L = 1282 (1294) μm ; Max. diam. = 57 (57) μm (near midbody); Buccal capsule = 16 (20) μm ; Pharynx = 193 (173) μm ; Corpus = 100 (91) μm ; Isthmus and bulb = 93 (82) μm ; Max. diam. bulb = 33 (28) μm ; Nerve ring = 141 (135), excretory pore = 176 (170), ant. flex. ovary 409 (310), blind end ovary 868 (619) and vulva 1227 (1214) μm from ant. end. Tail = 17 (14) μm ; a = 22.1 (22.7); b = 6.6 (7.5); c = 75.4 (92.4); V = 94.8 (94.6).

SPECIMENS

Voucher specimens from each locality have been deposited in the collection of the Canadian Museum of Nature, Ottawa, Canada.

Adult specimens from *Megalorchestia californica* from Bamfield, BC, Canada: males CMNPA 1996-0066, females CMNPA 1996-0067. Adults cultured from larvae collected from *M. corniculata* from Santa Barbara, CA, USA: males CMNPA 1996-0068, females CMNPA 1996-0069.

Discussion

The six well developed and well separated lips, the form of the bursa with its faint ridging and slightly fluted distal border, the arrangement of bursal papillae (in a 2/5 + 3 pattern with three papillae slightly more lateral in position) and the spicules fused in their distal two-thirds are sufficient to place this material in the subgenus *Rhabditis* (*Crustorhabditis*) Sudhaus, 1974. This genus includes four species (see Sudhaus, 1974, 1976; Belogurov, 1977): *R. (C.) oxypodis* (Chitwood, 1935) Sudhaus, 1974; *R. (C.) riemanni* Sudhaus, 1974; *R. (C.) scanica* (Allg n, 1949) Sudhaus, 1974, and *R. (C.) stasileonovi* (Belogurov, 1977) Sudhaus & Nimrich, 1989. The present material is easily distinguished from the first three by bursal ray disposition and by the shape of the female tail (although variants of *R. (C.) scanica* have a short rounded female tail). Up until now, *R. (C.) stasileonovi* has been known only on the basis of female anatomy, and in these respects resembles the present material especially in the presence of a short rounded tail in the female. We refer our material to this species because we have no clear way to distinguish it.

Belogurov (1977) proposed a new genus, *Marispelodera*, for *R. stasileonovi*, but Sudhaus and Nimrich (1989) placed it in *Rhabditis* (*Crustorhabditis*). The males described herein agree in all essential respects with the above subgenus and our findings therefore support the interpretation of Sudhaus and Nimrich (1989). One of the unique features that caused Belogurov to propose a new genus was a region of the reproductive system, translated as "dosator" in the english summary, but presumably referring to what we interpret herein as a sphincter between oviduct and ovary. It is not clear that this structure is unique to the species or even the genus.

Material from California agrees morphologically in most respects to that collected from Vancouver Island but differs in that the pharynx surrounds the posterior 8 μm of the buccal cavity in mature specimens. This pharyngeal extension was not present in newly moulted adults, however, and we are uncertain of its taxonomic import; it may represent an artifact. In some specimens there was an additional flexure near the blind end of the ovary. This was not observed in material from Vancouver Island.

Other differences in material collected from California include the fact that females carry eggs rather than

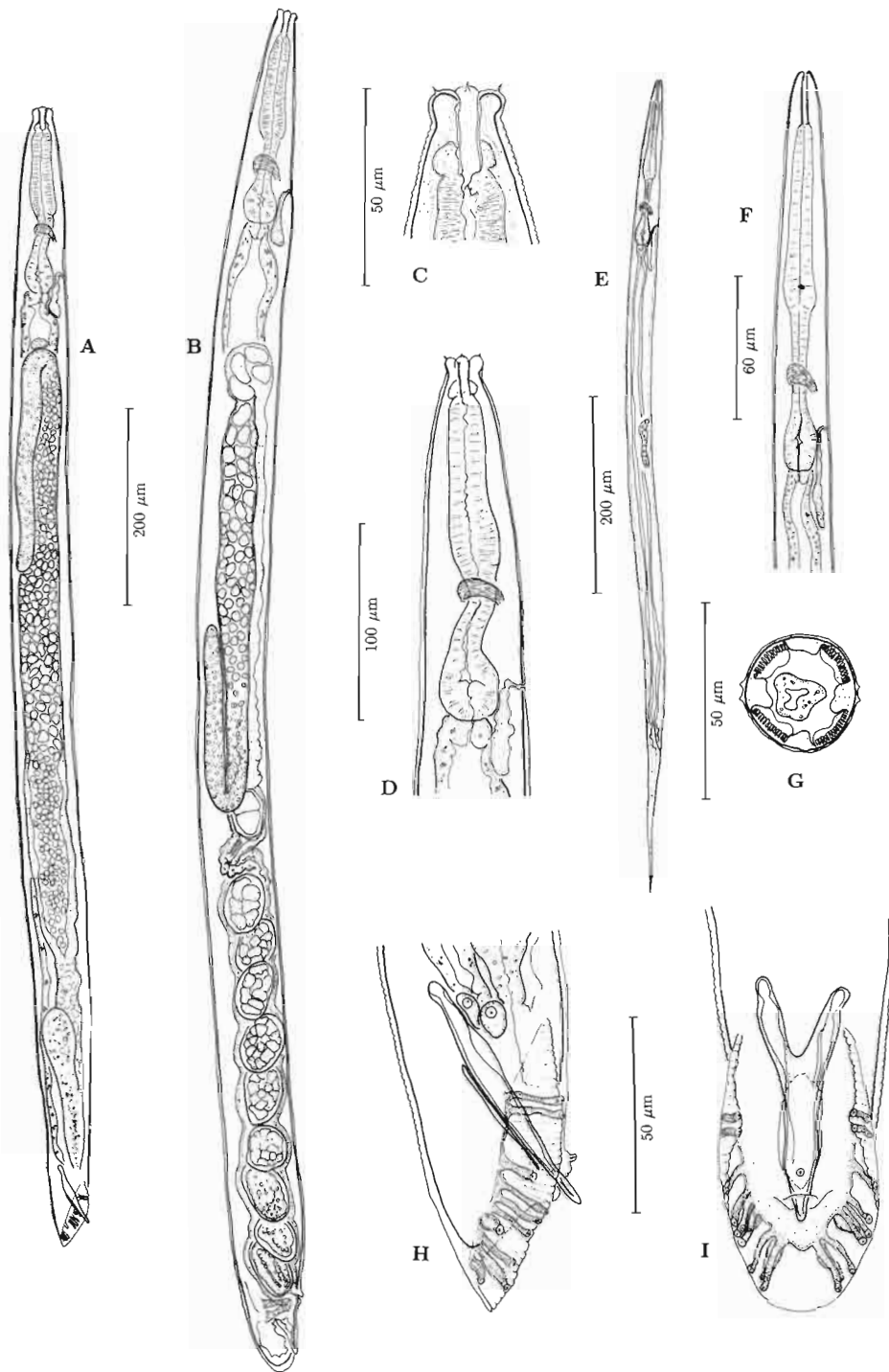


Fig. 2. *Rhabditis (Crustorhabditis) stasileonovi* (Belogurov), from California. A : Male, entire, lateral view; B : Female, entire, lateral view; C : Male, anterior extremity, lateral view; D : Male, pharyngeal region, lateral view; E : Parasitic larva, lateral view; F : Parasitic larva, pharyngeal region, lateral view; G : Parasitic larva, transverse section near level of midbody; H, I : Male, caudal extremity in lateral and ventral views, respectively.

larvae in the uterus, and that only larval stages were found on hosts from California. It is difficult to assess the taxonomic importance of these life history differences. Presence of eggs or larvae *in utero* may be a seasonally-dependent character. Although Californian material described herein was isolated from hosts in October and British Columbian material was isolated in July, Californian amphipods were sampled throughout much of the year and only larvae were ever recovered from these hosts. Belogurov's (1977) material was ovoviviparous, and practiced endotoky matricide, which we also observed in cultured material from Vancouver Island; these life history characters may be subject to latitudinal variation. Further collections and laboratory experimentation may be necessary to sort this out.

Sudhaus (1974) notes that the subgenus *Crustorhabditis* consists of beach-adapted species several of which having associations with crustaceans. The present material therefore agrees in life history with those previously described. Presumably worms live in decaying beach wrack and use amphipods for dispersal (Rigby, 1996). Thus, the association is probably phoretic. However, it is unusual to find adult nematodes involved in such associations; typically dispersal and therefore phoresy is practiced by larval stages.

Acknowledgments

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