

A NEW TRICHOSOMOIDID FROM THE SKIN OF *SEBASTES* SPP. (PISCES) FROM BRITISH COLUMBIA, CANADA

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ABSTRACT: A new species of trichosomoidid nematode, *Huffmanella canadensis* n. sp., is described from the skin of rockfish (*Sebastes* spp.) from the Pacific Ocean off the coast of Vancouver Island (Clayoquot Sound region), British Columbia, Canada, on the basis of the morphology of the adult worms and their eggs in the host's tissue and the biological characters. The species is characterized mainly by the shape and structure of the fully developed eggs (absence of surface envelope, surface with transverse ridges), by their small size (48–63 × 24–27 μm), and by the site of infection (skin). Besides *Huffmanella huffmanii*, this is the second *Huffmanella* species in which adult worms are known in addition to the eggs from the host's tissues (most *Huffmanella* spp. have been described only from their conspicuous eggs occurring in various tissues of fishes). Adults of *H. canadensis* differ morphologically from those of *H. huffmanii*, mainly in the structure of the male caudal end and in the distinctly elevated anterior vulvar lip of the female.

Conboy and Speare (2002) described *Huffmanella* eggs associated with linear black disfigurements and erosions of the skin, referred to as a “black-mould syndrome,” of a few species of rockfish (*Sebastes* spp.) off the coast of Vancouver Island in British Columbia, Canada. On comparing them with those of other species of *Huffmanella*, the authors found differences in size and morphology and believed that they probably belonged to a previously undescribed species. Initial attempts to recover whole-worm specimens from the fixed tissue were unsuccessful. After publishing the paper, the remaining fixed tissue from the case was thoroughly reexamined for the presence of adult nematodes. A few complete and incomplete specimens, including both males and females, were obtained. A detailed study of their morphology and a reexamination of the conspecific nematode's eggs from the fish tissue have confirmed that the specimens represent a new species, which is described herein.

MATERIALS AND METHODS

Rockfish (*Sebastes* spp.) with grossly visible skin lesions were caught by commercial fishermen in the Clayoquot Sound region of northern Vancouver Island. Fourteen of the rockfish were submitted live to a regional diagnostic laboratory. The fish varied in length from 15 to 46 cm, were in various stages of sexual maturity, and consisted of a mixed assemblage of several different species: yelloweye rockfish—*Sebastes ruberrimus* (Jordan & Gilbert); quillback rockfish—*Sebastes maliger* (Jordan & Gilbert); canary rockfish—*Sebastes pinniger* (Gill); and rougheye rockfish—*Sebastes aleutianus* (Jordan & Evermann). Visceral organs, gills, and skin tissue were collected from the fish, fixed in formalin, and submitted to the diagnostic services laboratory of the Atlantic Veterinary College in Charlottetown, Prince Edward Island, Canada. Species identification was not maintained when samples were submitted. All skin samples were examined subgrossly with a dissecting microscope. For light microscopy, both the nematodes and eggs from the tissue were cleared with glycerine. Drawings were made with the aid of a Zeiss microscope drawing attachment (Zeiss, Thornwood, New York). After examination, the specimens were stored in 70% ethanol. All measurements are in micrometers (μm) unless otherwise stated.

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DESCRIPTION

Huffmanella canadensis n. sp.

(Fig. 1)

Description: Small, filiform nematodes. Anterior end of body narrow, obtusely rounded, with indistinct cephalic papillae. Cuticle thin, with fine transverse striations. Two wide lateral bacillary bands extending along almost whole length of body. Esophagus consisting of narrow and short anterior muscular portion and simple posterior stichosome composed of single row of 32–42 stichocytes provided with large cell nuclei; stichocytes short, those in posterior part of stichosome usually subdivided into 2–3 transverse annuli; in larger specimens, 1–2 light-colored stichocytes alternating with darker (more granular) ones. Nerve ring encircling muscular esophagus approximately one-third posteriad, excretory pore not observed. Small postesophageal pseudocoelomocytes present. Entire esophagus representing 23–39% of body length. Males smaller than females.

Male (2 complete and 1 incomplete specimens; measurements of holotype in parentheses): Length of body 3,441–3,862 (3,441), maximum width 45–63 (45); width of cephalic end 12 (12), of posterior end 18–21 (18). Lateral bacillary bands 21–24 (21) wide. Length of entire esophagus 1,333–1,346 (1,333) (37–39% [39%] of body length), of muscular esophagus 147–153 (153), of stichosome 1,180–1,199 (1,180); stichocytes 40–42 (42) in number. Stichocytes in posterior part of stichosome usually 36–42 (36–42) long and 24 (24) wide. Nerve ring 48–69 (69) from anterior extremity. Single testis reaching anteriorly nearly to esophago-intestinal junction. Spicule and spicular sheath absent. Cloaca long (93 in holotype), with slightly thickened and cuticularized inner lining of its distal end. Caudal end slightly narrowed, rounded, bearing pair of large preanal papillae continuous with transverse, U-shaped band of darker hypodermal tissue surrounding laterally and dorsally distal end of cloaca; cloacal aperture slightly depressed; pair of minute subdorsal papillalike formations observed in holotype.

Female (3 complete specimens and 5 body fragments; measurements of allotype in parentheses): Body length of gravid specimens 7,711–8,160 (7,711), maximum width 90–105 (90); width of cephalic end 15–18 (15), of posterior end 33 (33). Lateral bacillary bands 36 (36) wide. Length of entire esophagus 1,768–2,054 (1,768) (23–26% [23%] of body length), of

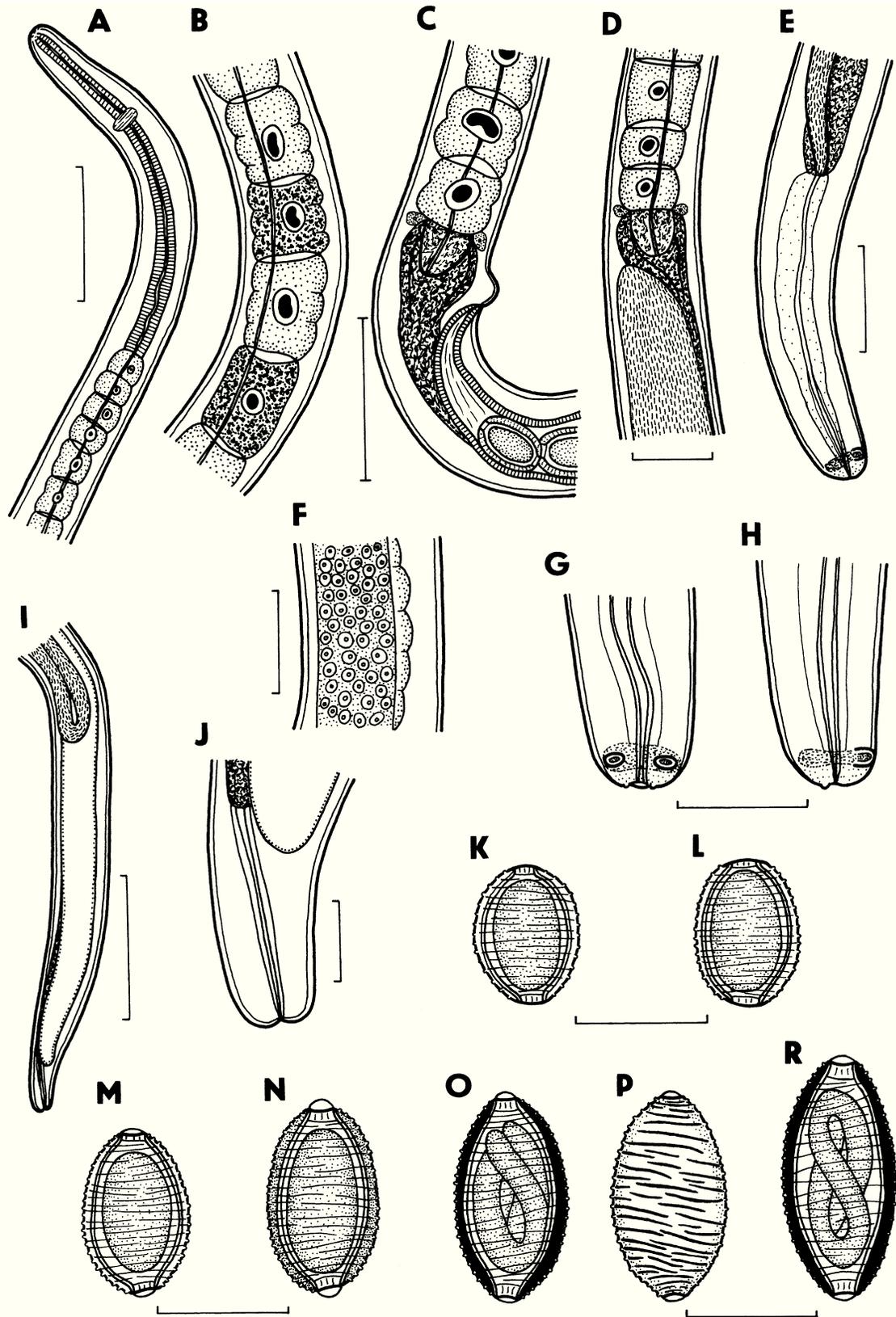


FIGURE 1. *Huffmanella canadensis* n. sp. **A.** Anterior end of male. **B.** Stichocytes in middle part of stichosome of male. **C.** Region of vulva. **D.** Region of esophago-intestinal junction of male. **E.** Posterior end of male, lateral view. **F.** Esophageal region with marked lateral bacillary band (male). **G-H.** Caudal end of male, ventral and lateral views. **I.** Posterior end of female, lateral view. **J.** Caudal end of female, lateral view. **K-L.** Most developed eggs from female uterus. **M-R.** Eggs from host's tissue (**M-N.** Light-colored, less developed eggs. **O-R.** Dark, fully developed eggs [**P.** Transverse ridges on egg surface]). Scale bars: **A** = 50 μ m; **B-C** = 100 μ m; **D-F, J-R** = 30 μ m; **G-H** = 20 μ m; **I** = 200 μ m.

muscular esophagus 180–216 (210), of stichosome 1,558–1,838 (1,558); stichocytes 32–37 (35) in number. Stichocytes in posterior part of stichosome usually 60–90 (60–90) long and 48–54 (48–54) wide. Nerve ring 75–78 (78) from anterior extremity. Vulva situated slightly posterior to esophago–intestinal junction, 30–39 (39) from end of esophagus; anterior vulvar lip distinctly elevated. Vagina short. Eggs oval, light-colored, non-embryonated, polar plugs of fully developed eggs not protruding. Eggshell appearing to be 2-layered; inner layer hyaline, outer layer somewhat thicker, with distinct narrow transverse ridges on surface. Size of eggs 32–39 × 24–27 (36–39 × 24–27), thickness of their wall being 2–3 (2–3); polar plugs 1–2 (1–2) long. Eggs arranged in 1 file in anterior part of uterus and in 2–3 files more posteriorly. Ovary single, extending posteriorly to about level of end of intestine. Oviduct forming marked coil filled with sperm some distance from posterior extremity. Rectum a colorless tube 0.111 (99) long; anus terminal.

Eggs from host's skin: Advanced eggs dark brown, oval, with 2-layered wall; inner layer thin, light-colored, outer layer dark, thick, with surface bearing numerous transverse and somewhat oblique, complete or incomplete ridges. Polar plugs light-colored, distinctly protruding; height of polar plug 6–9, width 6, height of its protruding part 2–3. Usually present thin transparent layer (envelope) covering entire egg, including polar plugs, not observed. Size of eggs including polar plugs 48–63 (mean 54) × 24–27 (26), their wall 3 thick. Eggs containing fully formed larva.

Less developed eggs (not yet containing larva) with colorless or light-brown shells. Outer egg layer with distinct transverse ridges. Polar plugs protruding. Size of eggs including polar plugs 45–57 × 24–27, thickness of their wall 2–3. Polar plug 6–9 long and 6 wide, height of its protruding part 1–3.

Taxonomic summary

Type host: Rockfish, *Sebastes* spp. (Sebastidae, Scorpaeniformes).

Other hosts: Some other *Sebastes* spp.

Site of infection: Skin of fins and that adjacent to fins.

Type locality: Clayoquot Sound region, northern Vancouver Island, British Columbia, Canada.

Deposition of specimens: Helminthological collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, in České Budějovice (cat. no. N-823).

Etymology: The specific name *canadensis* relates to the country of its origin (i.e., Canada).

Diagnosis: The genus *Huffmanella* Moravec, 1987 includes little-known histozoic parasites of various tissues (e.g., skin, gills, musculature, swimbladder, bones) of marine and, less often, freshwater fishes. Moravec (2001) in his monograph reported a total of 8 valid species in this genus: *H. carcharhini* (MacCallum, 1925), *H. banningi* Moravec, 1987, *H. huffmanii* Moravec, 1987, *H. schouteni* Moravec and Campbell, 1991, *H. japonica* Moravec, Koudela, Ogawa and Nagasawa, 1998, *H. shikokuensis* Moravec, Koudela, Ogawa and Nagasawa, 1998, *H. mexicana* Moravec and Fajer-Avila, 2000, and *H. paronai* Moravec and Garibaldi, 2000. Three additional species have recently been described by Justine (2004) from marine perciform fishes in New Caledonia: *H. branchialis* Justine, 2004 from the gills of *Nemipterus furcosus* Valenciennes, *H. fila-*

mentosa Justine, 2004 from the gills of *Gymnocranius grandoculis* (Valenciennes), and *H. ossicola* Justine, 2004 from the bones of *Bodianus loxozonus* (Snyder); he also recorded eggs of a *Huffmanella* sp., probably representing another new species, from the mucosa of the mouth of the marine perciform fish *Pentapodus* sp.

Remarks

Of the 11 above-mentioned *Huffmanella* species, 10 were described from their eggs only. Adult nematodes of this genus have so far been described only in *H. huffmanii*, a swimbladder parasite of freshwater centrarchids in Texas (Huffman and Moravec, 1988; Cox et al., 2004). Nematode specimens of uncertain developmental stage of *H. shikokuensis*, a parasite of the musculature of the marine tetraodontiform fish *Stephanolepis cirrhifer* (Temminck and Schlegel) in Japan, were found in histological sections as intracellular parasites by Moravec et al. (1998).

Since the great majority of *Huffmanella* spp. are known by their eggs only, it is necessary to make a comparison of *H. canadensis* sp. n. with its congeners on the basis of egg morphology. Also, the site of infection in the host appears to be a diagnostic characteristic of each respective *Huffmanella* species and can be considered a supplementary specific feature in this genus (Moravec, 2001).

The fully developed eggs (dark, larvated) of *H. canadensis* from the host's tissue differ from those of all other congeneric species in bearing distinct transverse ridges on the surface (the ridges are also present on light-colored eggs with an uncleaved content from the host's tissue or those in the female uterus). Moreover, the usual envelope covering the whole egg, often bearing small spines, which is reported for nearly all *Huffmanella* spp., was not observed in *H. canadensis*.

By the shape and size of fully developed eggs, *H. canadensis* somewhat resembles *H. branchialis*, *H. filamentosa*, *H. huffmanii*, *H. japonica*, and *H. paronai*; however, *H. branchialis* has a thin envelope forming a spindle-shaped balloon around the egg, *H. filamentosa* has numerous polar filaments, *H. huffmanii* has an envelope covered with spines, and the eggs of *H. mexicana* are slightly larger (63–69 × 30–33 μm). All these species, except for *H. paronai*, have a different localization in the host and are found in fish gills, swimbladder, or musculature. *Huffmanella paronai* was described from the skin of swordfish *Xiphias gladius* L. in the Ligurian Sea, Italy (Moravec and Garibaldi, 2000), and its eggs differ from those of *H. canadensis* in the absence of transverse ridges on their surface. The only other *Huffmanella* species parasitizing the host's skin is *H. carcharhini* from requiem sharks (*Carcharhinus melanopterus*) (Quoy and Gaimard) and *Carcharhinus plumbeus* (Nardo) off the Atlantic Coast of the United States, but its eggs are much larger (99–108 × 42–45 μm) and provided with a thin envelope covered with minute spines (Moravec, 2001).

Adults of *H. canadensis* can be compared only with those of *H. huffmanii*, from which they differ mainly in the shape and structure of the male tail and in the markedly elevated anterior vulvar lip in the female.

DISCUSSION

Huffman and Moravec (1988) observed that *H. huffmanii* eggs were laid with thin shells and uncleaved contents, and

then, in the host's tissue, they continued to develop. As the larva gradually developed, the egg somewhat increased in size, and the outer layer of the egg proper (chitinous layer) became thicker and gradually turned from colorless to brown or even black. The same was later observed in the eggs of *H. japonica* and *H. paronai* (Moravec et al., 1998; Moravec and Garibaldi, 2000). The present data on *H. canadensis* show that the eggs of this species are also laid in an early stage (colorless and unembryonated), whereas the larva develops in them, and their shells become dark only after the oviposition.

The eggs of *H. canadensis* are remarkable in that the transverse ridges on their surface are already present on eggs in the female uterus. The immature eggs of *H. japonica* have a superficial netlike sculpture (Moravec et al., 1998), whereas those of *H. huffmanii* are embedded in a thin envelope (vitelline layer) bearing numerous superficial spines (Huffman and Moravec, 1988; Žd'árská et al., 2001). No envelope was observed on the eggs of *H. canadensis*.

In spite of some recent attempts to experimentally study the life cycle of *H. huffmanii* (see Moravec, 2001; Cox et al., 2004), the biology of *Huffmanella* spp. remains unknown. The dark-shelled eggs of *Huffmanella* spp., which frequently occur in masses in various tissues of the infected fish hosts, can be liberated into the external environment after predation of infected fish by piscivorous animals and passage of the eggs through their digestive tract; this was demonstrated experimentally by Moravec (2001) and Cox et al. (2004) in *H. huffmanii*. However, Moravec and Garibaldi (2000) have assumed that the eggs of *H. carcharhini* and *H. paronai*, which occur in the host's epidermis or gill epithelium, are released into the environment during the normal turnover of epithelial cells (creeping of the host's several-layered epidermis). A similar mode of releasing the eggs can be supposed in *H. canadensis*. An intermediate host

is probably involved in the life cycles of *Huffmanella* spp. (Moravec, 2001).

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