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Larval *Hysterothylacium* sp. (Nematoda, Anisakidae) and trematode metacercariae from the amphipod *Paracorophium excavatum* (Corophiidae) in New Zealand

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Abstract

Previously undescribed fourth-stage larvae of anisakid nematodes were found in the haemocoel of the amphipod *Paracorophium excavatum* (Thomson, 1884) (Corophiidae) in New Zealand. Morphological examination by light microscopy showed that the worms belonged to a species of *Hysterothylacium* Ward et Magath, 1917, based on several characters including the presence of interlabia, the location of the excretory pore posterior to the nerve ring, and the characteristics of the intestinal caecum and ventricular appendix. Interestingly, several male specimens showed precocious sexual development. This is the first record of fourth larval stage and precocious adult male specimens of *Hysterothylacium* in an invertebrate host, as well as the first record of anisakid larvae in New Zealand crustaceans. In addition, metacercariae of two trematode species, *Coitocaecum parvum* and *Microphallus* sp., are recorded for the first time from the amphipod *P. excavatum*.

Key words

Anisakid larvae, *Hysterothylacium*, metacercariae, *Coitocaecum*, *Microphallus*, amphipods, *Paracorophium*, New Zealand

Introduction

Although its taxonomic status is under review (see Nadler *et al*. 2005 and references therein), the nematode family Anisakidae presently includes a wide range of parasites of aquatic organisms (fish, marine mammals, piscivorous birds, and even reptiles), some of which have considerable economic or medical importance (Anderson 2000). Although the life cycles of some species have been described in detail, basic information on larval stages and invertebrate intermediate hosts is missing for most species. In New Zealand, there has been almost no study of larval nematodes from invertebrate intermediate hosts, with only very few species being described (e.g., Moravec *et al*. 2003).

During recent investigations of amphipods in southern New Zealand, larval anisakids were found in the haemocoel of the corophiid amphipod *Paracorophium excavatum* (Thomson, 1884). Interestingly, some of the male specimens showed precocious development, i.e. the last two molts took place in the amphipod host. Here, we present the first survey of the metazoan parasites of *P. excavatum*, which is one of the most common crustacean species from coastal lagoons and brackish habitats in New Zealand. In addition, we provide a description of the larval anisakid nematodes they harbour, and report for the first time the presence of two trematode species in *P. excavatum* that were previously only known from other crustaceans.

Material and methods

The parasites were found in several samples of the amphipod *Paracorophium excavatum* collected from Lake Waihola (46°01´S, 170°05´E), near Dunedin, South Island, New Zealand, taken between March and November 2006.

The trematode metacercariae found in the amphipods belonged to previously known species, and are therefore not described here. Each metacercaria was identified under magnification, and the numbers of metacercariae of each species were recorded for each dissected amphipod.

All nematodes were fixed and stored in 70% ethanol. For light microscopy, the specimens were cleared in Amann's lac-

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tophenol (1:1:2:1 phenol:lactic acid:glycerine:water) in which they were kept during measuring and drawing. Drawings were made with the aid of a drawing tube attached to a light microscope. All measurements are given in millimeters (mm); the mean is followed by the range in parentheses. Specimens studied were deposited in the collection of the Otago Museum, Dunedin, New Zealand.

Results

Trematoda
Opecoelidae
***Coitocaecum parvum* Crowcroft, 1945**

Coitocaecum parvum Crowcroft was first described in New Zealand by Macfarlane (1939) and later redescribed by Holton (1984). Adults of this species are found in several species of freshwater fishes. The snail *Potamopyrgus antipodarum* serves as first intermediate host, and previously only two crustaceans were known as second intermediate hosts: the amphipod *Paracalliope fluviatilis* (Eusiridae) and the shrimp *Tenagomysis chiltoni* (Mysidae) (Macfarlane 1939, Holton 1984). The occurrence of *C. parvum* metacercariae in the amphipod *P. excavatum* is a new host record. All metacercariae were found in the haemocoelomic cavity, with a prevalence ranging from 7 to 15% among the different samples. Infection intensity was usually a single metacercaria per amphipod, with two metacercariae being found in the same host only rarely. Many metacercariae were progenetic and eggs were visible within the metacercarial cyst or within the haemocoelomic cavity in cases where the cyst was ruptured, as observed previously in other crustacean intermediate hosts of this species (Holton 1984, Poulin 2001).

Microphallidae
***Microphallus* sp.**

Metacercariae of an undescribed species of the genus *Microphallus* have previously been found in the amphipod *Para-*

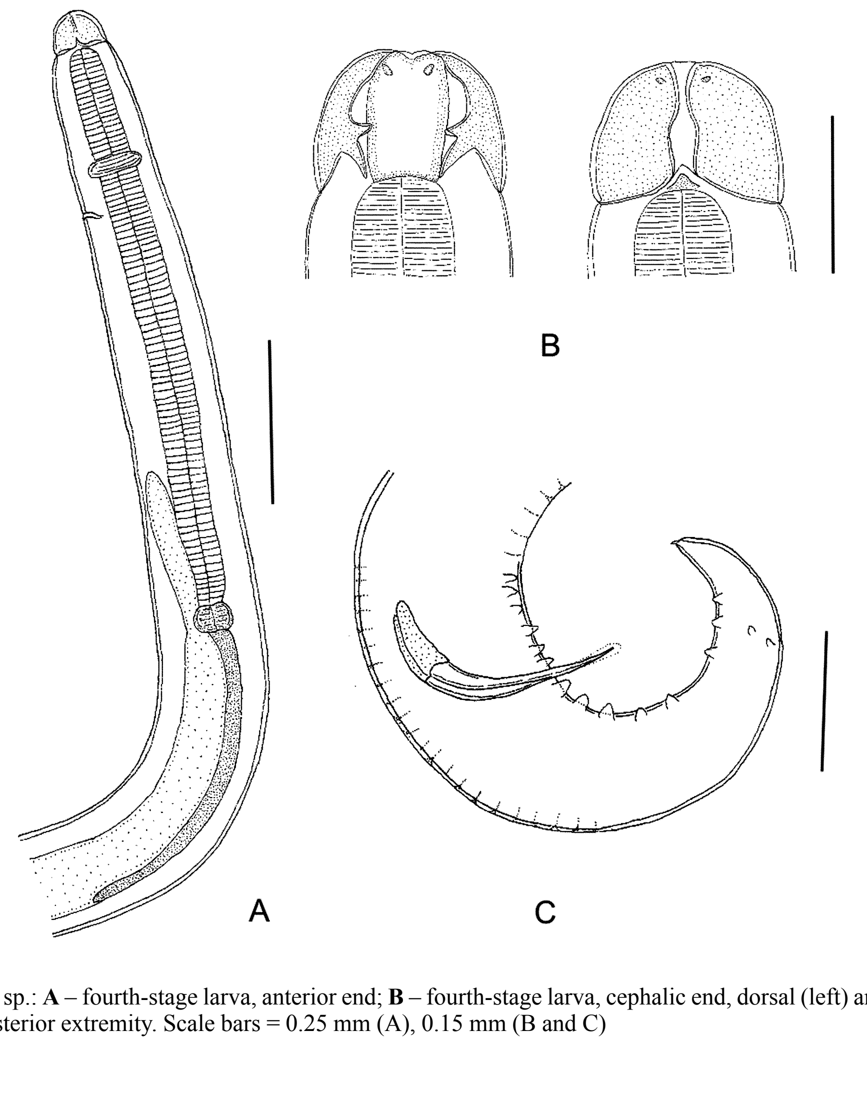

Fig. 1. *Hysterothylacium* sp.: **A** – fourth-stage larva, anterior end; **B** – fourth-stage larva, cephalic end, dorsal (left) and ventral (right) views; **C** – precocious male, posterior extremity. Scale bars = 0.25 mm (A), 0.15 mm (B and C)

calliope fluviatilis (Eusiridae) and the isopod *Austridotea annectens* (Idoteidae) from Lake Waihola (Hansen and Poulin 2005, 2006). Their occurrence in the amphipod *P. excavatum* is a new host record. All metacercariae were found in the haemocoelomic cavity, with a prevalence ranging from 65 to 80% among the different samples. Infection intensity ranged from 1 to 18 metacercariae per host.

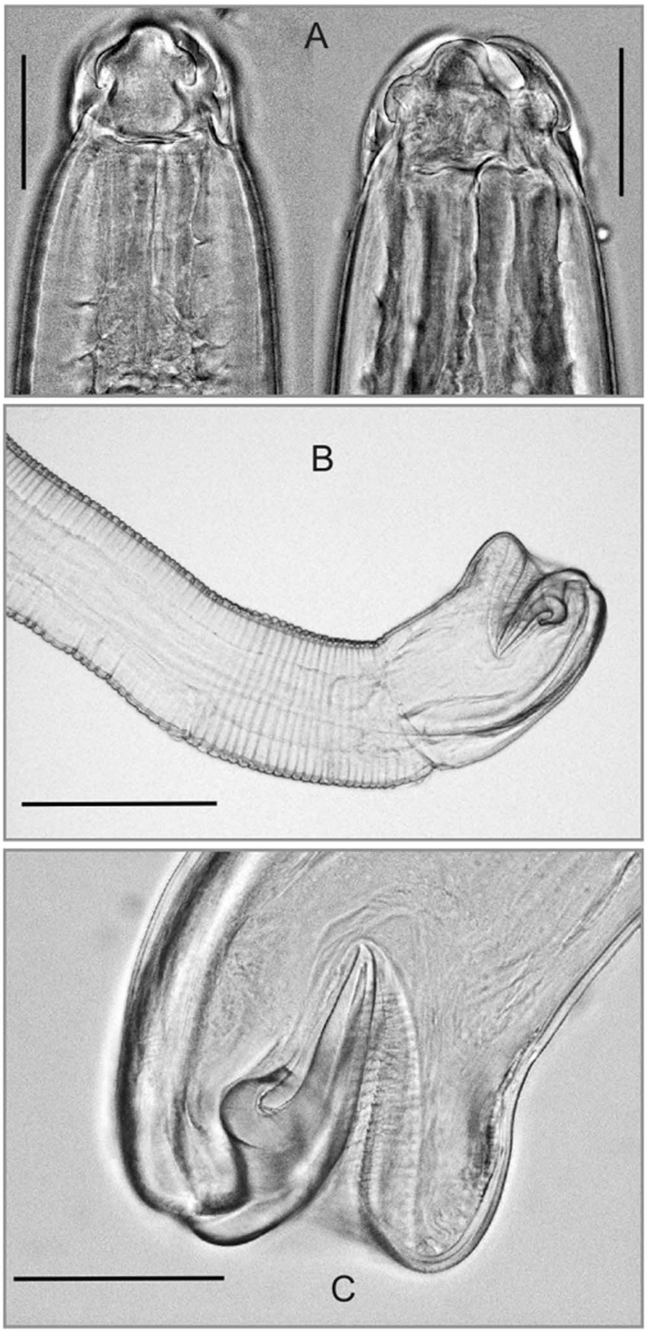

Fig. 2. *Hysterothylacium* sp.: **A** – fourth-stage larva, cephalic end, dorsal (left) and ventral (right) views; **B** – fourth-stage larva showing retracted posterior end shedding cuticle during moulting; **C** – detail of posterior end in the same condition. Scale bars = 0.25 mm (A), 0.2 mm (B), 0.1 mm (C)

Nematoda
Anisakidae
***Hysterothylacium* sp.** (Figs 1 and 2)

Description (based on nine specimens): Fourth larval stage, 4.717 (4–5.5) total length; 0.200 (0.175–0.225) maximum width. Cuticle transversally striated. No lateral alae. Lips longer than wide, approximately equal in size, pulp of subventral lips with lateral flanges forming two distinct spikes on laterodorsal borders. Interlabia do not quite reach half of the lip length. Oesophagus 0.999 (0.940–1.100) long, 21.4% (17.3–25%) of total body length. Ventriculus 0.039 (0.032–0.050) long, 0.072 (0.062–0.080) wide. Ventricular appendix 0.620 (0.550–0.690, n = 5) long. Oesophagus/ventricular appendix length ratio 1.635 (1.507–1.709, n = 5).

Intestinal caecum 0.239 (0.200–0.290, n = 7) long; ventricular appendix/intestinal caecum length ratio 2.517 (1.931–2.864, n = 5). Oesophagus/intestinal caecum length ratio 4.274 (3.276–4.900, n = 7). Excretory pore posterior to the level of the nerve ring. Distance from anterior end of body to the nerve ring, 0.384 (0.360–0.400, n = 5). Distance from anterior end of body to the excretory pore, 0.404 (0.360–0.460). Tail subconical, 0.046 (0.037–0.062, n = 5) length, tip covered with inconspicuous cuticular spines. Six specimens showed retracted posterior end shedding their cuticles during moulting. Precocious male adult specimens showed characteristic spicules and papillae on curved caudal end. Spicules ventrally alated, equal in size, 0.199 (0.191–0.206, n = 6) long. Eight pairs of postcloacal papillae arranged in two subventral rows and six pairs of precloacal papillae. Pair of papilla-like phasmids present slightly posterior to the last pair of postcloacal papillae.

Host: *Paracorophium excavatum* (Thomson, 1884) (Amphipoda, Corophiidae).

Prevalence and intensity: Between 1 and 10% of hosts were infected, depending on the sample, usually with 1 worm, but sometimes with 2.

Site of infection: Haemocoelomic cavity.

Voucher specimens: Three precocious male specimens (Otago Museum collection no. IV16246 to IV16248) and six fourth-stage larval specimens (no. IV16249 to IV16254), all stored in ethanol 70%.

Discussion

The present study provides new host records for two trematode species, and more importantly it gives a description of larval *Hysterothylacium* nematodes showing precocious sexual development. Whereas the amphipod *Paracorophium excavatum* represents merely an alternative intermediate host for the two previously-known trematodes, it becomes the first New Zealand amphipod known to harbour larval anisakids.

The combination of morphological characters including the presence of interlabia, the location of the excretory pore posterior to the nerve ring, the presence of both an intestinal caecum and a ventricular appendix, identify the nematodes collected from *P. excavatum* as belonging to the genus *Hysterothylacium* Ward et Magath, 1917. According to Gopar-Merino *et al*. (2005), *Hysterothylacium* includes 61 species, nevertheless the genus probably contains many complexes of sibling and cryptic species (Balbuena *et al*. 2000, Martín-Sanchez *et al*. 2003). Species of *Hysterothylacium* are normally found in the adult stage in the gut of fishes, whereas larvae have been regularly reported in the tissues of a variety of marine and freshwater fishes and invertebrates (including amphipods, chaetognaths, gastropods, squid, crabs, shrimps, and starfish) serving as intermediate hosts (see Marcogliese 1996, Jackson *et al*. 1997a, Balbuena *et al*. 1998, Navone *et al*. 1998, Anderson 2000, Shih and Jeng 2002).

There are many records of *Hysterothylacium* species parasitic in New Zealand fishes. Hine *et al*. (2000) listed larval and adult specimens of *Hysterothylacium* parasitizing 57 fish species from New Zealand, the majority of them being marine fish. Larvae have been recorded in a few freshwater species: *Anguilla dieffenbachii*, *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, *Retropinna retropinna* and *Salmo trutta*. The low prevalence and intensity of larval *Hysterothylacium* found in the amphipod *Paracorophium excavatum* makes it difficult to obtain more information about the possible fish host or the life cycle. Several specimens of *Gobiomorphus cotidianus*, a natural predator of this amphipod in the lake, have been examined over the years but no anisakids were ever recovered from this fish.

Fagerholm and Butterworth (1988) and Jackson *et al*. (1997a, b) have recorded precocious adult specimens of *Ascarophis* sp. (Spirurida) in amphipods and listed many examples of this phenomenon in this group of nematodes. Anderson (2000) mentioned precocial development as a feature of some ascaridoids, which possess the ability to develop to the infective stage while still in an invertebrate host. These worms might thus have the possibility to eliminate the vertebrate intermediate host and replace it with what was originally an invertebrate paratenic host. This may have evolved as a result of the very low abundance of suitable fish hosts in the lake system we studied. Precocious development of the fourth larval stage has also been recorded in anisakids by Køie (1993) and Balbuena *et al*. (1998). This is the first record of fourth larval stage and precocious adult male specimens of *Hysterothylacium* in the invertebrate host, as well as the first record of anisakid larvae in New Zealand amphipods. The finding of male adult specimens of *Hysterothylacium* with precocious sexual development demonstrates the possibility of an abbreviated life cycle not requiring the use of a vertebrate as paratenic or intermediate host. This finding might have implications for the life cycle of this species and its integration in the ecosystem.

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