New Zealand Journal of Zoology, 2008, Vol. 35: 73–84
0301–4223/08/3501–0073

Four trematode cercariae from the New Zealand intertidal snail *Zeacumantus subcarinatus* (Batillariidae)

SERGIO R. MARTORELLI
Centro de Estudios Parasitólogicos y Vectores (CEPAVE)
2 Nro. 584, 1900 La Plata
Buenos Aires, Argentina

BRIAN L. FREDENSBORG
Department of Biology
University of Texas Pan-American
Edinburg, TX 78539, USA

TOMMY L. F. LEUNG

ROBERT POULIN*
Department of Zoology
University of Otago
PO Box 56
Dunedin 9054, New Zealand

*Author for correspondence:
robert.poulin@stonebow.otago.ac.nz

Abstract The cercariae and sporocysts (or rediae) of four trematode species are described from the intertidal snail *Zeacumantus subcarinatus*: a distome xiphidiocercaria assigned to the genus *Renicola* (family Renicolidae); a monostome xiphidiocercaria belonging either to the genus *Microphallus* or *Megalophallus* (family Microphallidae); a magnacercous cercaria of the genus *Galactosomum* (family Heterophyidae); and a cercaria of the genus *Philophthalmus* (family Philophthalmidae). The morphological features of these cercariae are compared to previously described cercariae of the same genera. In addition, since the philophthalmid cercaria encysts readily on artificial substrates in the laboratory, the metacercaria of this species is also described. These cercariae are part of a diverse community of at least six digenean species parasitising the snail *Z. subcarinatus* that, together, have a major impact on the ecology and evolution of this snail.

Keywords Cercariae; Heterophyidae; Microphallidae; Philophthalmidae; Renicolidae; parasites; trematodes; *Zeacumantus subcarinatus*

INTRODUCTION

Intertidal mollusks are regularly found harbouring larval stages of parasitic trematodes, or Digenea (Poulin & Mouritsen 2003). Our knowledge of these parasites from New Zealand coastal ecosystems is very poor, however. As part of long-term parasitological surveys of mollusks from intertidal areas of the South Island, the gastropod *Zeacumantus subcarinatus* (Sowerby, 1855) (Prosobranchia: Batillariidae) was examined for cercariae. This gastropod is common and highly abundant in New Zealand soft-sediment intertidal areas as well as sheltered rocky shores. It has already been reported as first intermediate host of five trematode species, two of which have been described: the microphallid, *Maritrema novaezealandensis* Martorelli, 2004, and the echinostomatid *Acanthoparyphium* sp. (Martorelli et al. 2004; Martorelli et al. 2006). The other three cercariae previously recorded (see Hay et al. 2005; Fredensborg & Poulin 2006), as well as a new one found in more recent samples, are less common and are as yet undescribed. The goals of the present study are to describe the larval stages of these four new Digenea found in *Z. subcarinatus*, in order to complete the description of all the more prevalent species afflicting this snail.

MATERIALS AND METHODS

Infected mud snails (*Z. subcarinatus*) were collected by hand in Lower Portobello Bay, Otago Harbour, South Island, New Zealand (45°47′S, 170°42′E), and kept alive in the laboratory individually in 5 ml

Z07030; Online publication date 22 February 2008
Received 29 June 2007; accepted 18 December 2007

petri dishes at 25°C and under constant illumination to promote cercarial emission.

Cercariae were studied alive, though some specimens were also killed with nearly boiling saline followed, after 2 min, by fixation in AFA solution (a mixture of 500 ml of 85% ethanol, 60 ml of formalin, and 40 ml of glacial acetic acid in 400 ml of distilled water) for approximately 24 h. The specimens were then preserved in 10% formalin. Infected snails were crushed and rediae or sporocysts were studied alive, with some again fixed in AFA and preserved in 10% formalin. Encysted metacercariae of a philophthalmid cercaria were obtained by putting coverslips at the bottom of containers where the cercariae were present. These metacercariae were kept alive for 15 days in petri dishes with seawater, and were teased out of their cysts with the aid of needles. Excysted metacercariae were fixed in hot saline solution, transferred to AFA, and then preserved in 5% formalin until their subsequent study.

Fixed rediae, sporocysts, cercariae and metacercariae were stained with Semichon's acetocarmine or Harris haematoxylin, dehydrated in an ethanol series, cleared in clove oil and mounted in Canada balsam to obtain permanent slides.

Live specimens of all four cercariae were also stained with neutral red for detailed examination of the cephalic glands.

Measurements of the rediae, sporocysts, cercariae, and metacercariae are based on heat-killed, formalin-fixed and whole mounted specimens and are given in millimeters as the range. Voucher specimens of cercariae and metacercariae have been deposited in the Museo de La Plata, La Plata, Argentina, Helminthological Collection (MPHC) under the numbers: 5634–5637.

DESCRIPTIONS

Family Renicolidae Dollfus, 1939

Larval stages of *Renicola* sp.

SPOROCYST: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA fixed specimens. Body with thin wall, elongate, 0.6–1.3 long by 0.011–0.014 wide, with narrow anterior end, each with up to 15 cercariae. Birth pore not observed (Fig. 1A).

CERCARIA: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA-fixed cercariae (Fig. 1B,C).

Distome xiphidiocercaria. Body elongate, 0.14–0.18 long by 0.075–0.08 wide, covered with tiny spines from the oral sucker (where they are denser) to the posterior end of the body. Oral sucker 0.030–0.035 long by 0.030–0.036 wide. Stylet 0.010–0.014 long and 0.004–0.006 wide. Ventral sucker 0.030–0.034 long by 0.030–0.035 wide. Six pairs of cephalic glands filled with fine granular inclusions, located between pharynx and ventral sucker. Ducts of three pairs open near the stylet, two pairs open dorsolateral to oral sucker, and other pair opens dorsally and posterior to oral sucker near the pharynx. Tail simple, 0.09–0.11 long by 0.015–0.020 wide at base. Pre-pharynx not observed, pharynx 0.010–0.015 long by 0.010–0.012 wide, oesophagus 0.02–0.03 long, short intestine extending to middle of ventral sucker, 0.05–0.06 long. Body with large amount of granular cystogenous glands from level of intestinal bifurcation to posterior end. Reproductive primordia observed anterior to ventral sucker. Excretory bladder Y-shaped, opening toward the posterior extremity. Arms of bladder reaching to the level of middle of ventral sucker. Flame cell formula could not be determined due numerous cystogenous cells. Only two pairs of flame cells seen posterior to oral sucker. Cercarial swimming consisted in bending posterior body end downward and vibrating tail laterally. On the bottom substrate, cercaria moves by alternatively extending anterior body end and stretching and contracting tail.

HOST: *Zeacumantus subcarinatus*

SITE OF INFECTION: Digestive gland and gonad.

LOCALITY: Otago Harbour, New Zealand.

PREVALENCE: Less than 2%.

VOUCHER SPECIMEN: Number 5634 (cercaria).

REMARKS: The features of this cercaria indicates that it belongs to the family Renicolidae, and it is morphologically similar to cercariae of the genus *Renicola* described by Stunkard (1964). Within this family, at least three different kinds of cercariae have been described (Martin 1971): cercariae without a stylet but with a large finned tail, cercariae without a stylet and without a finned tail, and cercariae with short tails and with or without a stylet. Within the group of cercariae with a stylet and short tails, the following cercariae have been reported to date: cercaria of *R. thaidus* (Stunkard 1964); cercaria of *R. roscovita* (Stunkard 1932); *Cercaria opaca* (Holliman 1961); *Cercaria queenslandae* III (Cannon 1978); *Cercaria caribbea* XXXII and XXXIII (Cable 1956); *Cercaria parvicaudata* (Stunkard & Shaw 1931); Cercaria sp. XXI and Cercaria sp.

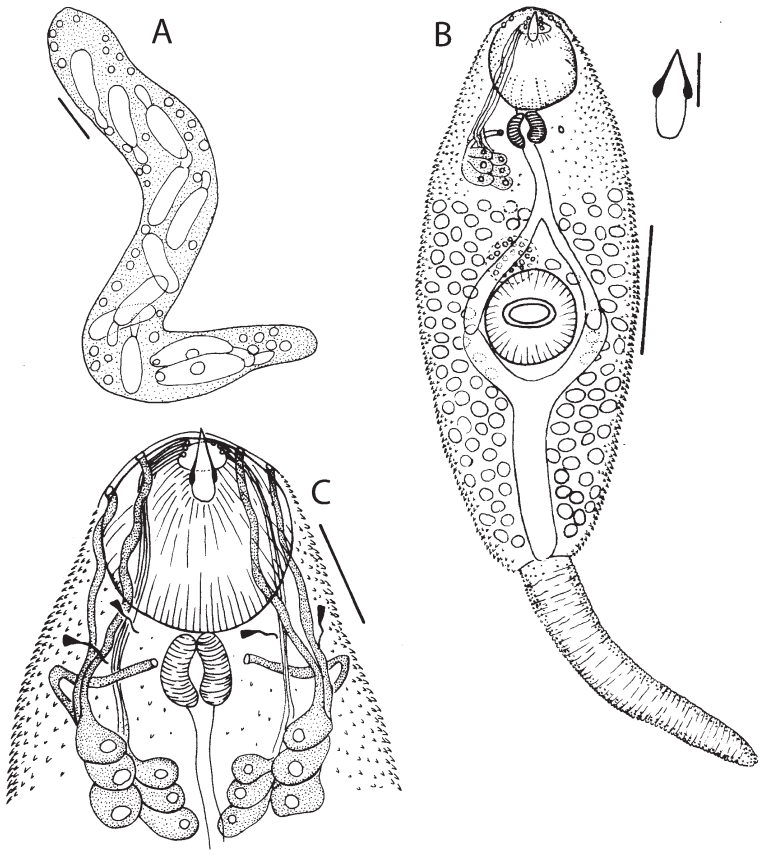


Fig. 1 *Renicola* sp. **A**, Sporocyst; scale = 0.009 mm. **B**, Cercaria, ventral view, with stylet shown separately; scale, whole cercaria = 0.04 mm, stylet = 0.06 mm. **C**, Cercaria showing detail of cephalic glands, oral sucker and stylet; scale = 0.015 mm.

XXII (Wardle 1974). The cercaria described here is similar to *Cercaria caribbea* XXXIII, Cercaria sp. XXI, and *Cercaria queenslandae* III, as all have in common the presence of a stylet and one pair of cephalic glands opening behind the oral sucker. However, the cercaria described here differs from *Cercaria queenslandae* III and Cercaria sp. XXII with respect to the total number of cephalic glands (five and eight pairs, respectively, instead of six pairs as in the cercaria described here), and to the shape of the sporocyst that is oval in those species instead of elongated. The cercaria described here differs from *Cercaria caribbea* XXXIII mainly by having larger suckers and stylet, and by having a well developed digestive system instead of no digestive system with only an embryonic pharynx. A comparison with the cercaria of *R. roscovita* will not be possible until the flame cell formula in the new cercaria can be determined (see Galaktionov & Skirnisson 2000). Overall, we conclude that the cercaria described here seems to be that of a species of *Renicola* not previously described.

Family Microphallidae Travassos 1920
Larval stages of *Microphallus* sp. or *Megalophallus* sp.

SPOROCYST: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA fixed specimens (Fig. 2A).

Sporocysts found in gonads of mud snail, *Z. subcarinatus*, as sacs more or less oval in shape, 0.028–0.035 long by 0.017–0.026 wide. Number of developed cercariae per sporocyst 10–15 in larger specimens, with similar number of embryonic masses. Birth pore not discernible.

CERCARIA: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA-fixed cercariae (Fig. 2B, 3).

Small monostome xiphidiocercaria. Body oval to elongate, 0.075–0.085 long by 0.020–0.035 wide, covered with minute spines from the oral sucker to the posterior end of the body. Tail simple, 0.060–0.075 long by 0.005–0.007 wide at base. Oral sucker 0.015–0.022 long by 0.018–0.022 wide.

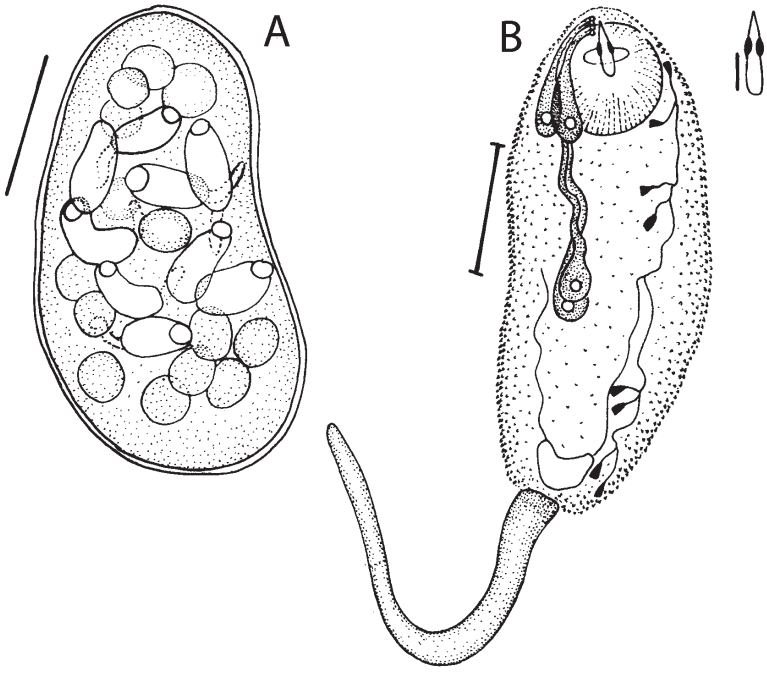


Fig. 2 Microphallidae. **A**, Sporocyst showing germ balls and fully developed cercariae; scale = 0.1 mm. **B**, Whole cercaria showing oral sucker and excretory system, with stylet shown separately; scale, whole cercaria = 0.02 mm, stylet = 0.006 mm.

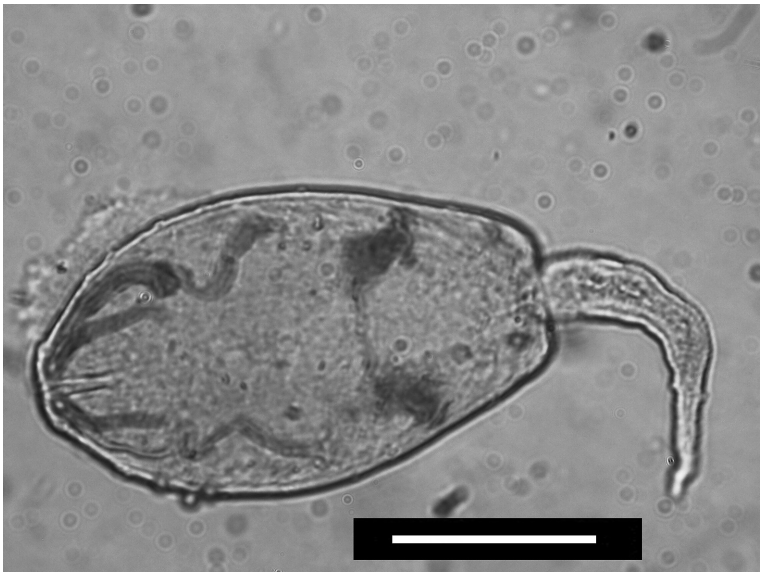


Fig. 3 Microphallidae, live cercaria stained with neutral red, showing penetration glands; scale = 0.15 mm.

Stylet 0.009–0.013 long and 0.003–0.004 wide, sub-cylindrical, with lateral protuberances (in dorsal view). Digestive system and ventral sucker not present. Four pairs of cephalic (penetration) glands stained well with neutral red (Fig. 3) and filled with fine granular inclusions. Two pairs lie near equatorial region of body, and other pairs are very short and extend from anterior body end to posterior margin of sucker. Ducts open in pairs into small pores lateral to stylet. Excretory bladder V-shaped, opening into posterior body extremity. Flame cell formula: 2[(2+2) + (2+2)] = 16.

HOST: *Zeacumantus subcarinatus*

SITE OF INFECTION: Digestive gland and gonad.

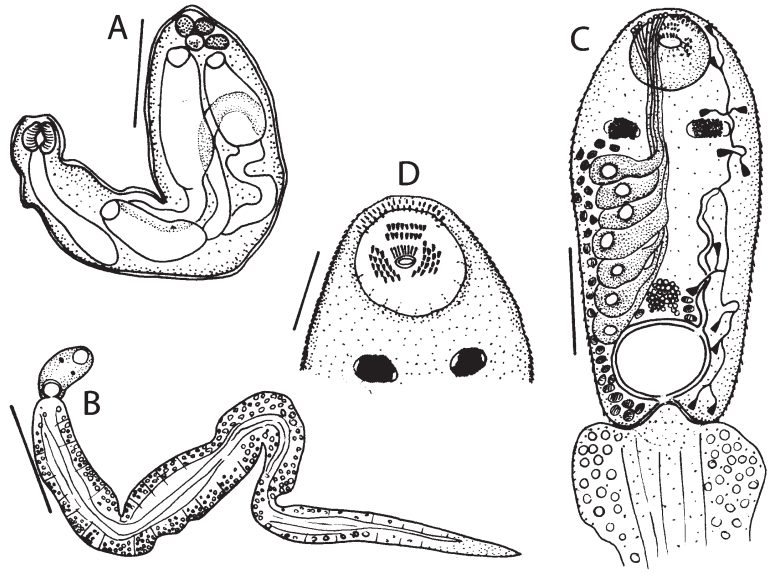


Fig. 4 *Galactosomum* sp. **A**, Redia showing cercarial embryos and germ balls; scale = 0.1 mm. **B**, Whole cercaria, lateral view; scale = 0.2 mm. **C**, Detail of cercarial body showing cephalic glands, cistogenous glands, and excretory system; scale = 0.05 mm. **D**, Detail of oral spines distribution at anterior end of the cercaria; scale = 0.03 mm.

LOCALITY: Otago Harbour, New Zealand.

PREVALENCE: Less than 2%.

VOUCHER SPECIMEN: Number 5637 (cercaria).

REMARKS: The morphological features of this cercaria (i.e., xiphidiocerca, monostome, without a digestive system, and from the ubiquita type) are consistent with the characteristics of microphallid cercariae. Furthermore, given the presence of two pairs of anterior cephalic glands situated just behind the posterior margin of the oral sucker, the cercaria described here appears related with cercariae belonging to the genera *Microphallus* Travassos 1920 and *Megalophallus* Cable, Connor & Balling, 1960. Within these genera, only eight cercariae have been reported to have a very short anterior pair of cephalic glands: *Cercaria caribbea* XXVI and XXVII (Cable 1956); cercaria of *Microphallus claviformis* (Deblock & Rose 1965); cercaria of *M. basodactylophallus* (Bridgeman 1969; Heard & Overstreet 1983); cercaria of *M. opacus* (Caveny & Etges 1971); *Cercaria queenslandae* (Cannon 1978); *Cercaria littorina saxatilis* VIII (Galaktionov & Skirnisson 2000); and cercaria of *Megalophallus carcini* (Prévot 1972). Of all these cercariae, only two have been reported with the anterior pairs of cephalic glands not extending beyond the posterior edge of the oral sucker, or just barely extending beyond it, as the cercaria described here: the cercaria of *Microphallus claviformis* and *Cercaria littorina saxatilis* VIII. The new cercaria can be distinguished from that of *M. claviformis* by its shorter stylet, by its different flame cell formula, and by its smaller sporocyst. The new cercaria differs from *Cercaria littorina saxatilis* VIII mainly by its smaller oral sucker, stylet, tail and sporocyst, and by its number of flame cells (eight in *Cercaria littorina saxatilis* VIII in contrast to 16 in the new cercaria). Until the life cycle of the new cercaria is elucidated and its other developmental stages are found, it will not be possible to determine whether it belongs to the genus *Microphallus* or *Megalophallus*.

Family Heterophyidae Odhner 1914

Larval stages of *Galactosomum* sp.

REDIA: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA fixed specimens (Fig. 4A).

Body elongated, 0.45–0.65 long by 0.10–0.14 maximum width. Pharynx spherical, 0.025–0.030 in diameter. Intestine short, 0.10–0.11 long. Birth-pore anterior but opening laterally. Body cavity filled with cercarial embryos, and germinal mass in posterior end.

CERCARIA: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA-fixed cercariae (Fig. 4B–D, 5).

Magnacercous, cercaria of opisthorchioid type. Body oval to elongate, 0.11–0.21 long by 0.09–0.1 of maximum width. Digestive system not visible. Tegument covered with spines. Sub-rectangular eyespots present with evident lateral lens. Cystogenous glands

Fig. 5 *Galactosomum* sp. live cercaria showing body and tail shape and pigmentation; scale = 0.10 mm.

numerous, from below level of eyespots to posterior body end. Oral sucker like a penetration organ, 0.014–0.038 long by 0.012–0.036 wide. Mouth surrounded by a sharp edge. Dorsal lip of mouth with three rows of spines. First row, close to mouth, with 8 spines, and other two rows with 8–10 small spines each, along anterior lip of mouth. Oral region with four lateral patches of enlarged spines on each side, with 3–6 spines each. Anterior border of oral sucker also bordered by two rows of enlarged spines (Fig. 4D). Penetration glands arranged in 7 pairs, with 5 pairs prevesicular between eyespots and genital primordium, 1 pair lateral to genital primordium and final pair lateral to excretory vesicle (paravesicular). The excretory duct runs to anterior body end and arises dorsal to oral sucker in an arrangement of 3+4+4+3 pores (Fig. 4C). Prepharynx, pharynx, oesophagus and caeca not visible. Tail 0.65–1.9 long, 10–12 times longer than body, with red-brown pigment granules and abundant vacuoles and muscular fibres (Fig. 5). Excretory vesicle rounded, thick-walled, 0.03–0.06 in diameter. Excretory arms extending to equatorial body area. Flame cell formula: 2[(2+2+2) + (2+2+2)] = 24. Excretory pore opens into apex of tail socket. Genital primordium visible anterior to excretory bladder.

HOST: *Zeacumantus subcarinatus*

SITE OF INFECTION: Gonad and digestive gland.

LOCALITY: Otago Harbour, New Zealand.

PREVALENCE: Less than 2%.

VOUCHER SPECIMEN: Number 5636.

REMARKS: Because of the presence of a penetration organ with rows of spines on the anterior lip, eyespots, seven pairs of cephalic glands, and an oval-shaped and thick-walled excretory vesicle, the new cercaria is clearly an opisthorchioid-type cercaria of the family Heterophyidae. These cercariae are common within the genera *Galactosomum* and *Condylocotyla* (Pearson & Prévot 1985; Beuret &

Pearson 1994). However, the cercaria of *C. pilodora* reported by Pearson & Prévot (1985) does not have the ventrolateral patches of enlarged spines that are present in most of the *Galactosomum* cercariae described to date, as well as in the cercaria described here. This places the latter within the genus *Galactosomum*. Among the cercariae reported as belonging to the genus *Galactosomum*, the cercaria from the gastropod *Z. subcarinatus* most closely resembles the following: *Cercaria caribbea* XVII, XVIII and XIX (Cable 1956), *Cercaria komiyai* (Ito 1956), *Cercaria caribbea* LXXI (Cable 1963), the cercariae of *G. timondavidi* and *G. nicolai* (= *Cercaria mirabilicaudata*) (Prévot 1967, 1973), *Cercaria melanocrucifera* (Reimer & Anantaraman 1968), *Cercaria queenslandae* IX (Cannon 1978), the cercaria of *G. ussurriese* (Rekharani & Madhavi 1985), and the cercaria of *G. bearupi* (Beuret et al. 2000).

The cercaria described here differs from *Cercariae komiyai* and *Cercaria melanocrucifera* by the presence of cephalic glands in paravesicular position instead of only prevesicular cephalic glands. With respect to all the remaining cercariae, only two, *Cercaria caribbea* XVIII and the cercaria of *G. bearupi*, were reported as having three rows of spines on the dorsal lip of the mouth, just like the cercaria described here. However, the new cercaria differs from *Cercaria caribbea* XVIII, found in the snail *Turritella exoleata* from Puerto Rico, mainly in the arrangement and number of spines on the dorsal lip of the mouth, in the ventrolateral patches of enlarged spines, by its flame cell formula, in the size and distribution of cephalic glands, and in the size and morphology of the tail. Although it resembles the cercaria of *G. bearupi* reported from Australia in the snail *Clypeomorus batillariaeformis*, the cercaria described here is different with respect to its number of flame cells and the position of the penetration glands, which are in a more anterior position in the new cercaria as it has only one gland in paravesicular position. The arrangement of the spines on the dorsal lip and the enlarged spines of the ventral patches, as well as the spines present on the anterior border of the penetration organ, are very similar in both cercariae. The new cercaria seems to belong to a species closely related to *G. bearupi*.

Family Philophthalmidae Travassos 1918

Larval stages of *Philophthalmus* sp. (probably *P. burrili* Howell & Bearup)

REDIA: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA fixed specimens (Fig. 6A,B).

Daughter rediae found in digestive gland and gonad of the host in two principal stages of development. Smallest redia (Fig. 6A) with numerous germinal balls in the posterior end, 0.400–0.480 long and 0.060–0.080 wide. Pharynx 0.045–0.050 long by 0.040–0.050 wide, connected to a long digestive sac, 0.250–0.350 long. Caudal appendage present at posterior end. Larger redia (Fig. 6B) with elongate body, 1.50–1.70 long and 0.250–0.360 wide, with prominent caudal appendages. Pharynx 0.050–0.060 long and 0.045–0.050 wide, followed by short intestinal sac, 0.300–0.500 long. Each redia contained 10–20 fully developed cercariae.

CERCARIA: Description based on live and fixed specimens. Measurements (mm) taken on 10 heat-killed and AFA-fixed cercariae (Fig. 6C).

Echinostome-like cercaria, with body spinous, 0.400–0.600 long by 0.170–0.200 wide. Abundant cystogenous glands, stained well with neutral-red, and filled with granular inclusions, in region from pharynx to posterior body end. Oral sucker (OS) circular, 0.060–0.080 in diameter, with mouth subterminal. Prepharynx short, 0.020–0.060 long; pharynx muscular, 0.030–0.040 long by 0.028–0.030 wide; oesophagus 0.050–0.060 long; intestinal caeca 0.200–0.250 long, reaching posterior end of body. Ventral sucker (VS) at mid-ventral level of body, 0.080–0.100 in diameter. Sucker ratio (OS/VS) 0.78. Eleven pairs of cephalic glands at level of intestinal bifurcation, each with a duct opening at anterior end of body. None of these glands stain well with neutral red. Genital primordia not present. Excretory vesicle oval, receiving main collecting canals through short medial duct. Main collecting canals extending to level of pharynx, without concretions inside. Flame cells 18 in total on each side of body; flame cell formula: 2[(3+3+3) + (3+3+3)] = 36. Excretory pores on both sides of proximal tail area.

METACERCARIA: Description based on 10 live encysted specimens (Fig. 7, 8). The cercariae emerged from the snail around midday, and encysted readily on the bottom of the petri dishes. After attachment to the substrate, secretions from cystogenous cells formed the cyst surrounding the metacercaria, which still had a tail at this stage. In some completed cysts, the tail remained attached to the cyst wall (Fig. 8).

Metacercarial cyst flask-like, 0.400–0.500 long and 0.180–0.260 wide, neck 0.12–0.15 long, with a bilayered wall. Largest external wall 0.040–0.045 thick. In live cysts, it was possible to see the metacercaria inside, folded upon itself and performing

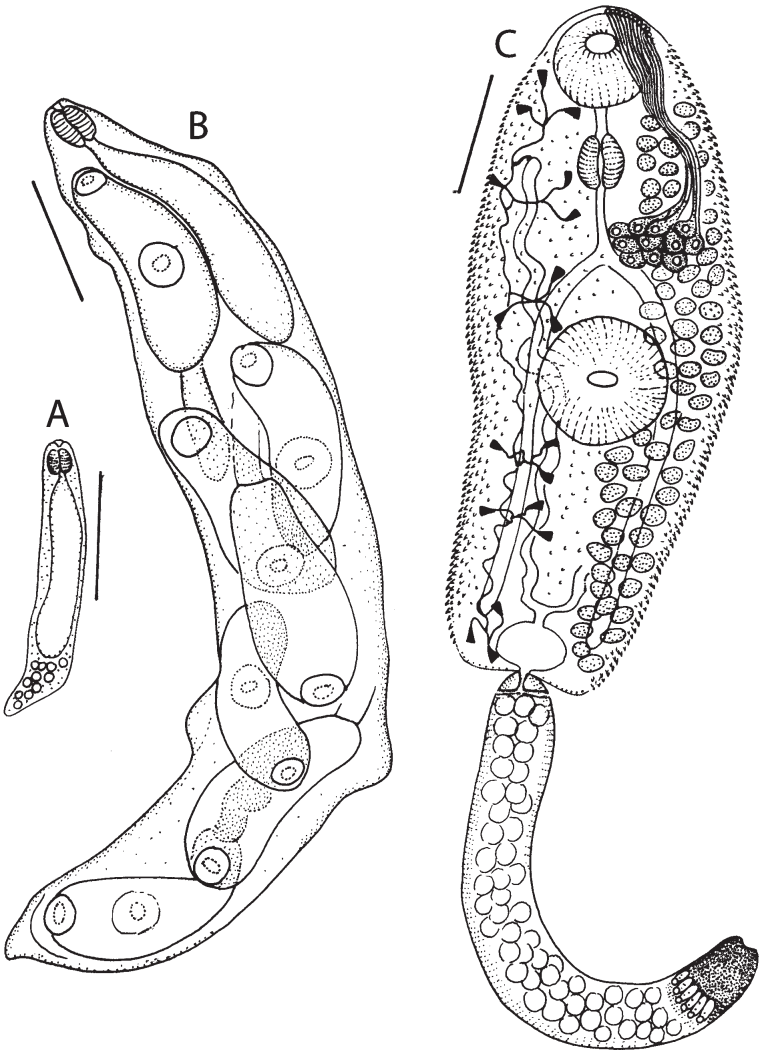


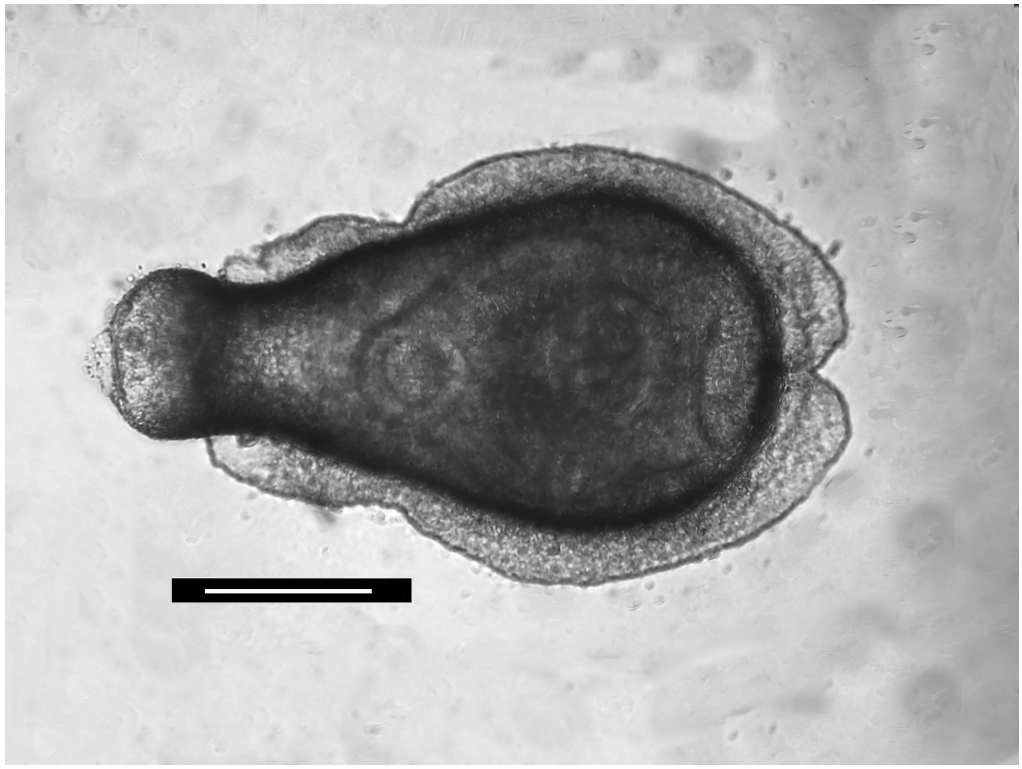
Fig. 6 *Philophthalmus* sp. **A**, Small daughter redia with germ balls; scale = 0.1 mm. **B**, Mature daughter redia containing fully developed cercariae; scale = 0.2 mm. **C**, Whole cercaria showing suckers, cephalic and cystogenous glands, excretory system and tail with body inclusions; scale = 0.1 mm.

slow movements. Encysted metacercariae were kept alive for over a month in a flask with sea water and still showed movements after that time.

EXCYSTED METACERCARIA: Description based on 10 formalin-fixed specimens obtained 10 days after encystment. Encysted metacercariae could readily be excysted under pressure or by breaking the cyst open with needles under cover slides. Body of metacercaria elongate, spinous, widest at ventral sucker level, 0.570–0.630 long by 0.116–0.220 wide. Oral sucker subterminal, 0.070–0.080 in diameter. Ventral sucker 0.080–0.110 in diameter. Sucker ratio (OS/VS) 0.80. Prepharynx 0.020–0.040 long; pharynx 0.030–0.040 long by 0.025–0.035 wide; oesophagus 0.050–0.090 long; intestinal caeca reaching posterior end of body. Excretory vesicle oval

Fig. 7 *Philophthalmus* sp., flask-like metacercarial cyst showing multilayered cyst wall; scale = 0.10 mm. ➤

Fig. 8 *Philophthalmus* sp., metacercaria showing tail still attached to cyst; scale = 0.10 mm. ➤



as in cercaria; main collecting canals extending to anterior level of pharynx.

HOST: *Zeacumantus subcarinatus*

SITE OF INFECTION: Digestive gland and gonad.

LOCALITY: Otago Harbour, New Zealand.

PREVALENCE: From less than 5% up to 30%, depending on site of collection.

VOUCHER SPECIMEN: Number 5635 (cercaria).

REMARKS: Based on its *Echinostoma*-like appearance but without a collar of spines, and because it possesses a glandular invaginated tail tip, and finally because it encysts outside the host on any substrate in a flask-like cyst, the cercaria described here clearly belongs to the family Philophthalmidae, and more specifically the genus *Philophthalmus*. Species of this genus are known to parasitise the eyes of birds and mammals, including man; adults of this genus have been found in gulls, *Larus dominicanus*, in New Zealand (Weekes 1982). A cercaria assigned to the genus *Philophthalmus* has been previously reported from the snail *Z. subcarinatus* in Wellington, New Zealand (Howell 1965). Philophthalmid cercariae have been described from freshwater and marine snails around the world. To date, five species of *Philopthalmus* cercariae have been reported from marine snails (Abdul-Salam et al. 2004): *P. hegenery* from the United States (Penner & Fried 1963); *P. burrili* from Australia (Howell & Bearup 1967); *P. larsoni* (Penner & Trimble 1970); *P. andersoni* (Dronen & Penner 1975); and *P. hegeneri* from Kuwait (Abdul-Salam et al. 2004). These last authors give a table comparing all the larval stages of *Philophthalmus* species from marine snails, and the measurements of all known larvae, including the cercaria described here, are all very similar. Nevertheless, the cercaria from *Z. subcarinatus* is more similar to those reported from Australia and Kuwait (Howell & Bearup 1967; Abdul-Salam et al. 2004). The cercaria described here differs from the Kuwait one, however, with respect to the number of cephalic glands, which are more numerous in the cercaria of *Z. subcarinatus*, as well as by its greater number of flame cells, by the morphology of the excretory bladder, and by the connection of the main excretory vessels that arise separately in the cercaria from Kuwait but from a common short base in the cercaria described here. When compared to the cercaria of *P. burrili* described from Australia, the New Zealand species is very similar in terms of its general body size, its number of cephalic glands (11 pairs can be counted in the illustration of the Australian cercaria), the size and morphology of the metacercarial cyst, and the characteristics of the mature daughter rediae. The only obvious difference is in the number of flame cells, which is greater in the cercaria from New Zealand. Although this difference might indicate that these two cercariae belong to similar and closely related species, their general similarity suggests that they may be the same species. It is also worth noting that *P. burrili* has been found in the snail *Velacumantus australis* (Quoy & Gaimard, 1834), a relative of the snail *Z. subcarinatus* that serves as host to the cercaria described here. Until the life cycle of this new cercaria can be elucidated, we tentatively assign it to *P. burrili*.

CONCLUSIONS

Adding to the previous descriptions of the microphallid *Maritrema novaezealandensis* (Martorelli et al. 2004) and the echinostomatid *Acanthoparyphium* sp. (Martorelli et al. 2006), the present investigation now brings up to six the number of described digenean species known to infect the snail *Zeacumantus subcarinatus*. The combined effect of these digeneans on populations of the snail hosts is considerable: the digeneans castrate the snails, cause changes in their shell morphology, and lower their population density and biomass (Fredensborg et al. 2005; Hay et al. 2005). The combined action of these six digenean species has also selected for faster growth rates and lower age at maturity in the most severely parasitised snail populations (Fredensborg & Poulin 2006). The great diversity of digenean parasites in *Z. subcarinatus* thus plays important ecological and evolutionary roles. We have only scratched the surface of the vast biodiversity of parasites afflicting New Zealand marine mollusks, and a huge effort will be needed if we are to understand the full role played by parasites and diseases in the functioning of marine ecosystems.

ACKNOWLEDGMENTS

We thank two anonymous referees for their very useful comments, and Felicia Cardillo for her excellent technical assistance in the laboratory. This work is an offshoot from research funded by a grant from the Marsden Fund of New Zealand to R. Poulin; this work was also in part funded by a reseach grant from CONICET to S. Martorelli.

REFERENCES

Abdul-Salam J, Sreelatha BS, Ashkanani H 2004. The eye fluke *Philophthalmus hegeneri* (Digenea: Philophthalmidae) in Kuwait Bay. Kuwait Journal of Science & Engineering 31: 119–134.

Beuret J, Pearson JC 1994. Description of a new zygocercous cercaria (Opisthorchioidea: Heterophyidae) from prosobranch gastropods collected at Heron Island (Great Barrier Reef, Australia) and a review of zygocercariae. Systematic Parasitology 27: 105–125.

Beuret J, Scott DA, Pearson JC 2000. The cercaria of *Galactosomum bearupi* Pearson, 1973 (Trematoda: Heterophyidae) at Heron and Masthead Islands, Great Barrier Reef, Australia. Systematic Parasitology 46: 69–77.

Bridgeman JF 1969. Life cycles of *Carneophallus choanophallus* n. sp. and *C. basodactylophallus* n. sp. (Trematoda: Microphallidae). Tulane Studies in Zoology and Botany 15: 81–104.

Cable RM 1956. Scientific survey of Porto Rico and the Virgin Islands: marine cercariae of Puerto Rico. Annals of the New York Academy of Sciences 16: 491–577.

Cable RM 1963. Marine cercariae from Curacao and Jamaica. Zeitschrift fur Parasitenkunde 23: 429–469.

Cannon LRG 1978. Marine cercariae from the gastropod *Cerithium moniliferum* Kiener at Heron Island, Great Barrier Reef. Proceedings of the Royal Society of Queensland 89: 45–57.

Caveny BA, Etges FJ 1971. Life history studies of *Microphallus opacus* (Trematoda: Microphallidae). Journal of Parasitology 56: 1215–1221.

Deblock FR, Rose F 1965. Contribution à l'étude des Microphallidae Travassos 1920 (Trematoda) des oiseaux de France XI- Identification de la cercaire de *Microphallus claviformis* (Brandes 1888). Bulletin de la Société Zoologique de France 90: 299–314.

Dronen NO, Penner IR 1975. Concerning *Philophthalmus andersoni* sp. n. (Trematoda: Philophthalmidae), another helminth from birds which develops in a marine gastropod. University of Connecticut Occasional Papers, Biological Science Series 2: 217–224.

Fredensborg BL, Mouritsen KN, Poulin R 2005. Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. Marine Ecology Progress Series 290: 109–117.

Fredensborg BL, Poulin R 2006. Parasitism shaping host life-history evolution: adaptive responses in a marine gastropod to infection by trematodes. Journal of Animal Ecology 75: 44–53.

Galaktionov KV, Skirnisson K 2000. Digeneans from intertidal molluscs of SW Iceland. Systematic Parasitology 47: 87–101.

Hay KB, Fredensborg BL, Poulin R 2005. Trematode-induced alterations in shell shape of the mud snail *Zeacumantus subcarinatus* (Prosobranchia: Batillariidae). Journal of the Marine Biological Association of the United Kingdom 85: 989–992.

Heard RW, Overstreet RM 1983. Taxonomy and life histories of two North American species of "*Carneophallus*" (*Microphallus*) (Digenea: Microphallidae). Proceedings of the Helminthological Society of Washington 50: 170–174.

Holliman RB 1961. Larval trematodes from the Apalachee Bay area, Florida, with a check list of known marine cercariae arranged in a key to their superfamilies. Tulane Studies in Zoology 9: l–74.

Howell M 1965. Notes on a potential trematode parasite of man in New Zealand. Tuatara 13: 182–184.

Howell MJ, Bearup AJ 1967. The life histories of two bird trematodes of the family Philophthalmidae. Proceedings of the Linnean Society of New South Wales 92: 182–194.

Ito J 1956. Studies on the brackish water cercariae in Japan. II. Two new longtailed cercariae, *Cercaria komiyai* n. sp. and *Cercaria nigrocaudata* n. sp. in Tokyo Bay (Trematoda). Japanese Journal of Medical Science and Biology 9: 235–242.

Martin WE 1971. Larval stages of renicolid trematodes. Transactions of the American Microscopical Society 90: 188–194.

Martorelli SR, Fredensborg BL, Mouritsen KN, Poulin R 2004. Description and proposed life cycle of *Maritrema novaezealandensis* n. sp. (Microphallidae) parasitic in red-billed gulls, *Larus novaehollandiae scopulinus*, from Otago Harbor, South Island, New Zealand. Journal of Parasitology 90: 272–277.

Martorelli SR, Poulin R, Mouritsen KN 2006. A new cercaria and metacercaria of *Acanthoparyphium (*Echinostomatidae) found in an intertidal snail *Zeacumantus subcarinatus* (Batillaridae) from New Zealand. Parasitology International 55: 163–167.

Pearson JC, Prévot G 1985. A revision of the subfamily Haplorchinae Looss, 1899 (Trematoda: Heterophyidae). III. Genera *Cercarioides* and *Condylocotyla* n.g. Systematic Parasitology 7: 1659–1697.

Penner LR, Fried B 1963. *Philophthalmus hegeneri* sp. n., an ocular trematode from birds. Journal of Parasitology 49: 974–977.

Penner LR, Trimble JJ 1970. *Philophthalmus larsoni* sp. n., an ocular trematode from birds. University of Connecticut Occasional Papers, Biological Science Series 1: 265–273.

Poulin R, Mouritsen KN 2003. Large-scale determinants of trematode infections in intertidal gastropods. Marine Ecology Progress Series 254: 187–198.

Prévot G 1967. Contribution à l'étude des cercaires de prosobranches de la région marseillaise: *Cercaria mirabilicauda* n. sp. (Trematoda: Digenea, Opisthorchioidea) de *Cerithium vulgatum* Brug. Bulletin de la Société Zoologique de France 92: 515–523.

Prévot G 1972. Contribution à l'étude des Microphallidae Travassos 1920 (Trematoda). Cycle évolutif de *Megalophallus carcini* Prévot et Deblock 1970 parasite du goéland (*Larus argentatus*). Bulletin de la Société Zoologique de France 97: 157–163.

Prévot G 1973. Cycle évolutif de *Galactosomum timondavidi* Pearson et Prévot, 1971 (Trematoda: Heterophyidae), parasite du Goéland à pieds jaunes, *Larus argentatus*. Annales de Parasitologie Humaine et Comparée 48: 457–467.

Reimer LW, Anantaraman S 1968. *Cercaria melanocrucifera*, a new magnacercous cercaria (Opisthorchioidea) from the marine gastropod, *Turritella attenuata* Reeve, 1897, from the Bay of Bengal, Madras. Current Science 37: 316–318.

Rekharani Z, Madhavi R 1985. The cercaria and metacercaria of *Galactosomum ussuriensis* Oshmarin, 1963 (Trematoda: Heterophyidae). Journal of Helminthology 59: 295–300.

Stunkard HW 1932. Some larval trematodes from the coast in the region of Roscoff, Finistere. Parasitology 24: 321–343.

Stunkard HW 1964. Studies on the trematode genus *Renicola*: observations on the life-history, specificity, and systematic position. Biological Bulletin 126: 468–489.

Stunkard HW, Shaw CR 1931. The effect of dilution of sea water on the activity and longevity of certain marine cercariae, with description of two new species. Biological Bulletin 61: 242–271.

Wardle WJ 1974. A survey of the helminth parasites of the marine molluscs of the Galveston Bay area, Texas. Department of Biology, Texas A&M University. 322 p.

Weekes PJ 1982. Checklist of helminth parasites of birds in New Zealand. New Zealand Journal of Zoology 9: 451–460.