

A new cercaria and metacercaria of *Acanthoparyphium* (Echinostomatidae) found in an intertidal snail *Zeacumantus subcarinatus* (Batillariidae) from New Zealand

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Abstract

A new 23-collar-spined cercaria and metacercaria are described from intertidal molluscs of the coast of New Zealand. The new cercaria found emerging from the mud snails *Zeacumantus subcarinatus* (Sowerby) (Prosobranchia: Batillariidae) is characterized mainly by the number and arrangement of the cephalic glands, the size of the suckers, and the size and number of the collar spines. The cercaria encysts in the cockle *Austrovenus stutchburyi* (Wood) (Bivalvia: Veneridae) which lives in sympatry with the first intermediate host throughout New Zealand. Laboratory infections of cockles by cercariae from naturally infected snails resulted in metacercariae identical to those found in naturally infected cockles. The main features of the cercaria and metacercaria are the presence of a reniform collar with 23 spines, two pairs of small cephalic glands at the oral sucker level, another two pairs of much longer ones posterior to the pharynx, and the excretory vesicle Y-shaped with the main collecting canals extending to the anterior level of the ventral sucker. Each of the main collecting canals had 10–11 pairs of bilateral diverticula between the anterior edge of the ventral sucker and anterior body end. A brief discussion of its possible life cycle and ecology is also provided. © 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Acanthoparyphium*; A new cercaria; Metacercaria; Mollusk; New Zealand

1. Introduction

The presence of cercariae of the family Echinostomatidae Looss, 1899, has been widely reported from different marine areas around the world. During a parasitological survey of molluscs from intertidal areas of South Island, New Zealand, 23-spined cercariae and metacercariae were recovered, respectively, from the endemic mud snail *Zeacumantus subcarinatus* (Sowerby) (Prosobranchia: Batillariidae) and the cockle *Austrovenus stutchburyi* (Wood) (Bivalvia: Veneridae). The morphological features of these larvae clearly place them in the genus *Acanthoparyphium* Dietz, 1909. This genus has been documented as parasitizing snails and birds in marine areas of

USA, Philippines, Korea, Kuwait, India, Puerto Rico, Japan and Australia. *Acanthoparyphium tyosenense* Yamaguti, 1939 has also been reported in humans in Korea [1–3]. In New Zealand two species have been reported from bird hosts: *Acanthoparyphium spinulosum* Johnston, 1917 from *Limosa lapponica baueri* [4] and *Acanthoparyphium* sp. from *Haematopus ostralegus finschi* [5].

Life cycles of species of *Acanthoparyphium* have been published for *A. spinulosum* [6,7], *Acanthoparyphium paracharadrii* Velazquez, 1964 [8], and *A. tyosenense* [9].

In this paper, a new cercaria and metacercaria of this genus are described and illustrated. A brief discussion of their possible life cycle and cercarial behavior information is also provided.

2. Materials and methods

Infected mud snails (*Z. subcarinatus*) were collected by hand in Lower Portobello Bay, Otago Harbour, South Island, New

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Zealand (45°47' S, 170° 42' E), and kept alive in the laboratory individually in 5-ml Petri dishes at 25 °C and under constant illumination to promote cercarial emission.

Cercariae were studied alive, and some specimens were fixed with nearly boiling saline and, after 2 min, they were transferred to AFA solution (containing 85% ethanol, formalin and glacial acetic acid), in which they were kept for approximately 24 h. The specimens were then preserved in 5% formalin. Infected snails were crushed and rediae were fixed in 5% formalin. The rediae and cercariae described herein were taken from four mud snails. One additional individual of *Z. subcarinatus* was kept alive in the laboratory during 7 months for the study of live cercariae and their patterns of emergence.

Cockles (*A. stutchburyi*) were collected in the same place (Otago Harbour in Lower Portobello Bay) as the mud snail, and dissected for metacercariae. Metacercariae with 23 collar spines were found encysted in the foot of the cockles. Encysted metacercariae of *Curtuteria australis* Allison, 1979 (Echinostomatidae) with 31 collar spines also were obtained.

Encysted metacercariae ($n=44$) collected from three cockles were placed in small Petri dishes containing saline solution at 40–42 °C to facilitate excystment. Excysted metacercariae were fixed in hot saline solution, transferred to AFA, and then preserved in 5% formalin until their subsequent study.

Fixed rediae, 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.

Live specimens of cercariae were also stained with neutral red for cephalic gland observation.

Measurements of the rediae, cercariae, and metacercariae were based on whole-mounted specimens and given in micrometers as the range. The voucher specimens of cercariae and excysted metacercariae were deposited in the Museo de La Plata, La Plata, Argentina, Helminthological Collection (MPHC) under the numbers: 5537–5538.

3. Results

Larval stages of *Acanthoparyphium* sp.

3.1. Redia

Redia probably in daughter stage with collar, and elongate body, 740–870 long and 160–180 wide. Pharynx 50–55 long and 48–55 wide, followed by a short intestine 200–230 long. One pair of posterior ambulatory buds was present at 430–620 from anterior end. Birth pore was evident at 120–140 from mouth. More developed or mature rediae were yellowish, including well-developed cercariae, immature forms and numerous germ bodies (Fig. 3).

3.2. Cercaria

Description was based on 10 live cercariae. Measurements were based on 10 fixed specimens. Echinostome cercariae with simple tail were 230–325 long by 20–30 wide. Body spinous

was 400–520 long by 105–180 wide, with abundant cystogenous glands filled with rod-like bodies in region from pharynx to posterior body end. Collar reniform; collar spines 23 in total number, similar in shape and size (7–8 long by 3–4 wide at basal level), were arranged in a dorsally uninterrupted single row. Oral sucker (OS) was circular, 35–50 in diameter, with mouth subterminal; prepharynx short, 23–45 long; pharynx muscular, 20–26 long by 18–24 wide; esophagus 86–150 long; intestinal caeca reaching posterior end of the body. Ventral sucker (VS) at mid-ventral level of the body was 52–72 in diameter. Sucker ratio (OS/VS) was 0.69. Two pairs of small cephalic glands at oral sucker level were almost median, each with a duct opening at anterior end of body. Another two pairs of much larger ones were submedian, posterior to pharynx, each with a duct opening at anterior end of body. Each duct has a

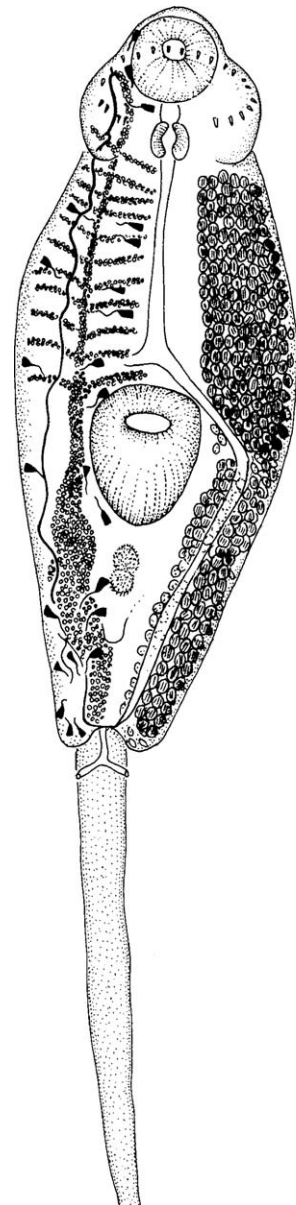


Fig. 1. Cercaria from *Zeacumantus subcarinatus*, ventral view. Scale bar=45 µm.

prominent reservoir at oral sucker level. All these glands were stained well with neutral red. Two genital primordia were present, tandem, posterior to ventral sucker (Figs. 1–2, 6).

Excretory vesicle was Y-shaped, with main collecting canals extending to anterior level of ventral sucker. Main collecting canals have 10–11 pairs of bilateral diverticula between anterior edge of ventral sucker and anterior end of body. Excretory vesicle, main collecting canals and diverticula were filled with spherical concretions. Flame cells were 24 in total on each side of body; flame cell formula $2[(3+3+3+3)+(3+3+3+3)]=48$. Tail excretory canal extends short in tail, bifurcating, opening at 2 lateral pores near base.

These cercariae emerged from the snail around the mid-day, lived for 24–36 h, and showed a positive phototaxis. Cercariae also swam on along the bottom, showing a negative geotaxis. In two instances, some specimens encysted on the bottom of the dish, forming a delicate, 200–250 μm diameter cyst (Figs. 4–5, 7).

3.3. Metacercaria

Based on 10 whole-mounted specimens. Metacercarial cyst was spherical, 380–470 in diameter, with a bilayered wall. In live and fixed metacercariae it was possible to see the collar spines, the suckers and the excretory organs filled with concretions through the cyst wall (Figs. 5–7).

Excysted metacercariae with well-developed reniform collar with 23 spines. Body elongate, spinous, widest at ventral sucker level, 350–450 long by 100–142 wide. Spines were 18–26 long by 7–10 wide at basal level. Oral sucker was subterminal, 36–45 in diameter. Ventral sucker was 50–67 long by 50–67 wide. Sucker ratio (OS/VV) was 0.66. Prepharynx was 27–42 long; pharynx 28–31 long by 19–22 wide; esophagus 90–118 long; intestinal caeca reaching posterior end of body. Testes tandem

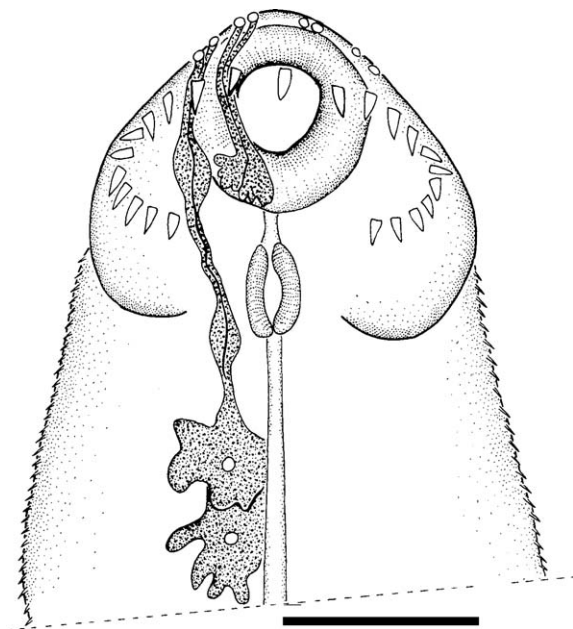


Fig. 2. Cercaria from *Zeacumantus subcarinatus* showing cephalic glands, ventral view. Scale bar=50 μm .

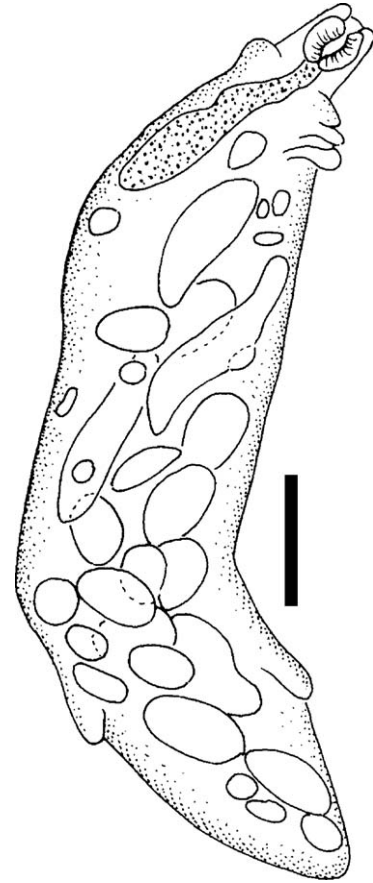


Fig. 3. Redia from *Zeacumantus subcarinatus*. Scale Bar=100 μm .

was median. Ovary median was posterior to ventral sucker. Terminal genitalia were dorsal to ventral sucker. Genital pore was anterior to ventral sucker. Excretory vesicle was Y-shaped as in cercaria; main collecting canals extending to anterior level of ventral sucker, each with 10–11 pairs of bilateral diverticula.

Comments: The metacercarial stage was found in the foot of the cockle *A. stutchburyi*, in the same area where the infected snails were collected, and preliminary laboratory infections of cockles with cercariae resulted in metacercariae identical to

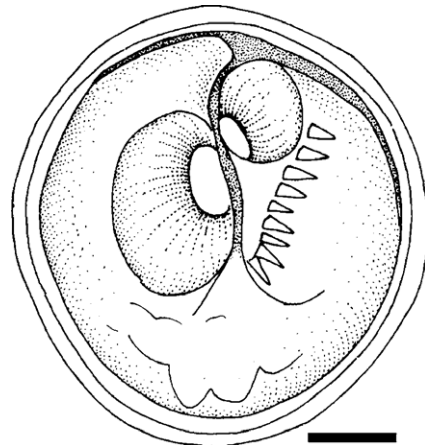


Fig. 4. Encysted from *Astrovenus stutchburyi*. Scale bar=100 μm .

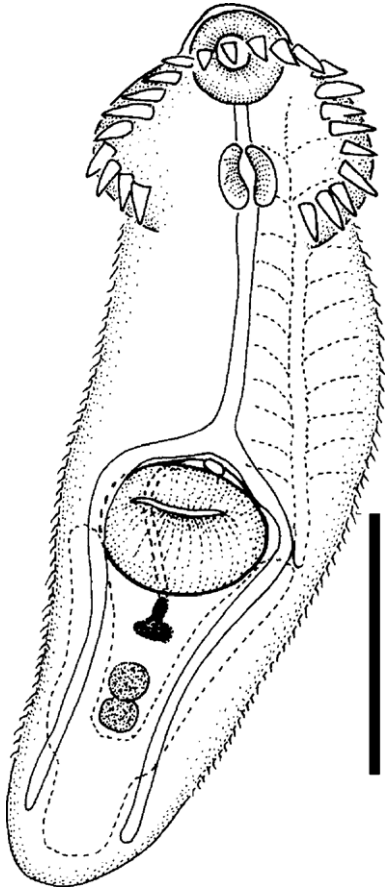


Fig. 5. Excysted metacercaria from *Austrovenus stutchburyi*, ventral view. Scale bar=100 μ m.

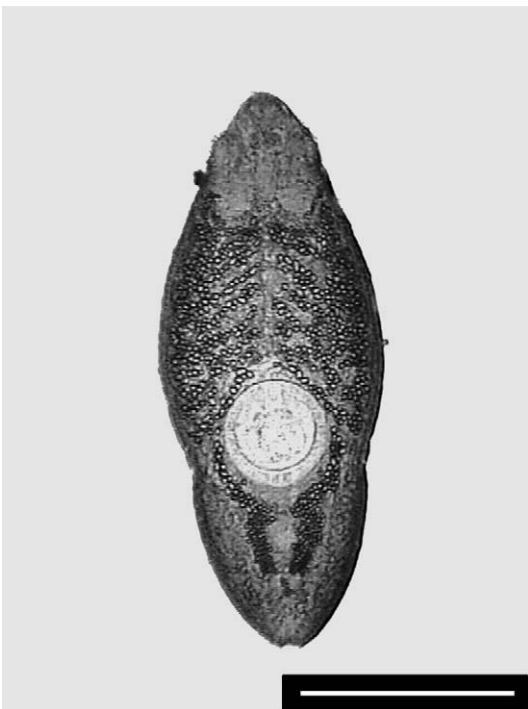


Fig. 6. Cercaria from *Zeacumantus subcarinatus*, showing concretions in excretory organs stained with neutral red. Scale bar=150 μ m.

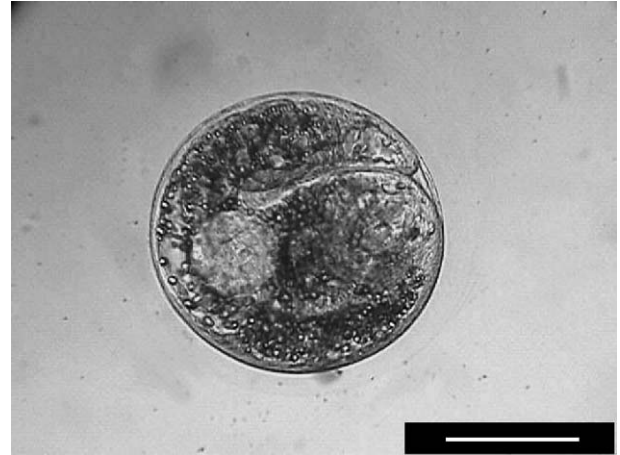


Fig. 7. Encysted metacercaria on Petridishes. Scale bar=200 μ m.

those found in naturally infected cockles. Based on the lack of other suitable bivalve hosts for the cercariae in the study area, and on the morphological similarities between the cercariae and metacercariae, they are assumed to represent different stages of the same species. This has now been confirmed by a comparison of micro satellite DNA from both the cercariae and metacercariae in naturally infected hosts, indicating that they belong to the same species (Leung T, Keeney D, Poulin R, unpublished data).

This new species appears to have the same life cycle as *C. australis*, already described from New Zealand (Allison 1979) [4]. The oystercatchers *H. ostralegus* Linnaeus, and *H. unicolor* Forster are likely to be the definitive hosts because no other bird feeds on cockles in the Otago Harbour area except occasionally gulls, *Larus* spp.

4. Discussion

The present cercaria had a reniform collar. The collar spines were 23 in total number, all similar in shape and size, and arranged in a dorsally uninterrupted single row. The main excretory canals had bilateral diverticula. Consequently, it can be placed in *Acanthoparyphium* Dietz, 1909. According to Fried [10], the best known species in this genus is *A. spinulosum* Johnston, 1917, with its life cycle first described in Australia [6]. Several cercariae belonging to this genus have been reported from marine snails worldwide: Cercaria of *Acanthoparyphium* sp. [11], cercaria of *A. paracharadrii* [8], cercaria of *A. spinulosum* [6], *Cercaria caribea* II [12], *Cercaria bengalensis* II [13], *C. queenslandae* I [14], *C. yamagutii* [15] (=cercaria of *A. tyosenense* [9]), *Cercaria kuwaitae* XI [16], and *Cercaria* sp. XVI [17]. All these cercariae are very similar in relation to their body size, general body shape, and general aspect of the main collecting canals with diverticula. The number of flame cells differs among related cercariae, but this number has not always been given by the previous authors. The present cercaria is similar in relation to its flame cell formula to the cercaria of *A. spinulosum* [6] and *Cercaria yamagutii* [15]. With respect to the number and position of the cephalic glands, some differences could be established between all previously described cercariae and the present one (Table 1). In addition,

Table 1
Number of pairs of the cephalic glands in cercariae of *Acanthoparyphium*

Cercaria	Oral sucker level	Pharyngeal level
<i>Cercaria</i> sp. XVI [17]	6	2
<i>Cercaria queenslandae</i> [14]	1	2
<i>Cercaria</i> of <i>A. spinulosum</i> [6]	0	6
<i>C. yamagutii</i> [15]	0	3
<i>Cercaria</i> of <i>A. paracharadrii</i> [8]	0	0
<i>Cercaria caribea</i> II [12]	1	3
<i>Cercaria kuwaitae</i> XI [16]	1	2
<i>Cercaria bengalensis</i> II [13]	1	6
<i>Cercaria</i> of <i>Acanthoparyphium</i> sp. (present work)	2	2

the number of the glands in the oral and pharyngeal positions can be used to differentiate some cercariae from the others. In this context the present cercaria is different from all previous ones in having the four pairs of the cephalic glands, two pairs of which at the oral sucker level and the other two at the post pharyngeal level.

Adults belonging to *Acanthoparyphium* which were reported from New Zealand as *Acanthoparyphium* sp. in the godwit *L. lapponica baueri* Nauman [4] and the oystercatcher *H. ostralegus finschi* Martens [5]. Most likely these represent the adult stage of the present new cercariae.

Comparisons can be made between the new cercaria and the cercariae described from Australia, *Cercaria queenslandae* I [14] and *Cercaria* of *A. spinulosum* [6]. The new cercaria can be differentiated from *Cercaria queenslandae* I mainly by number of the collar spines, 23 instead of 25 [14]. The latter also has smaller suckers and only one pair of the cephalic glands at the oral sucker level, unlike two in the new. In relation to the cercaria of *A. spinulosum* [6] the new cercaria can be distinguished by its bigger oral and ventral suckers (35–50 and 52–72 instead 21 and 49 in *A. spinulosum*), and its different number of the cephalic glands (2+2 instead 0+6 in the cercaria from Australia). In summary, the principal differences between the new cercaria and all previously described cercariae of *Acanthoparyphium* are in the number and disposition of the cephalic glands, the size of suckers, and the size and number of collar spines.

Finally this newly described *Acanthoparyphium* metacercaria appears to be roughly as abundant as another echinostome, *C. australis* (see [18]). Practically 100% of cockles harbour a mixture of metacercariae from the two species, with numbers of metacercariae per cockle ranging from just a few to well over one thousand. Thus metacercariae of both *C. australis* and the new *Acanthoparyphium* species impair the ability of cockles to burrow under the sediment, and then cause the increase of the predation by the oystercatcher on cockles ([19,20]. Both parasites also might contribute to the changes in the benthic community that result from the accumulation of cockles on the sediment surface after they lose their ability to burrow [21].

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