Identification and maturation of the metacercaria of *Indodidymozoon pearsoni*

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Abstract

The metacercaria of *Indodidymozoon pearsoni* (Digenea: Didymozoidae) is identified by DNA sequencing of second internal transcribed spacer (ITS2) ribosomal DNA and described. Its development into the adult is documented. Morphological features that may assist the matching of didymozoid metacercariae to adults are discussed. Those that may be of greatest use are the size of the oral and ventral suckers and the presence or absence of a chamber at the posterior extremity of the oesophagus.

Introduction

Didymozoids are a large family of trematodes that are found, as adults, in the tissues of fish. The life-cycle of didymozoids is thought, in most cases, to include four hosts where the metacercaria initially develops in a crustacean second intermediate host and is then transferred, via ingestion, to a third intermediate host, a small fish (Nikolaeva, 1965). Didymozoid metacercariae assume a characteristic form easily recognized by the structure of their gut; the caeca consist of a series of connected chambers. Unlike most digeneans, didymozoids change dramatically as they mature into adults in the definitive host; there are no clear characteristics that identify these metacercariae as didymozoids other than the structure of their excretory system (the configuration of the anterior excretory tubules) (Cable, 1955, 1956). Many authors have described didymozoid metacercariae and presented taxonomic systems for classifying them (e.g. Linton, 1905; Nikolaeva, 1965; Yamaguti, 1970, 1975; Kurochkin & Nikolaeva, 1978; Køie & Lester, 1985). However, none has been able to identify the species to which the metacercariae belong. The identification of didymozoid metacercariae is further complicated because the definitive hosts of didymozoids are generally difficult to keep in captivity which makes infection experiments difficult. Thus, there is a clear need to find a reliable method for matching metacercariae to adults.

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Køie & Lester (1985) collected nine types of didymozoid metacercariae from small fishes from Moreton Bay and postulated that some mature in platycephalid fishes. Anderson & Barker (1993) identified species level variation in the internal transcribed spacer (ITS1 and ITS2) and 5.8S ribosomal DNA (rDNA) of didymozoid species collected from platycephalid fishes from Moreton Bay. This region of DNA would therefore seem ideal to use for the identification of the sub-adult life stages of didymozoids.

Materials and methods

Small *Sillago maculata* were collected in intertidal areas at Boat Passage (27°24′S 153°10′E) and Sandgate (27°19′S 153°05′E) using a beach seine and in Moreton Bay (27°15′S 153°15′E) by otter trawl. Metacercariae were also collected from *Pseudorhombus arsius* caught by seine at Sandgate. Guts were removed from fish and the mesenteries around the gut were broken. The body cavity, mesenteries and outside of the gut were examined as was the saline in which the procedure was performed. Parasites were removed live from host tissue and kept in 0.85% saline until fixation. Specimens intended for morphological examination were fixed in hot (near boiling) 10% formalin and stored in 70% alcohol. Specimens intended as sources of DNA were frozen at –70°C in Tris-ethylenediaminetetra-acetate (TE) buffer.

Specimens for light microscopy were stained in Mayer's acid haematoxylin, destained in 3% hydrochloric acid (HCl) and neutralized in 3% ammonia (NH_3). They were then dehydrated in a graded series of ethanol (70%, 90% and absolute). Following dehydration, worms were

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cleared in methyl salicylate and mounted in Canada balsam. Sections (7 μm) were stained with haematoxylin and eosin and mounted in thin Canada balsam. Measurements were made using an ocular micrometer. In the description measurements are given as a range followed, in parentheses, by a mean value. Measurements are in micrometres unless otherwise stated. Drawings were made with the aid of a camera lucida.

DNA sequencing of metacercariae was carried out as detailed in Anderson & Barker (in press). A sample of four metacercariae was used. The sequence obtained from the metacercariae was compared to the sequences obtained from adults as reported in the above paper.

Observations on the maturation of the fluke were made from 17 specimens of *I. pearsoni*, at various stages of development, collected from the eyes of *Platycephalus endrachtensis*.

Results

The identity of one type of metacercaria was determined using DNA sequencing of the ITS2. The sequence of the metacercaria (the same for two samples) was 338 bases long and was identical in both length and nucleotide composition to the sequence of the adult of *Indodidymozoon pearsoni* as reported in Anderson & Barker (in press). Since there is variation in the ITS2 of even closely related species of didymozoid (see Anderson & Barker, in press), the lack of variation between the adult of *I. pearsoni* and the metacercaria indicates that *I. pearsoni* has the form of metacercaria described below.

Metacercaria of Indodidymozoon pearsoni *Anderson & Cribb, 1994*

(= Species 1 of Køie & Lester, 1985)
(fig. 1)

Host. *Sillago maculata* Quoy & Gaimard, 1824.

Site in host. Body cavity and mesenteries.

Material examined. ex *S. maculata*, Moreton Bay, Mar. 1991 (9); Dec. 1991 (1).

Description. (Measurements are of 10 specimens.) Body elongate, 655–777 (713) long and 84–148 (119) wide at mid-point. Oral sucker elliptical, 42–58 (49) long and 26–38 (35) wide. Prepharynx absent. Pharynx 9–16 (12) long and 13–16 (15) wide. Oesophagus 75–122 (91) long. Chamber formed between oesophagus and caeca, 32–67 (53) long, surrounded by gland cells. Caeca two, consist of small anterior tubular section and five distinct chambers (off-set against chambers of other caecum), extend length of body, terminate asymmetrically 26–116 (72) from posterior extremity of body. Ventral sucker 205–270 (244) from anterior extremity of body, 48–64 (56) wide. Excretory duct extends medially through hindbody, forms thick-walled bladder at posterior extremity. Excretory pore terminal.

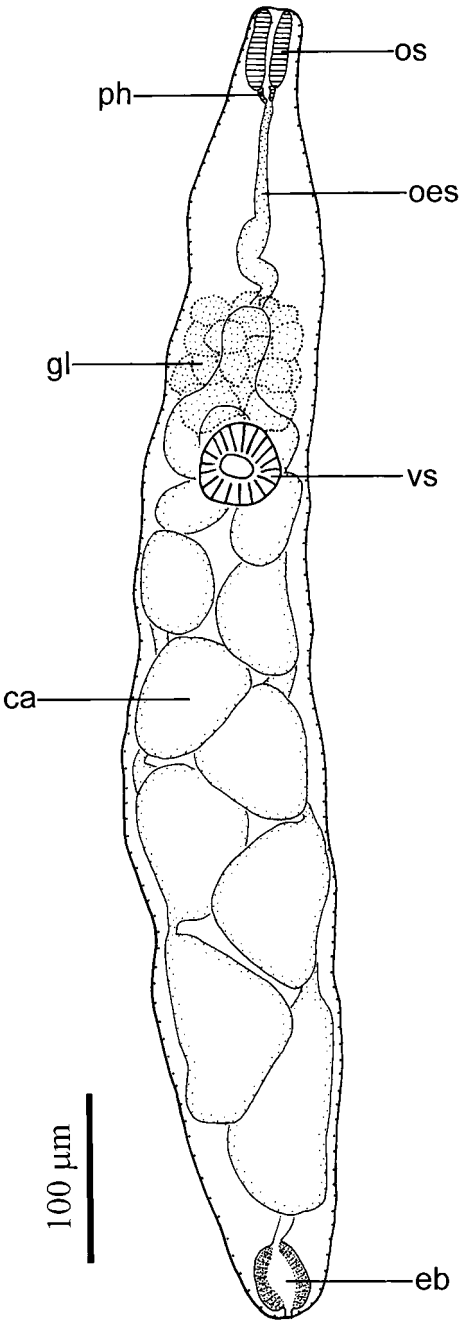

Fig. 1. Metacercaria of *Indodidymozoon pearsoni*. ca, caecum; eb, excretory bladder; gl, gland cells; oes, oesophagus; os, oral sucker; ph, pharynx; vs. ventral sucker.

Comparison. The metacercaria described here does not differ significantly from that described by Køie & Lester (1985) as 'species 1'. The dimensions given above mostly fall within the ranges given by Køie & Lester but are consistently in the upper end of the ranges and sometimes exceed them (for examples see table 1). It is unclear why the two sets of measurements differ. The variation

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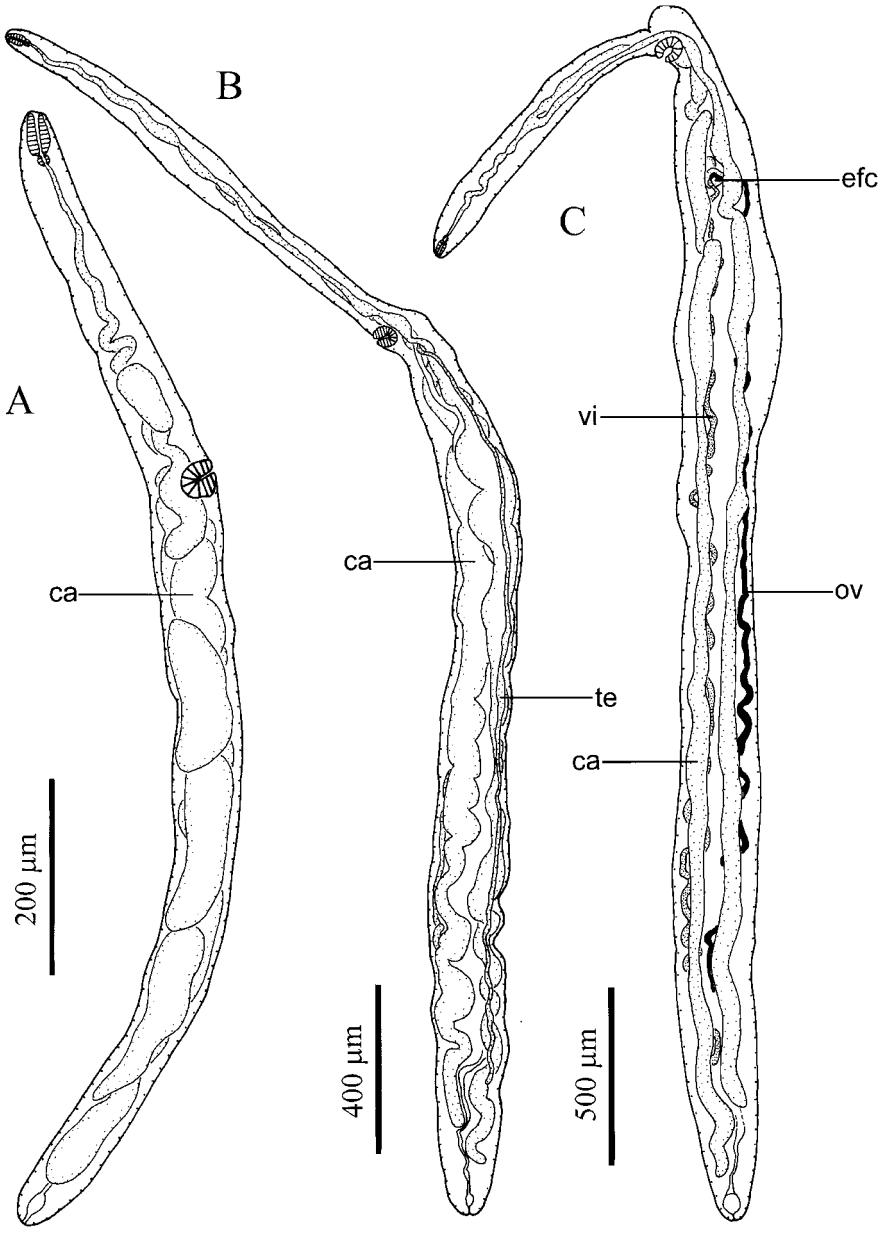

Fig. 2. *Indodidymozoon pearsoni.* A. Immature. B. Immature male. C. Immature female. ca, caecum; efc, egg-forming complex; ov, ovary; te, testis; vi, vitellarium.

recorded by Køie & Lester may simply be an artefact of the cold fixation method that they used. There are, however, other explanations. Køie & Lester may have measured metacercariae that were collected from a number of host species (they did not state the number of host species from which metacercariae were measured but found the metacercaria in 12 species of fish) whereas the metacercariae measured for the description above were all collected from *S. maculata*. The species of host probably influences the growth of metacercariae so that larger size ranges will be observed for specimens collected from a number of hosts than for specimens from one host species. For example, although the measurements of metacercariae collected from *Pseudorhombus arsius* also fall within a discrete part of the ranges given by Køie & Lester, they differ from those of the metacercariae from *S. maculata* (table 1). Thus, when the two data sets are combined, larger ranges are produced than from either set of data alone.

The metacercariae from *S. maculata* might fall at the upper end of the size ranges given by Køie & Lester because *S. maculata* is an optimal host. Køie & Lester recorded that of the 12 host species that harboured metacercaria species 1, *S. maculata* and unidentified gobies (Gobiidae) hosted the highest numbers. It is therefore possible that these are the most

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Table 1. Selected measurements of the metacercaria of *Indodidymozoon pearsoni*. Measurements are from Køie & Lester (1985) and metacercariae collected from *Sillago maculata* and *Pseudorhombus arsius*.

Source	Number measured	Length	O.S. length	O.S. width	V.S. width
Køie & Lester (1985)	20	200–800 (450)	20–60 (40)	10–40 (26)	18–56 (36)
Sillago maculata	10	655–777 (713)	42–58 (49)	26–38 (35)	48–64 (56)
Pseudorhombus arsius	16	441–659 (521)	40–48 (43)	27–32 (30)	38–53 (45)

Means are in parentheses. O.S., oral sucker; V.S., ventral sucker.

suitable hosts and that the metacercariae grow to their full potential in these hosts. Accordingly, the metacercariae may not grow as large in hosts less suitable than *S. maculata*. Hence, the ranges observed for metacercariae collected from *S. maculata* will be at the upper end of those presented by Køie & Lester because Køie & Lester included measurements of metacercariae from both optimal and suboptimal hosts.

Maturation

Once the metacercaria of *I. pearsoni* has reached an appropriate site in the tissues around the eye of the definitive host, it begins to elongate. As it grows, the gut extends and begins to lose the distinct chambers present in the metacercaria (fig. 2A). When the immature fluke is about six times longer than the metacercaria, a lobe develops on the dorsal surface at about the level of the ventral sucker. The dorsal lobe grows into the anterior section of the hindbody of the mature fluke. The region anterior to where the lobe develops becomes the forebody of the adult. By this stage, the caeca have lost any signs of the chambers that they possessed formerly and the reproductive organs have appeared. Males have testes that, although not fully developed, are apparently starting to function (fig. 2B). Females similarly have incomplete but partially functioning reproductive organs (fig. 2C). Both the forebody and hindbody continue to extend as the fluke matures and the reproductive systems become fully functional. The hindbody of females continues to enlarge throughout the life of the adult as the uterus becomes distended with eggs.

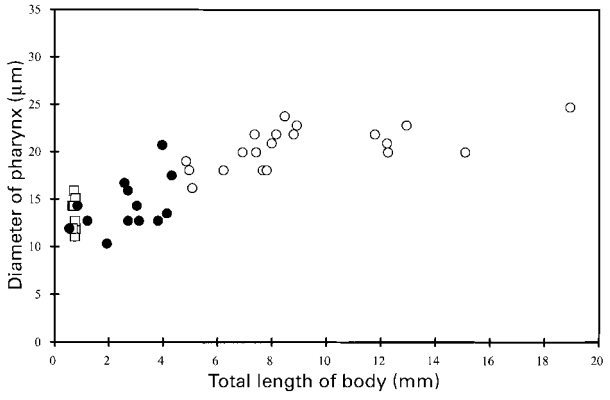

Fig. 3. Relationship of the diameter of the pharynx to the total length of the body of *Indodidymozoon pearsoni*. Note: the diameter of the pharynx was calculated by averaging the length and width, the total length of body for immatures and adults was calculated by adding the length of the forebody to the length of the hindbody (□ metacercaria; ● immature; ○ adult).

Although the fluke changes dramatically as it matures, some features change little or remain the same. For instance, although the pharynx of *I. pearsoni* grows during maturation (see fig. 3), the oral sucker apparently fails to grow; measurements of the oral sucker of immature and adult flukes all fall within or below the range of measurements recorded for the metacercariae used in the description above (see fig. 4). It is puzzling that the measurements for some adults and immatures should fall below the range observed for the metacercariae. It is probably not a result of atrophy of the sucker; the suckers of immatures and adults show no signs of degradation. Measurements may be smaller in some cases because the size of the body prevents flattening of the sucker during mounting. Perhaps, however, the smaller measurements reflect the presence of flukes that, as metacercariae, inhabited suboptimal hosts. All measurements recorded here for adults and immatures fall within the range of sizes (20–60 (40)×10–40 (26)) recorded by Køie & Lester (1985) for metacercariae probably collected from various hosts including hosts that may be suboptimal. Hence, the majority of flukes have oral suckers that fall within the size range of metacercariae that are from the most commonly parasitized fishes whereas a minority have suckers that, although falling within the overall range for metacercariae, fall below the size range for metacercariae from common hosts.

The ventral sucker also fails to grow with the fluke (see fig. 5). It is not entirely clear from the data that the sucker has not atrophied to some extent. However, again, the suckers of immatures and adults show no sign of degradation and the measurements all fall within the range recorded by Køie and Lester for metacercariae. Certainly any decrease in size of the ventral sucker is minor.

Discussion

Didymozoid metacercariae have no obvious morphological features that can be used to identify the species

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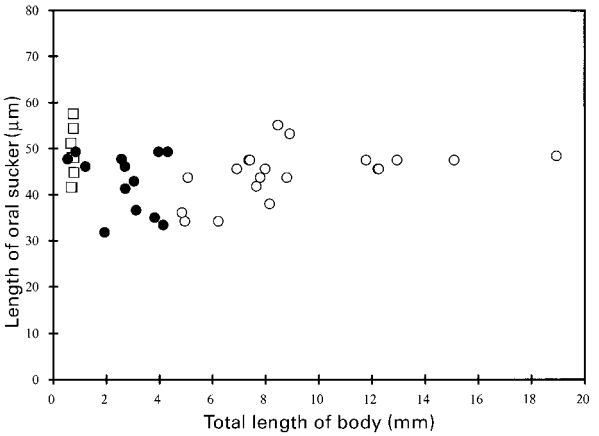

Fig. 4. Relationship of the length of the oral sucker to the total length of the body of *Indodidymozoon pearsoni*. Note: the total length of body for immatures and adults was calculated by adding the length of the forebody to the length of the hindbody (□ metacercaria; ● immature; ○ adult).

to which they belong. Body shape is of no help because of the enormous differences between metacercariae and adults. The characteristic chambered caeca of the metacercariae, although useful for distinguishing between metacercarial types, is of no use in the association of metacercariae with adults because the chambers are lost as the fluke matures. Similarly, the glandular mass present around the anterior part of the gut of some metacercariae is of limited value because the distribution of the gland cells changes as the fluke grows.

There are, however, some features that may be of some, if limited, use in identifying metacercariae. For instance, the 'stomach' that is present in some metacercariae probably forms the chamber at the posterior end of the oesophagus of some adults; the metacercaria of *I. pearsoni* has a 'stomach' and the adult has a chamber. The presence or absence of the chamber in metacercariae and adults might therefore be used as one piece of evidence to support a preliminary identification. The size of the suckers is probably more suitable for identifying metacercariae. The oral and ventral suckers of *I. pearsoni* are about the same size in both metacercariae and adults. If this is true in all species, it may be possible to make a preliminary identification of a metacercaria by matching the sizes of its suckers with those of an adult. To examine this idea, I compared the dimensions of the suckers of the nine species of metacercaria described by Køie & Lester (1985) with the dimensions of the adults found in flathead fishes from Moreton Bay. The size of the suckers of metacercaria sp. 2 are similar to those of *H. helicis*. Further, neither the metacercaria nor the adult have a chamber at the end of the oesophagus. Although this may be useful as a preliminary identification, confirmation is needed. The molecular technique used here has proved effective for such purposes.

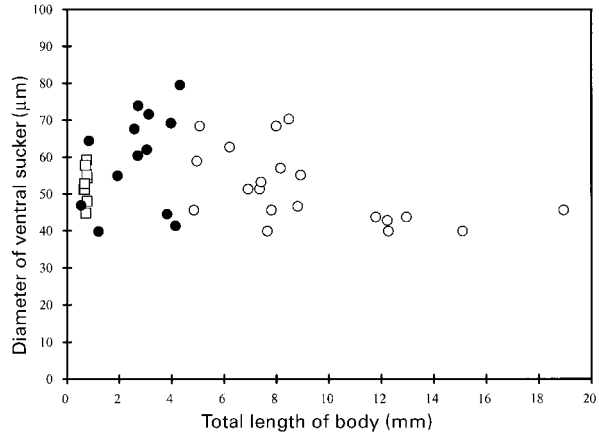

Fig. 5. Relationship of the diameter of the ventral sucker to the total length of the body of *Indodidymozoon pearsoni*. Note: the diameter of the ventral sucker was calculated by averaging the length and width, the total length of body for immatures and adults was calculated by adding the length of the forebody to the length of the hindbody. (□ metacercaria; ● immature; ○ adult).

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References

Anderson, G.R. & Barker, S.C. (1993) Species differentiation in the Didymozoidae (Digenea): restriction fragment length differences in internal transcribed spacer and 5.8S ribosomal DNA. *International Journal for Parasitology* **23,** 133–136.

Anderson, G.R. & Barker, S.C. (in press) Inference of phylogeny and taxonomy within the Didymozoidae (Digenea) from the second internal transcribed spacer (ITS2) of ribosomal DNA. *Systematic Parasitology.*

Cable, R.M. (1955) Affinities of the trematode family Didymozoidae. *Journal of Parasitology* **41** (supplement), 25.

Cable, R.M. (1956) Marine cercariae of Puerto Rico. *Scientific Survey of Puerto Rico and the Virgin Islands* **16,** 491–577.

Køie, M. & Lester, R.J.G. (1985) Larval didymozoids (Trematoda) in fishes from Moreton Bay, Australia. *Proceedings of the Helminthological Society of Washington* **52,** 196–203.

Kurochkin, Y.V. & Nikolaeva, V.M. (1978) [A classification of didymozoid metacercariae.] *First All-Union Congress of Parasito-Coenologists, Kiev: Naukova Dumka* **3,** 82–84. (In Russian.)

Linton, E. (1905) Parasites of fishes of Beaufort, North Carolina. *Bulletin US Bureau of Fisheries* **24,** 321–428.

Nikolaeva, V.M. (1965) [On the developmental cycle of the trematode family Didymozoidae (Monticelli, 1888) Poche, 1907.] *Zoologicheskii Zhurnal* **44,** 1317–1327. (In Russian.)

Yamaguti, S. (1970) *Digenetic trematodes of Hawaiian fishes*. Tokyo, Keigaku Publishing Company.

Yamaguti, S. (1975) *A synoptic review of life histories of digenetic trematodes of vertebrates*. Tokyo, Keigaku Publishing Company.

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