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VERSITA

Phylogenetic studies explain the discrepant host distribution of *Allopodocotyle heronensis* sp. nov. (Digenea, Opecoelidae) in Great Barrier Reef serranids

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Abstract

We report a new species of *Allopodocotyle* Pritchard, 1966 from the intestine of two species of Serranidae, *Cromileptes altivelis* and *Epinephelus fuscoguttatus*, from the southern Great Barrier Reef. Despite the examination of eight other species of *Epinephelus* from the same region this species appears anomalous in its distribution in one species of *Epinephelus* and the single species of *Cromileptes*. Molecular phylogenetic studies of the Epinephelinae suggest, however, that these two species are closely related so that the host specificity demonstrated by this species is actually stenoxenic (phylogenetically related hosts) rather than euryxenic.

Keywords

Digenea, Opecoelidae, *Allopodocotyle*, Serranidae, Epinephelinae, the Great Barrier Reef, Australia

Introduction

Epinepheline serranids (groupers) have a rich fauna of trematodes of which the Opecoelidae Ozaki, 1925 has by far the greatest genus-level richness (Cribb *et al*. 2002). Despite this richness, only a handful of studies have reported the opecoelid fauna of the serranids of tropical Australia (Durio and Manter 1968, Bray and Cribb 1989, Lester and Sewell 1989, Rigby *et al*. 1997). These studies have reported just five genera and five species of Opecoelidae from two genera and five species of epinephelines. Here we describe a new species from specimens collected from two serranids, *Cromileptes altivelis* Valenciennes and *Epinephelus fuscoguttatus* (Forsskål), from the Great Barrier Reef.

Materials and methods

Serranids were collected by line at Heron Island (23°27′S, 151°55′E) and the Swain Reefs Complex (21°54′S, 152°22′E and 21°36′S, 152°22′E) on the southern Great Barrier Reef. Trematodes were collected following methods described by Cribb and Bray (2010). Specimens were removed from freshly killed hosts, washed in vertebrate saline, and fixed in near-boiling 0.85% saline for preservation in 5% formalin. Whole-mounts were stained in Mayer's haematoxylin, cleared in methyl salicylate and mounted in Canada balsam. In some cases the ventral sucker was cut off and mounted separate from the body so as to allow the body to be mounted ventral side up. Measurements are given as ranges followed by means in parentheses, and are in micrometres unless otherwise stated. Drawings were made with the aid of a drawing tube and then digitised using Adobe Illustrator.

Results

Family: Opecoelidae Ozaki, 1925
Subfamily: Plagioporinae Manter, 1947
Genus: *Allopodocotyle* Pritchard, 1966
Allopodocotyle heronensis sp. nov.
Syn.: *Allopodocotyle* sp. 3 of Lucas *et al*. (2005).

Type-host: *Cromileptes altivelis* Valenciennes (Serranidae).

Other host: *Epinephelus fuscoguttatus* (Forsskål) (Serranidae).

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Sites in host: Pyloric caeca and intestine.

Type locality: Heron Island (23°27′S, 151°55′E), southern Great Barrier Reef.

Other localities: The Swain Reefs Complex (21°54′S, 152°22′E and 21°36′S, 152°22′E) on the southern Great Barrier Reef.

Prevalence: Heron Island and Swain Reefs combined *C. altivelis* 3/6 and *E. fuscoguttatus* 2/3.

Material examined: Holotype: QM G 233037; paratypes: QM G 233038–233049.

Etymology: This species is named for the type-locality, Heron Island.

***Allopodocotyle heronensis* sp. nov.** (Fig. 1)

Measurements are of 12 gravid, unflattened, whole-mount worms. Four specimens from *Cromileptes altivelis* (two each from Heron Island and the Swain Reefs complex) were mounted dorso-ventrally, four specimens from *C. altivelis* (all from Heron Island) were mounted ventrally and four specimens from *Epinephelus fuscoguttatus* (all from Heron Island) were mounted ventrally.

Body elongate, with almost parallel sides, sometimes curved dorsoventrally with curvature of body and proturberance of ventral sucker typically causing specimens to mount laterally, with maximum width in hindbody, typically in region of gonads, 1903–3287 (2643) long, 222–380 (310) wide, 103–305 (189) deep; length to width ratio 7.2–11.0 (8.8). Tegument unarmed. Forebody 338–705 (568) long, occupying 17.0–25.0 (22.0)% of body length.

Oral sucker opening ventro-subterminally, 78–151 (123) long, 99–149 (128) wide and 83–141 (110) deep. Ventral sucker subglobular, protuberant, in anterior third of body, 164–301 (233) long, 177–296 (256) wide and 147–276 (196) deep. Sucker length ratio 1.2–2.2 (1.9). Prepharynx very short, usually entirely within posterior cavity of oral sucker. Pharynx subglobular with anterior extremity protruding slightly into base of oral sucker, 59–114 (92) long, 72–106 (93) wide and 64–111 (82) deep. Oesophagus muscular, 39–118 (73) long, occupying 2.0–4.4 (3.0)% of body length. Intestinal bifurcation in forebody, immediately anterior to anterior margin of ventral sucker. Caeca long, ending blindly close to posterior extremity.

Testes 2, tandem, spherical to subspherical, in middle third of hindbody, separated by 108–310 (204). Anterior testis 100–246 (181) long, 85–216 (160) wide and 84–239 (149) deep. Posterior testis 98–246 (183) long, 81–226 (162) wide and 84–202 (141) deep. Posterior testis to end of body 617–1151 (822). Cirrus-sac extending from midway between ovary and posterior margin of ventral sucker to genital pore, distinctly swollen posterior to ventral sucker, narrower dorsal to ventral sucker, containing winding internal seminal vesicle posteriorly, 166–246 (217) long, 92–124 (104) deep and 67–135 (92) wide. Distinct pars prostatica not observed. Ejaculatory duct a straight, narrow tube apparently extending length of anterior narrow portion of cirrus-sac. Genital atrium small but distinct. Genital pore sinistral, midway between pharynx and ventral sucker.

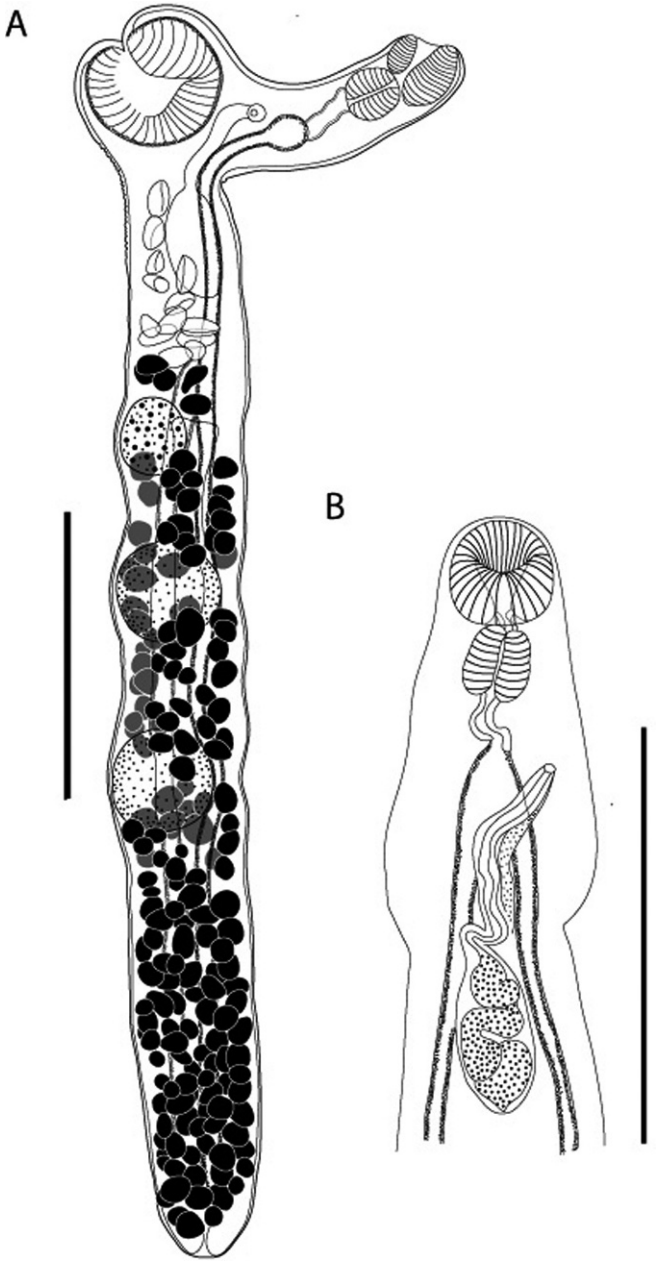

Fig. 1. *Allopodocotyle heronensis* sp. nov. from *Cromileptes altivelis* from Heron Island. **A.** Lateral view of full specimen. **B.** Terminal genitalia of specimen with ventral sucker removed. Scale bars = 500 μm

Ovary 76–187 (134) long, 71–144 (101) wide and 70–178 (109) deep. Canalicular seminal receptacle anterodorsal to ovary. Uterine coils between ovary and ventral sucker. Vitellarium follicular; follicles extend from posterior extremity to just anterior to ovary, 389–1089 (811) from anterior extremity, partially interrupted at level of ovary and testes. Eggs 48–68 (59) long, 30–44 (37) wide.

Excretory vesicle tubular, extends to level of ovary, sometimes strongly inflated.

Discussion

The present material agrees well with the concept of *Allopodocotyle* Pritchard, 1966, established by Pritchard (1966) for *A. plectropomi* (Manter, 1963) as part of her revision of *Podocotyle* Odhner, 1905. The genus presently contains approximately 25 species and is characterized by a combination of character states, none of which is uniquely diagnostic (Cribb 2005). The testes are tandem to oblique and well-separated from the posterior end of body, the genital pore is sinistral in the mid-forebody, the ovary is entire, and the vitelline follicles are essentially restricted to the hindbody. The genus forms a matrix with other genera in these characters. It differs from *Macvicaria* Gibson et Bray, 1982 in which the vitelline follicles extend into the forebody, from *Peracreadium* Nicoll, 1909 in which the genital pore is median, from *Podocotyloides* Yamaguti, 1934 in which the ventral sucker is pedunculate, and from *Podocotyle*, in which the ovary is deeply lobed. It seems highly unlikely that all these genera (and several others) are entirely natural units.

Gibson (1996) drew attention to the fact that some species of *Allopodocotyle* reported from freshwater fishes have short excretory vesicles and may require separation from marine species of *Allopodocotyle* in the same way that *Macvicaria* (long excretory vesicle and parasitic in marine fishes) was separated from *Plagioporus* Stafford, 1904 (short excretory vesicle and parasitic in freshwater fishes). The species he listed were *A. boleosomi* (Pearse, 1924), *A. lepomis* (Dobrovolny, 1939) and *A. virens* (Sinitsin, 1931). Of these, *A. virens* as originally described by Sinitsin (1931) has a long excretory vesicle and is here retained in *Allopodocotyle*. *A. chiliticorum* Barger et Esch, 1999 described from a freshwater cyprinid by Barger and Esch (1999) also has a very short excretory vesicle. It is concluded here that the possession of a short excretory vesicle and the parasitism of freshwater fishes suggests a relationship to *Plagioporus* as restricted by Gibson and Bray (1982). For this reason, and because we think it undesirable to divide opecoelid genera further on the basis of the anterior extent of the vitellarium, these three species are here incorporated in *Plagioporus*, requiring the modification of the concept of that genus to include species with vitelline follicles restricted to the hindbody. *Plagioporus lepomis* was originally described in *Plagioporus* and is here returned to that genus and the two new combinations *P. boleosomi* n. comb. and *P. chiliticorum* n. comb. are proposed.

Bray (1987) proposed that the marine *Allopodocotyle* species could be divided into three groups based on the arrangement of the testes, the length of the cirrus-sac and the extent of the vitelline follicles, recognising a fourth group for species of uncertain status. Since that work, which recognised 21 species of *Allopodocotyle*, four new species have been proposed, *A. margolisi* Gibson, 1995, *A. coniusi* Singh, 2009, *A. skoliorchis* Aken'Ova, 2003 and *A. tunisiensis* Derbel et Neifar, 2009. With respect to the groups proposed by Bray (1987), the present form clearly belong to Group C as the testes are tandem. Among species in this group it is distinguished from all but *A. israelense* (Fischthal, 1980) Bray, 1987 by having the testes well-separated by vitelline follicles; *A. lutianusi* Gupta et Ahmad, 1976 and *A. mecopera* (Manter, 1940) Pritchard, 1966 have the testes just slightly separated. *A. israelense* and *A. lutianusi* differ from the present material in having the vitelline follicles extend well anterior to the ovary. *A. mecopera*, from an unnamed serranid from the Galapagos Islands (Manter 1940), generally resembles the present species, especially in the swollen posterior portion of the cirrus-sac, but has the testes much closer together and the ovary is contiguous with, rather than well-separated from, the anterior testis.

Despite the utility of Bray's (1987) subdivision of the genus, we conclude that the present material is generally most similar to some of the five existing species that infect other serranids: *A. epinepheli* (Yamaguti, 1942) Pritchard, 1966 reported initially from *Epinephelus chlorostigma* (Valenciennes) from Japan, *A. manteri* (Saoud et Ramadan, 1984) from *Epinephelus summana* (Forsskål) from the Red Sea; *A. mecopera* and *A. plectropomi* (Manter, 1963) Pritchard, 1966 reported from Fiji from a species of *Plectropomus* Oken; and *A. serrani* (Yamaguti, 1952) Pritchard, 1966 from, initially, a "*Serranus* sp." from Celebes, Indonesia. *Allopodocotyle heronensis* can be distinguished from these five species as follows: *A. epinepheli* is less elongate than the present species and has testes which are much closer to each other and the ovary than in the present species. This species occurs sympatrically with *A. heronensis* on the Great Barrier Reef (Bray and Cribb 1989), but in different serranid species. *A. manteri* has a squat form entirely unlike the other species reported from serranids. As discussed above, *A. mecopera* has a larger cirrus-sac than the present species, the testes are closer together and it has a generally less elongated body shape. *A. plectropomi* generally resembles the present species but differs in that the post-testicular zone is far larger (at least 1/3 body length) than it is in the present species. In addition the cirrus-sac is distinctly narrower. *A. serrani* has testes which are closer to each other and to the ovary than in the present species.

Host specificity

We have examined 4 genera, 17 species and 451 specimens of epinepheline serranids on the southern Great Barrier Reef but have found infections of *Allopodocotyle heronensis* sp. nov. only in *Cromileptes altivelis* and *Epinephelus fuscoguttatus*. Thus, the shared parasitism of these species is initially surprising because the parasite has not been found in any of eight other species of *Epinephelus* occurring sympatrically with *C. altivelis* and *E. fuscoguttatus*. In this context it is of interest that the cryptogonimid *Mitotrema anthostomatum* Manter,

1963 has also been found exclusively in these two fishes on the Great Barrier Reef (Cribb *et al*. 1996). Recent molecular phylogenetic analysis of the Epinephelinae using 2 mitochondrial and 2 nuclear regions by Craig and Hastings (2007) suggests that, despite their present classification in separate genera, *C. altivelis* and *E. fuscoguttatus* are closely related and that *Cromileptes* is nested within *Epinephelus*. The apparent restriction of this species to *C. altivelis* and *E. fuscoguttatus* thus represents an interesting case of stenoxenicity (restriction to phylogenetically related hosts) rather than an erratic euryxenous distribution. The analysis by Craig and Hastings (2007) suggested that *C. altivelis* and *E. fuscoguttatus* form a clade with three other species, *E. lanceolatus* (Bloch), *E. itajava* (Lichtenstein) and *E. polyphekadion* (Bleeker). Of these three species we have examined only a single individual of *E. polyphekadion* on the southern Great Barrier Reef without finding an infection of *A. heronensis*. The present analysis suggests that these species might also be infected with *A. heronensis* sp. nov.

In the context of these observations, it is of interest to note that Rückert *et al*. (2010) reported *Allopodocotyle epinepheli* from *Epinephelus fuscoguttatus* in Indonesian waters. This paper refers to a figure in Rückert's PhD thesis which resembles *A. heronensis* more than *A. epinepheli* in the elongation of the body and the separation of the gonads. If the form reported from Indonesia is indeed *A. heronensis* then the species is evidently quite widespread and appears to maintain its distinct host-specificity over that range.

The first intermediate host of *A. heronensis* sp. nov. is already known. Lucas *et al*. (2005) reported a match of ITS2 rDNA sequences between an adult (named as "*Allopodocotyle* sp. 3") taken from one of the infections reported here and asexual stages taken from the haliotid gastropod *Haliotis asinina* Linnaeus, also from Heron Island on the southern Great Barrier Reef. This represents the first named opecoelid for which an intermediate host is known on the Great Barrier Reef. Rice *et al*. (2006) described patterns of modified gene expression associated with infection of a species of *Allopodocotyle* in *Haliotis asinina* at Heron Island, but the study did not distinguish whether it was the present species or a second morphologically indistinguishable species, described by Lucas *et al*. (2005), that is so far known only from its cercaria, also in *Haliotis asinina*.

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