

A new species of *Stephanostomum* Looss, 1899 (Digenea, Acanthocolpidae) with a bizarre oral sucker: *S. adlardi* sp. nov. from the common coral trout *Plectropomus leopardus* (Lacepède, 1802) (Perciformes, Serranidae) from Lizard Island, Great Barrier Reef

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Abstract

A new species of Acanthocolpidae, *Stephanostomum adlardi* is described from the serranid *Plectropomus leopardus* from Lizard Island in the northern Great Barrier Reef. It differs from all previously described acanthocolpids in the structure of the oral sucker which is extended into dorsal and ventral lobes each bearing a row of spines. A phylogenetic tree estimated from combined nuclear small and partial large ribosomal RNA gene sequences shows that, despite the unusual oral sucker structure, the species is a true member of the genus *Stephanostomum*. The molecular results also suggest that *Monostephanostomum nolani* is derived from within *Stephanostomum*.

Key words

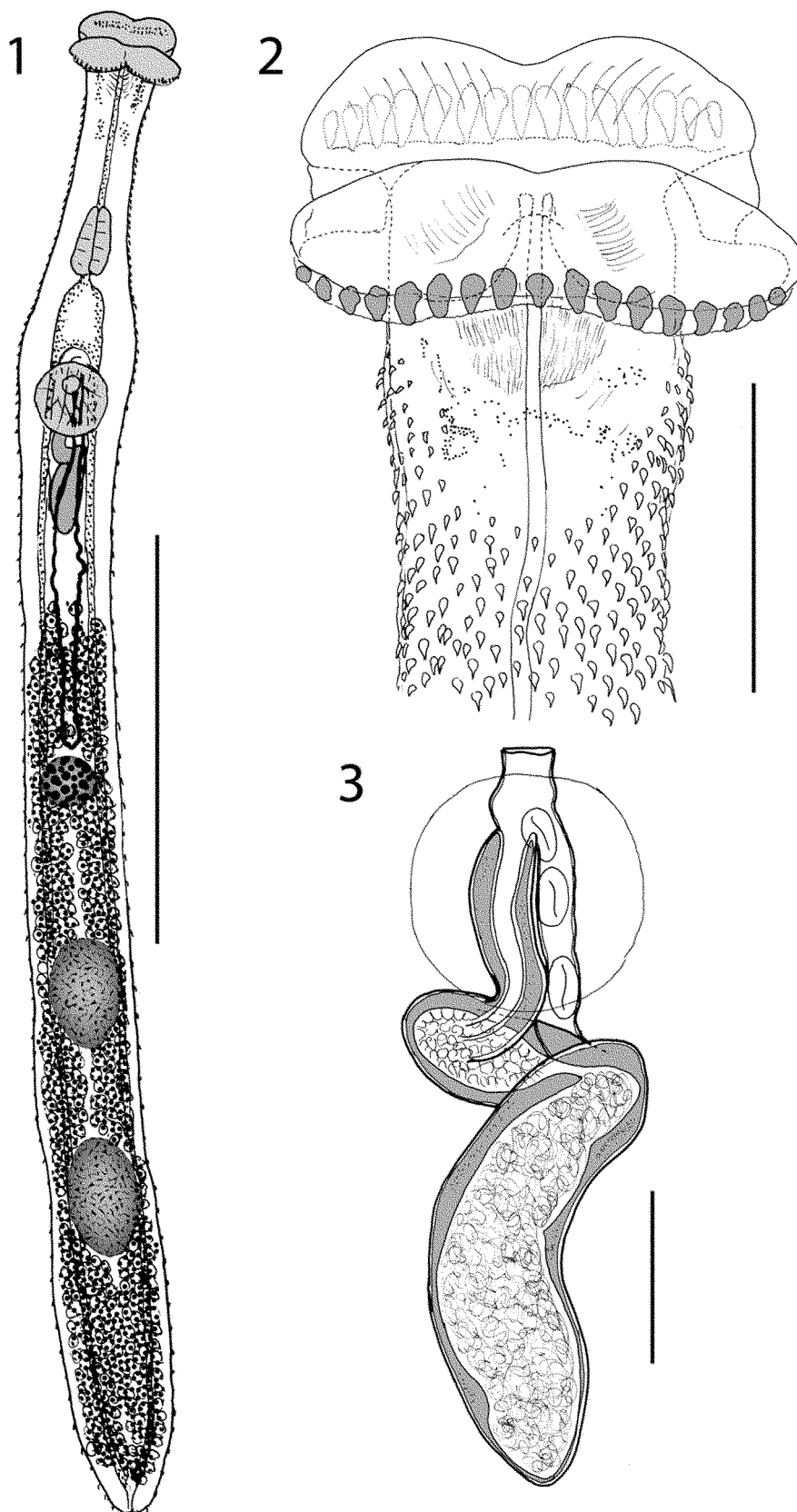
Digenea, Acanthocolpidae, *Stephanostomum adlardi* sp. nov., *Plectropomus leopardus*, Great Barrier Reef, *Monostephanostomum*, phylogeny

Introduction

According to the checklist in Cribb *et al.* (2002) the common coral trout *Plectropomus leopardus* harbours digeneans of the families Bucephalidae Poche, 1907, Hemiuridae Looss, 1899, Lecithasteridae Odhner, 1905, Opecoelidae Ozaki, 1925 and Sanguinicolidae von Graff, 1907. In addition, Lester and Sewell (1990) reported unnamed Bivesiculidae Yamaguti, 1934 and Didymozoidae Monticelli, 1888 in this host. We have recovered a most unusual digenean from *P. leopardus* from the waters around Lizard Island, which is not identifiable as belonging to any of the above families. Despite its uniquely developed oral sucker, its general morphology indicates a relationship with a family not previously reported from this species, the Acanthocolpidae Lühe, 1906. Molecular results show that it is an anomalous species of *Stephanostomum* Looss, 1899.

Materials and methods

Digeneans collected from freshly-killed fish were fixed by being pipetted into nearly boiling saline and immediately preserved in formalin or 100% ethanol. Whole-mounts were stained with Mayer's paracarmine, cleared in beechwood creosote and mounted in Canada balsam. Measurements were made through a drawing tube on an Olympus BH-2 microscope, using a Digidad Plus digitising tablet and Carl Zeiss KS100 software adapted by Imaging Associates, and are quoted in micrometres. Differential interference contrast images were captured using a Leica DM5000B microscope and 480 digital camera. The following abbreviations are used: BMNH, the British Museum (Natural History) collection at the Natural History Museum, London; QM, Queensland Museum Collection, Brisbane, Australia.



Figs 1–3. *Stephanostomum adlardi* sp. nov. 1. Holotype. 2. Anterior extremity. 3. Terminal genitalia, with ventral sucker outlined. Scale-bars = 1 mm (1), 200 μ m (2 and 3)

In order to assess the phylogenetic position of the new species, we reanalysed a subset of taxa from a combined small and large ribosomal RNA gene data set described in Bray *et al.* (2005), with new gene sequences. Complete small subunit (SSU) and partial (D1-D3) large subunit (LSU) rDNA fragments were PCR amplified and characterised for *Monostephanostomum nolani* and the new species, using the same procedures and protocols described in Bray *et al.* (2005). New SSU and LSU sequences were deposited with GenBank under the following accession numbers:

Stephanostomum adlardi sp. nov. ex *Plectropomus leopardus*, Lizard Island, SSU EF506760, LSU EF506761.

Monostephanostomum nolani Bray et Cribb, 2007 ex *Carangoides plagiotænia*, Lizard Island, SSU EF506762, LSU EF506763.

New sequences of SSU and LSU rDNA were combined with previously published and aligned sequences (Bray *et al.* 2005). The new sequences were incorporated into the existing alignments with adjustments to the alignments made by eye using MacClade (Maddison and Maddison 2002). Sequences for both genes were concatenated in MacClade and regions of ambiguous alignment defined in a character exclusion set. From the original taxon set, all the acanthocolpids and an assortment of taxa were chosen as outgroups (full details represented in Fig. 6). Following Bray *et al.* (2005), a Bayesian analysis of the combined data set was performed using the general time reversible model, with estimates of invariant sites and gamma distributed among-site rate variation (GTR+I+G). Posterior probabilities were approximated over 5,000,000 generations via 4 simultaneous Metropolis-Coupled Markov Chain Monte Carlo chains (nchains = 4) with every 1000th tree saved. Default values were used for the MCMCMC parameters. Consensus trees with mean branch lengths were constructed using the 'sumt' command option and ignoring the initial topologies saved during 'burnin'; the initial *n*-generations before log-likelihood values and substitution parameters plateau. For the analysis we plotted log-likelihood values against generation number and used a conservative 'burnin' of 2000 for estimating sumt and sump.

Results

Family Acanthocolpidae Lühe, 1906
Genus *Stephanostomum* Looss, 1899

Stephanostomum adlardi sp. nov. (Figs 1–5)

Description: Based on 17 whole-mounted specimens, some immature, some fragmentary, some mounted laterally, measurements on 10 dorso-ventrally mounted worms. Body elongate, widest in posterior forebody, 3,191–4,952 × 283–493 (3,982 × 371); width 7.06–11.7 (9.42) of length (Fig. 1). Tegument with strong spines reaching to posterior extremity, sparser in hindbody. Eye-spot pigment scattered in dense patches in parenchyma lateral to anterior part of oesophagus,

sparser lateral to mid-oesophagus. Oral sucker consisting of dorsal and ventral transversely elongated lobes, each bearing transverse row of spines, each lobe indented in middle of anterior margin, posterior part of sucker extended as semicircular muscular lobes either side of prepharynx (Figs 2 and 4), narrow flange between lobes laterally (Fig. 5a), aperture between lobes (Fig. 5c), 152–202 × 331–415 (179 × 359), length 3.58–5.68 (4.54)% of body-length. Oral spines 30–32 (31) in number, in two rows, one on each lobe; dorsal spines 14–15 (14.9) in number, claviform, mid-dorsal spines 28–49 (38) long, reducing in size laterally, ventral spines 15–18 (16.4) in number, peg-like to subglobular, mid-ventral spines 14–36 (27) long, reducing in size laterally. Ventral sucker oval, in anterior third of body, 181–256 × 162–249 (220 × 209), length 4.51–6.51 (5.59)% of body-length. Sucker-length ratio 1:1.06–1.46 (1.24); width ratio 1:0.47–0.66 (0.58). Forebody 792–1,178 (1,001) long, 22.7–29.0 (25.2)% of body-length. Hindbody 2,173–3,534 (2,760) long, 64.9–71.4 (69.2)% of body-length. Prepharynx long, narrow, 271–472 (376) long, 8.10–11.8 (9.46)% of body-length; surrounded by hemispherical patch of fibrous tissue anteriorly. Pharynx pyriform, 169–218 × 75–109 (196 × 93), 4.39–5.42 (4.97)% of body-length. Oesophagus short to very short, 3–66 (27) long. Intestinal bifurcation 137–296 (219) anterior to ventral sucker. Caeca narrow, reaching close to posterior extremity, where they form uroproct.

Testes 2, tandem, separated, in posterior half of hindbody, oval; anterior 261–364 × 163–235 (295 × 191), 6.59–8.25 (7.45)% of body-length; posterior 276–402 × 167–236 (339 × 192), 7.81–9.27 (8.55)% of body-length. Distance

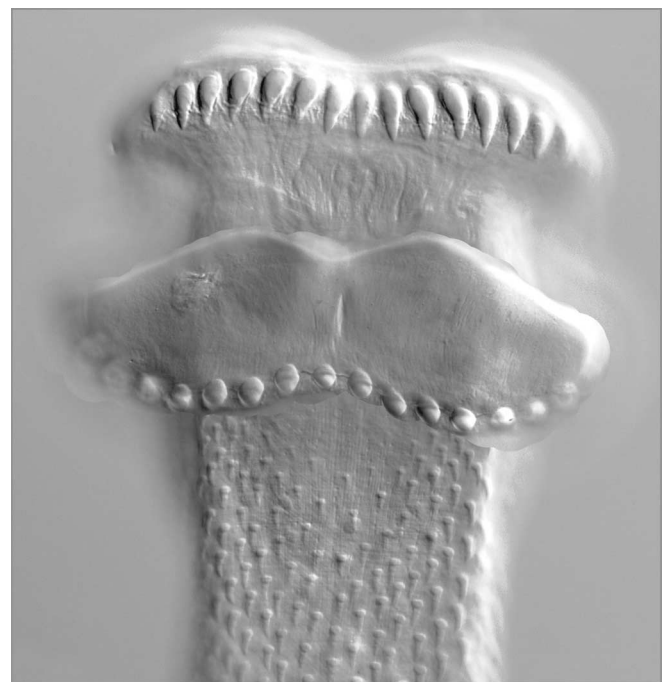


Fig. 4. *Stephanostomum adlardi* sp. nov. Composite picture of anterior extremity

between testes 45–334 (167), 1.35–7.78 (4.06)% of body-length. Post-testicular region 428–835 (586) long, 12.7–16.9 (14.6)% of body-length. Cirrus-sac elongate claviform, 511–904 × 79–128 (678 × 102), length 14.7–20.8 (17.1)% of body-length, overlaps 291–479 (368) into hindbody, 36.9–50.3 (42.1)% of ventral sucker to ovary distance (Fig. 3). Internal male duct 531–970 (702) long. Internal seminal vesicle large, curved oval, 241–543 (347) long, 38.8–56.0 (49.0)% of internal male duct. Pars prostatica elongate, but wider proximally, virtually vesicular in this region, lined with numerous non-nucleated cell-like bodies, 92–178 (137) long, 16.2–26.8 (19.7)% of internal male duct. Ejaculatory duct long, with fairly thick, smooth walls, no sign of ornamentation or arma-

ment, 148–282 (218) long, 26.9–42.7 (31.3)% of body-length. Genital atrium relatively small, about 14–99 (46) long. Genital pore median, immediately anterior to ventral sucker.

Ovary oval, median, pretesticular, 148–201 × 124–195 (173 × 151), length 3.39–5.25 (4.42)% of body-length; separated from ventral sucker by 683–1,024 (877), 20.4–23.3 (22.0)% of body-length; separated from anterior testis by 180–425 (283), 4.29–9.79 (7.10)% of body-length. Mehlis' gland antero-dorsal to ovary. Laurer's canal opens dorsally to ovary. Uterine seminal receptacle in proximal uterus. Uterus pretesticular, intercaecal. Metraterm short, with muscular wall, reaches to about mid-ventral sucker. Eggs tanned, operculate, 59–74 × 33–41 (67 × 36). Vitellarium follicular; anterior extremity in hindbody, 238–397 (298), 6.09–8.68 (7.49)% of body-length, 8.83–12.8 (10.8)% of hindbody, from ventral sucker; lateral, ventral and dorsal to caeca, confluent ventral and dorsal to uterus, virtually confluent between gonads, confluent dorsally and ventrally in post-testicular region.

Excretory pore terminal; excretory vesicle I-shaped, reaches almost to ovary.

Type-host: *Plectropomus leopardus* (Lacepède, 1802) leopard coral grouper (Perciformes, Serranidae).

Site: Intestine.

Type-locality: Lizard Island, Australia. (14°40'S, 145°28'E, Nov., 1988, April, 2006).

Prevalence: 3 of 22 (14%).

Deposition of specimens: Holotype – Queensland Museum QM G223067, paratypes – Queensland Museum QM G223068–223077, BMNH 2007.4.13.1–6.

Etymology: The species is named in honour of our colleague Dr Rob Adlard of Queensland Museum, in recognition of his help over many years and of his contribution to marine parasitology.

Discussion

Identification

Stephanostomum adlardi sp. nov. is unique in the Acanthocolpidae in the structure of the oral sucker (Bray 2005, Bray and Cribb 2006). This structure is not mentioned in the key in Bray (2005) and, therefore, it might be considered that this species belongs to an as yet undescribed genus. The molecular results discussed below suggest strongly, however, that this species is nested within the genus *Stephanostomum*, and in our present state of knowledge of the subclades of this genus, the erection of a new higher taxon is not appropriate. The oral sucker structure, therefore, distinguishes this species from all other species of *Stephanostomum*. Other noteworthy features of *S. adlardi* are the somewhat vesicular proximal part of the pars prostatica and the unarmed and unornamented ejaculatory duct.

As far as we are aware the only genus added to the Acanthocolpidae since the review of Bray (2005) is *Ningalootia* Bray et Cribb, 2006, from the Waigieu seaperch *Psammoderca waigiensis* (Cuvier) from Ningaloo Reef, off Western

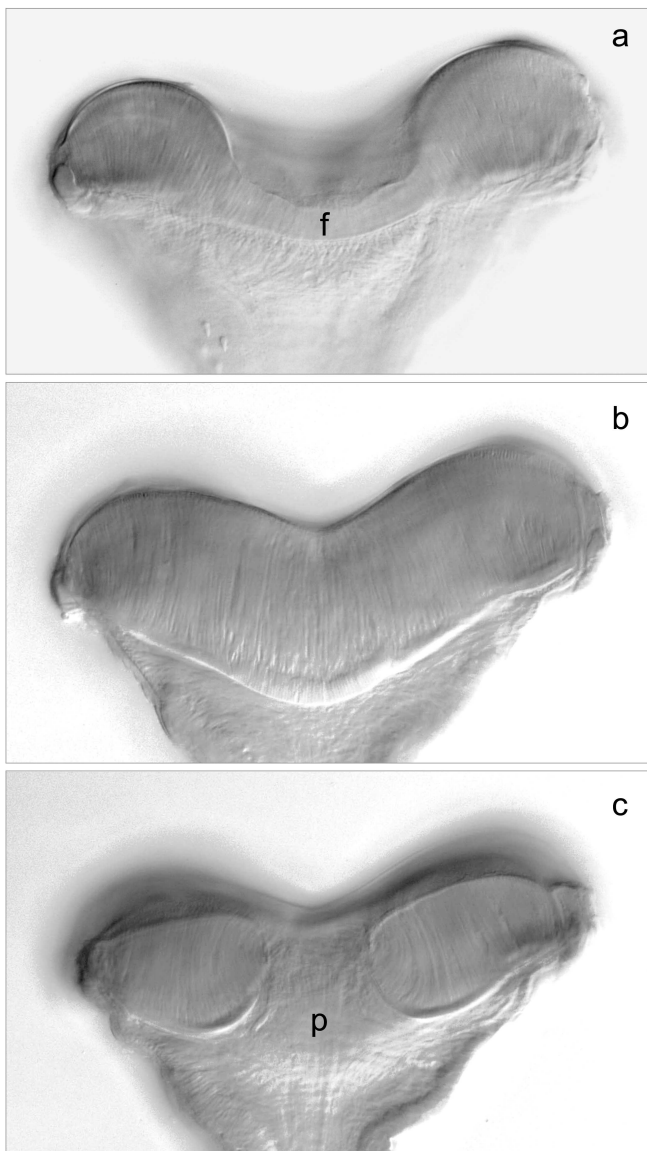


Fig. 5. *Stephanostomum adlardi* sp. nov. Three optical sections of lateral view of oral sucker: **a.** Marginal view, showing narrow flange (f); **b.** Submarginal view; **c.** Median view showing mouth aperture and prepharynx (p)

Australia (Bray and Cribb 2006). That genus is characterised mainly by the H-shaped intestine, and has a conventional (unspined) oval oral sucker. Bray *et al.* (2005) presented evidence that the marine mammal parasite family Brachycladiidae Odhner, 1905 (syn. Campulidae Odhner, 1926) is inseparable phylogenetically from the Acanthocolpidae. None of the brachycladiid genera as defined and keyed by Gibson (2005) have oral suckers similar to that of *S. adlardi* and it appears that none have been more recently described.

Molecular phylogeny

A molecular phylogeny has been inferred using SSU and partial LSU rDNA sequences (Fig. 6). The topology as reported in Bray *et al.* (2005) is altered by the addition of the two species to the study, both of which are nested within the genus *Stephanostomum*. The genus *Stephanostomum* is large (more than 110 described species) and the sample used in this study relatively small, less than 10% of known species. Nodal support is poor in some parts of the tree (<95% posterior probabilities), suggesting the need for additional more variable molecular phylogenetic markers, but the data set provides a suitable foundation for verifying the placement of *S. adlardi*. In the combined SSU and LSU data set a total number of 2828 characters were unambiguously alignable for the 17 ingroup and 11 outgroup taxa. Of these characters 772 were variable, of which 501 were informative under the principles of parsimony; 213 from SSU and 288 from LSU, respectively.

According to this analysis, *S. adlardi* is clearly a member of *Stephanostomum*, and is associated as a putative sister species to *Stephanostomum tantabiddii* Bray et Cribb, 2004, known only from the carangid, the yellowspotted trevally *Carangoides fulvoguttatus* (Forsskål, 1775), from the Ningaloo Coral Reef off northwestern Australia (Bray and Cribb 2004). No morphological features of this large species of *Stephanostomum* appear to substantiate this relationship, nor does host or locality give any clues to the relationship. The oral sucker is wider than long, but the circum-oral spines are in a conventional double row with a ventral hiatus. The ejaculatory duct is relatively short, but is armed with blunt conical spines and, in particular contrast to *S. adlardi* sp. nov., the pars prostatica is long and narrow. The addition of *S. adlardi* sp. nov. has resulted in the change of position of *S. tantabiddii* in the new tree relative to that in Bray *et al.* (2005), but the nodal support for much of the new topology is negligible and need not be discussed.

The second species added to the study, *Monostephanostomum nolani* from the carangid, the barcheek trevally, *Carangoides plagiotaenia* Bleeker, 1857 off Lizard Island (Bray and Cribb 2007), is associated with *Stephanostomum pristis* (Deslongchamps, 1824), a parasite mainly of gadids and other gadiforms from the north-eastern Atlantic and Mediterranean basin (Bartoli and Bray 2001). The genera *Monostephanostomum* Kruse, 1979 and *Stephanostomum* are very similar, reliably separated only on the number of circum-oral spine rows (Bray 2005). It is not at all surprising, therefore, that *Mono-*

stephanostomum is indicated to have arisen within *Stephanostomum*. If the genus *Monostephanostomum* should prove to have any validity as a monophyletic taxon, it is likely that other members of the genus will also prove close to *S. pristis*, because some [e.g. *M. mesospinosum* (Madhavi, 1976), *M. gazzae* (Shen, 1990) and *M. georgianum* Bray et Cribb, 2002] have comparable general morphology, having a relatively elongate body, with separated gonads and vitelline fields interrupted at the levels of the gonads. The addition of *M. nolani* has altered the topology of the tree slightly from that in Bray *et al.* (2005), but with negligible support.

The addition of the two new species to the study has shown unequivocally that both *S. adlardi* and *M. nolani* are acanthocolpids, but has raised more questions than answers in assessing their relationships. A denser sampling of the large genus *Stephanostomum*, and the sampling of more variable markers, perhaps from mitochondrial DNA, is required to shed light on the problem of the status of *Monostephanostomum* and whether *Stephanostomum* is readily divisible into morphologically recognisable subclades.

Distribution

This species was found in three of 22 (14%) individuals of *Plectropomus leopardus* examined at Lizard Island. This fish has been studied for digeneans from China (Ku and Shen 1975, Shen 1990) to the southern Great Barrier Reef (e.g. Bray *et al.* 1993, Overstreet and Kjøie 1989). Strikingly, however, *S. adlardi* has so far been found only at Lizard Island. In particular it has been seen in none of 72 individuals of the same species examined by the present authors at Heron Island on the southern Great Barrier Reef. A sample of 72 gives 95% confidence of finding any species present at just over 4%, so it seems likely that this species is absent at Heron Island, and perhaps on the southern Great Barrier Reef as a whole.

Genera in the Acanthocolpidae

There is little doubt that, had molecular results not been available, *S. adlardi* would have been considered unusual enough to merit the recognition of a new genus. The molecular results must cast considerable doubt, not only of the distinct status of *Monostephanostomum*, but also on the status of other similar acanthocolpids with oral spine rings, such as *Stephanostomoides* Mamaev et Oshmarin, 1966 and *Manteria* Caballero, 1950 (see Bray 2005). We are not, at this stage, formally synonymizing *Monostephanostomum* with *Stephanostomum* as *M. nolani* is not the type-species of the genus, which is now considered to contain a further seven species (Bray and Cribb 2007). Those genera lacking enlarged oral spines, represented in the molecular study by *Tormopsolus* Poche, 1926 and *Pleorchis* Railliet, 1896, appear to belong in a separate clade within the Acanthocolpidae and there is no evidence that they are not valid and monophyletic.

An alteration to the diagnosis of *Stephanostomum* is appropriate (changes from that in Bray 2005 in bold):

Diagnosis: Body elongate. **Oral sucker usually oval, terminal or subterminal, with two rings of enlarged oral spines, occasionally divided into dorsal and ventral lobes each with one row of enlarged oral spines.** Ventral sucker rounded, in anterior third of body. Prepharynx long. Pharynx oval to pyriform. Oesophagus absent or short. Intestinal bifurcation in posterior forebody. Caeca reach posterior extremity, open via uroproct or end blindly. Testes two, oval, entire, tan-

dem, in posterior half of hindbody. Cirrus-sac elongate claviform. Internal seminal vesicle oval. Pars prostatica present. Ejaculatory duct long, may be lined with spines. Genital atrium deep, may be armed. Genital pore median, immediately anterior to ventral sucker. Ovary rounded, pre-ovarian. Uterus pretesticular, intercaecal. Metraterm long, may be lined with spines similar to those lining ejaculatory duct. Vitellarium usually restricted to hindbody, may reach into forebody. Ex-

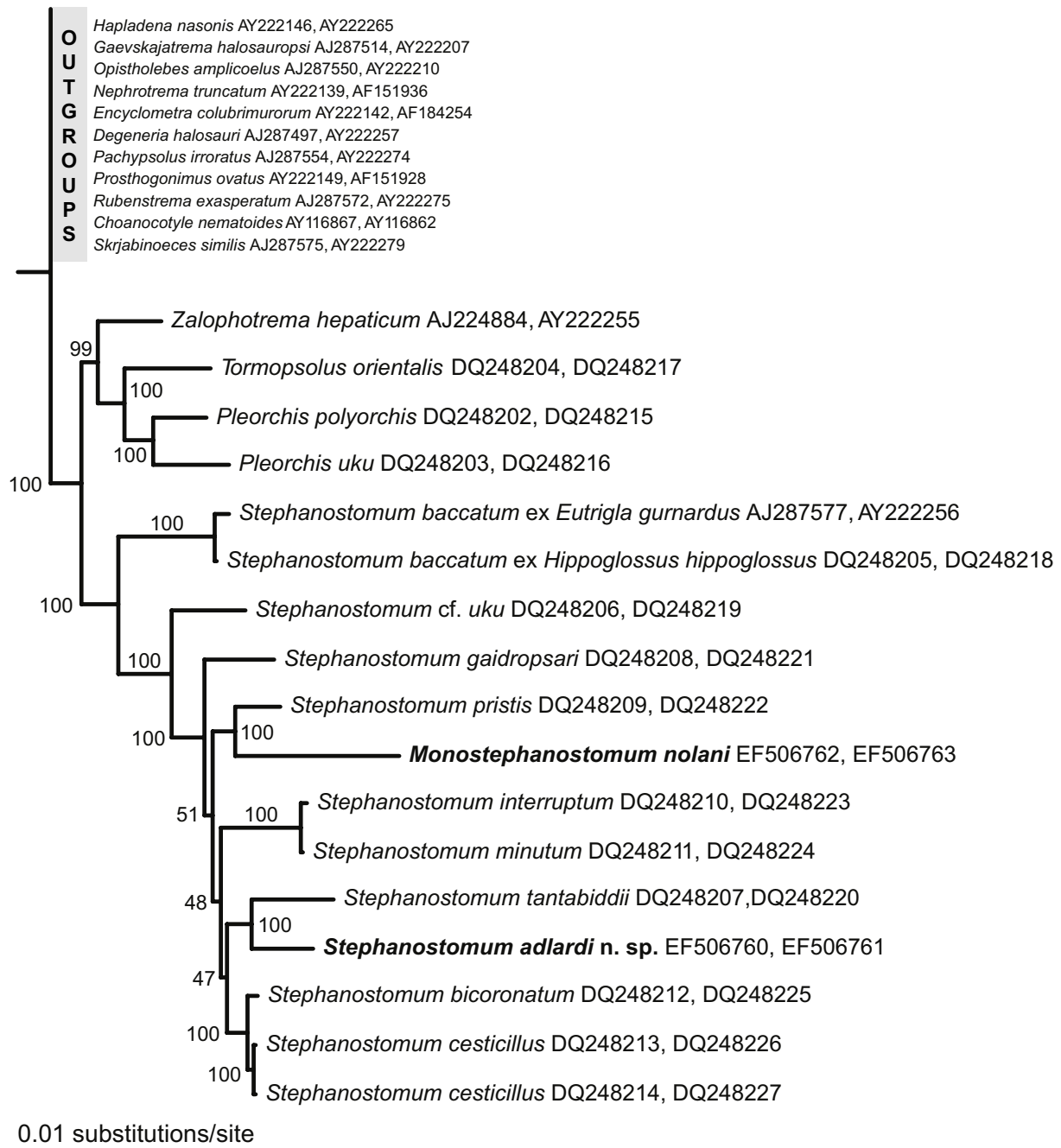


Fig. 6. Phylogeny of Acanthocolpidae estimated from combined complete SSU and partial LSU rDNA. The tree is derived from Bayesian inference, employing the GTR+I+G model of substitution for each data partition, with nodal support provided by posterior probabilities; see text for further details. Numbers after species names indicate GenBank accession numbers for complete SSU and partial LSU rDNA sequences respectively. Taxa in bold represent species newly sequenced for the present study

cretory vesicle I-shaped, reaches to various levels. In marine teleosts; cosmopolitan. Type-species *Stephanostomum cestiticillus* (Molin, 1858).

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