Parasitology

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A new genus and species of the trematode family Gyliauchenidae Fukui, 1929 from an unexpected, but plausible, host, *Kyphosus cornelii* (Perciformes: Kyphosidae)

Daniel C. Huston[1], Terrence L. Miller[2], Scott C. Cutmore[1] and Thomas H. Cribb[1]

[1]The University of Queensland, School of Biological Sciences, Brisbane, QLD 4072, Australia and [2]Department of Primary Industries and Regional Development, Fish Health Laboratory, South Perth, WA 6151, Australia

Research Article

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Author for correspondence:
Daniel Huston, E-mail: Daniel.Huston@uqconnect.edu.au

Abstract

The Enenteridae Yamaguti, 1958 and Gyliauchenidae Fukui, 1929 exhibit an interesting pattern of host partitioning in herbivorous fishes of the Indo-West Pacific. Enenterids are known almost exclusively from fishes of the family Kyphosidae, a group of herbivorous marine fishes common on tropical and temperate reefs. In contrast, gyliauchenids are found in most of the remaining lineages of marine herbivorous fishes, but until the present study, had never been known from kyphosids. Here we report on the first species of gyliauchenid known from a kyphosid. *Endochortophagus protoporus* gen. nov., sp. nov. was recovered from the Western buffalo bream, *Kyphosus cornelii* (Whitley, 1944), collected off Western Australia. *Kyphosus cornelii* also hosts an enenterid, *Koseiria allanwilliamsi* Bray & Cribb, 2002, and is thus the first fish known in which enenterids and gyliauchenids co-occur. Molecular phylogenetic analyses place the new species close to those of *Affecauda* Hall & Chambers, 1999 and *Flagellotrema* Ozaki, 1936, but there is sufficient morphological evidence, combined with the unusual host, to consider it distinct from these genera. We discuss factors which may have contributed to the host partitioning pattern observed between enenterids and gyliauchenids.

Introduction

The Gyliauchenidae Fukui, 1929 is a peculiar lineage of the Lepocreadioidea Odhner, 1905, comprising 13 genera and 41 species (Bray *et al.*, 2009; Bray and Cribb, 2012; WoRMS, 2018). Gyliauchenids are found almost exclusively in the intestines of herbivorous marine, and mostly tropical, teleosts and approximately 75% of species are known only from the Indo-West Pacific region (Hall and Cribb, 2005*a*; Cribb *et al.*, 2016). Species of the Gyliauchenidae reach their definitive hosts *via* cercariae which encyst on vegetation (Al-Jahdali and Hassanine, 2012), rather than infecting a second intermediate host. Such external encystment is the typical life-cycle strategy for digeneans which exploit herbivorous fishes, e.g. species of the Atractotrematidae Yamaguti, 1939, Gorgocephalidae Manter, 1966, Haplosplanchnidae Poche, 1926 and Microscaphidiidae Looss, 1900 (Cable, 1954; Hassanine *et al.*, 2016; Huston *et al.*, 2016, 2018).

Gyliauchenids are most readily recognized by their unusual morphology, which appears to represent specializations for life in herbivorous fishes. The oral sucker is thought to have been replaced by the pharynx as the anterior muscular structure, in many species the oesophagus has become coiled and greatly elongated, and there is a muscular oesophageal bulb that acts as a valve between the oesophagus and caeca (Jones *et al.*, 2000; Hall and Cribb, 2005*a*). The loss of the oral sucker is thought to be an indication of a transition to a fluidic diet, and the position of the ventral sucker near the posterior extremity in most species, is thought to facilitate better grazing on the hosts' luminal contents (Jones *et al.*, 2000). Gyliauchenids also appear to be closely associated with other intestinal symbionts, hosting complex ecto-commensal communities of microorganisms (Hughes-Stamm *et al.*, 1999), as well as ingesting free-swimming species (Jones *et al.*, 2000). The relationships between gyliauchenids and other endosymbionts are virtually unknown. Ecto-commensals are not thought to be harmful to gyliauchenids, though it is unknown if the relationship is beneficial to either the gyliauchenids or the microorganisms (Hughes-Stamm *et al.*, 1999). Similarly, it is unknown whether the ingestion of intestinal microorganisms by gyliauchenids is purposeful or incidental.

The major host groups for gyliauchenids in their adult form are herbivorous fishes from the surgeonfishes (Acanthuridae), butterflyfishes (Chaetodontidae), angelfishes (Pomacanthidae), rabbitfishes (Siganidae), parrotfishes (Scaridae), porgies (Sparidae) and the Moorish idol (Zanclidae) (Hall and Cribb, 2005*a*). Herbivorous species of damselfishes (Pomacentridae) have also been reported to host gyliauchenids occasionally (Dyer *et al.*, 1988; Nahhas and Wetzel, 1995). There are also scattered records from non-herbivores in the families Ariidae, Blenniidae, Clupeidae, Engraulidae and Synodontidae (Nahhas and Wetzel, 1995; Hall and Cribb, 2005*a*). Oddly, just one major group of large herbivorous marine fishes, the drummers or sea chubs (Kyphosidae), have never been reported to host gyliauchenids, although they have

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been extensively studied for parasites. Instead, they host species of the Enenteridae, the sister taxon of the Gyliauchenidae. This pattern of host-partitioning remains unexplained.

In December 2017, we collected specimens of *Kyphosus cornelii* (Whitley, 1944), *Kyphosus gladius* Knudsen & Clements, 2013, and *Kyphosus sydneyanus* (Günther, 1886) from off southwestern Australia. From *K. cornelii* we recovered a species of gyliauchenid, the first known from a kyphosid host. Here, using morphological and molecular data, we describe this species as new, propose a new genus to accommodate it and discuss the colonization of a kyphosid by a gyliauchenid.

Materials and methods

Specimen collection

This is the second in a series of reports on the trematode fauna of kyphosid fishes of the Indo-West Pacific (Perciformes: Kyphosidae). Between 1994 and 2018 we have collected 116 individual *Kyphosus* fishes of seven species from multiple localities across the Indo-West Pacific. The majority of these fishes were collected in Australian waters (Great Barrier Reef and Moreton Bay, Queensland; Yorke Peninsula, South Australia; Ningaloo Reef and off Perth, Western Australia), but the collection also includes fishes collected from French Polynesia, Japan, Palau and South Africa. For more specific information with regards to this collection, including collection numbers, localities and identification of specimens, readers are referred to the first paper of the series, Martin *et al.* (in press). Additionally, numerous other herbivorous marine fishes have been collected from the same localities during this period.

Morphological analyses

Herbivorous marine fishes were collected by spear and examined for trematodes following Cribb and Bray (2010). Trematodes were removed live from the host, fixed without pressure in near boiling saline and preserved in 70% ethanol. Trematode specimens used for morphological examination were removed from their preservative, washed in fresh water, overstained in Mayer's haematoxylin, destained in a solution of 1.0% hydrochloric acid and neutralized in a 0.5% ammonium hydroxide solution. Specimens were then dehydrated in a graded ethanol series. Some dehydrated specimens were selected for scanning electron microscopy (SEM); these specimens were transferred to hexamethyldisilazane, air-dried overnight and mounted on 12.5 mm pin-stubs using an adhesive carbon tab. Before SEM, specimens were coated with 15 nm of iridium with a Quorumtech Q150TS sputter coater. SEM images were obtained on a Hitachi SU3500 scanning electron microscope in secondary electron mode. Specimens for whole mounts were cleared for ~24 h in a 1:1 methyl salicylate/100% ethanol solution, then transferred to 100% methyl salicylate for ~24 h. After the 48 h clearing period, Canada balsam was added incrementally to the methyl salicylate over several days. Specimens were then permanently mounted on slides in Canada balsam. Both laterally and dorso-ventrally mounted specimens were used in this study; measurements for length, width and depth are provided. Length is taken from both dorso-ventrally and laterally mounted specimens, whereas width is taken only from dorso-ventrally mounted specimens and depth is taken only from laterally mounted specimens. Measurements were made with cellSens standard imaging software paired with an Olympus SC50 digital camera and drawings were made with a camera lucida, both mounted on an Olympus BX-53 compound microscope. Drawings were digitized in Adobe Illustrator. All vouchers are lodged in the Western Australian Museum (WAM), Perth, Australia; accession numbers are presented in the taxonomic section of this paper.

Molecular sequencing

Three molecular markers were targeted in this study, the second internal transcribed spacer region (ITS2), 28S rRNA and the mitochondrial cytochrome *c* oxidase I (COI). DNA was extracted from small tissue samples excised from individual specimens, with the remainder of the specimen being processed for morphological study as described above to serve as both a morphological and molecular voucher [hologenophore *sensu* Pleijel *et al.* (2008)]. Total genomic DNA was extracted from trematodes using phenol/chloroform extraction techniques (Sambrook and Russell, 2001). The D1–D3 regions of 28S nuclear ribosomal DNA (D1–D3 regions) were amplified using the primers LSU5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC-3′) (Littlewood, 1994) and 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Snyder and Tkach, 2001) and the ITS2 region using 3S (5′-GGTACC GGT GGATCA CGT GGC TAG TG-3′) (Morgan and Blair, 1995) and ITS2.2 (5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′) (Cribb *et al.*, 1998). Partial COI mtDNA was amplified using the primers Dig_cox1Fa (5′-ATG ATW TTY TTY TTY YTD ATG CC-3′) and Dig_cox1R (5′-TCN GGR TGH CCR AAR AAY CAA AA-3′) (Wee *et al.*, 2017).

Polymerase chain reaction (PCR) for the ITS2 and 28S regions was performed with a total volume of 20 μL consisting of 2 μL DNA template (~10 ng), 5 μL of 5× MyTaq Reaction Buffer (Bioline), 0.75 μL of each primer (10 μm), 0.25 μL of Taq DNA polymerase (Bioline MyTaq™ DNA Polymerase) and 11.25 μL H_2O (Invitrogen™ ultraPURE™ distilled water). PCR for the COI region was performed in the same manner using 4 μL DNA template, 5 μL reaction buffer, 2 μL of each primer, 0.25 μL Taq and 6.25 μL H_2O. Amplification was carried out on a MJ Research PTC-150 thermocycler with the following profiles: ITS2: an initial single cycle of 95 °C denaturation for 3 min, 45 °C annealing for 2 min, 72 °C extension for 90 s, followed by four cycles of 95 °C denaturation for 45 s, 50 °C annealing for 45 s, 72 °C extension for 90 s, followed by 30 cycles of 95 °C denaturation for 20 s, 52 °C annealing for 20 s, 72 °C extension for 90 s, followed by a final 72 °C extension for 5 min; 28S: an initial 95 °C denaturation for 4 min, followed by 30 cycles of 95 °C denaturation for 1 min, 56 °C annealing for 1 min, 72 °C extension for 2 min, followed by a single cycle of 95 °C denaturation for 1 min, 55 °C annealing for 45 s and a final 72 °C extension for 4 min; COI: an initial 94 °C denaturation for 3 min, followed by 40 cycles of 94 °C denaturation for 30 s, 50 °C annealing for 30 s and 72 °C extension for 30 s, with a final extension at 72 °C for 10 min. Sequence data were generated using the amplification primers, and the additional 28S internal primers 300F (5′-CAA GTA CCG TGA GGG AAA GTT-3′) (Littlewood *et al.*, 2000) and ECD2 (5′-CTT GGT CCG TGT TTC AAG ACG GG-3′) (Littlewood *et al.*, 1997) *via* cycle sequencing with an capillary sequencer and ABI Big Dye™ v.3.1 chemistry, performed by the Australian Genome Research Facility, Brisbane. Collection data and GenBank accession numbers for taxa sequenced are presented in the taxonomic section of this paper.

Phylogenetic analyses

The partial 28S rRNA sequences generated in this study were aligned with sequences of species of Gyliauchenidae and selected outgroup taxa available on GenBank (Table 1). Species of the Enenteridae were chosen as outgroup taxa as the family has been repeatedly demonstrated as the sister group to the Gyliauchenidae (Bray *et al.*, 2009, 2018; Bray and Cribb, 2012).

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Table 1. Gyliauchenidae and selected outgroup taxa from GenBank used in phylogenetic analyses, including host, provenance data, accession number and original reference

Parasite Taxon	Host	Collection locality	GenBank ID	Reference
Gyliauchenidae				
Affecauda annulata	*Naso tuberosus*	Lizard Island, QLD, Australia	FJ788501	Bray *et al.* (2009)
Flagellotrema amphitrite	*Prionurus maculatus*	Heron Island, QLD, Australia	AY382702	Hall and Cribb (2008)
Flagellotrema reburrus[a]	*Siganus vulpinus*	Heron Island, QLD, Australia	AY382703	Hall and Cribb (2008)
Paragyliauchen arusettae	*Pomacanthus sexstriatus*	Ningaloo Reef, WA, Australia	FJ788503	Bray *et al.* (2009)
Paragyliauchen sp.	*Centropyge bicolor*	Lizard Island, QLD, Australia	FJ788502	Bray *et al.* (2009)
Petalocotyle adenometra	*Prionurus microlepidotus*	North Stradbroke Island, QLD, Australia	FJ788504	Bray *et al.* (2009)
Petalocotyle diverticulata	*Acanthurus nigrofuscus*	Heron Island, QLD, Australia	AY382727	Hall and Cribb (2004)
Ptychogyliauchen leucothea	*Siganus argenteus*	Ningaloo Reef, WA, Australia	AY382714	Hall and Cribb (2004)
Ptychogyliauchen thetidis	*Siganus vulpinus*	Heron Island, QLD, Australia	AY382715	Hall and Cribb (2004)
Ptychogyliauchen thistilbardi	*Siganus spinus*	Noumea, New Caledonia	AY382720	Hall and Cribb (2004)
Robphildollfusium fractum	*Sarpa salpa*	Mediterranean Sea, Perpignan, France	FJ788505	Bray *et al.* (2009)
Outgroup				
Enenterum aureum	*Kyphosus vaigiensis*	Moorea, French Polynesia	AY222232	Olson *et al.* (2003)
Proenenterum isocotylum	*Aplodactylus arctidens*	Tasmania, Australia	FJ788500	Bray *et al.* (2009)

[a]As *Flagellotrema amphitrite* on GenBank (see Hall and Cribb, 2004).

We note that there are currently several gyliauchenid sequences on GenBank which relate to *nomina nuda*; these sequences were excluded from analyses. Alignment for the 28S rRNA sequences were performed with MUSCLE (Edgar, 2004) as implemented in MEGA7 (Kumar *et al.*, 2016). The resultant alignment was trimmed to the maximal length of the shortest 50% of sequences. Phylogenetic trees based on the 28S dataset were constructed with maximum likelihood and Bayesian inference analyses on the CIPRES portal (Miller *et al.*, 2010) implementing best-fit nucleotide substitution models selected using jModelTest 2 (Darriba *et al.*, 2012) with the Akaike information criterion and Bayesian information criterion. Maximum likelihood analyses were performed using RAxML (Stamatakis, 2014) with 1000 bootstrap pseudoreplicates. Bayesian inference was performed using MrBayes v3.2.6 (Ronquist *et al.*, 2012). Four chains were sampled every 1000 generations for 10 000 000 generations with the first 2500 samples being discarded as burn-in, at which point average standard deviation of split frequencies were <0.01 for all analyses.

Results

Of the 116 *Kyphosus* fishes collected between 1994 and 2018, trematodes of the family Gyliauchenidae were recovered from only six individuals, and only from *K. cornelii* collected from locations off Perth, Western Australia. This species of gyliauchenid is the first known from the family Kyphosidae, and based on combined morphological and molecular evidence, the species is considered new to science, and a new genus is proposed to accommodate it.

Phylogenetic results

The final 28S alignment consisted of 1325 nucleotide positions; no regions of alignment ambiguity were detected. Bayesian inference and maximum likelihood analyses produced trees with identical topologies (Fig. 1). Species of six genera, including representatives of all three of the currently recognized subfamilies were included, and all currently recognized morphological groupings formed highly-supported clades in the phylogenetic analyses.

The results of our molecular phylogenetic analyses were much the same as the most recent for the Gyliauchenidae (Bray *et al.*, 2009). *Robphildollfusium fractum* (Rudolphi, 1819), the sole representative of the Robphildollfusiinae Paggi & Orecchia, 1963 in our analyses, was found sister to species of the Petalocotylinae Ozaki, 1934. *Robphildollfusium fractum* + species of Petacotylinae were sister to the remaining genera, which are all members of the Gyliaucheninae Fukui, 1929. Species of *Paragyliauchen* Yamaguti, 1934 were sister to the remaining genera of the Gyliaucheninae represented in our analyses, which formed two clades, one comprising species of *Ptychogyliauchen* Hall & Cribb, 2004 and another further subdivided into three small clades representative of *Flagellotrema* Ozaki, 1936, *Affecauda* Hall & Chambers, 1999 and the gyliauchenids collected from *K. cornelii* in Western Australia. The molecular data, combined with morphological evidence (see below), warrants the erection of a new genus and the description of a new species.

Taxonomy

Family Gyliauchenidae Fukui, 1929

Subfamily Gyliaucheninae Fukui, 1929

Genus *Endochortophagus* gen. nov. Figures 2 and 3

Zoobank Life Science Identifier: urn:lsid:zoobank.org:act:0EC999B9-B0A7-415E-A77E-3FFD83E04A8C

Type and only known species: *Endochortophagus protoporus* sp. nov

Diagnosis. Body elongate, fusiform. Tegument smooth. Pharynx spheroid to dolioform. Ventral sucker ellipsoidal, near posterior extremity. Oesophagus muscular, with one or two loops, with direction of looping variable, length representing less than 50% of body length. Oesophageal bulb muscular, ellipsoidal, smaller than pharynx. Caeca two, blind, terminate in mid-body. Testes two, diagonal or tandem, in posterior third of body. Seminal vesicle external, distinct. Pars prostatica partially external to cirrus sac. Cirrus sac robust, in mid-body. Genital atrium indistinct. Ovary subglobular, smaller than testes. Uterus convoluted, heavily coiled. Eggs thin-shelled, unembryonated *in utero*. Vitellarium follicular, profuse; fields extend from pharynx to

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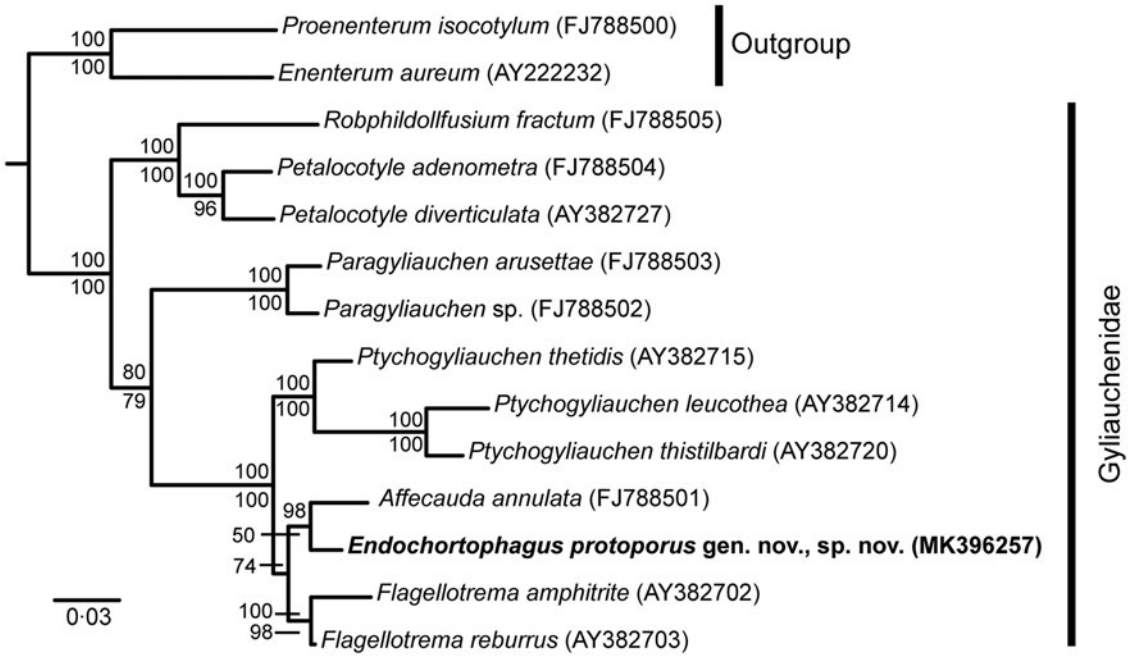

Fig. 1. Phylogenetic relationships of the Gyliauchenidae based upon Bayesian inference (BI) and maximum likelihood (ML) analyses of the 28S rRNA dataset. BI posterior probabilities are shown above the node and ML bootstrap support is shown below. Support values less than 50 not shown.

posterior testis. Excretory vesicle elongate, claviform, reaching to about level of posterior margin of posterior testis, but dependent on preparation/orientation of specimens, subterminal. Excretory pore opening on a small, conspicuous, subterminal papilla. In intestine of Indo-West Pacific herbivorous teleosts (Kyphosidae).

Etymology. The name of the new genus is constructed from Latinized Greek: 'endo' = within, and 'chortophagus' = vegetarian/vegetable eater, and is treated as masculine. The name is in reference to the algal cells often visible in the guts of these worms, which suggests that they may be herbivores living within herbivores.

Remarks. There are many unpublished sequences of gyliauchenids on GenBank that refer to taxa which are presently *nomina nuda*. The diversity among these unverified and otherwise unpublished sequences suggests that the genus-level classification of the Gyliauchenidae is far from settled. Analysis of these sequences however, shows that none of these unpublished taxa are especially close to *E. protoporus*; certainly none has been detected from a kyphosid.

The results of our molecular phylogenetic analyses (Fig. 1) place *E. protoporus* sp. nov. (see description below) as sister to *Affecauda annulata* Hall & Chambers, 1999. However, following the key to the Gyliauchenidae provided by Hall and Cribb (2005*a*) and the subsequently updated diagnoses of *Flagellotrema* Ozaki, 1936, *Ichthyotrema* Caballero & Bravo-Hollis, 1952 and *Telotrema* Ozaki, 1933 (Hall and Cribb, 2005*b*, 2007, 2008), *E. protoporus* is the most similar morphologically to species of *Flagellotrema* Ozaki, 1936. We note that in our phylogenetic analyses (Fig. 1) the branch lengths separating species of *Flagellotrema* and *Ptychogyliauchen* Hall and Cribb, 2004 are longer than those separating *E. protoporus* from *A. annulata*, but there is sufficient morphological evidence to recognize these species in separate genera.

Although *E. protoporus* is quite similar morphologically to species of *Flagellotrema*, it can be distinguished from species of that genus by having a short, relatively straight ejaculatory duct rather than one which is long and convoluted, a genital atrium without cilia-like lining, a uterus which may reach past the ovary rather than being entirely pre-ovarian, and having vitelline follicles reaching to the posterior testis, rather than being restricted entirely to the pre-testicular region. Species of *Affecauda* differ from *E. protoporus* primarily in having far more slender bodies, tegument with annulation, rather than being smooth, a large convoluted genital atrium rather than one which is short and unspecialized, and having vitelline follicles restricted to the pre-testicular region rather than extending to the posterior testis. *Endochortophagus protoporus* is readily distinguished from species of the remaining genera of the Gyliauchenidae using the key provided by Hall and Cribb (2005*a*).

Endochortophagus protoporus sp. nov. Figures 2 and 3.

Zoobank Life Science Identifier: urn:lsid:zoobank.org:act:7AA3D447-F945-4A40-B228-BBF89BEBCFF9.

Type-host: *Kyphosus cornelii* (Whitley, 1944) Western buffalo bream (Perciformes: Kyphosidae).

Type-locality: off Pt. Peron, WA, Australia (32°15′S, 115°41′E).

Other localities: off Garden Island, WA, Australia (32°13′S, 115°40′E).

Site in host: hindgut fermentation chamber (see Fig. 4).

Prevalence: six of seven (86%).

Type material: Holotype (WAM V9322), 27 paratypes, including three hologenophores (WAM V9323–9349).

Representative DNA sequences: three identical sequence replicates each of COI mtDNA, ITS2 rDNA and 28S rDNA (one of each submitted to GenBank, COI: MK396256, ITS2: MK396258, 28S: MK396257).

Etymology: 'protoporus' is a Latinized Greek word meaning pioneer. The name is chosen because the new species is the first gyliauchenid known from a species of the teleost fish family Kyphosidae.

Description (Figs 2 and 3), measurements given in Table 2.

Based on 28 specimens, five dorso-ventral wholemounts, 20 lateral wholemounts and three lateral hologenophores. Body elongate, fusiform, often strongly bent at midbody. Tegument smooth. Ventral sucker ellipsoidal, longitudinally oblate, near posterior extremity, ventro-subterminal; aperture longitudinal, attenuating posteriorly. Mouth surrounded by seven indistinct lobes with coarse tegument (Fig. 3). Pharynx spheroid to dolioform, ventro-subterminal; ventral portion with anteriorly

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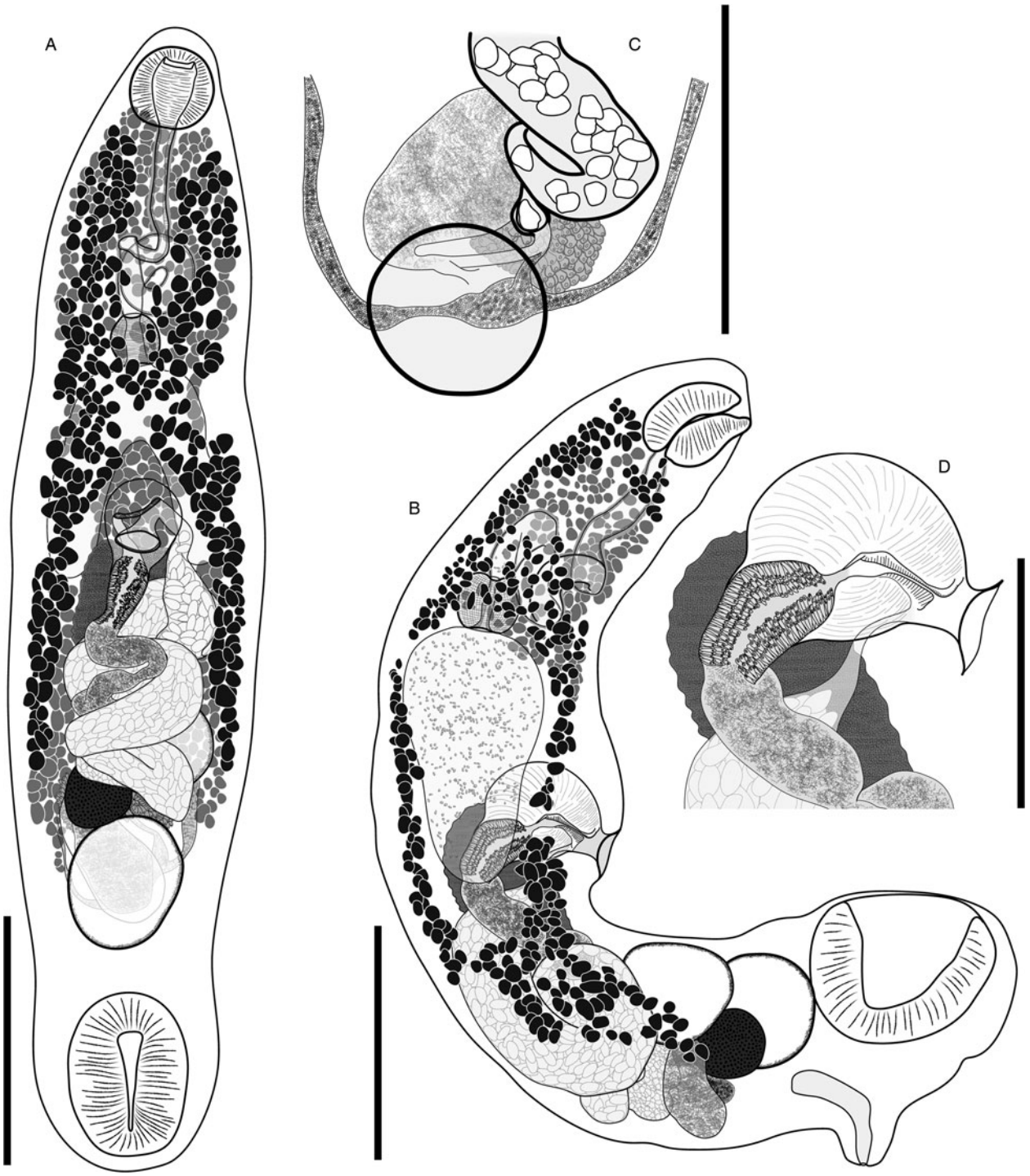

Fig. 2. *Endochortophagus protoporus* gen. nov., sp. nov. (A) Holotype, dorso-ventral view. (B) Paratype, lateral view. (C) Ovarian complex; dark circle is outline of ovary situated ventrally. (D) Terminal genitalia. Scale bars: A, B, 1000 μm; C, D, 500 μm.

protruding 'lip'. Oesophagus muscular, with one or two loops; direction of looping variable; prominent gland cells surround caeca along entire length. Oesophageal bulb in anterior third of body, muscular, ellipsoidal, smaller than pharynx. Intestine bifurcates immediately posterior to oesophageal bulb; caeca two, blind; gastrodermis heavily developed. Algal cells commonly visible in oesophagus and intestine.

Testes two, subglobular, diagonal or tandem, in posterior third of body. Seminal vesicle swollen, arises near anterior testis, passes anteriorly among uterine coils, uniting with pars prostatica. Pars prostatica amphoriform, penetrates cirrus sac; dense field of prostate gland cells surround pars prostatica and external to cirrus sac. Cirrus sac conspicuous, ellipsoidal to reniform, muscular, medial in midbody, ventral to caeca; ejaculatory duct broad or narrow, canalicular, relatively straight, passes directly to genital atrium. Genital atrium short, indistinct, unspecialized. Genital pore ovoid, medial, at level of caecal termination.

Ovary subglobular with position variable though generally intertesticular and contiguous with both testes. Seminal receptacle large, swollen, lachrymiform, antero-dorsal to ovary. Mehlis' gland and oötype dorsal or anterior to ovary. Uterus long, convoluted, heavily coiled, medial portions broad, typically pre-ovarian, rarely proximal portions coil just posterior to ovary, joins genital atrium ventral to cirrus sac; opening simple. Vitellarium follicular, profuse, fields confluent, extend from pharynx to posterior testis; ventral field forms X shape, with anterior arms extending along

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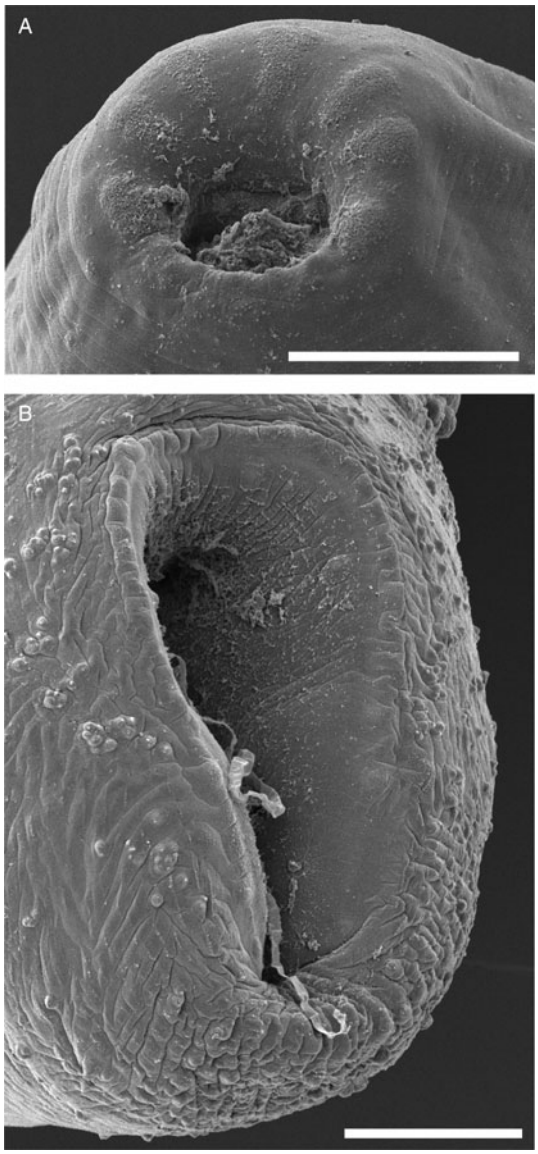

Fig. 3. Scanning electron micrographs of *E. protoporus* gen. nov., sp. nov. (A) Anterior extremity, showing seven indistinct lobes with coarse tegument surrounding mouth. (B) Posterior extremity and ventral sucker. Scale bars: 100 *μ*m.

either side of oesophagus, posterior arms diverging at caecal bifurcation; sinistral and dextral fields pass posteriorly from pharynx, diverge around caeca, re-join posterior to cirrus sac; dorsal field continuous, reaches from pharynx to posterior third of body. Vitelline reservoir dorsal to ovary; two main collecting ducts pass anteriorly, becoming indistinguishable from vitellarium anterior to cirrus sac. Eggs numerous, unembryonated *in utero*.

Remarks. This is the first gyliauchenid known from a kyphosid, but is otherwise morphologically unremarkable relative to the rest of the Gyliauchenidae. The worms were found only in a restricted region of the so called 'caecal pouch' or 'hindgut fermentation chamber' (see Rimmer and Wiebe, 1987; Clements and Choat, 1997) (Fig. 4), with the majority of specimens being collected from the lateral lobes of this structure. The enenterid *Koseiria allanwilliamsi* Bray and Cribb, 2002 was also found in *K. cornelii* in this study, from the same individuals infected with *E. protoporus*. However, they were found only in the midintestine (Fig. 4), demonstrating clear niche partitioning between *E. protoporus* and *K. allanwilliamsi*.

Discussion

The discovery of a gyliauchenid in a kyphosid was unexpected, but not unlikely. The most important host groups for species of the Gyliauchenidae are the rabbitfishes (Siganidae), parrotfishes (Scaridae) and surgeonfishes (Acanthuridae) (see Hall and Cribb, 2005*a*), and these three groups often represent the largest component of herbivore biomass in reef ecosystems (Cheal *et al*., 2012). Correspondingly, the gyliauchenid life cycle has adapted to exploit these herbivorous fishes through the omission of a second intermediate host, with metacercariae encysting in the environment (Al-Jahdali and Hassanine, 2012). However, species of the genus *Kyphosus* are also significant herbivores in terms of biomass on tropical and temperate reefs (Knudsen and Clements, 2013, 2016), and are thus likely to routinely consume gyliauchenid metacercariae. It seems likely then, that repeated encounter over time would create the opportunity for colonization. The present discovery was unexpected however, because there is a clear pattern in which gyliauchenids utilize all the major herbivore groups in the Indo-West Pacific except for species of the Kyphosidae, which are instead utilized by species of the sister family to the Gyliauchenidae, the Enenteridae (Bray and Cribb,

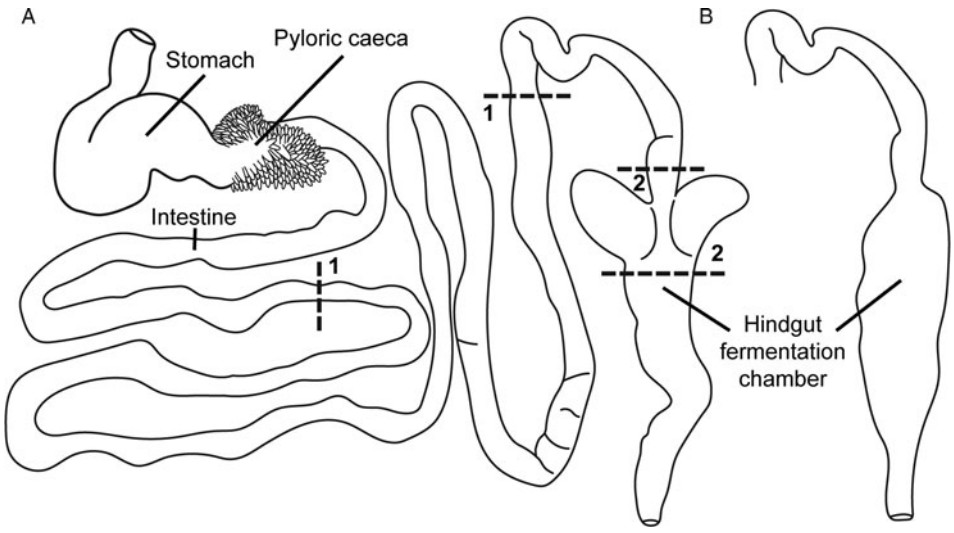

Fig. 4. Diagram of (A) the alimentary tract of *Kyphosus cornelii* and (B) a representation of the distal portion of the intestine of other *Kyphosus* species collected by the authors. (1) Section of *K. cornelii* intestine in which the enenterid *Koseiria allanwilliamsi* is found. (2) Section of *K. cornelii* intestine (subsection of hindgut fermentation chamber) in which *E. protoporus* gen. nov., sp. nov. is found.

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Table 2. Morphometric data for *Endochortophagus protoporus* gen. nov., sp. nov. expressed in micrometres or as percentages

Feature	*n*	Min	Max	Mean	Feature	*n*	Min	Max	Mean
BL	25	3302	4723	3834	AtW	5	385	469	432
BW	5	842	1073	944	AtD	20	305	537	414
BD	20	529	993	741	At–Pt	25	0	89	7
BL/BW	5	3.8	5.3	4.3	PtL	25	331	520	447
BL/BD	20	4	6.6	5.1	PtW	5	373	482	436.4
PhL	25	266	401	335	PtD	20	320	554	453
PhW	5	288	339	317	Pt–VS	25	0	475	71
PhD	20	262	328	296	AtL % of BL	25	0.08	0.15	0.11
PhL/PhW	5	0.85	1.01	0.96	PtL % of BL	25	0.10	0.15	0.12
PhL/PhD	20	0.83	1.46	1.17	Pt–VS % of BL	25	0	0.11	0.02
OeL	25	908	2048	1357	Pts	25	614	1160	769
OeL % of BL	25	0.29	0.46	0.36	Pts % of BL	25	0.17	0.27	0.20
Oeb	25	157	229	202	OvL	25	184	310	248
OebW	5	178	246	207	OvW	5	231	267	252.2
OebD	20	217	262	239	OvD	20	197	319	263
OebL/OebW	5	0.83	1.08	0.99	OvL % of BL	25	0.05	0.08	0.07
OebL/OebD	20	0.67	0.98	0.85	Ov–At	25	0	59	5.44
VSL	25	552	737	638	Ov–Pt	25	0	33	1
VSW	5	399	497	463	Ov–Ph	25	1745	3035	2209
VSD	20	457	599	517	Ov–Ph % of BL	25	0.47	0.70	0.58
VSL/VSW	5	1.27	1.38	1.33	Ov–VS	25	0	642	329
VSL/VSD	30	1.16	1.37	1.25	Ov–VS % of BL	25	0	0.14	0.09
VS–AE	25	2286	3896	3041	SrL	25	213	641	340
VS–PE	25	24	120	64	SrW	5	166	270	212
VS–AE % of BL	25	0.69	0.85	0.79	SrD	20	174	535	254
VS–PE % of BL	25	0.007	0.04	0.02	SrL % of BL	25	0.06	0.17	0.09
OebL/PhL	25	0.45	0.78	0.61	OvL/SrL	25	0.36	1.21	0.79
OebW/PhW	5	0.59	0.85	0.66	CsL	25	289	470	365
OebD/PhD	20	0.66	0.90	0.81	CsW	5	278	343	317
VSL/PhL	25	1.56	2.40	1.91	CsD	20	253	496	371
VSW/PhW	5	1.35	1.73	1.46	CsL % of BL	25	0.08	0.13	0.10
VSD/PhD	20	1.49	1.96	1.77	PPL	25	172	580	261
VSL/OebL	25	2.85	3.73	3.14	PPW	5	144	358	193
VSW/OebW	5	2.02	2.42	2.24	PPD	20	116	385	171
VSD/OebD	20	1.97	2.36	2.17	V–Ae	25	238	451	345
CL	25	579	1356	885	V–Pe	25	940	1584	1233
CW ab	5	518	723	591	V–Ae % of BL	25	0.07	0.13	0.09
Pcs	25	812	2438	1954	V–Pe % of BL	25	0.26	0.42	0.32
CL % of BL	25	0.17	0.33	0.23	Voc % of BL	25	0.47	0.67	0.59
Pcs % of BL	25	0.23	0.64	0.51	EvL	20	377	612	511
Ph–Oe	25	393	798	590	EVD	20	77	315	153
Ph–O % of BL	25	0.12	0.19	0.16	EPL	20	135	321	203
Ph–VS	25	1390	3569	2653	EPD	20	90	228	144
Ph–VS % of BL	25	0.59	0.77	0.70	Eggs L	50	75	91	82
Oeb–VS	25	1309	2622	2033	Eggs width	50	38	50	43
Oeb–VS % of BL	25	0.47	0.59	0.54	Egg L % of BL	50	0.02	0.03	0.02
AtL	25	281	517	425					

n, number measured; B, body; L, length; W, width; D, depth; Ph, pharynx; Oe, oesophagus; Oeb, Oesophageal bulb; VS, ventral sucker; Ae, anterior extremity; Pe, posterior extremity; C, caeca; ab, at caecal bifurcation; Pcs, post-caecal space; At, anterior testi; Pt, posterior testi; Pts, post-testicular space; Ov, ovary; Sr, seminal receptacle; Cs, cirrus sac; PP, *pars prostatica*; V, vitellarium; Voc, vitellarium occupies; Ev, excretory vesicle; EP, excretory papilla.
A dash (–) indicates distance between two features.

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2001, 2002, 2012; Bray *et al.*, 2009). Enenterids are restricted almost entirely to kyphosid fishes, and until now there was no host overlap between enenterids and gyliauchenids (Bray and Cribb, 2001, 2002; Bray *et al.*, 2009). The finding of *E. protoporus* from *K. cornelii* is a significant exception that deserves consideration.

How it is that a gyliauchenid has colonized a kyphosid in Western Australia, but not in other Indo-West Pacific localities, is unclear. Colonization of a new host requires that the infective stage of a parasite pass successfully through two filters: encounter and compatibility (Combes, 2001). In Western Australia, *K. cornelii* and *K. sydneyanus* occur sympatrically, but partition food resources, with *K. cornelii* feeding almost exclusively on benthic red algae and *K. sydneyanus* feeding almost exclusively on benthic brown algae (Rimmer and Wiebe, 1987). We examined specimens of *K. gladius* and *K. sydneyanus* from the same localities as *K. cornelii* (including on the same day), and gyliauchenids were not found in either of these species. Routine encounter between gyliauchenid metacercariae and many kyphosid species throughout the Indo-West Pacific seems highly likely. However it is conceivable that gyliauchenid cercariae have some level of specificity for the type of algae on which they encyst. If the cercariae of *E. protoporus* encyst only on red algae, then encounter between *E. protoporus* metacercariae and other kyphosid species might be rare.

Although a level of specificity for aquatic vegetation may explain the lack of gyliauchenids in *K. sydneyanus* and *K. gladius* in Western Australia, this idea is not convincing in other parts of the Indo-West Pacific. On the Great Barrier Reef for example, *Kyphosus cinerascens* feeds on the same algae species as *Acanthurus lineatus*, *Acanthurus nigricans*, *Naso tuberosus* and *Zebrasoma scopas*, and the diet of *Kyphosus vaigiensis* is comparable with that of *Naso unicornis* (Choat *et al.*, 2002). We have collected gyliauchenids from all of these acanthurid fishes on the Great Barrier Reef (unpublished data), but never from any kyphosids. If an explanation based on dietary distinction is not compelling in the tropics, then it may also be unconvincing for southwestern Australia. If it is likely that gyliauchenids routinely pass the encounter filter, but have not colonized other kyphosids of the Indo-West Pacific, compatibility may be a better explanation.

The site of infection in *K. cornelii*, the hindgut fermentation chamber, is unusual among kyphosids, in having two blind, lateral lobes, rather than being just an enlargement of the posterior portion of the intestine (Rimmer and Wiebe, 1987; Clements and Choat, 1997). This distinct morphological difference seems an important clue. *Kyphosus azureus* (Jenkins & Evermann), which occurs only along the Pacific coast of North America (Knudsen and Clements, 2013, 2016), has a similar hindgut fermentation chamber morphology, having a single blind lobe (Sturm and Horn, 1998; Fidopiastis *et al.*, 2006), but apparently no trematodes have been reported from this species. Understanding the function of these unique intestinal morphologies may explain why *K. cornelii* is the only kyphosid known to host gyliauchenids, but at present, it is unclear what physiological purpose the specialized hindgut configurations in *K. azureus* and *K. cornelii* serves (Clements and Choat, 1995; Mountfort *et al.*, 2002; Fidopiastis *et al.*, 2006). This difference may be due to diet and/or differences in the biochemical processes required for fermentation of different algal types.

There is little reported difference between acanthurids and kyphosids in the chemical composition, amount and proportion of short-chain fatty acids (SCFAs) produced as a result of microbial fermentation in the hindguts of these fishes (Rimmer and Wiebe, 1987; Clements and Choat, 1995; Choat and Clements, 1998; Choat *et al.*, 2002, 2004). However, little is known about the biodiversity and richness of the microbial communities which inhabit the intestinal tracts of marine herbivorous fishes. Although it is not entirely clear from where gyliauchenids derive their nutrition, they are thought to have a fluidic diet and to have adapted to feeding on the host's luminal content rather than the mucosa (Jones *et al.*, 2000). Furthermore, species of both the Enenteridae and the Gyliauchenidae consume algal cells, either purposefully or incidentally, as the cells are often apparent in mounted specimens. We have also observed enenterids expelling large numbers of live ciliate protists through the anus when they are removed from the host and placed in saline. In their study of microbial fermentation in *K. cornelii* and *K. sydneyanus*, Rimmer and Wiebe (1987) found that the greatest number and diversity of microorganisms occurred in the hindgut fermentation chamber, and that the proportions of bacteria and protists differed between the two fish species. Perhaps gyliauchenids and enenterids feed on some of the fermenting endosymbionts producing SCFAs, or ingest them incidentally while consuming a nutritive slurry containing the SCFAs. Conceivably, the gyliauchenids and enenterids diverged based upon physiological differences in the bacteria and protist endosymbiont communities associated with different host lineages.

At present there seems little evidence to explain the observed host-specificity patterns, but further insights might be gained through detailed study of the interactions between digeneans and other endosymbionts of herbivorous marine fishes. *Kyphosus cornelii* also hosts a species of enenterid, *K. allanwilliamsi* Bray and Cribb, 2002, and thus represents the first known host in which both an enenterid and gyliauchenid occur. Further study of *K. cornelii* and its endosymbiotic community may reveal factors which have led to the host-partitioning pattern observed between the Enenteridae and Gyliauchenidae.

Endochortophagus protoporus is the southernmost gyliauchenid yet reported. The range of *E. protoporus* may extend further south than the type locality, to Cape Leeuwin, Western Australia (34°22′S, 115°6′E), which is the southern range of the host, *K. cornelii* (Knudsen and Clements, 2013). At a minimum, the new record extends the range of gyliauchenids approximately 700 km further south, as the previous southernmost record was *Petalocotyle adenometra* from Amity Pt. in Queensland, Australia (27°23′S, 153°26′E). Gyliauchenids appear to range approximately as far north of the equator as south, with the northernmost record being near Tokyo, Japan (~35°N; Goto and Matsudaira, 1918). The exception to this pattern is the two species of the unusual Gyliauchenidae genus *Robphildollfusium*, which parasitize non-typical hosts, omnivorous fishes of the family Sparidae, in the Mediterranean and Atlantic. Species of *Robphildollfusium* have been reported as far north as the Adriatic Sea (~45°N). The centre of richness and diversity for the major host groups of the Gyliauchenidae is the tropical Indo-West Pacific (Nelson, 2006), but clearly a few gyliauchenids have managed to expand into subtropical zones [such as southwestern Australia where acanthurids, siganids and scarids are mostly absent (Gomon *et al.*, 2008)] through the colonization of other herbivorous host groups.

Author ORCIDs. Daniel C. Huston, 0000-0002-1015-4703

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Conflict of interest. None.

Ethical approval. This study was conducted in compliance with all institutional, national and international guidelines on the care and use of animals.

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