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# Two known and one new species of *Proctoeces* from Australian teleosts: variable host-specificity for closely related species identified through multi-locus molecular data

Nicholas Q-X. Wee [a], Thomas H. Cribb [a,*], Rodney A. Bray [b], and Scott C. Cutmore [a]

[a] The University of Queensland, School of Biological Sciences, St Lucia, Queensland, 4072, Australia

[b] Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom

* Corresponding author at: The University of Queensland, School of Biological Sciences, St Lucia, Queensland, 4072, Australia. Email: T.cribb@uq.edu.au (T. H. Cribb)

**Abstract**

Species of *Proctoeces* Odhner, 1911 (Trematoda: Fellodistomidae) have been reported from a wide range of marine animals globally. Members of the genus tend to lack strongly distinguishing morphological features for diagnosis, making identification difficult and the true number of species in the genus contentious. Combined morphological and molecular analyses were used to characterise three species of *Proctoeces* from Moreton Bay and the southern Great Barrier Reef. Data for two ribosomal regions and one mitochondrial region were generated for specimens collected from Australia. Three unique 18S-genotypes were identified which corresponded to subtle, but reliable, morphological differences. Two species of *Proctoeces* were identified from fishes of Moreton Bay, *Proctoeces insolitus* (Nicoll, 1915) Yamaguti, 1953 and *P. major* Yamaguti, 1934, and a third, *P. choerodoni* n. sp. from off Heron Island on the southern Great Barrier Reef. Phylogenetic analyses of partial 18S and partial 28S rDNA indicated that these three species differ from the four species reported outside of Australia for which sequence data are available. Phylogenetically, *Proctoeces* proved to be a reliable concept, with all species of *Proctoeces* that have been characterised genetically forming a well-supported clade in all analyses. Dramatically different patterns of host-specificity were identified for each of the three Australian species; *P. insolitus* apparently infects a single species of fish, *P. choerodoni* n. sp. infects multiple species of a single genus of fish, and *P. major* infects multiple species of two teleost orders.

**Keywords**
Fellodistomidae
*Proctoeces*
Host-specificity
18S rDNA

28S rDNA
*cox*1 mtDNA
Moreton Bay
Great Barrier Reef

## 1. Introduction

*Proctoeces* Odhner, 1911 is an enigmatic genus in the family Fellodistomidae Nicoll, 1909, comprising species reported from a wide range of marine animals. As sexually mature adults, species of the genus have been reported from many families of teleost fishes globally; however, the genus is strongly concentrated in two families, the Sparidae and Labridae [1-3]. Unusually for the Fellodistomidae, species of *Proctoeces* may also develop into sexually mature adults in the second [4-7] and even the first intermediate host [2, 8-10]; in some instances, the definitive host has been apparently completely excluded from the life-cycle [11] and several species have been described solely on the basis of infections from invertebrates [5, 7, 12, 13].

*Proctoeces* has a complicated taxonomic history. Since its erection by Odhner [14], to accommodate *Proctoeces maculatus* (Looss, 1901) from three labrids from Trieste, Italy [15], a further 20 species have been added to the genus. Due to a lack of distinguishing morphological features and the incidence of exceptional intraspecific morphological variation, Bray [16] synonymized 12 *Proctoeces* species with *P. maculatus.* The incorporation of molecular data in recent studies has since shown that this interpretation underestimates true richness [17-19]. The reinstatement of several of the synonymised species, and the description of several new species following Bray [16], means that currently 15 species are recognized as valid [20].

There are several reports of species of *Proctoeces* from Australia. *Proctoeces insolitus* (Nicoll, 1915) Yamaguti, 1953 was described from the sparid *Acanthopagrus australis* (Günther) [as *Xenopera insolita*] from Cleveland Bay off Northern Australia [21] and *P*. *maculatus* was reported from the labrid *Choerodon cyanodus* (Richardson) from the southern Great Barrier Reef (GBR) [16, 22]. Sporocysts and metacercariae interpreted as unidentified species of *Proctoeces* have been reported from oysters (*Saccostrea* Dollfus & Dautzenberg spp.) from Queensland and New South Wales [9, 23]. Here we combine morphological and molecular analyses to identify the richness of species of *Proctoeces* infecting teleosts from Queensland waters and explore host-specificity.

## 2. Materials and methods

### *2.1 Host and trematode collection*

Teleost fishes were collected from Moreton Bay and from off Heron Island, on the southern GBR, via tunnel or seine netting, line fishing and spear fishing. Fishes were killed via an overdose of anaesthetic (AQUI-S®) and the gastrointestinal tract was examined for parasites using the gut-wash approach described by Cribb & Bray [24]. Trematodes were washed in vertebrate saline, fixed by pipetting into near-boiling saline, and preserved in 70% ethanol for parallel morphological and molecular characterisation. Several specimens were cut at the midline and processed for both morphological (anterior portion) and molecular (posterior portion) analyses (hologenophores *sensu* Pleijel et al [25]).

### *2.2 Morphological analysis*

Specimens for morphological analysis were washed in fresh water, stained in Mayer's haematoxylin, destained in a solution of 1.0% HCl and neutralised in 0.5% ammonium hydroxide solution. Specimens were dehydrated through a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Measurements were made using an Olympus SC50 digital camera mounted on an Olympus BX-53 compound microscope using cellSens Standard imaging software. Measurements are in micrometres (µm) and are presented as a range, followed by a mean in parentheses. Where length is followed by breadth, the two measurements are separated by '×'. Drawings were made using an Olympus BX-53 compound microscope and drawing tube and digitized using Adobe Illustrator CS6 software. Regression functions were generated in Microsoft Excel. Type and voucher specimens are lodged in the Queensland Museum (QM), Brisbane. Specimens of *P. insolitus* of Nicoll [21] lodged in the QM were examined for comparative analysis.

*2.3 Molecular sequencing and phylogenetic analyses*

Total genomic DNA was extracted using phenol/chloroform extraction techniques [26]. The V4 region of the 18S nuclear ribosomal DNA region was amplified using SB3a (5'-GGA GGG CAA GTC TGG TGC-3';[22]) and A27a (5'-CCA TAC AAA TGC CCC CGT CTG-3';[22]) and the partial D1-D3 fragment of the 28S rDNA region using the primers LSU5 (5'-TAG GTC GAC CCG CTG AAY TTA AGC A-3'; [27]) and 1200R (5'-GCA TAG TTC ACC ATC TTT CGG-3'; [28]) or 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3'; [29]). Partial *cox*1 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').

PCR for both the 28S and 18S regions was performed with a total volume of 20 µl consisting of 5 µl of 5x MyTaq Reaction Buffer (Bioline), 0.75 µl of each primer (10 µM), 0.25 µl of Taq polymerase (Bioline MyTaq™ DNA Polymerase) and 2 µl of DNA template (approximately 10 ng), made up to 20 µl with Invitrogen™ ultraPURE™ distilled water. PCR for the *cox*1 region was performed with a total volume of 20 µl consisting of 5 µl of 5x MyTaq Reaction Buffer (Bioline), 2 µl of each primer (10 µM), 0.25 µl of Taq polymerase (Bioline MyTaq™ DNA Polymerase) and 2 µl of DNA template (approximately 10 ng), made up to 20 µl with Invitrogen™ ultraPURE™ distilled water. Amplification was carried out on a MJ Research PTC-150 thermocycler. The following profile was used to amplify the 18S region: an initial 94°C denaturation for 2 min 30 seconds, followed by 30 cycles of 94°C denaturation for 20 seconds, 50°C annealing for 30 seconds and 65°C extension for 1 min, with a final extension at 65°C for 10 min. The following profile was used to amplify the 28S region: 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. The following profile was used to amplify the *cox*1 region: an initial 94°C denaturation for 3 min, followed by 40 cycles of 94°C denaturation for 30 seconds, 50°C annealing for 30 seconds and 72°C extension for 30 sec, with a final extension at 72°C for 10 min. Amplified DNA was purified using a Bioline ISOLATE II PCR and Gel Kit according to the manufacturer's protocol. Cycle sequencing of purified DNA was carried out using ABI Big Dye™ v.3.1 chemistry following the manufacturer's recommendations, using the same primers used for PCR amplification as well as the additional 28S primers 300F (5'-CAA GTA CCG TGA GGG AAA GTT G-3'; [30]) and ECD2 (5'-CCT TGG TCC GTG TTT CAA GAC GGG-3'; [31]). Cycle sequencing was carried out at the Australian Genome Research Facility. Sequencher™ version 4.5

(GeneCodes Corp.) was used to assemble and edit contiguous sequences. Collection data and GenBank accession numbers for *Proctoeces* taxa sequenced for this study are presented in Table 2.

Newly generated 18S and 28S rDNA sequences were aligned with sequences of species of *Proctoeces* and other fellodistomid taxa available on GenBank (Table 3). Alignments were performed using MUSCLE version 3.7 [32] with ClustalW sequence weighting and UPGMA clustering for iterations 1 and 2. The resultant alignments were refined by eye using MESQUITE [33] and the ends of each fragment were trimmed to match the shortest sequence in each alignment.

Bayesian inference and Maximum Likelihood analyses were conducted for both 18S and 28S rDNA datasets to investigate species diversity and phylogenetic relationships. Bayesian inference analyses was performed using MrBayes version 3.2.6 [34] and Maximum Likelihood analysis was performed using RAxML version 8.2.6 [35], both run on the CIPRES portal [36]. The software jModelTest version 2.1.10 [37] was used to estimate the best nucleotide substitution model for the dataset. Both the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) calculated that the nucleotide substitution model TPM2uf+Γ was the most suitable for the 18S rDNA dataset and the model TPM2uf+I+Γ for the 28S rDNA dataset. Thus, Bayesian inference and Maximum Likelihood analyses were conducted using the closest approximation of these models, GTR+Γ and GTR+I+Γ for the 18S and 28S rDNA datasets, respectively. Bayesian inference analysis was run over 10,000,000 generations (ngen = 10000000) with two runs each containing four simultaneous Markov Chain Monte Carlo (MCMC) chains (nchains = 4) and every 1000$^{th}$ tree saved (samplefreq = 1000). Bayesian inference analysis used the following parameters: nst = 6, rates = invgamma (28S)/gamma (18S), ngammacat = 4, and the priors parameters of the combined dataset

were set to ratepr = variable. Samples of substitution model parameters, and tree and branch lengths were summarised using the parameters 'sump burnin = 3000' and 'sumt burnin = 3000'. Nodal support in the Maximum Likelihood analyses was estimated by performing 100 bootstrap pseudoreplicates. Species of *Coomera* Dove & Cribb, 1995 and *Fellodistomum* Stafford, 1904 (Fellodistomidae) were for designated as functional outgroups for 18S rDNA analyses, and species of Tandanicolidae Johnston, 1927 and Gymnophallidae Odhner, 1905 for the 28S rDNA analyses.

## 3. Results

### *3.1 Overview*

Specimens consistent with the genus *Proctoeces* were collected from 15 species, six families and two orders of fishes from Moreton Bay and off Heron Island (Table 1). Host/parasite combinations represented only by immature worms are not reported here on the basis that they do not reliably demonstrate infection. Preliminary morphological examination suggested the presence of multiple species of *Proctoeces*.

18S rDNA sequence data were generated for 12 of the Australian *Proctoeces*/host species combinations reported here (Table 2). There were three unique, replicated genotypes. The first 18S-genotype was from specimens found only in *A*. *australis* from Moreton Bay. The second 18S-genotype was from specimens found only in *C*. *cyanodus* from Heron Island. The third 18S-genotype was the most replicated, and was generated from specimens collected from a wide range of teleosts in Moreton Bay. The three 18S-genotypes differed from each other by 14–28 bases and from all other *Proctoeces* sequences available on GenBank by a minimum of seven bases. Complimentary 28S rDNA

sequences generated for the three Australian 18S-genotypes showed no variation within each type and differed from each other by 38–74 bases. Complimentary *cox*1 mtDNA sequences were generated for two specimens relating to each of the three Australian 18S-genotypes. These *cox*1 sequences showed intra-type variation of 0–2 bases and inter-type variation of 73–99 bases.

Additionally, five 18S and three 28S sequences were generated from samples of *P. maculatus* (*sensu* Antar and Gargouri [19]) from the Mediterranean. The 18S sequences, generated from infections from three hosts, were 100% identical and distinct from the three 18S-genotypes from Australia. This Mediterranean sequences were identical to an 18S-genotype identified as *Proctoeces maculatus* reported from specimens infecting *Mytilus edulis* Linnaeus from off New York (GenBank accession number KR052815). The 28S sequences matched those generated by Antar and Gargouri [19], with new sequences from specimens from *Mytilus galloprovincialis* Lamarck differing from sequences from specimens infecting *Sparus aurata* Linnaeus by 4 bases. These new Mediterranean 28S data differed from the three Australian genotypes by 41-62 bases.

Exploration of the morphology of specimens corresponding to the three Australian 18S-genotypes revealed consistent differences. Specimens from *A. australis* from Moreton Bay were distinctly narrower than those from all the other fishes (Fig. 1, 2A). Separation of the other two forms was less obvious. However, a difference was observed in the shape of the intestinal bifurcation. Specimens from species of *Choerodon* lack recognisable 'shoulders' at the intestinal bifurcation, whereas those relating to the third 18S-genotype (from multiple fishes from Moreton Bay) possess distinctly squared 'shoulders' (Fig. 1). This distinction is reflected in comparison of body length relative to the width of the gut at the intestinal bifurcation (Fig. 2B).

On the basis of differences in molecular data, morphology and host-specificity, we conclude that these forms can be reliably distinguished and should be considered distinct species. The two species collected from Moreton Bay were identified as known species described from elsewhere in the tropical Indo-west Pacific. The form found infecting *A. australis* is identified as *P. insolitus*, originally described from *A. australis* from Cleveland Bay, Queensland, Australia [21]. The form infecting a wide range of Moreton Bay fishes is identified as *P. major* Yamaguti, 1934, described from *Chrysophrys auratus* (Forster) from Japan [38]. These two reports represent significant range extensions for both species and new morphological and molecular data are provided. The single species from the GBR was found infecting four labrid fishes. Although molecular data could only be generated from specimens from one host (*C. cyanodus*), specimens from three other *Choerodon* species were morphologically consistent and are thus interpreted as the same species. This species is considered new to science and is described below.

*3.2. Morphology*

**Class Trematoda Rudolphi, 1808**

**Subclass Digenea Carus, 1863**

**Order Plagiorchiida La Rue, 1957**

**Suborder Bucephalata La Rue, 1926**

**Superfamily Gymnophalloidea Odhner, 1905**

**Family Fellodistomidae Nicoll, 1909**

**Genus *Proctoeces* Odhner, 1911**

### *3.3. Proctoeces insolitus* (Nicoll, 1915) Yamaguti, 1953

*Synonym*: *Xenopera insolita* Nicoll, 1915

Description (Fig. 1A, B)

[Based on seven unflattened specimens]. Body elongate, tapering at posterior end, widest towards posterior end of hindbody, 2009–2671 (2342) × 218–335 (295); forebody 372–540 (475) long, occupying 15.5–24.7 (21)% of body length. Tegument smooth. Oral sucker subterminal, globular, ovoid to nearly spherical, 204–314 (245) × 200–284 (241). Ventral sucker ovate to spheroid, 171–229 (196) × 236–332 (278). Oral to ventral sucker width ratio 1:1.04–1.24 (1:1.15). Prepharynx absent. Pharynx globular, well developed, muscular, leads to short oesophagus, 137–190 (171) × 141–187 (161). Intestine bifurcates in mid-forebody; caeca extend to post-testicular region, terminating close to posterior extremity. Testes ovoid to spherical, entire, oblique, overlapping caeca; anterior testis 122–175 (143) × 127–175 (154); posterior testis 118–173 (152) × 138–178 (157). Posterior margin of posterior testis to posterior body margin 632–1027 (855), occupying 29.7–48 (36.6)% of body length. Cirrus-sac elongate, mostly in hindbody, terminating variably dorsal to ventral sucker, 451–584 (515) long. Internal seminal vesicle in posterior region of cirrus-sac, tubular, coiled. Pars prostatica wide, occupying more than half of cirrus-sac, straight to slightly curved, surrounded by profuse gland cells. Muscular papilla at distal end of cirrus-sac. Ejaculatory duct short, muscular, leads to genital atrium. Genital atrium long. Genital pore antero-sinistral to ventral sucker. Ovary globular, margins unlobed, in mid-hindbody, immediately pre-testicular, 131–175 (157) × 123–188 (152); distance from ventral sucker to ovary 217–642 (404), occupying 10.0–24.0 (17.1)% of body length. Uterus convoluted, restricted to hindbody with main coils posterior to testes, extending from close to posterior extremity to

past testes and ovary, leading into genital atrium. Vitelline follicles in two lateral irregular fields, at level of gonads, occasionally extending past posterior testes. Excretory pore terminal; arms of excretory vesicle terminating just anterior to ventral sucker. Eggs ovoid, numerous, 30–40 (33) × 13–19 (15).

*3.4. Taxonomic summary*

*Type-host*: *Acanthopagrus australis* (Günther), Yellowfin bream (Perciformes: Sparidae).

*Type-locality*: Cleveland Bay, Queensland, Australia

*New records*

*Host*: *Acanthopagrus australis* (Günther), Yellowfin bream (Perciformes: Sparidae).

*New localities*: eastern Moreton Bay, Queensland, Australia (27°26'S, 153°24'E); western Moreton Bay, Queensland, Australia (27°22'S, 153°13'E).

*Site of infection*: rectum.

*Prevalence*: 16/87 (18%).

*Voucher material*: nine voucher specimens (QM G235189–97) deposited in the Queensland Museum (Hologenophores QM G235196–7).

*Molecular sequence data*: 28S rDNA, three identical replicates (one submitted to GenBank KX671300); 18S rDNA, seven identical replicates (two submitted to GenBank KX671311–2); *cox*1 mtDNA, two identical replicates (one submitted to GenBank KY073873).

***3.5. Proctoeces major* Yamaguti, 1934**

Description (Fig. 1C, D)

[Based on 13 unflattened specimens]. Body robust, widest at level of ventral sucker, 1310–3297 (2534) × 392–918 (689); forebody 381–828 (666) long, occupying 23.7–35.5 (26.2)% of body length. Tegument smooth. Oral sucker subterminal, globular, ovoid to spherical, 183–395 (338) × 212–454 (350). Ventral sucker transversely oval, 256–489 (420) × 350–689 (584). Oral to ventral sucker width ratio 1:1.45–1.8 (1:1.67). Prepharynx absent. Pharynx well developed, globular, muscular, leads to short oesophagus, 179–374 (264) × 151–335 (238). Intestine bifurcates at broad angle, immediately anterior to anterior margin of ventral sucker; caeca terminate posterior to testes, noticeably short of posterior extremity. Testes globular, oblique, occasionally overlapping either caecum; anterior testis 131–283 (209) × 117–269 (192); posterior testis 176–286 (240) × 171–259 (216). Posterior margin of posterior testis to posterior extremity 224–988 (641), occupying 20.8–30.0 (26.4)% of body. Cirrus-sac elongate, mostly dorsal to ventral sucker, posterior part extending into anterior hindbody, 392–821 (648) long. Internal seminal vesicle in proximal end of cirrus-sac, tubular, highly coiled; spherical chamber at proximal end of seminal vesicle visible in some specimens. Pars prostatica elongate, occupying approximately half of cirrus-sac length, surrounded by profuse gland-cells. Muscular papilla at distal end of cirrus-sac. Ejaculatory duct short, leads to genital atrium. Genital atrium elongate, opening at genital pore antero-sinistral to ventral sucker. Ovary globular, margins unlobed, immediately pre-testicular, in anterior hindbody, 176–337 (255) × 183–290 (225); distance from ventral sucker to ovary 59–312 (203), occupying 2.8–11.0 (7)% of body length. Uterus convoluted, restricted to hindbody, with main coils posterior to testes, leading into genital atrium. Vitelline follicles in two lateral fields in hindbody, generally restricted to level between ovary and posterior testis, occasionally extending just past posterior testis. Excretory pore terminal; arms of

excretory vesicle terminating just anterior to ventral sucker. Eggs ovoid, numerous, 37–51 (44) × 17–23 (20).

*3.6. Taxonomic summary*

*Type-host*: *Chrysophrys auratus* (Forster), Snapper (Sparidae).

*Type-locality*: Tarumi, Seto Inland Sea, Japan.

*New records*

*Hosts*: Perciformes: *Abudefduf bengalensis* (Bloch), Bengal sergeant (Pomacentridae); *Chrysophrys auratus* (Forster), Snapper (Sparidae); *Lethrinus laticaudis* Alleyne & MacLeay, Grass emperor; *L. nebulosus* (Forsskål), Spangled emperor (Lethrinidae); *Monodactylus argenteus* (Linnaeus), Silver moony (Monodactylidae); *Thalassoma hardwicke* (Bennett), Sixbar wrasse; *T. jansenii* (Bleeker), Jansen's wrasse; *T. lunare* (Linnaeus), Moon wrasse; *T. purpureum* (Forsskål), Surge wrasse (Labridae). Tetraodontiformes: *Chaetodermis penicilligerus* (Cuvier), Prickly leatherjacket (Monacanthidae).

*Localities*: western Moreton Bay, Queensland, Australia (27°22'S, 153°13'E); eastern Moreton Bay, Queensland, Australia (27°26'S, 153°24'E).

*Site of infection*: rectum.

*Prevalence*: see Table 1 for species specific prevalence.

*Voucher material*: 15 voucher specimens (QM G235159–73) deposited in the Queensland Museum (Hologenophores QM G235172–3).

*Molecular sequence data*: 28S rDNA, seven identical replicates (all submitted to GenBank KX671303–9); 18S rDNA, 16 identical replicates (10 submitted to GenBank KX671316–25); *cox*1 mtDNA, two replicates (submitted to GenBank KY073874–5).

### *3.7. Proctoeces choerodoni* n. sp.

*Synonyms*: *Proctoeces maculatus* (Looss, 1901) from *Choerodon cyanodus* of Bray [16] (as *C. albigena*) and Hall et al. [22]

Description (Fig. 1E, F)

[Based on 33 unflattened specimens] Body elongate, widest at level of ventral sucker, tapering slightly towards posterior end, 1417–2338 (1907) × 369–571 (466); forebody 239–653 (486) long, occupying 16.7–40.3 (27)% of body length. Tegument smooth. Oral sucker subterminal, globular, ovoid to almost spherical, 169–248 (214) × 152–246 (206). Ventral sucker transversely oval, 220–407 (284) × 294–485 (379). Oral to ventral sucker width ratio 1:1.61–2.43 (1:1.84). Prepharynx absent. Pharynx well developed, globular, muscular, 118–189 (148) × 128–200 (159). Oesophagus short. Intestine bifurcates at acute angle, just anterior to ventral sucker; caeca terminate near posterior extremity. Testes globular, slightly oblique, occasionally overlapping caeca; anterior testis 93–189 (154) × 108–258 (162); posterior testis 122–221 (168) × 105–285 (169). Posterior margin of posterior testis to posterior body margin, 286–864 (530), occupying 15.9–42.0 (27.4)% of body length. Cirrus-sac elongate, mostly in hindbody, terminating variably dorsal to ventral sucker, 217–600 (471) long. Internal seminal vesicle at posterior end of cirrus-sac, highly coiled, tubular; spherical chamber at proximal end of seminal vesicle visible in some specimens. Pars prostatica elongate, occupying approximately half of cirrus-sac, straight to slightly curved,

surrounded by profuse gland cells. Muscular papilla at distal end of cirrus-sac. Ejaculatory duct short, leads to genital atrium. Genital atrium elongate. Genital pore antero-sinistral to ventral sucker. Ovary globular, margins unlobed, immediately pre-testicular, in mid-hindbody, 134–187 (162) × 115–194 (155); distance from ventral sucker to ovary 44–423 (182), occupying 2.6–19.0 (9.2)% of body length. Uterus highly coiled, overlapping testes, restricted to hindbody, extending anterior to ovary, leading into genital atrium. Vitelline follicles in two irregular lateral fields in hindbody, at level of ovary to just posterior to posterior testis. Excretory pore terminal; arms of excretory vesicle terminating just anterior to ventral sucker. Eggs ovoid, numerous, 31–44 (37) × 14–21 (18).

*3.8. Taxonomic summary*

*Type-host*: *Choerodon cyanodus* (Richardson), Blue tuskfish (Perciformes: Labridae).

*Other hosts*: *Choerodon graphicus* (De Vis), Graphic tuskfish; *Choerodon schoenleinii* (Valenciennes), Blackspot tuskfish; *Choerodon venustus* (De Vis), Venus tuskfish (Perciformes: Labridae).

*Type-locality*: Heron Island, southern GBR, Queensland, Australia (23°27'S, 151°55'E).

*Site of infection*: rectum.

*Prevalence*: 27/36 (75%) ex *C. cyanodus*; 1/19 (5%) ex *C. graphicus*; 1/2 (50%) ex *C. schoenleinii*; 3/58 (5%) ex *C. venustus*.

*Type-material*: Holotype (QM G235174) and 14 paratypes (QM G235175–88) deposited in the Queensland Museum.

*Molecular sequence data*: 28S rDNA, two identical replicates (one submitted to GenBank KX671299); 18S rDNA, three identical replicates (one submitted to GenBank KX671310); *cox*1 mtDNA, two replicates (submitted to GenBank KY073876–7).

Etymology: This species is named for the genus of fish it infects.

*3.9. Phylogenetic analysis*

Alignment of the 18S and 28S rDNA datasets resulted in 310 and 917 characters for analysis, respectively. Bayesian inference and Maximum Likelihood analyses of partial 18S rDNA dataset produced phylograms with different topologies (Fig. 3). In both analyses, all *Proctoeces* genotypes formed a well-supported clade relative to the fellodistomid outgroup taxa (*Coomera* and *Fellodistomum*). Nodal support was poor for most relationships inferred by 18S analyses, however *P. insolitus*, *P. choerodoni* n. sp. and *Proctoeces* sp. from Chile formed a clade in both analyses. Bayesian inference and Maximum Likelihood analyses of partial 28S rDNA dataset produced phylograms with identical topologies (Fig. 4) and strong support on most major nodes. In both analyses, all *Proctoeces* species form a well-supported clade sister to all other fellodistomid genera for which molecular data are available (Species of *Coomera*, *Fellodistomum*, *Oceroma* Cribb, Miller, Bray & Cutmore, 2014, *Olssonium* Bray & Gibson, 1980, *Steringophorus* Odhner, 1905 and *Tergestia* Stossich, 1899). *Proctoeces insolitus* + *P. choerodoni* n. sp. formed a clade with *Proctoeces* from Mississippi, USA, which was sister to the two genotypes of *Proctoeces* from the Mediterranean. *Proctoeces major* was sister to all other *Proctoeces* sequences.

**4. Discussion**

*4.1. Taxonomy*

The literature relating to the taxonomy of species of *Proctoeces* is extensive. The most thorough reviews were those of Bray & Gibson [39] and Bray [16]. These reviews led to a view that, at the time, the best interpretation was that just a single widespread and highly variable species should be recognised. The recent application of molecular approaches to taxonomy in this genus by Munoz et al. [10], Oliva et al. [17] and Valdivia et al. [18], as well as in the present study, demonstrates unequivocally that this interpretation was mistaken. However, the taxonomic difficulties with the genus remain substantial.

For any genus the status of the type-species is critical. Fortunately, there has been some progress in the understanding of the type-species of *Proctoeces*, *P*. *maculatus*. The species was described originally from three labrids from the Adriatic Sea at Trieste [15]. Antar and Gargouri [19] recently published molecular data for specimens which they interpreted as *P*. *maculatus*. 28S rDNA sequence data was generated for *Proctoeces* specimens from sparid and carangid fishes, and invertebrates, from Tunisia. Two aspects of the report are significant. First, because *P*. *maculatus* was originally described from labrids and at some distance from where Antar and Gargouri [19] worked, we cannot be certain that specimens reported from sparid and carangid fishes are necessarily the same species. However, labrids have been reported as hosts for *P*. *maculatus* off Tunisia [40] and we know that, at least some, species of *Proctoeces* exhibit low-host specificity. Secondly, Antar and Gargouri [19] reported intraspecific variation for 28S sequences in relation to different host taxa, and raised the possibility that cryptic species are present. Here we generated 18S and 28S rDNA sequences derived from specimens collected in the same study as those of Antar and Gargouri [19]. Significantly, 18S rDNA data were identical for specimens from three host

species (a polychaete, a mussel and a sparid fish). However, complementary 28S rDNA data showed the same level of variation reported by Antar and Gargouri [19]. We have no explanation for these conflicting molecular results. Although we think that sequencing of specimens from the type-host and type-locality remains highly desirable, as the two 28S genotypes identified by Antar and Gargouri [19] form sister clades in all analyses, their work still creates a reference point for comparison of other species from the genus.

In our view it is clear that seven species of *Proctoeces* can now be distinguished on the basis of molecular data – *P. maculatus sensu* Antar and Gargouri [19], *P.* cf. *lintoni* Siddiqi & Cable, 1960 of Valdivia et al. [18], *Proctoeces* "*maculatus*" ex *A. probatocephalus* (Walbaum) from the United States, *Proctoeces* sp. of Munoz et al. [10] ex *S. sanguineus* Müller & Troschel from Chile, and the three species recognised here. However, the question of to which, if any, of the existing named species the five unnamed taxa can be identified is exceptionally problematic, given the issues identified by Bray & Gibson [39], Bray [16] and others - morphological plasticity, uncertainty regarding the extent to which species may be widespread and the reliability of their patterns of host-specificity. The ultimate solution to these issues can only lie in further study, ideally in the form of combined morphological and molecular analyses incorporating multiple biomarkers [41]. Studies that examine material from type-hosts and type-localities of existing species will be especially important. Until such studies are completed we are substantially hamstrung in our capacity to put reliable names on new samples. In the present study we contemplated identifying the three forms recognised here as *Proctoeces* sp. 1, 2 and 3, to avoid the possibility of applying wrong names. However, we decided against this course of action; we think the identifications that we propose are convincing and failure to use proper binomials tends to paralyse the

literature. It should be clear that the names that we propose are taxonomic hypotheses subject to testing and revision.

*4.2 Proctoeces insolitus*

The new specimens identified here as *P*. *insolitus* are broadly consistent with the description of the species by Nicoll [21]. The illustration of *P*. *insolitus* in the original description of Nicoll [21] shows a distinctive elongate body with a constriction of the anterior hindbody and a bulbous posterior hindbody, whereas the specimens from Moreton Bay have an elongated and tapered hindbody. The specimen figured by Nicoll does not appear to exist in the lodged type-material. The heavily flattened apparent paratype of *P*. *insolitus* from the QM illustrated by Bray [16] is seemingly the only existing complete specimen from Nicoll's original material. This specimen has a hindbody more consistent with that found for the new specimens. The two forms are otherwise broadly consistent. Both are reported from Queensland waters and the same fish, *A*. *australis*. We thus identify the present material from *A*. *australis* as *P. insolitus* but emphasise the need for DNA sequencing of specimens from the type-host/type-locality to test this conclusion.

*4.3. Proctoeces major*

The specimens here identified as *P*. *major* are the most problematic to identify. We conclude that this species is distinct from *P*. cf. *lintoni* and *P*. *maculatus* on the basis of molecular distinction, and from *P*. *magnorus* Manter, 1940 (from the Atlantic), in that this species has an oral sucker which is larger than the ventral sucker. There are eight plausible

nominal species of *Proctoeces* from the Indo-west Pacific. [We here exclude *P*. *parapistipomae* Wang, 1987 from consideration as its spiny tegument and negligible genital atrium render it inconsistent with *Proctoeces*.] Of these eight we distinguish the present form from *P*. *insolitus* on the basis of both molecular data and differences in proportions of the suckers. We accept the view of Shimazu [42] that *P*. *ostreae* Fujita, 1925 should be considered a *species inquirendae*. *Proctoeces gohari* Ramadan, 1983 is described as having the vitelline follicles extend well into the forebody which, if true, immediately distinguishes it from the present form. *Proctoeces ichiharai* Shimura & Egusa, 1979 was described as sexual adults from a turbinid gastropod from Japan. This species may be relatively huge, reaching almost 9 mm in length [7], which appears to distinguish it from the present form. Five species, *P*. *erythraeus* Odhner, 1911, *P*. *hawaiiensis* Yamaguti, 1970, *P*. *longisaccatus* Wang, 1991, *P*. *major* and *P*. *orientalis* Cao, 1989 are not readily distinguishable from the present form; no molecular data are available for any of them. Of these, the oldest, *P*. *erythraeus*, is arguably the least well known. It was not figured and only a few distinguishing characters were mentioned when it was described [14]. It was reported from a sparid and a labrid from the Red Sea and distinguished from *P*. *maculatus* on the basis of egg size, sucker size and configuration of the vitelline follicles [14]. The species was subsequently reported from Florida by Manter [43]; we consider a distribution incorporating the Red Sea and Florida to be inherently unlikely. Multiple subsequent authors have considered *P*. *erythraeus* a synonym of *P*. *maculatus* [11, 16, 44]. However, given that *P*. *maculatus* was described from the Mediterranean Sea and *P*. *erythraeus* from the Red Sea and in the context of our developing understanding of diversity within *Proctoeces*, we think this synonymy is unlikely. It is possible that *P*. *erythraeus* is a widespread species in the Indo-Pacific and that the present form is conspecific with it. However, in the absence of a

detailed description or molecular data, we consider this presently unknowable. We thus conclude that *P. erythraeus* is best considered a *species inquirendae* until it can be better characterised.

The next oldest species reported from the Indo-Pacific is *P. major*, which was described by Yamaguti [38] from *C. auratus* (one of the hosts reported here) from the Seto Inland Sea. The present specimens are broadly consistent with *P. major*, especially in the possession of a large ventral sucker which extends beyond the body margins and in the shape of the divergence of the intestinal caeca. Given the broadly shared morphology and overlapping host ranges we cautiously identify the present specimens as *P. major*. We do, however, draw attention to four issues with this identification. There are two notable differences between Yamaguti's description and our specimens. First, the Japanese specimens are reported to reach 5.96 mm in length, substantially larger than the largest specimen seen here which was only 3.30 mm long. Secondly, Yamaguti reported *P. major* as having a distinctly tri-lobed ovary whereas the ovary in the present specimens appears consistently globular. In this context we note that Yamaguti's drawing depicts a worm that was clearly somewhat flattened which would likely exacerbate the appearance of any tendency towards lobation. In addition, Ichihara [45] reported that lobation of the ovary of an unidentified species of *Proctoeces* from Japan was highly variable, so that this distinction may be unimportant. Thirdly, we note that there may be a discrepancy between the host specificity of *P. major* and the present form. Yamaguti [38] described *P. major* only from *C. auratus*, whereas the present form has also been collected from several other families of fishes. The level of sampling that has been done in Japanese waters would lead to an expectation that if *P. major* infected other fishes it would have been found and reported from them. In this context we observe that there are records of *P. maculatus*, *P.*

*longisaccatus* and *P. orientalis* from the same region (the Warm Temperate Northwest Pacific of Spalding et al. [46]) from multiple families (Centrolophidae, Labridae, Monacanthidae, Serranidae, Sparidae and Triacanthidae) [38, 47-50]; it remains possible that at least some of these will prove conspecific with *P. major*, thus rendering its overall pattern of host-specificity comparable to that reported here from Moreton Bay. Finally, we note that in the absence of comparative molecular studies we have no real understanding of whether fellodistomid species can have distributions as extensive as from Japan to Moreton Bay [51]. Significantly, several hosts reported for *P. major* have been examined in some numbers on the GBR (sites between Moreton Bay and Japan) without any infections consistent with *P. major* being found. However, in this connection we also note that 18S sequences of sporocysts and cercaria reported as *Proctoeces maculatus* from off New York perfectly matched the newly generated sequence data for *Proctoeces maculatus* from the Mediterranean, a distance similar to that between Japan to Moreton Bay. Thus, it appears that at least some species of *Proctoeces* can have extensive geographical distributions.

*4.4 Proctoeces choerodoni n. sp.*

The form described here as *P. choerodoni* n. sp. has been previously reported by Bray [16] and Hall et al. [22] as *P. maculatus,* however the present study shows that it genetically distinct from *P. maculatus sensu* Antar and Gargouri [19]. *Proctoeces choerodoni* n. sp. has few distinguishing morphological characters, although the narrowness of its body appears distinctive. This species has its greatest distinctiveness in its host-specificity, being evidently restricted to species of the labrid genus *Choerodon*. We have examined 868 specimens of 43 species of Labridae from the southern GBR and have found this species of

*Proctoeces* in only four species of *Choerodon*. It has also not been detected in 8427 individuals of 462 species of another 59 families examined on the GBR [52]. Sequence data generated for specimens collected from Heron Island indicate that *P. choerodoni* n. sp. is distinct from all other *Proctoeces* species for which sequence data are available. 18S sequences of *P. choerodoni* n. sp. generated during this study differ from those of Hall et al. [22] by a single base; this variation is attributed to intraspecific variation or sequencing error. There is no other report of a species of *Proctoeces* from a species of *Choerodon* and we conclude that the combination of molecular and host-specificity distinction justifies the proposal of a new species

*4.5. Host-specificity*

The current study shows that species of *Proctoeces* may exhibit dramatically different host-specificities. Of the three species, *P. insolitus* is oioxenous, infecting a single fish species (*A. australis*), *P. choerodoni* n. sp. is stenoxenous, infecting four species of a single genus (*Choerodon*), and *P. major* is euryxenous, found in 10 species, from 6 families, and two orders of fishes. These patterns have been identified following extensive sampling from the southern GBR and Moreton Bay and there appears no basis to doubt their reliability. In the case of *P. insolitus*, our group has found no evidence of infection in two co-occurring sparids in Moreton Bay; no infections were found from examination of 56 individuals of *Rhabdosargus sarba* (Forsskål) or 24 individuals of *C. auratus*. In the case of *P. choerodoni* n. sp., we have examined 868 specimens of 43 species of labrids from the southern GBR and *P. choerodoni* n. sp. has been found only in species of *Choerodon*. It is exceptional for species of a single genus to exhibit such dramatically different patterns of

host-specificity. The implications of these findings are that it is impossible to make reliable assumptions or predictions about the nature of host-specificity of individual species of *Proctoeces*.

A striking aspect of the contrasts in host-specificity exists in the specificity of *P. major* (infecting *C. auratus*) and *P. insolitus* (infecting *A. australis*). Both fish species belong to the family Sparidae and were examined from the same localities in Moreton Bay, yet *P. major* was not found to infect *A. australis* and *P. insolitus* was not found in *C. auratus*. Absence of *P. major* from *A. australis* is especially intriguing given its evidently otherwise low host-specificity. The basis of these patterns is not understood and warrants future exploration.

In their analysis of host-specificity of fish trematodes of the GBR, Miller et al. [3] found that euryxenous trematodes are rare; just 23 of 290 trematode species studied fell in this category, including *P. maculatus* on the basis of reports in a sparid [21] and a labrid [16]. This study has shown that neither of these forms relates to *P. maculatus* and that they represent separate species with oioxenous or stenoxenous specificity. However, our genetic data does demonstrate that *P. major* is euryxenous. True euryxenous specificity has rarely been demonstrated from marine fish trematodes on the basis of molecular data [53-56], and the specificity of other *Proctoeces* species is thus of great interest. *Proctoeces maculatus* has currently been shown to infect three species of fishes from two families from Tunisia on the basis of molecular data [19], suggesting euryxenicity, but more research is needed to test for the possible presence of cryptic species in that system.

*4.6. Phylogeny*

Phylogenetic trees produced for the partial 18S and the partial 28S rDNA datasets suggest that the generic concept is reliable in that all species of *Proctoeces* form a well-supported clade relative to the other fellodistomid taxa, with high nodal support in all analyses. Analyses of the 18S dataset provided little resolution for relationships within the genus, with poor bootstrap support for most nodes. However, relationships inferred from the analyses of the partial 28S rDNA dataset have high nodal support on most major nodes. Interestingly, *P*. *insolitus* (AUS) + *P*. *choerodoni* n. sp. (AUS), *Proctoeces* "*maculatus*" from Mississippi (USA) and *P. maculatus* from the Mediterranean formed a well-supported sister to *P*. *major*. Given that *P*. *insolitus* and *P*. *major* occur sympatrically, it appears that speciation has not been driven by geographical isolation. However, this inference is based on few taxa and the genetic characterisation of more species is necessary for a more comprehensive exploration of the phylogenetic relationships within the genus and their implications.

*4.7. Conclusion*

Although species of *Proctoeces* are some of the most extensively studied of marine trematodes, the genus remains surprisingly poorly understood in terms of its species level richness. In our view, the recent series of molecular studies [17-19], including the present work, suggest that molecular approaches are the key to unravelling this problem. In particular, the V4 region of 18S rDNA and *cox*1 mtDNA appears to be highly effective in distinguishing species in this genus. We thus strongly advocate that, where possible, future studies should sequence and report these regions, even if other markers are to be explored. This recommendation is not to deny the continued importance of morphological study. In

the present study we were able to distinguish co-occurring species by morphology and we think it unrealistic and probably unnecessary to suggest that specimens of *Proctoeces* should always need to be sequenced to be identified. The most surprising result of the present study was the finding of contrasting patterns of host-specificity for the three Australian species. We imagine that only studies focused on the life-cycle of these forms and those elsewhere will lead to a real understanding of these patterns.

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**Compliance with ethical standards** All applicable institutional, national and international guidelines for the care and use of animals were followed.

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**Fig. 1.** Line drawings of *Proctoeces* species and their terminal genitalia, from Moreton Bay and Heron Island, Queensland, Australia. **A, B**: *P. insolitus* (voucher, QM G235189); **C, D**: *P. major* (voucher, QM G235165); **E, F**: *P. choerodoni* n. sp. (holotype, QM G235174). *Scale-bars*: A, C, E, 500 µm; B, D, F, 200 µm.

**Fig. 2.** Regression functions for Australian *Proctoeces* species. **A**. demonstrating the relationship between the maximum body length and width for the three species characterised morphologically in this study: *P. insolitus* (Circle), *P. major* (Square), *P. choerodoni* n. sp. (Triangle); **B**. demonstrating the relationship between the width of the caeca and body length for *P. major* (Triangle) and *P. choerodoni* n. sp. (Square).

**Fig. 3.** Relationships between species of *Proctoeces* based on Maximum Likelihood (**A**) and Bayesian inference (**B**) analyses of the 18S rDNA dataset. Australian sequences are shown in bold. Out = outgroups. Bootstrap support values (ML) and posterior probabilities (BI) are shown above the nodes. Nodal support below 80 (ML) and 0.8 (BI) are not shown.

**Fig. 4.** Relationships between species of *Proctoeces* and other fellodistomid taxa based on Bayesian inferences (BI) and Maximum Likelihood (ML) analyses of the 28S rDNA dataset. Australian sequences are shown in bold. Bootstrap support values (BI) shown above the nodes and posterior probabilities (ML) shown below the nodes. Nodal supports below 0.8 (BI) and 80 (ML) are not shown.

Figure 1

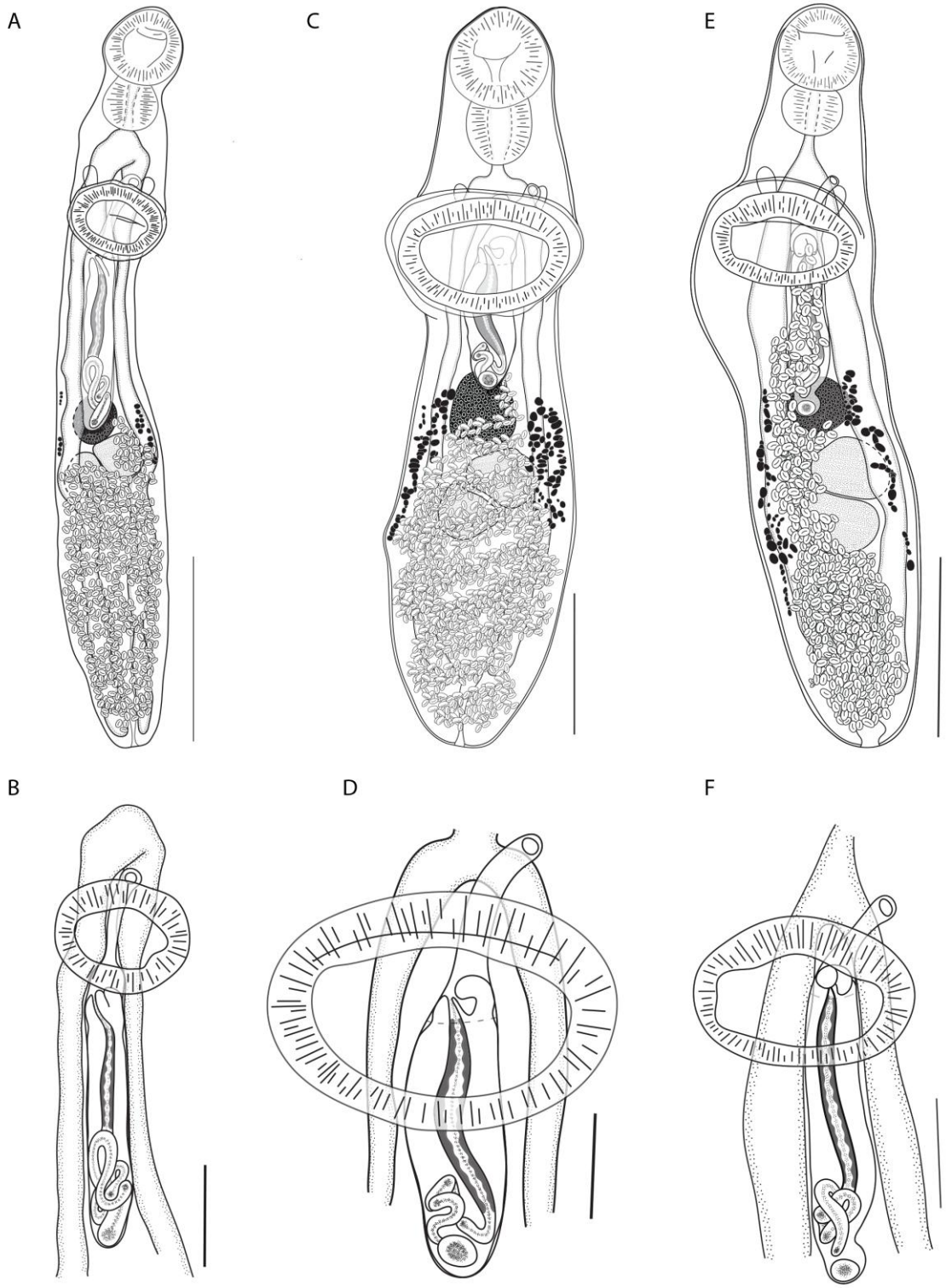


Figure 2

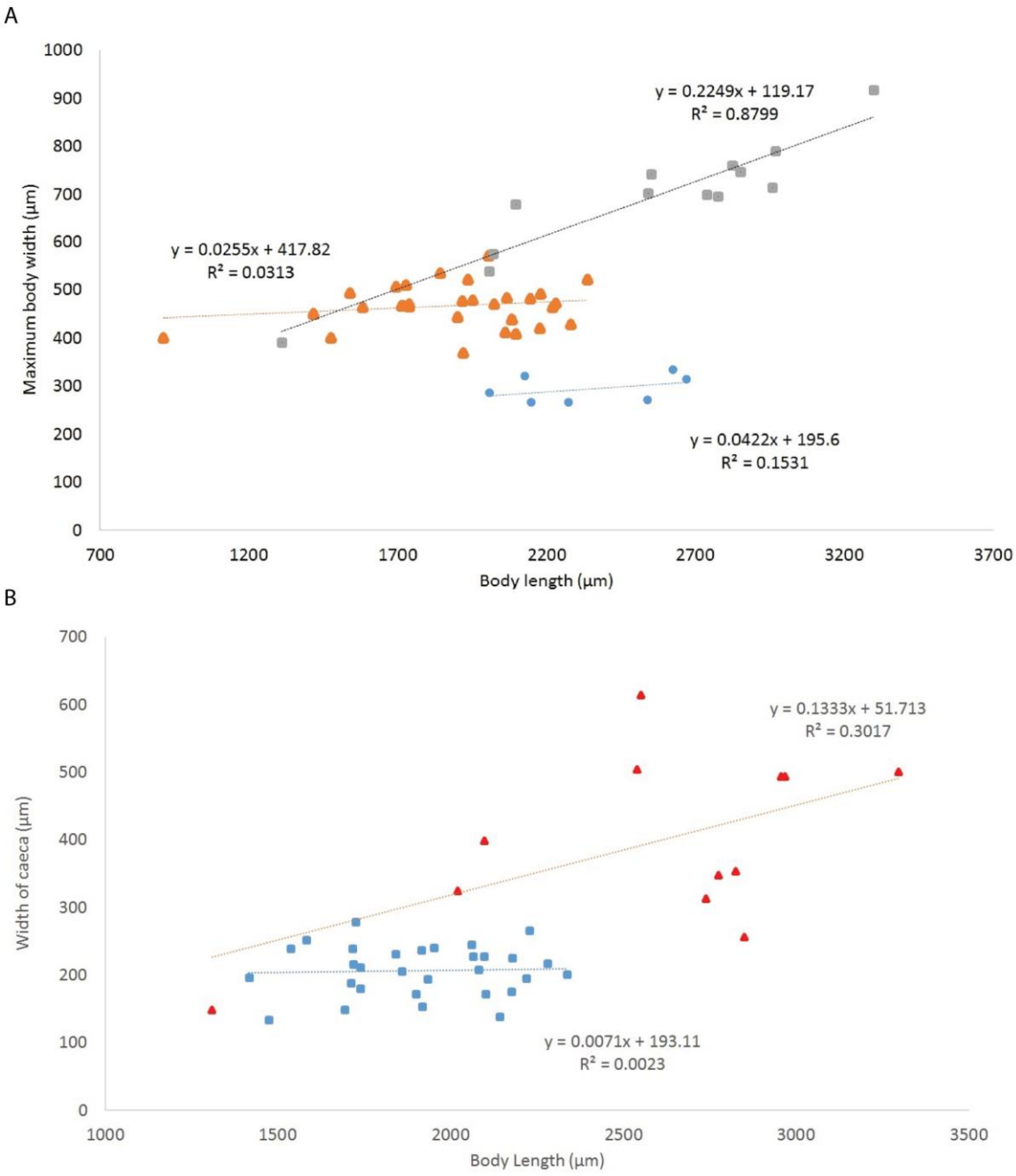


Figure 3

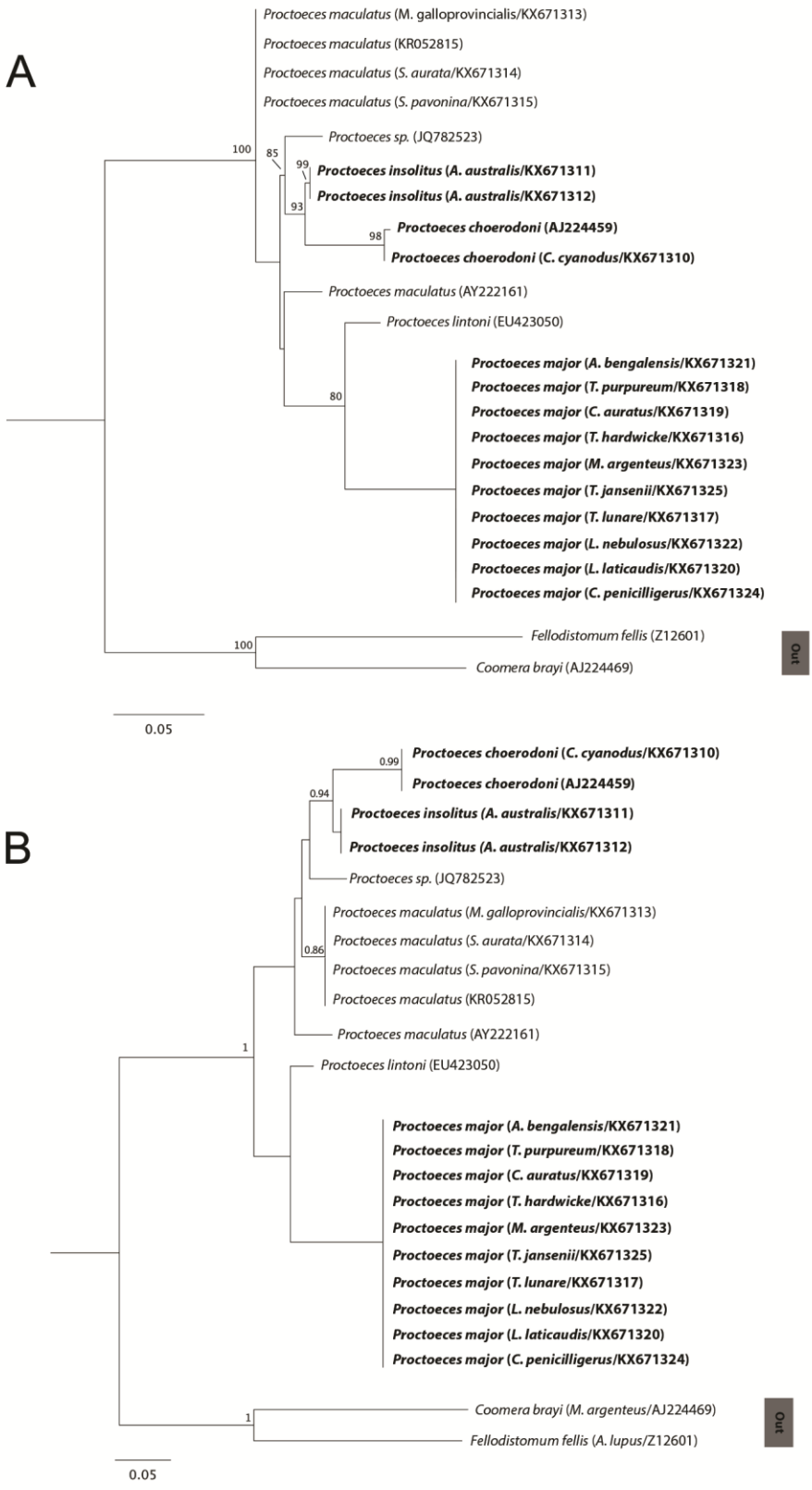


Figure 4

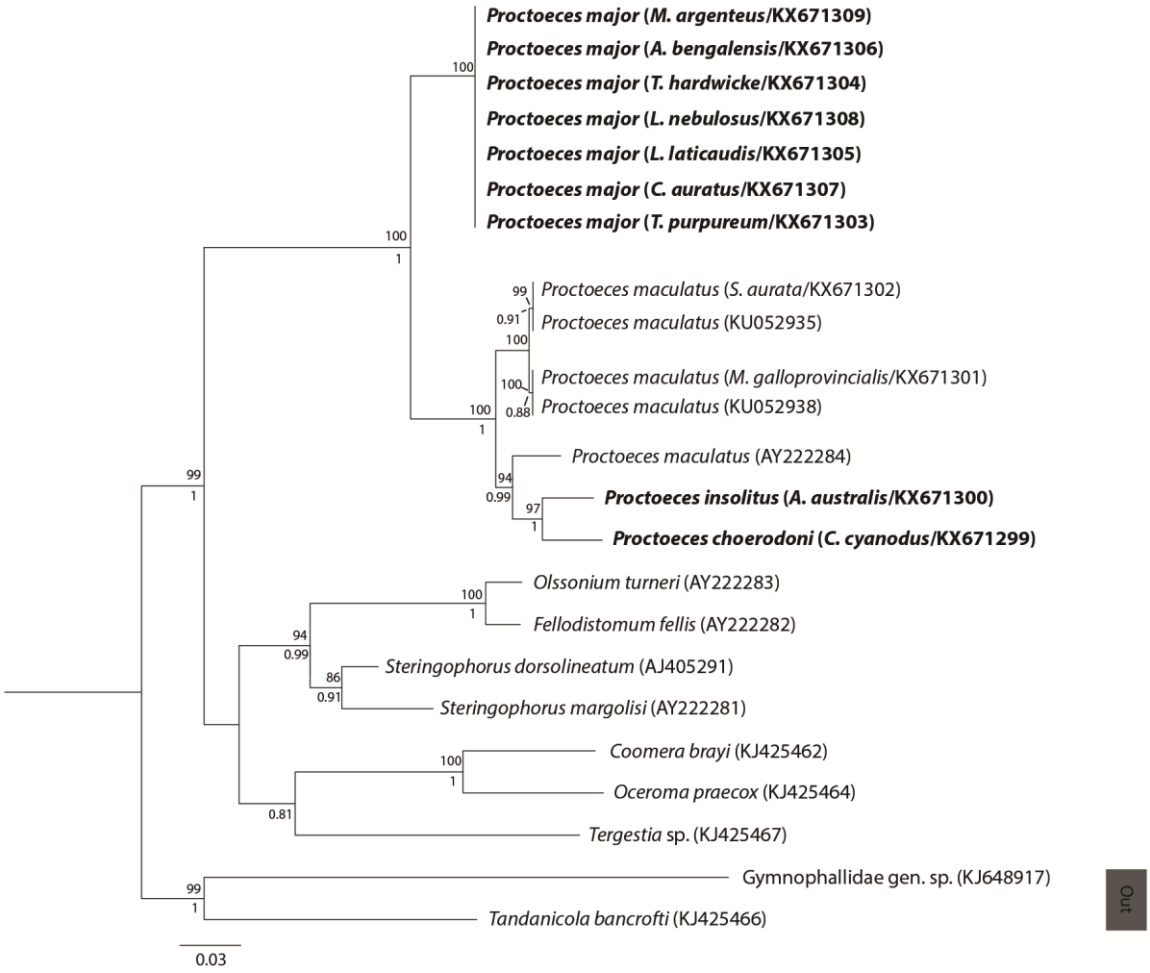


**Table 1** Teleost fishes infected by species of *Proctoeces* identified in this study. Numbers of host examined/infected.

| Host species | Moreton Bay | Heron Island |
|---|---|---|
| **Order Perciformes** | | |
| **Labridae** | | |
| *Choerodon cyanodus* | - | 36/27 |
| *Choerodon graphicus* | 1/0 | 19/1 |
| *Choerodon schoenleinii* | - | 2/1 |
| *Choerodon venustus* | - | 58/3 |
| *Thalassoma hardwicke* | 1/1 | 8/0 |
| *Thalassoma jansenii* | 7/1 | 8/0 |
| *Thalassoma lunare* | 15/2 | 296/0 |
| *Thalassoma purpureum* | 1/1 | - |
| **Lethrinidae** | | |
| *Lethrinus laticaudis* | 25/9 | - |
| *Lethrinus nebulosus* | 2/1 | 35/0 |
| **Monodactylidae** | | |
| *Monodactylus argenteus* | 15/2 | - |
| **Pomacentridae** | | |
| *Abudefduf bengalensis* | 13/1 | 43/0 |
| **Sparidae** | | |
| *Acanthopagrus australis* | 87/16 | - |
| *Chrysophrys auratus* | 11/5 | - |
| **Order Tetraodontiformes** | | |
| **Monacanthidae** | | |
| *Chaetodermis penicilligerus* | 3/3 | - |

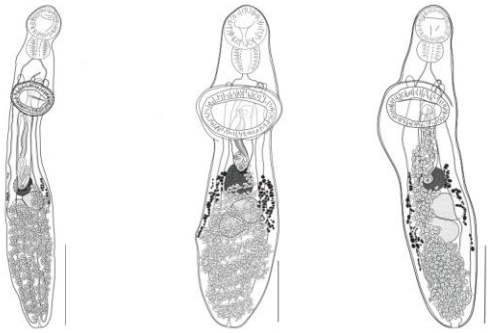
**Table 2** Sequence data for *Proctoeces* specimens genetically characterised during this study

| | | GenBank accession # | | |
|---|---|---|---|---|
| **Species/ ex host species** | **Location** | **18S rDNA** | **28S rDNA** | ***cox*1 mtDNA** |
| ***Proctoeces insolitus*** | | | | |
| *Acanthopagrus australis* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671312 | - | - |
| | western Moreton Bay, Queensland (27°22'S, 153°13'E) | KX671311 | KX671300 | KY073873 |
| ***Proctoeces maculatus*** | | | | |
| *Mytilus galloprovincialis* | Shellfish farm, Menzel Jemil (37°13'N, 9°55'E) | KX671313 | KX671301 | - |
| *Sparus aurata* | Fish Market, Bizerte Lagoon, Tunisia | KX671314 | KX671302 | - |
| *Sabella pavonina* | Bizerte Lagoon, Tunisia | KX671315 | - | - |
| ***Proctoeces major*** | | | | |
| *Abudefduf bengalensis* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671321 | KX671306 | - |
| *Chaetodermis penicilligerus* | western Moreton Bay, Queensland (27°22'S, 153°13'E) | KX671324 | - | - |
| *Chrysophrys auratus* | western Moreton Bay, Queensland (27°22'S, 153°13'E) | KX671319 | KX671307 | KY073874–5 |
| *Lethrinus laticaudis* | western Moreton Bay, Queensland (27°22'S, 153°13'E) | KX671320 | KX671305 | - |
| *Lethrinus nebulosus* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671322 | KX671308 | - |
| *Monodactylus argenteus* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671323 | KX671309 | - |
| *Thalassoma hardwicke* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671316 | KX671304 | - |
| *Thalassoma jansenii* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671325 | - | - |
| *Thalassoma lunare* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671317 | - | - |
| *Thalassoma purpureum* | eastern Moreton Bay, Queensland (27°26'S, 153°24'E) | KX671318 | KX671303 | - |
| ***Proctoeces choerodoni* n. sp.** | | | | |
| *Choerodon cyanodus* | off Heron Island, southern Great Barrier Reef (23°27'S, 151°55'E) | KX671310 | KX671299 | KY073876–7 |

**Table 3** Sequences from GenBank analysed in this study.

| Species/ex host species | Location | GenBank accession # 18S rDNA | GenBank accession # 28S rDNA | Reference |
|---|---|---|---|---|
| **Fellodistomidae** | | | | |
| ***Coomera brayi*** | | | | |
| *Monodactylus argenteus* | Moreton Bay, Australia | AJ224469 | KJ425462 | [22, 57] |
| ***Fellodistomum fellis*** | | | | |
| *Anarhichas lupus* | North Sea, UK | Z12601 | AY222282 | [58, 59] |
| ***Oceroma praecox*** | | | | |
| *Scorpis lineolata* | Moreton Bay, Australia | - | KJ425464 | [57] |
| ***Olssonium turneri*** | | | | |
| *Alepocephalus agassizi* | North-eastern Atlantic | - | AY222283 | [59] |
| ***Proctoeces* cf. *lintoni*** | | | | |
| *Fissurella costata* | Concepción, Chile | EU423050 | - | [18] |
| ***Proctoeces maculatus*** | | | | |
| *Archosargus probatocephalus* | Mississippi, USA | AY222161 | AY222284 | [59] |
| *Lithognathus mormyrus* | Bizerte Lagoon, Tunisia | - | KU052937 | [19] |
| *Mytilus edulis* | New York, USA | KR052815 | - | |
| *Mytilus galloprovincialis* | Bizerte Lagoon, Tunisia | - | KU052939 | [19] |
| *Sabella pavonina* | Bizerte Lagoon, Tunisia | - | KU052941 | [19] |
| *Sparus aurata* | Bizerte Lagoon, Tunisia | - | KU052934 | [19] |
| *Trachinotus ovatus* | Bizerte Lagoon, Tunisia | - | KU052936 | [19] |
| ***Proctoeces choerodoni* n. sp.** | | | | |
| *Choerodon cyanodus* | Heron Island, Australia | AJ224459 | - | [22] |
| ***Proctoeces* sp.** | | | | |
| *Sicyases sanguineus* | Chile | JQ782523 | - | [10] |
| ***Steringophorus dorsolineatum*** | | | | |
| *Bathypterois dubius* | North-eastern Atlantic | - | AJ405291 | [60] |
| ***Steringophorus margolisi*** | | | | |
| *Spectrunculus grandis* | North-eastern Atlantic | - | AY222281 | [59] |
| ***Tergestia* sp.** | | | | |
| *Selaroides leptolepis* | Bali, Indonesia | - | KJ425467 | [57] |
| **Gymnophallidae** | | | | |
| **Gymnophallidae gen. sp.** | | | | |
| *Paphies elongata* | Bribie Island, Australia | - | KJ648917 | [61] |
| **Tandanicolidae** | | | | |
| ***Tandanicola bancrofti*** | | | | |
| *Tandanus tandanus* | Brisbane River, Australia | - | KJ425466 | [57] |

Graphical abstract



**Highlights**

- Morphological and molecular data were used to characterise species of *Proctoeces*
- Three species of *Proctoeces* are identified from Moreton Bay and Heron Island
- Unique patterns of host-specificity were identified for each *Proctoeces* species