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Two new cryptogonimid genera *Beluesca* n. gen. and *Chelediadema* n. gen. (Digenea: Cryptogonimidae) from tropical Indo-West Pacific Haemulidae (Perciformes)

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Abstract

A survey of the parasites of Indo-West Pacific Haemulidae revealed the presence of three new cryptogonimid (Digenea: Cryptogonimidae) species warranting two new genera, *Beluesca littlewoodi* n. gen., n. sp. and *B. longicolla* n. sp. from the intestine and pyloric caeca of *Plectorhinchus gibbosus* and *Chelediadema marjoriae* n. gen., n. sp. from the intestine and pyloric caeca of *Diagramma labiosum*, *P. albovittatus* and *P. gibbosus* from Heron and Lizard Islands off the Great Barrier Reef, Australia. *Beluesca* n. gen. is distinguished from all other cryptogonimid genera by the combination of an elongate body, funnel-shaped oral sucker, relatively small number of large oral spines, highly lobed ovary, opposite to slightly oblique testes, uterine loops that are restricted to the hindbody and extend well posterior to the testes and vitelline follicles that may extend from the ovary into the forebody, but do not extend anterior to the intestinal bifurcation. *Pseudallacanthochasmus plectorhynchi* Mamaev, 1970 is transferred to *Beluesca* as *B. plectorhyncha* (Mamaev, 1970) n. comb. based on morphological and ecological (host preference) characteristics. *Chelediadema* n. gen. is distinguished from all other cryptogonimid genera by the combination of a lanceolate body, relatively small number of large oral spines, prepharynx that is much longer than the oesophagus, tandem testes, uterine loops that are extensive in the hindbody and extend well posterior to the testes and vitelline follicles that extend from the ovary to the pharynx. Morphological analysis of the three species described here was augmented with DNA sequence analyses utilizing data from the large subunit (LSU) and the internal transcribed spacers (ITS) 1 and 2, and 5.8S nuclear ribosomal DNA. Sequence data from the LSU and ITS (encompassing the ITS1, 5.8S and ITS2) of the taxa examined here were aligned with those reported for other cryptogonimids, *Caulanus thomasi*, *Latuterus tkachi*, *Neometadena ovata*, four representative species of *Retrovarium* and an undescribed species of *Siphoderina*, for comparative purposes and to explore levels of interspecific and intergeneric variation among these taxa. Minimum evolution analysis was conducted on a combined (LSU and ITS) dataset to explore relationships among these genera. Despite their superficial morphological and host preference similarities, species of *Beluesca* and *Chelediadema* were genetically distant from each other. Interspecific and intergeneric variation among the species described here is similar to that reported for other cryptogonimids.

Key words: Cryptogonimidae, *Beluesca*, *Chelediadema*, *Caulanus*, Haemulidae, Digenea, *Diagramma*, internal transcribed spacers, ITS, Indo-West Pacific, Great Barrier Reef, *Latuterus*, LSU, Lutjanidae, *Plectorhinchus*, *Siphoderina*, *Retrovarium*.

Introduction

The Haemulidae is a relatively large cosmopolitan family of fishes which currently comprises 150 species in 19 genera that inhabit tropical and subtropical waters (primarily marine, but some taxa live in fresh or euryhaline environments) (Froese & Pauly 2007). This family is closely related to species of the Lutjanoidea and Sparoidea (Miller & Cribb in press-a; Orrell & Carpenter 2004), but is distinguished from these by the lack of

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canine and vomerine teeth and the presence of sensory pits on the chin (Johnson 1980). The diet of many of these species consists largely or entirely of benthic invertebrates, but some prey opportunistically on fishes, making them susceptible to infection by trematodes that utilise these prey items as intermediate hosts.

The Cryptogonimidae Ward is one of the many digenean families reported to infect species of Haemulidae. It is a large family of trematodes that currently comprises 66 genera (Miller & Cribb in press-c, d) found worldwide in marine and freshwater fishes and reptiles. Cryptogonimids are distinctly concentrated in marine fishes of the Lutjanidae and Haemulidae; approximately 1/4 of the over 200 species of cryptogonimids infect these families almost exclusively. Although a much higher proportion of cryptogonimid reports in the literature have been from the Lutjanidae, at least eleven species of Cryptogonimidae have been reported from the Haemulidae. Six species are known from the Indo-Pacific: *Biovarium pomadaysidis* Shen & Tong, 1985 was described by Shen & Tong (1985) from *Pomadasys hasta* (Bloch) off the coast of China; *Metadena hapalogenyos* (Yamaguti, 1958) Miller & Cribb, in press was described by Yamaguti (1958) from *Hapalogenys* sp. off the coast of Japan; *Neometadena ovata* (Yamaguti, 1952) Miller & Cribb, in press was reported from *Pomadasys hasta* from the Bay of Bengal by Madhavi (1976); *Pseudallacanthochasmus grandispinus* Velasquez, 1961 was reported from *Pomadasys hasta* off the coast of India by Hafeezullah & Siddiqi (1970) and Chilka Lake, eastern India by Dutta (1995); *Pseudallacanthochasmus plectorhynchi* Mamaev, 1970 was described from *Plectorhinchus cinctus* (Temminck & Schlegel) from the Gulf of Tonkin; *Siphoderina microvata* (Tubangui, 1928) Miller & Cribb, in press was described by Tubangui (1928) from *Pomadasys hasta* off the Philippines.

Five species are known from the eastern Atlantic and western Pacific: *Metadena vancleavei* (Manter, 1940) Miller & Cribb, in press was described by Manter (1940) from *Orthostoechus* (now *Haemulon*) *maculicauda* (Gill) off the Pacific coast of Colombia; *Siphodera vinaledwardsii* Linton was reported from *Orthopristis chrysoptera* (Linnaeus) by Linton (1905) from Beaufort, North Carolina, USA and *Haemulon flavolineatum* (Desmarest) by Vélez (1978) off Columbia; *Siphoderina ackerti* (Watson, 1976) Miller & Cribb, in press was described by Watson (1976) from *Pomadasys boucardi* (Steindachner) (now *P. croco* Cuvier) from Lake Nicaragua; *Stegopa globosa* Linton was reported from *Haemulon scudderii* (Gill) by Bravo-Hollis & Sogandares-Bernal (1956) near Puerto Vallarta, off the Pacific coast of Mexico. Lastly, *Siphoderina ghanensis* (Fischthal & Thomas, 1968) Miller & Cribb, in press was reported by Fischthal & Thomas (1972) from *Pomadasys jubelini* (Cuvier) from Ghana.

The cryptogonimid fauna of haemulids on the Great Barrier Reef (GBR) has been poorly explored. Only a single species, *Retrovarium mariae* Miller & Cribb, 2007, from *Diagramma labiosum* (Macleay) has been reported (Miller & Cribb in press-b). This is in contrast to the 14 species reported from Lutjanidae in the same region so far (Miller & Cribb 2005 in press-b, d). Here we describe three new species in two new genera from the intestine and pyloric caeca of *Diagramma labiosum*, *Plectorhinchus albovittatus* (Rüppell) and *P. gibbosus* (Lacepède) collected from Heron and Lizard Islands in the southern and northern (respectively) GBR. We augment our morphologically based taxonomic approach with analysis of molecular data from multiple nuclear ribosomal DNA regions, the LSU, 5.8S and internal transcribed spacers 1 and 2 (ITS1 and ITS2), to explore the integrity of these species and to compare levels of intra- or interspecific and generic variation with that observed in other cryptogonimids. Relationships of the taxa examined here relative to other recently reported cryptogonimid taxa (Miller & Cribb in press-b, d) was assessed by minimum evolution analysis of a combined (LSU and ITS) rDNA dataset.

Materials and methods

Host and parasite collection

Fishes were collected using spears from Heron Island (23°26'S; 151°54'E) in the southern GBR and Lizard Island (14°40'S; 145°27'E) in the northern GBR. Fish were euthanased by neural pithing and the intestine

immediately removed, washed in vertebrate saline (0.85%), and examined for the presence of endohelminths. Trematodes were washed in saline and killed by pipetting them into nearly boiling saline. Specimens for morphological analysis were then stored in 10% formalin and specimens for DNA extraction and analysis were stored in 95–100% ethanol at -20°C.

Morphological samples

Preserved specimens for morphological analysis were washed in fresh water and placed in Mayer's haematoxylin for staining. The specimens were overstained and then destained by placing them in a solution of 1.0% HCl and subsequently neutralized in a 0.5–1.0% ammonium hydroxide solution. Stained specimens were then dehydrated through a graded series of ethanol for at least half an hour at each dehydration step, cleared in methyl salicylate and mounted in Canada balsam. Drawings were made with the aid of a drawing tube. All measurements were made using an ocular micrometre and are in micrometres with the mean followed by the range in parentheses. Type-specimens were deposited in the Queensland Museum, Brisbane, Australia.

Molecular samples

Total genomic DNA from species of *Beluesca* n. gen. and *Chelediadema* n. gen. was isolated by a standard proteinase K and phenol : chloroform extraction procedure (Sambrook *et al*. 2001). Amplification of the LSU rDNA region was performed with the primers LSU5 (5'-TAGGTCGACCCGCTGAAYTTAAGCA-3' Littlewood *et al*. 2000) and ECD2 (5'-CCTTGGTCCGTGTTTCAAGACGGG-3' Littlewood *et al*. 2000), the ITS1 region with the primers BD1 (5'-GTCGTAACAAGGTTTCCGTA-3' Bowles & McManus 1993) and 4S (5'-TCTAGATGCGTTCGAARTGTCGATG-3' Bowles & McManus 1993) and the ITS2 region with the primers 3S (5'-GGTACCGGTGGATCACGTGGCTAGTG-3' Anderson & Barker 1993) and ITS2.2 (5'-CCTGGTTAGTTTCTTTTCCTCCGC-3' Anderson & Barker 1993). PCR was conducted for all rDNA regions with a total volume of 20 µl consisting of approximately 10 ng of template DNA, 0.75 µl of each primer (10 pmols), 1.6 µl $MgCL_2$, 2 µl of 10 × reaction buffer, 0.8 µl deoxyribonucleotide triphosphate (dNTP) (each 2.5 mM), and 0.25 µl of *Taq* DNA polymerase. Amplifications of the LSU, ITS1 and ITS2 rDNA regions were carried out on a MJ Research PTC-150 thermocycler (Waltham, MA). The following profile was used to amplify the LSU and ITS2 rDNA regions: an initial 96°C denaturation for 5 min, followed by 25 cycles of 96°C denaturation for 1 min, 54°C annealing for 15 s, 72°C extension for 30 s, and a final 72°C extension for 4 min. The following profile was used to amplify the ITS1 region: an initial 95°C denaturation for 5 min, followed by 30 cycles of 95°C denaturation for 30 s, 55°C annealing for 30 s, 72°C extension for 1 min, and a final 72°C extension for 10 min. Amplified DNA was purified using QIAGEN® QIAquick™ PCR purification kit according to manufacturer's protocol. Cycle sequencing was conducted using the same primers utilized for PCR amplification with ABI Big Dye™ v.3.1 chemistry following manufacturer's recommendations. Precipitation with 3 M Sodium Acetate (pH approximately 5) and alcohol was done to remove dye terminators, and the pellets were then dried for 30–60 min at 39°C and sequenced using an ABI 3730xl automated sequencer. The resulting sequences were edited and contigs constructed using Sequencher™ version 4.5 (GeneCodes Corp.). GenBank accession numbers for all taxa sequenced in this study are provided in Table 1. The consensus sequences for each taxon utilized in this study were constructed from multiple replicates (each replicate being both a forward and reverse sequence from a single individual from different infections when possible) from different host/parasite/location combinations whenever possible. The total number of replicates for each rDNA region sequenced in this study are shown in Table 1.

Comparative DNA analyses

The LSU and ITS rDNA regions from taxa sequenced in this study were initially aligned using ClustalX version 1.83 (Thompson *et al.* 1997) under the following parameters: pairwise alignment parameters= gap

opening 10.00, gap extension 0.10, DNA weight matrix International Union of Biochemistry (IUB); multiple alignment parameters= gap opening 10.00, gap extension 0.20, delay divergent sequences 30%, DNA weight matrix IUB. The resulting sequence alignments were exported from ClustalX in FASTA and NEXUS formats, and refined by eye using MacClade version 4.08 (Maddison & Maddison 2005). After alignments of the rDNA regions were edited, the ends of each fragment were trimmed to match the shortest sequence in the alignment. Distance matrices for the rDNA regions were constructed with the absolute pairwise character difference and the percentage of uncorrected "p" pairwise character differences. Pairwise comparisons of absolute sequence divergence for all taxa were calculated with gaps treated as missing data.

The LSU and ITS rDNA regions for species of *Beluesca* and *Chelediadema* were also aligned with those reported for the species *Caulanus thomasi*, *Latuterus tkachi*, *Retrovarium amplorificium*, *R. gardneri*, *R. sablae*, *R. snyderi*, *Neometadena ovata* and an undescribed species of *Siphoderina* by Miller & Cribb (in press-b, d) for comparative purposes and to explore levels of intergeneric and interspecific variation. The LSU and ITS rDNA regions were then combined and assigned partitions in a single NEXUS file. Minimum evolution analyses of the combined (LSU and ITS) dataset of these taxa was performed using PAUP* version 4.0b10 (Swofford 2003). Nodal support was inferred by bootstrap analysis using a heuristic search of 10,000 replicates.

TABLE 1. Number of molecular sequence replicates for each of the rDNA regions produced during this study followed by the corresponding GenBank accession numbers for species of *Beluesca* n. gen. and *Chelediadema* n. gen.

Species	Number of replicates and GenBank Accession #			
	LSU		ITS	
Beluesca littlewoodi n. sp.	3	EF566867	3	EF566870
Beluesca longicolla n. sp.	2	EF566868	2	EF566871
Chelediadema marjoriae n. sp.	4	EF566866	4	EF566869

Results

Morphological data

Family Cryptogonimidae Ward, 1917

Beluesca n. gen.

Type-species: *B. littlewoodi* n. sp.

Diagnosis: Body elongate, widest near level of testes or slightly posterior; length/width ratio *c.* 2.8–8.7. Tegument with small to minute spines. Oral sucker funnel-shaped, with large oral spines, opens terminally. Ventral sucker unspecialised, embedded in ventrogenital sac. Ratio oral/ventral sucker width *c.* 1.6–2.6. Forebody occupies *c.* 1/2–2/3 body length. Prepharynx shorter than oesophagus. Oesophagus long. Intestinal bifurcation in approximately mid-forebody, well anterior to ventral sucker. Caeca blind, terminate close to posterior end of body. Testes two, opposite to oblique, in anterior hindbody. Seminal vesicle tubulosaccular. Common genital pore immediately anterior to ventral sucker. Gonotyl absent. Ovary deeply lobed, ventral to or immediately anterior to testes. Seminal receptacle tubulosaccular, posterior to ovary, may extend well posterior to testes. Vitelline follicles in two lateral groups, may extend from ovary to near intestinal bifurcation. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Excretory vesicle Y-shaped; arms reach to level of pharynx or between pharynx and oral sucker. In marine fishes (Haemulidae); tropical Indo-West Pacific.

Etymology: The name *Beluesca* is derived from the Latin *belua*, meaning beast or monster and *esca*, meaning bait. It is proposed in recognition of the original purpose for capturing the host individual found to harbour the type-species, which was as bait for a large serranid off Lizard Island. It is to be treated as feminine.

Differential diagnosis: *Beluesca* n. gen. is distinguished from all other cryptogonimid genera by the combination of an elongate body, funnel-shaped oral sucker, the relatively small number of large oral spines, highly lobed ovary, opposite to slightly oblique testes, uterine loops that are restricted to the hindbody and extend well posterior to the testes, and vitelline follicles that may extend from the ovary into the forebody, but do not extend anterior to the intestinal bifurcation.

***Beluesca littlewoodi* n. sp.**
(Figs 1–3)

Type host: *Plectorhinchus gibbosus* (Lacepède).

Type locality: Lizard Island, Great Barrier Reef (14°40'S; 145°27'E), Queensland, Australia.

Site: Intestine and pyloric caeca.

Prevalence: 1 of 1 (100%) at Lizard Island, 0 of 5 at Heron Island.

Deposited specimens: holotype G227654, eleven paratypes G227655–G227665.

Etymology: The epithet *littlewoodi* is in honour of Dr Tim Littlewood, in recognition of his contributions to platyhelminth systematics.

Description: Based on 12 specimens. Body elongate, longer than wide, 2083 (1744–2472) long by 285 (240–368) wide; length/width ratio 7.4 (6.7–8.7). Oral sucker funnel-shaped, 234 (202–273) long by 207 (182–250) wide. Oral spines 21 (20–22), length 51 (40–64). Ventral sucker 91 (78–111) long by 108 (91–127) wide. Ratio oral/ventral sucker width 1.9 (1.6–2.2). Forebody occupying 52 (48–58)% of body length. Prepharynx 127 (59–182) long. Pharynx 68 (55–78) long by 67 (59–75) wide. Ventral sucker/pharynx width ratio 1.6 (1.4–1.8). Oesophagus 304 (241–384) long. Intestinal bifurcation at mid-forebody, well anterior to ventral sucker. Intestinal caeca blind, 1227 (1024–1456) long, terminate close to posterior end of body. Testes two, opposite to slightly oblique, in mid- to anterior hindbody, 181 (137–247) long by 116 (94–137) wide. Seminal vesicle tubulosaccular, between testes and ventral sucker. Genital pore immediately anterior to ventral sucker. Ovary deeply lobed 145 (114–189) long by 131 (104–182) wide, ventral to or immediately anterior to testes. Laurer's canal present. Seminal receptacle tubulosaccular, posterior to ovary, may extend well posterior to testes. Vitelline follicles in two lateral groups, extend from level of ovary to midway between ventral sucker and intestinal bifurcation. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Eggs small, darkly tanned, 17 (16–21) long by 9 (7–10) wide. Excretory vesicle Y-shaped, bifurcates dorsal to ovary; arms extend anterior to pharynx, 1746 (1456–2064) long.

***Beluesca longicolla* n. sp.**
(Figs 4 and 5)

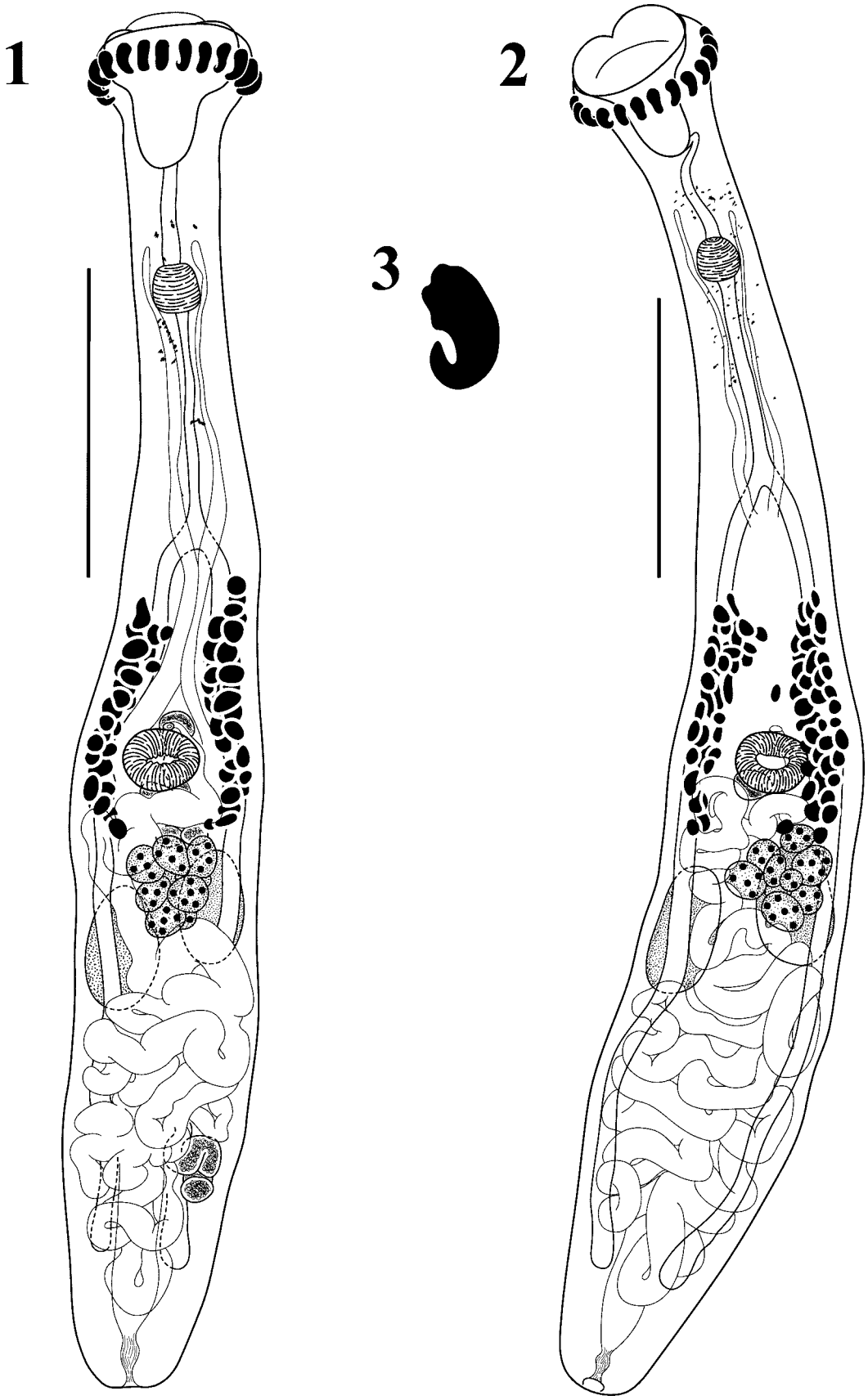
Type host: *Plectorhinchus gibbosus* (Lacepède).

Type locality: Heron Island, Great Barrier Reef (23°26'S; 151°54'E), Queensland, Australia.

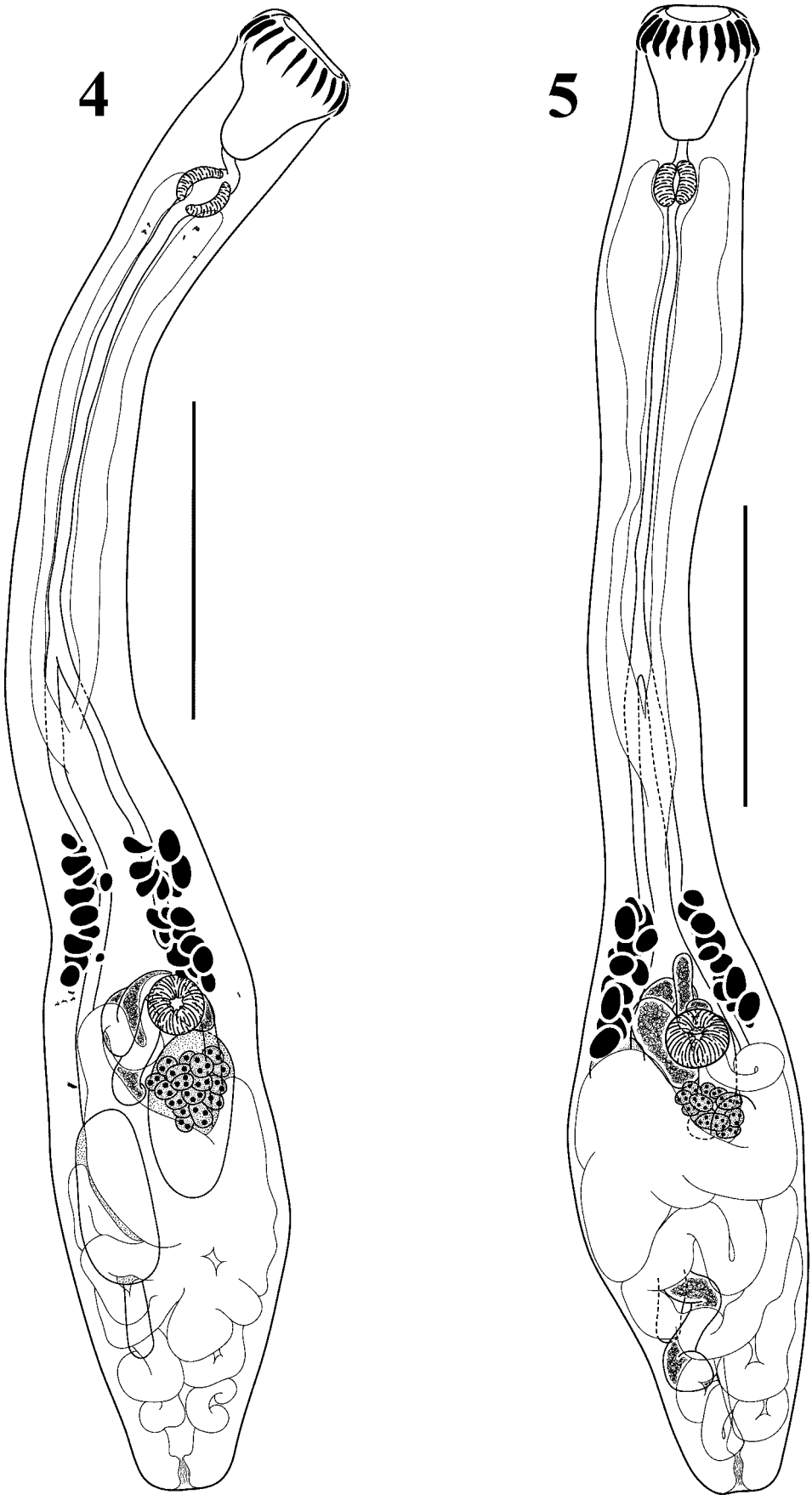
Site: Intestine and pyloric caeca.

Prevalence: 1 of 5 (20%) at Heron Island, 0 of 1 at Lizard Island.

Deposited specimens: holotype G227666, ten paratypes G227667–227676.



FIGURES 1–3. *Beluesca littlewoodi* n. sp. from the intestine of *Plectorhinchus gibbosus* off Lizard Island, Great Barrier Reef, Australia. 1. Ventral view of holotype. 2. Ventral view of paratype G227655. 3. Lateral view of an oral spine from the paratype, illustrating curvature. Scale bar for figures 1 and 2 = 500 μm.



FIGURES 4–5. *Beluesca longicolla* n. sp. from the intestine of *Plectorhinchus gibbosus* off Heron Island, Great Barrier Reef, Australia. 4. Ventral view of holotype. 5. Ventral view of paratype G227667. Scale bar for both figures = 500 µm.

Etymology: The epithet *longicolla* is derived from the Latin *longus*, meaning long and *collum*, meaning neck, referring to the long forebody of this species.

Description: Based on 11 specimens. Body elongate, longer than wide, 2361 (2216–2536) long by 357 (320–384) wide; length/width ratio 6.7 (6–7.5). Oral sucker funnel-shaped, 226 (208–244) long by 186 (176–195) wide. Oral spines 19 (19–21), length 64 (51–82). Ventral sucker 94 (91–96) long by 98 (94–109) wide. Ratio oral/ventral sucker width 1.9 (1.8–2). Forebody occupying 66 (64–67)% of body length. Prepharynx much shorter than oesophagus, 56 (46–85) long. Pharynx 72 (68–78) long by 71 (68–75) wide. Ventral sucker/pharynx width ratio 1.4 (1.3–1.5). Oesophagus 687 (592–774) long. Intestinal bifurcation near mid-forebody, well anterior to ventral sucker. Intestinal caeca blind, 1099 (1053–1157) long, terminate close to posterior end of body. Testes two, opposite to oblique, in mid- to anterior hindbody, 259 (228–304) long by 152 (125–189) wide. Seminal vesicle tubulosaccular, between testes and ventral sucker. Genital pore immediately anterior to ventral sucker. Ovary deeply lobed 138 (99–163) long by 132 (109–144) wide, ventral to or immediately anterior to testes. Laurer's canal present. Seminal receptacle tubulosaccular, posterior to ovary, may extend well posterior to testes. Vitelline follicles in two lateral groups, extend from level of ventral sucker to midway between ventral sucker and intestinal bifurcation, do not extend posterior to ventral sucker. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Eggs small, darkly tanned, 16 (13–18) long by 7 (7–8) wide. Excretory vesicle Y-shaped, bifurcates dorsal to ovary; arms extend to pharynx, 2038 (1888–2224) long.

***Chelediadema* n. gen.**

Type-species: *C. marjoriae* n. sp.

Diagnosis: Body lanceolate, widest near level of ovary; length/width ratio *c.* 3.7–6. Tegument with small to minute spines. Oral sucker funnel-shaped, with large oral spines, opens terminally. Ventral sucker unspecialised, embedded in ventrogenital sac. Ratio oral/ventral sucker width *c.* 1.6–2.2. Forebody occupies *c.* 1/2 body length. Prepharynx long, much longer than oesophagus. Oesophagus short. Intestinal bifurcation dorsal to or immediately anterior to ventral sucker. Caeca blind, terminate close to posterior end of body. Testes two, entire, tandem, adjacent, in mid-hindbody. Seminal vesical saccular, between ovary and ventral sucker. Common genital pore immediately anterior to ventral sucker. Gonotyl absent. Ovary entire, anterior and adjacent to or ventral to anterior testis. Laurer's canal present. Seminal receptacle saccular, between ovary and ventral sucker. Vitelline follicles in two lateral groups, extend from ovary to pharynx. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Excretory vesicle Y-shaped; arms reach to near pharynx. In marine fishes (Haemulidae); tropical Indo-West Pacific.

Etymology: The name *Chelediadema* is derived from the Greek *chele*, meaning nail or claw and *diadema*, meaning crown, referring to the crown of large oral spines that are analogous to claws. It is to be treated as neuter.

Differential diagnosis: *Chelediadema* n. gen. is distinguished from all other cryptogonimid genera by the combination of a lanceolate body, the relatively small number of large oral spines, a prepharynx that is much longer than the oesophagus, caeca that terminate close to the posterior end of the body, tandem testes, uterine loops that are extensive in the hindbody and extend well posterior to the testes, and vitelline follicles that extend from the ovary to the pharynx.

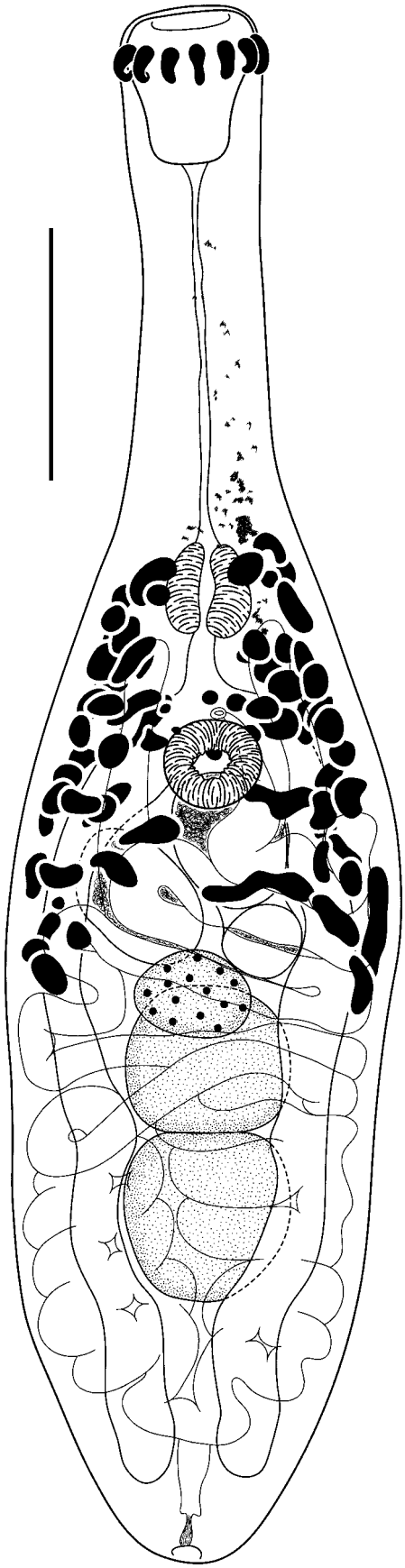


FIGURE 6. *Chelediadema marjoriae* n. sp. from the intestine of *Diagramma labiosum* off Heron Island, Great Barrier Reef, Australia. Ventral view of holotype. Scale bar = 200 μm.

***Chelediadema marjoriae* n. sp.**
(Fig. 6)

Type host: *Diagramma labiosum* (Macleay) Perciformes, Haemulidae.

Additional hosts: *Plectorhinchus albovittatus* (Rüppell); *P. gibbosus* (Lacepède).

Type locality: Heron Island, Great Barrier Reef (23°26'S; 151°54'E), Queensland, Australia.

Additional localities: Lizard Island, Great Barrier Reef (14°40'S; 145°27'E), Queensland, Australia.

Site: Intestine and pyloric caeca.

Prevalence: *Diagramma labiosum*, 7 of 43 (16%) at Heron Island, 0 of 7 at Lizard Island; *P. albovittatus*, 1 of 1 (100%) at Lizard Island; *P. gibbosus*, 1 of 1 (100%) at Lizard Island, 0 of 5 at Heron Island.

Deposited specimens: holotype G227677, nine paratypes G227678–G227686.

Etymology: The epithet *marjoriae* is in honour of Mrs Marjorie Neill in recognition of her support and encouragement of this work.

Description: Based on 10 specimens. Body lanceolate, longer than wide, 1150 (930–1368) long by 240 (195–309) wide; length/width ratio 4.9 (3.7–6.0). Oral sucker funnel-shaped, 117 (102–134) long by 92 (83–106) wide. Oral spines 14 (14–15), length 32 (24–47). Ventral sucker 64 (56–69) long by 67 (54–75) wide. Ratio oral/ventral sucker width 1.37 (1.24–1.59). Forebody occupying 50 (46–53)% body length. Prepharynx 305 (227–381) long. Pharynx 67 (54–80) long by 66 (58–85) wide. Ventral sucker/pharynx width ratio 1.03 (0.87–1.11). Oesophagus 46 (26–70) long. Intestinal bifurcation at level of or immediately anterior to ventral sucker. Intestinal caeca blind, 591 (501–670) long, terminate close to posterior end of body. Testes two, tandem, adjacent, in mid-hindbody; anterior testis 106 (90–122) long by 112 (86–136) wide, posterior testis 122 (102–147) long by 111 (90–144) wide. Seminal vesicle saccular, between ovary and ventral sucker. Genital pore immediately anterior to ventral sucker. Ovary entire, anterior and adjacent to or ventral to anterior testis, 74 (67–80) long by 70 (58–90) wide. Laurer's canal present. Seminal receptacle saccular, between ovary and ventral sucker. Vitelline follicles in two lateral groups, extend from level of ovary to pharynx. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Eggs small, darkly tanned, 21 (18–24) long by 9 (7–11) wide. Excretory vesicle Y-shaped, bifurcates dorsal to ovary; arms extend to posterior end of pharynx, 661 (520–767) long.

Molecular data

LSU rDNA

Sequencing of the LSU rDNA yielded an average of approximately 870 base pairs (bp) for all taxa. The aligned and trimmed sequences incorporated a total of 859 characters (base pairs and gaps) for analysis. Intra- and intergeneric variation in the number of base pair differences observed between these taxa over this region are shown in Table 2.

ITS rDNA

Variation in the 5' half of the ITS1 made alignment in this region between the taxa examined here impossible, so only the 3' half of the ITS1 were included as in Miller & Cribb (in press-a), because this region was alignable. Alignment of the ITS dataset, which included the 3' end of the ITS1, the entire 5.8S and ITS2 and 51 bp of the 5' end of the LSU yielded 911 characters for analysis. Genetic variation in the ITS dataset between these taxa is shown in Table 2. Additionally, the number of base pair differences in the 3' end of the ITS1 only and in the ITS2 region only are shown in Table 2.

Minimum evolution analysis (ME score = 597.14) of the combined dataset resulted in a phylogram with species of *Beluesca* forming a clade with the undescribed species of *Siphoderina* and *Chelediadema marjoriae* was sister to the remainder of the taxa analysed (Fig. 7). Nodal support was relatively high for the observed clades.

TABLE 2. Genetic variation among species of *Beluesca* n. gen. and *Chelediadema* n. gen. and the additional cryptogonimid taxa examined here over the partial large subunit (LSU), the internal transcribed spacer (ITS) (includes the 3' end of the ITS1, and the complete 5.8S and ITS2), the 3' end of the ITS1 only and the ITS2 only rDNA datasets. Values to the left represent the number of base pair differences and those in parentheses the percentage of uncorrected "p" pairwise distance.

	Beluesca littlewoodi n. sp.		*Beluesca longicolla* n. sp.		*Chelediadema marjoriae* n. sp.	
LSU						
Beluesca littlewoodi	-	-	31	(3.6)	99	(11.6)
Beluesca longicolla	31	(3.6)	-	-	85	(10)
Chelediadema marjoriae	99	(11.6)	85	(10)	-	-
Caulanus thomasi	54	(6.3)	36	(4.2)	80	(9.4)
Latuterus tkachi	62	(7.2)	53	(6.2)	90	(10.5)
Neometadena ovata	76	(8.9)	66	(7.7)	88	(10.3)
Retrovarium spp.	67–71	(7.8–8.3)	58–63	(6.8–7.4)	81–84	(9.5–9.8)
Siphoderina sp.	53	(6.2)	42	(4.9)	79	(9.3)
ITS						
Beluesca littlewoodi	-	-	30	(3.4)	99	(11.6)
Beluesca longicolla	30	(3.4)	-	-	96	(11.2)
Chelediadema marjoriae	99	(11.6)	96	(11.2)	-	-
Caulanus thomasi	69	(7.9)	65	(7.5)	74	(8.6)
Latuterus tkachi	62	(7.1)	60	(6.8)	76	(8.8)
Neometadena ovata	97	(11.3)	90	(10.5)	105	(12.3)
Retrovarium spp.	81–89	(9.4–10.2)	81–89	(9.4–10.2)	81–91	(9.4–10.7)
Siphoderina sp.	64	(7.3)	61	(7)	82	(9.6)
3' ITS1 only						
Beluesca littlewoodi	-	-	13	(3.4)	48	(13.2)
Beluesca longicolla	13	(3.4)	-	-	49	(13.1)
Chelediadema marjoriae	48	(13.2)	49	(13.1)	-	-
Caulanus thomasi	32	(8.5)	30	(7.9)	37	(9.9)
Latuterus tkachi	29	(7.7)	26	(6.9)	37	(9.9)
Neometadena ovata	47	(13.1)	46	(12.7)	51	(14.1)
Retrovarium spp.	39–46	(10.4–12.3)	41–46	(10.8–12.1)	37–49	(9.9–13.3)
Siphoderina sp.	27	(7.1)	22	(5.8)	42	(11.3)
ITS2 only						
Beluesca littlewoodi	-	-	17	(5.9)	49	(17.5)
Beluesca longicolla	17	(5.9)	-	-	45	(16.2)
Chelediadema marjoriae	49	(17.5)	45	(16.2)	-	-
Caulanus thomasi	35	(12.1)	33	(11.5)	37	(13.2)
Latuterus tkachi	31	(10.7)	32	(11.1)	39	(13.9)
Neometadena ovata	44	(15.3)	38	(13.3)	50	(18)
Retrovarium spp.	39–44	(13.8–15.2)	37–43	(13.2–14.9)	39–47	(14.3–16.9)
Siphoderina sp.	34	(11.9)	36	(12.6)	39	(14.2)

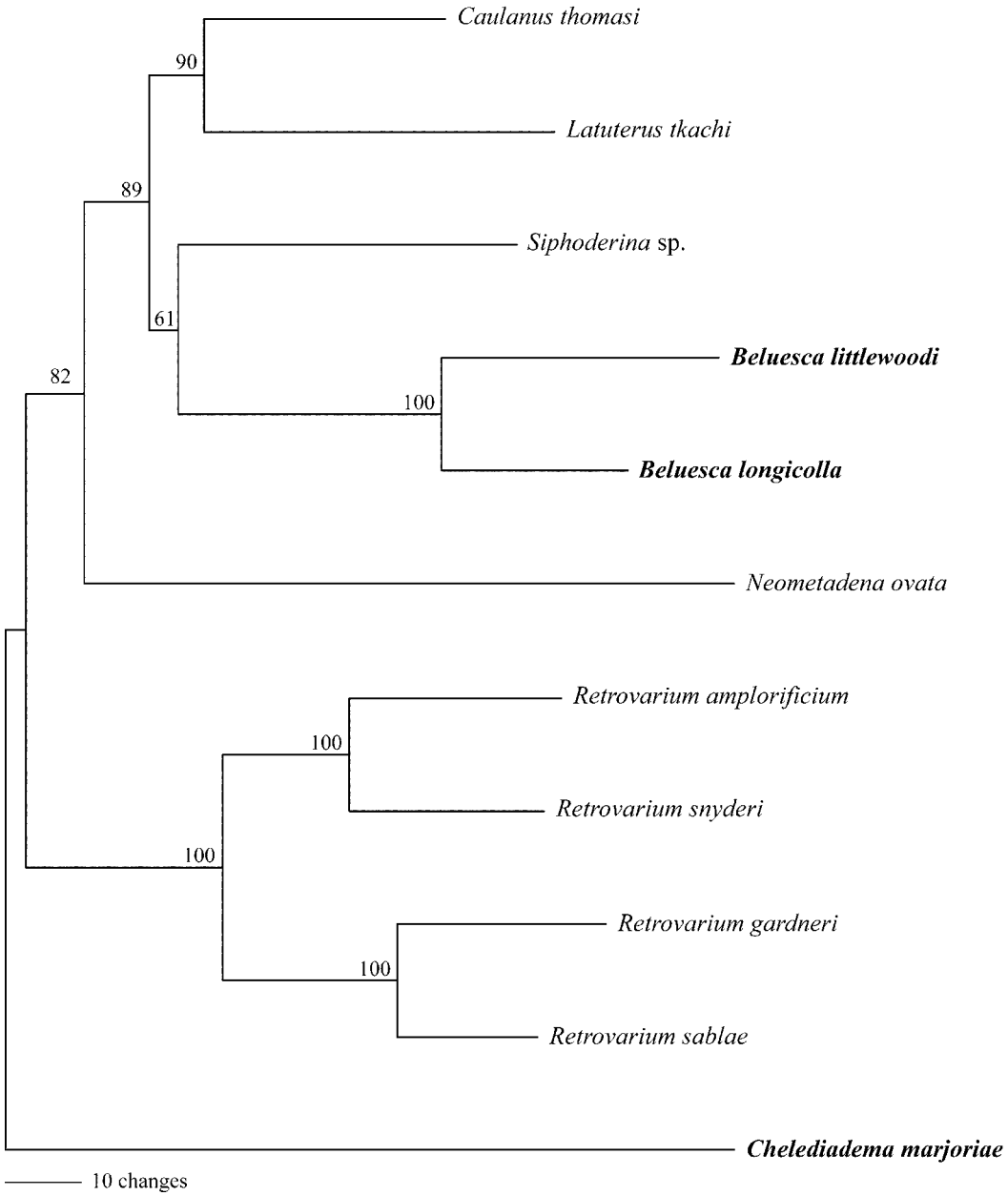


FIGURE 7. Relationships between species of *Beluesca* n. gen., *Chelediadema* n. gen., *Caulanus*, *Latuterus*, *Neometadena*, *Retrovarium* and an undescribed species of *Siphoderina* inferred from minimum evolution analysis of total genetic distance for the combined LSU and ITS (includes partial ITS1 and complete 5.8S and ITS2) rDNA dataset. Bootstrap values are indicated at the nodes. Phylogram is midpoint rooted.

Discussion

Beluesca n. gen.

The species *Pseudallacanthochasmus plectorhynchi* Mamaev, 1970 was described by Mamaev (1970) from the haemulid *Plectorhinchus cinctus* in the Gulf of Tonkin and later placed as a *species incertae sedis* by Miller & Cribb (in press-c) because it did not fit within the revised concept of *Pseudallacanthochasmus* or any other cryptogonimid genus. A new genus was not proposed to accommodate this species because the description and figures provided by Mamaev (1970) were inadequate. The obvious host and morphological similarities of *P. plectorhynchi* to species of *Beluesca* imply that these species are closely related and warrant

placement together, so we here transfer *P. plectorhynchi* to *Beluesca* as *B. plectorhyncha* (Mamaev, 1970) n. comb.

We were unable to obtain the specimens described by Mamaev (1970), but despite the apparent poor quality of the specimens figured and described, we are confident that the specimens described here are not those reported by Mamaev (1970). This is because the characters important in distinguishing between the morphologically and molecularly distinct species *Beluesca littlewoodi* and *B. longicolla* (the distribution of the vitelline follicles in combination with the length of the prepharynx and oesophagus) also distinguish these two from *B. plectorhyncha*. The vitelline follicles of *B. plectorhyncha* do not extend posterior to the ventral sucker, which is similar to *B. longicolla*, but the oesophagus of the latter is much longer than that of the former, even taking into consideration that the specimens described by Mamaev (1970) did not appear to be fully extended when fixed. Also, the oral and ventral suckers are distinctly smaller and the prepharynx and oesophagus of *B. plectorhyncha* are much shorter than those of both *B. littlewoodi* and *B. longicolla* (Table 3). Furthermore, the type-host of *B. plectorhyncha*, *Plectorhinchus cinctus*, is distributed in the northern Indo-Pacific and has not been reported from Australian waters. This combination of host and morphological differences supports our hypothesis that the species described by Mamaev (1970) is distinct from both *B. littlewoodi* and *B. longicolla*.

TABLE 3. Comparison of morphometric variables reported for *B. plectorhyncha* (Mamaev, 1970) n. comb. to *Beluesca littlewoodi* n. sp. and *B. longicolla* n. sp. to illustrate differences between these three species. All values are in micrometres and those to the left under B. littlewoodi and B. longicolla represent the mean and in parentheses the range.

Measurement, number or ratio	*B. plectorhyncha*	*B. littlewoodi*	*B. longicolla*
Body length	1370	2083 (1744–2472)	2361 (2216–2536)
Body width	440	285 (240–368)	357 (320–384)
Oral sucker length	180	234 (202–273)	226 (208–244)
Oral Sucker width	120	207 (182–250)	186 (176–195)
Number of oral spines	18–20	21 (20–22)	19 (19–21)
Oral spine length	100	51 (40–64)	64 (51–82)
Ventral sucker length		91 (78–111)	94 (91–96)
Ventral sucker width	70	108 (91–127)	98 (94–109)
Forebody as % of body length	44%	52 (48–58)%	66 (64–67)%
Pharynx length	70	68 (55–78)	72 (68–78)
Pharynx width	40	67 (59–75)	71 (68–75)
Testes length	210–220	181 (137–247)	259 (228–304)
Testes width	130–150	116 (94–137)	152 (125–189)
Egg length	17–20	17 (16–21)	16 (13–18)
Egg width	11–13	9 (7–10)	7 (7–8)

Species of *Beluesca* most closely resemble those of the genera *Allacanthochasmus* Van Cleave, *Pseudallacathochasmus* Velasquez and *Stemmatostoma* Cribb. Species of *Allacanthochasmus* are restricted to freshwater moronids from North America and differ from those of *Beluesca* in that they have a gonotyl and the ovary extends nearly the entire width of the body. Species of *Pseudallacathochasmus* differ from those of *Beluesca* in having tandem testes, a distinctly shorter forebody and smaller oral spines. The type-and only species of *Stemmatostoma*, *S. pearsoni* Cribb, 1986, which is found in freshwater terapontids in Australia, differs from species of *Beluesca* because the caeca terminate near the anterior end of the testes and the uterus extends well into the forebody.

TABLE 4. Numbers and localities of individuals of Haemulidae collected and examined for cryptogonimids. Species in bold were infected with species of *Beluesca* n. gen. or *Chelediadema* n. gen.

	Locality				
Haemulidae	1	2	3	4	Totals
Diagramma labiosum	43	7			50
Plectorhinchus albovittatus		1			1
Plectorhinchus chrysotaenia		3			3
Plectorhinchus gibbosus	5	1			6
Plectorhinchus mulitivittatus	4				4
Plectorhinchus picus	3				3
Plectorhinchus schotaf			1		1
Pomadasys kaakan	1				1
Pomadasys maculatus				1	1
Total	56	12	1	1	70

Localities: 1. Heron Island, GBR; 2. Lizard Island, GBR; 3. Ningaloo Reef, Western Australia; 4. Cleveland Bay, Townsville, Queensland.

Chelediadema n. gen.

Chelediadema n. gen. most closely resembles *Acanthostomoides* Szidat, *Acanthostomum* Looss, *Caimanicola* Teixeira de Freitas & Lent, *Gymnatrema* Morozov, *Proctocaecum* Baugh and *Timoniella* Rebecq, which all contain species with oral spines, entire ovaries and tandem testes. *Chelediadema* differs from all of these genera by the presence of uterine coils that extend well posterior to the testes and vitelline follicles that extend well into the forebody. Also, species of *Acanthostomum*, *Caimanicola*, *Gymnatrema*, *Proctocaecum* and *Timoniella* all have one or both caeca open via ani at or near the posterior end of the body, further distinguishing them from *Chelediadema*. The type- and only species of *Acanthostomoides*, *A. apophalliformis* Szidat, 1956 differs from *C. marjoriae* in having an oesophagus that is much longer than the prepharynx and the vitelline follicles are confluent throughout the hindbody and extend to the posterior extremity.

DNA sequence data for *Chelediadema marjoriae* was recovered only from Lizard Island, so future studies will need to incorporate samples from Heron Island and other localities where this species is discovered.

Relationships between lutjanid and haemulid cryptogonimid taxa

Species of *Beluesca* and *Chelediadema* have not been recovered from any of the numerous lutjanid species that have been sampled from the same localities (Miller & Cribb in press-b), suggesting that these genera are restricted to the Haemulidae. Although fewer individual haemulids than lutjanids have been examined for the presence of cryptogonimids during this survey (Table 4 and Miller & Cribb in press-b), there does not appear to be the concentration of cryptogonimids within the Haemulidae that is seen in Lutjanidae. Because cryptogonimids apparently always utilize fish as their second intermediate host, the lack of a concentration of taxa infecting the Haemulidae is probably explained by dietary preference, which in lutjanids is primarily fish, whereas fish are a lesser component of the diet of haemulids (Kulbicki *et al*. 2005).

The intergeneric and interspecific variation among the taxa analysed here is similar to that observed in previous reports from rDNA sequence data from cryptogonimids infecting the Indo-West Pacific lutjanid and haemulid ichthyofauna (Miller & Cribb in press-b, d). Surprisingly, despite the host preference (Haemulidae) and superficial morphological similarities (elongate body, funnel-shaped oral sucker and few large oral spines), species of *Beluesca* and *Chelediadema* were among the most genetically distant of the taxa analysed. Species of *Beluesca* formed a clade with three genera of cryptogonimids that are known only from lutjanids.

This pattern recalls the cryptogonimid genus *Retrovarium*, which has twelve species in lutjanids and one species in a haemulid (Miller & Cribb in press-b). Also, six of the seven genera reported from haemulids (*Metadena* Linton, *Neometadena* Hafeezullah & Siddiqi, *Pseudallacanthochasmus* Velasquez, *Siphodera* Linton, *Siphoderina* Manter, and *Stegopa* Linton) are comprised entirely or mostly of species that also occur in lutjanids. Taken together, the observed host distribution of cryptogonimid genera whose species infect the Haemulidae and Lutjanidae and the observation that taxa infecting haemulids are nested well within clades whose hosts are exclusively lutjanids, suggests that host switching between the two closely related families Lutjanidae and Haemulidae has been common in the evolutionary history of this system.

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