

# ***Adlardia novaecaledoniae* n. g., n. sp. (Digenea: Cryptogonimidae) from the fork-tailed threadfin bream *Nemipterus furcosus* (Val.) (Perciformes: Nemipteridae) off New Caledonia**

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**Abstract** *Adlardia novaecaledoniae* n. g., n. sp. (Digenea: Cryptogonimidae) is described from the fish *Nemipterus furcosus* (Val.) (Perciformes: Nemipteridae) from off New Caledonia (South Pacific). *Adlardia* n. g. is distinguished from all other cryptogonimid genera by the combination of an elongate body, the presence of oral spines, intestinal caeca that open via ani at the posterior end of the body, a highly lobed ovary, oblique testes that are located in the mid-hindbody, vitelline follicles that extend from midway between the testes and ovary to midway between the ovary and ventral sucker, and an excretory vesicle that bifurcates dorsal to the ovary

and reunites immediately anterior to the pharynx. *A. novaecaledoniae* n. sp. is the only cryptogonimid that has been reported with an excretory vesicle that reunites anterior to the pharynx. *Siphoderina elongata* (Gu & Shen, 1979) Miller & Cribb, 2008 is transferred to *Adlardia* as *A. elongata* (Gu & Shen, 1979) n. comb. based on morphological and ecological (host group) agreement with *A. novaecaledoniae*. Bayesian inference analysis of LSU rDNA revealed that *A. novaecaledoniae* nested well within a clade containing cryptogonimid taxa known almost exclusively from haemulid and lutjanid fishes, suggesting that host-switching between teleosts of the Haemuloidea, Lutjanoidea and Sparoidea may have been common in the evolutionary history of this system.

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**Résumé** *Adlardia novaecaledoniae* n. g., n. sp. (Digenea: Cryptogonimidae) est décrit du poisson *Nemipterus furcosus* (Val.) (Perciformes: Nemipteridae) de Nouvelle-Calédonie (Pacifique Sud). *Adlardia* n. g. est distingué de tous les autres genres de Cryptogonimidae par la combinaison d'un corps allongé, la présence d'épines orales, des caeca intestinaux qui s'ouvrent par des anus dans la partie postérieure du corps, un ovaire très lobé, des testicules obliques qui sont situés au centre de la partie postérieure du corps, des follicules vitellins qui s'étendent d'entre les testicules et l'ovaire jusqu'entre l'ovaire et la ventouse ventrale et une vésicule excrétrice qui bifurque dorsalement par rapport à l'ovaire et se réunit juste en avant du

pharynx. *A. novaecaledoniae* n. sp. est le seul Cryptogonimidae qui a été décrit avec une vésicule excrétrice qui se réunit juste en avant du pharynx. *Siphoderina elongata* (Gu & Shen, 1979) Miller & Cribb, 2008 est transféré vers *Adlardia* comme *A. elongata* (Gu & Shen, 1979) n. comb. sur la base de ressemblances morphologiques et écologiques (groupe hôte) avec *A. novaecaledoniae*. Une analyse d'inférence bayésienne de l'ADNr LSU a révélé que *A. novaecaledoniae* trouvait sa place dans un clade comprenant des taxons de Cryptogonimidae connus presque exclusivement de poissons Haemulidae et de Lutjanidae, ce qui suggère que des changements d'hôtes entre des téléostéens Haemuloidea, Lutjanoida et Sparoidea ont pu être communs dans l'histoire évolutive du système.

## Introduction

The Nemipteridae (threadfin or whiptail breams) is a medium sized family of teleosts, currently comprising five genera and 62 species, which are distributed throughout the tropical and sub-tropical Indo-West Pacific (Froese & Pauly, 2008); no species occur in the eastern Pacific or Atlantic. *Nemipterus japonicus* (Bloch) was recently reported from the Mediterranean Sea by Golani & Sonin (2006), who suggested that its presence was probably due to migration from the Red Sea via the Suez Canal. Like many other species of the superfamily Sparoidea (which includes the Centranchthidae, Nemipteridae, Lethrinidae and Sparidae), nemipterids form a considerable and important component of commercial and artisanal fisheries throughout their range (Froese & Pauly, 2008; Russell, 1990). A large proportion of the diet for many of the species in this family consists of small reef-dwelling fish, cephalopods, crustaceans, polychaetes and other nekton, some of which are known to harbour metacercariae of numerous trematode groups (Froese & Pauly, 2008; Kulbicki et al., 2005; Rigby et al., 1999; Russell, 1990).

A survey of the cryptogonimid fauna of Indo-West Pacific teleosts revealed the presence of a new species from *Nemipterus furcosus* (Valenciennes) off New Caledonia that does not agree with any previously reported genus or species. Only one other cryptogonimid has been reported from a nemipterid; Gu & Shen (1979) described *Siphoderina elongata*

(Gu & Shen, 1979) Miller & Cribb, 2008 from *Nemipterus virgatus* (Houttuyn) off China and distinguished it from other species of *Paracryptogonimus* Yamaguti, 1934 (now *Siphoderina* Manter, 1934) based on the elongate body, number of oral spines and smooth tegument.

We augment our morphologically based taxonomic approach to the taxon recovered here with an analysis of genetic data from the large subunit (LSU) nuclear ribosomal DNA region with the aim of exploring the integrity of the new species and its phylogenetic relationships with other cryptogonimid taxa. The LSU rDNA data obtained for the specimens reported here were aligned with data available from previously reported cryptogonimids (Miller & Cribb, 2007b, c, d; 2008b; Miller, Downie & Cribb, 2009) and analysed using Bayesian inference analysis to explore intergeneric relationships between these species.

## Materials and methods

### *Host and parasite collection*

Individuals of the fish *Nemipterus furcosus* were collected using baited line from the following sites around Nouméa, New Caledonia: Baie Maa (22°13'S, 166°20'E) and (22°12'S, 166°20'E); Grande Rade, Nouméa, Nouméa City (22°14'S, 166°23'E); and off Baie des Citrons, Nouméa (22°17'S, 166°25'E). Digeneans were collected live, immediately fixed in hot seawater and then transferred to 70% ethanol. Specimens for DNA extraction and analysis were fixed and then stored in 70% or 95–100% ethanol at –20°C. Two voucher specimens of *N. furcosus* were deposited in the Muséum National d'Histoire Naturelle (MNHN), Paris under the accession numbers MNHN 2005-0768 and 2006-1330.

### *Morphological samples*

Preserved digenean specimens for morphological analysis were placed in Mayer's paracarmine for staining. The specimens were overstained and then destained by placing them in 50% acid alcohol solution. Stained specimens were then dehydrated through a graded series of ethanol for at least half an hour at each dehydration step, cleared in beechwood creosote and mounted in Canada balsam. Drawings were made with the aid of a drawing tube on an

Olympus BH-2 microscope. All measurements were made using an ocular micrometer or a Digicad Plus digitising tablet and Carl Zeiss KS100 software adapted by Imaging Associates, and are quoted in micrometres with the mean followed by the range in parentheses. For two-dimensional measurements, length is given before breadth. Type-specimens were deposited in the MNHN and the British Museum of Natural History (BMNH) collection at the Natural History Museum, London.

#### *Molecular sample processing*

Total genomic DNA for the new species was isolated from single individual worms following the removal of alcohol from preserved specimens by evaporation on a heating block prior to extraction. The samples were then incubated at 55°C overnight in cell lysis solution (10 mM Tris, 100 mM EDTA, 2% SDS, pH 8.0). Proteins were removed with 7.5 M ammonium acetate. DNA was then precipitated with isopropanol and resuspended in Tris–EDTA buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). The partial large subunit (LSU) nuclear ribosomal DNA region was amplified using the primers reported by Littlewood et al. (2000). PCR was conducted with a total volume of 25 µl, consisting of 1 µM of each primer combined with 1–10 µM of template DNA, 5 µl 5× *Taq* buffer, 4 mM MgCl<sub>2</sub>, 5 mM dNTP and 0.6 µl *Go Taq* DNA polymerase. Each reaction was performed in an ABI Veriti thermocycler programmed for 35 cycles of 95°C for 1 min, 58°C for 30 sec, 72°C for 1 min, and a final extension step at 72°C for 7 min. Amplified DNA was then purified from bands excised from ethidium bromide stained 1.5% agarose TBE gels using a Qiagen Gel extraction kit according to manufacturer's protocols. Cycle sequencing was conducted using the same primers utilised for PCR amplification with ABI Big Dye™ v.3.1 chemistry following the manufacturer's recommendations. Precipitation with 3 M Sodium Acetate (pH c.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. Sequencing was performed by Macrogen Inc., Seoul, Korea. The resulting sequences were edited and contigs constructed using Sequencher™ version 4.5 (GeneCodes Corp.). The GenBank accession number for the LSU rDNA region sequenced is provided in the Results section. The consensus

sequence for the LSU rDNA region utilised in this study was constructed from multiple replicates (each replicate contig being both a forward and a reverse sequence from a single individual). Additional replicate contigs were sequenced from specimens obtained from different host/parasite/location combinations whenever possible.

#### *Comparative DNA analyses*

The LSU rDNA region was aligned with data reported for species of the cryptogonimid genera *Beluesca* Miller & Cribb, 2007, *Caulanus* Miller & Cribb, 2007, *Chelediadema* Miller & Cribb, 2007, *Latuterus* Miller & Cribb, 2007, *Lobosorchis* Miller & Cribb, 2005, *Neometadena* Hafeezullah & Siddiqi, 1970, *Retrovarium* Miller & Cribb, 2007 and *Siphoderina* Manter, 1934 by Miller & Cribb (2007b, c, d; 2008b) and Miller, Downie & Cribb (2009) for comparative purposes and to explore levels of intergeneric variation. The LSU rDNA dataset was initially aligned using ClustalX version 2.0.9 (Larkin et al., 2007) under the following parameters: pairwise alignment parameters = gap opening 10.00, gap extension 0.10, DNA weight matrix International Union of Biochemistry (IUB); and multiple alignment parameters = gap opening 10.00, gap extension 0.20, delay divergent sequences 30%, DNA weight matrix IUB. The resulting sequence alignment was exported from ClustalX in FASTA and NEXUS formats, and refined by eye using MacClade version 4.08 (Maddison & Maddison, 2005). After alignment of the LSU dataset was edited, the ends of each fragment were trimmed to match the shortest sequence in the alignment. Bayesian inference analysis of the LSU dataset was performed using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) to explore relationships between these taxa. Modeltest version 3.7 (Posada & Crandall, 1998) was used to estimate the best substitution model for the LSU dataset. Bayesian inference analysis was conducted on the combined dataset using the TVM+I+G model predicted as the best estimator by the Akaike Information Criterion (AIC) in Modeltest. Bayesian inference analysis was run over 1,000,000 generations (ngen = 1000000) via four simultaneous Markov Chain Monte Carlo (MCMC) chains (nchains = 4) and every 100th tree saved (samplefreq = 100). Bayesian analysis used the following parameters: nst = 6, rates = invgamma,

ngammacat = 4, and the MCMC parameters were left at the default settings. Samples of substitution model parameters, and tree and branch lengths were summarised using the parameters ‘sump burnin = 3000’ and ‘sumt burnin = 3000’. These ‘burnin’ parameters were chosen because the log likelihood scores ‘plateaued’ well before 300,000 replicates in the Bayesian inference analysis.

### Family Cryptogonimidae Ward, 1917

#### *Adlardia* n. g.

##### *Diagnosis*

Body elongate; length/width ratio *c.*5.2–7.3. Tegument armed with small to minute spines. Oral sucker nearly round, with 21–30 oral spines, opens subterminally to terminally. Ventral sucker unspecialised, embedded in ventrogenital sac. Oral/ventral sucker width ratio *c.*1.3–2.2. Forebody occupies *c.*18–25% of body length. Prepharynx slightly longer than or nearly equal to length of oesophagus, rarely shorter. Oesophagus short. Intestinal bifurcation slightly posterior to pharynx or midway between ventral sucker and pharynx. Caeca open via ani at posterior end of body. Testes two, oblique, in mid-hindbody. Cirrus and cirrus-sac absent. Seminal vesicle tubulosaccular, anterior to seminal receptacle, between ovary and ventral sucker. Common genital pore immediately anterior to ventral sucker. Gonotyl absent. Ovary deeply lobed, in anterior hindbody anterior to testes. Laurer’s canal present. Seminal receptacle saccular, immediately anterior to ovary, posterior to seminal vesicle. Vitelline follicles in two lateral groups, extend from slightly anterior to testes to midway between ovary and ventral sucker. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Excretory vesicle bifurcates dorsal to ovary and reunites immediately anterior to pharynx, reaches to level between anterior to pharynx to oral sucker; excretory pore terminal at posterior end of body. *Type-species*: *A. novaecaledoniae* n. sp.

*Etymology*: The name *Adlardia* is for Dr Robert Adlard, in recognition of his contributions to the study of marine parasites of Australia. It is to be treated as feminine.

#### Differential diagnosis

*Adlardia* n. g. is distinguished from all other cryptogonimid genera by the combination of an elongate body, the presence of oral spines, intestinal caeca that open via ani at the posterior end of the body, a highly lobed ovary, oblique testes that are located in the mid-hindbody, vitelline follicles that extend from midway between the testes and ovary to midway between the ovary and ventral sucker, and an excretory vesicle that bifurcates dorsal to the ovary and reunites immediately anterior to the pharynx. Like most cryptogonimid genera, *Adlardia* is not diagnosed by any single character, but a combination of characters, although the unique reunion of the excretory vesicle reported in species of this genus immediately distinguish them from other cryptogonimids.

#### *Adlardia novaecaledoniae* n. sp.

Syn. *Siphoderina elongata* (Gu & Shen, 1979) of Quilichini et al. (2009)

*Type-host*: *Nemipterus furcosus* (Valenciennes) (Nemipteridae).

*Type-locality*: Baie Maa, New Caledonia (22°13’S, 166°20’E; 30/08/2007).

*Other localities*: Baie Maa (22°12’S, 166°20’E; 13/11/2007); Grande Rade, Nouméa, Nouméa City (22°14’S, 166°23’E; 09/01/2006, 10/01/2006); near Baie des Citrons, Nouméa (22°17’S, 166°25’E; 10/10/2006, 16/10/2007), all off New Caledonia.

*Site*: Intestine and pyloric caeca.

*Type-material*: Holotype MNHN JNC2291-1, 16 paratypes MNHN JNC2289-1-5, JNC2291-2-4, JNC2331B-1-4, JNC2288-1-3, JNC2398-1, 10 paratypes BMNH 2008.12.30.1-3.

*Prevalence*: 9 of 13 (69%).

*Molecular sequence data*: LSU, 3 replicates.

*GenBank accession number*: FJ554632.

#### Description (Figs. 1–2)

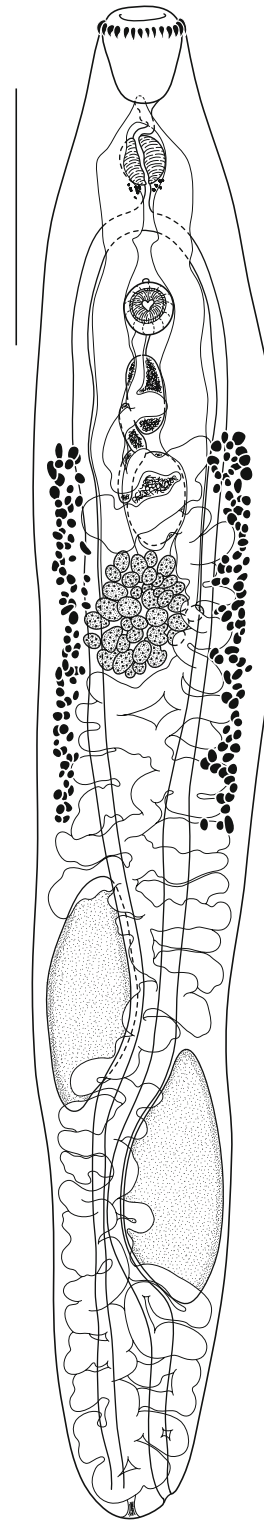
[Based on 17 specimens.] Body elongate, longer than wide, 2,385 (1,787–2,987) × 394 (306–483); length/width ratio 6.1 (5.3–7.3). Oral sucker 178 (147–217) × 158 (125–199). Oral spines 27 (24–30), length 30 (21–49). Ventral sucker 98 (86–109) × 99 (78–119). Oral sucker/ventral sucker width ratio 1.6

(1.3–1.9). Forebody occupies 21 (18–25)% of body length. Prepharynx slightly longer than or nearly equal to length of oesophagus, rarely shorter, 64 (40–91) long. Pharynx 86 (70–104)  $\times$  88 (73–100). Ventral sucker width/pharynx width ratio 1.1 (1–1.4). Oesophagus 51 (34–75) long. Intestinal bifurcation slightly posterior to pharynx or midway between ventral sucker and pharynx. Caeca pass between testes, open via ani at posterior end of body. Testes 2, oblique, in mid-hindbody, 336 (195–498)  $\times$  183 (131–254). Seminal vesicle tubulosaccular, anterior to seminal receptacle, between ovary and ventral sucker. Common genital pore immediately anterior to ventral sucker. Gonotyl absent. Ovary deeply lobed, in anterior hindbody, pre-testicular, 231 (190–265)  $\times$  214 (150–282). Laurer's canal present. Seminal receptacle saccular, immediately anterior to ovary, posterior to seminal vesicle. Vitelline follicles in 2 lateral groups, extend from slightly anterior to testes to midway between ovary and ventral sucker. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Eggs small, darkly tanned, 18 (14–21)  $\times$  10 (8–12) wide. Excretory vesicle bifurcates dorsal to ovary and reunites dorsally immediately anterior to pharynx, reaches to oral sucker; excretory pore terminal at posterior end of body.

#### Comparative DNA analysis

Alignment of the LSU rDNA region for *Adlardia novaecaledoniae* n. sp. and the remainder of the cryptogonimid taxa examined yielded 861 characters for analysis. No intraspecific variation was observed in *A. novaecaledoniae* over the LSU rDNA region sequenced here.

Bayesian inference analysis of the LSU rDNA dataset resulted in a phylogram with *A. novaecaledoniae* forming a well-supported clade with species of *Lobosorchis* (Fig. 3). The clade containing *A. novaecaledoniae* and species of *Lobosorchis* was sister to species of *Retrovarium*. Another well-supported clade was formed between species of *Beluesca*, *Caulanus*, *Latuterus* and *Siphoderina*. All genera were resolved with strong posterior probability support in the Bayesian analysis, although many intergeneric relationships were not well supported.



**Fig. 1** *Adlardia novaecaledoniae* n. sp. from the intestine of *Nemipterus furcosus* off Baie Maa, New Caledonia. Ventral view of holotype. Scale-bar: 500  $\mu$ m



**Fig. 2** Immature specimen of *Adlardia novaecaledoniae* n. sp. from the intestine of *Nemipterus furcosus* off New Caledonia. Ventral view of paratype MNHN JNC2289. Scale-bar: 500  $\mu$ m

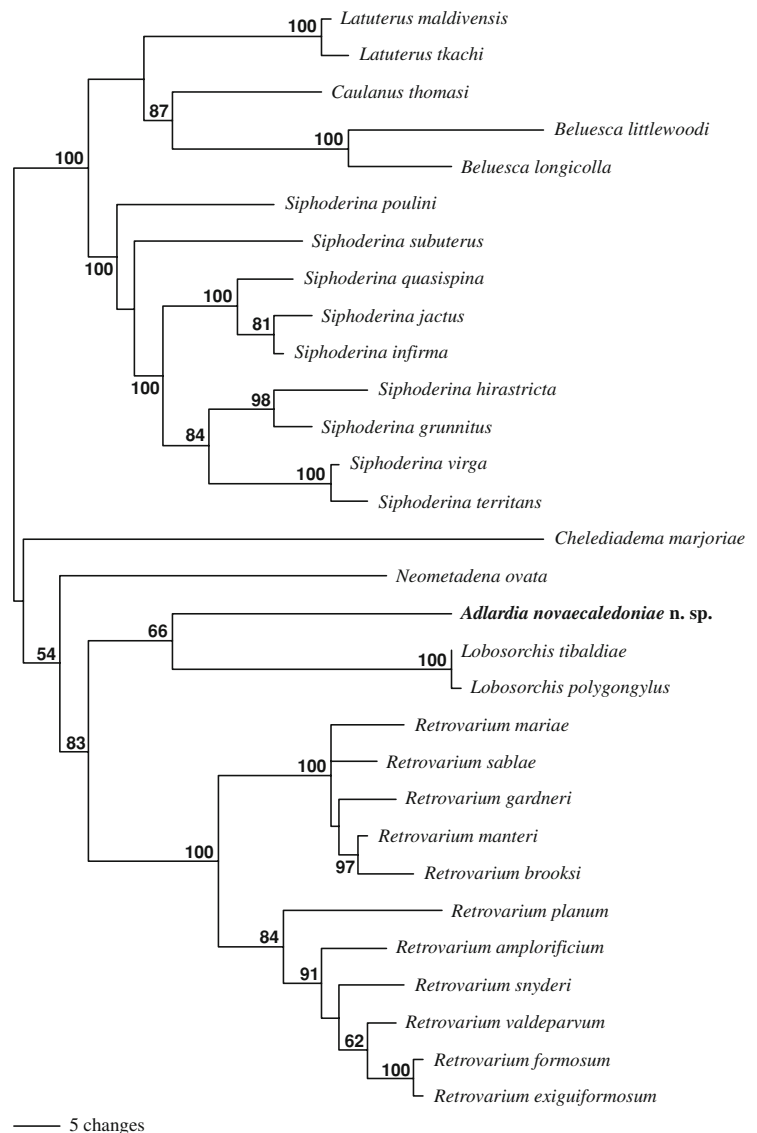
## Discussion

### Systematics

*Adlardia novaecaledoniae* n. sp. is the only cryptogonimid that has been reported with an excretory vesicle which reunites anterior to the pharynx. This apparent apomorphy is an important diagnostic character for the genus, independent of the combination of other distinguishing morphological features. Seven other cryptogonimid genera have species which do not have a reuniting excretory vesicle but do have caeca that open via ani at or near the posterior end of the body; these are *Acanthostomum* Looss, 1899, *Caimanicola* Freitas & Lent, 1938, *Caulanus* Miller & Cribb, 2007, *Gymnatrema* Morozov, 1955, *Orientodiploproctodaemum* Bhutta & Khan, 1970, *Novemtestis* Yamaguti, 1942 and *Timoniella* Rebecq, 1960. *A. novaecaledoniae* is easily distinguished from species of *Acanthostomum*, *Caimanicola*, *Gymnatrema* and *Timoniella* by its highly lobed ovary (entire in these genera), uterine distribution and testicular position and location (oblique at mid-hindbody as opposed to tandem at the posterior end of the body). Species of *Orientodiploproctodaemum* are immediately distinguished from *A. novaecaledoniae* by their lack of oral spines, the large collar surrounding the oral sucker, intercaecal testes and vitelline follicle distribution. The type- and only species of *Novemtestis*, *N. armatus* Yamaguti, 1942, differs from *A. novaecaledoniae* in that it has a distinctly large oral sucker, a pharynx that is much larger than the ventral sucker, nine testes and vitelline follicles that extend well into the forebody. *Caulanus thomasi* Miller & Cribb, 2007, the type- and only species of *Caulanus*, is distinguished from *A. novaecaledoniae* by the uterus that extends well into the forebody, tandem testes located in the mid-hindbody and vitelline follicles that extend to the level of the pharynx.

The discovery of *A. novaecaledoniae* brings the number of cryptogonimids known from nemipterids to two; the other, *Siphoderina elongata* reported by Gu & Shen (1979), is strikingly similar in morphology. Notable morphological characteristics shared by these two species are the body shape, number of oral spines, ventral sucker location, testicular configuration, highly lobed ovary near the mid-body, vitelline distribution, seminal vesicle and seminal receptacle

**Fig. 3** Relationships between *Adlardia novaecaledoniae* n. sp. and the remainder of the cryptogonimid taxa examined here based on Bayesian inference analysis of the LSU rDNA dataset. Posterior probabilities are shown at the nodes with values < 50 not shown. Phylogram is mid-point rooted



shape and location, and the distribution of the uterus. Gu & Shen (1979) did not describe the terminal morphology of or provide measurements of the caeca of *S. elongata*, possibly due to the caeca being obscured by eggs within the uterus. We have found that the caeca and anal openings are often obscured by the numerous eggs in many of our mature specimens of *A. novaecaledoniae*, which can lead to these characters being overlooked (Fig. 1). Additionally, Gu & Shen (1979) did not characterise the anterior extent of the excretory vesicle very well, and in the figure provided show it terminating near the anterior end of the pharynx. We have also found that

the anteriormost portion of the excretory vesicle (anterior to the pharynx) is difficult to see and can be easily overlooked as it passes dorsal to the oral sucker. The specimens described by Gu & Shen (1979) from *Nemipterus virgatus* off China are distinctly larger and appear to have a smaller oral to ventral sucker ratio (Table 1), distinguishing them from *A. novaecaledoniae*. Unfortunately, we were unable to borrow the specimens deposited by Gu & Shen in the Institute of Oceanology, Chinese Academy of Sciences, but are confident that there is sufficient evidence from the morphological and host group similarities to suggest that these species are

**Table 1** Comparison of morphometric variables reported for the species *Adlardia novaecaledoniae* n. sp. and *Adlardia elongata* (Gu & Shen, 1979) n. comb. All values are in micrometres

|                           | <i>Adlardia novaecaledoniae</i> n. sp. ex <i>Nemipterus furcosus</i> |        |        | <i>Adlardia elongata</i> n. comb. ex <i>N. virgatus</i> |               |
|---------------------------|--|--------|--------|---|---------------|
|                           | Mean   | Min.   | Max.   | Min.  | Max.          |
| Body length               | 2,385  | 1,787  | 2,987  | 2,238   | 5,461         |
| Body width                | 394  | 306    | 483    | 434   | 885           |
| Oral sucker (OS) length   | 178  | 147    | 217    | 117   | 267           |
| Oral sucker width         | 158  | 125    | 199    | 150   | 334           |
| Oral spine number         | 27   | 24     | 30     | 21  | 27            |
| Oral spine length         | 30   | 21     | 49     | 30  | 48            |
| Ventral (VS) sucker width | 158  | 125    | 199    | 84  | 150           |
| OS/VS width ratio         | 1:0.63   | 1:0.54 | 1:0.79 |   | Mean c.1:0.32 |
| Prepharynx length         | 64   | 40     | 91     | 67  | 200           |
| Pharynx length            | 86   | 70     | 84     | 67  | 200           |
| Oesophagus length         | 51   | 34     | 75     | 50  | 200           |
| Ovary length              | 231  | 190    | 265    | 217   | 451           |
| Ovary width               | 214  | 150    | 282    | 251   | 534           |
| Anterior testis length    | 314  | 195    | 424    | 267   | 618           |
| Anterior testis width     | 169  | 131    | 224    | 200   | 317           |
| Posterior testis length   | 358  | 255    | 498    | 301   | 701           |
| Posterior testis width    | 196  | 156    | 254    | 200   | 284           |
| Egg length                | 18   | 14     | 21     | 15  | 18            |
| Egg width                 | 10   | 8      | 12     | 9   | 12            |

congeners, so we transfer *Siphoderina elongata* (Gu & Shen, 1979) Miller & Cribb, 2008 to *Adlardia* as *A. elongata* (Gu & Shen, 1979) n. comb.

#### Geographical distribution

*Adlardia* n. g. appears to be widely distributed in the Indo-West Pacific. The type-hosts for *A. novaecaledoniae* n. sp. and *A. elongata*, *Nemipterus furcosus* and *N. virgatus*, are widely distributed throughout the Indo-Australasian region, so it is possible that these parasites are not restricted to New Caledonia and China (respectively), particularly in view of the wide biogeographical distributions reported recently for other cryptogonimid taxa in the Indo-West Pacific region (Miller & Cribb 2007b, c). *N. furcosus* is also one of the most common and highly abundant fish of its size-class in the lagoon of New Caledonia (referred to as *N. peronii* in Laboute & Grandperrin, 2000), so *A. novaecaledoniae* is probably distributed throughout the entire New Caledonian coral reef system.

#### Relationships between lutjanid and nemipterid cryptogonimid taxa

Species of *Adlardia* n. g. have not been recovered in any of the many lutjanid taxa examined in this and other surveys (Durio & Manter, 1969; Miller & Cribb, 2007b, d) off Australia or New Caledonia, so it is likely that species of this genus do not infect lutjanids, at least on the Great Barrier Reef and off New Caledonia. We do not yet know whether species of *Adlardia* are found in Australian waters, although we have examined 244 individuals of nine species of nemipterids and have not found any cryptogonimid taxa inhabiting these fish. In addition, we have examined 12 individuals of the nemipterid *Pentapodus aureofasciatus* (Russell) from off New Caledonia and found no cryptogonimids. It seems clear, therefore that there has been no major radiation of cryptogonimids in nemipterids.

A recent study by Quilichini et al. (2009) examined the ultrastructure of spermatozoa of *A. novaecaledoniae* (referred to in their paper as *Siphoderina*



*elongata*) from specimens collected from *Nemipterus furcosus* off New Caledonia and showed that the spermatozoon of *A. novaecaledoniae* is very similar in structure to that of *Stemmatostoma pearsoni* Cribb, 1986 reported by Jamieson & Daddow (1982). The homogeneity in spermatozoon structure of these two cryptogonimids (e.g. external ornamentation and presence of two mitochondria) observed by Quilichini et al. (2009) suggests a relatively close phylogenetic relationship between these taxa, but they found that the number of cortical microtubules could consistently distinguish the species.

Phylogenetic analysis of the taxa examined here showed *A. novaecaledoniae* nested within a clade of cryptogonimids known exclusively from lutjanids and haemulids (Fig. 3). This pattern is similar to that observed for species of *Beluesca* and *Chelediadema* (whose species are found only in haemulids) and *Retrovarium* (found in haemulids and lutjanids) (Miller & Cribb 2007b, d). The observation that taxa infecting nemipterids are nested well within clades of cryptogonimid species known from lutjanids and haemulids further suggests that host-switching between the sister superfamilies Haemuloidea (Haemulidae), Lutjanoidea (Lutjanidae) and Sparoidea (Miller & Cribb, 2007a; Orrell & Carpenter, 2004) was common in the evolutionary history of this system. No cryptogonimids have been reported from centrarchids (Miller & Cribb, 2008a), one species is known from a lethrinid (Manter, 1963) and a few taxa are known from sparids (Bartoli & Bray, 1987; Yamaguti, 1971). Phylogenetic analyses of DNA sequence data from the cryptogonimid taxa infecting lethrinids and sparids with the taxa examined here will certainly give more insight into the evolution of this group.

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