Molecular characterisation of acanthocephalans from Australian marine teleosts: proposal of a new family, synonymy of another and transfer of taxa between orders

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This article is part of the Topical Collection Acanthocephala

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Abstract We provide molecular data (*cox1*, 18S rDNA and 28S rDNA) for 17 acanthocephalan species and 20 host-parasite combinations from Australian marine teleosts

collected from off Queensland, Australia. Fourteen of these acanthocephalans are characterised with molecular data for the first time and we provide the first molecular data for a species of each of the genera *Heterosentis* Van Cleave, 1931, *Pyriproboscis* Amin, Abdullah & Mhaisen, 2003 and *Sclerocollum* Schmidt & Paperna, 1978. Using 18S and 28S rDNA sequences, the phylogenetic position of each newly sequenced species is assessed with both single-gene and concatenated 18S+28S maximum likelihood and Bayesian inference analyses. Additional phylogenetic analyses focusing on the genus *Rhadinorhynchus* Lühe, 1912 and related lineages are included. Our phylogenetic results are broadly consistent with previous analyses, recovering previously identified inconsistencies but also providing new insights and necessitating taxonomic action. We do not find sufficient evidence to recognise the Gymnorhadinorhynchidae Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014 as distinct from the Rhadinorhynchidae Lühe, 1912. The family Gymnorhadinorhynchidae and its sole genus, *Gymnorhadinorhynchus* Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014, are here recognised as junior synonyms of Rhadinorhynchidae and *Rhadinorhynchus*, respectively. The two species currently assigned to *Gymnorhadinorhynchus* are recombined as *Rhadinorhynchus decapteri* (Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014) n. comb. and *Rhadinorhynchus mariserpentis* (Steinauer, Garcia-Vedrenne, Weinstein & Kuris, 2019) n. comb. In all of our analyses, *Rhadinorhynchus biformis* Smales, 2014 is found basal to the Rhadinorhynchidae + Transvenidae Pichelin & Cribb, 2001, thus resulting in a paraphyletic Rhadinorhynchidae. It appears that *R. biformis* may require a new genus and family; however, morphological data for this species are currently insufficient to adequately distinguish it from related lineages, thus we defer the proposal of any new higher-rank names for this species. Species of the genus *Sclerocollum*, currently assigned to the Cavisomidae Meyer, 1932, are found nested within the family Transvenidae. We transfer the genus *Sclerocollum* to the Transvenidae and amend the diagnosis of the family accordingly. The genera *Gorgorhynchoides* Cable & Linderoth, 1963 and *Serrasentis* Van Cleave, 1923, currently assigned to the Rhadinorhynchidae, are supported as sister taxa and form a clade in the Polymorphida. We transfer these genera and *Golvanorhynchus* Noronha, Fabio & Pinto, 1978 to an emended concept of the Isthomosacanthidae Smales, 2012 and transfer this family to the Polymorphida. Lastly, *Pyriproboscis heronensis* (Pichelin, 1997) Amin, Abdullah & Mhaisen, 2003, currently assigned to the Pomphorhynchidae Yamaguti, 1939, falls under the Polymorphida in our analyses with some support for a sister relationship with the Centrorhynchidae Van Cleave, 1916. As this species clearly does not belong in the

Pomphorhynchidae and is morphologically and molecularly distinct from the lineages of the Polymorphida, we propose the Pyriproboscidae n. fam. to accommodate it.

Introduction

The Acanthocephala is a small, monophyletic group of obligate endoparasites comprising some 12,000 species divided among four classes (Amin, 2013; Smales, 2015).

Acanthocephalans have indirect, two-host life-cycles involving invertebrate intermediate and vertebrate definitive hosts and they have been convincingly demonstrated as sister to the rotifers (Wallace & Colburn, 1989; Garey et al., 1996, 1998; Giribet et al., 2000; Near, 2002; Sørensen & Giribet, 2006). While there has been much interest in the higher order relationships between the Acanthocephala, the Rotifera and the remaining lineages of the Gnathifera, phylogenetic study of acanthocephalan diversity has lagged far behind that of other parasitic groups. There is still a paucity of molecular data for most acanthocephalan lineages, especially amongst marine species, and broader sampling is needed to achieve a more robust understanding of acanthocephalan relationships (García-Varela et al., 2000, 2002; Herlyn et al., 2003; García-Varela & Nadler, 2005, 2006; Verweyen et al., 2011; Braicovich et al., 2014).

Despite the many species of acanthocephalan reported from marine and terrestrial vertebrates of Australia (Edmonds, 1989; Barton, 1994; Pichelin et al., 1999; Smales, 2003; Smales & Weaver, 2015) molecular data have been generated for only a single species from this vast region, *Serrasentis sagittifer* (Linton, 1889) Linton, 1932 (Barton et al., 2018). The primary objective of this study was to provide new molecular data for acanthocephalans of marine fishes collected across over more than a decade from off the coast of Queensland, Australia, and place these species in the greater acanthocephalan phylogeny. We also aimed to evaluate the utility of 28S rDNA sequences for future molecular phylogenetic studies of the Acanthocephala. Our results prompted further analyses of sequences for species of the genus *Rhadinorhynchus* Lühe, 1912 and related genera and have resulted in revisions to the taxonomy of several acanthocephalan lineages as discussed below.

Materials and methods

Specimen collection and identification

Acanthocephalan specimens used for generation of molecular data in this study were obtained opportunistically between 2005 and 2018 from marine fishes collected off Queensland, Australia, primarily in Moreton Bay and on the Great Barrier Reef. Fishes were collected *via* angling, netting, spearing, trapping, or were obtained from commercial fishers. All fishes were dissected fresh and examined for parasites following Cribb & Bray (2010) and acanthocephalans found were fixed in 70–80% ethanol for parallel morphological and molecular analyses.

Specimens used for morphological identifications were cleared with and temporarily mounted in beechwood creosote or lactophenol and studied under an Olympus BH-2 compound microscope. Hook measurements and morphometric data for the body of each specimen examined as above were obtained with an ocular micrometer. Specimens were initially identified to genus following the keys to acanthocephalans of Australian fishes provided by Smales & Weaver (2015) and identifications were refined following the pertinent literature for each acanthocephalan lineage present in the collection. Where possible, specimens selected for molecular analyses were taken from infrapopulations in which multiple specimens had been identified morphologically, although sufficient specimens were not available to perform this for all species examined in this study. All specimens used for DNA extraction were processed as hologenophores (*sensu* Pleijel et al., 2008), with a small piece of tissue excised from the specimen for DNA extraction and the remainder of the specimen studied as above for identification and retained as a morphological and molecular voucher. Host and collection data are presented in Table 1. Hologenophores and their source infrapopulations (where available) are lodged in the Queensland Museum, Brisbane, Australia; accession numbers are listed in Table 1.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tissue samples using phenol/chloroform extraction techniques (Sambrook & Russell, 2001). Three molecular markers were targeted in this study, the small subunit ribosomal RNA (18S rDNA), the large subunit ribosomal RNA (28S rDNA) and the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*). The 18S rDNA gene region was amplified using the forward (5'-AGA TTA AGC CAT GCA TGC GTA AG-3') and reverse (5'-TGA TCC TTC TGC AGG TTC ACC TAC-3') primers of Garey et al. (1996). The 28S rDNA gene region was amplified using the forward primer LSU5 (5'-TAG GTC GAC CCG CTG AAY TTA AGC-3') (Littlewood, 1994) paired with 1200R (5'-GCA

TAG TTC ACC ATC TTT CGG-3') (Lockyer et al., 2003) or 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') (Snyder & Tkach, 2001). The *cox1* region was amplified using the forward (5'-AGT TCT AAT CAT AAR GAT ATY GG-3') and reverse (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') primers of Folmer et al. (1994).

PCR was performed with a total volume of 20 µl consisting of 2 µl DNA template (~10 ng), 5 µl of 5× MyTaq Reaction Buffer (Bioline, Alexandria, Australia), 0.75 µl of each primer (10 µM), 0.25 µl of Taq DNA polymerase (Bioline MyTaq™ DNA Polymerase), and 11.25 µl H₂O (Invitrogen™ ultraPURE™ distilled water). Amplification was carried out on a TaKaRa thermocycler Dice™ with the following profiles: (18S rDNA), an initial denaturation for 3 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 54°C, 2 min at 72°C and a 10 min extension at 72°C; (28S rDNA), an initial denaturation for 4 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 56°C, and 2 min at 72°C, 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; (*cox1*), an initial denaturation for 5 min at 94°C, followed by 35 cycles of 1 min at 94 °C, 1 min at 40–55 °C (optimised to mediate non-specific amplification) and 1 min at 72 °C, with a final extension at 72 °C for 5min. Sequence data were generated using the amplification primers, and the additional 28S rDNA internal primers 300F (5'-CAA GTA CCG TGA GGG AAA GTT-3') (Littlewood et al., 2000) and ECD2 (5'-CTT GGT CCG TGT TTC AAG ACG GG-3') (Littlewood et al., 1997) *via* cycle sequencing with an capillary sequencer and ABI Big Dye™ v.3.1 chemistry, performed by the Australian Genome Research Facility (AGRF), Brisbane. Representative sequences have been lodged on GenBank (Table 1).

Phylogenetic analyses

All analyses were performed on XSEDE (Towns et al., 2014) accessed through the CIPRES portal (Miller et al., 2010). To explore the congruence of phylogenies derived from 18S and 28S rDNA separately and to provide robust concatenated analyses, sequences from GenBank were included in the Acanthocephala-wide analyses only if matching 18S and 28S rDNA sequences were available (i.e. both sequences came from the same specimen). Preliminary analyses indicated taxonomic changes to the Rhadinorhynchidae Lühe, 1912 may be required, therefore additional phylogenetic analyses were performed for available sequences of this and related lineages alone using only 18S rDNA sequences, including taxa for which 28S rDNA sequences were not available. Both Amin et al. (2019) and Lisitsyna et al. (2019) commented on some sequences available on GenBank which have been incorrectly assigned

to the genus *Rhadinorhynchus*; we have followed these authors regarding currently accepted sequences of rhadinorhynchids. Rotifers were used for outgroup taxa for the Acanthocephala-wide analyses and a single species each of the Archiacanthocephala and Eoacanthocephala were used as outgroup taxa for the rhadinorhynchid sub-analyses. The newly generated 18S and 28S rDNA sequences were aligned with those selected from GenBank (Table 2) using MAFFT v. 7.402 (Kato & Standley, 2013), and each alignment was trimmed to the median sequence length. The resultant alignments were used to manually construct a concatenated 18S + 28S rDNA sequence alignment. Alignments were not filtered, and no sites were masked (Tan et al., 2015). Nucleotide substitution models were selected for the single-gene and concatenated alignments based on the Akaike's information criterion, Bayesian information criterion, the greedy algorithm (Lanfear et al., 2012) and PhyML (Guindon et al., 2010) as implemented in PartitionFinder v.2.1.1 (Lanfear et al., 2017). The GTR + I + G model was selected for all single-gene datasets and for both partitions of the concatenated dataset. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI) analyses. Maximum likelihood analyses were performed using RAxML (Stamatakis, 2014) with 1,000 bootstrap pseudoreplicates. Bayesian inference was performed using MrBayes v3.2.6 (Ronquist et al., 2012) with four chains being sampled every 1,000 generations for 10,000,000 generations and the first 2,500 samples being discarded as burn-in. Trees presented are those generated through Bayesian analyses, with maximum likelihood support also indicated for each node. Alignment and tree files have been uploaded to the Zenodo data repository (doi: 10.5281/zenodo.3451473).

Results

General results

We obtained sequence data for 20 host-parasite combinations and 17 acanthocephalan species. Fourteen of these acanthocephalans had not been characterised with molecular data previously. The majority of specimens examined were morphologically consistent with described species, although some could be identified to genus only because of lack of specimens for comparison. We identified 13 acanthocephalans to species, and four to genus. Combined morphological and molecular evidence for specimens of the genera *Gorgorhynchoides* Cable & Linderth, 1963 and *Sclerocollum* Schmidt & Paperna, 1978 obtained from *Pseudocaranx dentex* (Bloch & Schneider) and *Zebrasoma veliferum* (Bloch),

respectively, suggest these specimens may represent undescribed species. We also obtained the first molecular data for species of the genera *Heterosentis* Van Cleave, 1931, *Pyriproboscis* Amin, Abdullah & Mhaisen, 2003 and *Sclerocollum* Schmidt & Paperna, 1978.

Several new host records were obtained in this study. *Acanthurus olivaceus* Bloch & Schneider is a new host for *Sclerocollum robustum* (Edmonds, 1964) Schmidt & Paperna 1978; this is the first record of a species of the genus *Sclerocollum* parasitising a species of the genus *Acanthurus*. *Seriola dumerili* (Risso) and *Seriola hippos* Günther are new hosts for *Gorgorhynchoides queenslandensis* Smales, 2014 and *Auxis thazard* (Lacépède) is a new host for *Rhadinorhynchus johnstoni* Golvan, 1969. *Rhadinorhynchus johnstoni* is also recorded from Queensland waters for the first time.

Sequence data for all three targeted markers were obtained for 15 of the 17 acanthocephalan species studied. Clean sequences of *cox1* for *Neoechinorhynchus agilis* (Rudolphi, 1819) Van Cleave, 1916 were not obtained because of consistent co-amplification of an unknown bacterium and attempts to amplify the 18S rDNA region for *Neoechinorhynchus tylosuri* Yamaguti, 1939 failed.

Phylogenetic results: Acanthocephala-wide analyses

The trimmed 18S rDNA sequence alignment for the Acanthocephala-wide analyses included 85 sequences comprising three rotifer outgroup species and 78 acanthocephalan species and consisted of 2,315 total positions. Excluding gap-positions, the mean sequence length was 1669, the longest sequence was that of *Polyacanthorhynchus caballeroi* Diaz-Ungria & Gracia-Rodrigo, 1960 (AF388660) at 2,159 positions, and the shortest was that of *Filisoma caudatum* Costa Fernandes, Amin, Borges & Santos, 2019 at 442 positions. The trimmed 28S rDNA sequence alignment included the same number of sequences and taxa, but with the addition of *Neoechinorhynchus tylosuri* (MN705844), and consisted of 3,815 total positions. Excluding gap-positions, the mean sequence length was 2,090, and again, the longest and shortest sequences were those of *P. caballeroi* and *F. caudatum* at 3,378 and 361 positions, respectively.

No significant regions of alignment ambiguity were detected in the 18S rDNA sequence alignment and in general there were few gaps other than a number of indels due to many insertions in the sequence of *P. caballeroi*. Furthermore, all but three of the sequences

in the alignment were within 300 bp of the mean sequence length, and only two were shorter than 1,000 bp. In the 28S rDNA sequence alignment, a number of indels were again introduced because of insertions in the sequence of *P. caballeroi*, but in contrast to the 18S rDNA sequence alignment, there were a significant number of additional alignment gaps. The most affected region was the leading portion of the alignment, from around position 100–850. Furthermore, ~30% of the sequences in the trimmed alignment, including those generated in this study, included less than 1,000 non-gap positions and for most, these positions were found in the leading portion of the alignment. The middle to end of the alignment was relatively conserved, although again a number of the shortest sequences had gap-only positions in this region. Despite these apparent problems in the 28S rDNA sequence alignment, the trees obtained were well-supported and largely consistent with those obtained from analyses of the 18S rDNA sequence alignment (Fig. 1).

Acanthocephala-wide analyses: single-gene datasets

Maximum likelihood and Bayesian inference analyses for both single-gene datasets produced nearly congruent trees, although there were some minor differences in branching order, mostly in the 28S rDNA trees (Fig. 1). Both single-gene trees followed the higher-order topology found in previous molecular studies using rotifers as outgroup taxa (e.g. García-Varela & Nadler, 2005, 2006; Verweyen et al., 2011). In both trees, the Archiacanthocephala was sister to the remaining clades Eoacanthocephala + Polyacanthocephala and Palaeacanthocephala. The same topology was recovered for the taxa of the Archiacanthocephala in both trees. For the clade consisting of Eoacanthocephala + Polyacanthocephala, *P. caballeroi* (Polyacanthorhynchidae) was found sister to the species of the Neoechinorhynchidae in both trees, but the neoechinorhynchid clade differed between the 18S and 28S rDNA tree, likely due to the inclusion of *N. tylosuri* in the 28S rDNA dataset only. In the Bayesian 28S rDNA tree, *Neoechinorhynchus agilis* and *N. tylosuri* formed a polytomy with *Neoechinorhynchus* sp., although in the ML 28S rDNA tree *N. agilis* and *N. tylosuri* are sister to *Neoechinorhynchus* sp. + *Neoechinorhynchus saginatus* Van Cleave & Bangham, 1949 + *Floridosentis mugilis* (Machado Filho, 1951) Bullock, 1962. In both trees, the placement of *F. mugilis* results in a polyphyletic *Neoechinorhynchus* Stiles & Hassall, 1905.

The Illiosentidae is the earliest divergent clade of the Palaeacanthocephala in both single-gene trees, and, along with the Arhythmacanthidae, Cavisomidae, Echinorhynchidae, Pomphorhynchidae, Rhadinorhynchidae and Transvenidae, comprise the order Echinorhynchida. In the 18S rDNA tree, *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 was placed in the Illiosentidae with strong-support; this is consistent with previous phylogenies using 18S rDNA data (e.g. García-Varela & Nadler, 2005; Gregori et al., 2013). However, in the 28S rDNA tree *P. bulbocolli* was found in a more derived clade, sister to another putative member of the Pomphorhynchidae, *Longicollum pagrasomi* Yamaguti, 1935. In both trees, the Rhadinorhynchidae + Transvenidae is sister to the remaining taxa of the Palaeacanthocephala with strong support. However, neither the Rhadinorhynchidae nor *Rhadinorhynchus* were supported as monophyletic in either tree, with *Rhadinorhynchus biformis* Smales, 2014 basal to the Rhadinorhynchidae + Transvenidae. The genus *Sclerocollum*, previously considered a member of the Cavisomidae (see Pichelin et al., 2016), was supported as a member of the Transvenidae in our analyses, but the interrelationships of the genera *Transvena* Pichelin & Cribb, 2001, *Pararhadinorhynchus* Johnston & Edmonds, 1947 and *Sclerocollum* are not clearly resolved in either tree and differences in branching order exist between trees recovered using BI and ML for both datasets. We note that the genus *Pararhadinorhynchus* was previously considered a member of the Diplosentidae Meyer, 1932 (Amin, 2013; Pichelin et al., 2016) but now falls within the Transvenidae (see Lisitsyna et al. (2019) for discussion). The interrelationships of the remaining taxa of the Echinorhynchida were not congruent between the 18S and 28S rDNA single-gene trees. In the 18S rDNA tree, the Echinorhynchidae and Pomphorhynchidae are polyphyletic, and the Arhythmacanthidae and Cavisomidae are monophyletic, whereas in the 28S rDNA tree the Echinorhynchidae and Cavisomidae are polyphyletic and the Pomphorhynchidae and Arhythmacanthidae are monophyletic.

Plagiorhynchus cylindraceus (Goeze, 1782) Schmidt & Kuntz, 1966, the only representative of the Plagiorhynchidae in our analyses, was found sister to the remaining taxa of the order Polymorphida in both single-gene trees. In both trees, the remaining taxa of the Polymorphida formed two major sister-clades, the Centrorhynchidae + the clade containing *Gorgorhynchoides* and *Serrasentis* and a monophyletic Polymorphidae. A key difference between the 18S and 28S rDNA tree was the placement of *Pyriproboscis heronensis* (Pichelin, 1997) Amin, Abdullah & Mhaisen, 2003, previously considered a member of the Pomphorhynchidae in the order Echinorhynchida (Amin et al., 2003; Amin, 2013; Smales, 2015). In both trees, *P. heronensis* was placed in the order Polymorphida, but in the 18S

rDNA tree it is supported as sister to the Centrorhynchidae, whereas in the 28S rDNA tree it was found in polytomy with the two major clades of the Polymorphidae. *Gorgorhynchoides* and *Serrasentis* are currently classified as members of the Rhadinorhynchidae in the order Echinorhynchida (Amin, 2013; Smales, 2015). In both single-gene trees these taxa were placed in a strongly supported monophyletic clade sister to the Centrorhynchidae in the order Polymorphida. This is in agreement with previous molecular analyses (Verweyen et al., 2011; Lisitsyna et al., 2019). In both the 18S and 28S rDNA tree, *Serrasentis sagittifer* was placed among species of *Gorgorhynchoides*. Ignoring the polytomy created by *P. heronensis*, the Polymorphidae was strongly supported as monophyletic in both single-gene trees; however, a number of the constituent genera appear polyphyletic.

Acanthocephala-wide analyses: concatenated dataset

The topology of the trees resulting from maximum likelihood and Bayesian analyses of the concatenated 18S + 28S rDNA dataset (Fig. 2) were congruent, and were largely similar to those produced by the single-gene datasets, but with higher support for most nodes. The genus *Neoechinorhynchus* was still recovered as polyphyletic. The Pomphorhynchidae was paraphyletic in the concatenated tree; this is possibly an artefact of the family being found polyphyletic in the 18S and monophyletic in the 28S rDNA tree. The lack of monophyly for the genus *Filisoma* (Cavisomidae) in the concatenated tree may be the result of a similar phenomenon as the genus was recovered as monophyletic in the 18S rDNA tree, but polyphyletic in the 28S rDNA tree. The Echinorhynchidae, Rhadinorhynchidae and the genus *Rhadinorhynchus* were all again found to not be monophyletic. However, the concatenated analyses did provide some resolution for the Transvenidae, recovering the three genera represented as monophyletic. The concatenated tree also provides robust support for the clade *Gorgorhynchoides* + *Serrasentis* Van Cleave, 1923 as a member of the order Polymorphida rather than the Echinorhynchida, but again recovered *S. sagittifer* among species of *Gorgorhynchoides*. *Pyriproboscis heronensis* was placed as sister to the Centrorhynchidae, as in the 18S rDNA tree, but without support. The topology of the Polymorphidae was broadly consistent with the results from single-gene trees, but with higher support for all nodes.

Phylogenetic analyses of the genus *Rhadinorhynchus* and related taxa

The trimmed 18S rDNA sequence alignment for analyses of the genus *Rhadinorhynchus* included 22 sequences comprising a species each of the Archiacanthocephala and Eoacanthocephala as outgroup taxa, and 20 species of rhadinorhynchids or thought to be closely related taxa. The alignment consisted of 1,660 total positions. Excluding gap-positions the mean sequence length was 1,456 positions, the longest sequence was that of *Pararhadinorhynchus* sp. (HM545903) at 1,640 positions and the shortest sequence was that of *Rhadinorhynchus laterospinosus* Amin, Heckman & Ha, 2011 at 767 positions. No significant regions of alignment ambiguity were detected, and outgroup choice resulted in only a few minor indels.

The analyses for the genus *Rhadinorhynchus* and related taxa (Fig. 3) included sequences of three species not included in our Acanthocephala-wide analyses, *Rhadinorhynchus laterospinosus*, *Rhadinorhynchus pristis* (Rudolphi, 1802) and *Rhadinorhynchus* sp. (JQ061133), as matching 28S rDNA sequences are not available for those specimens. Despite the additional taxa, the trees obtained for the analyses of the Rhadinorhynchidae were consistent with the Acanthocephala-wide analyses of this study, and show that the Rhadinorhynchidae is not monophyletic. *Rhadinorhynchus biformis* was again found basal to two sister clades, one containing species of the genera *Pararhadinorhynchus*, *Sclerocollum* and *Transvena*, and the other containing species of the genera *Rhadinorhynchus* and *Gymnorhadinorhynchus* Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014. Species of the genus *Pararhadinorhynchus* were not well-differentiated from those of the genus *Sclerocollum*, but these two genera were sister to species of the genus *Transvena*. The other clade, containing all other species of the genus *Rhadinorhynchus* and those of the genus *Gymnorhadinorhynchus*, formed two subclades, with a species of *Gymnorhadinorhynchus* placed in each.

Based on the overall results of the phylogenetic analyses, we propose several taxonomic changes to the Acanthocephala.

Taxonomy

Class Palaeacanthocephala

Order Echinorhynchida

Family Rhadinorhynchidae Lühe, 1911

Syn. Gymnorhadinorhynchidae Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014 (new synonym)

Genus *Rhadinorhynchus* Lühe, 1911

Syn. *Gymnorhadinorhynchus* Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014 (new synonym)

***Rhadinorhynchus decapteri* (Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014) n. comb.**

***Rhadinorhynchus mariserpentis* (Steinauer, Garcia-Vedrenne, Weinstein & Kuris, 2019) n. comb.**

Remarks

The family Gymnorhadinorhynchidae and genus *Gymnorhadinorhynchus* were erected by Braicovich et al. (2014) based on combined morphological and molecular analyses of specimens from the carangid *Decapterus punctatus* (Cuvier), collected from off Brazil. Braicovich et al. (2014) noted that although the type-species, *Gymnorhadinorhynchus decapteri* Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014, was morphologically consistent with species of the genus *Rhadinorhynchus*, it lacked trunk spines, placing it closer to the genus *Neorhadinorhynchus* Yamaguti, 1939 (Cavisomidae), and precluding assignment to either the Rhadinorhynchidae or the Cavisomidae. Braicovich et al. (2014) presented further support for a new genus and family *via* their molecular phylogenetic analyses. They observed that *G. decapteri* was not sister to two species of the genus *Rhadinorhynchus* in their analyses (*R. pristis* and *Rhadinorhynchus lintoni* Cable & Mafarachisi, 1970), but sister to the sequence of *Rhadinorhynchus* sp. provided by García-Varela et al. (2002) which Braicovich et al. (2014) interpreted as a misidentification and possibly representative of another lineage distinct from the Rhadinorhynchidae. However, as summarised by Amin et al. (2019), the sequences of *R. pristis* and *R. lintoni* of Verweyen et al. (2011), used in the analyses of Braicovich et al. (2014), are not valid rhadinorhynchids and other analyses (Gregori et al., 2013; Amin et al., 2019; Lisitsyna et al., 2019) have demonstrated them to be closer to pomphorhynchids. Thus, only a single valid species of the

genus *Rhadinorhynchus* was included in the molecular phylogeny of Braicovich et al. (2014), and it was the sister taxon to *G. decapteri*. Inclusion of additional rhadinorhynchid taxa in subsequent phylogenies has not supported the genus *Gymnorhadinorhynchus* as distinct from *Rhadinorhynchus* (Amin et al., 2019; Lisitsyna et al., 2019). In all analyses of the present study, both species of *Gymnorhadinorhynchus* are placed within a clade containing all other species of *Rhadinorhynchus* (except for *R. biformis*; see below). In their molecular phylogeny Lisitsyna et al. (2019) found two acanthocephalan species described without trunk spines, including *G. decapteri*, clustering with spined species, and thus concluded that the presence or absence of trunk spines is not a strong family-level character. We agree, and as the molecular evidence also suggests that gymnorhadinorhynchids are not distinct from rhadinorhynchids, we propose formally recognising the family Gymnorhadinorhynchidae and the genus *Gymnorhadinorhynchus* as junior synonyms of Rhadinorhynchidae and *Rhadinorhynchus*, respectively.

Family Transvenidae Pichelin & Cribb, 2001

Amended diagnosis

Palaeacanthocephala. Echinorhynchida. Small to medium-sized worms. Trunk elongate, cylindrical to fusiform, aspinose or with single ring of spines at anterior end; sclerotised plaques may be present. Proboscis claviform to cylindrical with longitudinal rows of hooks decreasing in length from apex to base. Proboscis receptacle double-walled; associated cerebral ganglion about mid-proboscis receptacle. Lemnisci shape variable, not extending beyond anterior testis. Testes two, elongate, ovoid, in mid-body. Cement glands two (*Transvena*, *Trajectura* and *Pararhadinorhynchus*) or four (*Sclerocollum*), pyriform to tubular. Seminal vesicle and Säfteggen's pouch present. Genital pore subterminal to terminal. Eggs elongate, with polar prolongations of intermediate membranes. Intestinal parasites of marine teleosts.

Type-genus: Transvena Pichelin & Cribb, 2001.

Included genera: Pararhadinorhynchus Johnston & Edmonds, 1947; *Trajectura* Pichelin & Cribb, 2001; *Sclerocollum* Schmidt & Paperna, 1978.

Remarks

In all analyses, species of the genus *Sclerocollum* were found within the Transvenidae, rather than the Cavisomidae under which the genus is currently classified (Pichelin et al., 2016). Thus, we propose transfer of the genus *Sclerocollum* to the Transvenidae and have revised the diagnosis of the family to reflect this. The major change of note to this diagnosis is that previously all species of the Transvenidae had two cement glands, but species of the genus *Sclerocollum* have four. The number of cement glands has been proposed as useful family level character for the Echinorhynchida (Pichelin & Cribb, 2001), however the molecular results of this study suggest that the Rhadinorhynchidae and Transvenidae cannot be distinguished based on cement gland number.

Order Polymorphida

Family Isthmosacanthidae Smales, 2012

Amended diagnosis

Palaeacanthocephala: Polymorphida. Medium to large worms. Trunk more or less cylindrical with anterior field of spines and with or without anterior swelling that may or may not be spined; ventral trunk with or without spinous cuticular combs placed transversely. Proboscis elongate to claviform, armed with longitudinal rows of numerous hooks. Proboscis receptacle double-walled, inserted at base of proboscis; cerebral ganglion at about mid-region. Lemnisci long, tubular. Testes two, ovoid to elongate. Cement glands, four or six, elongate. Eggs ovoid to fusiform, with or without polar prolongations of fertilization membrane. Genital pore subterminal. Parasites of freshwater, estuarine and marine fishes.

Type-genus: Isthmosacanthus Smales, 2012.

Included genera: Gorgorhynchoides Cable & Linderoth, 1963; *Golvanorhynchus* Noronha, Fabio & Pinto, 1978; *Serrasentis* (Linton, 1889) Van Cleave, 1923.

Remarks

Given the morphological similarities of the genera *Gorgorhynchoides* and *Golvanorhynchus* to each other (Pichelin & Cribb, 2001) and to *Isthmosacanthus*, the two genera were moved to the Isthmosacanthidae when it was erected by Smales (2012). This decision was not supported by Amin (2013) because one of the defining characters was the number of cement glands. The molecular phylogenetic analyses of this study, however, clearly place the genus

Gorgorhynchoides in a sister clade to the Centrorhynchidae, together with the genus *Serrasentis*. This latter genus has previously been considered a member of the Rhadinorhynchidae. Placing *Gorgorhynchoides* and *Serrasentis* in the Polymorphida is also supported by previous molecular phylogenetic studies (García-Varela & Nadler, 2005, 2006; García-Varela & González-Oliver, 2008; Verweyen et al., 2011; Gregori et al., 2013; Lisitsyna et al., 2019). Although sequences of the genera *Isthmosacanthus* and *Golvanorhynchus* were not available to include in the analyses, the most parsimonious interpretation, using a combination of morphological and sequence data, is to return *Gorgorhynchoides* and *Golvanorhynchus* to the family Isthmosacanthidae and to remove *Serrasentis* from the Rhadinorhynchidae and place it also in the Isthmosacanthidae.

Order Polymorphida

Family Pyriproboscidae n. fam.

Diagnosis

Palaeacanthocephala: Polymorphida. Medium-sized worms. Trunk wider anteriorly, aspinose, with many small hypodermal nuclei. Proboscis pyriform with anterior series of spirally arranged rooted hooks and posterior series of longitudinal rows of rootless hooks. Neck with anterior symmetrical, spherical bulb. Proboscis receptacle double-walled, not extending beyond bulb of neck; cerebral ganglion in region of posterior third. Lemnisci slender. Testes two, pre-equatorial, contiguous; cement glands two, elongate, tubular. Seminal vesicle and Safftigen's pouch present. Genital pore sub-terminal. Eggs ellipsoid with polar prolongation of fertilisation membrane. Parasites of marine and estuarine carnivorous fishes.

Type-genus: Pyriproboscis Amin, Abdullah & Mhaisen, 2003.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new name have been submitted to ZooBank. The Life Science Identifier (LSID) for Pyriproboscidae n. fam. is urn:lsid:zoobank.org:act:66C0990A-8020-4AAE-8A96-DCEE1883140F.

Remarks

The type- and only species *Pyriproboscis heronensis* was originally placed in the genus *Pomphorhynchus* (Echinorhynchida: Pomphorhynchidae) by Pichelin (1997). Subsequently Amin et al. (2003) determined that the species did not belong in *Pomphorhynchus* or any other existing genus and erected a new genus, *Pyriproboscis*, to accommodate the species which they retained in the Pomphorhynchidae. Our molecular analyses indicate that the genus does not fall within the Pomphorhynchidae, but instead falls within the Polymorphida. Although the prominent bulb on the distal portion of the neck appears to be a convergent morphological feature between pomphorhynchids and *P. heronensis*, there are differing proportions of the proboscis receptacle between the two groups. The proboscis receptacle extends at least the full length of the neck and may extend into the trunk in pomphorhynchids but does not extend beyond the bulb in *P. heronensis*. Our 18S rDNA single-gene and the 18S + 28S rDNA concatenated dataset provided some support for a sister relationship between *P. heronensis* and species of the genus *Centrorhynchus*. Species of both *Pyriproboscis* and *Centrorhynchus* have bipartite proboscides and a proboscis armature of rooted hooks anteriorly and rootless hooks posteriorly. However, all species of the family Centrorhynchidae differ from *P. heronensis* in lacking the distinct bulb on the distal portion of the neck, in having cylindrical, rather than pyriform, probosces, and in having three to six, rather than two, cement glands. Furthermore, the definitive hosts of centrorhynchids are birds and mammals, rather than fish as for *P. heronensis*. In addition, the position of *P. heronensis* within the Polymorphida was not resolved in our 28S rDNA single-gene analyses, and in the 18S single-gene analyses the sister-relationship between *P. heronensis* and the species of the genus *Centrorhynchus* was supported by Bayesian inference alone. In the concatenated 18S + 28S analyses the sister relationship between *P. heronensis* and the species of *Centrorhynchus*, was again found, but without support. The combined morphological and molecular evidence available thus suggests that *P. heronensis* represents a distinct lineage within the Polymorphida, justifying the proposal of the Pyriproboscidae n. fam.

Discussion

Nearly every study of the Acanthocephala that has provided new molecular data for more than a few species finds evidence of paraphyletic and polyphyletic taxa (e.g. García-Varela et al., 2000; García-Varela & Nadler, 2005; Verweyen et al., 2011; Lisitsyna et al., 2019). Although misidentification of specimens used for sequencing has clearly caused some issues

in phylogenetic studies within this group (see Amin et al., 2019), accumulating evidence suggests that the morphological characters used to classify acanthocephalans are not reliable in all cases. For example, our analyses provide robust support for *Sclerocollum* as a member of the Transvenidae rather than the Cavisomidae. This invalidates one of the defining morphological characters used when the family was proposed, that all species have only two cement glands (Pichelin & Cribb, 2001); *Sclerocollum* has four. Furthermore, the absence of trunk spines does not appear to reflect phylogenetic difference between gymnorhadinorhynchids and rhadinorhynchids (Amin et al., 2019; Lisitsyna et al., 2019; present study). The species *R. biformis*, with two fields of morphologically different spines may represent a lineage basal to the Rhadinorhynchidae + Transvenidae requiring the proposal of a new family and genus. As the molecular coverage for the Acanthocephala becomes more comprehensive, it is likely that further patterns of paraphyly and polyphyly will emerge as diagnoses for some families have not always relied on shared, derived characters (García-Varela & Nadler, 2005).

Although our primary aim was to provide new molecular resources for acanthocephalans from marine fishes of eastern Australia, our assessment of the phylogenetic position for these parasites warranted some taxonomic action. The interrelationships of the Rhadinorhynchidae are incompletely resolved and here we provide some preliminary, albeit conservative, amendments to the composition of the group. Although previous studies have provided evidence that the genus *Gymnorhadinorhynchus* is not phylogenetically distinct from *Rhadinorhynchus* (Amin et al., 2019; Lisitsyna et al., 2019), none have formally called for taxonomic action; we do so here. A different scenario is the issue presented by *Rhadinorhynchus biformis*. On one hand, in all our phylogenetic analyses this species was found basal relative to two sister clades, which we recognise as the Rhadinorhynchidae and Transvenidae here. If we choose to retain both of these families as valid then a new genus and family should be proposed to accommodate *R. biformis*. On the other hand, the original description of this species was based on only three specimens in which the proboscis was not fully extended (Smales, 2014). Although Smales (2014) noted that the pattern of trunk spines had not been described for any other species of *Rhadinorhynchus*, phylogenetic data suggest the presence or absence of trunk spines is not a strong family-level character (Lisitsyna et al., 2019), hence our proposal to recognise *Gymnorhadinorhynchus* (two species without trunk spines) as a junior synonym of *Rhadinorhynchus* (all species with trunk spines). Because we do not have data for a strong morphological concept for *R. biformis*, it seems best to defer proposal of a new genus and family until more specimens can be examined.

Our analyses support a close relationship between *Gorgorhynchoides* and *Serrasentis* and the placement of these taxa in the order Polymorphida. Sequences for a species of *Gorgorhynchoides* have been available for nearly 15 years and the original study providing these data suggested that the genus did not belong to the Rhadinorhynchidae (see García-Varela & Nadler, 2005). Subsequent studies, including some incorporating sequences of *Serrasentis*, have provided similar evidence (García-Varela & González-Oliver, 2008; Verweyen et al., 2011; Gregori et al., 2013; Lisitsyna et al., 2019). Despite this, these two genera are currently classified as members of the Rhadinorhynchidae (see Amin, 2013; Smales, 2015). We feel that the weight of the accumulated evidence justifies the formal transfer of these two genera to the Polymorphida, but the most suitable family is not clear. Rather than proposing a new family we think it more conservative to use an existing family, providing a least-change option. Smales (2012) presented morphological evidence for the inclusion of *Gorgorhynchoides* and *Golvanorhynchus* with *Isthomosacanthus* in the Isthomosacanthidae. The most parsimonious decision using all available evidence is to place the Isthomosacanthidae in the Polymorphida with a revised diagnosis, while returning *Gorgorhynchoides* and *Golvanorhynchus* to, and placing *Serrasentis* in, this family.

Although only a few members of the Pomphorhynchidae were included in our analyses, *Pyriproboscis heronensis* was never found sister to any of these taxa and was not even within the Echinorhynchida, always falling within the Polymorphida. This was somewhat unexpected as both Pichelin (1997), in the original description, and Amin et al. (2003), when proposing a new genus for the species, interpreted *P. heronensis* as an unequivocal pomphorhynchid. However, *P. heronensis* was found to represent a distinct lineage within the Polymorphida in all analyses, though its relationship with other polymorphid lineages was unclear. Our 18S-only and concatenated Acanthocephala-wide analyses suggested a potential sister relationship with the Centrorhynchidae. There appears some morphological evidence for this relationship as both *Pyriproboscis heronensis* and species of *Centrorhynchus* have bipartite proboscides and a proboscis armature of rooted hooks anteriorly and rootless hooks posteriorly. Centrorhynchids, however, are primarily parasites of birds and mammals (Dimitrova & Gibson, 2005). Generation of further molecular data for additional polymorphid taxa is likely to clarify host-colonisation history and the interrelationships of the order (García-Varela et al., 2019). Despite the lack of a full understanding of the phylogenetic relationship of *P. heronensis* to polymorphids, this species has been well-characterised morphologically and combined morphological and molecular evidence justifies proposal of the Pyriproboscidae as a new family.

Most molecular phylogenetic analyses of acanthocephalan taxa have been based on 18S rDNA sequences alone (Garey et al., 1996; García-Varela et al., 2000; Herlyn et al., 2003; Verweyen et al., 2011; Gregori et al., 2013). A number of studies have included combined analyses of 18S and near complete 28S rDNA sequences (e.g. García-Varela & Nadler, 2005, 2006; García-Varela & González-Oliver, 2008; Braicovich et al., 2014; García-Varela et al., 2019) and most have included *cox1* sequence data in the analyses as well. However, a number of modern studies characterising acanthocephalan taxa (e.g. Rosas-Valdez et al., 2012; Pinacho-Pinacho et al., 2015, 2017, 2018; Presswell et al., 2018; García-Varela & Pinacho-Pinacho, 2020) have omitted generation of 18S rDNA sequences, opting instead to produce ITS and/or *cox1* barcodes and short 28S rDNA sequences. Although the alignment of 28S rDNA sequences in the present study includes far more gap-positions and short sequences than the 18S sequence alignment, the trees recovered from analyses of the 28S dataset are largely congruent with those obtained from the 18S dataset. This suggests that the phylogenetic position of taxa characterised using only 28S rDNA sequences can still be robustly determined in most cases, even when only relatively short sequences are available. Although we chose not to delete or mask gaps in our sequence alignments prior to phylogenetic analyses (see Tan et al., 2015) careful filtering or masking of sites in 28S rDNA sequence alignments in future work may improve resolution.

We generated new *cox1* sequence data for all but one of the acanthocephalan species from our collection as a molecular resource for future work, but because *cox1* sequence data are not available for all species which were included in the Acanthocephala-wide analyses we did not include these data in our concatenated analyses. Although most clades in our analyses had strong support, relationships within some genera were not fully resolved (i.e. paraphyly of *Filisoma* and placement of *Serrasentis* within *Gorgorhynchoides*). Inclusion of *cox1* sequence data in future work is likely to improve resolution within families and genera, and will be useful, especially in analyses of smaller numbers of taxa. The *cox1* gene is the most widely available barcode for acanthocephalans although we found that this gene was difficult to amplify for some species and consistent co-amplification of other microorganisms prevented us from obtaining sequences of one species. A number of recent studies have provided sequences of the internal transcribed spacers (ITS) (e.g. Li et al., 2017, 2018; Fernandes et al., 2019; García-Varela & Pinacho-Pinacho, 2020) and such data may provide a viable barcoding alternative to *cox1* for acanthocephalans for which amplification is difficult.

Continued effort generating molecular data for the Acanthocephala holds great promise for clarifying relationships, leading to the identification of new morphological

characters which can be used to evaluate taxa for which molecular data are lacking (Monks, 2001; Amin, 2013). Priority should be placed on re-collection and generation of sequences for species of lineages for which no molecular data are available (García-Varela & Nadler, 2005). Marine species are particularly underrepresented. Furthermore, molecular data are still available only for a single species for many families and genera. Sequences for additional species of these lineages are needed to reach a mature understanding of acanthocephalan relationships.

Acknowledgments We thank the staff of the Lizard Island (Australian Museum), Heron Island and Moreton Bay (University of Queensland) research stations for their ongoing support of our work. We thank Dr Scott Cutmore and the many students of the Marine Parasitology Laboratory at the University of Queensland who have assisted in the collection of specimens over the years. We thank two anonymous reviewers for their constructive criticisms which improved the content of the manuscript.

Funding This work was funded in part by grants from the Holsworth Wildlife Research Endowment, PADI foundation and Systematics Research Fund to DCH. Collection within Moreton Bay was part of a larger effort to characterise the metazoan parasite fauna of fishes from that region, funded by an ABRS grant (RF215-40) awarded to THC and Dr Scott Cutmore (University of Queensland).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

References

Amin, O. M. (2013). Classification of the Acanthocephala. *Folia Parasitologica*, 60, 273–305.

- Amin, O. M., Abdullah, S. M. A., & Mhaisen, F. T. (2003). Description of *Pomphorhynchus spindlet truncatus* n. sp. (Acanthocephala: Pomphorhynchidae) from freshwater fishes in northern Iraq, with the erection of a new pomphorhynchid genus, *Pyriproboscis* n. g., and keys to genera of the Pomphorhynchidae and the species of *Pomphorhynchus* Monticelli, 1905. *Systematic Parasitology*, 54, 229–235.
- Amin, O. M., Heckmann, R. A., Dallarés, S., Constenla, M., & Ha, N. V. (2019). Morphological and molecular description of *Rhadinorhynchus laterospinosus* Amin, Heckmann & Ha, 2011 (Acanthocephala, Rhadinorhynchidae) from marine fish off the Pacific coast of Vietnam. *Parasite*, 26, 1–14.
- Amin, O. M., Heckmann, R.A., Wilson, E., Keele, B., & Khan, A. (2015). The description of *Centrorhynchus globirostris* n. sp. (Acanthocephala: Centrorhynchidae) from the pheasant crow, *Centropus sinensis* (Stephens) in Pakistan, with gene sequence analysis and emendation of the family diagnosis. *Parasitology Research*, 114, 2291–2299.
- Bao, M., Roura, A., Mota, M., Nachón, D. J., Antunes, C., Cobo, F., et al. (2015). Macroparasites of allis shad (*Alosa alosa*) and twaite shad (*Alosa fallax*) of the Western Iberian Peninsula Rivers: ecological, phylogenetic and zoonotic insights. *Parasitology Research*, 114, 3721–3739.
- Barton, D. (1994). A checklist of helminth parasites of Australian amphibia. *Records of the South Australian Museum*, 27, 13–30.
- Barton, D. P., Smales, L., & Morgan, J. A. T. (2018). A redescription of *Serrasentis sagittifer* (Rhadinorhynchidae: Serrasentinae) from *Rachycentron canadum* (Rachycentridae) with comments on its biology and its relationship to other species of *Serrasentis*. *Journal of Parasitology*, 104, 117–133.
- Braicovich, P. E., Lanfranchi, A. L., Farber, M. D., Marvaldi, A., Luque, J. L., & Timi, J. T. (2014). Genetic and morphological evidence reveals the existence of a new family, genus and species of Echinorhynchida (Acanthocephala). *Folia Parasitologica*, 61, 377–384.
- Cribb, T. H., & Bray, R. A. (2010). Gut wash, body soak, blender and heat-fixation: approaches to the effective collection, fixation and preservation of trematodes of fishes. *Systematic Parasitology*, 76, 1–7.
- Dimitrova, Z. M., & Gibson, D. I. (2005). Some species of *Centrorhynchus* Lühe, 1911 (Acanthocephala: Centrorhynchidae) from the collection of the Natural History Museum, London. *Systematic Parasitology*, 62, 117–134.

- Edmonds, S. (1989). A list of Australian Acanthocephala and their hosts. *Records of the South Australian Museum*, 23, 127–133.
- Fernandes, V. S. C., Amin, O. M., Borges, J. N., & Santos, C. P. (2019). A new species of the acanthocephalan genus *Filisoma* (Cavisomidae) from perciform fishes in Rio de Janeiro, Brasil. *Acta Parasitologica*, 64, 176–186.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- García-Varela, M., Cummings, M. P., Pérez-Ponce de León, G., Gardner, S. L., & Laclette, J.P. (2002). Phylogenetic analysis based on 18S ribosomal RNA gene sequences supports the existence of class Polyacanthocephala (Acanthocephala). *Molecular Phylogenetics and Evolution*, 23, 288–292.
- García-Varela, M., & González-Oliver, A. (2008). The systematic position of *Leptorhynchoides* (Kostylew, 1924) and *Pseudoleptorhynchoides* (Salgado-Maldonado, 1976), inferred from nuclear and mitochondrial DNA gene sequences. *Journal of Parasitology*, 94, 959–962.
- García-Varela, M., & Nadler, S. A. (2005). Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. *Journal of Parasitology*, 91, 1401–1409.
- García-Varela, M., & Nadler, S.A. (2006). Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution*, 40, 61–72.
- García-Varela, M., Park, J.-K., Hernández-Orts, J.S., & Pinacho-Pinacho, C.D. (2019). Morphological and molecular data on a new species of *Plagiorhynchus* Lühe, 1911 (Acanthocephala: Plagiorhynchidae) from the long-billed curlew (*Numenius americanus*) from northern Mexico. *Journal of Helminthology*, in press.
- García-Varela, M., Pérez-Ponce de León, G., Aznar, F. J., & Nadler, S. A. (2009). Systematic position of *Pseudocorynosoma* and *Andracantha* (Acanthocephala, Polymorphidae) based on nuclear and mitochondrial gene sequences. *Journal of Parasitology*, 95, 178–185.
- García-Varela, M., Pérez-Ponce de León, G., Aznar, F. J., & Nadler, S.A. (2011). Erection of *Ibirhynchus* gen. nov. (Acanthocephala: Polymorphidae), based on molecular and morphological data. *Journal of Parasitology*, 97, 97–105.

- García-Varela, M., Pérez-Ponce de León, G., Aznar, F. J., & Nadler, S. A. (2013). Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution*, 68, 176–184.
- García-Varela, M., Pérez-Ponce de León, G., De La Torre, P., Cummings, M. P., Sarma, S. S., & Laclette, J. P. (2000). Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution*, 50, 532–540.
- García-Varela M., Henández-Orts J. S., Pinacho-Pinacho C. D. (2017). A morphological and molecular study of *Pseudocorynosoma* Aznar, Pérez-Ponce de León and Raga, 2006 (Acanthocephala: Polymorphidae) from Mexico with the description of a new species and the presence of cox 1 pseudogenes. *Parasitology International*, 66, 27–36.
- García-Varela, M., & Pinacho-Pinacho, C. D. (2020). Molecular characterization of *Neoechinorhynchus cylindratus* Van Cleave, 1913 (Acanthocephala: Neoechinorhynchidae), a parasite of the largemouth bass (*Micropterus salmoides*) in northern Mexico. *Journal of Helminthology*, 94, 1–9.
- Garey, J. R., Near, T. J., Nonnemacher, M. R., & Nadler, S. A. (1996). Molecular evidence for Acanthocephala as a subtaxon of Rotifera. *Journal of Molecular Evolution*, 43, 287–292.
- Garey, J. R., Schmidt-Rhaesa, A., Near, T. J., & Nadler, S. A. (1998). The evolutionary relationships of rotifers and acanthocephalans. *Hydrobiologia*, 387/388, 83–91.
- Giribet, G., Distel, D. L., Polz, M., Sterrer, W., & Wheeler, W. C. (2000). Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cycliophora, Plathelminthes, and Chaetognatha: a combined approach of 18S rDNA sequences and morphology. *Systematic Biology*, 49, 539–562.
- Gregori, M., Aznar, F. J., Abollo, E., Roura, A., González, A. F., & Pascual, S. (2013). *Nyctiphanes couchii* as intermediate host for *Rhadinorhynchus* sp. (Acanthocephala, Echinorhynchidae) from NW Iberian Peninsula waters. *Diseases of Aquatic Organisms*, 105, 9–20.
- Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59, 307–321.

- Herlyn, H., Piskurek, O., Schmitz, J., Ehlers, U., & Zischler, H. (2003). The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution*, 26, 155–164.
- Kamimura, K., Yonemitsu, K., Maeda, K., Sakaguchi, S., Setsuda, A., Varcasia, A., & Sato, H. (2018). An unexpected case of a Japanese wild boar (*Sus scrofa leucomystax*) infected with the giant thorny-headed worm (*Macracanthorhynchus hirudinaceus*) on the mainland of Japan (Honshu). *Parasitology Research*, 117, 2315–2322.
- Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.
- Keidel, L., García-Varela, M., Brener, B., Perez-Ponce de Leon, G., & Santos, C. P. (2019). Integrative taxonomy reveals a new species of *Dollfusentis* (Acanthocephala: Illiosentidae), in *Orthopristis ruber* (Osteichthyes: Haemulidae) from Rio de Janeiro, Brazil. *Parasitology International*, 71, 132–142.
- Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29, 1695–1701.
- Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34, 772–773.
- Li, L., Chen, H.-X., & Yang, Y. (2018). Morphological and molecular study of *Neorhadinorhynchus nudus* (Harada, 1938) (Acanthocephala: Cavisomidae) from *Auxis thazard* Lacépède (Perciformes: Scombridae) in the South China Sea. *Acta Parasitologica*, 63, 479–485.
- Li, L., Chen, H. X., Amin, O. M., & Yang, Y. (2017). Morphological variability and molecular characterization of *Pomphorhynchus zhoushanensis* sp. nov. (Acanthocephala: Pomphorhynchidae), with comments on the systematic status of *Pomphorhynchus* Monticelli, 1905. *Parasitology International*, 66, 693–698.
- Lisitsyna, O. I., Kudlai, O., Cribb, T. H., & Smit, N. J. (2019). Three new species of acanthocephalans (Palaeacanthocephala) from marine fishes collected off the East Coast of South Africa. *Folia Parasitologica*, 66, 012.
- Littlewood, D. T. J. (1994). Molecular phylogenetics of cupped oysters based on partial 28S rDNA sequences. *Molecular Phylogenetics and Evolution*, 3, 221–229.

- Littlewood, D. T. J., Curini-Galletti, M., & Herniou, E. A. (2000). The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution*, 16, 449–466.
- Littlewood, D. T. J., Rohde, K., & Clough, K. A. (1997). Parasite speciation within or between host species? Phylogenetic evidence from site-specific polystome monogeneans. *International Journal for Parasitology*, 27, 1289–1297.
- Lockyer, A. E., Olson, P. D., & Littlewood, D. T. J. (2003). Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society*, 78, 155–171.
- Miller, M. A., Pfeiffer, W., & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, 2010. pp 1–8.
- Monks, S. (2001). Phylogeny of the Acanthocephala based on morphological characters. *Systematic Parasitology*, 48, 81–115.
- Near, T. J. (2002). Acanthocephalan phylogeny and the evolution of parasitism. *Integrative and Comparative Biology*, 42, 668–677.
- Near, T. J., Garey, J. R., & Nadler, S. A. (1998). Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution*, 10, 287–298.
- Pichelin, S. (1997). *Pomphorhynchus heronensis* sp. nov. (Acanthocephala: Pomphorhynchidae) from *Lutjanus carponotatus* (Lutjanidae) from Heron Island, Australia. *Records of the South Australian Museum*, 30, 19–27.
- Pichelin, S., & Cribb, T. H. (2001). The status of the Diplosentidae (Acanthocephala: Palaeacanthocephala) and a new family of acanthocephalans from Australian wrasses (Pisces: Labridae). *Folia Parasitologica*, 48, 289–303.
- Pichelin, S., Smales, L. R., & Cribb, T. H. (2016). A review of the genus *Sclerocollum* Schmidt & Paperna, 1978 (Acanthocephala: Cavisomidae) from rabbitfishes (Siganidae) in the Indian and Pacific Oceans. *Systematic Parasitology*, 93, 101–114.
- Pichelin, S., Thomas, P. M., & Hutchinson, M. N. (1999). A checklist of helminth parasites of Australian reptiles. *Records of the Australian Museum Monograph Series*, 5, 1–61.
- Pinacho-Pinacho, C. D., García-Varela, M., Sereno-Urbe, A. L., & Pérez-Ponce de León, G. (2018). A hyper-diverse genus of acanthocephalans revealed by tree-based and non-tree-based species delimitation methods: Ten cryptic species of *Neoechinorhynchus* in

- Middle American freshwater fishes. *Molecular Phylogenetics and Evolution*, 127, 30–45.
- Pinacho-Pinacho, C. D., Hernández-Orts, J. S., Sereno-Uribe, A. L., Pérez-Ponce de León, G., & García-Varela, M. (2017). *Mayarhynchus karlae* n. g., n. sp. (Acanthocephala: Neoechinorhynchidae), a parasite of cichlids (Perciformes: Cichlidae) in southeastern Mexico, with comments on the paraphyly of *Neoechinorhynchus* Stiles & Hassall, 1905. *Systematic Parasitology*, 94, 351–365.
- Pinacho-Pinacho, C. D., Sereno-Uribe, A. L., Pérez-Ponce de León, G., & Garcia-Varela, M. (2015). Checklist of the species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) in fishes and turtles in Middle-America, and their delimitation based on sequences of the 28S rDNA. *Zootaxa*, 3985, 98–116.
- Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C., et al. (2008). Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution*, 48, 369–371.
- Presswell, B., García-Varela, M., & Smales, L.R. (2018). Morphological and molecular characterization of two new species of *Andracantha* (Acanthocephala: Polymorphidae) from New Zealand shags (Phalacrocoracidae) and penguins (Spheniscidae) with a key to the species. *Journal of Helminthology*, 92, 740–751.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.
- Rosas-Valdez, R., Morrone, J. J., & García-Varela, M. (2012). Molecular phylogenetics of *Floridosentis* Ward, 1953 (Acanthocephala: Neoechinorhynchidae) parasites of mullets (Osteichthyes) from Mexico, using 28S rDNA sequences. *Journal of Parasitology*, 98, 855–862.
- Sambrook, J., & Russell, D. W. (2001). *Molecular cloning: a laboratory manual*, Vol 1. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
- Smales, L. R. (2003). An annotated checklist of the Australian Acanthocephala from mammalian and bird hosts. *Records of the South Australian Museum*, 36, 59–82.
- Smales, L. R. (2012). A new acanthocephalan family, the Isthmosacanthidae (Acanthocephala: Echinorhynchida), with the description of *Isthmosacanthus fitzroyensis* n. g., n. sp. from threadfin fishes (Polynemidae) of northern Australia. *Systematic Parasitology*, 82, 105–111.

- Smales, L.R. (2014). The genus *Rhadinorhynchus* (Acanthocephala: Rhadinorhynchidae) from marine fish in Australia with the description of four new species. *Acta Parasitologica*, 59, 721–736.
- Smales, L. R. (2015). Acanthocephala. In: Schmidt-Rhaesa A. (Ed.) *Handbook of Zoology. Cycloneuralia Gastrotricha and Gnathifera. Vol 3. Gastrotricha and Gnathifera*. Berlin: De Gruyter, pp. 317–336.
- Smales, L. R., & Weaver, H. J. (2015). An annotated checklist of Acanthocephala from Australian fish. *Zootaxa*, 3985, 349–374.
- Snyder, S. D., & Tkach, V. V. (2001). Phylogenetic and biogeographical relationships among some Holarctic frog lung flukes (Digenea: Haematoloechidae). *Journal of Parasitology*, 87, 1433–1440.
- Sørensen, M. V., & Giribet, G. (2006). A modern approach to rotiferan phylogeny: combining morphological and molecular data. *Molecular Phylogenetics and Evolution*, 40, 585–608.
- Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
- Steinauer, M. L., Garcia-Vedrenne, A. E., Weinstein, S. B., & Kuris, A. M. (2019). Acanthocephalan parasites of the oarfish, *Regalecus russelii* (Regalecidae), with a description of a new species of *Gymnorhadinorhynchus* (Acanthocephala: Gymnorhadinorhynchidae). *Journal of Parasitology*, 105, 124–132.
- Steinauer, M. L., Flores, V., & Rauque, C. (2020). *Centrorhynchus nahuelhuapensis* n. sp. (Acanthocephala: Centrorhynchidae) from rufous-legged owl (*Strix rufipes* King) in Patagonia. *Journal of Helminthology*, 94, 1–7.
- Tan, G., Muffato, M., Ledergerber, C., Herrero, J., Goldman, N., Gil, M., & Dessimoz, C. (2015). Current methods for automated filtering of multiple sequence alignments frequently worsen single-gene phylogenetic inference. *Systematic Biology*, 64, 778–791.
- Towns, J., Cockerill, T., Dahan, M., Foster, I., Gaither, K., Grimshaw, A., et al. (2014). XSEDE: accelerating scientific discovery. *Computing in Science & Engineering*, 16, 62–74.
- Verweyen, L., Klimpel, S., & Palm, H. W. (2011). Molecular phylogeny of the Acanthocephala (class Palaeacanthocephala) with a paraphyletic assemblage of the orders Polymorphida and Echinorhynchida. *PloS One*, 6, e28285.

Wallace, R. L., & Colburn, R. A. (1989). Phylogenetic relationships within phylum Rotifera: orders and genus *Notholca*. *Hydrobiologia*, 186/187, 311–318.

Figure captions

Fig. 1 Majority-rule Bayesian phylogram of the 18S and 28S rDNA Acanthocephala-wide single-gene datasets. Bayesian inference (BI) posterior probabilities for nodes represented by circles, maximum likelihood (ML) bootstrap support represented by squares. Support values less than 70 not shown. An asterisk indicates different topology recovered in ML analysis. Tip labels in bold are those sequences generated in the present study

Fig. 2 Majority-rule Bayesian phylogram of the 18S + 28S rDNA Acanthocephala-wide dataset. Bayesian inference (BI) posterior probabilities for nodes represented by circles, maximum likelihood (ML) bootstrap support represented by squares. Support values less than 70 not shown. An asterisk indicates different topology recovered in ML analysis. Tip labels in bold are those sequences generated in the present study

Fig. 3 Majority-rule Bayesian phylogram of the 18S rDNA dataset for the genus *Rhadinorhynchus* and related taxa. The genus *Gymnorhadinorhynchus* is recognised as a junior synonym of *Rhadinorhynchus*. Bayesian inference (BI) posterior probabilities for nodes represented by circles, maximum likelihood (ML) bootstrap support represented by squares. Support values less than 70 not shown. An asterisk indicates different topology recovered in ML analysis. Tip labels in bold are those sequences generated in the present study

Fig. 1

Figure 2

Figure 3

Table 1. Collection data and GenBank accession numbers for acanthocephalans sequenced in this study. For geographic locality: MB = Moreton Bay, Queensland, Australia (27° 28'S, 153° 15'E); HI = Heron Island, Great Barrier Reef, Queensland, Australia (23° 27'S, 151° 55'E); LI = Lizard Island, Great Barrier Reef, Queensland, Australia (14° 40'S, 145° 27'E); SP = Square Patch, off Queensland, Australia (27° 14.6'S, 153° 36.9'E).

Taxon	Host	Geographic locality	GenBank Accession Numbers			Queensland Museum Accession Numbers	
			<i>cox1</i>	18S	28S	Hologenophore(s)	Source population voucher(s)
Eoacanthocephala							
Neoechinorhynchida							
Neoechinorhynchidae Ward, 1917							
<i>Neoechinorhynchus agilis</i> (Rudolphi, 1819)	<i>Mugil cephalus</i> Linnaeus	MB		MN705824	MN705843	G238251–53	G238254–56
<i>Neoechinorhynchus tylosuri</i> Yamaguti, 1939	<i>Tylosurus gavialoides</i> (Castelnau)	MB	MN692675		MN705844	G238257–59	G238260–63
Palaeacanthocephala							
Echinorhynchida							
Arhythmacanthidae Yamaguti, 1935							
<i>Heterosentis</i> sp.	<i>Acanthopagrus australis</i> (Günther)	MB	MN692676–7	MN705825	MN705845	G238264–65	G238266
Cavisomidae Meyer, 1932							
<i>Filisoma filiforme</i> Weaver & Smales, 2013	<i>Kyphosus bigibbus</i> Lacepède	MB	MN692678–9	MN705826	MN705846	G238267–69	G238270
Rhadinorhynchidae Lühe, 1912							
<i>Rhadinorhynchus johnstoni</i> Golvan, 1969	<i>Auxis thazard</i> (Lacepède)	MB	MN692680	MN705827	MN705847	G238271	
<i>Rhadinorhynchus</i> sp.	<i>Auxis thazard</i> (Lacepède)	MB	MN692681	MN705828	MN705848	G238272	
<i>Rhadinorhynchus biformis</i> Smales, 2014	<i>Helotes sexlineatus</i> (Quoy & Gaimard)	MB	MN692682–3	MN705829	MN705849	G238273–75	
<i>Rhadinorhynchus carangis</i> Yamaguti, 1939	<i>Trachinotus coppingeri</i> Günther	HI	MN692684	MN705830	MN705850	G238276–77	G238278
Transvenidae Pichelin & Cribb, 2001							
<i>Sclerocollum australe</i> Pichelin, Smales & Cribb, 2016	<i>Siganus argenteus</i> (Quoy & Gaimard)	LI	MN692685–6	MN705831	MN705851	G238279–80	G238281–83
<i>Sclerocollum robustum</i> (Edmonds, 1964) Schmidt & Paperna, 1978	<i>Siganus lineatus</i> (Valenciennes)	LI	MN692687	MN705832	MN705852	G238284–86	G238289–92
	<i>Acanthurus olivaceus</i> Bloch & Schneider	LI	MN692688	MN705833	MN705853	G238287–88	G238293
<i>Sclerocollum</i> sp.	<i>Zebrasoma veliferum</i> (Bloch)	LI	MN692689	MN705834	MN705854	G238294–95	G238296
<i>Transvena annulospinosa</i> Pichelin & Cribb, 2001	<i>Thalassoma lunare</i> (Linnaeus)	HI	MN692690	MN705835	MN705855	G238297–98	

Polymorphida

Isthomosacanthidae Smales, 2012

<i>Gorgorhynchoides queenslandensis</i> Smales, 2014	<i>Seriola dumerili</i> (Risso)	SP	MN692691–2	MN705836	MN705856	G238299–301	G238302
	<i>Seriola hippos</i> Günther	SP	MN692693–4	MN705837	MN705857	G238303–04	G238305–07
	<i>Seriola lalandi</i> Valenciennes	SP	MN692695–6	MN705838	MN705858	G238308–10	G238311
<i>Gorgorhynchoides gnathanodontos</i> Smales, 2014	<i>Gnathanodon speciosus</i> (Forsskål)	LI	MN692697	MN705839	MN705859	G238312–14	G238315–16
<i>Gorgorhynchoides</i> sp.	<i>Pseudocaranx dentex</i> (Bloch & Schneider)	MB	MN692698–9	MN705840	MN705860	G238317–19	G238320
<i>Serrasentis sagittifer</i> (Linton, 1889) Linton, 1932	<i>Rachycentron canadum</i> (Linnaeus)	SP	MN692700–02	MN705841	MN705861	G238321–23	G238324
Pyriproboscidae n. fam.							
<i>Pyriproboscis heronensis</i> (Pichelin, 1997) Amin, Abdullah & Mhaisen, 2003	<i>Lutjanus carponotatus</i> (Richardson)	HI	MN692703–04	MN705842	MN705862	G238325–27	G238328

Table 2. GenBank accession numbers for sequence data used in phylogenetic analyses, including host and original reference.

Taxon	Host	GenBank ID (18S)	GenBank ID (28S)	Reference
Archiacanthocephala				
Gigantorhynchida				
Gigantorhynchidae Hamann, 1892				
<i>Mediorhynchus</i> sp.	<i>Cassidix mexicanus</i>	AF064816	AY829087	García-Varela et al. (2000); García-Varela & Nadler (2005)
Oligacanthorhynchidae Southwell & Macfie, 1925				
<i>Macracanthorhynchus hirudinaceus</i> (Pallas, 1781)	<i>Sus scrofa leucomystax</i>	LC350000	LC350000	Kamimura et al. (2018)
<i>Macracanthorhynchus ingens</i> (von Linstow, 1897)	<i>Procyon lotor</i>	AF001844	AY829088	Near et al. (1998); García-Varela & Nadler (2005)
<i>Oligacanthorhynchus tortuosa</i> (Leidy, 1850)	<i>Didelphis virginiana</i>	AF064817	AY829090	García-Varela et al. (2000); García-Varela & Nadler (2005)
<i>Oncicola</i> sp.	<i>Nasua narica</i>	AF064818	AY829089	García-Varela et al. (2000); García-Varela & Nadler (2005)
Eoacanthocephala				
Neoechinorhynchida				
Neoechinorhynchidae Ward, 1917				
<i>Floridosentis mugilis</i> (Machado Filho, 1951)	<i>Mugil cephalus</i>	AF064811	AY829111	García-Varela et al. (2000); García-Varela & Nadler (2005)
<i>Neoechinorhynchus</i> sp.	<i>Siganus fuscescens</i>	HM545898	HM545899	Wang et al. unpublished
<i>Neoechinorhynchus saginatus</i> Van Cleave & Bangham, 1949	unknown	AY830150	AY829091	García-Varela & Nadler, 2005
Palaeacanthocephala				
Echinorhynchida				
Arhythmacanthidae Yamaguti, 1935				
<i>Acanthocephaloides propinquus</i> (Dujardin, 1845)	<i>Gobius bucchichii</i>	AY830149	AY829100	García-Varela & Nadler, 2005
Cavisomidae Meyer, 1932				
<i>Filisoma bucerium</i> Van Cleave, 1940	<i>Kyphosus elegans</i>	AF064814	AY829110	García-Varela et al. (2000); García-Varela & Nadler (2005)
<i>Filisoma caudatum</i> Costa Fernandes, Amin, Borges & Santos, 2019	<i>Kyphosus incisor</i>	MH004443	MH004455	Fernandes et al. (2019)
Echinorhynchida				
Echinorhynchidae Cobbold, 1876				
<i>Acanthocephalus dirus</i> (Van Cleave, 1924)	<i>Asellus aquaticus</i>	AY830151	AY829106	García-Varela & Nadler, 2005

<i>Acanthocephalus lucii</i> (Müller, 1777)	<i>Perca fluviatilis</i>	AY830152	AY829101	García-Varela & Nadler, 2005
<i>Echinorhynchus truttae</i> Schrank, 1788	<i>Thymallus thymallus</i>	AY830156	AY829097	García-Varela & Nadler, 2005
Illiosentidae Golvan, 1960				
<i>Dollfusentis bravoae</i> Salgado-Maldonado, 1976	<i>Eugerres plumieri</i>	MK282759	MK282754	Keidel et al. (2019)
<i>Illoesentis</i> sp.	unknown	AY830158	AY829092	García-Varela & Nadler, 2005
<i>Koronacantha mexicana</i> Monks & Ponce de Leon, 1996	<i>Pomadasys leuciscus</i>	AY830157	AY829095	García-Varela & Nadler, 2005
<i>Koronacantha pectinaria</i> (Van Cleave, 1940) Monks & Pérez-Ponce de León, 1996	<i>Microlepidotus brevipinnis</i>	AF092433	AY829094	García-Varela et al. (2000); García-Varela & Nadler (2005)
<i>Leptorhynchoides thecatus</i> (Linton, 1891) Kostylev, 1924	<i>Lepomis cyanallus</i>	AF001840	AY829093	Near et al. (1998); García-Varela & Nadler (2005)
<i>Pseudoleptorhynchoides lamothei</i> Salgado-Maldonado, 1976	<i>Ariopsis guatemalensis</i>	EU090950	EU090951	García-Varela & González-Oliver (2008)
Pomphorhynchidae Yamaguti, 1939				
<i>Longicollum pagrosomi</i> Yamaguti, 1935	<i>Pagrus major</i>	LC195887	LC195888	Mekata et al. unpublished
<i>Pomphorhynchus bulbocolli</i> Linkins in Van Cleave, 1919	unknown	AF001841	AY829096	Near et al. (1998); García-Varela & Nadler (2005)
Rhadinorhynchidae Lühe, 1912				
<i>Rhadinorhynchus decapteri</i> (Braicovich, Lanfranchi, Farber, Marvaldi, Luque & Timi, 2014) n. comb.	<i>Decapterus punctatus</i>	KJ590123	KJ590124	Braicovich et al. (2014)
<i>Rhadinorhynchus gerberi</i> Lisitsyna, Kudlai, Cribb & Smit, 2019	<i>Trachinotus botla</i>	MN105739	MN105745	Lisitsyna et al. (2019)
<i>Rhadinorhynchus laterospinosus</i> Amin, Heckman & Ha, 2011	<i>Auxis rochei</i>	MK457183		Amin et al. (2019)
<i>Rhadinorhynchus mariserpentis</i> (Steinauer, Garcia-Vedrenne, Weinstein & Kuris, 2019) n. comb.	<i>Regalecus russelii</i>	MK014866	MK014867	Steinauer et al. (2020)
<i>Rhadinorhynchus pristis</i> (Rudolphi, 1802)	<i>Alosa alosa</i>	KR349116		
<i>Rhadinorhynchus</i> sp.	unknown sp. of Scianidae	AY062433	AY829099	García-Varela et al. (2002, 2005)
<i>Rhadinorhynchus</i> sp.	<i>Nyctiphanes couchii</i>	JQ061133		Gregori et al. (2013)
Transvenidae Pichelin & Cribb, 2001				
<i>Pararhadinorhynchus sodwanensis</i> Lisitsyna, Kudlai, Cribb & Smit, 2019	<i>Pomadasys furcatus</i>	MN105738	MN105744	Lisitsyna et al. (2019)
<i>Pararhadinorhynchus</i> sp.	<i>Siganus fuscescens</i>	HM545903	HM545904	Wang et al. unpublished
<i>Transvena annulospinosa</i> Pichelin & Cribb, 2001	<i>Anampses neoguinaicus</i>	AY830153	AY829098	García-Varela & Nadler, 2005

<i>Transvena pichelinae</i> Lisitsyna, Kudlai, Cribb & Smit, 2019	<i>Thalassoma purpureum</i>	MN105737	MN105743	Lisitsyna et al. (2019)
Polymorphida				
Centrorhynchidae Van Cleave, 1916 (Golvan 1960)				
<i>Centrorhynchus globirostris</i> Amin, Heckmann, Wilson, Keele & Khan, 2015	<i>Centropus sinensis</i>	KM588206	KM588207	Amin et al. (2015)
<i>Centrorhynchus nahuelhuapensis</i> Steinauer, Flores & Rauque, 2019	<i>Strix rufipes</i>	MK411249	MK411250	Steinauer et al. (2019)
<i>Centrorhynchus</i> sp.	<i>Falco peregrinus</i>	AY830155	AY829104	García-Varela & Nadler, 2005
Isthmosacanthidae Smales, 2012				
<i>Gorgorhynchoides bullocki</i> Cable & Mafarachisi, 1970	<i>Eugerres plumiere</i>	AY830154	AY829103	García-Varela & Nadler, 2005
<i>Serrasentis sagittifer</i> (Linton, 1889) Linton, 1932	<i>Lethrinus laticaudis</i>	MF426933	MF426926	Barton et al. (2018)
Plagiorhynchidae Golvan, 1960				
<i>Plagiorhynchus cylindraceus</i> (Goeze, 1782)	<i>Armadillidium vulgare</i>	AF001839	AY829102	Near et al. (1998); García-Varela & Nadler (2005)
Polymorphidae Meyer, 1931				
<i>Andracantha gravis</i> (Alegret, 1941) Schmidt, 1975	<i>Phalacrocorax auritus</i>	EU267802	EU267814	García-Varela et al. (2009)
<i>Arhythmorhynchus brevis</i> (Van Cleave, 1916)	<i>Botaurus lentiginosus</i>	JX442171	JX442183	García-Varela et al. (2013)
<i>Arhythmorhynchus brevis</i> (Van Cleave, 1916)	<i>Nycticorax nycticorax</i>	AF064812	AY829105	García-Varela et al. (2000); García-Varela & Nadler (2005)
<i>Arhythmorhynchus frassoni</i> (Molin, 1858)	<i>Uca spinicarpa</i>	JX442164	JX442176	García-Varela et al. (2013)
<i>Arhythmorhynchus frassoni</i> (Molin, 1858)	<i>Eudocimus albus</i>	JX442165	JX442177	García-Varela et al. (2013)
<i>Bolbosoma turbinella</i> (Diesing, 1851)	<i>Eschrichtius robustus</i>	JX442166	JX442178	García-Varela et al. (2013)
<i>Bolbosoma</i> sp.	<i>Callorhinus ursinus</i>	JX442167	JX442179	García-Varela et al. (2013)
<i>Corynosoma australe</i> Johnston, 1937	<i>Phocarcos hookeri</i>	JX442168	JX442180	García-Varela et al. (2013)
<i>Corynosoma enhydri</i> Morozov, 1940	<i>Enhydra lutris</i>	AF001837	AY829107	Near et al. (1998); García-Varela & Nadler (2005)
<i>Corynosoma magdaleni</i> Montreuil, 1958	<i>Phoca hispida saimensis</i>	EU267803	EU267815	García-Varela et al. (2009)
<i>Corynosoma obtusens</i> Lincicome, 1943	<i>Callorhinus ursinus</i>	JX442169	JX442181	García-Varela et al. (2013)
<i>Corynosoma strumosum</i> (Rudolphi, 1802) Lühe, 1904	<i>Phoca vitulina</i>	EU267804	EU267816	García-Varela et al. (2009)

<i>Corynosoma validum</i> Van Cleave, 1953	<i>Callorhinus</i>	JX442170	JX442182	García-Varela et al. (2013)
<i>Hexaglandula corynosoma</i> (Travassos, 1915)	<i>ursinus</i>			
	<i>Nyctanassa</i>	EU267808	EU267817	García-Varela et al. (2009)
	<i>violacea</i>			
<i>Ibirhynchus dimorpha</i> (Schmidt, 1973) García-Varela, Pérez-Ponce de León, Aznar & Nadler, 2011	<i>Eudocimus albus</i>	GQ981436	GQ981437	García-Varela et al. (2011)
<i>Polymorphus obtusus</i> Van Cleave, 1918	<i>Ahythya affinis</i>	JX442172	JX442184	García-Varela et al. (2013)
<i>Polymorphus minutus</i> (Zeder, 1800) Lühe, 1911	<i>Gammarus pulex</i>	EU267806	EU267819	García-Varela et al. (2009)
<i>Polymorphus trochus</i> Van Cleave, 1945	<i>Fulica americana</i>	JX442173	JX442185	García-Varela et al. (2013)
<i>Profilicollis altmani</i> (Perry, 1942) Van Cleave, 1947	<i>Enhydra lutris</i>	AF001838	AY829108	Near et al. (1998); García-Varela & Nadler (2005)
				García-Varela et al. (2013)
<i>Profilicollis bullocki</i> (Mateo, 1982)	<i>Emerita analoga</i>	JX442174	JX442186	García-Varela et al. (2013)
<i>Profilicollis botulus</i> (Van Cleave, 1916)	<i>Somateria</i>	EU267805	EU267818	García-Varela et al. (2009)
	<i>mollissima</i>			
<i>Profilicollis botulus</i> (Van Cleave, 1916)	<i>Anas</i>	AF064815	AY829109	García-Varela et al. (2000); García-Varela & Nadler (2005)
	<i>platyrhynchos</i>			
<i>Pseudocorynosoma anatarium</i> (Van Cleave, 1945) Aznar, Pérez-Ponce de León & Raga, 2006	<i>Bucephala albeola</i>	EU267801	EU267813	García-Varela et al. (2009)
<i>Pseudocorynosoma constrictum</i> (Van Cleave, 1918) Aznar, Pérez-Ponce de León & Raga, 2006	<i>Anas clypeata</i>	EU267800	EU267812	García-Varela et al. (2009)
<i>Pseudocorynosoma tepehuanesi</i> García-Varela, Henández-Orts & Pinacho-Pinacho, 2017	<i>Oxyura</i>	JX442175	JX442187	García-Varela et al. (2013); García-Varela et al. (2017)
<i>Southwellina hispida</i> (Van Cleave, 1925) Witenberg, 1932	<i>jamaicensis</i>			
	<i>Tigrisoma</i>	EU267807	EU267811	García-Varela et al. (2009)
	<i>mexicanum</i>			
Polyacanthocephala				
Polyacanthorhynchida				
Polyacanthorhynchidae Golvan, 1956				
<i>Polyacanthorhynchus caballeroi</i> Diaz-Ungria and Gracia-Rodrigo, 1960	<i>Caiman yacare</i>	AF388660	DQ089738	García-Varela et al. (2002); García-Varela & Nadler (2006)
Rotifera (Outgroup)				
<i>Brachionus urceolaris</i> Müller, 1773	free-living	DQ089734	DQ089740	García-Varela & Nadler, 2006
<i>Rotaria rotatoria</i> (Pallas, 1766)	free-living	DQ089736	DQ089743	García-Varela & Nadler, 2006
<i>Seison nebaliae</i> Grube, 1861	<i>Nebaliae bipes</i>	DQ089737	DQ089744	García-Varela & Nadler, 2006