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An identity crisis in the Indo-Pacific: molecular exploration of the genus *Koseiria* (Digenea: Enenteridae)

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ABSTRACT

We explore the growing issue of cryptic speciation in the Digenea through study of museum material and newly collected specimens consistent with the enenterid genus *Koseiria* from five species of the Kyphosidae and *Chaetodontoplus meredithi* Kuiter (Pomacanthidae) collected in the Indo-Pacific. We use an integrated approach, employing traditional morphometrics, principal components analysis (PCA), and molecular data (ITS2 and 28S rDNA). Our results support recombination of *Koseiria allanwilliamsi* Bray & Cribb, 2002 as *Proenenterum allanwilliamsi* (Bray & Cribb, 2002) n. comb. and transfer of *Koseiria huxleyi* Bray & Cribb, 2001 to a new genus as *Enenterageitus huxleyi* (Bray & Cribb, 2002) n. comb. Molecular data indicate the presence of four further species consistent with *Koseiria*, one from Western Australia (sequence data only) and three from eastern Australia. All three eastern Australian species are morphologically consistent with *Koseiria xishaensis* Gu & Shen, 1983, but distinct from all other previously described species. Although *K. xishaensis* has been reported from Australia, we conclude that the similarity of the present forms to the original description of *K. xishaensis* means records of this species from Japan, Palau and Australia are unreliable. Because the eastern Australian forms cannot be reliably ascribed to *K. xishaensis*, we describe *Koseiria argalea* n. sp., *Koseiria laiphopharophora* n. sp., and *Koseiria pyknophora* n. sp., following application of PCAs and iterative refinement of species concepts and type series. These analyses did not allow convincing identification hypotheses for all specimens examined. In this genus, both morphological and molecular data, together with reliable host identifications, are essential for species recognition, and thus we refrain from attempting to name samples lacking molecular data. The issues presented by these taxa encapsulate those of trematodes in the region as a whole. Many records require dramatically improved supporting data, leading to substantial uncertainly in the identification of this fauna.

1. Introduction

The adoption of molecular methods for species identification has led to the discovery of biodiversity at levels far greater than had been presumed on the basis of morphology alone (e.g. Bickford et al., 2007; Pfenninger and Schwenk, 2007; Poulin, 2011). Cryptic species are detected routinely among parasitic helminths, especially amongst the digenetic trematodes, likely because species of most digenean lineages lack hard or sclerotised structures which facilitate species-specific diagnoses (Poulin, 2011; Pérez-Ponce de León and Poulin, 2018). Once detected via molecular means, many putatively cryptic species can be distinguished morphologically after careful re-evaluation (Pérez-Ponce de León and Nadler, 2010; Nadler and Pérez-Ponce de León, 2011; Bray and Cribb, 2015), thus facilitating description and naming. In situations where multiple species are masked under a single name, such a posteriori delineation should be relatively straightforward if original descriptions are highly detailed, new specimens are available from the type locality, or name-bearing types are available and of sufficient quality for comparative study. In practice, however, discovering a cryptic complex hidden under a single name presents significant challenges. Many descriptions are generalised or of poor quality, and lack the detail necessary for distinguishing between morphologically similar species. Morphological comparison between newly collected material and name-bearing types is often futile; type specimens may be poorly preserved, become lost or are otherwise unavailable. Re-collection from type hosts and type localities is critical, but this is frequently logistically difficult or impractical. The challenges associated with delineation of cryptic species complexes in such less than ideal scenarios have only been cursorily explored.

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The Enenteridae Yamaguti, 1958 is a small family of the Lepocreadioidea Odhner, 1905 (Bray et al., 2009; Bray and Cribb, 2012), presently comprising five genera and 23 species according to the World Register of Marine Species (http://www.marinespecies.org/aphia.php?p=taxdetails&id=393684 accessed on 2019-09-23). Enenterids are known mostly from the intestines of herbivorous marine teleosts in the Indo-West Pacific marine region (IWP), and overwhelmingly from species of the family Kyphosidae (Bray and Cribb, 2001, 2002; Bray, 2005; Bray et al., 2016; Cribb et al., 2016). The phylogenetic placement of the Enenteridae, and the interrelationships of its constituent genera, were long uncertain (Bray, 1978; Brooks et al., 2000; Bray and Cribb, 2001; Bray, 2005). This was due, in part, to the few morphological synapomorphies exhibited by enenterid species. Beyond most species having a spiny tegument (which is often difficult to observe) and being associated with kyphosid fishes, the major identifying feature of the group is specialisation of the posterior caeca, forming a cyclocoel with or without an anus (Bray and Cribb, 2001, 2002; Bray, 2005; Bray and Cribb, 2012), but these conditions are not restricted to the Enenteridae.

Molecular phylogenetic analyses have demonstrated the Enenteridae as a strongly supported monophyletic group, sister to the Gyliauchenidae Fukui, 1929 (Cribb et al., 2001; Bray et al., 2009, 2018). It has recently been demonstrated, however, that species of *Cadenatella* Dollfus, 1946, which had long been considered members of the Enenteridae, are actually haploporoids (Bray et al., 2014). The original confusion was caused by apparent convergent evolution, as species of both *Cadenatella* and *Enenterum* Linton, 1910 are found in fishes of the genus *Kyphosus* Lacepède, and have complex, ornamented oral suckers. This confusion highlights problems brought about by using only morphological characters to consider evolutionary history.

Despite the reported richness of the Enenteridae in the IWP (Cribb et al., 2016), the family has yet to be well characterised with molecular data. The position of *Koseiria* Nagaty, 1942 within the Enenteridae is supported by molecular and morphological data and, with their unspecialised oral suckers, cyclocoel with dorso-subterminal anus, and concentration in species of *Kyphosus*, species of *Koseiria* are thought to represent the pleisiomorphic condition for the family (Bray et al., 2009). Thus, systematic evaluation of species of *Koseiria* is a logical first step towards improved understanding of the evolutionary history and host relationships of the Enenteridae. However, two species of *Koseiria* are anomalously known from non-kyphosid fishes. *Koseiria huxleyi* Bray & Cribb, 2001, is found in the pomacanthid *Chaetodontoplus meredithi* Kuiter, and *Koseiria manteri* Ahmad, 1987 is found in the halfbeak *Rhynchorhamphus georgii* (Valenciennes) (Hemiramphidae). The other four species are known only from species of *Kyphosus*. *Koseiria xishaensis* Gu & Shen, 1983 has been reported from three species of *Kyphosus* across a wide geographic range: the Xisha Islands in the South China Sea (Gu and Shen, 1983), Japan (Machida, 1993), the Great Barrier Reef, Australia (Bray and Cribb, 2001) and Palau (Bray and Cribb, 2002), but with no molecular data from any collection locality other than Australia. It is also difficult to have complete confidence that the kyphosids from these various reports were correctly identified. Most species of *Kyphosus* form mixed schools, have similar colouration and overlapping meristic characteristics, and thus can be exceptionally difficult to identify to species (Knudsen and Clements, 2013a,b, 2016). In addition, new species of *Kyphosus* have been recognised as recently as 2013 (Knudsen and Clements, 2013a). This creates doubt in our understanding of host specificity for the Enenteridae, as it is possible that some reported hosts have been misidentified.

Here, we evaluate a number of putative species of *Koseiria* collected in Australian, Japanese and Micronesian waters with morphological data, evaluate Australian material with molecular data, and produce an updated molecular phylogeny for the Enenteridae. Our findings have general relevance to our capacity to be confident in the identification of Indo-Pacific fish trematodes.

2. Materials and methods

2.1. Specimen collection

Between 1994 and 2018 we collected 116 individuals of seven species of *Kyphosus* from multiple localities across the Indo-West Pacific. Most were collected in Australian waters (Great Barrier Reef and Moreton Bay, Queensland; Yorke Peninsula, South Australia; Ningaloo and off Perth, Western Australia), but also from French Polynesia, Japan, Palau and South Africa. For specific information regarding this collection, including collection numbers, localities and identification of fish specimens, readers are referred to the previous parasitological studies of this collection (Huston et al., 2019; Martin et al., 2019). Additionally, 10 individuals of the Queensland angelfish, *Chaetodontoplus meredithi*, were collected from Heron Island, Great Barrier Reef, between 2014 and 2018.

Fishes were collected mainly by spear, although some were caught on line or purchased from local fishermen or fish markets. The gut of each fish was excised and examined for trematodes following the recommendations of Cribb and Bray (2010). rematodes were collected alive, and fixed without pressure in near-boiling saline. Most of the material collected before 2015 was preserved in 10% formalin, with some molecular vouchers being preserved in 70–100% ethanol. Material collected between 2015 and 2018 was preserved in 70–80% ethanol for subsequent parallel morphological and molecular analyses. Molecular data were generated for fishes collected between 2015 and 2018 to confirm identifications.

2.2. Morphological analyses

Newly collected trematode specimens used for morphological examination were removed from their preservative, washed in fresh water, overstained in Mayer's haematoxylin, destained in a solution of 1.0% hydrochloric acid and neutralised in a 0.5% ammonium hydroxide solution. Specimens were then dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted on slides in Canada balsam. In addition, vouchers of *Koseiria* spp. lodged in the Queensland Museum, Brisbane, Australia (QM), British Museum of Natural History, London, United Kingdom (BMNH), and National Science Museum, Tokyo, Japan (NSMT), from the studies of Bray and Cribb (2001, 2002) and Machida (1993) were re-examined. Measurements of 40 features (Table 1) were made with cellSens standard imaging software paired with an Olympus SC50 digital camera. Measurements are reported in micrometers (μm).

Morphometric data were log transformed and explored with principal components analyses (PCAs) of the covariance matrix in R (https://www.R-project.org) and visualised with the package ggfortify (Tang et al., 2016). PCA followed a holistic iterative approach, and was used to help refine specimen identification and subsequent taxonomic hypotheses. We note that PCA cannot accommodate missing values, therefore PCA was also performed with a reduced dataset designed to include some hologenophores. Features which were included in PCA are listed in Table 1.

Drawings of holotypes, vouchers and other features of interest were made with a camera lucida, mounted on an Olympus BX-53 compound microscope. Drawings were digitised in Adobe Illustrator. Vouchers are lodged in the Western Australian Museum

Table 1
Morphometric data for three new species of *Koseiria* expressed as a range and mean in micrometres or as percentages. A dash (-) indicates distance between two features.

Feature	*Koseiria argalea* n. sp. (*n* = 10) min–max (mean)	*Koseiria laiphopharophora* n. sp. (*n* = 22) min–max (mean)	*Koseiria pyknophora* n. sp. (*n* = 21) min–max (mean)
BL[a]	4668–6159 (5494)	3891–7647 (5929)	2779–5973 (4039)
BW[ab]	749–928 (842)	446–1253 (958)	499–1103 (768)
BL/BW	6.07–6.78 (6.4)	5.07–6.76 (5.96)	4.74–6.08 (5.28)
BW % BL	0.15–0.17 (0.16)	0.15–0.19 (0.17)	0.16–0.21 (0.19)
FL[a]	1135–1537 (1328)	1046–1941 (1498)	781–1485 (1009)
FL % BL	0.21–0.27 (0.25)	0.24–0.27 (0.25)	0.22–0.31 (0.26)
OSL[ab]	364–448 (401)	345–636 (533)	252–444 (321)
OSW[ab]	313–496 (429)	414–717 (587)	296–495 (355)
OSL/OSW	0.86–1.19 (0.95)	0.83–1.07 (0.91)	0.77–1.00 (0.91)
VSL[ab]	244–327 (285)	200–391 (310)	202–322 (257)
VSW[ab]	280–329 (293)	199–401 (324)	198–331 (261)
VSL/VSW	0.86–1.05 (0.97)	0.85–1.07 (0.96)	0.89–1.08 (0.99)
VSW/OSW	0.62–0.93 (0.69)	0.52–0.61 (0.56)	0.62–0.85 (0.73)
VSL/OSL	0.62–0.78 (0.71)	0.54–0.66 (0.59)	0.66–0.92 (0.79)
PPhL	51–176 (120)	up to 65 (9.67)	61–194 (108)
PhL[ab]	276–362 (321)	282–507 (398)	210–330 (262)
PhW[ab]	300–346 (319)	233–427 (361)	210–358 (263)
PhL % BL	0.04–0.06 (0.05)	0.06–0.08 (0.07)	0.05–0.08 (0.06)
PhL/PhW	0.88–1.10 (1.00)	0.97–1.23 (1.10)	0.92–1.11 (0.99)
PhW/OSW	0.69–1.06 (0.76)	0.57–0.69 (0.62)	0.67–0.79 (0.74)
OesL	39–74 (54)	up to 87 (23)	up to 19 (1.0)
CSL[ab]	439–640 (550)	300–827 (591)	289–514 (423)
CSW[ab]	349–491 (403)	181–594 (443)	240–581 (397)
CSL/CSW	1.17–1.70 (1.37)	1.14–1.77 (1.35)	0.88–1.22 (1.07)
CSL % BL	0.08–0.13 (0.10)	0.09–0.11 (0.10)	0.09–0.13 (0.11)
IBD[ab]	78–177 (129)	51–217 (136)	58–181 (109)
IB-VS[a]	122–330 (244)	178–528 (334)	90–395 (202)
IB-VS % BL	0.02–0.06 (0.05)	0.04–0.07 (0.06)	0.03–0.07 (0.05)
Vit-VS[ab]	206–474 (379)	127–539 (358)	169–619 (335)
Vit-VS % BL	0.03–0.08 (0.06)	0.05–0.08 (0.06)	0.06–0.10 (0.08)
OS-IB[ab]	242–714 (582)	423–738 (564)	361–625 (463)
OS-IB % BL	0.05–0.13 (0.11)	0.09–0.11 (0.1)	0.09–0.15 (0.11)
CaeL[a]	3648–5012 (4402)	2428–6422 (4621)	2043–5058 (3216)
CaeW	78–268 (182)	77–351 (163)	56–203 (110)
CaeL/CaeW	18.4–46.8 (27.3)	16.6–44.7 (29.5)	20.0–47.7 (31.1)
CaeL % BL	0.78–0.81 (0.80)	0.76–0.84 (0.79)	0.74–0.85 (0.79)
ATL[ab]	528–855 (743)	391–908 (689)	381–922 (646)
ATW[ab]	387–642 (471)	214–539 (449)	326–650 (489)
AT-PT[ab]	79–250 (182)	19–386 (176)	16–134 (60)
AT-PT % BL	0.02–0.04 (0.03)	0.004–0.06 (0.03)	0.005–0.03 (0.01)
PTL[a]	690–910 (824)	408–976 (760)	462–1131 (779)
PTW[a]	355–590 (446)	205–613 (455)	304–619 (471)
AT-VS	578–833 (695)	366–1164 (753)	208–628 (419)
AT-VS % BL	0.12–0.15 (0.13)	0.10–0.16 (0.13)	0.07–0.12 (0.10)
Ptes[a]	1160–1976 (1412)	799–1979 (1592)	607–1530 (943)
Ptes % BL	0.22–0.32 (0.26)	0.26–0.31 (0.28)	0.19–0.25 (0.23)
ATL % BL	0.13–0.15 (0.14)	0.09–0.14 (0.12)	0.12–0.18 (0.15)
PTL % BL	0.14–0.16 (0.15)	0.12–0.15 (0.13)	0.16–0.21 (0.19)
OV-VS[ab]	166–225 (202)	111–453 (245)	34–279 (131)
OV-VS % BL	0.03–0.05 (0.04)	0.03–0.06 (0.04)	0.01–0.05 (0.03)
OV-AT[ab]	79–611 (241)	64–442 (219)	0–103 (26)
OV-AT % BL	0.02–0.05 (0.03)	0.02–0.06 (0.04)	0–0.01 (0.005)
OVL[ab]	254–349 (299)	183–375 (301)	179–339 (263)
OVW[ab]	220–340 (264)	120–308 (252)	151–357 (256)
OVL % BL	0.05–0.07 (0.06)	0.04–0.07 (0.5)	0.05–0.07 (0.06)
OS-VS[ab]	711–1044 (879)	608–1915 (946)	491–997 (664)
OS-VS % BL	0.14–0.19 (0.16)	0.14–0.28 (0.16)	0.14–0.20 (0.17)
OS-Vit[ab]	364–644 (504)	356–745 (530)	234–483 (336)
OS-Vit % BL	0.08–0.11 (0.09)	0.08–0.11 (0.09)	0.05–0.12 (0.09)
OS-OV[ab]	731–1593 (1316)	1001–2042 (1462)	761–1579 (1038)
OS-OV % BL	0.21–0.28 (0.25)	0.23–0.28 (0.25)	0.23–0.29 (0.26)
Previt[ab]	817–1116 (951)	795–1409 (1143)	552–896 (677)
Posvit[a]	106–168 (130)	95–272 (189)	43–221 (94)
Previt % BL	0.16–0.19 (0.18)	0.18–0.21 (0.19)	0.14–0.23 (0.17)
Posvit % BL	0.02–0.04 (0.02)	0.02–0.05 (0.03)	0.01–0.04 (0.02)
VitOcc % BL	0.78–0.81 (0.79)	0.75–0.79 (0.77)	0.73–0.84 (0.80)
EggL	40–66 (56)	52–67 (59)	44–62 (54)
EggW	23–30 (27)	23–34 (28)	25–34 (28)

B, body; L, length; W, width; D, diameter; F, forebody; OS, oral sucker; VS, ventral sucker, PPh, prepharynx; Ph, pharynx, Oe, oesophagus; CS, cirrus sac; IB, intestinal bifurcation; Vit, vitellarium; Previt, previtelline region; Postvit, postvitelline region; VitOcc, vitellarium occupies; Cae, caecum; AT, anterior testis; PT, posterior testis; Ptes, post-testicular space; OV, ovary.

[a] Feature included in Principal components analyses.

[b] Feature used in principal components analysis of the reduced dataset designed for the inclusion of hologenophores.

(WAM) and Queensland Museum (QM); accession numbers are presented in the taxonomic section of this manuscript.

2.3. Molecular sequencing

Two markers were targeted in this study, the second internal transcribed spacer region (ITS2) and the lsrRNA (28S rDNA). DNA was extracted from small tissue samples excised from individual specimens, with the remainder of the specimen being processed for morphological study, as described above, to serve as both a morphological and molecular voucher ((hologenophore sensu Pleijel et al. (2008)). Total genomic DNA was extracted from tissue samples using phenol/chloroform extraction techniques (Sambrook and Russell, 2001). PCR and sequencing for the ITS2 and 28S rDNA gene regions followed the protocols of Huston et al. (2016). Collection data and GenBank accession numbers for taxa sequenced are presented in the taxonomic section of this manuscript.

2.4. Phylogenetic analyses

The partial 28S rRNA gene sequences generated in this study were aligned with sequences of species of Enenteridae and selected outgroup taxa available on GenBank (Table 2). Species of the family Gyliauchenidae have been repeatedly demonstrated as the sister group of the Enenteridae (Bray et al., 2009, 2018; Bray and Cribb, 2012), and were thus chosen as the outgroup for analyses. The alignment for the 28S rRNA gene sequences was performed with MUSCLE (Edgar, 2004) as implemented in MEGA7 (Kumar et al., 2016) and the resultant alignment was trimmed to the shortest sequence length. Phylogenetic trees based on the 28S rDNA dataset were constructed with maximum likelihood and Bayesian inference analyses on the CIPRES portal (Miller et al. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, pp. 1–8.) implementing best-fit nucleotide substitution models selected using jModelTest 2 (Darriba et al., 2012) with the Akaike information criterion and Bayesian information criterion. Maximum likelihood analyses were performed using RAxML (Stamatakis, 2014) with 1,000 bootstrap pseudoreplicates. Bayesian inference was performed using MrBayes v3.2.6 (Ronquist et al., 2012) with four chains being sampled every 1000 generations for 10,000,000 generations and the first 2500 samples being discarded as burn-in, at which point the average standard deviation of split frequencies was <0.01.

2.5. Data accessibility

The raw morphometric data underpinning descriptions and comparisons, R code and datasets used for PCA, and the 28S rDNA alignment used in the phylogenetic analyses, are freely accessible at https://doi.org/10.17632/n6jhg87rsg.1.

3. Results

Approximately 70 of the 116 (~60%) *Kyphosus* fishes collected between 1994 and 2018 hosted trematodes of the family Enenteridae. Most of these parasite infrapopulations were mixed, and often included one or more species of the genera *Enenterum* and/or *Koseiria*. Despite collection of *Kyphosus* specimens from off Japan, French Polynesia and South Africa, putative specimens of *Koseiria* spp. were recovered from kyphosid fishes only in Australian and Micronesian (Palau) waters. The specimens collected from off Palau were recovered from *Kyphosus bigibbus* Lacepède, and were studied previously by Bray and Cribb (2002), who identified them as *Koseiria xishaensis*. In Australia, specimens consistent with *Koseiria xishaensis* were obtained from *Kyphosus cinerascens* (Forsskål) and *K. vaigiensis* (Quoy & Gaimard) off Lizard Island (northern Great Barrier Reef), *K. bigibbus* off Heron Island (southern Great Barrier Reef), and from *K. bigibbus* in Moreton Bay (southeast Queensland). Additionally, specimens consistent with *Koseiria allanwilliamsi* were collected from *Kyphosus cornelii* (Whitley), off Perth, Western Australia, and specimens consistent with *Koseiria huxleyi* were obtained from *Chaetodontoplus meredithi* from off Heron Island. Lastly, three specimens consistent with *Koseiria* were obtained from *Kyphosus gladius* from off Perth.

ITS2 sequence data generated for the putative specimens of *Koseiria* indicated the presence of six distinct species in Australian waters, with sequences of putative species differing from one another by 6–64 bp. No intraspecific variation was detected in the ITS2 sequences. Specimens morphologically consistent with *Koseiria allanwilliamsi*, *Koseiria huxleyi*, and the additional species of *Koseiria* from off Perth were each found to be distinct, but those consistent with *Koseiria xishaensis*, collected from the Great Barrier Reef and southeast Queensland, were found to represent a complex of three species. Molecular data suggested that one of these species was restricted to *Kyphosus cinerascens* from Lizard Island, one was found only in *K. vaigiensis* at Lizard Island and *K. bigibbus* at Heron Island, and the last was found only in *K. bigibbus* from Heron Island and Moreton Bay.

BLAST analyses using the 28S rRNA gene sequences generated for specimens from the complex of three species from the Great Barrier Reef and southeast Queensland against GenBank data resulted in a match for one of these species with a sequence reported as *Koseiria xishaensis* (AY222233). The specimen from which this sequence was generated was collected from Heron Island, Great Barrier Reef (Olson et al., 2003). However, when we compared the morphology of the specimens of *Koseiria* from eastern Australia with the original description of *Koseiria xishaensis* by Gu and Shen (1983), we found that none of the three species in the eastern Australian complex could be reliably differentiated. Furthermore, we found that the specimens of Machida (1993), identified as *K. xishaensis*, were prepared in a manner (flattened) that prevented direct morphological comparison with material from Australia and Palau (Fig. 1). Here we choose to describe the three

Table 2
Enenteridae and selected gyliauchenid outgroup taxa from GenBank used in phylogenetic analyses including host, provenance data, accession number and original reference.

Parasite Taxon	Host	Collection locality	GenBank ID	Reference
Enenteridae				
Enenterum aureum	*Kyphosus vaigiensis*	Moorea, French Polynesia	**AY222232**	Olson et al. (2003)
Koseiria argalea n. sp.[a]	*Kyphosus vaigiensis*	Heron Island, QLD, Australia	**AY222233**	Olson et al. (2003)
Proenenterum isocotylum	*Aplodactylus arctidens*	Tasmania, Australia	**FJ788500**	Bray et al. (2009)
Proenenterum ericotylum	*Aplodactylus arctidens*	Tasmania, Australia	**FJ788499**	Bray et al. (2009)
Outgroup				
Affecauda annulata	*Naso tuberosus*	Lizard Island, QLD, Australia	**FJ788501**	Bray et al. (2009)
Paragyliauchen arusettae	*Pomacanthus sexstriatus*	Ningaloo Reef, WA, Australia	**FJ788503**	Bray et al. (2009)

[a] As *Koseiria xishaense* on GenBank.

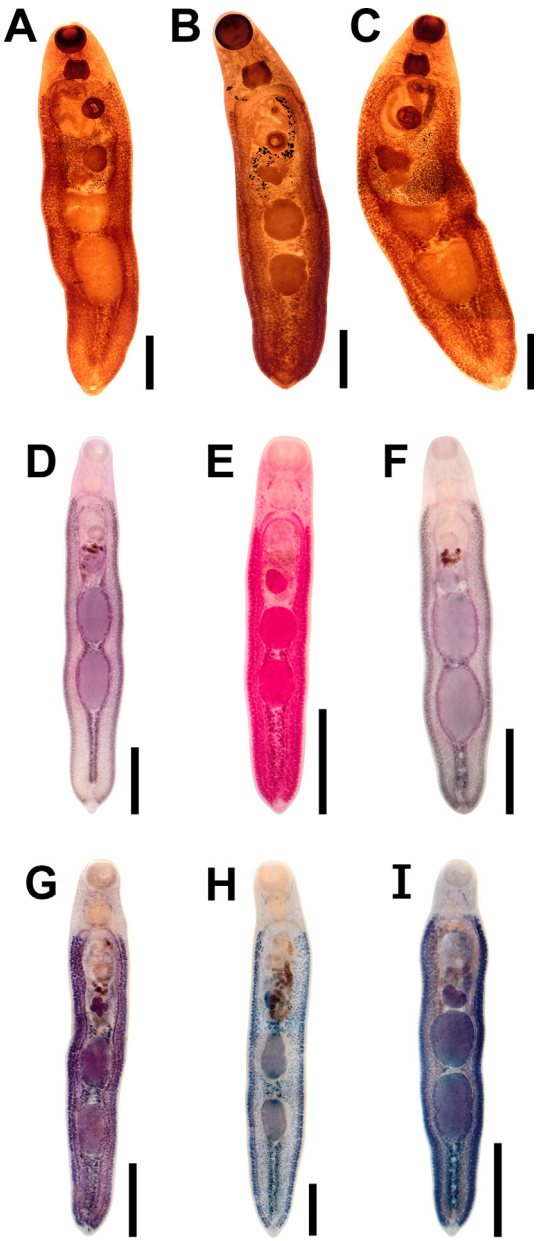

Fig. 1. Microphotographs obtained in the present study of specimens of *Koseiria* prepared by different authors. (A–C) Flattened specimens from Machida (1993). (D–F) Specimens of Bray and Cribb (2001, 2002). (G–I) Holotypes of *Koseiria argalea*, *Koseiria laiphopharophora* and *Koseiria pyknophora*, respectively, from the present study. Scale bars = 1000 μm.

species from the east Australian complex as new, because each is clearly distinct, but none of the three can be reliably ascribed to *K. xishaensis*. To facilitate explanation of the results that follow, we introduce the names of the species described herein: *Koseiria argalea* n. sp., *Koseiria laiphopharophora* n. sp., and *Koseiria pyknophora* n. sp.

3.1. Phylogenetic results

No regions of alignment ambiguity were detected in the 28S rDNA alignment, which consisted of 1265 base positions. Bayesian inference and maximum likelihood analyses generated trees with identical topologies (Fig. 2). Our phylogenetic analyses demonstrate the family Enenteridae as a well-supported monophyletic group containing three clades, although the interrelationships between these three clades are not well resolved. Significantly, however, *Koseiria huxleyi* was sister to the *Proenenterum* clade and, with the inclusion of morphological evidence, is transferred to a new genus as *Enenterageitus huxleyi* n. gen., n. comb. *Koseiria allanwilliamsi* was nested in the *Proenenterum* clade, and with a revaluation of morphological characters, is recombined as *Proenenterum allanwilliamsi* n. comb. The remaining four species were supported as distinct, and formed a well-supported monophyletic group consistent with the generic concept of *Koseiria*.

3.2. Morphometric analyses

PCA was employed to determine if the three species of *Koseiria* detected via molecular means could be differentiated on the basis of morphology. Preliminary study indicated that specimens of *Koseiria* from Lizard Island could be differentiated into two species by host and on a morphological basis with traditional morphometrics (Table 1); this is also reflected in PCA involving only these specimens (Fig. 3A). However, inclusion of additional morphometric data from newly collected specimens of *Koseiria* from Heron Island and Moreton Bay, plus museum material (collectively referred to as *Koseiria xishaensis* '*sensu lato*'), blurred the morphometric distinction between the putative species of *Koseiria* detected (Fig. 3B). Closer examination of data input, examination of reduced datasets and the study of the distribution of individuals in the full dataset ultimately revealed informative patterns as well as unresolved problems.

We found that PCA was highly '0' sensitive, meaning that results were heavily skewed based on whether individuals included a 0 μm value for any of their feature measurements. In our PCAs, inclusion of oesophagus length and prepharynx length, for which many specimens had measurements of 0 μm (this does not mean absence, just the result of the feature not being observable due to three dimensionality), resulted in four well-differentiated, but taxonomically spurious, groupings (Fig. 3C). These spurious groupings were detected because in a reduced dataset (not shown), hologenophores were not clustering with their presumed species groupings (based on host and geography). Thus, measurements of prepharynx and oesophagus length were removed from further analyses. In PCAs performed on a reduced dataset without prepharynx and oesophagus length (Fig. 3D), hologenophores clustered reasonably well with their presumed species group.

Specimens collected from off Lizard Island still formed two distinct clusters even when all material was included (Fig. 3B). Additionally, all the specimens from Palau, originally identified as *K. xishaensis* by Bray and Cribb (2002), formed a distinct cluster, separated from the two Lizard Island clusters (Fig. 3E). Conversely, the remaining specimens of *Koseiria* from Australia were widely spread within the PCA plot; this led us to question whether these specimens were conspecific. One of the museum specimens originally identified as *K. xishiaensis* (QM G219380), collected from Heron Island, was placed well apart from other presumptive conspecifics, within the *K. pyknophora* cluster (1 in Fig. 3B). Although we cannot be certain of the host identification, it was reported as *Kyphosus vaigiensis* (the host for *K. pyknophora*) and our molecular data suggests that the range of *K. pyknophora* includes Heron Island. Furthermore, careful re-examination of this specimen revealed qualitative and meristic qualities which we found sufficiently convincing to consider it a member of the *K. pyknophora* group. The reassignment of this specimen to *K. pyknophora* allows for a more likely host distribution (Fig. 3E).

To develop more conservative species hypotheses, we relied on data for which there was supporting molecular information, or highly plausible morphological evidence. We consider the identity of the specimens from Palau as essentially unidentifiable because no molecular data are available for them, and thus refrain

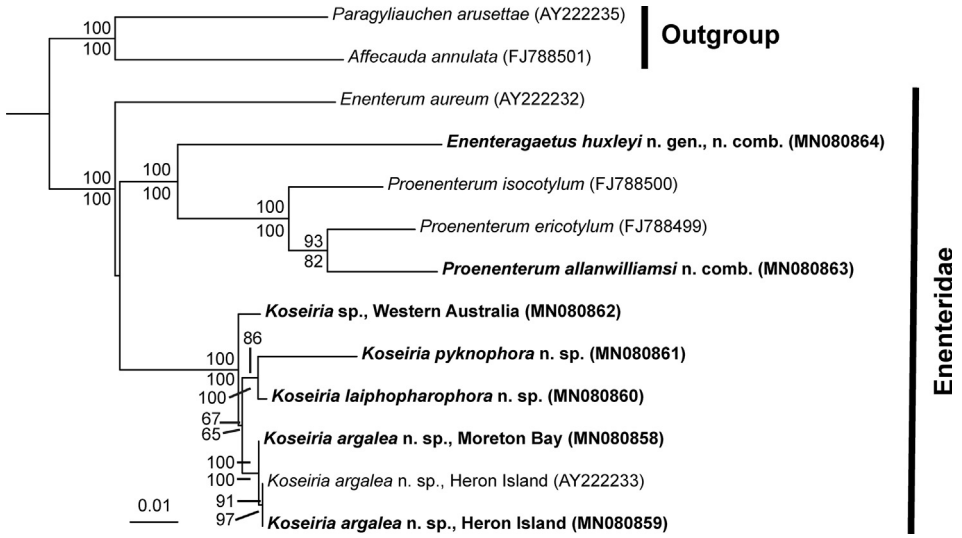

Fig. 2. Phylogenetic relationships of the Enenteridae based on maximum likelihood analyses of the 28S rDNA dataset. ML bootstrap support is shown above the node, and Bayesian inference posterior probabilities are shown below. Values <50 not shown.

from assigning them to a named species. Four Australian specimens (QM G217721, BMNH2000.3.15.7, BMNH2000.3.15.8-9, BMNH2002.7.17.22-24) were not assigned to species, as they appear as outliers when the group from Palau is considered distinct (2 in Fig. 3E). To keep our morphological concept of *Koseiria argalea* conservative, we base our description only on those whole mounts and hologenophores which clustered closely together. Although this resulted in a complete overlap between *K. laiphopharophora* and *K. argalea* in PCA, a distinct difference in the structure of the ovary can be used to differentiate these two species in the conservative hypothesis (see descriptions). Fig. 3F represents the final morphometric dataset on which we base our species hypotheses.

3.3. Taxonomy

3.3.1. Genus Koseiria Nagaty, 1942 (Lepocreadioidea Odhner, 1905: Enenteridae Yamaguti, 1958)

Amended diagnosis: Modified from Bray (2005). Body elongate, cylindrical. Tegument finely spinose; spines often indistinct. Oral sucker subglobular or bell-shaped, subterminal, lacking lobes. Ventral sucker round. Prepharynx short, often broad. Pharynx ellipsoidal to dolioform. Oesophagus short. Intestinal bifurcation in posterior forebody. Caeca form cyclocoel near posterior extremity giving rise to single, dorso-subterminal anus. Testes two, margins entire or lobed, tandem, in area of mid-body to posterior hindbody. External seminal vesicle absent. Cirrus-sac ellipsoidal to spheroid. Internal seminal vesicle saccular or tubular, convoluted. Pars prostatica narrow, distinct. Ejaculatory duct short. Genital pore median, in posterior region of forebody. Canalicular seminal receptacle present. Laurer's canal present. Uterus entirely preovarian or post-ovarian. Eggs thin-shelled; not connected via medium or casing. Vitelline fields extensive, restricted to hindbody or extend into forebody. Excretory pore terminal or dorsally subterminal. Excretory vesicle tubular, passes ventral to cyclocoel, bifurcate or not, extends to ovary or as far as oral sucker. In herbivorous marine teleosts (principally kyphosids), Indo-West Pacific.

Type species: Koseiria tahmeli Nagaty, 1942.

Other species: K. argalea n. sp., *K. laiphopharophora* n. sp., *K. manteri* Ahmad, 1987, *K. nagatyi* Ahmad, 1984, *K. pyknophora* n. sp., *K. xishaensis* Gu & Shen, 1983.

Remarks: Minor refinements have been made to the diagnosis of Bray (2005) to accommodate the transfer of *Koseiria allanwilliamsi* to *Proenenterum* and *Koseiria huxleyi* to *Enenterageitus* n. gen. An amendment of note is that we do not think that any species of *Koseiria* has an accessory sucker associated with the genital pore. Such structures are sometimes present in species of *Enenterum* (see Bray and Cribb, 2001), but we think the notion that such a structure may be present in species of *Koseiria* originated with Yamaguti (1970). When describing the distal portions of the terminal genitalia and uterus of *K. kyphosi* (considered a junior synonym of *K. tahmeli*; see Bray and Cribb (2001)), Yamaguti (1970) referred to a 'genital sucker', but never used the term 'genital pore'. No other author describing a species of *Koseiria* has reported an accessory sucker associated with the genital pore, and we have not observed such a feature either. We suggest that the term 'genital sucker' used by Yamaguti (1970) in his description originated in error and should be considered synonymous with 'genital pore'.

3.3.2. Koseiria argalea n. sp. (Table 1; Figs. 4A and 5A)

Zoobank LSID: urn:lsid:zoobank.org:act:021D28CC-32E3-4D16-952F-0A6841EF81AC.

Type host and locality: Kyphosus bigibbus Lacepède, 1801 (Kyphosidae), Amity Point, Moreton Bay, Queensland, Australia (27° 23′S 153° 26′E) (AP).

Other localities: off Heron Island, Great Barrier Reef, Queensland, Australia (23° 27′S, 151° 55′E) (HI).

Material examined: Holotype: from intestine of *Kyphosus bigibbus*, off AP, coll. D. Huston, 2017, (QM G238145). Paratypes: two from intestine of *K. bigibbus*, off AP, coll. D. Huston, 2016, 2017, (QM G238146–7); three from *K. vaigiensis* (?) off HI, coll. Lo & Pichelin, 1999 (QM G219379, G219381; BMNH.2002.7.17.22-24); Hologenophores: three from intestine of *K. bigibbus*, off AP, coll. D. Huston, 2016, 2017, (QM G238148–50); one from intestine of *K. bigibbus* off HI, coll. D. Huston, 2018 (QM G238151).

Representative DNA sequences: ITS2: four identical replicates, all from above hologenophores. One replicate submitted to GenBank (**MN080850**). Partial 28S rRNA gene sequences: four replicates; all from above hologenophores (HI replicate differs by 1 bp). Two replicates submitted to GenBank (**MN080858**; **MN080859**).

Description: Based on six whole mounts and four hologenophores; measurements in Table 1. Body elongate, cylindrical, rounded at anterior end, attenuating at posterior end. Tegument finely spinose; spines indistinct in larger specimens. Oral sucker simple, subglobular, subterminal. Ventral sucker round, smaller

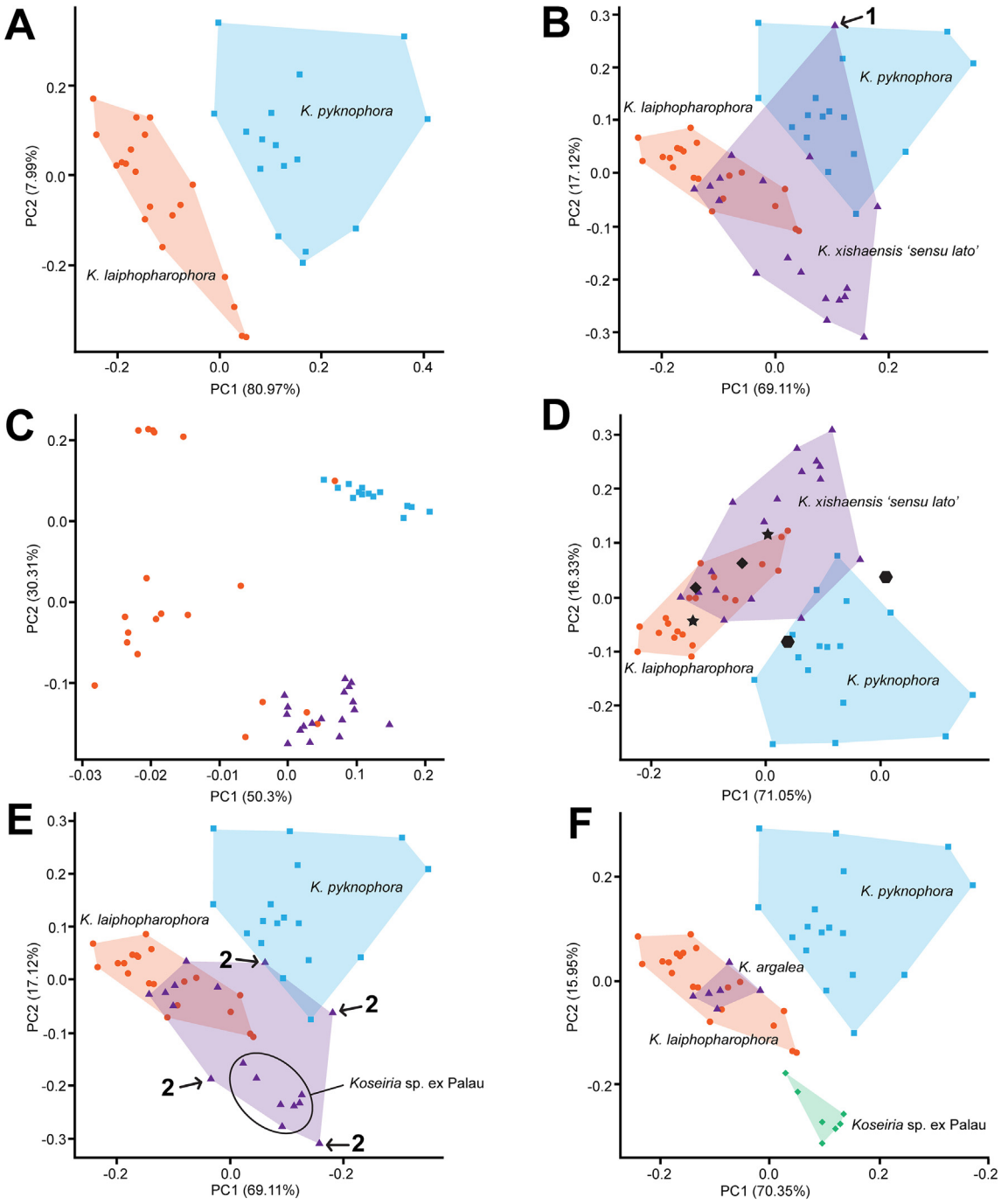

Fig. 3. Principle components analyses (PCAs) on morphometric data from specimens of *Koseiria* spp. (A) *Koseiria laiphopharophora* and *Koseiria pyknophora* from Lizard Island, Great Barrier Reef, Australia, only. (B) Morphometric data for all specimens examined in this study, with specimens from localities other than Lizard Island assigned to *Koseiria xishaensis 'sensu lato'*. (1) Specimen reassigned to *Koseiria pyknophora*. (C) Erroneous species groupings resulting from the inclusion of prepharynx and oesophagus length in the PCA. (D) PCA with reduced dataset allowing inclusion of hologenophores. Hologenophores are: *K. laiphopharophora* – black stars; *K. pyknophora* – black hexagons; *K. xishaensis 'sensu lato'* (*Koseiria argalea*) – black diamonds. (E) Re-assignment of specimens from Palau to *Koseiria* sp. (2) Putative *K. xishaensis* specimens from Heron Island, Australia, removed for conservative hypothesis. (F) Final conservative species hypothesis.

than oral sucker, in anterior third of body; aperture rhomboid. Prepharynx distinct, wide. Pharynx well-developed, in mid-forebody, ellipsoidal to pyriform. Oesophagus distinct, short. Intestine robust, gastrodermis heavily developed. Intestine bifurcates anterior to ventral sucker, caeca reunite near posterior extremity, forming short rectum and dorso-subterminal anus with wide opening.

Testes two, ellipsoidal, with margins entire in immature specimens, lobed in larger specimens, approximately equal in size, tandem, separated, medial, in mid-hindbody, ventral to caeca. Cirrus-sac ellipsoidal, thick-walled, ventral to intestine, posterior half dorsal to ventral sucker, anterior margin ventral to caecal bifurcation. Internal seminal vesicle long, convoluted, swollen. Pars prostatica indistinct, short, vesicular. Ejaculatory duct long, coiled, muscular, occasionally containing eggs. Genital atrium large. Genital pore ventral, at level of intestinal bifurcation.

Ovary weakly to distinctly three, four or five lobed, intercaecal, midway between ventral sucker and anterior testis. Mehlis' gland profuse, intercaecal, dorsal, extends dorsally from ovary to

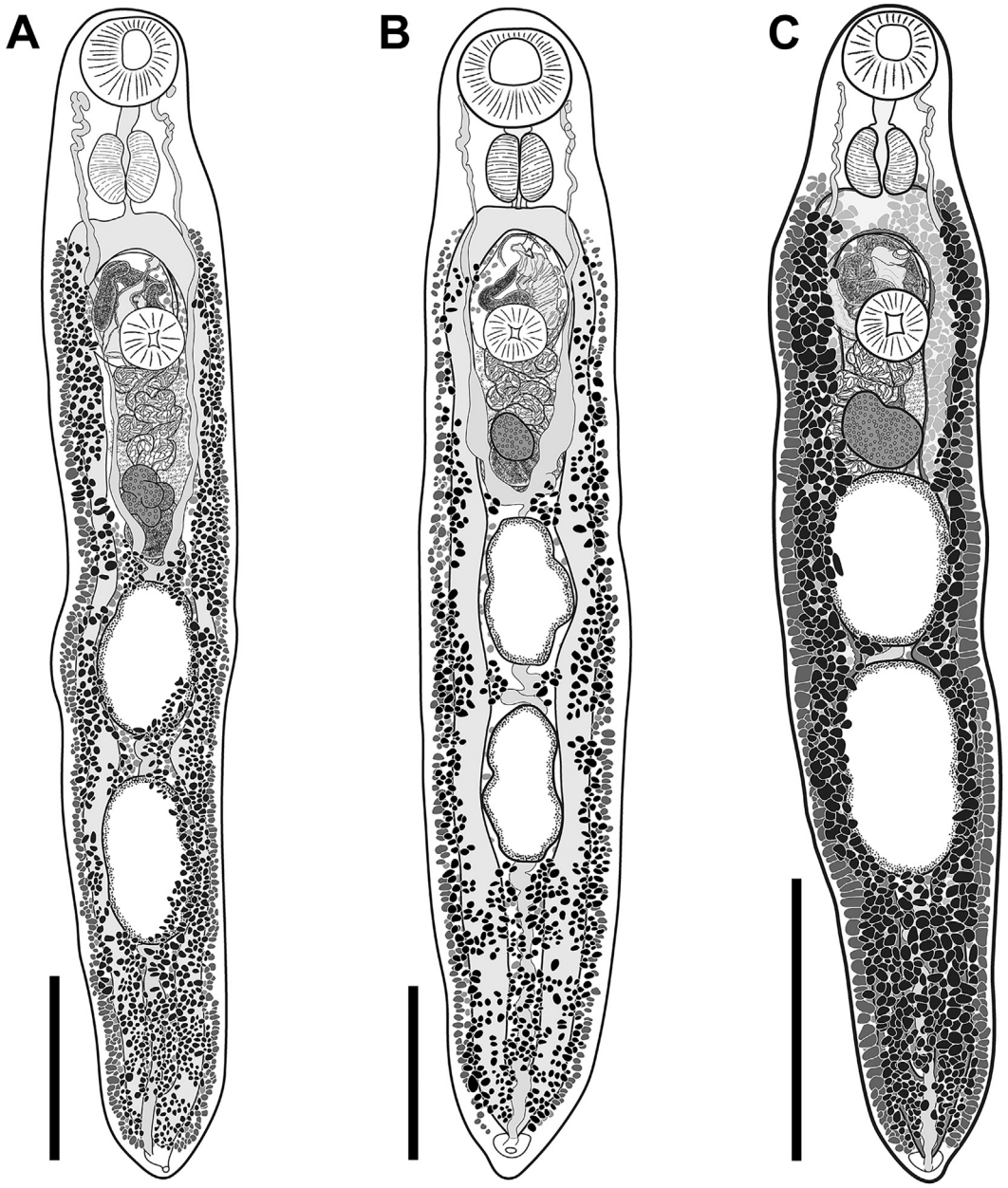

Fig. 4. Illustrations of new species of *Koseiria* described in the present study. (A) *Koseiria argalea* n. sp., holotype. (B) *Koseiria laiphopharophora* n. sp., holotype. (C) *Koseiria pyknophora* n. sp., holotype. Scale bars = 1000 μm.

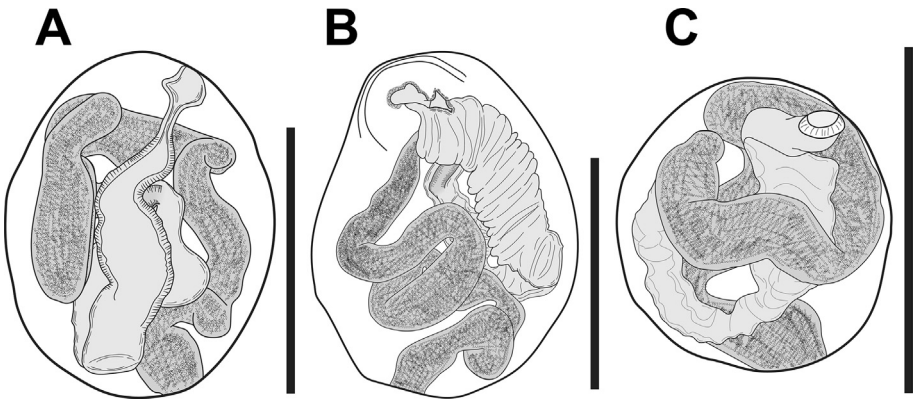

Fig. 5. Illustrations of cirrus-sacs of new species of *Koseiria* described in the present study. (A) *Koseiria argalea* n. sp., cirrus-sac. (B) *Koseiria laiphopharophora* n. sp., cirrus-sac. (C) *Koseiria pyknophora* n. sp., cirrus-sac. Scale bars = 500 μm.

posterior margin of cirrus-sac. Canalicular seminal receptacle posterio-dorsal to ovary. Laurer's canal opens dorsally to seminal receptacle. Uterus highly convoluted, intercaecal, coils dorsally to ovary and seminal receptacle, extends posteriorly to anterior margin of anterior testis, majority of coils between ovary and ventral sucker, proximal portion passes along dextral margin of cirrus-sac to genital pore. Eggs elongate, operculate, numerous.

Vitellarium follicular, fields confluent, extending from pharynx to near posterior extremity; small and large medial gap dorsally and ventrally, respectively, anterior to testes, fields encroach dextral and sinistral portions of testes, fill entire body between and posterior to testes. Vitelline reservoir dorso-dextral to ovary; two collecting ducts pass laterally from reservoir, one sinistral and one dextral, each branching to pass anteriorly and posteriorly before becoming indistinguishable from vitelline follicles.

Excretory pore dorsally subterminal, posterior to anus. Excretory vesicle originates as a swollen pyriform chamber with attenuating portion pointing posteriorly; vesicle duct arises ventrally from chamber, passes anteriorly ventral to caeca, gradually enlarging, passes dorsally to testes, bifurcates just anterior to anterior testis; collecting ducts trace path of intestinal caeca, extend as far as oral sucker.

Etymology: The name of this species come from Greek word 'argaleos' which means troublesome or vexing. The name is chosen in memory of the trials and tribulations of delineating species of *Koseiria* in Australia.

Remarks: Kyphosus bigibbus is a confirmed host for this species, and although we include specimens in the type series that were reported from *Kyphosus vaigiensis* (which closely resembles *K. bigibbus*), the identity of the fishes concerned cannot be known for certain. Thus, we consider the status of *K. vaigiensis* as a host for *Koseiria argalea* as doubtful.

Koseiria argalea is reliably distinguished from *K. tahmeli* and *K. nagatyi* in having vitelline fields that extend into the forebody, rather than being restricted to the hindbody. *Koseiria argalea* can be distinguished from *K. manteri* in utilising a kyphosid, rather than hemiramphid, host, having a cirrus-sac dorsal to the ventral sucker rather than being entirely anterior to the ventral sucker, and having vitelline fields that extend into the forebody, rather than being restricted to the hindbody. At first no reliable features could be found to differentiate this species from *K. xishaensis*, but refinement of the range of measurements for *K. argalea* in our conservative hypothesis resulted in some morphometric differences. From the original description of *K. xishaensis* there appears to be a distinct difference in the sucker width ratio, (1:0.56 in *K. xishaensis* versus 1:0.62–0.93 in *K. argalea*) which, given the flattening technique clearly employed by Gu and Shen (1983), would seem to be a reliable feature for differentiating these species. This is also the case with the oral sucker to pharynx width ratio, though the difference is less distinct (1:0.68 in *K. xishaensis* versus 1:0.7–1.1 in *K. argalea*). There is also a difference in host species (*Kyphosus bigibbus* and *K. vaigiensis* for *Koseiria argalea* versus *Kyphosus cinerascens* for *Koseiria xishaensis*), although there remains some doubt as to whether host identity is a reliable method for differentiating species of *Koseiria*.

3.3.3. *Koseiria laiphopharophora* n. sp. (Table 1; Fig. 4B and 5B)

ZOOBANK LSID: urn:lsid:zoobank.org:act:255C718B-6542-4FD2-9AD4-2B987106A6F4.

Type host and locality: Kyphosus cinerascens (Forsskål, 1775) (Kyphosidae), off Lizard Island, Great Barrier Reef, Queensland, Australia (14°40′S, 145°27′E) (LI).

Material examined: Holotype: from intestine of *Kyphosus cinerascens*, off LI, coll. D. Huston, 2016, (QM G238152). Paratypes: 18 from intestine of *K. cinerascens*, off LI, coll. D. Huston, 2016, (QM G238153–238170). Hologenophores: three from intestine of *K. cinerascens*, off LI, coll. D. Huston, 2016, (QM G238171–73).

Representative DNA sequences: ITS2: three identical replicates, all from above hologenophores. One replicate submitted to GenBank (**MN080851**). Partial 28S rRNA gene sequences: two identical replicates; all from above hologenophores. One replicate submitted to GenBank (**MN080860**).

Description: Based on 19 dorso-ventral whole mounts and 3 hologenophores; measurements in Table 1. Body elongate, cylindrical, rounded at anterior end, attenuating at posterior end. Tegument finely spinose; spines indistinct in larger specimens. Oral sucker subglobular, subterminal. Ventral sucker round, smaller than oral sucker, in anterior third of body; aperture rhomboid. Prepharynx very short. Pharynx well-developed, ellipsoidal to dolioform. Oesophagus short. Intestine robust, gastrodermis heavily-developed. Intestine bifurcates anterior to ventral sucker, caeca reunite near posterior extremity, attenuating to form short rectum and broad dorso-subterminal anus.

Testes two, ellipsoidal, with margins irregular to deeply lobed, approximately equal in size, tandem, separated, medial, in mid-hindbody, ventral to caeca. Cirrus-sac ellipsoidal to pyriform, thick-walled, ventral to intestine, posterior half dorsal to ventral sucker, anterior margin near intestinal bifurcation. Internal seminal vesicle swollen, long, convoluted. Pars prostatica relatively inconspicuous, short, vesicular, walls muscular. Ejaculatory duct long, broad, coiled; walls muscular. Genital atrium large. Genital pore ventral, near intestinal bifurcation.

Ovary ellipsoidal, intercaecal, approximately midway between ventral sucker and anterior testis. Mehlis' gland profuse, intercaecal, dorsal, extends from ovary to posterior extremity of cirrus-sac. Canalicular seminal receptacle posterio-dorsal to ovary. Laurer's canal opens dorsal to seminal receptacle. Oötype anterio-dorsal to ovary. Uterus long, intercaecal, lightly coiled and pre-ovarian in smaller specimens, highly convoluted and pre- and post-ovarian in larger specimens, coiled most extensively just posterior to ventral sucker, proximal portion curving directly along dextral margin of cirrus-sac to genital atrium. Eggs elongate, operculate, numerous.

Vitellarium follicular, profuse; fields confluent, extending from pharynx to near posterior extremity; distinct medial gap dorsally and ventrally anterior to testes, fields encroach along testicular margins in testicular region, nearly confluent dorsally and ventrally between testes, fill entire body posterior to testes. Vitelline reservoir dorsal to ovary, small; two collecting ducts pass laterally from reservoir, one sinistral and one dextral, each branching to pass anteriorly and posteriorly before becoming indistinguishable from vitelline follicles.

Excretory pore dorsally subterminal, posterior to anus. Excretory vesicle originating as swollen ellipsoidal to pyriform chamber; vesicle duct arises from posterior portion of chamber, passes anteriorly ventral to caeca, gradually enlarging and undulating dorsoventrally, passes dorsal to testes, bifurcates at anterior margin of anterior testes; collecting ducts trace path of intestinal caeca, extend as far as oral sucker.

Etymology: The name of this species is constructed from Greek: laiphos (=tattered) + phoros (=cloak) + phera (=bearing/having). The name refers to the relatively sparse distribution of the vitelline follicles relative to the other species of *Koseiria* described herein.

Remarks: As with *K. argalea*, *K. laiphopharophora* can be differentiated from *K. tahmeli* and *K. nagatyi* in having vitelline fields extending into the forebody, rather than being restricted to the hindbody. *Koseiria laiphopharophora* can be distinguished from *K. manteri* in utilising a kyphosid, rather than hemiramphid, host, having a cirrus-sac dorsal to the ventral sucker rather than being entirely anterior to the ventral sucker, and having vitelline fields that extend into the forebody, rather than being restricted to the

hindbody. From *K. argalea*, *K. laiphopharophora* can be differentiated in having less dense vitelline fields, having an ellipsoidal, rather than distinctly lobed, ovary, having a smaller sucker width ratio (1:0.52–61 versus 1:0.62–0.93), and a smaller oral sucker to pharynx width ration (1:0.57–0.69 versus 1:0.7–1.1). *Koseiria laiphopharophora* is essentially indistinguishable from the type description of *K. xishaensis* morphometrically, other than the eggs being somewhat larger in the former species (54–67 × 23–34 versus 39–42 × 21–24). Of the species described here, *Koseiria laiphopharophora* is certainly the most similar to *K. xishaensis*, and future work might demonstrate that they are conspecific. However, we prefer to consider *K. laiphopharophora* as distinct for the present.

3.3.4. Koseiria pyknophora n. sp. (Table 1; Fig. 4C and 5C)

ZOOBANK LSID: urn:lsid:zoobank.org:act:2FC9A8D5-7F38-41FF-9CA6-7CC35B19DB20.

Type-host and locality: Kyphosus vaigiensis (Quoy & Gaimard, 1825) (Kyphosidae), off Lizard Island, Great Barrier Reef, Queensland, Australia (14° 40′ S, 145° 27′ E) (LI).

Other localities: off Heron Island, Great Barrier Reef, Queensland, Australia (23° 27′ S, 151° 55′ E) (HI).

Material examined: Holotype: from intestine of *Kyphosus vaigiensis*, off LI, coll. D. Huston, 2016, (QM G238174). Paratypes: 15 from intestine of *K. vaigiensis*, off LI, coll. D. Huston, 2016, (QM G23175–238189); one from *K. vaigiensis* off HI, coll Lo & Pichelin, 1999 (QM G219380). Hologenophores: three from intestine of *K. vaigiensis*, off LI, coll. D. Huston, 2016, (QM G238190–2); one from intestine of *K. bigibbus* off HI, coll. D. Huston, 2018 (QM G238193).

Representative DNA sequences: ITS2: four identical replicates, all from above hologenophores. One replicate submitted to GenBank (**MN080852**). Partial 28S rRNA gene sequences: two identical replicates; from two LI hologenophores. One replicate submitted to GenBank (**MN080861**).

Description: Based on 17 dorso-ventral whole mounts and 4 hologenophores. Measurements in Table 1. Body elongate, cylindrical, rounded at anterior end, attenuating slightly at posterior end. Tegument finely spinose; spines indistinct in larger specimens. Oral sucker simple, subglobular, subterminal. Ventral sucker simple, round, smaller than oral sucker, in anterior third of body; aperture rhomboid. Prepharynx short. Pharynx well developed, ellipsoidal to dolioform, often situated in shallow bowl-shaped depression in anterior portion of intestine. Oesophagus very short, usually not visible. Intestine robust, gastrodermis heavily developed. Intestine bifurcates anterior to ventral sucker, caeca reunite near posterior extremity, attenuating to form short rectum and dorso-subterminal anus.

Testes two, ellipsoidal, with margins irregular, often deeply lobed, anterior testis noticeably shorter than posterior testis, tandem, separated, medial, in mid-hindbody, ventral to caeca. Cirrus-sac spheroid, thick walled, ventral to intestine, posterior half dorsal to ventral sucker, anterior margin bordering intestinal bifurcation. Internal seminal vesicle swollen, convoluted. Pars prostatica relatively inconspicuous, short, vesicular; walls muscular. Ejaculatory duct long, broad, coiled. Genital atrium large. Genital pore ventral, near intestinal bifurcation.

Ovary ellipsoidal to cordiform, intercaecal, pre-testicular, close to anterior testis. Mehlis' gland profuse, intercaecal, dorsal, extends from ovary to posterior margin of cirrus-sac. Canalicular seminal receptacle dorsal to ovary. Laurer's canal opens dorsal to seminal receptacle. Oötype antero-dorsal to ovary. Uterus long, intercaecal, lightly coiled and pre-ovarian in smaller specimens, highly convoluted and pre- and post-ovarian in larger specimens, coiled most extensively posterior to ventral sucker, proximal portion passes ventral or dextral to cirrus-sac to genital atrium. Eggs elongate, operculate, numerous.

Vitellarium exceptionally dense, follicular to dendritic, fields confluent, extending from pharynx to near posterior extremity; distinct medial gap dorsally and ventrally anterior to testes, fields overlap dextral and sinistral portions of testes, fill entire body between and posterior to testes. Vitelline reservoir small, distinct, dextral to ovary; two collecting ducts pass laterally from reservoir, one sinistral and one dextral, each branching to pass anteriorly and posteriorly before becoming indistinguishable from vitelline follicles.

Excretory pore dorsally subterminal, posterior to anus. Excretory vesicle originates as a swollen, triangular to pyriform chamber with attenuating portion pointing posteriorly; vesicle duct arises from posterior portion of chamber, passes anteriorly ventral to caeca gradually enlarging, passes dorsally to testes, bifurcates just anterior to anterior testis; collecting ducts trace path of intestinal caeca, extend as far as oral sucker.

Etymology: The name of this species is constructed from the Greek words Pykno (=thick/dense) + Phoros (=cloak). The name is in reference to the exceptionally dense vitellarium of this species.

Remarks: Two immature specimens of *Koseiria* were recovered from a specimen of *Kyphosus bigibbus* collected from Heron Island in 2018. The identity of this fish was confirmed with molecular data. Molecular data obtained from the worms showed that one was a specimen of *Koseiria argalea* and the other was *K. pyknophora*. This demonstrates that the range of *K. pyknophora* extends to the southern Great Barrier Reef, and may suggest that this species can utilise both *Kyphosus bigibbus* and *K. vaigiensis* as a host, although this record may represent an incidental infection given the immaturity of the specimens.

Koseiria pyknophora is readily differentiated from *K. tahmeli* and *K. nagatyi* in having vitelline fields extending into the forebody, rather than being restricted to the hindbody. *Koseiria pyknophora* can be distinguished from *K. manteri* in utilising a kyphosid, rather than hemiramphid, host, having a cirrus-sac dorsal to the ventral sucker rather than being entirely anterior to the ventral sucker, and having vitelline fields that extend into the forebody, rather than being restricted to the hindbody. *Koseiria pyknophora* can most readily be differentiated from *K. argalea* in having far more dense vitelline fields, but also in having an ovary which is ellipsoidal or cordiform rather than distinctly lobed, an extremely short oesophagus vs a distinct oesophagus, a posterior testis occupying a greater percentage of the body length (16–21 versus 14–16%), and less distance between the ovary and the anterior testis (0–1 versus 2–5% of body length). *Koseiria pyknophora* can be differentiated from *K. laiphopharophora* most readily by having far more dense vitelline fields, but also in having greater oral sucker width and length ratios (1:0.62–0.85 and 1:0.66–0.92 versus 1:0.52–0.61 and 1:0.54–0.66, respectively). *Koseiria pyknophora* is difficult to distinguish from the type-description of *K. xishaensis* on the basis of morphometrics, but qualitatively, has distinctly more dense vitelline fields. *Kosieria pyknophora* also has somewhat larger eggs than *K. xishaensis* (44–62 × 24–34 versus 39–42 × 21–24).

3.3.5. Koseiria sp. 'Western Australia'

Host and locality: Kyphosus gladius Knudsen & Clements, 2013 (Kyphosidae), off Point Peron, Western Australia (14°40′S, 145°27′E) (PP).

Material examined: Vouchers: one from intestine of *Kyphosus gladius*, off PP, coll. D. Huston, 2017, (WAM V9476). Hologenophore vouchers: two from intestine of *K. gladius* off PP, coll. D. Huston, 2017, (WAM V9477–8).

Representative DNA sequences: ITS2: two identical replicates, both from above hologenophores; one replicate submitted to GenBank (**MN080853**). Partial 28S rRNA gene sequences: two identical replicates from above hologenophores; one replicate submitted to GenBank (**MN080862**).

Remarks: This species is represented in our collection by only three specimens, two of which had their posterior extremities removed for DNA extraction. Unfortunately these specimens are of poor quality. In these specimens the vitelline fields reach just beyond the anterior margin of the ventral sucker, whereas in *K. argalea*, *K. laiphopharophora*, *K. pyknophora* and *K. xishaensis* the vitelline follicles reach the level of the pharynx. However, at present not enough data are available to definitively separate the present forms from *K. nagatyi* and *K. tahmeli*. Because *K. nagatyi* and *K. tahmeli* have not been evaluated with molecular data, and adequate morphological data for the present specimens are not available, we refrain from naming this species.

3.3.6. Koseiria sp. 'Palau'

Synonym: Koseiria xishaensis of Bray and Cribb (2002).

Host and locality: Kyphosus bigibbus Lacepède, 1801 (Kyphosidae), off Palau, Micronesia (7°21′N, 134°31′E) (PA).

Material examined: Seven from intestine of *Kyphosus bigibbus*, off PA, coll. Adlard & Bray, 2001, (QM G219383–219386, BMNH 2002.7.17.25–28).

Remarks: Bray and Cribb (2002) identified these specimens as *K. xishaensis*, however, on the basis of morphology alone, we cannot determine with any certainty if these specimens are representative of *K. xishaensis* or conspecific with one of the species from Australia. We think it likely that these specimens represent a distinct species. However, because no molecular data are available, their identity is uncertain, and we refrain from assigning them a name.

3.3.7. Koseiria sp. 'Australia' unassigned specimens

Synonym: Koseiria xishaensis of Bray and Cribb (2001, 2002)

Hosts and localities: Kyphosus cinerascens (Forsskål, 1775) and *K. vaigiensis* (Quoy & Gaimard, 1825) (Kyphosidae), off Heron Island, Great Barrier Reef, Australia (23° 27′S, 151° 55′E) (HI).

Material examined: Two from intestine of *Kyphosus cinerascens*, off HI, coll. Ernst & Bray 1998, (QM G217721, BMNH 2000.3.15.7); two from intestine of *Kyphosus vaigiensis* off HI, coll. Ernst & Bray (BMNH 2000.3.15.8-9, 2002.7.17.22-24).

Remarks: As for specimens from Palau, in line with our conservative species hypotheses, these specimens are not assigned to a species.

3.3.8. Koseiria sp. 'Japan' (Fig. 1).

Synonym: Koseiria xishaensis of Machida (1993)

Host and locality: Kyphosus cinerascens (Forsskål, 1775) (Kyphosidae), off Kushimoto and Okinawa, Japan.

Material examined: One from intestine of *K. cinerascens*, off Kushimoto (NSMT 2233), coll. Machida, 1979; five from intestine of *K. cinerascens*, off Okinawa (NSMT 3838; 4200a, b; 4303a, b), coll. Machida, 1983, 1990, 1991, 1992.

Remarks: Machida (1993) identified these specimens as *Koseiria xishaensis*. However, due to the flattening technique employed (see Fig. 1), these specimens are not directly comparable with others of *Koseiria* examined in this study; we were unable to conclude with any certainty if they were conspecific with any others. The host for these specimens is the same as that reported by Gu and Shen (1983) for *K. xishaensis*, and geographically the Xisha Islands and Okinawa are separated by only approximately 2000 km. Thus, we think it plausible that these specimens from Japan are representatives of the true *K. xishaensis*. In the absence of molecular data for specimens of *Koseiria xishaensis* from the type locality and for specimens of *Koseiria* from Japan, however, we consider their identity unknowable.

3.3.9. Koseiria xishaensis Gu & Shen, 1983

Host and locality: Kyphosus cinerascens (Forsskål, 1775) (Kyphosidae), off Xisha Islands, South China Sea.

Remarks: We have been unable to locate the holotype of this species, and no molecular data are available from the type locality. Furthermore, the original description of this species is generalised such that all of the specimens examined in this study from eastern Australia, Japan and Palau are diagnosable as *K. xishaensis*. Thus, we propose that the 'range' of *K. xishaensis* be considered restricted to the type locality for the time being, until further data become available.

3.4. Genus Proenenterum Manter, 1954

Amended diagnosis: Modified from Bray (2005). Body elongate, ellipsoidal, narrow or robust. Tegument finely spinose. Eye-spot pigment present or absent. Oral sucker subglobular, without lobes, subterminal. Ventral sucker round. Prepharynx short, distinct. Pharynx oval. Oesophagus very short. Intestinal bifurcation in mid-forebody. Caeca form cyclocoel; anus absent or, present as a short common duct opening laterally, sinistrally or dextrally subterminal (Fig. 6C). Testes two, tandem, margins entire or lobed, contiguous to well separated, in mid- to posterior regions of hindbody. External seminal vesicle absent. Cirrus-sac ovoid to reniform. Internal seminal vesicle tubular, convoluted. Ejaculatory duct short or long. Pars prostatica short. Genital pore ventral, post-bifurcal, anterior to ventral sucker. Ovary ovaloid or lobed. Canalicular seminal receptacle present. Laurer's canal present. Eggs thin-shelled; oriented in series in long, thin or thickened casings (Fig. 7). Vitelline fields extend from near posterior extremity to mid-forebody, or restricted to hindbody. Excretory pore terminal or dorsally subterminal. Excretory vesicle tubular, extends as far as level of ovary. In herbivorous marine teleosts, Indo-west pacific.

Type species: Proenenterum isocotylum Manter, 1954.

Other species: Proenenterum allanwilliamsi (Bray & Cribb, 2002) n. comb., *Proenenterum ericotylum* Manter, 1954, *Proenenterum manteri* Ahmad, 1985.

Remarks: We have made minor modifications to the diagnosis of Bray (2005) to accommodate the transfer of *Koseiria allanwilliamsi* to *Proenenterum*. While studying newly collected specimens of *Proenenterum allanwilliamsi* we discovered that the eggs are enclosed in long, thin casings (Fig. 7). These casings were visible when processing specimens, and in some whole mounted specimens the egg strings can be traced exiting the genital pore. These casings are not apparent in the posterior reaches of the uterus in whole mounted specimens. Furthermore, in dissected specimens it seemed that only eggs in the anterior reaches of the uterus are enclosed. This pattern suggests that the eggs enter the casings after they are formed, although it is possible that the heavy coiling of the uterus obscures the feature, or dissection damaged them. We also examined specimens of the type species *Proenenterum isocotylum* from the Natural History Museum in London (BNHM 2002.7.17.28-31) and were able to confirm that the eggs were in a similar, even thicker, casing. We have not observed egg casing in any of the specimens of *Koseiria* we have examined, including multiple specimens of *K. laiphopharophora* which we dissected. This feature constitutes a generic level difference between *Koseiria* and *Proenenterum*. We have not observed casing in the eggs of any specimens of *Enenterageitus huxleyi* n. comb., but did not have sufficient specimens for dissection.

In addition to the four species of *Proenenterum* included above, three additional species have been reported, all of questionable validity. One of these species was not formerly named, being referred to only as '*Proenenterum* n. sp.' (Morsy et al., 2011). This species was reported from a non-herbivore, the seabream *Pagrus pagrus* (Linnaeus) (Sparidae) collected from the Red Sea, which is an unlikely host. The illustration and images provided in the manuscript suggest that this species is probably a member of the Opecoelidae, species of which are commonly found in sparids

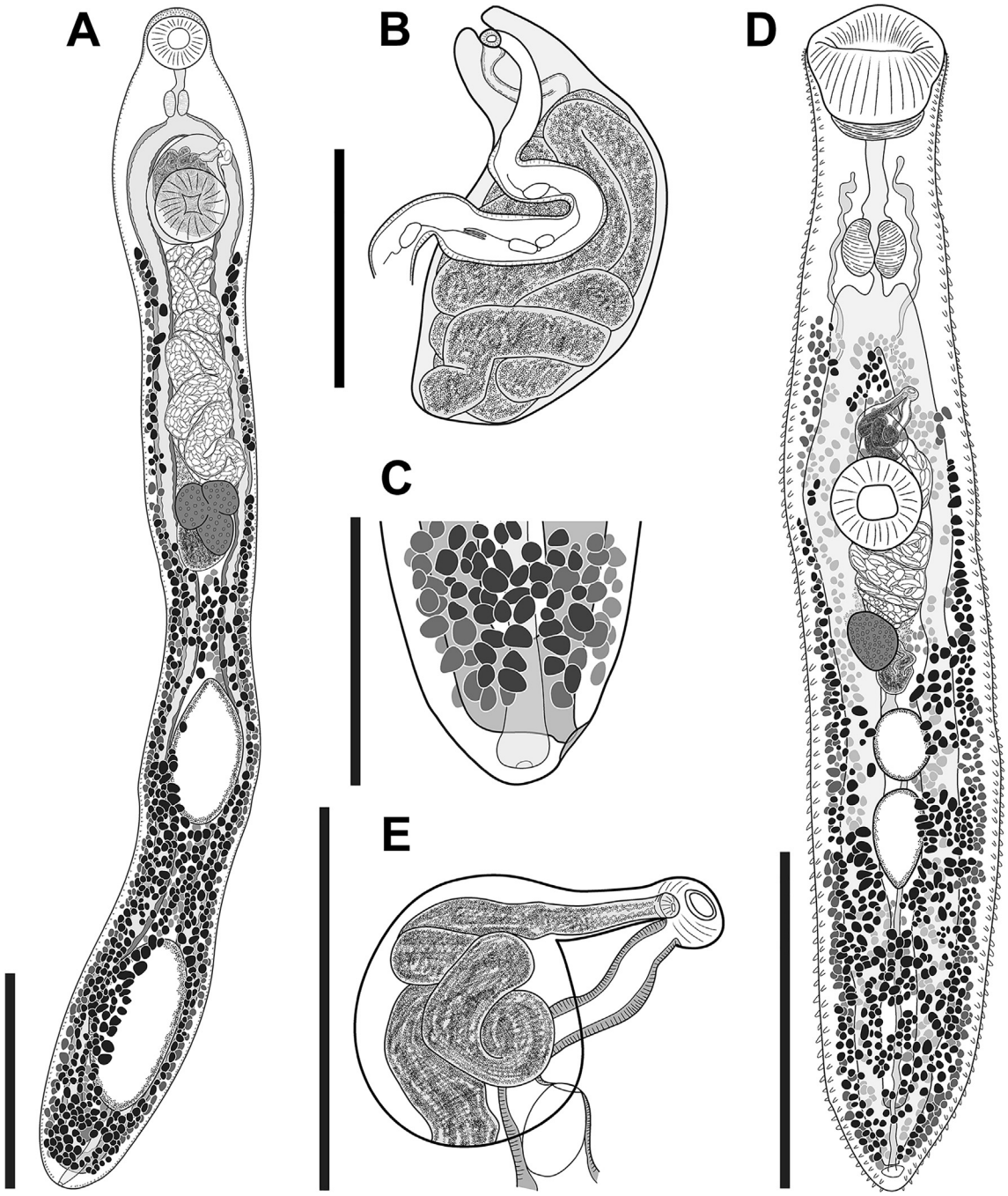

Fig. 6. Illustrations of enenterids recombined in the present study. (A) *Proenenterum allanwilliamsi* n. comb. (B) *Proenenterum allanwilliamsi* n. comb. cirrus-sac. (C) *Proenenterum allanwilliamsi* n. comb., distal portion of caeca showing excretory pore. (D) *Enenterageitus huxleyi* n. gen., n. comb. (E) *Enenterageitus huxleyi* n. gen., n. comb., cirrus-sac. Scale bars: (A, D) 1000 μm; (B, C, E) 500 μm.

(e.g. Manter, 1940; Nahhas and Cable, 1964; Bray, 1987; Bartoli et al., 1988). We suggest that this record be considered erroneous and not representative of an enenterid.

Two additional putative *Proenenterum* species do not meet the requirements to be considered valid. As pointed out by Bray and Cribb (2001), *Proenenterum vindoae* Ahmad, 1983 is a *nomen nudum*. This species was reported from a cornetfish *Fistularia villosa* Klunzinger (Fistulariidae) collected in the Arabian Sea (Ahmad 1983. On a new species of the trematode genus *Proenenterum* Manter, 1954 (Digenea: Lepocreadiidae) from *Fistularia villosa* (Klunzinger) from the Arabian Sea, off the Panaji coast, Goa., Proceedings of the Indian Science Congress, Section VII, 124.). Cornetfish are ambush predators rather than herbivores, so the validity of this record is questionable. Abdel-Gaber et al. (2015) described *Proenenterum myripristiae* Abdel-Gaber, Abdel-Ghaffar & Bashtar, 2015 from a non-herbivore, the soldierfish *Myripristis murdjan* Forsskål (Holocentridae) collected from the Red Sea, and again a non-herbivorous host suggests the record should be treated as dubious. The cirrus-sac of *P. myripristiae*, as it appears in the illustration and photograph, strongly suggest that this species is also a member of the Opecoelidae, species of which are commonly found in holocentrids (e.g. Nahhas and Cable, 1964; Yamaguti, 1970; Bray and Justine, 2014). Opecoelids have even been reported from *M. murdjan* from the Red Sea previously (Parukhin, 1970).

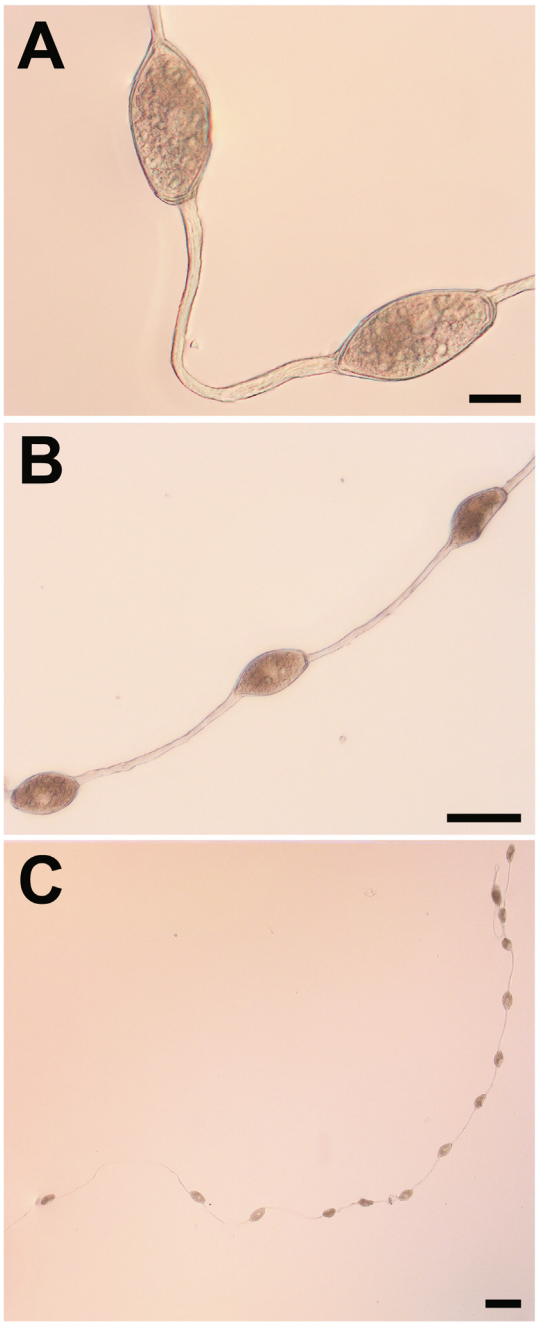

Fig. 7. Eggs of *Proenenterum allanwilliamsi* n. comb. connected via casing. Scale bars: (A) 20 μm; (B) 50 μm; (C) 100 μm.

Because the description of this species was published in an online-only journal and was not registered in ZooBank at the time of publication (see Abdel-Gaber et al., 2015), the name does not meet the requirements of the ICZN (article 8.5.3) to be considered available.

3.4.1. Proenenterum allanwilliamsi (Bray & Cribb, 2002) n. comb. (Figs. 6 and 7A–C)

Synonym: Koseiria allanwilliamsi Bray & Cribb, 2002.

Type-host and locality: Kyphosus cornelii (Whitley, 1944) (Kyphosidae), off Red Bluff, Kalbarri, Western Australia (27°42′S, 114°10′E) (RB).

Additional localities: off Point Peron, Western Australia (14°40′S, 145°27′E) (PP).

Material examined: Paratypes: seven whole mounts, from intestine of *Kyphosus cornelii*, off RB, coll. R. Bray & A. Williams, 1988, (BMNH 1988.6.20.1–7). Additional vouchers: Fifteen whole mounts, three hologenophores and two slides of dissected specimens from intestine of *Kyphosus cornelli*, off PP, coll. D. Huston, 2017 (WAM V9479–9498).

Representative DNA sequences: ITS2: three identical replicates, all from above hologenophores; one replicate submitted to GenBank (**MN080854**). Partial 28S rRNA gene sequences: two identical replicates; from above hologenophores. One replicate submitted to GenBank (**MN080863**).

Remarks: The original assignment of this species to *Koseiria* was justified; only in light of molecular data can we rationalise placing it in *Proenenterum*. The spines of *P. allanwilliamsi* are much more distinct than in species of *Koseiria*, and the filamented eggs certainly ally *P. allanwilliamsi* with *P. isocotylum*. The presence of an anus in *P. allanwilliamsi* rather than a closed cyclocoel, as in other species of *Proenenterum*, is somewhat puzzling. The anus in *P. allanwilliamsi* manifests as a small duct to an anal pore which can open laterally, dorso-sinistrally or dorso-dextrally (Fig. 6C), rather than as the distinct rectum and broad anus opening dorso-medially as found in species of *Koseiria*.

3.5. Genus Enenterageitus n. gen.

ZOOBANK LSID: urn:lsid:zoobank.org:act:D0685F50-347E-4DD4-B0CB-FDFDE8326F17.

Diagnosis: Body elongate, ellipsoidal. Tegument spinous; spines large, scale-like. Eyespot pigment present. Oral sucker weakly infundibuliform, without lobes, terminal. Muscular post-oral ring present. Ventral sucker round. Prepharynx distinct. Pharynx oval. Oesophagus short. Intestinal bifurcation in mid-forebody. Caeca form cyclocoel near posterior extremity, gives rise to single, dorso-subterminal anus. Testes two, oval, margins entire, tandem, in mid-hindbody. External seminal vesicle absent. Cirrus-sac oval. Internal seminal vesicle tubular, convoluted. Pars prostatica small, vesicular, indistinct. Ejaculatory duct narrow. Genital pore medial to sinistral in posterior forebody. Ovary oval, pretesticular. Canalicular seminal receptacle present. Laurer's canal present. Uterus preovarian. Eggs large, operculate. Vitellarium follicular, fields extend from mid-forebody to near posterior extremity. Excretory pore dorsally subterminal, opens close to anus. Excretory vesicle tubular, bifurcate, reaches anterior to pharynx. In marine teleosts (Pomacanthidae), Indo-West Pacific (Great Barrier Reef).

Type and only species: Enenterageitus huxleyi (Bray & Cribb, 2001) n. comb.

Etymology: The name for the proposed genus means 'isolated enenterid' and is formed from the name of the type-genus of the family, *Enenterum* (which itself is formed from the Greek words, *en* and *enterum*, =in intestine), combined with the Greek 'ageiton' (=isolated or solitary). The name is chosen because the genus is monotypic, and the type species is known from only a single non-kyphosid host species, which is geographically restricted. The genus is treated as masculine.

3.5.1. Enenterageitus huxleyi (Bray & Cribb, 2001) n. comb. (Fig. 7D and E)

Synonym: Koseiria huxleyi Bray & Cribb, 2001.

Type- and only known host: Chaetodontoplus meredithi Kuiter, 1990, Queensland yellowtail angelfish. (Perciformes: Pomacanthidae).

Type- and only known locality: off Heron Island, Great Barrier Reef, Australia (23° 27′S, 151° 55′E) (HI).

Material examined: Paratypes: 15 whole mounts, from intestine of *Chaetodontoplus meredithi*, off HI, coll. Barker & Adlard, 1998 (QM G217706–217720). Additional vouchers: one whole mount,

from intestine of *Chaetodontoplus meredithi*, off HI, coll. T. Cribb, 2014 (QM G238194). Hologenophores: one from intestine of *Chaetodontoplus meredithi*, off HI, coll. T. Cribb, 2014 (QM G238195); two from intestine of *Chaetodontoplus meredithi*, off HI, coll. S. Cutmore, 2017 (QM G238196–7).

Representative DNA sequences: ITS2: three identical replicates, all from above hologenophores; one replicate submitted to GenBank (**MN080855**). Partial 28S rRNA gene sequences: three identical replicates; all from above hologenophores; one replicate submitted to GenBank (**MN080864**).

Remarks: The new material which we examined, including the hologenophores from which molecular data were generated, were consistent with the description of this species by Bray and Cribb (2001). *Enenterageitus huxleyi* n. comb. was given a high-quality description previously by Bray and Cribb (2001) who provide measurements and a description of the morphology. In light of the molecular data and phylogenetic results, the unique host and morphological distinctiveness of this species warrant proposal of a new genus.

Some distinct features of *Enenterageitus huxleyi* are the terminal, weakly infundibuliform oral sucker, and the muscular post-oral ring. Among the Enenteridae, only the monotypic *Pseudozakia hatampo* Machida & Araki, 1977 shares a non-lobed, terminal, infundibuliform oral sucker, although it does not possess a muscular post-oral ring. Although still classed as an enenterid, the inclusion of *P. hatampo* in the Enenteridae family has been questioned (Brooks et al., 2000; Bray and Cribb, 2001, 2002; Bray, 2005); molecular data will be required to confirm its position. *Koseria nagatyi* has a bell-shaped oral sucker somewhat similar to that of *E. huxleyi*, but it is illustrated as being subterminal and the species also lacks a post-oral ring (Ahmad, 1984). Another distinct characteristic of *E. huxleyi* is the large scale-like spines of the tegument, quite unlike those of other enenterids which are small and fine when visible at all.

Enenterageitus huxleyi was found sister to, but distinct from, *Proenenterum* in our phylogenetic analyses. *Enenterageitus huxleyi* is also most similar to species of *Proenenterum* morphologically, but differs in having a terminal, infundibuliform oral sucker with a muscular post-oral ring rather than a subterminal, subglobular oral sucker; in having a medial, wide dorso-subterminal anus, rather than no anus, or a small, dorso-lateral anus (*P. allanwilliamsi*); in having a long prepharynx and distinct oesophagus rather than a short prepharynx and a very short oesophagus; in having distinct 'shoulders' on the anterior portion of the intestine; and, in having a bifurcate excretory vesicle which reaches anterior to the pharynx rather than a non-bifurcate vesicle which reaches only to the ovary. *Enenterageitus huxleyi* differs from the monotypic *P. hatampo* in having a cirrus-sac and an anus, both of which are absent in *P. hatampo*. From species of *Enenterum*, *Enenterageitus huxleyi* differs distinctly in lacking lobes on the oral sucker. Lastly, from *Koseiria*, *E. huxleyi* differs in having a terminal, infundibuliform oral sucker with a muscular post-oral ring, rather than a subterminal, subglobular or bell-shaped oral sucker with no post-oral ring.

4. Discussion

Transfer of *P. allanwilliamsi* and *E. huxleyi* from *Koseiria* allows refinement of the concept of the genus. However, the systematics of *Koseiria* continue to be plagued by three major issues: an incomplete understanding of host specificity, unequal molecular coverage, and strong morphological similarity between genetically distinct species. Taken together, these issues can be framed as essentially a cryptic species problem within a greater identification dilemma across the Indo-Pacific region.

Of the seven named species of *Koseiria* recognised here, only *K. manteri* is known from a non-kyphosid (Ahmad, 1984), and this species may yet prove to represent a distinct lineage as we found with *Enenterageitus huxleyi*. Prior to the present study, only *K. xishaensis* had been reported from a kyphosid other than *Kyphosus cinerascens* (although the type-host is *Kyphosus cinerascens*), and with the exception of *Koseiria tahmeli* (which includes an additional record in the form of a junior synonym), is the only species of *Koseiria* that has been reported more than once (Bray and Cribb, 2001).

Our evidence points to a pattern of distinct host specificity in species of *Koseiria*, and host identity certainly appears useful for delineating species of *Koseiria* in Australia. However, at present, the host-parasite combination data available are not sufficiently comprehensive to reach definitive conclusions about host specificity of species of this genus.

We found no host overlap between *K. laiphopharophora* and *K. pyknophora* at Lizard Island in robust samples of their respective hosts, *Kyphosus cinerascens* and *Kyphosus vaigiensis*. In Moreton Bay, we found *K. argalea* only in *Kyphosus bigibbus*, despite examining seven *Kyphosus cinerascens* from the same locality. On the other hand, our molecular data from Heron Island revealed an immature specimen of *K. pyknophora* in *Kyphosus bigibbus*, suggesting that *K. pyknophora* may not be specific to one fish host. This record is problematic, however, because larval trematodes might succeed in reaching a host, but fail to develop to maturity because that host is unsuitable (Poulin and Keeney, 2008). In lower latitudes, *Kyphosus bigibbus* and *Kyphosus vaigiensis* form mixed schools and have partially overlapping diets (Clements and Choat, 1997; Knudsen and Clements, 2013b). Thus, the metacercariae of *Koseiria pyknophora* (presumably found associated with algae) are likely ingested by *Kyphosus bigibbus* routinely, but we do not yet know if these worms are capable of reaching maturity in this host.

Species of *Koseiria* have been reported from the Red and South China Seas, off Hawaii, India, Japan and Palau, yet the only molecular data available come from specimens collected from off Australia (Olson et al., 2003; present study). Thus, we have no real understanding of the genetic variability between populations of these species over large ranges. Considering that three species of *Koseiria* were detected in eastern Australia alone, the lineage may be far more diverse than is presently recognised. Notably, Bray and Cribb (2001) recognised *Koseiria kyphosi* Yamaguti, 1970 as a junior synonym of *K. tahmeli* based upon strong morphological similarity and that both were described from *Kyphosus cinerascens*. However, *K. tahmeli* was described from the Red Sea, whereas *K. kyphosi* was described from Hawaii. These localities are on the extreme western and eastern ends of the Indo-West Pacific marine eco-region, respectively (Spalding et al., 2007), and this vast geographic distance may suggest that these two species are distinct from one another. The understanding that many presumably widespread trematodes are actually complexes of morphologically similar, or 'cryptic', species has become an established paradigm (Pérez-Ponce de León and Nadler, 2010; Nadler and Pérez-Ponce de León, 2011; Poulin, 2011; Pérez-Ponce de León and Poulin, 2018) and reports of cryptic species of trematode are accumulating quickly (e.g. Curran et al., 2013; Cribb et al., 2014; McNamara et al., 2014; Rima et al., 2017; Huston et al., 2018; Martin et al., 2018).

In the absence of a definitive understanding of patterns of host specificity, and without molecular data for any specimens of *Koseiria* collected outside of Australia, we have only morphological data for species delineation in this genus across the Indo-West Pacific. *Koseiria manteri*, *K. nagatyi* and *K. tahmeli* are certainly morphologically distinct from one another, and from the species of the *K. xishaensis* complex, but species within the *K. xishaensis* complex are very similar morphologically. In the strict sense, 'cryptic' refers to species which cannot be diagnosed based solely

on morphological characters, although it is often more loosely applied in reference to species which are simply difficult to identify with traditional systematic methods (Pérez-Ponce de León and Nadler, 2010; Nadler and Pérez-Ponce de León, 2011). When considered in isolation, the morphological data for species of *Koseiria* from eastern Australia is consistent with this 'loose' concept, but when the *K. xishaensis* complex is considered in full, a truly cryptic species problem emerges.

The type description of *K. xishaensis* was based on only a single specimen and is somewhat generalised, i.e. there are almost no qualitative aspects of the description which are useful for distinguishing *K. xishaensis* from the other species of *Koseiria* described in this study. We have been unable to locate the holotype; Gu and Shen (1983) provided no information as to where it was deposited, if at all, and although we have contacted likely institutions the specimen has not been found. Although we cannot examine the type-specimen, it is clear from the illustration provided by Gu and Shen (1983) that it was flattened. Flattening results in inconsistent preparation of morphological specimens between (and by individual) workers, who might apply varying amounts of pressure, and affect various anatomical features differently. For example, the oral and ventral suckers may not flatten in the same way, which skews normal sucker ratios, and flattening tends to push internal organs into atypical positions. Thus, in our view, flattened specimens are not suitable for taxonomic purposes, and instead specimens should be fixed without pressure (Cribb and Bray, 2010).

Because the type-specimen of *K. xishaensis* was flattened, it is impossible to be confident that comparisons between the qualitative features and reported measurements of this specimen and others collected in the Indo-Pacific reflect true metrical relationships rather than artefacts of the flattening process. Although we think it possible that one of the three species of *Koseiria* detected in eastern Australia is representative of the true *K. xishaensis*, the morphological data available are not sufficient to determine which of the three it is. We thus consider it unwise, at present, to associate the name *K. xishaensis* with any specimens other than those collected at the type locality.

Even among the non-flattened material examined in this study, not all specimens could be reliably diagnosed on a morphological basis, even when PCA was employed. PCA has been used to aid delineation of species in cryptic species complexes in a variety of taxa (e.g. Kappes and Sinsch, 2002; Schönrogge et al., 2002; Klimov et al., 2009; Prié and Bichan, 2009), but has not always been successful (e.g. Baker et al., 2003; Fontoura and Morais, 2011; Murphey et al., 2015). PCA of our dataset was successful in separating *K. laiphopharophora* from *K. pyknophora*, but when morphometric data was added from specimens from other localities, species boundaries became less clear. Notably, there were several specimens which could not be reliably assigned to any particular group, qualitatively, with traditional morphometrics, or in PCA. It appears then, that in the *Koseiria xishaensis* complex, species cannot always be diagnosed on a purely morphological basis. Thus, species of *Koseiria* found in eastern Australia can be considered truly cryptic.

At present, molecular data are the only reliable basis for distinguishing species in this complex, but such data are available for only a few individuals from Australia. To obtain a proper understanding of parasite host specificity, systematics, biogeography and evolution, and to be able to quantify biodiversity in general, cryptic species of parasites need to be described and named (Cook et al., 2010; Pérez-Ponce de León and Nadler, 2010; Nadler and Pérez-Ponce de León, 2011; Jörger and Schrödl, 2013; Delić et al., 2017). However, delineation and description of cryptic species in situations such as that encountered here present both logistical and philosophical challenges.

We noted previously that among the species described here, *K. laiphopharophora* was closest morphologically to the description of *K. xishaensis*, and shares the same host, but we do not think it wise to report these specimens as *K. xishaensis*, while describing the other two as new. This would be little more than a 'best guess', based on general morphometric similarities and a shared host. We could refrain from naming any of the species collected in Australia, but this is unsatisfactory as not giving names to what we recognise as distinct species hinders efforts to improve understanding of parasite biodiversity, biogeography, co-phylogeny and systematics and would require future workers to deal with less informative species 'labels' (Pérez-Ponce de León and Nadler, 2010; Jörger and Schrödl, 2013). Ultimately, we conclude that describing the three species as new is the least problematic option. The molecular data that we have generated and lodged on GenBank will facilitate rapid synonymy of one of these newly described species with *K. xishaensis* if needed. Although our solution does not resolve the identity crisis associated with *K. xishaensis*, we think it the best compromise between the needs of taxonomic stability and the imperative for rapid characterisation of Earth's biodiversity before it is lost.

A detailed morphological description is desirable even for cryptic species, because it is evidence of the features studied and demonstrates that the species belongs to the higher taxonomic group in which it is placed (Cook et al., 2010; Jörger and Schrödl, 2013; Delić et al., 2017). To determine which specimens should be added to the type series for each of the species described herein, we sought to ensure conspecificity while encompassing as much morphological variability as possible. With the benefit of hindsight from molecular data, but in the absence of such data for all specimens of *Koseiria* examined, PCAs were useful for aiding the selection of specimens included in the type series for each new species. We centred our species hypotheses on hologenophores and paragenophores (whole mounts which came from infrapopulations from which molecular data had been obtained). We then expanded our morphological concept based on iterative refinements informed by PCA. These refinements resulted in morphological concepts for *K. argalea*, *K. laiphopharophora* and *K. pyknophora* which were distinguishable from one another. This approach did not, however, permit taxonomic hypotheses for all specimens examined, and we consider multiple specimens as unidentifiable. These results are not fully satisfactory, but with the paucity of data available for the genus *Koseiria* as a whole, are the best that can be achieved presently.

Determining how deep to delve when attempting to delineate species of trematodes in cryptic complexes requires consideration of the large-scale goals of systematics, the need for a complete inventory of global biodiversity and, prosaically, the project's budget. Taxonomists can certainly spend as long as they like testing multiple methods of species delineation; this can result in better species concepts and better understanding of the evolution of the group being studied. However, dwelling on cryptic species problems for too long may be counterproductive in the context of the goals listed above, by tying the hands of taxonomists who might otherwise describe further species (Jörger and Schrödl, 2013). It is thought that at the current rate of ~15,000 new species being described each year, 300–500 years will be required to describe all of Earth's diversity (Carbayo and Marques, 2011; Mora et al., 2011). At the same time it has been suggested that we are unlikely to describe most species before they become extinct (Pimm et al., 1995; Dirzo and Raven, 2003). It is important then, to keep the overall goals in perspective when faced with a problem such as that encountered in this study. Although time and resources do not yet allow a fully satisfactory conclusion to the story of *Koseiria* in the Indo-Pacific, the present work demonstrates yet another digenean lineage to be more diverse and species rich than

previously thought, contributing to our overall understanding of global biodiversity and species interactions.

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