A new microbothriid monogenean *Dermopristis pterophilus* n. sp. from the skin of the Critically Endangered green sawfish *Pristis zijsron* Bleeker, 1851 (Batoidea: Pristidae) in Western Australia

Jack Ingelbrecht [*], David L. Morgan, Karissa O. Lear, Travis Fazeldean, Alan J. Lymbery, Bradley M. Norman, Storm B. Martin

Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University, 90 South Street, Murdoch, WA, 6150, Australia

ABSTRACT

A new microbothriid monogenean *Dermopristis pterophilus* n. sp. is described from the skin of the Critically Endangered green sawfish *Pristis zijsron* Bleeker, 1851 in the Ashburton River delta, northern Western Australia. Analyses of the 28S ribosomal DNA marker and the molecular barcoding markers Histone 3 and Elongation Factor 1 α confirmed position among the Microbothriidae, with close affinity to the only other sequenced representative of *Dermopristis* Kearn, Whittington and Evans-Groing, 2010. The new species is morphologically consistent with the concept of *Dermopristis*; it has two testes, lacks a male copulatory organ and has a simple haptor. It is smaller than its two congeners *D. paradoxus* Kearn, Whittington and Evans-Gowing, 2010 and *D. cairae* Whittington and Kearn, 2011 and is most similar to the former, distinguished only in that it lacks the strong, transverse, parallel ridges on the ventral body surface that characterise that species. It is more easily distinguished from *D. cairae*, differing in body shape, possession of a seminal receptacle, and relative position and size of the haptor. It may further differ from both species by fine details of the gut diverticula, although these details are difficult to ascertain. Spermatophores were observed in the new species, similar to those previously reported for *D. cairae*. The new species exhibits site attachment preference: infections were greatest on and immediately adjacent to the host pelvic fins (including male reproductive organs, i.e. claspers), moderate in proximity to the dorsal and pectoral fins, few on the caudal fin and peduncle, and infrequently, isolated worms occurred elsewhere on the dorsal and ventral surfaces of the body. There was no incidence of infection on the head (including rostrum). We presume *D. pterophilus* is restricted to *P. zijsron* and thus likely faces the same threat of extinction.

1. Introduction

The sawfishes (Batoidea: Pristidae) are a small group of large, charismatic and vulnerable elasmobranchs. All five extant species currently recognised within the family (Faria et al., 2013) are designated as Endangered or Critically Endangered by the International Union for the Conservation of Nature, and continue to face significant threats throughout much of their distributions (Dulvy et al., 2016). Northern Australia is a stronghold for four of the five sawfish species, accounting for roughly half of the protected area across their combined ranges (Thorburn et al., 2007; Morgan et al., 2011, 2015, 2017, 2021; Dulvy et al., 2016). Effective conservation of sawfishes may substantially improve outcomes for a myriad of other species via flow-on protection.

The species to indirectly benefit most are likely those most intimately associated with the sawfishes: their parasites. Conversely, host-specific parasites exploiting, and thus reliant upon, threatened hosts like sawfishes are themselves inherently at risk of extinction.

Microbothriid monogeneans (=monogenoids) are a family of parasitic flatworms that attach to the skin of elasmobranchs with a hookless, unsclerotised haptor (Kearn, 1965; Whittington and Chisholm, 2008). At present, the Microbothriidae is comprised of 22 species from 12 genera, with the family best represented in waters of Australia, New Zealand, and the United States of America. The microbothriid genus *Dermopristis* Kearn, Whittington and Evans-Groing, 2010 currently comprises two species known only from Queensland waters, *D. paradoxus* Kearn et al., 2010 from the largetooth sawfish *Pristis pristis* Linnaeus, 1758 (as *P.*

---

* Corresponding author.
E-mail address: jack.ingelbrecht@murdoch.edu.au (J. Ingelbrecht).

Fig. 1. *Dermopristis pterophilus* n. sp. attached to the skin adjacent to the base of left pectoral fin of juvenile green sawfish *Pristis zijsron* Bleeker, 1851 (photograph DLM, 2011).

microdon), and *D. cairae* Whittington and Kearn, 2011 from the giant shovelnose ray *Glaucostegus typus* [anonymous (Bennett, 1830)] (Kearn et al., 2010; Whittington and Kearn, 2011). Species of *Dermopristis*, *Dermophthirius* MacCallum, 1926 and *Dermophthirioides* Cheung and Nigrelli, 1983 are easily distinguished from other microbothriids by possession of two testes, but species of *Dermopristis* are further differentiated, among all microbothriids, by lacking a male copulatory organ (Kearn et al., 2010; Whittington and Kearn, 2011). Species of *Dermopristis* have a large voluminous male reproductive tract and Whittington and Kearn (2011) discovered spermatophores attached to the external ventral surface in specimens of *D. cairae*, adjacent to the vagina and male pore.

While surveying juvenile green sawfish *Pristis zijsron* Bleeker, 1851 in the Ashburton River delta, Western Australia, and nearby tidal creeks and lagoons (see Morgan et al., 2015, 2017), numerous ectoparasitic flukes were found attached to the skin denticles (Fig. 1). Parasites of *P. zijsron* are largely unknown, although two of the five species of sawfish, the smalltooth sawfish *Pristis pectinata* Latham, 1794 and the largetooth sawfish *P. pristis*, are known hosts of microbothriids (Cheung and Nigrelli, 1983; Kearn et al., 2010), and the parasite fauna of the former has been previously characterised (Bakenhaster et al., 2018). Samples of these ectoparasitic flukes were collected and are proposed to be a new species of microbothriid.

2. Materials and methods

2.1. Ethical clearance

Handling and sampling of sawfish was conducted under Murdoch University Animal Ethics Approval: RW2397/11 and RW3191/19, Western Australian Government Department of Primary Industries and Regional Development (DPIRD) fisheries exemption no. 3378 and 3553, Department of Fisheries Regulation 178 (SPA 11-11), Department of Environment and Conservation Permit SF007889.

2.2. Host and parasite collection

Targeted sampling for *P. zijsron* occurred during April and October 2011, October 2019, October and December 2020, and April and October 2021. Sample sites included the Ashburton River mouth and

nearby tidal creeks of the Onslow region, Western Australia (see Morgan et al., 2017). Sawfish were collected with monofilament gillnets using methodologies detailed in Morgan et al. (2015), and upon capture, were held on their backs in the extreme shallows with their gills submerged, inducing a state of tonic immobility. Examinations for ectoparasites were conducted first on the ventral surface and then the dorsal surface, after which sawfish were righted before release. Parasite attachment sites were recorded according to general body location: first and second dorsal fins (grouped together), pectoral fins, pelvic fins (including the claspers, male reproductive organs), caudal fin (including caudal peduncle), general dorsal body surface, general ventral body surface, and head (anterior to host's gills, including rostrum). Dorsal and ventral body surface categories describe isolated infections not in the immediate vicinity of any fins and excluding the head and rostrum. Parasites were removed using forceps and immediately preserved in either 100% ethanol or 10% formalin, allowing for both genetic sequencing and morphological study.

2.3. Morphological study

Most specimens used in morphological analyses were examined as uncleared and unstained wet mounts, in absolute ethanol. Specimens initially preserved in formalin were later transferred to absolute ethanol using a graded ethanol series: 40, 60, 75, 100, and 100%, at approximately 1 h per stage. Selected specimens were cleared, stained and mounted in Canada balsam. Several approaches were attempted: some specimens were cleared in lactophenol and mounted unstained; others were stained with Semichon's acetocarmine, de-stained in hydrochloric acid, and dehydrated in a graded ethanol series (70, 90, 95, 100, and 100%); one of these was cleared in Hoyer's solution prior to staining and the others in methyl salicylate after dehydration. The anatomies of mounted specimens were examined and photographed using an Olympus BX50 compound microscope, with Nomarski interference contrast, fitted with an Olympus DP71 digital microscope camera and U-CMAD3 adaptor (Olympus Inc., Tokyo, Japan). Morphometric data from wholemounts were collected with the same microscope and camera, using the measurement function of the Olympus platform cellSens standard imaging software. Dimensions from wet mounts were taken using an Olympus SZX7 stereo microscope fitted with an Olympus DP27 digital microscope camera and cellSens. All measurements were taken
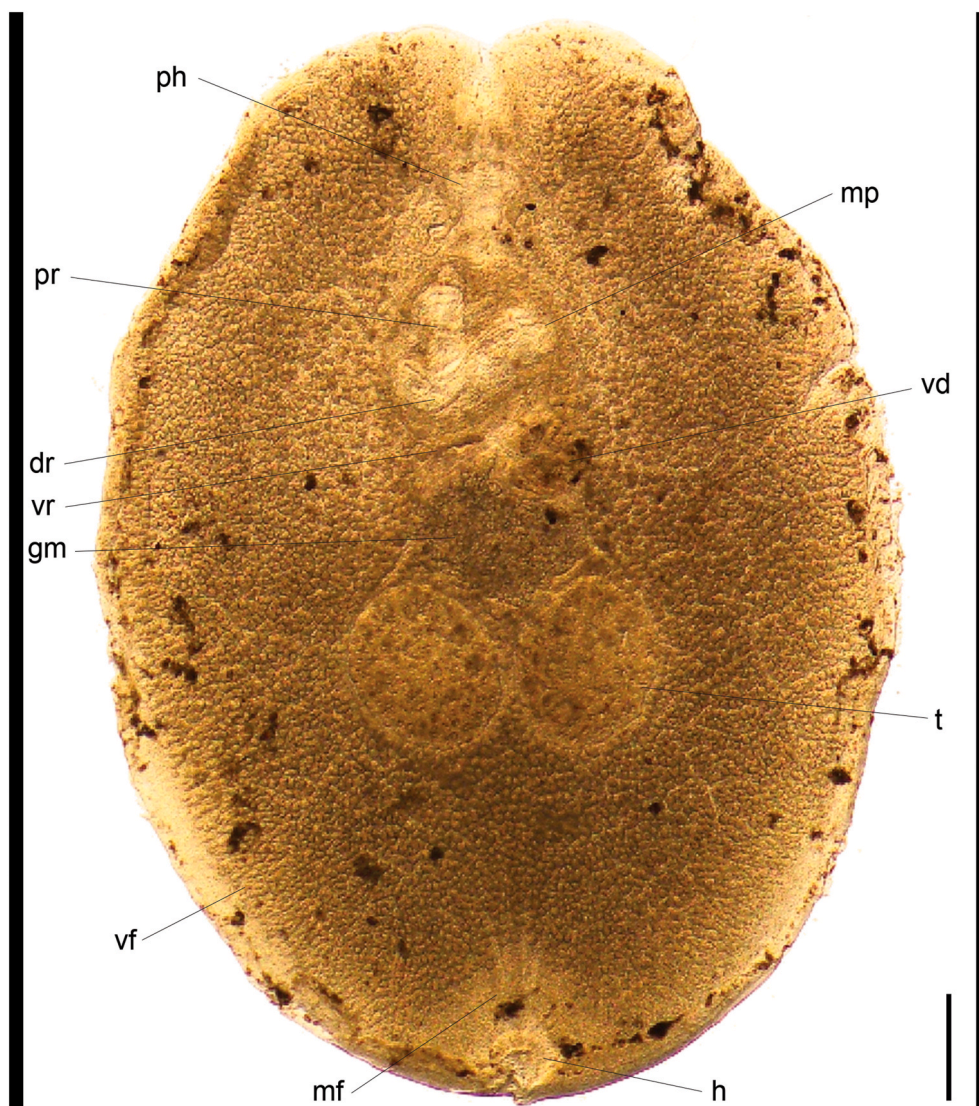
Fig. 2. *Dermopristis pterophilus* n. sp. holotype, ventral perspective. Abbreviations: dr, distal region of tubular male reproductive tract; gm, germarium; h, haptor; mf, muscle fibres; mp, male pore; ph, pharynx; pr, proximal region of tubular male reproductive tract; t, testis; vd, vas deferens; vf, vitelline follicles; vr, vitelline reservoir. Scale bar: 500 μm.

from the ventral perspective. Line drawings were made with a drawing tube attached to an Olympus BHA phase contrast compound microscope and digitised in Adobe Illustrator CS6. Type material has been deposited with the Crustacea and Worms collection of the Western Australian Museum (WAM). To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2021), details of the new taxon have been submitted to ZooBank; the Life Science Identifier (LSID) is reported in the taxonomic summary.

2.4. Molecular sequencing

Partial sequence data were generated for 28S rDNA, Histone 3 (H3) and Elongation Factor 1 α (EF1α). Genomic DNA was extracted from eight hologenophores using a QIAGEN DNeasy Blood & Tissue Extraction Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The three target marker regions were amplified by PCR with the following primer sets: C1/D2 for 28S rDNA (~900 bp), H3aF/H3R2 (~350 bp) and G926/G927 (~300 bp) for H3, and G959/G960 (~800 bp) and G1050/G1051 (~720 bp) for EF1α. Primers and denaturation-annealing-extension cycles are detailed in Chisholm et al. (2001) and

Perkins et al. (2009); the G959/G960 primer combination for EF1α failed to yield viable amplicons. Genetic sequence data were produced by the Western Australian State Agricultural Biotechnology Centre, Murdoch University. Contiguous sequences were constructed and examined for intragenomic polymorphisms in Geneious v.9.1.4 (Kearse et al., 2012). GenBank (GB) accession numbers for novel sequences are provided in the taxonomic summary. Genetic sequence data for each target marker were compared against all comparable data from microbothriids publicly available on GenBank. Data were aligned using MUSCLE v.3.8.31 (Edgar, 2004) in MEGA v.11 (Tamura et al., 2021) with a ClustalW sequence weighting and the UPGMB clustering algorithm for iterations 1 and 2.

2.5. Data analyses

Attachment site preference was investigated using R (R core team, 2021), by comparing the number of worms found on or adjacent to major features of host external anatomy: first and second dorsal fins (grouped together), pectoral fins, pelvic fins (including the claspers, male reproductive organs), caudal fin (including caudal peduncle), general dorsal body surface, general ventral body surface, and head
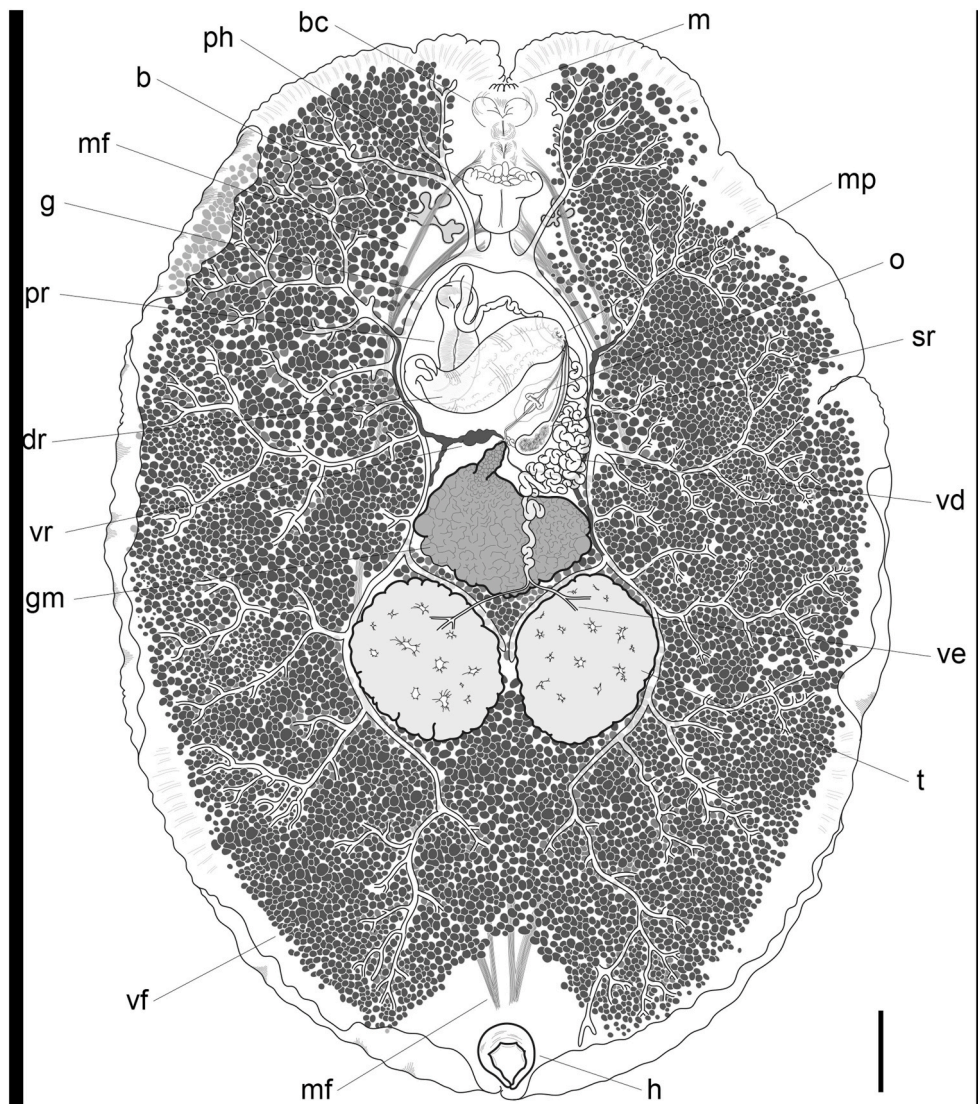
Fig. 3. *Dermopristis pterophilus* n. sp. holotype, ventral perspective. Abbreviations: b, bladder; bc, buccal cavity; dr, distal region of tubular male reproductive tract; g, gut; gm, germarium; h, haptor; m, mouth; mf, muscle fibres; mp, male pore; o, oötype; ph, pharynx; pr, proximal region of tubular male reproductive tract; sr, seminal receptacle; t, testis; vd, vas deferens; ve, vasa efferentia; vf, vitelline follicle; vr, vitelline reservoir. Scale bar: 500 μm.

(including rostrum). Dorsal and ventral body surface categories describe isolated infections not in the immediate vicinity of any fins. No correction was applied to control for discrepancy in available surface area between sites, as this information was not available. Differences in mean parasite abundance between attachment sites were compared using a bootstrap test with 2,000 bootstrap replications, implemented in QPweb v1.0.15 (Reiczigel et al., 2019). Ninety five percent confidence intervals (CIs) for mean parasite abundance at each attachment site were calculated using a resampling method with 2,000 bootstrap replications.

3. Results

3.1. Molecular results

No intragenomic polymorphisms were detected in generated molecular data for any of the three targeted markers. Data for the 28S rDNA and EF1α marker regions were most similar to that of *Dermopristis cairae*, differing by four and 123 base positions, respectively. However, data for H3 were more similar to that of *Dermophthirius penneri* Benz, 1987 than that of *Dermopristis cairae*, differing by seven *vs* 46 base-positions, respectively. No sequence data are publicly available for *Dermopristis*

paradoxus, nor any other species of *Dermophthirius* or *Dermophthirioides*.

3.2. Taxonomy

Family: Microbothriidae Price, 1936.
Subfamily: Microbothriinae Yamaguti, 1963.
Genus: *Dermopristis* Kearn, Whittington and Evans-Gowing, 2010.

3.2.1. Dermopristis pterophilus Ingelbrecht, Morgan and Martin n sp

3.2.1.1. Taxonomic summary. Type-host: *Pristis zijsron* Bleeker, 1851 (Batoidea: Pristidae), green sawfish.
   Type-locality: Ashburton River mouth (21°41′38″ S, 114°55′01″ E).
   Other localities: Hooley's Lagoon (21°40′35″ S, 114°59′06″ E), Hooley's Creek (21°41′08″ S, 115°02′08″ E), and Four Mile Creek (21°40′59″ S, 115°03′22″ E), Onslow region, Western Australia.
   Site of infection: Attached to skin, overwhelmingly on or immediately adjacent to fin bases, especially pelvic fins (including genital area), moderately so on pectoral and dorsal fins, less so around caudal fin, isolated infections elsewhere on dorsal and ventral body surface, no infections anterior to gills on the head or rostrum.
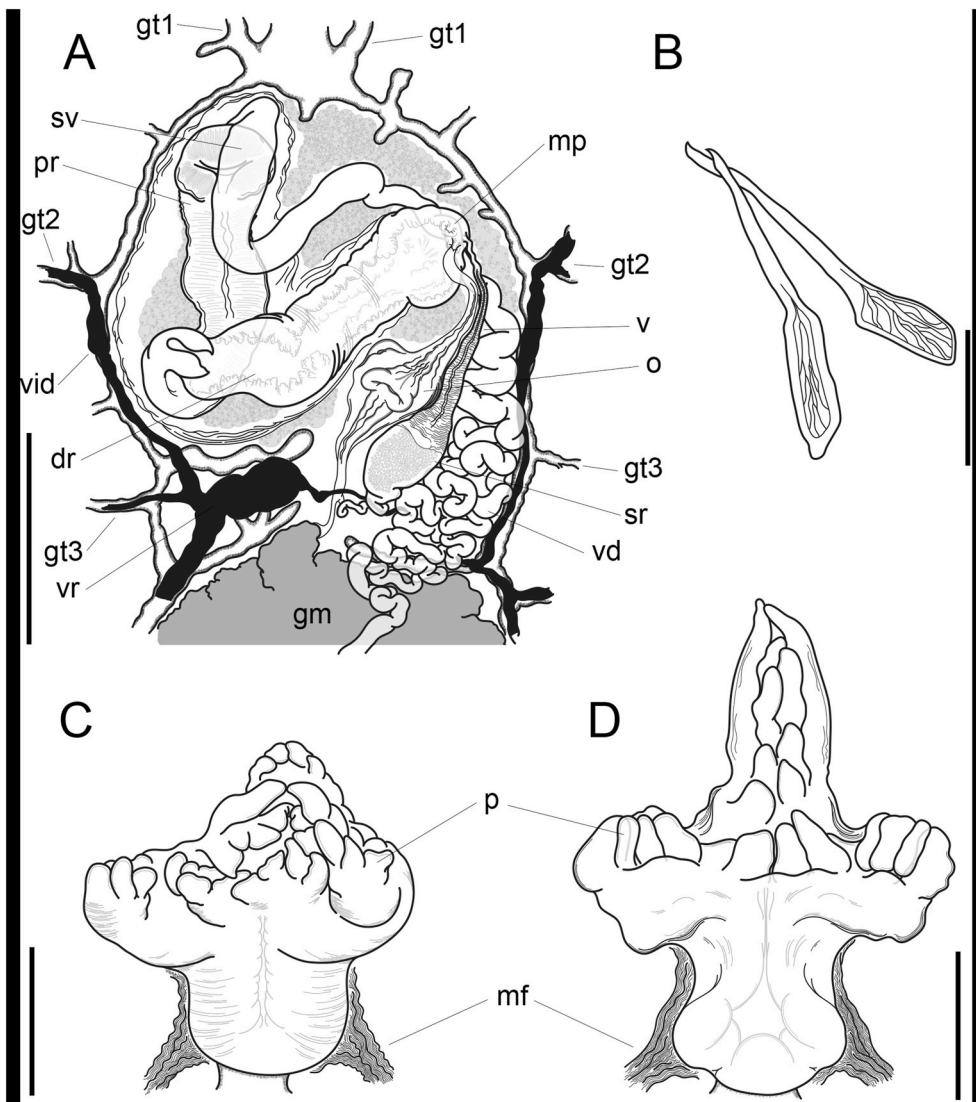
Fig. 4. *Dermopristis pterophilus* n. sp. various soft parts, ventral perspective. (A) Reproductive system (excluding testes). (B) Spermatophores. (C & D) Pharynx with papillae retracted vs extended. Abbreviations: dr, distal region of tubular male reproductive tract; gm, germarium; gt1–3, first through third major gut sub-trees; mf, muscle fibres; mp, male pore; o, oötype; p, digitiform papillae; pr, proximal region of tubular male reproductive tract; sr, seminal receptacle; sv, seminal vesicle; v, vagina; vd, vas deferens; vid, vitelline duct; vr, vitelline reservoir. Scale bars: (A) 250 μm; (B) 300 μm; (C & D) 100 μm.

Prevalence and intensity: 16 of 26 *P. zijsron* (62%); mean intensity 4.81 (95% CI 1–13).

Type-specimens: Holotype WAM V10840 (ventral wholemount, lactophenol) collected April 25, 2021 on a female *P. zijsron* 2524 mm total length (TL), in the Ashburton River mouth. Nine paratypes WAM V10841–10849 (nine adults): V10841 (ventral wholemount, Semichon's acetocarmine, methyl salicylate) and V10842 (wet mount) collected December 17, 2020 on a male *P. zijsron* of 751 mm TL (Hooley's Lagoon, Ashburton River delta), V10843 (wet mount) collected April 25, 2021 on a female *P. zijsron* of 1266 mm TL (Ashburton River mouth), V10844–10849 (V10844-10847 wet mounts; V10848 ventral wholemount, Semichon's acetocarmine, methyl salicylate; V10849 ventral wholemount, Hoyer's solution, Semichon's acetocarmine) collected April 25, 2021 on a male *P. zijsron* of 2595 mm (Ashburton River mouth). Material collected by DLM, TF and KOL.

Representative DNA sequences: Five identical replicates of partial 28S rDNA (GB OM320818), and two identical replicates each of H3 (GB OM320819) and EF1α (GB OM320820).

ZooBank registration: The LSID for *D. pterophilus* is: lsid:zoobank.org:act:BCB37141-9764-42C9-BCD7-3AA7E491DA96.

Etymology: The specific epithet *pterophilus* is a compound masculine adjective from Greek πτερον, *pteron* (wing) and φίλος, *philos* (having affinity for), after the affinity of this parasite to attach proximal to the host fins.

3.2.1.2. Description. Based on seven adult ventral wholemounts including one holotype (Fig. 2, Fig. 3) and three paratypes; WAM V10840 and V10841, V10848 and 10849 (Fig. 4, Fig. 5). Whole animal dimensions based on seven wholemounts and 35 wet mounts, organ dimensions based on three to seven wholemounts and, for germarium, also one wet mount. Measurements are in micrometres (μm) with length followed by width, and range followed by mean in parentheses.

Body dorsoventrally flattened, oval to almost round, broadest at level of gonads in mid-body, longer than wide with length 1.02–1.69 (1.26) times width, 2456–5570 (3863) × 2114–4521 (3085). Tegument without strong, transverse, ventral ridges. Haptor small, almost terminal, roughly circular, 124–332 (228) × 163–297 (209); inner cavity 55–172 (124) × 63–179 (105); sclerites absent; no host denticles observed. Eyes absent. Mouth anterior, sub-terminal, inconspicuous; buccal cavity deeply infolded. Pharynx prominent, somewhat cruciform polypoid, with approximately 14–22 anterior, apparently retractable, digitiform papillae projected into lumen. Prominent muscle fibres apparently associated with pharynx run posterolaterally length of body to near haptor. Isolated, irregular bladder either side of pharynx. Oesophagus absent or indiscernible from gut.

Gut dendritic, blind, thin, unpigmented, sometimes inconspicuous or obscured by vitellarium, bifurcates posterior to pharynx into roughly equal gut-trees; main arms gently sinusoidal, run posteriorly either side of and constrain gonadal and terminal genitalia zones; major gut sub-
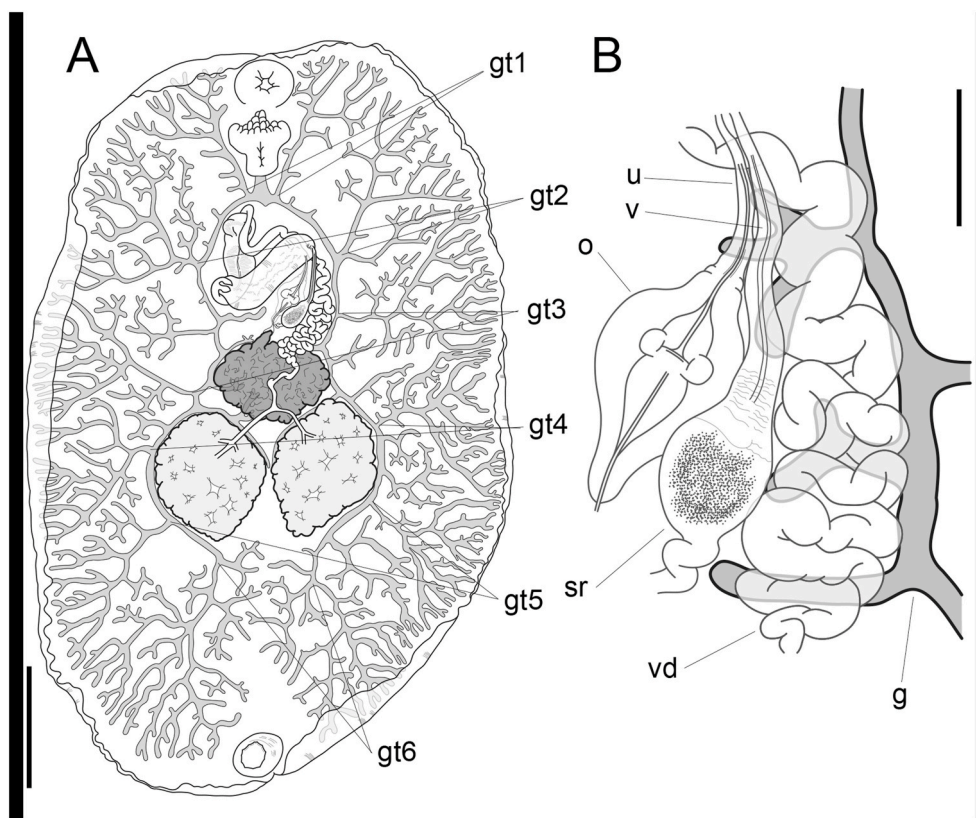
Fig. 5. *Dermopristis pterophilus* n. sp. detail of dendritic gut, ventral perspective. (A) Extensiveness and pattern of gut diverticula with position of the major gut sub-trees extending from each side of the main lateral gut arms. (B) Fine details of minor gut diverticula dorsal to the coiled vas deferens. Abbreviations: g, gut; gt1–6, first through sixth major gut sub-trees; o, oötype; sr, seminal receptacle; u, uterus; v, vagina; vd, vas deferens. Scale bars: (A) 500 μm; (B) 100 μm.

trees six, arise from main gut arms, extend outwards to edge of vitelline follicle field near to body margin, collectively fill much of available body area; first major sub-tree arises immediately after main gut bifurcation, runs anteriorly either side of pharynx; second major sub-tree arises at about level of male genital pore; third major sub-tree arises anterior to germarium; fourth major sub-tree arises at about level to first third of testes; fifth major sub-tree arises at about level to second third of testes; sixth major sub-tree continues from posterior end of main gut arm, extends posteriorly; minor gut sub-trees (not reaching edge of vitellarium near to body margin) arise between second and third, third and fourth, and fifth and sixth major sub-trees; inward gut diverticula include one long diverticulum either side protruding between germarium and either testis and reaching posteriorly to midway along inner testis margin and meeting (but not joining) medially, and two lateral diverticula anterior to germarium on either side, with or without minor branching, reaching close to midline, on right associated with right vitelline reservoir, on left dorsal to vas deferens; minor protrusions from left main gut arm invade inwards into terminal genitalia zone between first and second major sub-trees; no diverticula invading testicular material observed.

Testes two, opposite, with crenulated margins, situated at about second third of body, roughly of equal size; left testis 391–862 (606) × 298–821 (548); right testis 399–798 (598) × 339–892 (623); testicular muscle columns prominent, numerous. Post-testicular zone 23–38 (31) % of body length, 686–1770 (1211) long. Vasa efferentia narrow, arise from ventral testicular surface, multiple and apparently unequal (three associated with right testis and two with left in holotype, Fig. 3), short, connect to form a single duct which connects with vas deferens at level of posterior margin of germarium. Vas deferens prominent, proximal part densely coiled between germarium and genital pores, constrained laterally between vagina and left main gut arm, crosses midline dorsal to vagina, uterus, and distal tubular region of male tract, broadens distally to form thin-walled seminal vesicle immediately prior to proximal end of

tubular male reproductive tract. Male reproductive tract tubular with thick walls and voluminous lumen, apparently constrained by thin membranes, surrounded by gland cells, bipartite: proximal part glandular, dextro-submedial, roughly longitudinal, 303–631 (509) × 112–256 (160); distal part tubular, semi-transverse, crosses midline, similar size to proximal part, 334–751 (593) × 87–292 (185) (Fig. 4). Male pore ventral, sinistro-submedial, enclosed by main gut arms. Male copulatory organ apparently absent; no sclerites associated with terminal male genitalia. Spermatophores present in five specimens, four in one (WAM V10849), two in one (WAM V10848; subsequently dislodged during mounting), and one in three (not lodged, observed from wet mounts), fusiform capsule, attached to ventral surface adjacent to genital pores; capsule (excluding stalk) 270–374 (338) × 50–62 (56).

Germarium medial, in mid-body, anterior to and of similar size to testes, roughly rhomboid, with lobulated margin, tapers anteriorly to give rise to oviduct, 244–729 (491) × 344–983 (714). Vagina singular, sinistro-submedial, apparently opens close to male pore, widens proximally to form seminal receptacle. Seminal receptacle oval, sinistro-submedial, anterior to germarium, 124–244 (183) × 54–150 (102). Vitellarium extensive, comprised of two fields of follicles; fields separate anterior to germarium, confluent posterior to germarium, united by transverse ducts; vitelline reservoirs two, anterior to germarium, right always prominent, left sometimes inconspicuous or obscured by vas deferens, give rise to branched vitelline ducts; vitelline ducts apparently follow gut closely, extend anteriorly only to second major gut sub-tree; vitelline follicles small, compact, dense, dispersed extensively throughout body, extend near to body margins, excluded from around pharynx, mouth and haptor, excluded from zone between main gut arms anterior to germarium. Oviduct short, simple, roughly medial. Oötype prominent, tetrahedral, sinistro-submedial, adjacent and similar in size to seminal receptacle, 126–345 (261) × 70–161 (109). Uterus short, simple, continues anteriorly from oötype alongside vagina, apparently

Table 1

Mean length and width (µm) of main anatomical features of *Dermopristis pterophilus* n. sp., based on up to seven wholemounts and 35 wet mounts, as well as for *Dermopristis paradoxus* (up to six wholemounts) and *Dermopristis cairae* (up to four wholemounts). Wet mounts used for whole-body dimensions, germarium dimensions for a single specimen, and post-testicular zone. Relative dimensions taken from published drawings or photographs denoted by an asterisk (from Kearn et al., 2010; Whittington and Kearn, 2011). Abbreviations: BL, body length; MR, tubular male reproductive tract; PTZ, post-testicular zone; SPM, spermatophore. MR to testes is inclusive distance from MR (anterior) to testes (posterior).

Feature	*D. pterophilus* [n]	*D. paradoxus* [n]	*D. cairae* [n]
Body length	2456–5570 (3863) [42]	4091–6076 (4986) [6]	5174–6257 (5934) [3]
Body width	2114–4521 (3085) [42]	3489–5189 (4296) [6]	5354–7069 (6527) [3]
Body length/width	1.02–1.69 (1.26) [42]	1.17–1.17 [2] (1.16) [6]	0.88–0.97 [2] (0.91) [3]
Haptor outer length	124–332 (228) [5]	372–434 (388) [4]	722*
Haptor outer width	163–297 (209) [5]	337–396 (378) [5]	644–813 (748) [3]
Haptor inner length	55–172 (124) [5]	–	361*
Haptor inner width	63–179 (105) [5]	–	288–438 (359) [3]
Pharynx length	231–524 (365) [7]	345–517 (423) [6]	470–635 (572) [4]
Pharynx width	197–468 (321) [7]	365–463 (422) [6]	470–690 (601) [4]
Testis left length	391–862 (606) [6]	627–1113 (903) [6]	625–1075 (841) [4]
Testis left width	298–821 (548) [6]	588–963 (752) [6]	850–1188 (1036) [4]
Testis right length	399–798 (598) [6]	627–1113 (903) [6]	625–1075 (841) [4]
Testis right width	339–892 (623) [6]	588–963 (752) [6]	850–1188 (1036) [4]
PTZ/BL %	23–38 (31) [42]	32*	7*
MR proximal length	303–631 (509) [7]	979*	744*
MR distal length	334–751 (593) [7]	1277*	1279*
MR to testes/BL %	44–46 (45) [4]	42*	36*
Germarium length	244–729 (491) [8]	511*	605*
Germarium width	344–983 (714) [8]	766*	1209*
SPM capsule length	270–374 (338) [3]	–	450–500 (468*) [5]
SPM capsule width	50–62 (56) [3]	–	149–212 (182) [5]

opens adjacent to male pore and vaginal pore. Eggs not observed in any specimen.

3.2.1.3. Remarks. The new species, *Dermopristis pterophilus,* is recognisable as a microbothriid by its simple haptor, and as a species of *Dermopristis* because it has two testes and lacks a male copulatory organ; it is entirely consistent with the revised concept of that genus provided by Whittington and Kearn (2011). *Dermopristis pterophilus* bears strong resemblance to both previously described species of *Dermopristis*: *D. cairae* and *D. paradoxus* (see Table 1). It is smaller than both those species and can otherwise be reliably distinguished from *D. paradoxus* only in that it lacks the strong, transverse, ventral tegumental ridges characteristic of that species. It is more easily distinguished from *D. cairae* by its oval body longer (3863 ± 110 µm) than wide (3085 ± 91 µm) *vs* an inverted heart-shaped body wider than long; presence *vs* absence of a seminal receptacle; and the position (terminal *vs* subterminal) and smaller size of the haptor relative to the body (Table 1). The new species also differs from *D. paradoxus* and *D. cariae* in two fine details of the gut diverticula. First, the gut does not entirely encircle the testes as in both those species, and no diverticula were observed invading the testicular material, whereas these invasions were obvious

ventrally in *D. cairae* and discovered dorsally following targeted sectioning in *D. paradoxus* (Whittington and Kearn, 2011). Second, a minor inward diverticulum of the gut dorsal to the proximal coils of the vas deferens is present but simple, with only two or three short branches in the new material (Fig. 5) *vs* comparatively dendritic with multiple fine branches in *D. cairae*.

3.3. Preferred infection site

There was a significant difference in mean number of *D. pterophilus* infections on *P. zijsron* between attachment sites (p = 0.005), without correcting for site surface area. Number of infections was highest on or immediately adjacent to the pelvic fins (mean = 1.94; 95% CI = 1.19–3.56), whereas moderate infections were recorded on, or adjacent to, the dorsal fins (mean = 1.06; 95% CI = 0.50–1.69) and pectoral fins (mean = 1.19; 95% CI = 0.69–1.75). No infections were not recorded on head anterior to the gill slits or spiracles, or on the rostrum. Low intensity of infections was found on the caudal fin and peduncle (mean = 0.13; 95% CI = 0–0.25), as well as those isolated elsewhere on either the dorsal (mean = 0.31; 95% CI = 0–0.88) or ventral (mean = 0.19; 95% CI = 0–0.5) body surfaces (Fig. 6).

4. Discussion

Dermopristis pterophilus is the first microbothriid monogenean recorded from the green sawfish *Pristis zijsron*. The three species of *Dermopristis* are each known from only a single host-locality combination. Monogeneans have direct lifecycles and, although all sawfish examined here were presumed to be juveniles (see Morgan et al., 2015), *D. pterophilus* likely exploits *P. zijsron* at any stage of host development. We might therefore presume the distribution of *D. pterophilus* and other species of *Dermopristis* simply mirror that of their hosts. This may well be the case; however, two complicating factors are worth consideration. First, sawfishes have experienced substantial and rapid declines across much of their distributions over the past century (Dulvy et al., 2016), likely limiting connectivity and thus potentially causing local extinctions of supported parasites. Second, sawfishes are euryhaline (Thorburn et al., 2007; Peverell, 2010; Kyne et al., 2013; Simpfendorfer, 2013), and so the distribution of their monogenean parasites might be relatively restricted due to barriers imposed by considerable environmental gradients (see Morgan et al., 2010).

The site of infection varies between the three species of *Dermopristis*. All species attach to the skin, but *D. paradoxus* is found anterior to the gills, particularly around both the mouth and nasal fossae of *P. pristis* (Kearn et al., 2010), whereas for *D. pterophilus*, infections were found exclusively posterior to the gills. The infection sites for *D. cairae* are less clear; apparently the dorsal body surface and in the nasal fossae of *G. typus* (Whittington and Kearn, 2011). The overwhelming majority of *D. pterophilus* were found attached on (or immediately adjacent to) the fins of *P. zijsron*; only 10.4% (eight specimens) were found attached to the dorsal or ventral body surfaces not in the immediate vicinity of the fins, and none was found anterior to the gill slits on the head or rostrum. Some other monogeneans exhibit similar specificity for host fins, including a species of *Gyrodactylus* von Nordmann, 1832 (Gyrodactylidae) (Chen et al., 2020) and several benedeniines (Capsalidae) (Whittington and Kearn, 1993; Whittington and Horton, 1996). In evaluating site attachment preferences, our analysis did not correct for discrepancies in the surface area available between sites, e.g., the pectoral fins and dorsal fins are much larger than the pelvic fins, and the dorsal and ventral body surface categories each offer greater area than fin-associated sites. Furthermore, worms were found mostly around the base of the fins rather than on the fins and thus the borders between sites are soft and difficult to define. Nevertheless, we think our analysis justifiably suggests discrepant site usage, considering that: 1) all sites are relatively massive compared to the size of an individual worm, 2) in no fish did we observe any indication of a site nearing saturation with
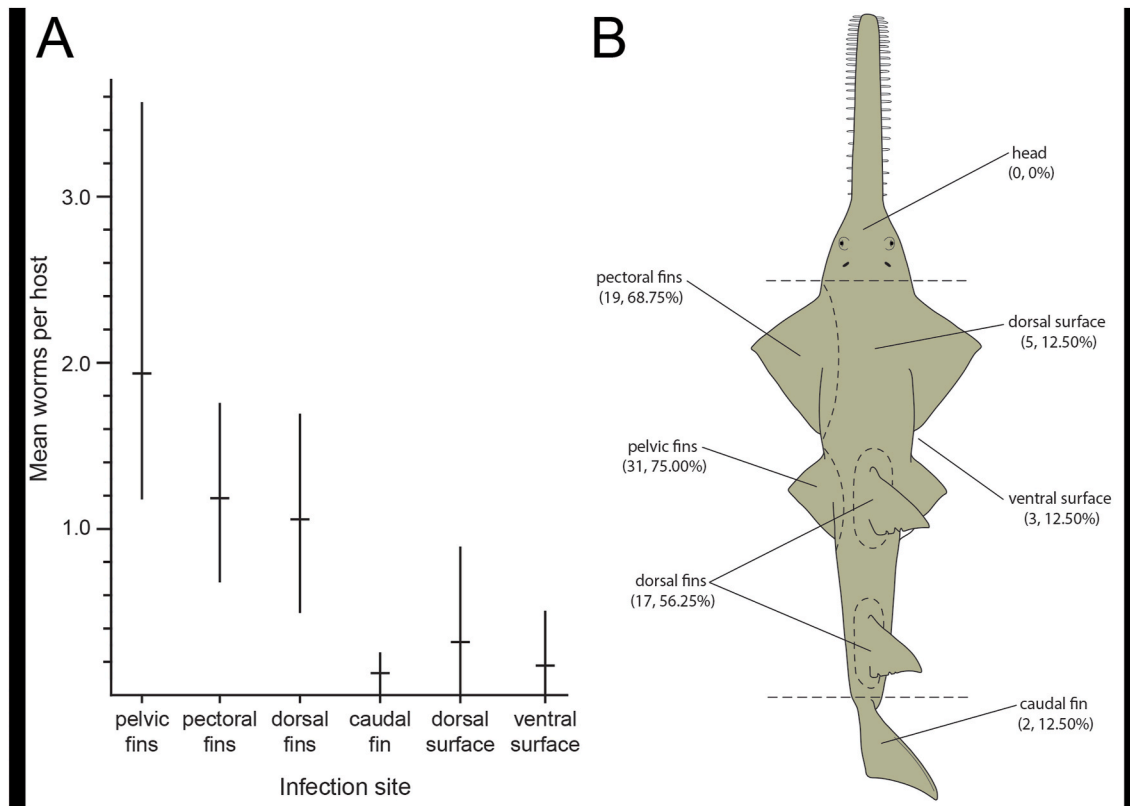
Fig. 6. (A) Mean abundance with 95% CI's of *Dermopristis pterophilus* n. sp. recorded on or adjacent to one of six attachment sites on *Pristis zijsron*. (B) Green sawfish *Pristis zijsron* gross morphology with combined total number of *D. pterophilus* n. sp. infections and the percentage of hosts infected with at least one worm per attachment site, from 16 fish examined. *No infections were found on the head (anterior to host gills, including the rostrum).

worms, 3) some monogeneans appear to have some capacity to seek a specific attachment site (e.g. *D. paradoxus*), and 4) it is clear, *a posteriori*, that sites with greater available surface area did not recruit greater infections: the body surface categories had only scanty infections and the pelvic fins recruited most infections despite being the smallest site.

Among microbothriids, the concept of *Dermopristis* is distinguished principally by the absence of a male copulatory organ or cirrus (Kearn et al., 2010; Whittington and Kearn, 2011). Kearn et al. (2010) first considered how insemination might occur, initially speculating that self-insemination might be the only means of conception. However, they also considered the possibility of spermatophore exchange, due to the relatively spacious lumen and glandular wall of the male reproductive tract. Whittington and Kearn (2011) discovered fusiform capsules on the ventral surface of *D. cairae* and Kearn et al. (2011) confirmed these capsules to be spermatophores, but could not determine whether worms with spermatophores attached were donors or recipients. Kearn et al. (2011) and Whittington and Kearn (2011) suggested that the presence of spermatophores in *D. cairae* might account for the absence of a seminal receptacle, but the presence of both a seminal receptacle and spermatophores observed here in *D. pterophilus* suggests this is not the case. The seminal receptacle was not always readily visible in specimens of *D. pterophilus* and the description of *D. cairae* was based on only five specimens, but having recently described *D. paradoxus* with a seminal receptacle (in Kearn et al., 2010), we think it unlikely that Kearn and Whittington (2011) would have overlooked the feature in *D. cairae*. Spermatophores have never been reported from *D. paradoxus*, but we think their production is likely in that species too, as initially predicted by Kearn et al. (2010); *D. pterophilus* provides evidence that the presence of a seminal receptacle in *D. paradoxus* does not preclude use of spermatophores.

A dendritic gut is common to all three species of *Dermopristis*. The gut of *D. cairae* was not described in complete detail, but that of *D. paradoxus*

appears to match *D. pterophilus* closely, specifically in the number and position of the major gut sub-trees extending from the main gut arms. Indeed, this gross gut structure is also similar to at least some species of *Dermophthirius* (see Cheung and Ruggieri, 1983; Benz, 1987). Nevertheless, the gut of *D. pterophilus* possibly differs from that of *D. paradoxus* and *D. cairae* (Whittington and Kearn, 2011) in fine details of the diverticula. Specifically, the gut does not entirely encircle the testes and we did not observe any invasion of diverticula among the testicular material; in those species, the gut does surround the testes and the diverticula invade among the testicular matter. However, these invading diverticula were readily observable in *D. cairae*, ventrally, due to brown pigment in the gut (Whittington and Kearn, 2011), but were only discovered in *D. paradoxus* following targeted serial resin sectioning (Kearn et al., 2010) and were dorsal to the testes in that species. The gut was not pigmented or readily visible in specimens of *D. pterophilus* and we did not take sections; thus, it is difficult to be certain that no similar interaction between the gut and testes occurs. Furthermore, in *D. cairae*, a dendritic diverticulum dorsal to the proximal coils of the vas deferens is clearly visible, whereas in *D. pterophilus*, diverticula are present in the same area but are less obvious and comparatively simple, with only two or three short, stout branches.

In addition to *P. zijsron*, we encountered and examined several other elasmobranch species in the estuarine waters of the Ashburton River and adjacent tidal creeks. We found no incidence of species of *Dermopristis* infecting any, including giant shovelnose rays *G. typus*, the type-host of *D. paradoxus*, nor on several of two carcharhinid sharks, specifically nervous sharks *Carcharhinus cautus* Whitley, 1945 and sicklefin lemon sharks *Negaprion acutidens* Rüppell, 1837. We presume that *D. pterophilus* is specific to, and thus dependent on, *P. zijsron* and, as with other host-specific parasites of threatened species, it should also be considered to face the same imminent threat of extinction as its host (e.g. Morgan et al., 2010; Simpfendorfer, 2013; Norman et al., 2021).

Declaration of competing interest

Acknowledgments

References

Bakenhaster, M.D., Bullard, S.A., Curran, S.S., Kritsky, D.C., Leone, E.H., Partridge, L.K., Ruiz, C.F., Scharer, R.M., Poulakis, G.R., 2018. Parasite component community of smalltooth sawfish off Florida: diversity, conservation concerns, and research applications. Endanger. Species Res. 35, 47–58.

Benz, G.W., 1987. *Dermophthirius penneri* sp. n. (Monogenea: Microbothriidae) an ectoparasite of carcharhinid sharks, *Carcharhinus brevipinna* and *Carcharhinus limbatus*. Proc. Helminthol. Soc. Wash 54, 185–190.

Chen, X., Wang, B., Nie, J., You, P., 2020. A survey of gyrodactylid parasites on the fins of *Homatula variegata* in central China. PLoS One 15, e0230320.

Cheung, P.J., Nigrelli, R., 1983. *Dermophthirioides pristidis* n. gen., n. sp. (Microbothriidae) from the skin and *Neoheterocotyle ruggierii* n. sp. (Monocotylidae) from the gills of the smalltooth sawfish, *Pristis pectinata*. Trans. Am. Microsc. Soc. 102, 366–370.

Cheung, P.J., Ruggieri, G.D., 1983. *Dermophthirius nigrellii* n. sp. (Monogenea: Microbothriidae), an ectoparasite from the skin of the lemon shark, *Negaprion brevirostris*. Trans. Am. Microsc. Soc. 102, 129–134.

Chisholm, L.A., Morgan, J.A.T., Adlard, R.D., Whittington, I.D., 2001. Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. Int. J. Parasitol. 31, 1253–1263.

Dulvy, N.K., Davidson, L.N.K., Kyne, P.M., Simpfendorfer, C.A., Harrison, L.R., Carlson, J.K., Fordham, S.V., 2016. Ghosts of the coast: global extinction risk and conservation of sawfishes. Aquat. Conserv. 26, 134–153.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Faria, V.V., McDavitt, M.T., Charvet, P., Wiley, T.R., Simpfendorfer, C.A., Naylor, G.J., 2013. Species delineation and global population structure of Critically Endangered sawfishes (Pristidae). Zool. J. Linn. Soc. 167, 136–164.

Kearn, G.C., 1965. The biology of *Leptocotyle minor*, a skin parasite of the dogfish, *Scyliorhinus canicula*. Parasitology 55, 473–480.

Kearn, G.C., Whittington, I.D., Evans-Gowing, R., 2010. A new genus and new species of microbothriid monogenean (Platyhelminthes) with a functionally enigmatic

reproductive system, parasitic on the skin and mouth lining of the largetooth sawfish, *Pristis microdon*, in Australia. Acta Parasitol. 55, 115–122.

Kearn, G.C., Whittington, I.D., Evans-Gowing, R., 2011. Spermatophores in *Dermopristis cairae* Whittington et Kearn, 2011 (Monogenea, Microbothriidae). Acta Parasitol. 56, 371–376.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Kyne, P.M., Rigby, C., Simpfendorfer, C.A., 2013. Pristis clavata. The IUCN Red List of Threatened Species 2013: e.T39390A141790455.

Morgan, D.L., Allen, M.G., Ebner, B.C., Whitty, J.M., Beatty, S.J., 2015. Discovery of a pupping site and nursery for critically endangered green sawfish *Pristis zijsron*. J. Fish Biol. 86, 1658–1663.

Morgan, D.L., Ebner, B.C., Allen, M.G., Gleiss, A.C., Beatty, S.J., Whitty, J.M., 2017. Habitat use and site fidelity of neonate and juvenile green sawfish *Pristis zijsron* in a nursery area in Western Australia. Endanger. Species Res. 34, 235–249.

Morgan, D.L., Lear, K.O., Dobinson, E., Gleiss, A.C., Fazeldean, T., Pillans, R.D., Beatty, S. J., Whitty, J.M., 2021. Seasonal use of a macrotidal estuary by the endangered dwarf sawfish (*Pristis clavata*). Aquat. Conserv. 31, 2164–2177.

Morgan, D.L., Tang, D., Peverell, S.C., 2010. Critically endangered *Pristis microdon* (Elasmobranchii), as a host for the Indian parasitic copepod, *Caligus furcisetifer* Redkar, Rangnekar et Murti, 1949 (Siphonostomatoida): new records from northern Australia. Acta Parasitol. 55, 419–423.

Morgan, D.L., Whitty, J.M., Phillips, N.M., Thorburn, D.C., Chaplin, J.A., McAuley, R., 2011. North-western Australia as a hotspot for endangered elasmobranchs with particular reference to sawfishes and the northern river shark. J. Roy. Soc. West Aust. 94, 345–358.

Norman, B.M., Reynolds, S., Morgan, D.L., 2021. Three-way symbiotic relationships in whale sharks. Pac. Conserv. Biol.

Perkins, E.M., Donnellan, S.C., Bertozzi, T., Chisholm, L.A., Whittington, I.D., 2009. Looks can deceive: molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. Mol. Phylogenet. Evol. 52, 705–714.

Peverell, S.C., 2010. Sawfish (Pristidae) of the Gulf of Carpentaria, Queensland, Australia. James Cook University, Townsville.

R Core Team, 2021. R: a language and environment for statistical computing, R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/.

Reiczigel, J., Marozzi, M., Fábián, I., Rózsa, L., 2019. Biostatistics for parasitologists – a primer to quantitative parasitology. Trends Parasitol. 35, 277–281.

Simpfendorfer, C., 2013. Green sawfish, *Pristis zijsron*. The IUCN red list of threatened species 2013 e.T39393A141792003.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027.

Thorburn, D.C., Morgan, D.L., Rowland, A.J., Gill, H.S., 2007. Freshwater sawfish *Pristis microdon* Latham, 1794 (Chondrichthyes: Pristidae) in the kimberley region of western Australia. Zootaxa 1471, 27–41.

Whittington, I.D., Chisholm, L.A., 2008. Diseases caused by Monogenea. In: Eiras, J.C., Segner, H., Wahli, T., Kapoor, B.G. (Eds.), Fish Diseases. Enfield (NH), USA. Science Publishers, pp. 683–816.

Whittington, I.D., Horton, M., 1996. A revision of *Neobenedenia Yamaguti*, 1963 (Monogenea: Capsalidae) including a redescription of *N. melleni* (MacCallum, 1927) Yamaguti, 1963. J. Nat. Hist. 30, 1113–1156.

Whittington, I.D., Kearn, G.C., 1993. A new species of skin-parasitic benedeniine monogenean with a preference for the pelvic fins of its host, *Lutjanus carponotatus* (Perciformes: Lutjanidae) from the Great Barrier Reef. J. Nat. Hist. 27, 1–14.

Whittington, I.D., Kearn, G.C., 2011. A new species of *Dermopristis* Kearn, Whittington & Evans-Gowing, 2010 (Monogenea: Microbothriidae), with observations on associations between the gut diverticula and reproductive system and on the presence of denticles in the nasal fossae of the host *Glaucostegus typus* (Bennett) (Elasmobranchii: Rhinobatidae). Syst. Parasitology 80, 41–51.