



Description of *Echthrogaleus spinulus* n. sp. (Copepoda: Pandaridae) parasitic on a torpedo ray from the central Pacific Ocean utilising a morphological and molecular approach

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Received: 3 July 2019 / Accepted: 24 September 2019
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Abstract A new species of parasitic copepod, *Echthrogaleus spinulus* n. sp. (Pandaridae), is described from the torpedo ray *Tetronarce tokionis* (Tanaka) (Torpedinidae) captured in pelagic Hawaiian waters. The new species has pediger 4 bearing large dorsal plates with denticles on posterior margin, genital complex with posterolateral lobes widely curved medially and overlapping, leg 4 exopod incompletely 3-segmented, and the largest body size (maximum length 16 mm from anterior rim of frontal

plates to tip of caudal rami, excluding setae). This morphology does not match any of the seven valid species of *Echthrogaleus* Steenstrup & Lütken, 1861. Analysis of 28S rDNA sequences separated the new material from the Central Pacific from samples of *E. coleopratus* in the Atlantic and Eastern Pacific Oceans. However, due to the lack of DNA sequences in the databases, the new 28S rDNA sequence cannot be used to confirm the species identity. The unique morphological characteristics of the Central Pacific female copepods combined with 28S rDNA sequencing was used as a basis to validate the new species.

This article was registered in the *Official Register of Zoological Nomenclature* (ZooBank) as 4F3B37A9-9717-43DF-9A1F-292CD29A490A. This article was published as an Online First article on the online publication date shown on this page. The article should be cited by using the doi number. This is the Version of Record.

This article is part of the Topical Collection Arthropoda.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11230-019-09885-5>) contains supplementary material, which is available to authorized users.

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Introduction

Copepods belonging to the order Siphonostomatoida are mostly parasites of fish. This order comprises 41 families of which 10 (Caligidae Burmeister, 1835; Dichelesthidae Milne Edwards, 1840; Dissonidae Yamaguti, 1963; Eudactylinidae Wilson, 1932;

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Kroyeriidae Kabata, 1979; Lernaeopodidae Milne Edwards, 1840; Pandaridae Milne Edwards, 1840; Pennellidae Burmeister, 1835; Sphyrriidae Wilson, 1919 and Trebiidae Wilson, 1905) may be found on elasmobranch hosts (Dippenaar, 2009). Members of the Pandaridae are almost exclusively parasites of a variety of elasmobranchs (Izawa, 2010; Bernot & Boxshall, 2017). Recently, a torpedo ray caught in Hawaiian waters was found infected with several individuals of a parasite copepod species (Fig. 1A, B). Morphological examination of these copepods revealed that they belong to the pandarid genus *Echthrogaleus* Steenstrup & Lütken, 1861. This genus has been reported from the following elasmobranch families: Alopidae, Carcharhinidae, Laminidae, Mitsukurinidae, Mobulidae, Sphyrnidae, Squalidae and Torpedinidae (see Wilson, 1907; Benz & Deets, 1987;

Henderson et al., 2002; Izawa, 2012). Currently, there are seven species of *Echthrogaleus* considered valid: *E. asiaticus* Ho, Liu & Lin, 2012; *E. coleopratus* (Guérin-Méneville, 1837); *E. denticulatus* Smith, 1873; *E. disciarai* Benz & Deets, 1987; *E. mitsukurinae* Izawa, 2012; *E. pellucidus* Shiino, 1963; and *E. torpedinis* Wilson, 1907. For a complete list of elasmobranch hosts of *Echthrogaleus* spp. see The World of Copepods (www.marinespecies.org/copepoda). Morphology of our specimens did not fit completely with any of these seven species. Therefore, in the present paper we describe a new species of *Echthrogaleus*. The morphological description is based on adult females because male specimens were not found at the time of collection.

A single DNA sequence for *E. coleopratus* was available in the GenBank and Barcode of Life Data System databases. We utilised this 28S rDNA sequence (GenBank: DQ 180344) to compare with two sequences published in Véliz et al. (2018), one unpublished sequence from Argentina and the new species from the Central Pacific (Table 1). We also investigated the biodiversity of *E. coleopratus* to see whether 28S rDNA reveals a cosmopolitan distribution for this species.

Materials and methods

On 19 May 2018 a torpedo ray, *Tetronarce tokionis* (Tanaka) was captured in the Hawaiian longline fishery (24°59.3'N, 156°17.3'W) on a longline set for bigeye tuna *Thunnus obesus* (Lowe) at an estimated set depth of 300–400 m with bottom depth over 3,000 m. The torpedo ray was an adult male, with a total length of 72.8 cm. It is a rare capture because only four torpedo rays have been captured in the Hawaiian longline fisheries since 2010 [Pacific Island Region Observer Programme (PIROP) data].

The specimen was secured by the NOAA/NMFS/PIFSC/PIROP staff of the Pacific Islands Regional Office, Honolulu, HI, frozen on board, brought to the NOAA/NMFS, Inouye Regional Center, maintained frozen and thawed for examination on 5 June 2018. Torpedo ray ventral pelvic fin muscle tissue was removed and placed in 90% ethanol for DNA sequencing and copepod parasites were removed from the dorsal and ventral surface of the ray and placed in either 5% buffered formalin or ethanol for species

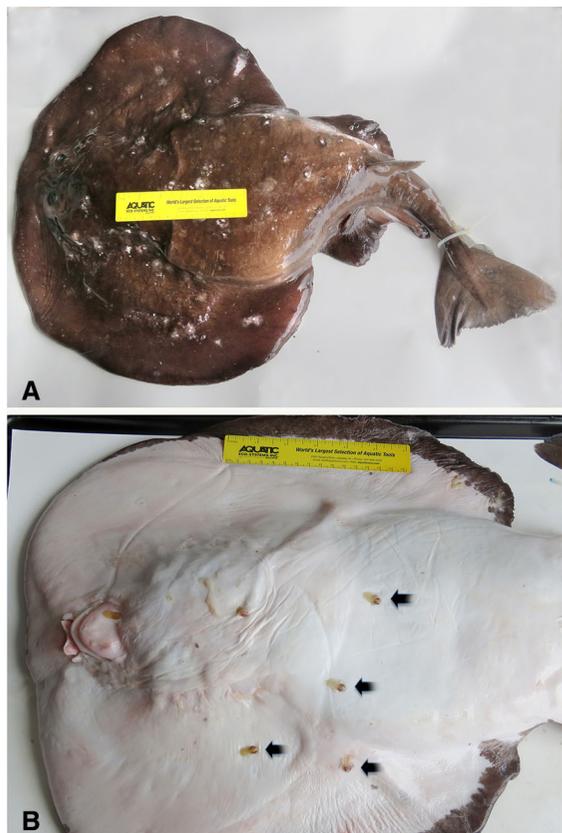


Fig. 1 Photographs of the Hawaii *Tetronarce tokionis* specimen. A, Dorsal body surface of a male specimen (total length 72.8 cm); white spots on body showing previous copepod attachment and feeding sites; B, Ventral surface with arrowheads designating *Echthrogaleus spinulus* n. sp.

Table 1 *Echthrogaleus* spp., *Caligus quadratus* and *Bomolochus bellones* sequences utilised in the molecular analysis based on the 28S rRNA gene

Copepod species	Host	Location	GenBank ID	Reference
<i>E. coleoptratus</i> (Guérin-Méneville, 1837)	Unknown	Off Norway	DQ180344	GenBank
	<i>Prionace glauca</i> (L.)	Off Chile	MN115791; MN115792	Véliz et al. (2018)
	<i>Lamna nasus</i> (Bonnaterre)	Off Rawson City, Argentina	MN089568	Present study
<i>E. spinulus</i> n. sp.	<i>Tetronarce tokionis</i> (Tanaka)	Off Hawaiian Islands	MN089569; MN089570	Present study
<i>C. quadratus</i> Shiino, 1954	<i>Hemilutjanus macrophthalmos</i> (Tschudi)	Off Chile	JX896377; JX896378	Véliz et al. (2018)
	Unknown	Off Korea	KR048863	GenBank
<i>B. bellones</i> Burmeister, 1833	Unknown	Off Korea	KR048853	GenBank

identification and DNA sequencing. All copepods used in the 28S rDNA analysis were placed in 90% ethanol (Table 1).

Five specimens fixed in formalin were later cleared in lactic acid for 2 h and one of them selected for dissection of the appendages on a slide under a Zeiss Stemi 305 dissection microscope. The body parts and appendages were mounted on slides in lactic acid, examined and drawn using a Leica DMLB compound microscope equipped with a drawing tube, at magnifications of up to 1000×. Measurements were made with an ocular micrometer and are given in millimetres and are provided as the range followed by the mean in parentheses. Anatomical images of a formalin-fixed specimen on a depression slide were created using a Leica TCS SP8X confocal laser scanning microscope (CLSM) with 10× and 20× objectives. The type-material and additional specimens were deposited in the Colección de Parásitos de Peces (CPPNP) at CIAD-Mazatlán, Sinaloa, Mexico and the Bernice P. Bishop Museum (BPBM), Honolulu, Hawaii, USA.

Copepods were assessed by 28S rDNA molecular sequence analysis (Table 1). Two specimens of the new species from the torpedo ray from off Hawaiian Islands plus one specimen of *E. coleoptratus* from the shark *Lamna nasus* (Bonnaterre) collected off Argentina were processed for DNA extraction. DNA was extracted using Wizard[®] Genomic DNA Purification Kit (Promega, Madison, USA) according to the manufacturer's protocol. The 28S rDNA gene fragment was amplified by polymerase chain reaction (PCR) on an Eppendorf Mastercycler thermal cycler using the forward primer LSU (5'-TAG GTC GAC

CCG CTG AAY TTA AGC A-3') and the reverse primer 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3'). The reactions were prepared using Green GoTaq 5× Buffer (Promega), 2.5 mM MgCl₂ (Promega), 0.2 mM of NEB Nucleotide Mix, and Flexi GoTaq polymerase enzyme (Promega).

The PCR reactions were performed according to protocols described by Tkach et al. (2003). The PCR products were analysed by electrophoresis in 1% agarose gel using TAE 1× buffer and observed on ultraviolet light. The PCR products were purified and sequenced using an ABI 3730XLs sequencer, Macrogen Inc. (Korea). Sequences of *Echthrogaleus* spp. obtained in this study were edited using the platform Geneious Pro v5.1.7 (Drummond et al., 2010), and then compared with available 28S sequences of *E. coleoptratus* from off central coast of Chile on the blue shark *Prionace glauca* (L.) (Véliz et al., 2018; MN115791, MN115792), off Norway (DQ180344), and the outgroups *Caligus quadratus* Shiino, 1954 (JX896377, JX896378, KR048863) and *Bomolochus bellones* Burmeister, 1833 (KR048853). All sequences were aligned using the online version of MAFFT v.7 (Kato & Standley, 2013). The Gblocks Website (Castresana, 2000; Talavera & Castresana, 2007) was used to detect ambiguously aligned hypervariable regions in the 28S rDNA dataset, according to a secondary structure model, that were excluded from the analyses using a less stringent selection (allowing smaller final blocks, gap position within the final blocks, and less strict flanking positions). The best partitioning scheme and substitution model for the 28S rDNA was chosen under the Bayesian Information Criterion (BIC; Schwarz,

1978) using the ‘greedy’ search strategy in Partition Finder v.1.1.1 (Lanfear et al., 2012, 2014).

The appropriate nucleotide substitution model implemented for the matrix resulting after the Gblock selection was K80 (Kimura, 1980). Additionally, genetic distance between copepod species (p-distance; Nei & Kumar, 2000) was calculated using MEGA v.7.0 (Kumar et al., 2016).

Phylogenetic reconstruction was carried out using Bayesian Inference (BI) through MrBayes v.3.2.3 (Ronquist et al., 2012). Phylogenetic trees were constructed using two parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMC) for 20×10^6 generations each, to estimate the posterior probability (pp) distribution. Topologies were sampled every 1,000 generations and the average standard deviation of split frequencies was observed to be less than 0.01, as suggested by Ronquist et al. (2012).

The robustness of the clades was assessed using Bayesian pp, where pp > 0.95 was considered strongly supported. A majority consensus tree with branch lengths was reconstructed for the two runs after discarding the first 5,000 sampled trees. The newly generated sequences of *Echthrogaleus* spp. were submitted to the GenBank database.

Order Siphonostomatoida Thorell, 1859
Family Pandaridae Milne Edwards, 1840
Genus *Echthrogaleus* Steenstrup & Lütken, 1861

***Echthrogaleus spinulus* n. sp.**

Type-host: *Tetronarce tokionis* (Tanaka) (Torpedinidae), torpedo ray deposited in California Academy of Sciences, Ichthyology Collection (CAS 244536).

Type-locality: North of the main Hawaiian Islands (24°59.3'N, 156°17.3'W), Central Pacific Ocean.

Type-material: Holotype female (CPPNP 1373) and 4 paratype females (CPPNP 1374) were deposited in the Colección de Parásitos de Peces at CIAD-Mazatlán, Sinaloa, Mexico. Four additional specimens (BPBM S17857) were deposited in the Bernice P. Bishop Museum (BPBM), Honolulu, Hawaii, USA.

Site on host: The dorsal and ventral surfaces of the torpedo ray had, respectively, about 40 and 15 copepods. The dorsal attachment sites had white

lesions around the copepod from presumed feeding areas (Fig. 1A).

Representative DNA sequences: GenBank numbers MN089569 and MN089570 (28S rDNA).

Etymology: the specific name refers to the numerous rows and patches of spinules present on legs.

Description (Figs. 2–5)

Adult female. [Based on 5 specimens.] Body flattened (Fig. 2A), 14.5–16.0 (15.3) long from anterior rim of frontal plates to tip of caudal rami (excluding setae). Frontal plates distinctly separated from cephalothoracic shield. Cephalothorax orbicular, 6.0–7.0 (6.3) long, 6.8–7.8 (7.1) wide, well divided by sutures into median lobe and bipartite lateral lobes, posteriorly extended, without marginal membrane. Cephalothorax ventrally bearing cephalic appendages, maxillipeds, and pediger 1. Pedigers 2 and 3 fused dorsally. Pediger 2 with paired dorsolateral plates. Pediger 3 with posterolateral protrusions but without dorsal plate. Pediger 4 narrowest, with paired, large dorsal plates (roughly rectangular) covering approximately two thirds of genital complex on both sides; its posterior rim and distal half of medial rim with fine denticles, and antero-outer corner with spinous process. Genital complex (Fig. 2A) slightly longer than wide, 8.0–9.0 × 5.5–7.2 (8.5 × 6.3), larger than cephalothorax with pedigers 2 and 3 combined, with large posterolateral lobes widely curved medially with their tips overlapping across the midline and carrying leg 5. Posterior plate between lobes (Fig. 2B), covering abdomen below. Genital field located anterior to abdomen on ventral side (Fig. 2C). Abdomen (Fig. 2C) 1-segmented, almost as long as wide, gradually narrowing posteriorly. Caudal ramus (Figs. 2C, 6D) about two times as long as wide, bearing spinules on distal inner corner and 6 apical setae.

Antennule (Fig. 2D) 2-segmented; proximal segment with 27 spinulose setae; distal segment with 1 subterminal seta on posterior margin and 10 setae plus 2 aesthetascs on distal margin. Antenna (Fig. 2E) 4-segmented; first to fourth segments unarmed; distal segment forming claw strongly recurved and bearing 2 basal setae. Mouth tube (Figs. 2F, 6A) as typical form in pandarids; distal part (Figs. 2G, 6B, Supplementary Figure S1 and Video S1) with 2 rows of serrated membranes and mandible with about 12 teeth on blade. Maxillule (Fig. 2H) lobate, indistinctly 2-segmented; first segment with 3 small setae anterodistally; second

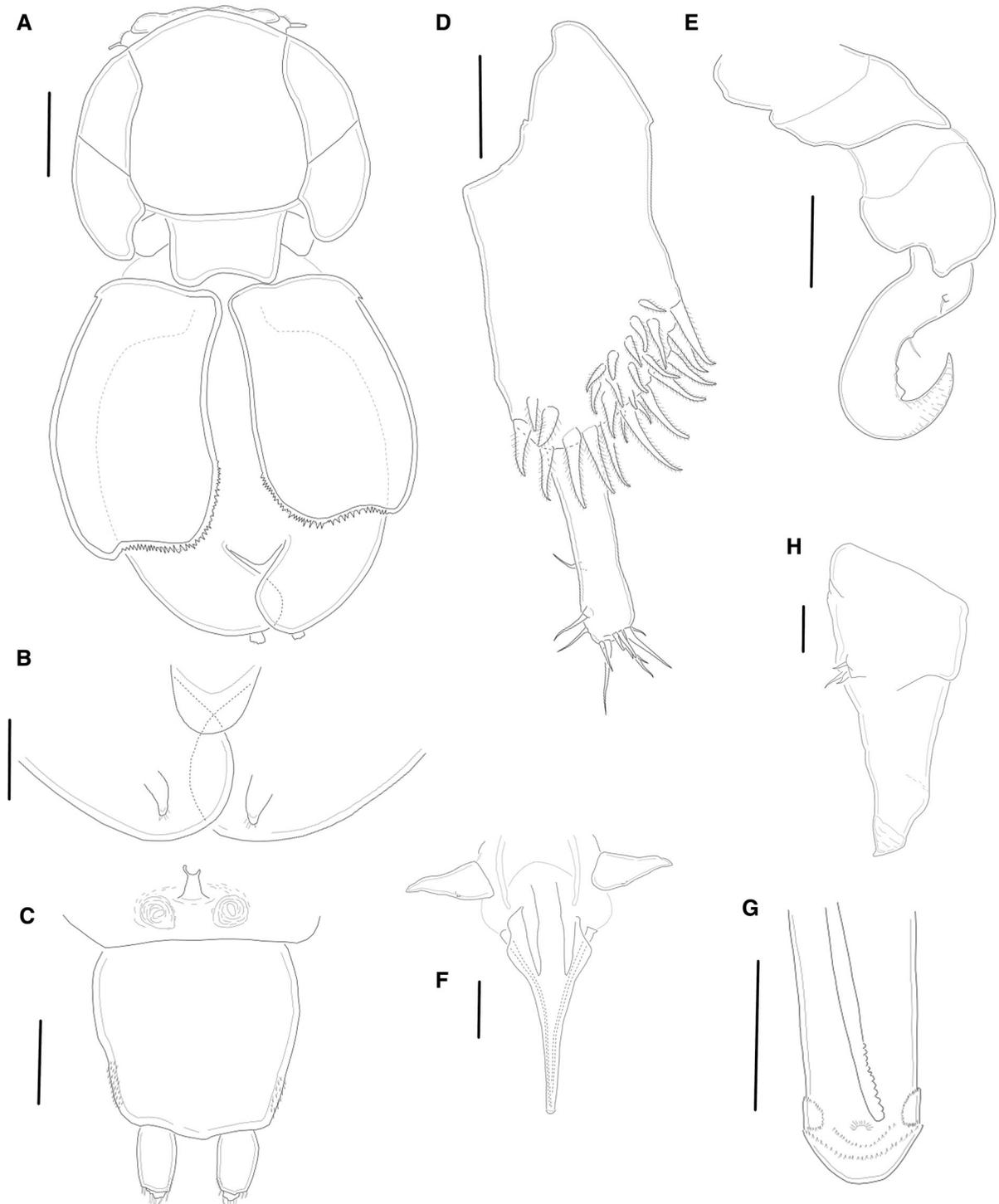


Fig. 2 *Echthrogaleus spinulus* n. sp., adult female. A, Habitus, dorsal view; B, Posterior part of genital complex, ventral view; C, Abdomen and caudal rami, ventral view; D, Left antennule, ventral view; E, Right antenna, anterior view; F, Oral region, anterior view; G, Distal part of mandible and oral cone, anterior view; H, Maxillule, anterior view. *Scale-bars*: A, 2 mm; B, C, 1 mm; D, 200 μ m; E, F, 500 μ m; G, 150 μ m; H, 100 μ m

segment narrowed distally to form papilla. Maxilla (Fig. 3A) 2-segmented; first segment (lacetus) unarmed; second segment (brachium) bearing subterminal crista covered by denticles and tipped with canna and calamus. Maxilliped (Fig. 3B) subchelate; corpus robust with 3 protuberances and small spinulose process; claw with seta medioproximally. Digitiform process (Fig. 3B) near basal area of maxilliped.

Legs 1–4 biramous; armature on rami as follows (Roman and Arabic numerals indicating spines and setae, respectively):

	Exopod	Endopod
Leg 1	I-0; III, I, 3	0-0; 3
Leg 2	I-1; I-1; IV, 4	0-1; 7, 1
Leg 3	I-1; I-1; III, 5	0-1; 0-0; 4, II
Leg 4	I-0; I-1; 3, 4	6

Leg 1 (Fig. 3C), protopod with 1 outer and 1 inner seta in addition to 3 adhesion pads on ventral surface. Exopod 2-segmented; first segment with 1 outer spine on distal corner and setules on inner margin; second segment with short row of spinules on inner margin, patch of spinules on outer margin, 4 spines fringed with setules, and 3 pinnate setae distally. Endopod 2-segmented; first segment with 1 adhesion pad on ventral surface and patch of spinules on distal outer corner; second segment with spinules along outer margin, patch of spinules on distal inner corner, and 1 inner and 2 apical pinnate setae.

Leg 2 (Fig. 4A), intercoxal bar modified into large plate fringed with spinules on posterior margin. Protopod with 1 outer pinnate seta and 1 small spiniform inner seta in addition to 2 adhesion pads, 3 patches of spinules, and row of setules on posterior margin. Exopod 3-segmented; first and second

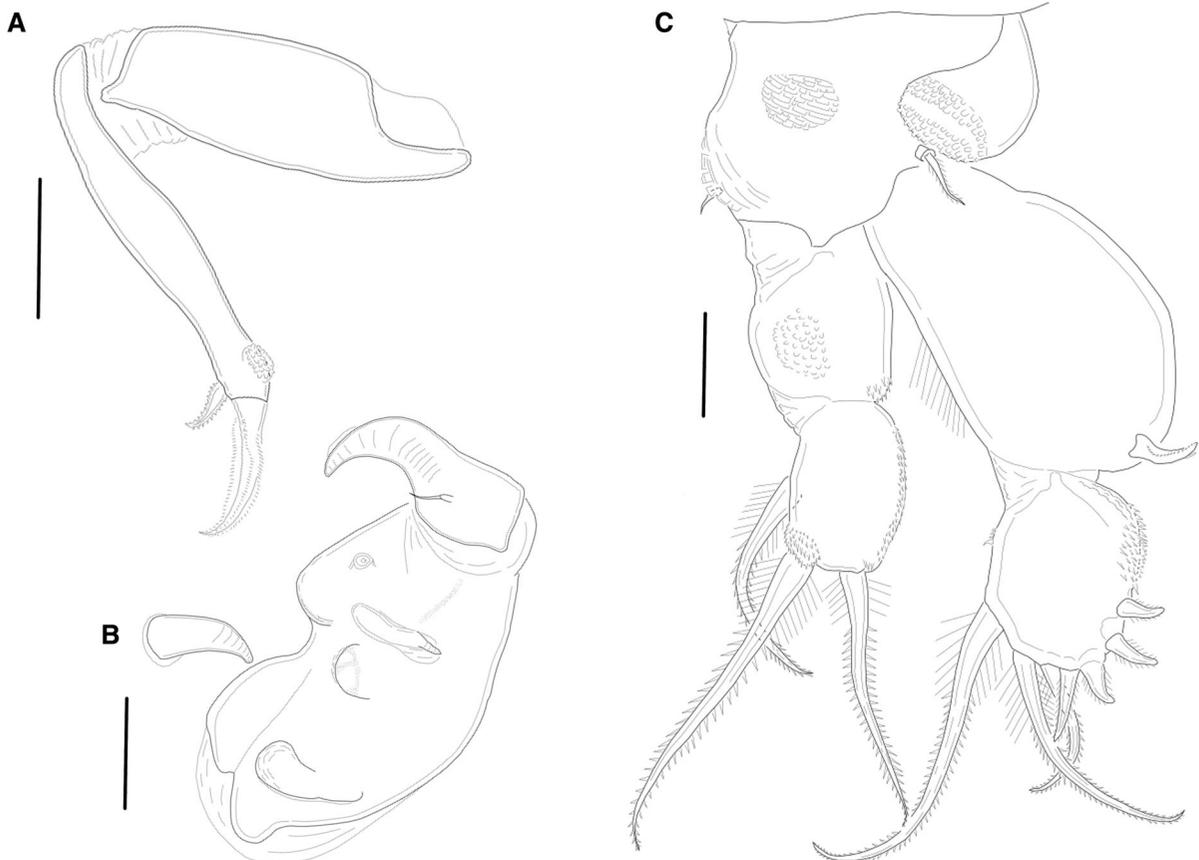


Fig. 3 *Echthrogaleus spinulus* n. sp., adult female. A, Right maxilla, anterior view; B, Left maxilliped, posterior view; C, Left leg 1, anterior view. Scale-bars: A, B, 500 µm; C, 200 µm



Fig. 4 *Echthrogaleus spinulus* n. sp., adult female. A, Right leg 2, anterior view; B, Right leg 3, ventral view. Scale-bars: 1 mm

segments bearing rows of spinules on outer margin, 1 outer spine fringed with setules, and 1 inner plumose seta;

spines fringed with spinules, and 4 pinnate setae. Endopod 2-segmented; first segment with rows of spinules on outer margin, and 1 inner pinnate seta;

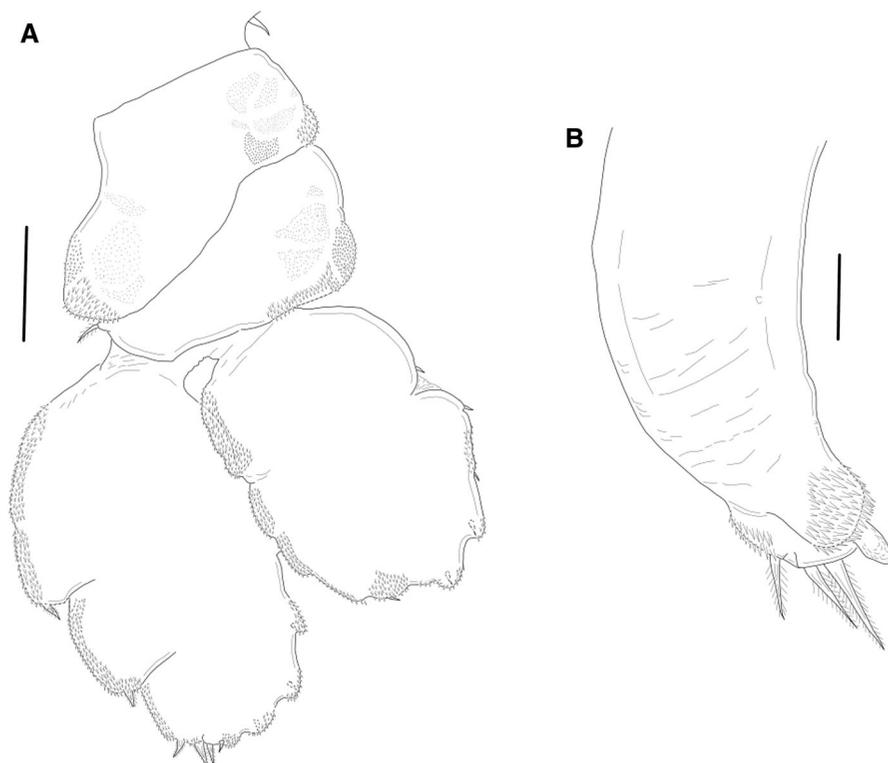


Fig. 5 *Echthrogaleus spinulus* n. sp., adult female. A, Left leg 4, anterior view; B, Left leg 5, ventral view. Scale-bars: A, 500 μ m; B, 100 μ m

second segment with row of setules on outer margin, patch of spinules on distal area, 7 pinnate setae, and 1 spine-like seta.

Leg 3 (Fig. 4B), protopod with 3 adhesion pads, rows of spinules on outer margin, 1 outer and 1 inner pinnate seta, and hyaline membrane along entire posterior margin. Exopod indistinctly 3-segmented; first and second segments bearing rows of spinules on outer margin, 1 outer spine, and 1 inner pinnate seta; third segment with rows of spinules on outer margin, 3 spines, and 5 pinnate setae (upper section Fig. 6C). Endopod indistinctly 3-segmented; first segment fringed with setules on outer margin and 1 inner pinnate seta; second segment unarmed; third segment with 4 pinnate setae and 2 spines.

Leg 4 (Figs. 5A, 6C), coxa with patches of spinules on distal corners and ventral surface, and 1 small seta at intersection with intercoxal bar. Basis with 1 outer pinnate seta, and patches of spinules on distal inner corner. Both rami with rows of spinules along outer margin. Exopod incompletely 3-segmented; first

segment with 1 outer pinnate seta; second segment with 1 outer pinnate seta and 1 inner small spine; third segment with 3 pinnate setae and 4 small spines. Endopod with 3 inner and 3 apical small setae.

Leg 5 (Figs. 5B, 6D) located ventrally on postero-lateral lobe of genital complex, behind caudal ramus in ventral view, represented by process with patches of spinules on distal end, and tipped with 1 spine and 3 setae.

Molecular data

A total of 3 consensus 28S rDNA sequences were assembled from three adult female *Echthrogaleus* spp.: *E. spinulus* n. sp. (1,029 nt and 1,053 nt) and *E. coleopratus* from off Argentina (1,054 nt). To evaluate the phylogenetic position of *E. spinulus* n. sp., two sequences were included in the Bayesian phylogenetic analysis of *E. coleopratus* [from off Argentina (MN089568), Chile (MN115791, MN115792) and

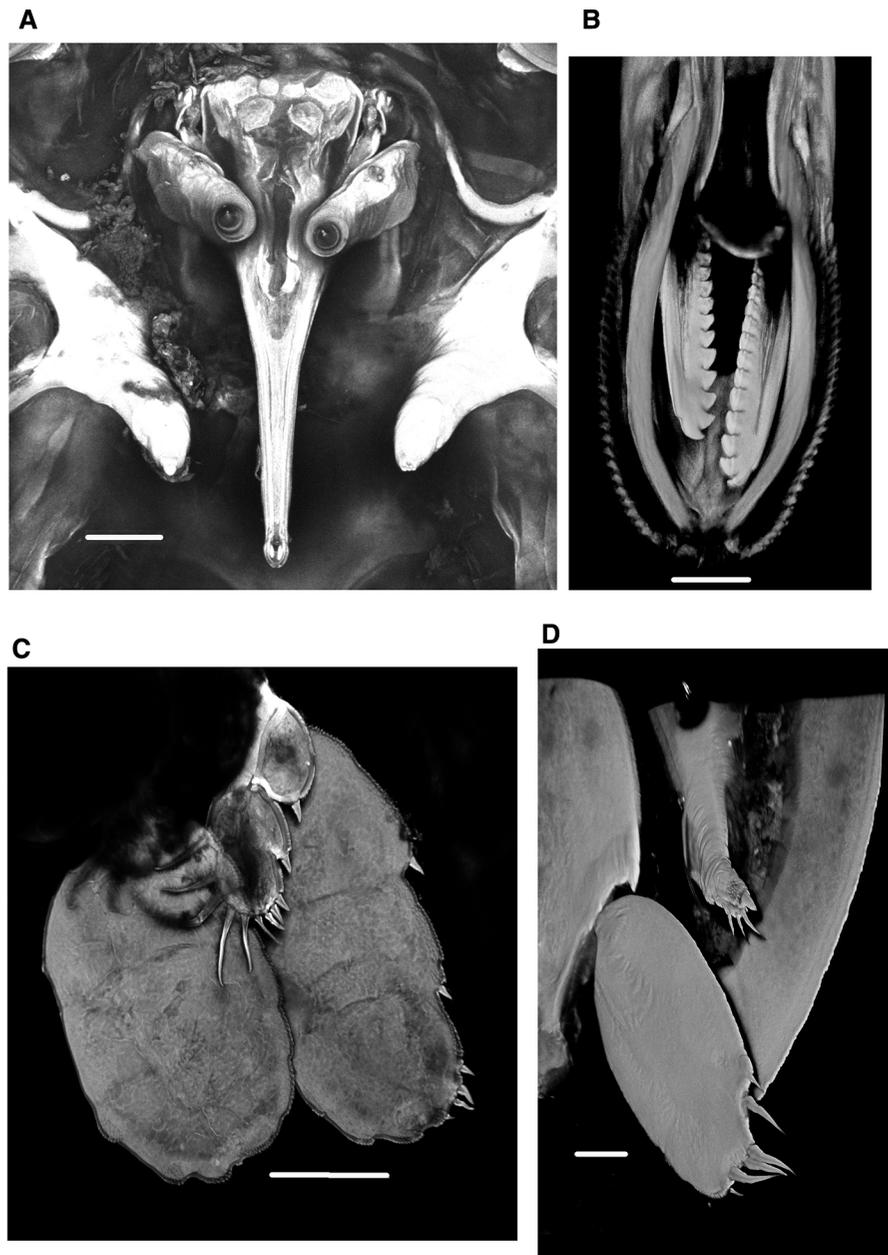


Fig. 6 Confocal laser scanning microscope (CLSM) images of mouth tube and various appendages of *Echthrogaleus spinulus* n. sp., adult female. A, Mouth tube; B, Tips of mandibles within oral opening at apex of mouth tube; C, Leg 3 exopod above (overlaps) leg 4 exopod and endopod; note incomplete segmentation of the exopod; D, Leg 5 and caudal ramus. Scale-bars: A, 200 μ m; B, 50 μ m; C, 500 μ m; D, 200 μ m

Norway (DQ180344)]. The Bayesian phylogenetic tree for *Echthrogaleus* based on 28S rDNA revealed that the genus *Echthrogaleus* and currently *E. coleopratus* and *E. spinulus* n. sp. form a monophyletic clade ($pp \geq 0.95$) (Fig. 7). The genetic distance values of *E.*

spinulus n. sp. relative to the other specimens of *E. coleopratus* was 1% compared with specimens from off Chile and Norway, and 2% with the specimen from off Argentina (Table 2).

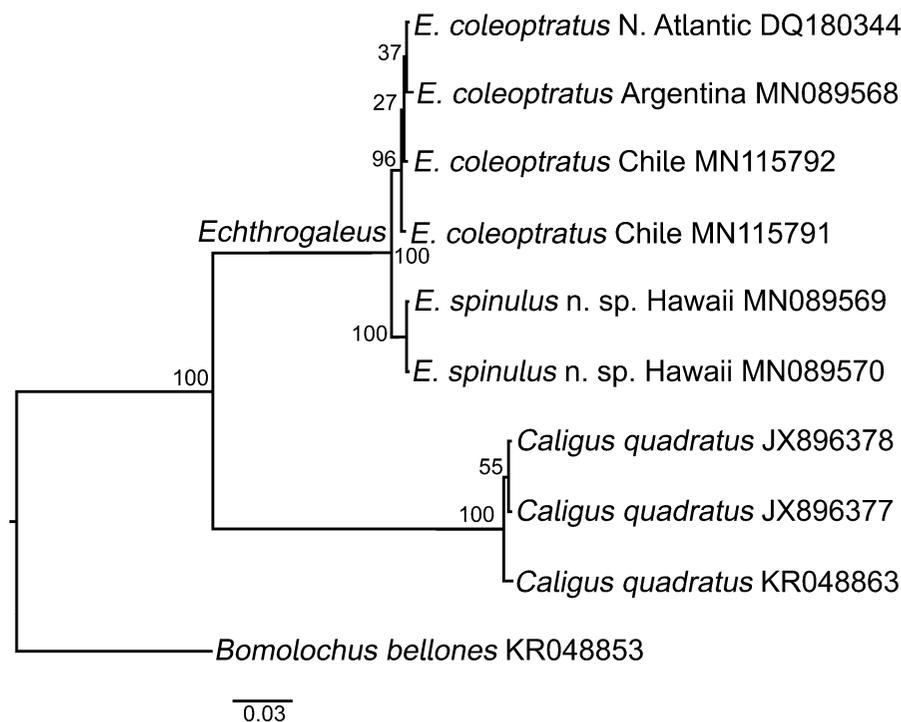


Fig. 7 Bayesian inference tree based on 28S rDNA sequences for *Echthrogaleus coleoptratus*, *Caligus quadratus*, *Bomolochus bellones* and *Echthrogaleus spinulus* n. sp.

Table 2 Genetic distances matrix (uncorrected p-distance) for *Echthrogaleus coleoptratus*, *E. spinulus* n. sp. and the outgroup (*Caligus quadratus* and *Bomolochus bellones*). The percentage values are derived from the 28S rDNA by Bayesian phylogenetic analysis

Species	1	2	3	4	5	6
1 <i>E. coleoptratus</i> (n = 1) (DQ180344)		0	1	2	32	32
2 <i>E. coleoptratus</i> (n = 2) (MN115791; MN115792)	0		1	1	27	32
3 <i>E. coleoptratus</i> (n = 1) (MN089568)	1	1		1	27	32
4 <i>E. spinulus</i> n. sp. (n = 2) (MN089569; MN089570)	1	1	2		27	32
5 <i>Caligus quadratus</i> (n = 3)	27	27	28	27		33
6 <i>Bomolochus bellones</i> (n = 1) (KR048853)	32	32	32	32	33	

Discussion

Overall, *Echthrogaleus spinulus* n. sp. can be morphologically distinguished from the other seven congeneric species by the combination of the following characteristics in the female: (i) pediger 4 bearing large dorsal plates with denticles on posterior margin; (ii) genital complex with posterolateral lobes widely curved medially and overlapping; (iii) leg 4 exopod

incompletely 3-segmented; and (iv) the largest body size. Additionally, *Echthrogaleus torpedinis* has pediger 4 bearing large dorsal plates but without denticles on margins, and genital complex with curved lobes but not overlapping (Wilson, 1907). Of the known species, *E. mitsukurinae* is the unique having a genital complex with lobes curved and overlapping as in the new species; however, *E. mitsukurinae* may be differentiated by the pediger 4 bearing shorter dorsal plates (3 vs

6 mm) with smooth margins, legs 2 and 3 with endopod completely 3-segmented, and leg 4 exopod incompletely 2-segmented (Izawa, 2012).

Additionally, there are other characteristics by which *E. spinulus* n. sp. can be easily differentiated from the congeneric species, such as the 2-segmented leg 2 endopod and short leg 5 in the new species vs 3-segmented leg 2 endopod and long leg 5 in *E. asiaticus* (Ho et al., 2012); the caudal ramus square (longer than wide) and legs with long setae in the new species vs the caudal ramus suboval and legs with shorter setae in *E. coleopratus* (Izawa, 2010; Ho et al., 2012); the shorter leg 5 (0.5 mm) in the new species than in *E. denticulatus* (0.9 mm) (Izawa, 2010); the leg 1 with longer setae (≥ 0.4 mm) in the new species than in *E. disciarai* (< 0.2 mm) (Benz & Deets, 1987); and the maxilliped with a well-defined claw in the new species vs maxilliped with claw relatively short, broad, flattened, and divided at the apex into two blunt unequal laminae in *E. pellucidus* (Shiino, 1963).

The torpedo ray *Tetronarce occidentalis* (Storer) is reported as the host for *E. torpedinis* in the Atlantic Ocean off Woods Hole and Princetown, USA (Wilson, 1907). The majority of *Echthrogaleus* spp. have been described in waters of the Pacific Ocean, particularly in the Asian region; however, *E. coleopratus* and *E. denticulatus* are also reported from the Atlantic and Indian Oceans (Ho & Kim, 1996) and *E. pellucidus* was reported from South African waters (Dippenaar, 2018).

The torpedo ray host *T. tokionis* is currently reported from Taiwan, Japan and Hawaiian waters (Haas & Ebert, 2006; Bandai et al., 2017; this report). Torpedo rays are typically thought to be a benthic species with most captures on bottom longlines or trawls. The capture in the pelagic Hawaiian longline fisheries reveals that dispersal for this ray species may include active swimming in the water column. *Echthrogaleus* spp. are parasites of oceanic, coastal and benthopelagic sharks as well as torpedo rays.

We utilised 28S rRNA gene sequencing in order to evaluate multiple geographical ocean basins [North Atlantic (off Norway), South Atlantic (off Argentina), Eastern Pacific (off Chile) locations] to compare with the Central Pacific Ocean sample of the new species. As of 3 May 2019 there was only one sequence for the *cox1* gene on GenBank and none in the Barcode of Life sequence database. Due to the lack of *cox1* sequences in the databases we selected 28S rRNA

gene for comparative purposes. This gene has a low mutation rate and thus has limitations for the species level identification beyond the basal branch in phylogenetic trees (González et al., 2016). In this study, the 28S rDNA phylogenetic tree clearly separated *E. spinulus* n. sp. from *E. coleopratus*. However, there are six additional described species of *Echthrogaleus* with no genetic sequence information to compare with our sequence. The 28S rDNA sequence alone cannot be used for species identification but combined with the morphological description can provide a basis for species identification. Based on morphology we designate *E. spinulus* as a new species from the torpedo ray *T. tokionis*.

Acknowledgements This paper is dedicated to George Benz, a true force in copepod discovery and friend. We thank K. Jurow of the Pacific Island Region Observer Programme of the NOAA/NMFS/PIFSC for collecting the torpedo ray and S. Arceneaux, K. Forney, J. Kelly for research assistance. The support from the FV Vui Vui longliner, Captain L. V. Pham and crew G. Villasrosa, D. Salvo, V. Quang, E. Ebiata and K. Herrera is appreciated. The NOAA/NMFS/PIFSC program for laboratory support especially J. O'Malley, Bruce Mundy, R. Humphreys, Jr and M. Lee. The Argentina copepod specimen was collected by Claudio Serrano (Museo de Historia Natural Patagonia) and processed for molecular analysis by Walter Ferrari. This research was supported by Ocean Research Explorations. The CLSM images were taken at the University of Hawaii at Manoa, PBRC, Biological Electron Microscope Facility with support from T. Carvalho and M. Dunlap and NSF grant # 1828262. We acknowledge D. Catania CAS, H. Bolick BPBM for the specimen curation and Jennifer Crites for her help with figures. This research was facilitated while (GLC) was a visiting scientist at PIFSC. This is Ocean Research Explorations Hawaiian Islands Biodiversity Project publication 01.

Compliance with ethical standards

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

Ethical approval All applicable institutional national and international guidelines for the care and use of animals were followed.

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