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ORIGINAL PAPER

Two new species of *Xouthous* Thomson, 1883 (Copepoda, Harpacticoida, Pseudotachidiidae) from the Caribbean

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Abstract

During a coastal biodiversity survey of meiofauna taxa in southwest Puerto Rico, specimens of the genus *Xouthous* Thomson, 1883 were recovered. Morphological and genetic studies were ensued and two new species, *Xouthous spinifurca* sp. nov. and *Xouthous dichroma* sp. nov. are herein described. The new species were recovered from epiphytic algae growing on red mangrove (*Rhizophora mangle*) roots. *Xouthous spinifurca* sp. nov. is closely related to *X. aemula* (Thompson & Scott, 1903), *X. wellsi* Huys, 2016, and *X. yeoghooni* Song, Lee, Lee & Khim, 2020 sharing with them the palisade-like spines on the female baseoendopod of the fifth pereopod and the seven-segmented female antennule. It differs from the first two species by the presence of longer baseoendopodal spines of the fifth pereopod, as long or longer than the baseoendopod, the baseoendopod reaching the middle of the longer exopod. It is closely related to *X. yeoghooni*, sharing the same female baseoendopod/exopod ratio of the fifth pereopod, a rectangular endopod-2 of the first pereopod, and a strong outer process in the endopod-2 of the second pereopod. However, in the new species, the baseoendopod spines are longer, the endopod-2 of the first pereopod has three inner setae instead of two present in *X. yeoghooni*, and the outer process on the endopod-2 of the second pereopod is stronger in *X. spinifurca* sp. nov. *Xouthous dichroma* sp. nov. does not belong to the palisade group and appears to be closely related to *X. simulans* (Brady, 1910), and *X. naroensis* Karanovic, 2023, sharing the presence of two inner setae on the endopod-2 of the second pereopod. It differs from *X. simulans* by the presence of a shorter baseoendopodal seta 1, even shorter than setae 3–5, not ending in a long flagellate portion, and the presence of a shorter female exopod of the fifth pereopod exp, not longer than twice its width (about three times as long as wide in *X. simulans*), and with five setae (six in *X. simulans*). *Xouhous naroensis* and *X. dichroma* share the same ratio and armature of the female exopod/baseoendopod of the fifth pereopod, differing, however, in the presence of serrate outer spines in the third exopod of the second to fourth pereopods. A gap between setae four and five of the female baseoendopod of the fifth pereopod occur in both *X. dichroma* and *X. naroensis*. A genetic analysis of available cytochrome oxidase subunit I (COI) Pseudotachidiidae sequences indicate that the two new *Xouthous* species share a closer genetic distance than to other available species of Pseudotachidiidae in GenBank. The 20% sequence divergence in COI between the two *Xouthous* species may be indicative of the phylogenetic separation of the species belonging to the palisade group (e.g., *X. spinifurca* sp. nov.) and those that do not (e.g., *X. dichroma* sp. nov.). Within species divergence in two different species of Pseudotachidiidae ranges from 0.7 to 1% and between species belonging to different genera can be as high as 36–37%.

Keywords Puerto Rico · Red mangroves · Meiofauna · Biodiversity · Pseudotachidiinae

Introduction

The Caribbean Sea is a biodiversity-rich region that washes the coasts of 30 countries and territories and stretches across nearly 4 million km^2 of sea. The island biogeography and complex geological history of the Caribbean have created a wide array of unique habitats and high species diversity (Miloslavich et al. 2010). As many as 10,676 metazoan

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species, including about 2916 crustaceans, were reported in the most recent, comprehensive census of Caribbean biodiversity (Miloslavich et al. 2010). Meiofauna taxa are not well represented in this biodiversity census because of limited taxonomic expertise and effort in the Caribbean despite new species and new lineages that are still being discovered regularly (Schizas et al. 2015; Veglia et al. 2018; Corgosinho et al. 2016). The ubiquitous meiobenthic taxon of harpacticoid copepods is represented in the Caribbean region by 178 species (Suárez-Morales et al. 2006), which is certainly just a portion of the true diversity of this micro-crustacean taxon.

The genus *Xouthous* Thomson, 1883, has a single record (*X. purpurocinctum* (Norman & Scott T., 1905) in the Caribbean from Mexico as *Idomene purpurocincta* (Suárez-Morales et al. 2006), but since then, the genus *Xouthous*, previously known as *Idomene* Philippi, 1843 was re-established by Huys (2009). *Xouthous purpurocinctum* was originally collected by dredging in Salcombe, southwest England in 1875 (Norman & Scott T. 1905). Such wide distribution raises some doubt about the real distribution of species, and more studies are needed to confirm this record.

Xouthous consists of 17 species, two of them appearing as *taxon inquirendum*, i.e., *X. australis* (Brady, 1910) and *X. kabylica* Monard, 1936. They can be associated with ascidians (*X. purpurocinctus*; Saito 2009; Song et al. 2020), bryozoans, mollusks (Song et al. 2020), the phytal community (Médioni and Soyer 1968; Song et al. 2020), and within tidal pools (Song et al. 2020). The genus is distributed worldwide (see Map in Song et al. 2020), with most of the records in the Indian and Pacific Ocean, and the northern Atlantic coast of Europe. *Xouthous novaezealandiae* Thomson, 1883 collected from Dunedin, New Zealand is the designated type species of the genus, which is tentatively divided into two groups, the guttiform (teardrop-shaped) and the clypeiform (shield-shaped) groups (Huys 2016). According to Huys (2016), a future analysis would probably restrict the generic concept to a core group of species belonging to the first group, also characterized by the discrete color pattern (first three free somites red or brownish). Within the guttiform group, the species can be ascribed to a palisade and a non-palisade group of species (Huys 2016; Song et al. 2020). The last new species of this genus (*Xouthous yeonghooni* Song et al. 2020, and *X. naroensis* Karanovic, 2023) were recently described from Korean waters (Song et al. 2020; Karanovic 2023).

Material and Methods

Collections

Specimens of *Xouthous* were collected from various islands and habitats of the northeastern Caribbean Sea (Fig. 1). Most specimens were collected from Puerto Rico because most of the collection efforts were made there. The habitats varied from algae overgrowing red mangrove (*Rhizophora mangle*) roots (Fig. 2) to *Thalassia testudinum* seagrass blades to dead coral rubble. The samples were collected from a 0.5- to 55-m depth. All samples regardless the geographic origin were treated the same. They were placed on a 1-mm sieve and were washed to a 0.063-mm sieve. The fraction of the sample retained on the 0.063-mm sieve was fixed in ethanol 90% for further observations and sorting under an Olympus dissection microscope. Type series deposited at Museum of Marine Invertebrates (MMI-UPRM) at Magueyes Island, Puerto Rico.

Morphology

Prior to light microscopic examination, specimens were cleared, dissected in lactic acid, and mounted on slides with glycerine. The specimens were dissected in lactic acid and mounted on slides with glycerine. All observations and drawings were conducted with the aid of an Olympus BX51 compound microscope equipped with Normarsky interference contrast and using a drawing tube, at 400× and 1000× magnifications.

Two adult specimens of each species (female and male of *X. dichroma* sp. nov. and *X. spinifurca* sp. nov.) were used for CLSM (confocal laser scanning microscopy) as indicated below. The specimens were stained with 1:1 solution of Congo Red and Acid Fuchsin overnight using procedures adapted from Michels and Büntzow (2010). The whole specimens were temporarily mounted in glycerin, and self-adhesive plastic reinforcement rings were used to support the coverslip (Kihara and Rocha 2009; Michels and Büntzow 2010). The material was examined using a Leica TCS SP5 equipped with a Leica DM5000 B upright microscope and three visible-light lasers (DPSS 10 mW 561 nm; HeNe 10 mW 633 nm; Ar 100 mW 458, 476, 488 and 514 nm), combined with the software LAS AF 2.2.1. (Leica Application Suite Advanced Fluorescence). Images were obtained using a 561-nm excitation wavelength with 80% acousto-optic tunable filter (AOTF). Series of stacks were obtained, collecting overlapping optical sections throughout the whole preparation with an optimal number of sections according to the software. The acquisition resolution was 2048 × 2048 pixels, and final images were obtained by maximum projection. To obtain a three-dimensional representation from selected body parts, the data produced during the CLSM scanning was processed with the free software ImageJ Fiji 2.1.0 (https://imagej.nih.gov/ij/), Drishtiimport v2.6.4, and Drishti v2.6.4 (http://anusf.anu.edu.au/Vizlab/drishti/), using a protocol adapted from Kamanli et al. (2017). Final plates were composed and adjusted for contrast and brightness using the software Adobe Photoshop CS4.

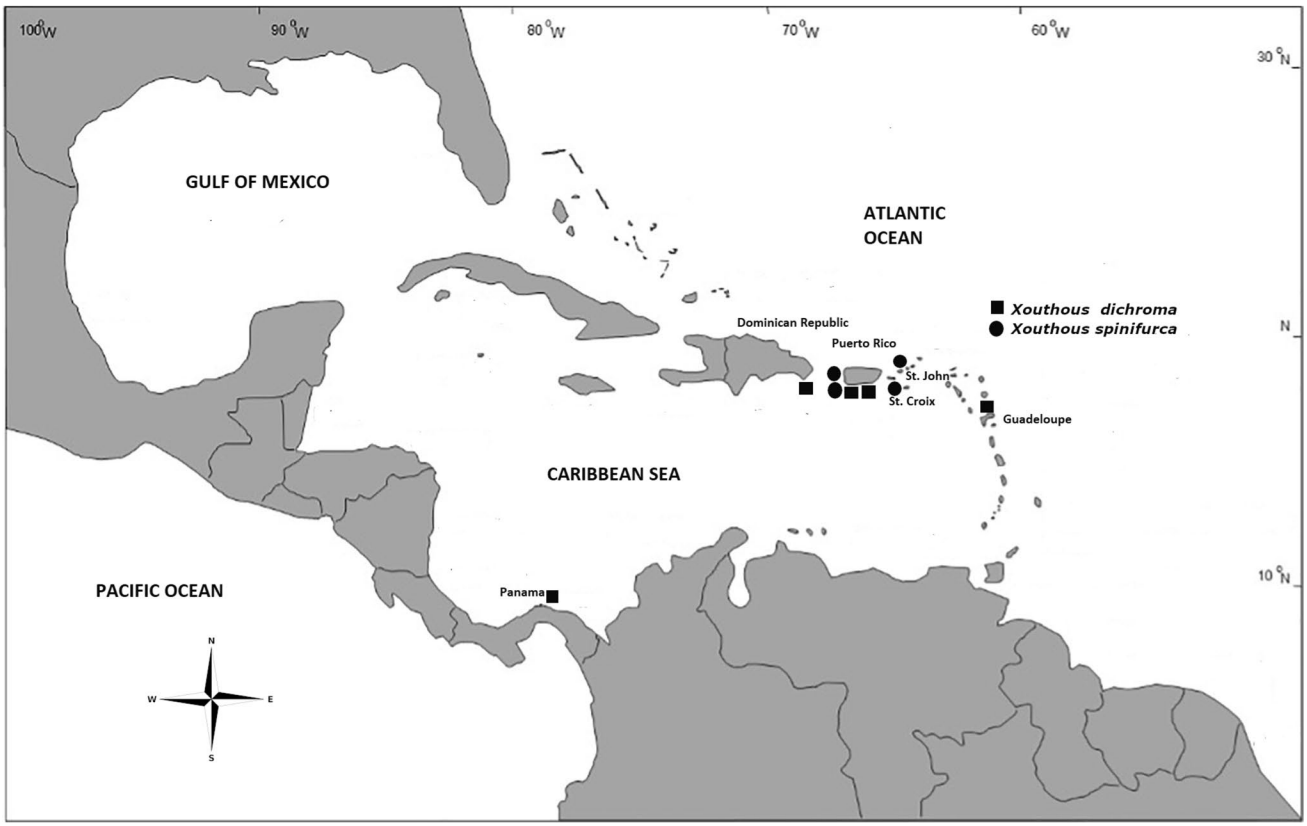


Fig. 1 Map of distribution of *X. dichroma* sp. nov. and *X. spinifurca* sp. nov. in the Caribbean

Terminology and homologization of maxillary and maxillipedal structures follow the methods of Ferrari and Ivanenko (2008). The terms seta, setules, spines, and spinules are used according to the terminology proposed by Huys and Boxshall (1991). The following abbreviations are used in the text: A1, antennule; A2, antenna; ap, apomorphy; baseoenp, baseoendopod; enp, endopod; enp-1 (2,3), proximal (middle, distal) segment of endopod; exp, exopod; exp-1 (2,3), proximal (middle, distal) segment of exopod; Md, mandible; Mx1, maxillule; Mx2, maxilla; Mxp, maxilliped; P1–P6, first to sixth thoracopod; pl, plesiomorphy; UPRM-MMI, University of Puerto Rico, Mayagüez—Museum of Marine Invertebrates, PR, USA (Department of Marine Sciences).

Molecular methods

We used the Chelex® 100 Resin (Bio-Rad, Inc.) protocol to extract DNA from individual copepods. The quantity and quality of DNA were obtained with the NanoDrop 2000™ Spectrophotometer. We amplified by PCR (polymerase chain reaction) fragments of ~ 650 bp of the mitochondrial cytochrome oxidase subunit I (COI) gene with the universal primers LCOI-1490 (5′ GGT CAA CAA ATC ATA AAG ATA TTG G 3′) and HCOI-2198 (5′ TAA ACT TCA GGG TGA CCA AAA AAT CA 3′) (Folmer et al. 1994). Each PCR reaction mixture (total volume 25 µl) contained 1 µl DNA as template, 0.3 µl of each primer forward and reverse (100 µM), 12.5 µl of BioMix (Bioline Company), and 10.9 µl water (ddH2O). PCR reactions were conducted on a Bio-Rad MyCycler ™ Thermal Cycler. The PCR protocol for COI was 95 °C for 3 min, followed by 35 cycles of 15 s at 95 °C, 30 s at 43 °C, 30 s 72 °C, followed by 1 cycle of 5 min at 72 °C, and finally keeping the PCR products at 15 °C. PCR products were loaded on a 1% agarose gel stained with ethidium bromide and visualized under UV fluorescence. All successful PCR products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) to eliminate the remainder dNTPs and unincorporated primers which were then sequenced in both directions using Sanger sequencing at MCLAB (San Francisco, CA, USA) with an ABI 3130xl 16-capillary Genetic Analyzer. All original DNA sequences have been submitted to GenBank and are included in Table 1 of this contribution (COI: Accession Numbers PP786541–PP786544). The accession numbers for the GenBank sequences of the other taxa included in the analysis are from Khodamis's et al. unpublished data (COI: Accession Numbers MF077872, MF077872, MH976521,

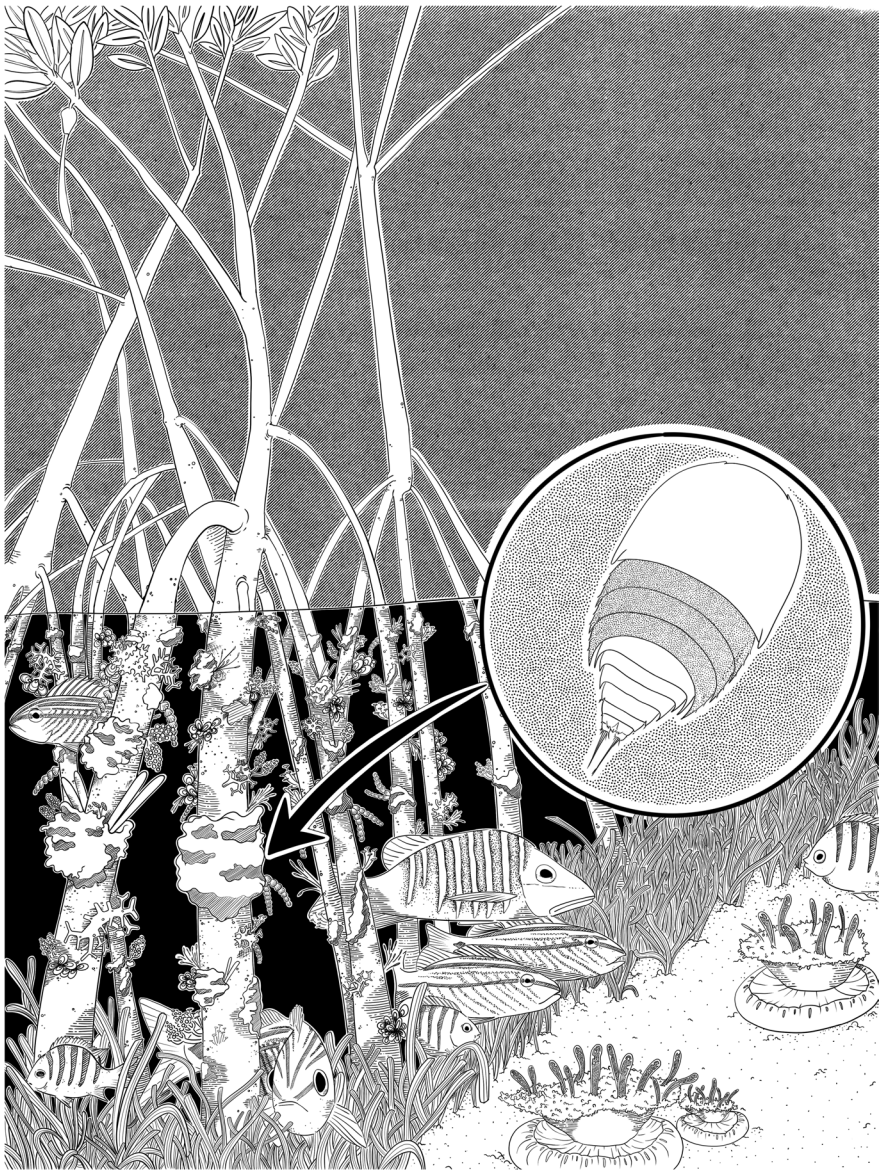


Fig. 2 Illustration of the environment in which *X. dichroma* sp. nov. can be found

MH976523–MH976528). The list of taxa and GenBank accession numbers for the COI data used in this work are also given in Table 1.

The resulting DNA traces were viewed and checked for quality control and verification of mutations in Codon Code Aligner v. 10.0.2 (Codon Code Corp.). Edited sequences were aligned within the MAFFT portal v.7 (Katoh et al. 2019). The genetic analysis of COI sequences were conducted with the maximum likelihood method as implemented in raxmlGUI 2.0.10 (Edler et al. 2020; Stamatakis 2014). The program ModelTest-ng (Darriba et al. 2020) was used within raxmlGUI to estimate the best model of nucleotide substitution for the COI alignment. The alignment is available in Supplementary material 1. The RaxML-ng algorithm (Kozlov et al. 2019) within raxmlGUI was used with the following line of commands: raxml-ng –all –msa < FILENAME > –model K81uf + I + G –prefix < FILENAME > –seed 259,370 –outgroup Sentiropsis_sp_MF077872 –bs-metric tbe –tree rand{10} –bs-trees 1000. Clade support was assessed with the transfer bootstrap expectation (Lemoine et al. 2018). The resulting tree file was uploaded to iTOL v6.6 (Letunic and Bork 2021) for tree visualization and editing. Final edits were made in Adobe Illustrator. Sequence divergence between and within species was assessed in PAUP* (Swofford 2002) with the HKY model of substitution (Hasegawa et al. 1985).

Results

Molecular methods

After cleaning and end-trimming, the final sequence COI alignments were 605 bp length. Nucleotide pairwise distance based on 600 bp of COI within and between species of Pseudotachidiidae Lang, 1936 are on Table 2. The genetic distances were estimated with the HKY (Hasegawa et al. 1985) nucleotide model of substitution. The final data set consisted of sequences of *Xouthous dichroma* sp. nov. and *X. spinifurca* sp. nov. and sequences from three other Pseudotachidiidae species available in GenBank at that time (all from (Khodami et al. unpublished data); Table 1). The phylogenetic analysis with maximum likelihood yielded a monophyletic clade comprised of the two new species of *Xouthous* (100% bootstrap transfer value; Fig. 19). The *Xouthous* species were more closely related to *Pseudotachidius bipartitus* Montagna, 1980 than to *Danielssenia typica* Boeck, 1873. Up to 1% sequence divergence was observed among the three specimens of *X. dichroma* sp. nov. (Table 2). In other Pseudotachidiidae species comparisons, the DNA divergence in *D. typica* ranged from 0% (identical sequences of *D. typica* specimens 4, 5, and 7) to 0.73% divergence. The between species comparisons yielded much larger differences. The two species of *Xouthous* differ as much as 20% in COI sequence divergence (Table 2). The biggest differences were observed between *D. typica* and the *Xouthous dichroma* sp. nov. specimens (~36–37%).

Table 1 Name of species used in the phylogenetic analyses, collection sites, Genbank Accession numbers and references

Species name	Collection site	GenBank accession numbers	Reference
Pseudotachidius bipartitus	Pacific Ocean: North Pacific	MF077873	(Khodami et al., unpublished data)
Sentiropsis sp.	Mediterranean Sea	MF077872	(Khodami et al., unpublished data)
*Danielssenia typica*4	North Sea	MH976524	(Khodami et al., unpublished data)
*Danielssenia typica*3	North Sea	MH976523	(Khodami et al., unpublished data)
*Danielssenia typica*7	North Sea	MH976525	(Khodami et al., unpublished data)
*Danielssenia typica*1	North Sea	MH976521	(Khodami et al., unpublished data)
*Danielssenia typica*8	North Sea	MH976528	(Khodami et al., unpublished data)
*Danielssenia typica*6	North Sea	MH976526	(Khodami et al., unpublished data)
*Danielssenia typica*5	North Sea	MH976527	(Khodami et al., unpublished data)
*Xouthous dichroma*1 sp. nov.	Puerto Rico	PP786541	This study
*Xouthous dichroma*3 sp. nov.	Puerto Rico	PP786542	This study
*Xouthous dichroma*4 sp. nov.	Puerto Rico	PP786543	This study
Xouthous spinifurca sp. nov.	Puerto Rico	PP786544	This study

Table 2 Nucleotide pairwise distance based on 600 bp of COI within and between species of Pseudotachidiidae

	1	2	3	4	5	6	7	8	9	10	11	12
1-*Xouthous dichroma*3	-											
2-*Xouthous dichroma*4	0.83	-										
3-*Xouthous dichroma*1	1.00	0.83	-									
4-*Xouthous spinifurca*	20.24	20.48	20.48	-								
5-*Pseudotachidius*	23.36	23.36	23.99	22.43	-							
6-*Sentiropsis* sp.	26.40	26.63	26.88	26.37	22.12	-						
7-*Danielssenia typica*4	36.93	36.41	37.61	33.76	35.10	31.89	-					
8-*Danielssenia typica*3	37.53	36.73	37.87	35.21	35.16	31.73	0.18	-				
9-*Danielssenia typica*7	37.23	36.43	37.56	34.90	35.16	31.45	0.00	0.17	-			
10-*Danielssenia typica*8	36.77	35.92	37.10	34.82	34.71	31.57	0.37	0.17	0.35	-		
11-*Danielssenia typica*1	36.26	35.44	36.58	35.04	34.36	31.52	0.73	0.51	0.68	0.18	-	
12-*Danielssenia typica*5	37.23	36.43	37.56	34.90	35.16	31.45	0.00	0.17	0.00	0.35	0.68	-
13-*Danielssenia typica*6	36.69	35.90	37.02	34.37	34.49	30.94	0.17	0.33	0.17	0.17	0.51	0.17

Genetic distances were estimated with the HKY (Hasegawa et al. 1985) nucleotide model of substitution in PAUP*. Numbers after the species indicate specimen IDs

Taxonomy

Order Harpacticoida Sars, 1903

Family Pseudotachidiidae Lang, 1936

Subfamily Pseudotachidiinae Lang, 1936

Genus *Xouthous* Thomson, 1883

Type species: *Xouthous novaezealandiae* Thomson, 1883 **(type by original designation)**

New taxa: *Xouthous dichroma* sp. nov. and *Xouthous spinifurca* sp. nov.

Description

Xouthous dichroma sp. nov.

Zoobank: https://zoobank.org/50BE8E28-A1BA-4DDA-BE0F-621C2A50F494.

Type material: 1 female from Bioluminescent Bay, La Parguera Natural Reserve (LPNR), southwest Puerto Rico dissected and mounted in 7 slides (holotype; MMI-UPRM 10007); 1 male from LPNR dissected and mounted in 7 slides (paratype; MMI-UPRM 10008); 1 male from LPNR dissected and mounted in 7 slides (paratype; MMI-UPRM 10009); 1 female from LPNR dissected and mounted in 6 slides (paratype; MMI-UPRM 10010); 1 undissected female from LPNR, mounted in 1 slide (paratype; MMI-UPRM 10011); two undissected males from Ponce, south Puerto Rico, mounted in 1 slide each (paratype; MMI-UPRM 10012, 10,013); three undissected females from Ponce, mounted in 1 slide each (paratype; MMI-UPRM 10014, 10,015, 10,016); two undissected males from LPNR (Cayo Media Luna), mounted in 1 slide each (paratype; MMI-UPRM 10017, 10,018); 1 undissected female from LPNR (Cayo Media Luna), mounted in 1 slide (paratype; MMI-UPRM 10019); 1 undissected female from Isla Grande in Panamá, mounted in 1 slide (paratype; MMI-UPRM 10020); 1 undissected male from Parque Nacional del Este Dominican Republic, mounted in 1 slide (paratype; MMI-UPRM 10021); 1 female from Guadeloupe, mounted in 1 slide (paratype; MMI-UPRM 10022). Coordinates, sampling date, depth, and substrate in Table 3.

Type locality: Samples recovered from the Bioluminescent Bay, La Parguera Natural Reserve, southwest Puerto Rico; taken from epiphytic algae on the roots of red mangroves, varying from 30 cm to 1 m in depth; other samples were collected from Cayo Media Luna, Cayo Caracoles and Cayo Enrique, also located inside the La Parguera Natural Reserve of Puerto Rico.

Other material: Additional samples were collected from Dominican Republic, Guadeloupe, and Panama (Table 3).

Etymology: *Xouthous dichroma* sp. nov. was named after the brownish color of the first three free prosomites, characteristic of the guttiform group (see Huys 2016).

Description of adult female holotype: Habitus shield-shaped (Figs. 3a and 4b, c). Total body length 412 µm

Table 3 Date of collection of the new species, with their respective locality sampling depths and associated substrata

Species	Locality	Latitude, longitude	Depth	Date	Substrate
Xouthous dichroma sp. nov.	Bioluminescent Bay—Puerto Rico	17.973611, – 67.014722	0.5 m	Not recorded	Algae from red mangrove roots
Xouthous dichroma sp. nov.	Media Luna—Puerto Rico	17.935556, – 67.0425	18 m	21/11/2008	Sediment/Coral rubble
Xouthous dichroma sp. nov.	Ponce—Puerto Rico	17.964167, – 66.610278	Shallow—up to 1 m	28/11/2004	*Acanthophora spinicifera*
Xouthous dichroma sp. nov.	Parque Del Este—Dominican Republic	18.205, – 68.753611	Shallow—up to 1 m	28/09/2008	Algae from red mangrove roots
Xouthous dichroma sp. nov.	Isla Grande—Panamá	9.629722, – 79.568056	Shallow—up to 1 m	31/01/2009	Coral rubble
Xouthous dichroma sp. nov.	Guadeloupe	16.356389, – 61.626944	Shallow—up to 1 m	02/05/2008	*Thalassia testudinum*
Xouthous spinifurca sp. nov.	Abrir La Sierra—Puerto Rico	18.76197, – 67.15696	50 m	25/04/2012	Wash from lithic substrate and corals
Xouthous spinifurca sp. nov.	S. Buoy 8—Puerto Rico	18.130556, – 67.334167	15 m	28/04/2012	Coral sample
Xouthous spinifurca sp. nov.	Lang Bank—St. Croix	17.83421, – 64.47584	50 m	06/05/2012	Wash from lithic substrate and corals
Xouthous spinifurca sp. nov.	North Star—St. Croix	17.76985, – 64.82173	23 m	01/05/2011	Wash from lithic substrate and corals
Xouthous spinifurca sp. nov.	East St. John	18.22186, – 64.67596	24–27 m	08/05/2012	Wash from lithic substrate and corals

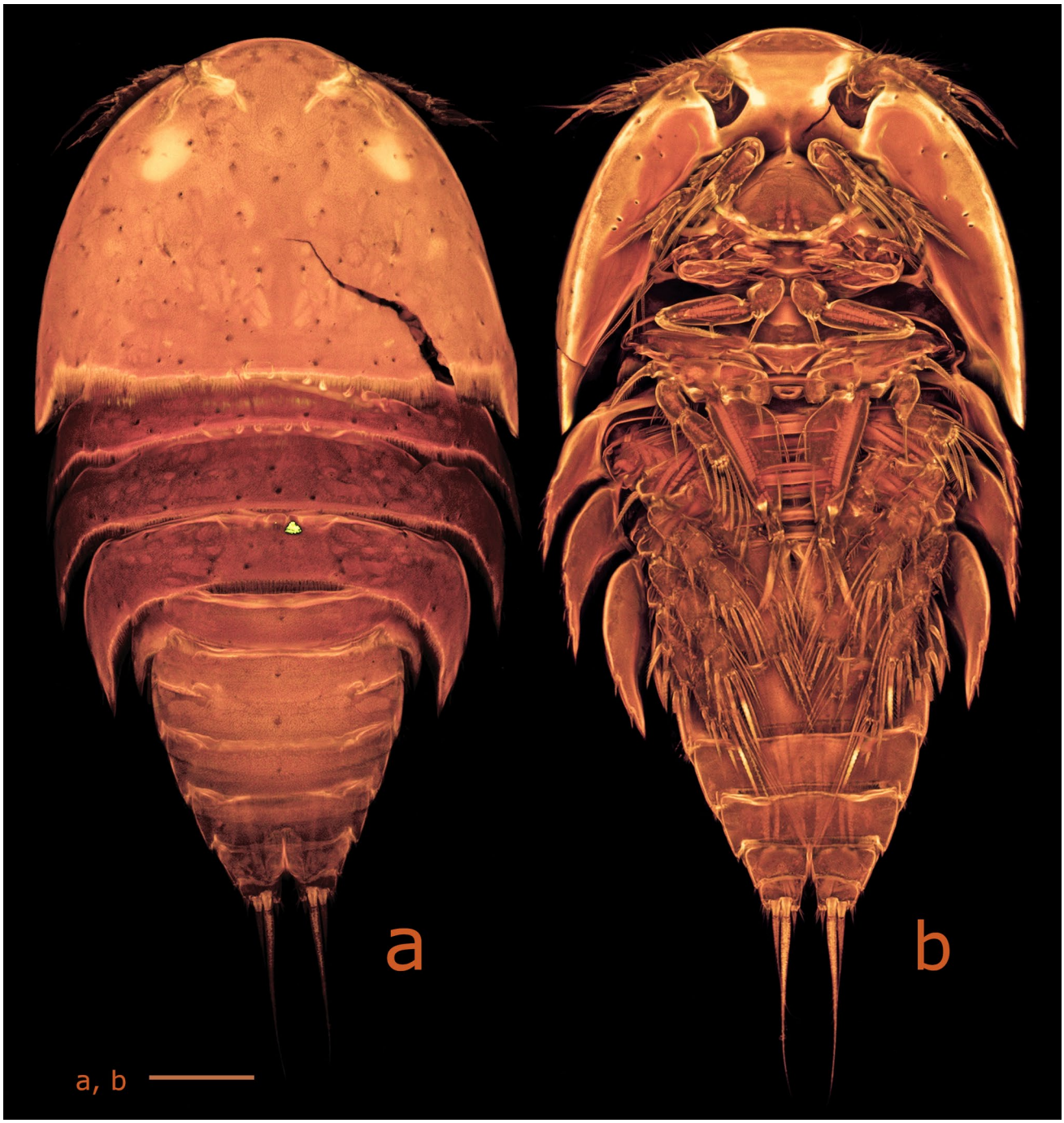
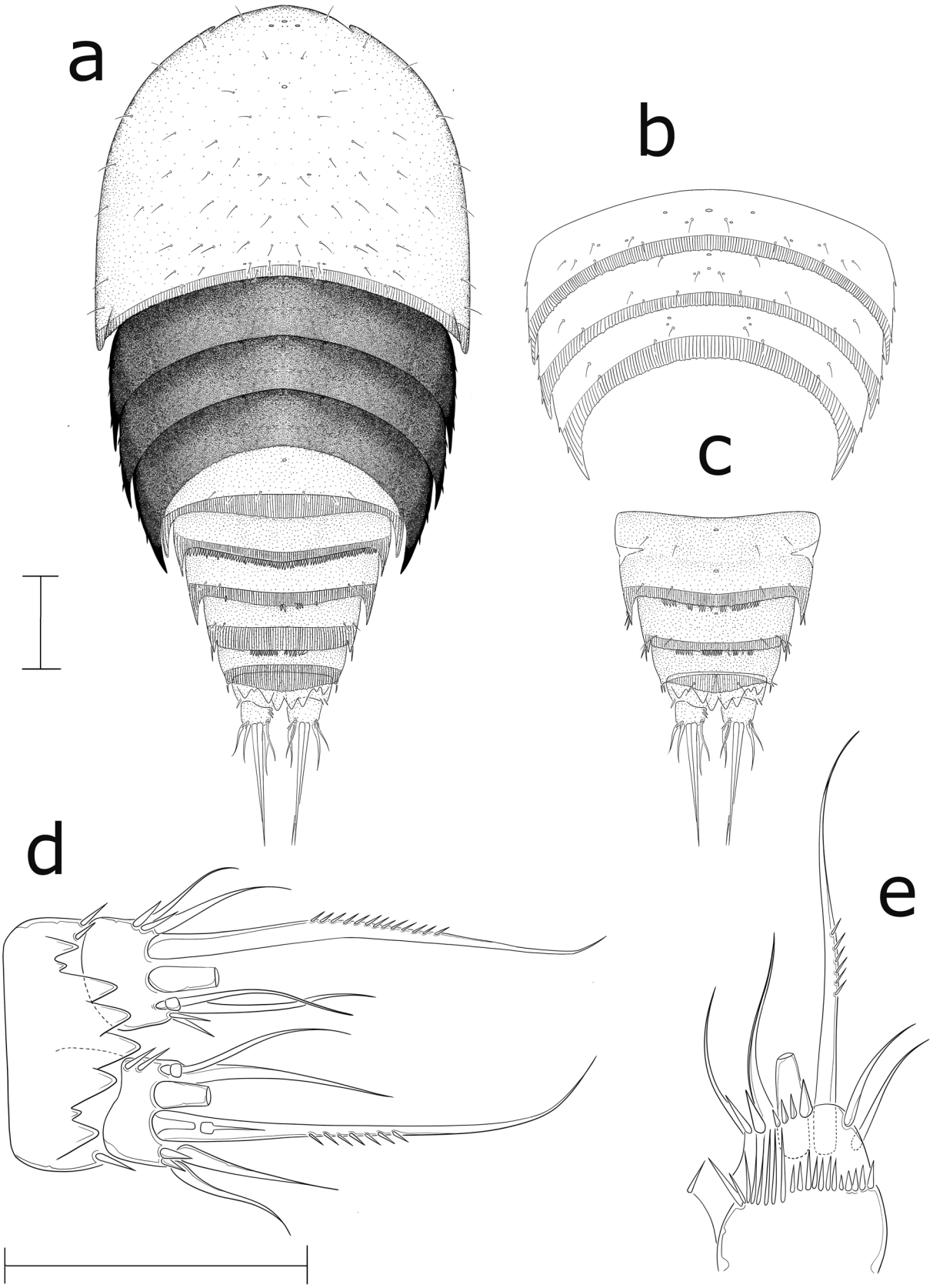


Fig. 3 *X. dichroma* sp. nov., female; confocal laser scanning microscopy of the dorsal (**a**) and ventral (**b**) habitus. Scale bar 50 µm

(mean = 374.6 µm ± 32 µm, $n = 7$), measured from the anterior margin of the rostrum to the posterior margin of the caudal rami. Body sensilla and pores as depicted in Figs. 3a, b and 4b, c). Rostrum absent. Cephalothorax wider than long, posterior border smooth with hyaline frill; relation width/length equal 1.09. First three free pedigerous somites dark red in colour, with serrulate outer margins (Figs. 3a and 5a). All body somites ending in well-developed hyaline frill. Urosome gradually tapering posteriorly, 5-segmented, comprising P5-bearing somite, genital-double somite, 2 free abdominal somites, and telson (Figs. 3a, b and 4c), dorsal margin of urosomites 2 and 3 with distal row of spinules. Genital double-somite (Figs. 3a and 4c) about 2.0 times wider than long, with transverse surface ridges dorsally and laterally, representing original segmentation. P6 (Fig. 6b) represented by smooth single plate over gonopore. Telson


a
b
c
d
e

◂**Fig. 4** *X. dichroma* sp. nov., male, dorsal habitus (**a**); female 1st to 3rd free pedigerous somites (**b**), and urosomite (without 5.th pedigerous somite) with telson and furca (**c**); female telson and furca, dorsal view (**d**); female furca, ventral view (**e**). Scale bars 50 µm; dorsal (**a**), (**c**), ventral (**d**), (**e**)

(Figs. 3a, b and 4c, d) with deep cleft medially, ventrally with a row of unequal spinules near the insertion of the furca. Anal operculum strongly serrulate, partially covered by the hyaline frill of the previous somite (Fig. 4c, d). Furca (Fig. 4d, e) about 1.65 times wider than long, with ventral, inner, and outer row of spinules; with 6 setae inserted distally, outer setae II and III almost the same size of seta VI and VII, setae IV and V the largest, seta V pinnated on the middle outer margin.

A1 (Fig. 6a) 7-segmented; segment 1 small; segment 2 longest; segments 5 and 6 shortest. All setae bare. Setal formula as follows: 1-[1], 2-[9], 3-[9], 4-[5 + 1 fused to the ae], 5-[4], 6-[2], 7-[3 + acrothek (2 + ae)].

A2 depicted for the male only, equal in both sexes.

Md (Fig. 7a) with well-developed gnathobase bearing several bicuspidate teeth distally and 1 long seta in dorsal corner; anterior surface with a short row of spinules; palp comprising basis and 1-segmented exp and enp; basis ornamented with short spinules on anterior surface, and with 4 long plumose setae; exp and enp subequal, exp with 2 lateral strong spines and 4 distal plumose setae, enp with 2 lateral setae, and 7 distal setae.

Mx1 (Fig. 7b, b'), praecoxa without ornamentation, arthrite well-developed, with 9 distal spines/setae, and 2 anterior surface setae, coxa with 5 plumose setae, basis with 6 bare setae, exp fused to basis with 4 plumose setae, enp shorter than exp, with proximal row of spinules, fused to basis with 2 plumose and 1 smooth setae.

Mx2 (Fig. 7c), syncoxa without ornamentation, and with 2 endites; proximal endite divided into 2 bulbs, proximally with 1 unipinnate large seta, distally with 2 long setae; second endite with 2 bipinnate large setae; basis with 1 endite with a long seta and a strong claw-like unipinnate seta (distal one broken); enp-1 drawn out into strong claw with 3 accessory setae; enp-2 represented by 3 setae.

Mxp (Fig. 7d) well-developed, subchelate, syncoxa with spinular rows as depicted, and with 1 long uniplumose seta distally, basis elongated with strong spinular row along

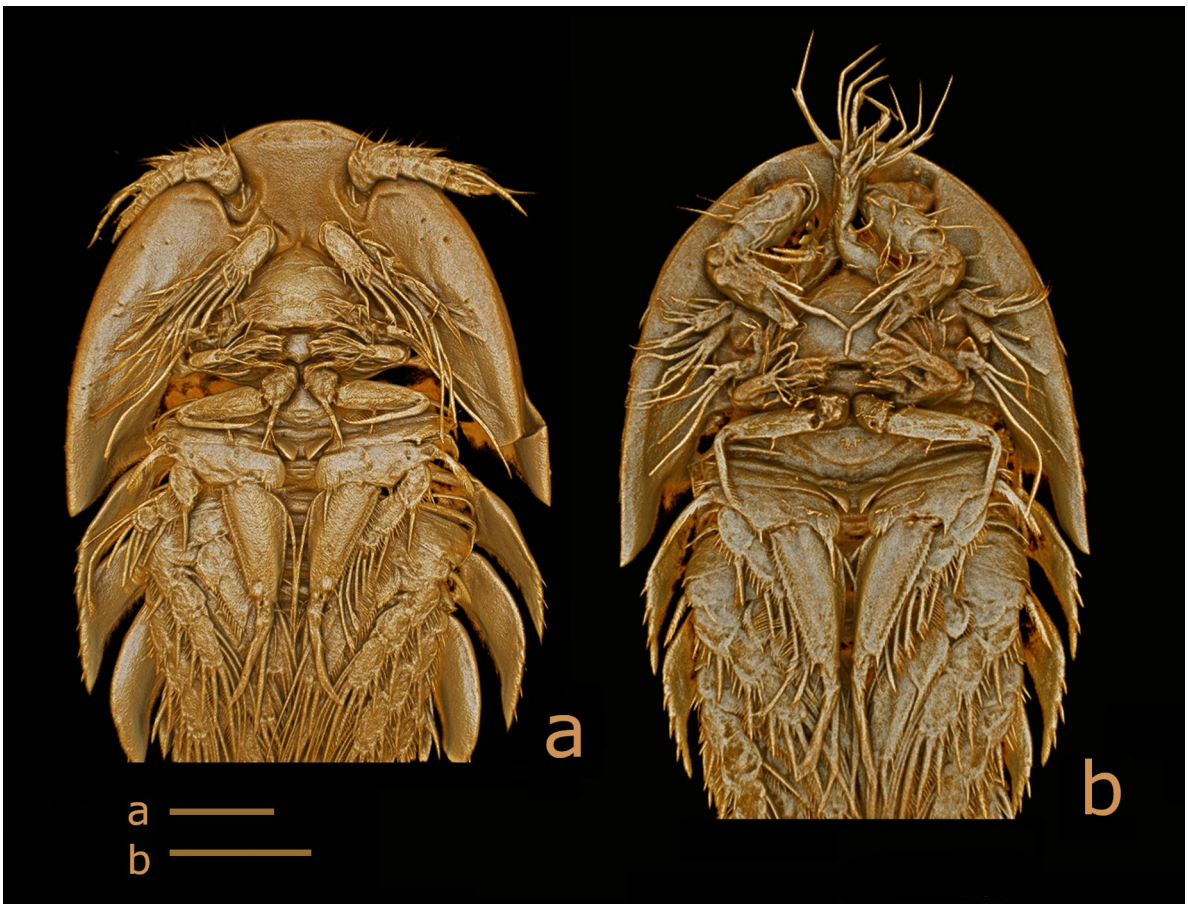


Fig. 5 Three-dimensional representation of the anterior half of a female (**a**) and a male (**b**), of *X. dichroma* sp. nov. Scale bars 50 µm

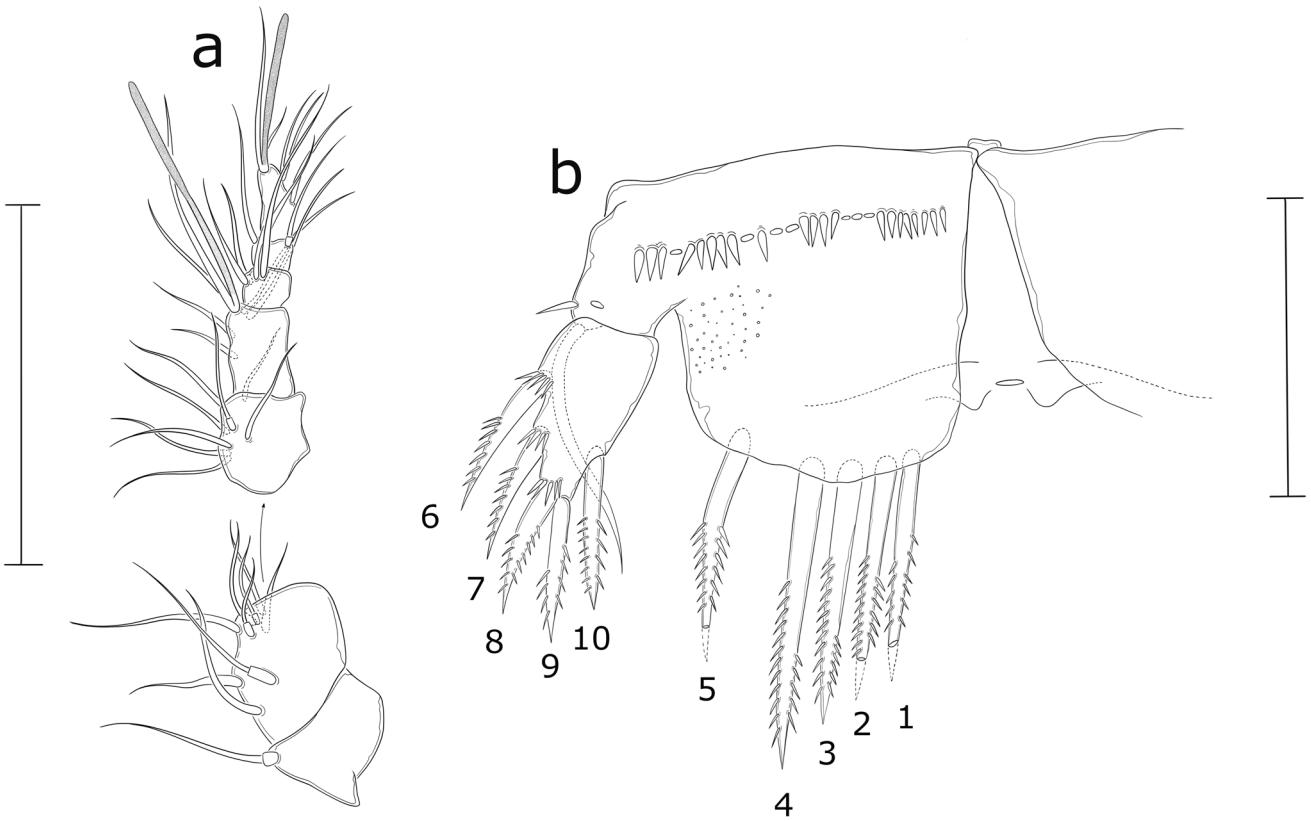


Fig. 6 *X. dichroma* sp. nov., female; A1 (**a**) and P5, P6, and genital field (**b**). Scale bars 50 µm

palmar margin, with 1 plumose seta on palmar margin, enp represented by a strong and unipinnate claw, with 1 long accessory seta.

P1 (Fig. 8a). Praecoxa smooth; coxa wider than long, ornamented with 2 spinular rows and setules along outer margin and on anterior surface, and a small pore on anterior inner surface; basis wider than long, with 1 bipinnate outer and 1 plumose inner spine, with spinules at insertion of both elements, on anterior proximal surface, between enp and exp, and a pore on anterior outer surface; exp 3-segmented, much shorter than enp-1; exp-1 with outer setules and spinules, and 1 outer unipinnate spine; exp-2 slightly longer than exp-1, with outer spinules, 1 outer unipinnate spine, and 1 plumose inner seta; exp-3 shortest, with outer spinules, 3 outer unipinnate spines, and 2 geniculate setae unipinnate at their distal halves; enp 2-segmented; enp-1 large, trapezoid and broad, with spinular rows on outer margin, with anterior median pore, and with 1 long plumose inner seta in proximal third; enp-2 much smaller than enp-1, squared, about 1.4 times as broad as long, with few spinules on outer margin, 2 plumose setae on the inner margin, 1 geniculate seta pinnate medially and proximally, and 1 claw pinnate proximally, rattail ornamented distally.

P2 (Fig. 8b), intercoxal sclerite with 2 median rows of spinules; praecoxa with outer row of spinules; coxa wider than long with anterior hyaline frill, inner surface pore, and outer setules and spinule rows; basis wider than long, with outer bipinnate spine, outer row of spinules, row of spinules between exp and enp, and pore on the outer surface margin; exp 3-segmented, all segments nearly equal in length; exp-1 with spinules on outer margin, spinules distally in the outer corner, and inner hyaline frill, with 1 bipinnate outer spine and 1 short plumose inner seta; exp-2 with strong outer spinules, spinules distally in the outer corner, inner hyaline frill, with 1 bipinnate outer spine and 1 long plumose inner seta; exp-3 with spinules on outer margin, spinules distally in the outer corner, 2 strong outer spines (heavily pectinate on inner side and pinnate outer side), 1 bipinnate outer spine, 1 bipinnate and 1 plumose long seta distally, and 2 plumose long inner setae; enp 3-segmented, longer than exp; enp-1 shortest, with outer spinules, 1 plumose short inner seta and inner hyaline frill; enp-2 and enp-3 subequal in length, enp-2 with outer spinules, distal hyaline frill, 2 outer spinules, 1 inner plumose short seta proximally, and 1 strong inner bipinnate outer seta; enp-3 with outer spinules, distal pore, 2 distal bipinnate spines, 1 distal weakly unipinnate seta, and 2 inner plumose setae.

P3 (Fig. 9a), praecoxa with strong outer spinules; coxa wider than long, with spinules on outer margin and on distal inner margin, and medially; basis wider than long, with

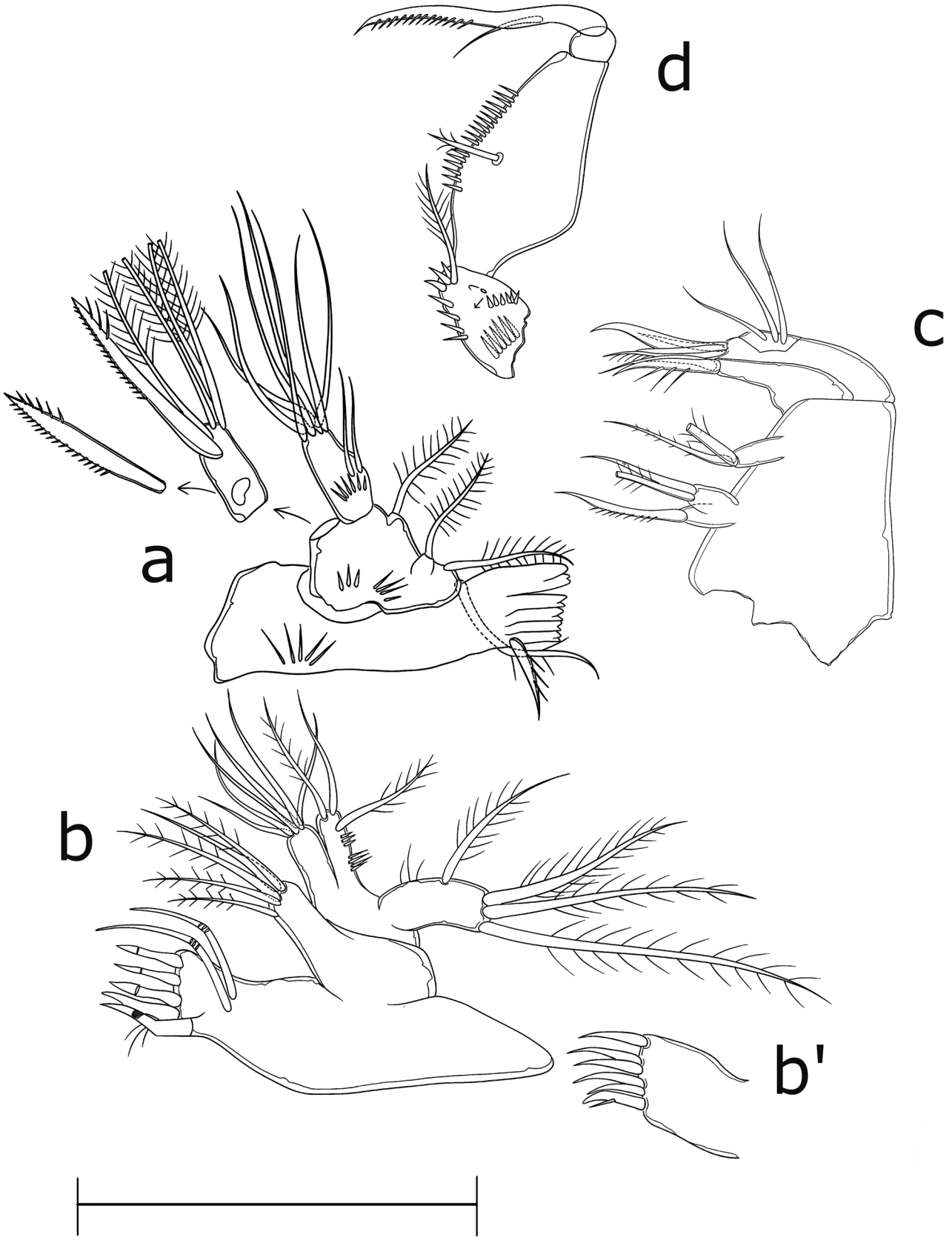


Fig. 7 *X. dichroma* sp. nov., female; Md (**a**); Mx1 (**b**, **b'**); Mx2 (**c**); Mxp (**d**). Scale bars 50 µm

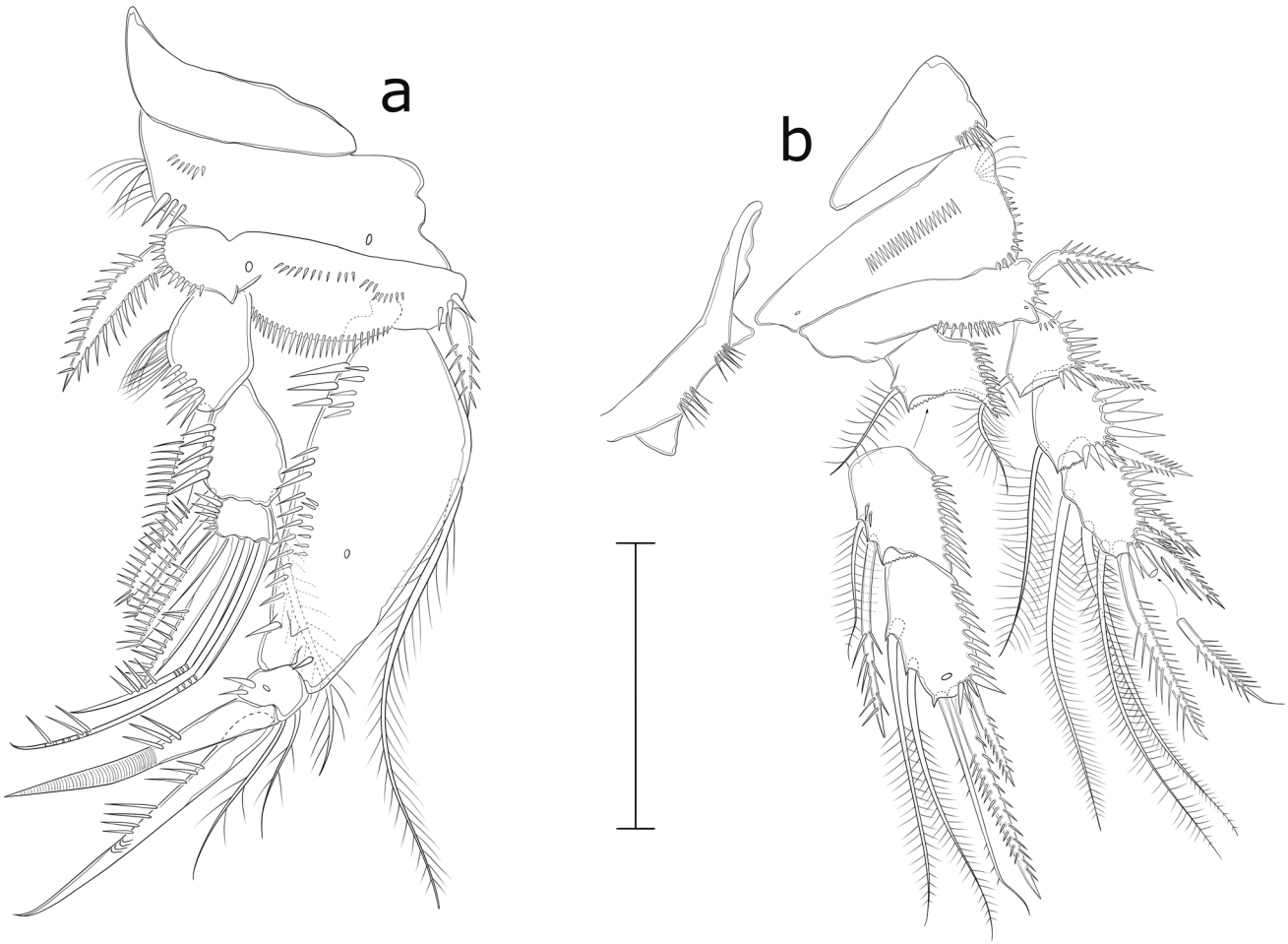


Fig. 8 *X. dichroma* sp. nov., female; P1 (**a**) and P2 (**b**). Scale bar 50 µm

outer spinules, outer pore and small outer seta, with row of spinules between enp and exp; exp 3-segmented; segments slightly different from P2 in fine ornamentation, similar to P2 in armature, except for 3 plumose inner setae on exp-3; enp 3-segmented, as long as exp; enp-1 as in P2; enp-2 with outer spinules and 1 plumose long inner seta; enp-3 longest, with spinules on outer margin, spinules on distal outer margin, distal anterior pore, 1 bipinnate outer spine, 1 bipinnate distal spine and 1 distal plumose seta, and 3 plumose long inner setae.

P4 (Fig. 9b), praecoxa with outer spinules; coxa wider than long with outer spinules, spinules distally on the inner margin, and inner pore; basis wider than long, with spinules near outer seta and between exp and enp, 1 pore on outer surface, and with 1 bare outer seta; exp 3-segmented; segments slightly different from P2 in fine ornamentation, similar to P2 in armature, except for 3 plumose inner setae on exp-3, as in P3; enp 3-segmented, slightly shorter than exp; enp-1 and enp-2 as in P3; enp-3 as in P3 except for the absence of the distal surface pore and the presence of only 2 plumose inner setae.

P5 (Fig. 6b) with completely separated baseoenp and exp. Baseoenp broad, with anterior row of spinules; outer basal seta long and smooth; endopodal lobe reaching the insertion of exp spine 9, slightly convex, with 5 spines and a gap between spine 4 and 5; exp 1.5 as long as wide, clearly protruding beyond apical margin of baseoenp, with 5 spines: 2 outer unipinate, 2 distal, and 1 inner bipinate spines.

P6 (Fig. 6b) represented by smooth and unarmed single plate over gonopore.

Description of adult male allotype. Total body length 375 µm (mean = 377 µm ± 5.7 µm, $n = 4$), measured from anterior margin of the cephalothorax to posterior margin of caudal rami (Figs. 4a and 10a, b). Urosome gradually

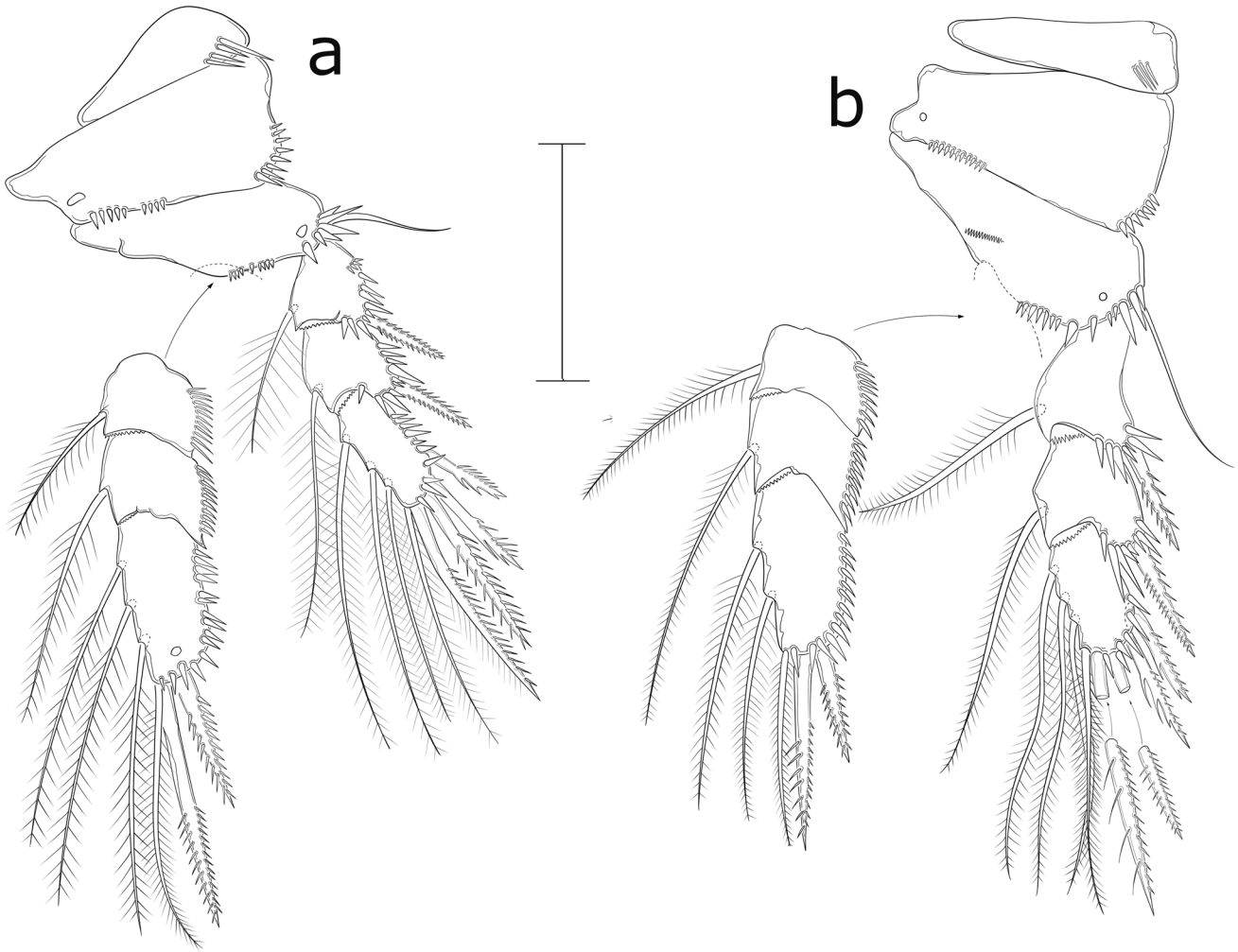


Fig. 9 *X. dichroma* sp. nov., female; P3 (**a**) and P4 (**b**). Scale bar 50 µm

tapering posteriorly (Figs. 4a and 10a,b). Cephalothorax wider than long, ornamented as in the female; relationship width/length equal 1.05. First three free pedigerous somites dark in color as in female, with serrulate outer margin (Figs. 4a, 10b, and 5b). Urosome (Figs. 4a and 10a,b) 6-segmented, comprising P5-bearing somite, genital somite, 3rd to 5th urosomites, and telson, with row of spinules on urosomites 2–4.

A1 (Fig. 11a) 8-segmented; subchirocer; segment 1 with 1 plumose seta; segment 2 shortest with 1 plumose seta; segment 3 with 6 setae anteriorly and 5 setae posteriorly, and an anterior ae; segment 4 small with 6 bare setae, and 1 ae; segment 5 with 2 bare setae; segment 6 strongly swollen with 5 bare setae and 1 ae; segment 7 elongate with 1 modified spinule, segment 8 with acrothek (2 setae + ae).

A2 (Fig. 11b) 3-segmented, comprising coxa, allobasis (fused basis and first endopodal segment), and free endopodal segment; coxa small, without ornamentation; allobasis longer than enp and bearing a abexopodal seta, and 2 inner spinular rows; enp with inner spinular rows; armature consisting of 8 spines/setae (1 swollen unipinnate spine with a distal flagellar portion, 4 geniculate setae, 1 naked seta, 1 unipinnate seta and 1 modified spine with pinnate tip); exp 2-segmented and slightly shorter than enp; proximal segment short with 2 plumose setae, distal one with a spinule row distally, 2 lateral bipinnate and 2 distal bipinnate setae.

Mouth appendages, P1, P2, P3, and P4 as in female.

P5 (Fig. 11c) with separated baseoenp and exp; baseoenp confluent, with row of spinules along anterior

Fig. 10 Confocal laser scanning microscopy of the dorsal (**a**) and ventral (**b**) habitus of a male of *X. dichroma* sp. nov. Scale bar 50 µm

Table 4 Setal formula of swimming legs of *Xouthous dichroma* sp. nov.

Thoracopod	Exopod	Endopod
P1	1 1 230	1 220
P2	1 1 223	1 2 221
P3	1 1 224	1 1 321
P4	1 1 224	1 1 221

margin, with undulated distal margin, and 2 long bipinnate distal setae accompanied by spinules around each seta; with outer basal seta in a long setophore; exp longer than wide, with 6 bipinnate setae, 3 outer, 2 distal, and 1 inner setae.

P6 (Fig. 11d) represented a single cuticular flap with 1 bare seta, and 1 strong bipinnate spine on each side. P1–P4 armature formulae in Table 4.

Xouthous spinifurca sp. nov.

Zoobank: https://zoobank.org/5CEE1575-2440-4A68-90EC-263B350C9E01.

Type material: 1 female from east St. John, (US Virgin Islands—USVI) dissected and mounted in 7 slides (holotype; MMI-UPRM 10023); 1 male from east St. John dissected and mounted in 7 slides (paratype; MMI-UPRM 10024); 1 undissected female from east St. John mounted in 1 slide (paratype; MMI-UPRM 10025); 1 undissected male from Abrir La Sierra in Puerto Rico, mounted in 1 slide (paratype; MMI-UPRM 10026); three undissected females from South Bouy 8 in Puerto Rico, mounted in 1 slide each (paratype; MMI-UPRM 10027); 1 undissected male from Lang Bank in St. Croix (USVI), mounted in 1 slide (paratype; MMI-UPRM 10028); two undissected male and 1 undissected female from Lang Bank and North Star respectively-St. Croix, mounted in 1 slide each (paratype; MMI-UPRM 10029); 1 undisected male from east St. John, mounted in 1 slide (paratype; MMI-UPRM 10030). Coordinates, sampling date, depth, and substrate in Table 3.

Type locality: St. John, U.S. Virgin Islands-. Other samples recovered from St. Croix, USVI, Abrir La Sierra, and S. Buoy 8, both locations west off Puerto Rico (Table 3).

Etymology: *Xouthous spinifurca* sp. nov. was named after its characteristic furca being more ornate with larger spinules.

Description of adult female holotype: Habitus shield-shaped (Fig. 12a). Total body length 400 µm (mean = 444.7 µm ± 58 µm, $n = 3$), measured from the anterior margin of the cephalothorax to the posterior margin of the caudal rami. Body sensilla and pores as depicted in Figs. 12a, b and 13b, c). Rostrum absent. Cephalothorax wider than long, posterior border smooth with hyaline frill; relation width/length equal 1.13. First three free pedigerous somites dark red in color, with serrulate outer margins (Figs. 12b; 18a). All body somites ending in well-developed hyaline frill. Urosome gradually tapering posteriorly, 5-segmented, comprising P5-bearing somite, genital-double somite, 2 free abdominal somites, and anal somite (Fig. 12a, b; Fig. 13c). Dorsal margin of urosomites 2 (genital double somite) and 3 with distal row of spinules. Genital double-somite (Figs. 12a and 13c) about 2.0 times wider than long, with transverse surface ridges dorsally and laterally, representing original segmentation. Telson (Figs. 12a, b and 13c, d) with deep cleft medially, ventrally with a row of long spinules near the insertion of the furca. Anal operculum strongly serrulate, with sharp edges (Fig. 13c, d). Furca (Fig. 13d, e) about 1.76 times wider than long, with ventral, inner and outer row of spinules; with 6 setae inserted distally, outer setae II shorter than seta III; seta III almost the same size of seta VI and VII, setae IV and V the largest.

A1, A2, and mouth appendages as in *Xouthous dichroma* sp. nov..

P1 (Fig. 14a). Praecoxa and coxa damaged during dissection; basis wider than long, with 1 bipinnate outer and 1 plumose inner short and thick seta, with spinules at insertion of both elements, on anterior midline, running from the middle to inner margin, and a pore on anterior outer surface; exp 3-segmented, shorter than enp-1; exp-1 with outer spinules, and 1 outer unipinnate spine; exp-2 with outer spinules, 1 outer unipinnate spine, and 1 plumose inner seta; exp-3 shortest, with outer spinules, 3 outer unipinnate spines, and 2 geniculate setae unipinnate at their distal halves; enp 2-segmented; enp-1 large, trapezoid and broad, with spinular rows on outer margin, and with 1 long plumose inner seta in proximal third; enp-2 much smaller than enp-1, rectangular, about 1.7 times as long as wide, with few spinules on anterior surface, 3 plumose setae on the inner margin, 1 geniculate seta pinnate medially, and 1 claw pinnate medially, rat-tail ornamented distally.

P2 (Fig. 14b), praecoxa with outer row of spinules; coxa wider than long with outer row of spinules and inner pore; basis wider than long, with outer bipinnate seta, outer row of spinules, short row of spinules between exp and enp, and pore on the outer surface margin; exp 3-segmented, all segments subequal in length; exp-1 with spinules on outer margin, spinules distally in the outer corner, and inner hyaline frill, with 1 bipinnate outer spine and 1 short plumose inner seta; exp-2 with strong outer spinules, spinules distally in the outer corner, inner hyaline frill, with 1 bipinnate outer spine and 1 long plumose inner seta; exp-3 with spinules on outer margin, spinules distally in the outer corner, 3 bipinnate outer spines, 1 unipinnate and 1 plumose long seta distally, and 2 plumose long inner setae; enp 3-segmented, longer than exp; enp-1 shortest, with outer spinules, 1 plumose short inner seta and inner hyaline frill; enp-2 with outer spinules, a long outer spiniform process distally, 1 inner plumose short seta proximally,

and 1 bare long inner seta; enp-3 with outer spinules, 2 distal bipinnate spines, 1 distal plumose seta, and 2 inner plumose setae.

P3 (Fig. 15a), praecoxa and coxa damaged during dissection; basis wider than long, with outer spinules, outer pore and small outer seta, with row of spinules between enp and exp; exp 3-segmented; segments slightly different from P2 in fine ornamentation, similar to P2 in armature, except for 3 plumose inner setae on exp-3; enp 3-segmented, as long as exp; enp-1 as in P2; enp-2 with row of spinules along whole outer margin, inner hyaline frill, and 1 plumose long inner seta; enp-3 longest, with row of spinules on whole outer margin, 1 bipinnate outer spine, 1 bipinnate distal spine and 1 distal plumose seta, and 3 plumose long inner setae.

P4 (Fig. 15b), praecoxa smooth; coxa wider than long with outer spinules; basis wider than long, with spinules near outer seta, 2 small spinules between exp and enp, 1 pore on outer surface, and with 1 bare outer seta; exp 3-segmented; segments less ornamented than P2 and P3, similar to P2 in armature, except for 3 plumose inner setae on exp-3, as in P3; enp 3-segmented, slightly shorter than exp; enp-1 and enp-2 less ornate than in P3, but with the same armature; enp-3 less ornate than in P3, with a distal surface pore and with only 2 plumose inner setae.

P5 (Fig. 16b) with completely separated baseoenp and exp. Baseoenp broad, with anterior row of spinules; outer basal seta long and smooth; endopodal lobe not reaching the insertion of exp spine 10, straight, with 6 juxtaposed thick spines; exp 2.6 times as long as wide, with 3 bipinnate and 2 unipinnate spines: 2 outer, 2 distal, and 1 inner spine. P1–P4 armature formulae in Table 5.

P6 (Fig. 16b) represented by single plate over gonopore, armed on each side with a seta and a pectinated short spine.

Description of adult male allotype. Total body length 450. μm (mean = 438.8 μm ± 26 μm, $n = 4$), measured from anterior margin of cephalothorax to posterior margin of caudal rami (Figs. 13a and 17a, b). Urosome gradually tapering posteriorly. Cephalothorax wider than long, ornamented as in the female (Fig. 13a); relationship width/length equal 1.11. First three free pedigerous somites dark in color as in female, with serrulate outer margin (Figs. 13a, 17a, b and 18b). Urosome (Figs. 13a and 17a) 6-segmented, comprising P5-bearing somite, genital somite, 3rd to 5th urosomites, and telson.

Table 5 Setal formula of swimming legs of *Xouthous spinifurca* sp. nov.

Thoracopod	Exopod	Endopod
P1	1 1 230	1 220
P2	1 1 223	1 2 221
P3	1 1 224	1 1 321
P4	1 1 224	1 1 221

A1, A2, and mouth appendages as in *Xouthous dichroma* sp. nov. P1, P2, P3, and P4 as in female.

P5 (Fig. 16a) with separated baseoenp and exp; baseoenp confluent, with 2 rows of spinules along anterior margin, with strongly irregular distal margin, with 2 long bipinnate distal setae; with outer basal seta in a long setophore; exp longer than wide, with 6 spines, 3 outer, 2 distal, and 1 inner spine, outer proximal missing.

P6 (Fig. 16a) represented a single cuticular flap with 1 bare seta, and 1 strong bipinnate spine on each side.

Discussion

The new species belong to the guttiform group of species, sharing the following characters that we consider apomorphic for this group (plesiomorphies within brackets): a discrete color pattern of red or brownish on the first 3 free somites (absent in the clypeiform group), the teardrop-shaped (guttiform) habitus (shield-shaped in the clypeiform group), the presence of 2 enlarged spines on the Md exp (3 in the ground pattern of the clypeiform group), and the 2-segmented P1 enp (3 in the clypeiform group) with a distinctly trapezoidal-shaped proximal segment. The coloration persists even in specimens stored in ethanol.

It is challenging to establish a clear phylogenetic structure for the species in the guttiform group of the genus *Xouthous* due to the lack of complete descriptions, absence of illustrations, and insufficient information in the existing literature. Determining the phylogenetic significance of certain traits, especially those newly observed or previously undescribed, is particularly difficult. Despite the significant contributions by Huys in 2016, who redefined the genus and introduced three new species with detailed character descriptions in the identification key, understanding the phylogenetic relationships within *Xouthous* remains complex. However, the palisade group within the monophyletic guttiform group appears to be well-defined by a unique feature: the palisade arrangement of spines on the female P5 baseoenp, which is one of the most distinct modifications observed within *Xouthous* and is considered an apomorphic trait for this group. In contrast, the baseoenp setae within harpacticoids are seldom tightly juxtaposed, often showing significant gaps between setae. It is not possible to address, with the current knowledge of the group and the incomplete descriptions available in the literature if there is any apomorphy for the non-palisade group. As far as we can know, this group lacks clear apomorphies, suggesting that it may be paraphyletic.

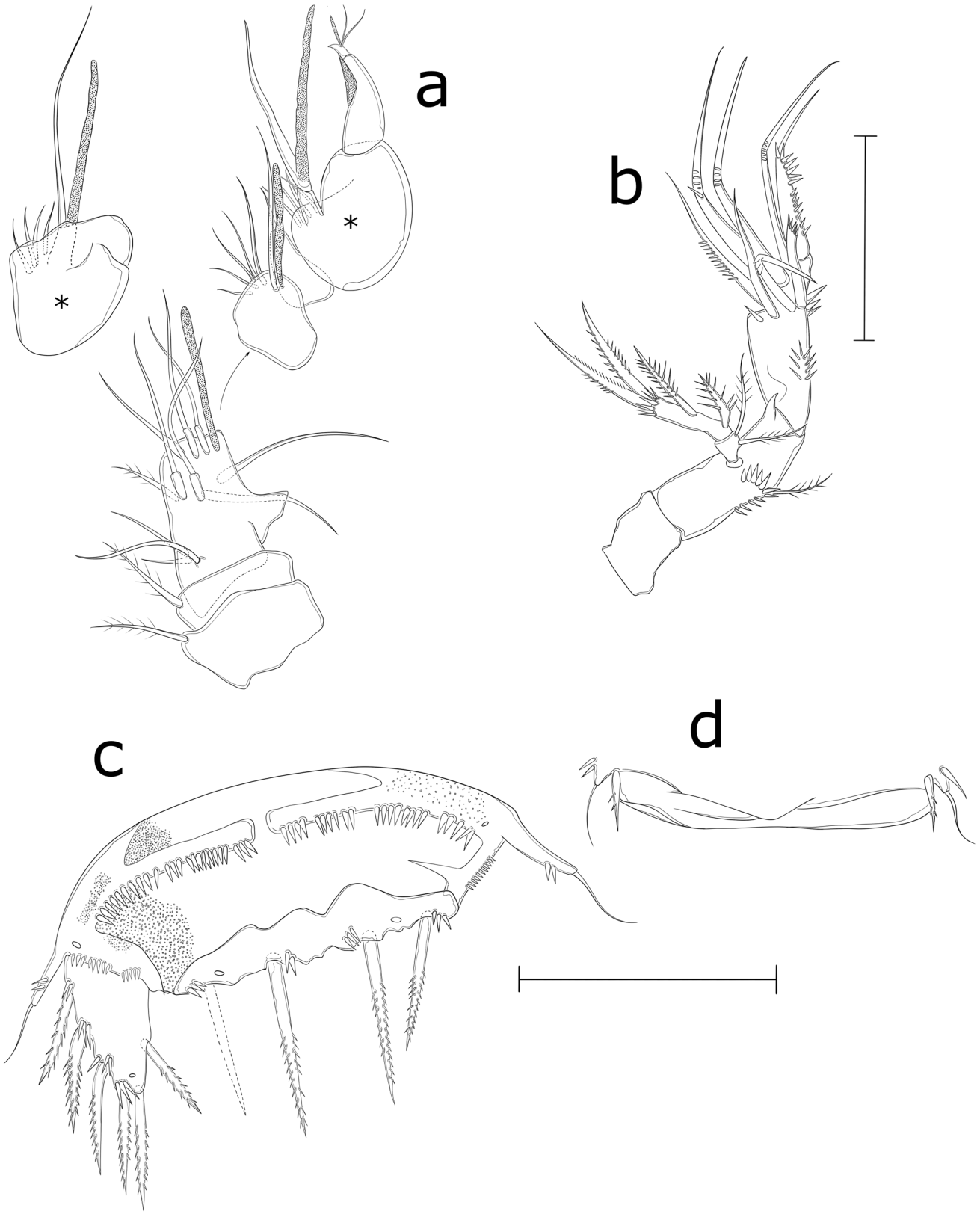


Fig. 11 *X. dichroma* sp. nov., male; A1 (**a**); A2 (**b**); P5 (**c**); P6 (**d**). Scale bars 50 µm; vertical (**a**), (**b**), horizontal (**c**), (**d**)

The palisade group is composed of *X. laticaudatus* (Thompson & Scott, 1903), *X. maldiviae* Sewell, 1940, *X. andamanensis* Huys, 2016, *X. aemula*, *X. wellsi*, *X. yeonghooni*. Its monophyly is supported by a female P5 baseoenp "with broad, spatulate, parallel-sided setae that are tapered or rounded only towards the extreme apex; setae set very close together and approximately equal in length, giving the appearance of a palisade" (Huys 2016) (ap). *Xouthous spinifurca* sp. nov. has all of these characters and can be easily accommodated within the palisade group. Within this group, *Xouthous spinifurca* sp. nov. differs from *X. laticaudatus*, *X. maldiviae*, *X. andamanensis* by the presence of a 7-segmented A1 in the female (pl). It differs from *X. laticaudatus* by having a longer female P5 exp (pl), extending at least or beyond the middle of the baseoenp setae, which are as long or longer than the exp. It also differs from *X. maldiviae*, and *X. andamanensis* by the presence of a female P5 baseoenp reaching at least the middle of the exp (pl?; ap?); the seta 10 of the female P5 exp is not fused as in *X. maldiviae* (pl), and the female P5 exp has only 5 setae (ap), instead of 6 as in *X. andamanensis. Xouthous spinifurca* sp. nov. may be closely related to *X. laticaudatus* and *X. yeonghooni* sharing with them rectangular enp-2 of P1 (ap), the quadratic enp-2 observed in other species such as *X. aemula*

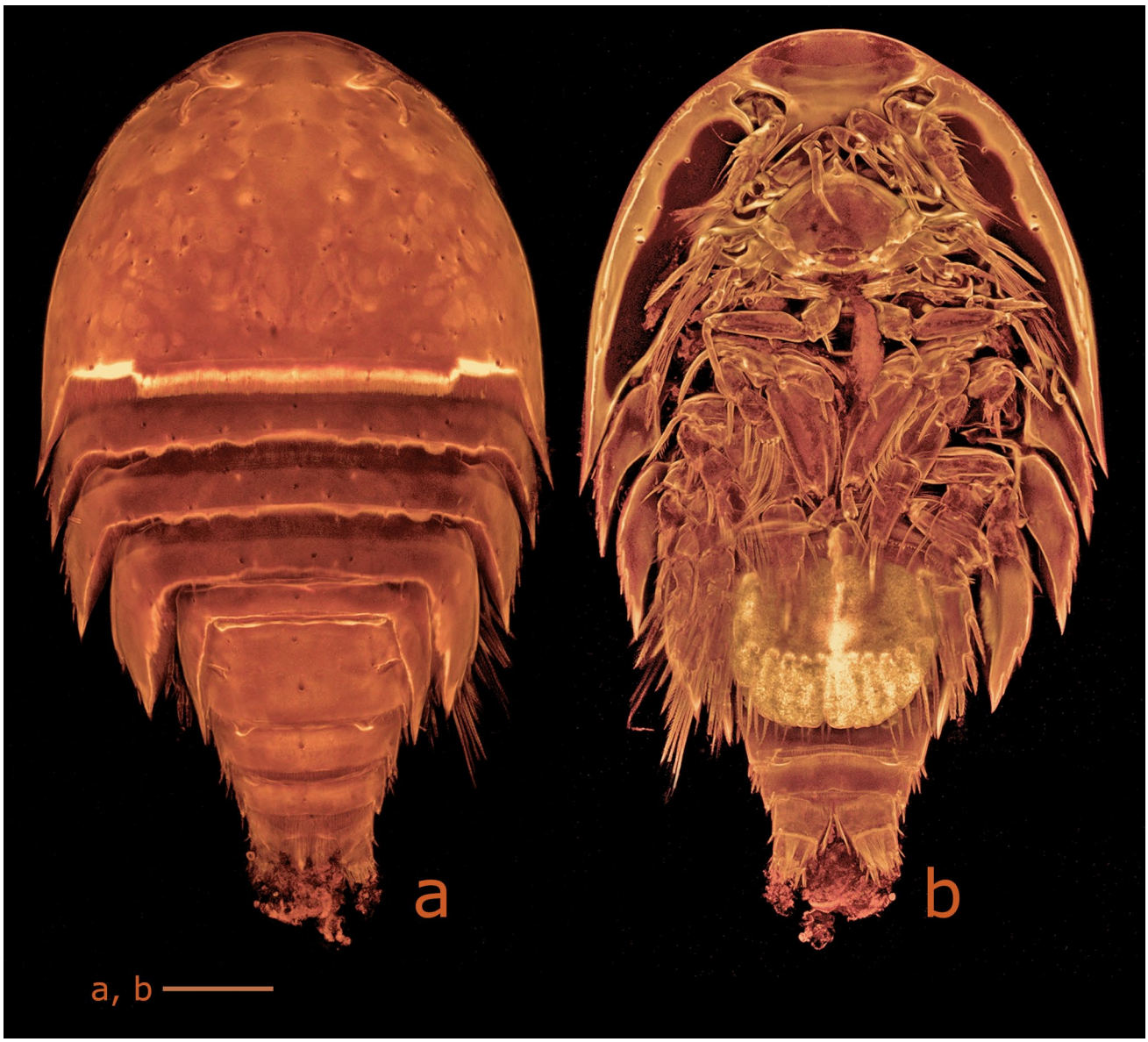


Fig. 12 *X. spinifurca* sp. nov., female; confocal laser scanning microscopy of the dorsal (**a**) and ventral (**b**) habitus. Scale bar 50 µm

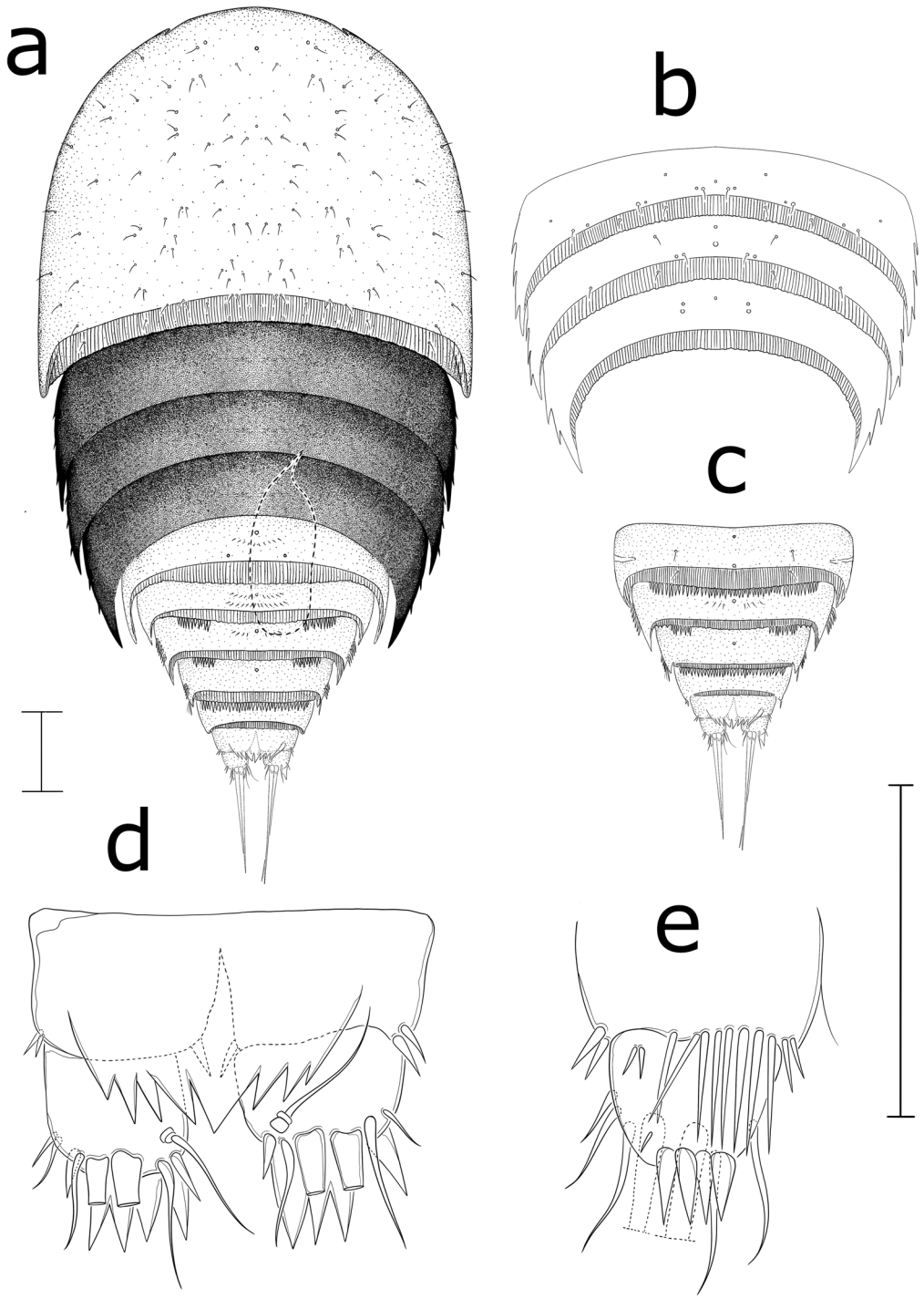


Fig. 13 *X. spinifurca* sp. nov., male, dorsal habitus (**a**); female 1st to 3rd free pedigerous somites (**b**), and urosomite (without 5.th pedigerous somite) with telson and furca (**c**); female telson and furca, dorsal view (**d**); female furca, ventral view (**e**). Scale bars 50 μm; left (**a**), (**c**), right (**d**), (**e**)

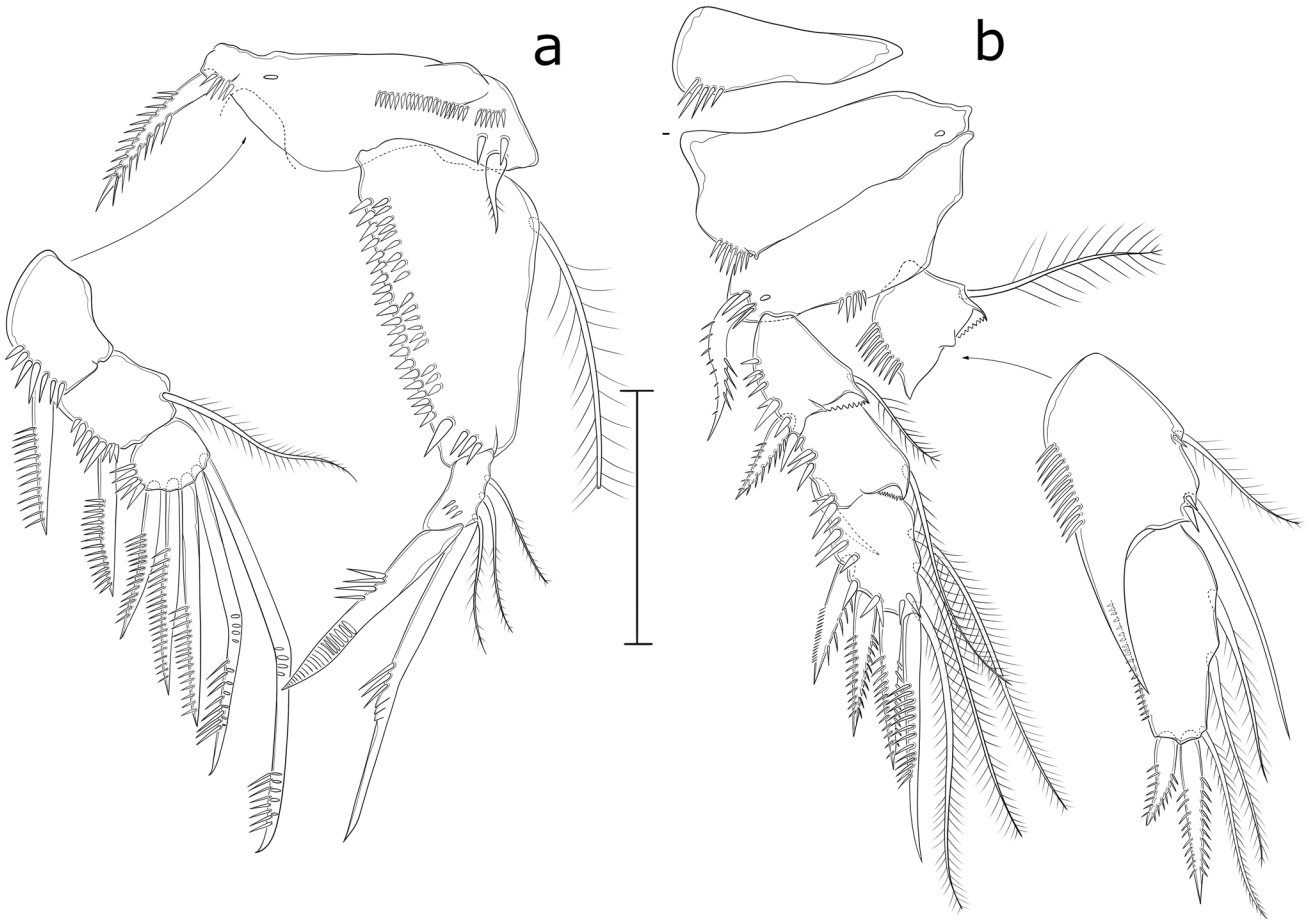


Fig. 14 *X. spinifurca* sp. nov., female; P1 (**a**) and P2 (**b**). Scale bar 50 µm

and *X. maldiviae* is considered plesiomorphic. Nothing can be said about the proximity of *Xouthous spinifurca* sp. nov. and *X. andamanensis* and *X. wellsi*, because they were established by Huys (2016) without illustrations or reference to this character. It differs from the first two species by the presence of longer P5 baseoenp spines, as long as or longer than the baseoenp (pl), the baseoenp reaching the middle of the longer exp (pl); in *X. aemula*, *X. wellsi* the female P5 baseoenp is longer than the exp (ap) and the baseoenp setae are short and truncate (ap). The P1 enp-2 is also longer, with 3 inner setae (pl) in *Xouthous spinifurca* sp. nov. It is most closely related to *X. yeonghooni*, sharing the same female P5 baseoenp/exp ratio with the baseoenp not reaching the insertion of the exopodal seta 10 (pl). With *X. yeonghooni* it shares a rectangular P1 enp-2 (ap), and a strong outer process in the P2 enp-2 (ap), convergently appearing in *X. naroensis* from the non-palisade group. However, in the new species the baseoenp spines are longer (pl), the P1 enp-2 has 3 inner setae (pl) instead of 2 present in *X. yeonghooni* and *X. aemula* (ap), the outer process on the P2 enp-2 is much stronger in *X. spinifurca* sp. nov. (ap), and the outer spines of the P2–P4 exp-3 are not serrate (pl) (these two last characters could not be accessed for *X. aemula* in the literature).

Xouthous purpurocinctus, *X. parasimulans* (Médioni & Soyer, 1968), *X. simulans*, *X. pectinatus* (Scott & Scott, 1898), *X. namibiensis* Huys, 2016, *X. naroensis* Karanovic, 2023 , and *Xouthous dichroma* sp. nov. compose the group outside of the palisade group. *Xouthous dichroma* sp. nov. has 2 inner setae on the P2 enp-2 (pl), a character not shared with *Xouthous purpurocinctus* and *X. parasimulans* which share only 1 inner seta on this segment (ap). It differs from *X. pectinatus*, and *X. namibiensis* by the presence of 5 setae on the female P5 exp (ap), instead

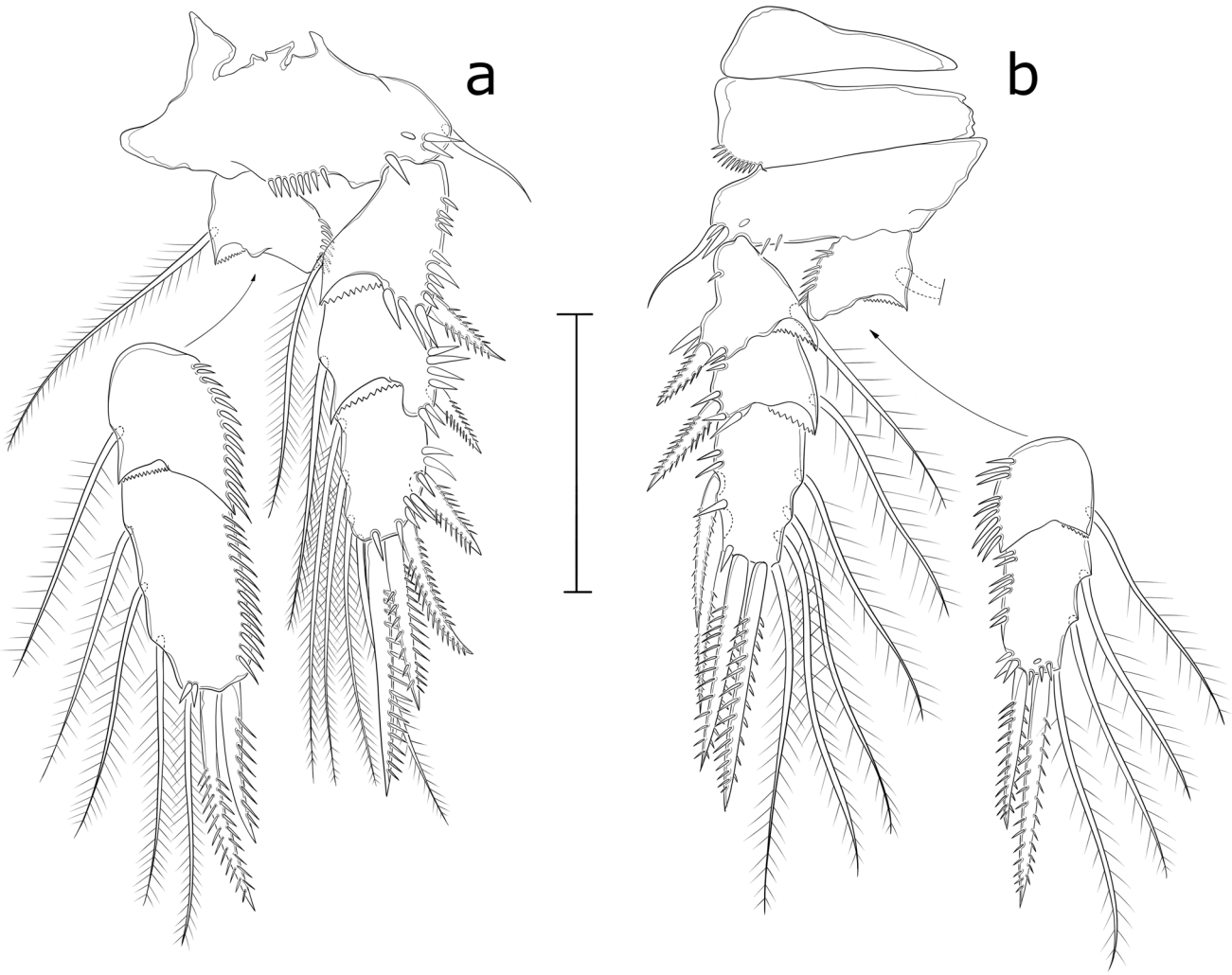


Fig. 15 *X. spinifurca* sp. nov., female; P3 (**a**) and P4 (**b**). Scale bar 50 µm

of 6 setae (pl). *Xouthous dichroma* sp. nov. resembles *X. simulans,* and *X. naroensis*, sharing the presence of 2 inner setae on the P2 enp-2. It differs from *X. simulans* by the presence of a shorter baseoenp seta 1 (ap), even shorter than setae 3–5, not ending in a long flagellate portion (pl) as in *X. simulans* and *X. naroensis* (ap). *X. dichroma* sp. nov. and *X. naroensis* seem to be closely related, sharing a female P5 exp with 5 setae (ap), and no longer than twice its width (ap), whereas in *X. simulans* the female P5 exp is also elongate, "about 3 times as long as wide" (Huys 2016) (ap?), but with 6 setae (pl). *X. naroensis, X. dichroma* sp. nov. also share the same female P5 exp/baseoenp ratio and armature, differing, however, in the presence of serrate outer spines in the exp-3 of P2–P4 of *X. dichroma* sp. nov. (ap). In addition, in *X. simulans* the setae of female P5 baseoenp are evenly spaced (pl), occurring a gap between setae 4 and 5 in both *X. dichroma* sp. nov. and *X. naroensis* (ap). It also differs from *X. naroensis* by the presence in this species of a strong outer process in the endopod-2 of the second pereopod (ap), a character not found in *X. dichroma* sp. nov. (pl).

The genetic distance analysis (Table 2) is based on very few species of Pseudotachidiidae; nevertheless, some useful conclusions can be drawn from the resulting topology (Fig. 19) which must be viewed not as a phylogenetic cladogram, but as representing the genetic relationships of different Pseudotachidiidae taxa given a chosen genetic distance. As expected, the two congeneric

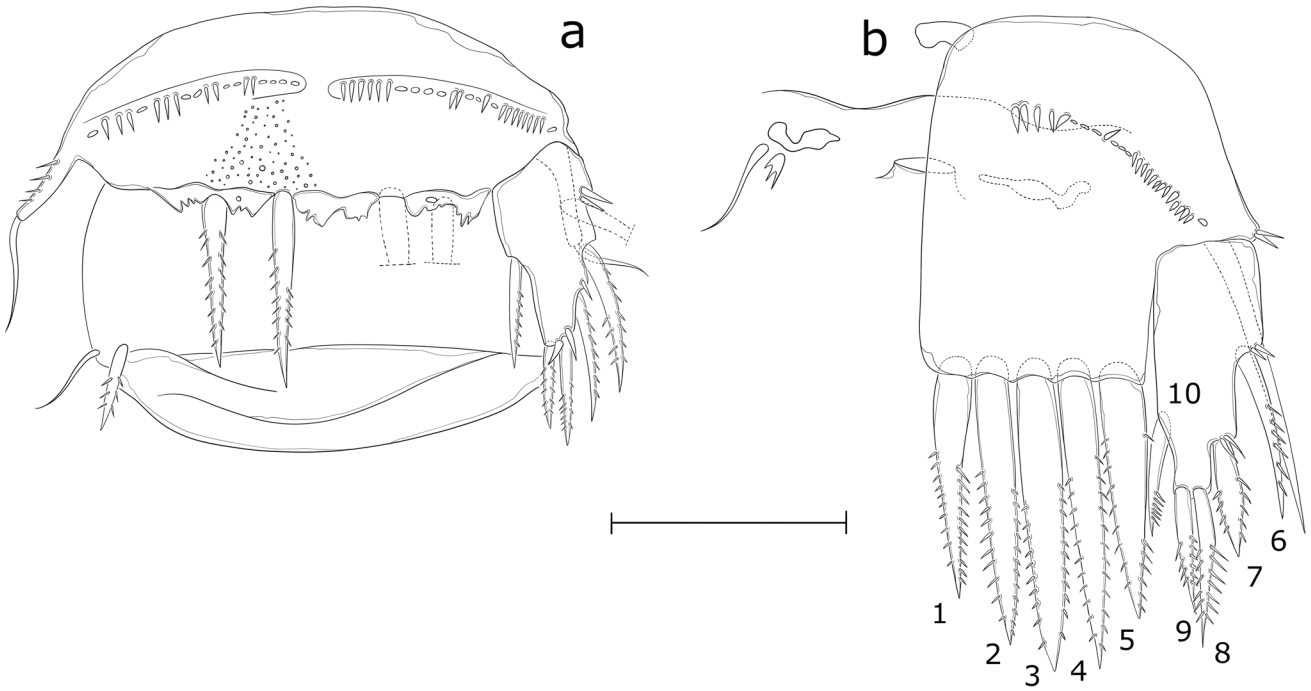


Fig. 16 *X. spinifurca* sp. nov., male P5 and P6 (**a**); female P5, P6, and genital field (**b**). Scale bars 50 µm

species of *Xouthous* closely related to each other than to other available species of Pseudotachidiidae in GenBank. The 20% sequence divergence in COI between the two *Xouthous* species may be indicative of the phylogenetic separation of the species belonging to the palisade group (e.g., *X. spinifurca* sp. nov.) and those that do not (e.g., *X. dichroma* sp. nov.). As more copepod species receive COI barcodes, the phylogenetic relationship between the two proposed taxonomic subdivisions of *Xouthous* can be further tested. The morphological resemblance of *X. spinifurca* sp. nov. to three species (*X. aemula*, *X. wellsi*, and *X. yeonghooni)* found in the Indo-Pacific may be indicative of a species radiation from the Pacific to the Caribbean Sea. If this is true, *X. spinifurca* sp. nov. has been isolated from its Pacific congeners at least 2.8 MYA, since the full formation of the Isthmus of Panama (O'Dea et al. 2016). The resemblance of *X. dichroma* sp. nov. with the species *X. simulans*, and *X. naroensis* is challenging to interpret because of the wide distance covering the distribution of these species; *Xouthous simulans* from Simons Bay (South Africa) and *X. naroensis* from South Korea. Although we cannot give a definite answer, if *X. dichroma* sp. nov. is closer to *X. naroensis*, we would have the same biogeographic and radiation pattern proposed for *X. spinifurca* sp. nov. also occurring outside of the palisade group. It is important to mention that the widespread distribution of *X. purpurocinctus* (UK, Namibia, Maldives, Micronesia, and California) and *X. simulans* (Portugal and Easter Island) presents an excellent opportunity to test with molecular markers whether these are truly cosmopolitan species or are comprised of cryptic species.

The geographic and depth distributions of the two new *Xouthous* species are different. *Xouthous dichroma* sp. nov. was collected from a much wider area in the Caribbean stretching from Puerto Rico to Panama whereas *X. spinifurca* sp. nov. was collected exclusively in the US Caribbean (Fig. 1, Table 3). *Xouthous dichroma* sp. nov. was sampled from shallow depths (1–18 m) but *X. spinifurca* sp. nov. attains a wider depth distribution from shallow (15 m) to mesophotic depths (50 m) (Table 3). The harpacticoid fauna and more generally the meiofauna of the mesophotic reefs of the Caribbean has not been studied and it will certainly yield many new species findings as indicated from preliminary work (e.g., Schizas et al. 2015; Corgosinho et al. 2016; Veglia et al. 2018). The reported species geographic and depth distribution should be regarded preliminary since they are based on a limited number of samples. The type of substrate also differs by species: *X. dichroma* sp. nov.

Fig. 17 *X. spinifurca* sp. nov., male; confocal laser scanning microscopy of the dorsal (**a**) and ventral (**b**) habitus. Scale bar 50 µm

seems to be a generalist since specimens were collected from phytal/coral/sediment substrata; *X. spinifurca* sp. nov. was limited to coral substrata. Because of the way we processed the substrata (i.e., washing whole pieces of corals, coral rubble with the underlying substrate over sieves) we cannot be certain of the exact habitat of each species and it is likely with more samples and more precise collections to find both species in additional habitats.

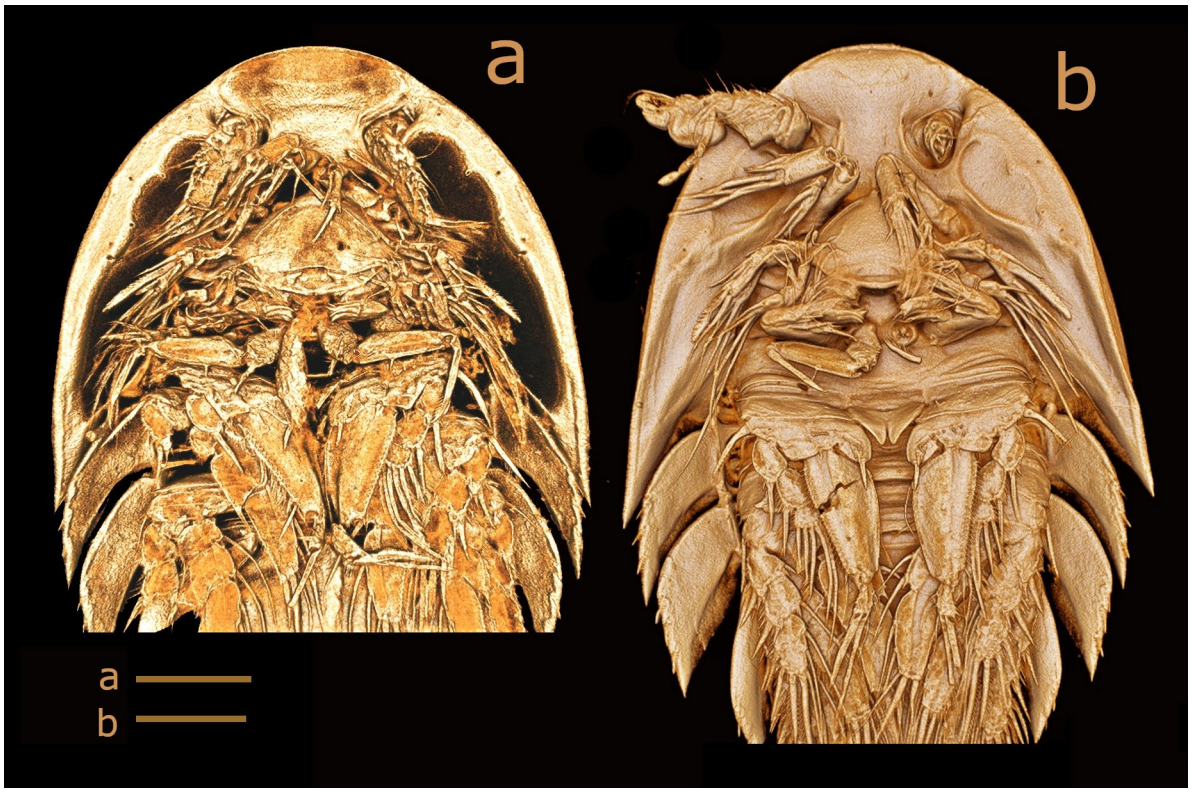


Fig. 18 Three-dimensional representation of the anterior half of a female (**a**) and a male (**b**), of *X. spinifurca* sp. nov. Scale bars 50 µm

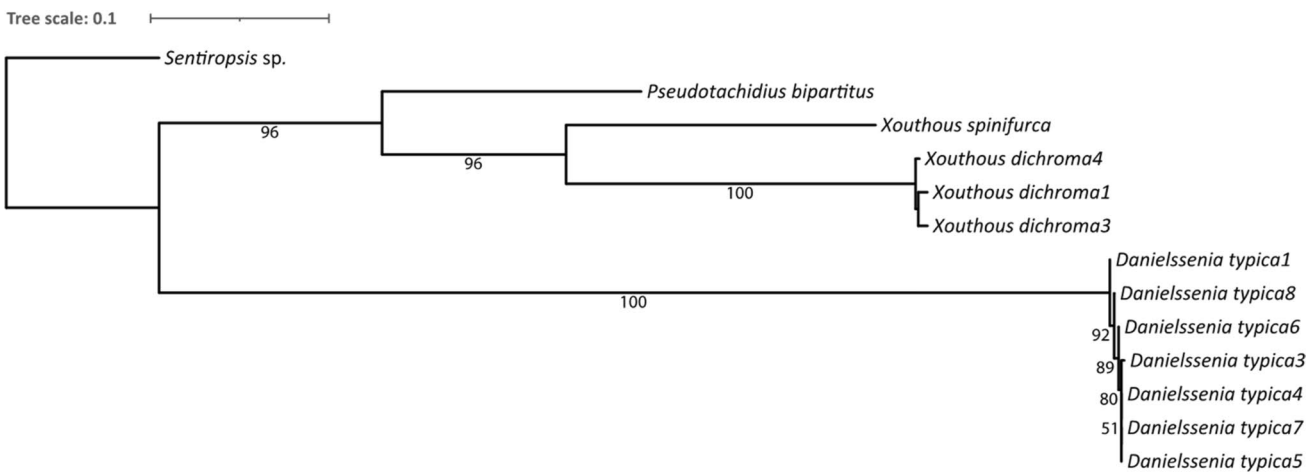


Fig. 19 Maximum likelihood dendrogram of available Pseudotachidiidae COI sequences. See Table 1 for GenBank accession numbers. Numbers below branches indicate transfer bootstrap expectation (Lemoine et al. 2018)

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Declarations

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

Ethical approval All applicable international, national, and/or institutional guidelines for animal testing, animal care and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable. The study is compliant with CBD and Nagoya protocols.

Data availability Voucher specimens are deposited at the Museum of Marine Invertebrates (MMI-UPRM) at Magueyes Island and can be accessed via de museum numbers provided in the description. Molecular sequences, both original from this work or used from other studies are deposited in GenBank and can be accessed by the accession numbers given in methods section and Table 1 of this contribution.

Author contributions PHCC field sampling, animal dissection, pencil illustration, writing of the manuscript; NVS field sampling, writing of the manuscript, genetic analysis, obtaining funding; GRVR digital illustration; TCK confocal microscopy; MAL manuscript correction, obtaining funding.

References

Brady GS (1910) Die marinen Copepoden der Deutschen Südpolar-Expedition 1901–1903.I. Über die Copepoden der Stämme Harpacticoida, Cyclopoida, Notodelphyoida und Caligoida. Deutsche Südpolar-Expedition 11 (=Zoologie, 3), 497–594

Corgosinho PHC, Schizas NV, Alfaro Lozano M (2016) A new species of *Atergopedia* (Copepoda: Harpacticoida: Novocriniidae) from a Caribbean mesophotic reef. Mar Biodivers 46(4):841–852. https://doi.org/10.1007/s12526-016-0446-9

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. Mol Biol Evol 37(1):291–294. https://doi.org/10.1093/molbev/msz189

Edler D, Klein J, Antonelli A, Silvestro D, Matschiner M (2020) raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol Evol 12(2):373–377. https://doi.org/10.1111/2041-210x.13512

Ferrari FD, Ivanenko VN (2008) The identity of protopodal segments and the ramus of maxilla 2 of copepods (Copepoda). Crustaceana 81(7):823–835. https://doi.org/10.1163/156854008784771702

Folmer O, Black M, Hoen W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22(2):160–174

Huys R (2009) Unresolved cases of type fixation, synonymy and homonymy in harpacticoid copepod nomenclature (Crustacea: Copepoda). Zootaxa 2183:1–99

Huys R (2016) Harpacticoid copepods - their symbiotic associations and biogenic substrata: A review. Zootaxa 4174(1):448–729. https://doi.org/10.11646/zootaxa.4174.1.28

Huys R, Boxshall GA (1991) Copepod evolution. The Ray Society, London, London

Kamanli AS, Kihara TC, Ball AD, Morritt D, Clark PF (2017) A 3D imaging and visualization workflow, using confocal microscopy and advanced image processing for brachyuran crab larvae. J Microsc 266(3):307–323. https://doi.org/10.1111/jmi.12540

Karanovic T (2023) Four rare harpacticoid copepods from shallow littoral habitats in South Korea. Zootaxa 5311(4):568–578. https://doi.org/10.11646/zootaxa.5311.4.4

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20(4):1160–1166. https://doi.org/10.1093/bib/bbx108

Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinform 35(21):4453–4455. https://doi.org/10.1093/bioinformatics/btz305

Lang K (1936) Copepoda Harpacticoida. In: Bock S (ed) Further Zoological results of the Swedish Antarctic expedition 1901-1903, vol 3(3). Stockholm, pp 1–68

Lemoine F, Domelevo Entfellner JB, Wilkinson E, Correia D, Davila Felipe M, De Oliveira T, Gascuel O (2018) Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature 556(7702):452–456. https://doi.org/10.1038/s41586-018-0043-0

Letunic I, Bork P (2021) Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 49(W1):W293–W296. https://doi.org/10.1093/nar/gkab301

Médioni A, Soyer J (1968) Copépodes Harpacticoïdes de Banyuls-sur-Mer. 6. Nouvelles formes associées à des Bryozoaires. Vie Et Milieu Série A 18:317–343

Miloslavich P, Diaz JM, Klein E, Alvarado JJ, Diaz C, Gobin J, Ortiz M (2010) Marine biodiversity in the Caribbean: regional estimates and distribution patterns. PLoS ONE 5(8):e11916. https://doi.org/10.1371/journal.pone.0011916

Monard A (1936) Note préliminaire sur la faune des Harpacticoïdes marins d'Alger. Bulletin De La Station D'aquiculture Et De Pêche De Castiglione 1935(1):45–85

Norman AM, Scott T (1905) Crustacea copepoda new to science from Devon and Cornwall. Ann Mag Nat Hist Series 7(15):284–300. https://doi.org/10.1080/03745480509443044

O'Dea A et al (2016) Formation of the Isthmus of Panama. Sci Adv 2(8):e16008883. https://doi.org/10.1126/sciadv.1600883

Philippi A (1843) Fernere Beobachtungen über die Copepoden des Mittelmeeres. Archiv Für Naturgeschichte 9(1):54–71

Sars GO (1903) Copepoda Harpacticoida. Parts I & II, Misophriidae, Longipediidae, Cerviniidae, Ectinosomidae (part). In: An Account of the Crustacea of Norway, with short descriptions and figures of all the species, vol. 5. Bergen Museum, pp 1–28

Schizas NV, Dahms H-U, Kangtia P, Corgosinho PHC, Galindo Estronza AM (2015) A new species of *Longipedia* Claus, 1863

(Copepoda: Harpacticoida: Longipediidae) from Caribbean mesophotic reefs with remarks on the phylogenetic affinities of Polyarthra. Mar Biol Res 11(8):789–803. https://doi.org/10.1080/17451000.2015.1013556
Scott T, Scott A (1898) Description of three apparently new copepods from the Clyde. Ann Mag Nat Hist Series 7(1):185–190
Sewell RBS (1940) Copepoda, Harpacticoida. The John Murray Expedition 1933–1934. Scientific Reports. 7(2):117–382
Song SJ, Lee S-K, Lee J-S, Khim JS (2020) A new species of *Xouthous* Thomson (Copepoda: Harpacticoida: Pseudotachidiidae), widely distributed in the Korean waters. Annal Zool 70(4). https://doi.org/10.3161/00034541anz2020.70.4.003
Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinform 30(9):1312–1313. https://doi.org/10.1093/bioinformatics/btu033
Suárez-Morales E, De Troch M, Fiers F (2006) A checklist of the marine Harpacticoida (Copepoda) of the Caribbean Sea. Zootaxa 1285:1–19
Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods) (Version 4.0b10). Sunderland, Mass.: Sinauer Associates
Thompson IC, Scott A (1903) Report on the Copepoda collected by Professor Herdman, at Ceylon, in 1902. In: Herdman WA (ed) Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar 1(Supplement 7):227–307
Thomson GM (1883) On the New Zealand Copepoda. Trans Proc New Zealand Inst 15(1882):93–116
Veglia AJ, Hammerman NM, Rivera Rosaly CR, Lucas MQ, Galindo Estronza A, Corgosinho PH, Schizas NV (2018) Characterizing population structure of coral-associated fauna from mesophotic and shallow habitats in the Caribbean. J Mar Biol Assoc UK 99(3):619–629. https://doi.org/10.1017/s0025315418000413

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