



A NEW SPECIES OF *ACANTHOCYCLOPS* KIEFER, 1927 (COPEPODA, CYCLOPOIDA) FROM EPHEMERAL AND PERMANENT WATERBODIES OF SIAN KA'AN AND CALAKMUL BIOSPHERE RESERVES, MEXICO

BY

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ABSTRACT

A new species of the genus *Acanthocyclops* is described from temporal freshwater bodies in the Sian Ka'an and Calakmul Biosphere Reserves in Southeast Mexico. *Acanthocyclops fiersi* n. sp. represents the third species of the genus described from the Neotropics, only after *A. rebecae* and *A. smithae*. *Acanthocyclops fiersi* n. sp., is recognized as a member of the *A. vernalis-robustus* species-complex, and its clear divergence with respect to its congeners was supported using comparative morphology and molecular methods. The mtCOI marker revealed a 0.54% K2P divergence within populations of *A. fiersi* n. sp. and 17.6-22.41% with respect to other members of the *A. vernalis-robustus* species-complex. *Acanthocyclops fiersi* n. sp. might be restricted to the Yucatán Peninsula. Comments about the likely underestimated regional diversity of *Acanthocyclops* and the need of further studies in the Neotropics are provided. An identification key to the species of *Acanthocyclops* distributed in Mexico is included.

Key words. — Morphology, *Acanthocyclops vernalis-robustus* species complex, Neotropics, freshwater, Yucatán Peninsula, COI, GMYC

RESUMEN

Se describe una nueva especie de *Acanthocyclops* de cuerpos de agua temporales en las Reservas de la Biosfera de Sian Ka'an y Calakmul en el sureste de México. *Acanthocyclops fiersi* n. sp.

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representa la tercera especie del género descrita para el Neotrópico, solo después de *A. rebecca* y *A. smithae*. *Acanthocyclops fiersi* n. sp. es reconocida como miembro del complejo de especies *A. vernalis-robustus*, y su diferenciación con respecto a sus congéneres se sostiene usando morfología comparativa y métodos moleculares. El gen mtCOI reveló una divergencia de K2P del 0.54% dentro de poblaciones de *Acanthocyclops fiersi* n. sp. y de 17.6–22.41% con respecto a otros miembros conocidos del complejo de especies *A. vernalis-robustus*. *Acanthocyclops fiersi* n. sp., podría estar restringida a la península de Yucatán. Se comenta acerca de la diversidad regional, probablemente subestimada, del género *Acanthocyclops* y de la necesidad de más estudios en el Neotrópico. También se incluye una clave de identificación de las especies de *Acanthocyclops* distribuidas en México.

Palabras clave. — Morfología, complejo *Acanthocyclops vernalis-robustus*, Neotrópico, aguas continentales, Península de Yucatán, COI, GMYC

## INTRODUCTION

The freshwater copepod genus *Acanthocyclops* Kiefer, 1927 is one of the most diverse within the subfamily Cyclopinae Rafinesque, 1815; it comprises more than 75 species and subspecies, most of which are known from the Holarctic region (Mirabdullayev & Defaye, 2002, 2004; Dussart & Defaye, 2006; Mercado-Salas & Álvarez-Silva, 2013). During the last decades, numerous efforts have been made to clarify the taxonomical status of some nominal species of the genus, especially those within the *A. vernalis-robustus* species complex. Many of these studies have focused on morphological data, but others included the use of molecular tools and the addition of new micro-characters in the separation of closely related species (Reid, 1992; Dodson, 1994; Dahms & Fernando, 1997; Mirabdullayev & Defaye, 2002, 2004; Dodson et al., 2003; Mercado et al., 2006; Mercado-Salas et al., 2009; Bláha et al., 2010; Mercado-Salas & Álvarez-Silva, 2013; Miracle et al., 2013; Alekseev et al., 2021).

The most recent taxonomic revisions concerning the status of the *A. vernalis-robustus* species complex were provided by Miracle et al. (2013) and Alekseev et al. (2021). The former work promoted important taxonomic changes. *Acanthocyclops americanus* (Marsh, 1893), considered a synonym of *A. robustus* (G.O. Sars, 1863) by Kiefer (1976) and accepted as such by most authors, was re-established as a valid species closely related to both *A. robustus* and *A. vernalis* (Fisher, 1853). These authors also synonymized *A. trajani* Mirabdullayev & Defaye, 2002 to *A. americanus* and *A. einslei* Mirabdullayev & Defaye, 2004 to *A. robustus*. Anufrieva et al. (2014) disagreed with the statements made by Miracle et al. (2013) and provided grounds to reject their proposals emphasizing the morphological differences between the synonymized species and those between the holotype original illustrations and the neotype specimen designated by Miracle et al. (2013). More recently Alekseev et al. (2021) provided a complete redescription of both *A. vernalis* and *A. robustus* from topotypic specimens, highlighting their main

morphological and ecological divergences with respect to *A. americanus*. Furthermore, they discussed about the clear genetic differences among these species and also how the work of Anufrieva et al. (2014) dismissed the evidence provided by molecular methods in distinguishing the three species. Overall, these studies (e.g., Miracle et al., 2013; Anufrieva et al., 2014; Alekseev et al., 2021) have focused on the European populations of the *A. vernalis-robustus* species-complex; hence, both the Nearctic but mostly the Neotropical members of this complex remain poorly studied and certainly deserve additional investigations.

In the Americas, up to 22 species and subspecies of the genus have been recorded (Dussart & Defaye, 2006; Mercado-Salas & Álvarez-Silva, 2013; Suárez-Morales et al., 2020) of which only four are distributed in the Neotropical region. Currently, there are only two Neotropical species of *Acanthocyclops* described: *Acanthocyclops smithae* Reid & Suárez-Morales, 1999 and *A. rebecae* Fiers, Ghenne & Suárez-Morales, 2000. The former species is known to occur in Southeast Mexico, Honduras, probably Cuba, and was subsequently reported from pools linked to crayfish burrows in Brazil (Reid & Suárez-Morales, 1999; Reid et al., 2006). *Acanthocyclops rebecae* is known from a single locality in Campeche, Yucatan Peninsula, Mexico (Fiers et al., 2000; Dussart & Defaye, 2006; Reid et al., 2006).

Here we describe a new Neotropical species of *Acanthocyclops* based on male and female adult individuals collected from Sian Ka'an and Calakmul Biosphere Reserves (Yucatan Peninsula, Mexico). The significance of this finding is discussed in terms of their morphological and genetic diversity, as well as the genus distributional patterns in the Americas.

#### MATERIAL AND METHODS

Study area.— The survey includes biological samplings in two protected areas of Mexico, both under the category of Biosphere Reserve: Sian Ka'an and Calakmul (fig. 1) (Collecting permit: PPF/DGOPA-003/15 SEMARNAT-CONAPESCA, Mexico). These reserves represent two of the most extensive areas of preserved tropical forests in Mexico. The Biosphere Reserve of Sian Ka'an comprises 652 000 ha (both terrestrial and marine). The reserve occupies a partially emerged limestone plateau that gradually descends to the sea, forming a gradient from dry to flooded areas. Within this gradient medium forest, lowlands, flooded plains, marshes, mangroves and seagrass can be found. The area comprises karstic-derived features like cenotes (sinkholes), hillocks, lagoons, cays and ephemeral springs. Calakmul Biosphere Reserve comprises 723 185 ha (terrestrial) and it is a mixture of high and medium forests with seasonally flooded lowlands and aquatic

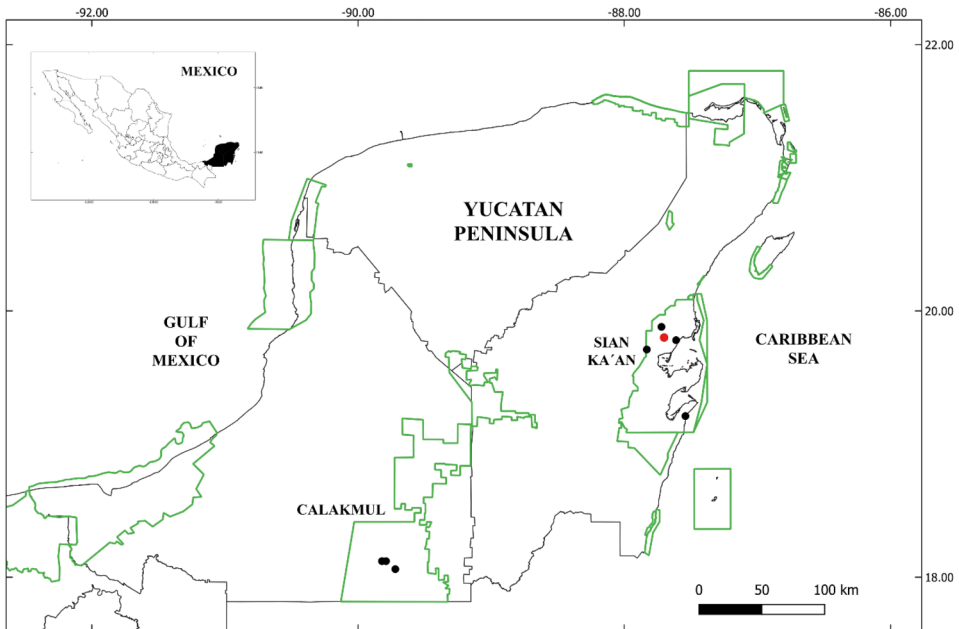


Fig. 1. Distribution of *Acanthocyclops fiersi* n. sp. in Calakmul and Sian Ka'an Biosphere Reserves, Mexico. Symbols: red circle – type locality (Savannah 2, Sian Ka'an); black circles, localities where *A. fiersi* n. sp. was recorded; green polygons, Natural Protected Areas.

vegetation. The hydrography of Calakmul surface is determined by the amount and distribution of rainfall, evapotranspiration, water bodies physiography, and soil types, and surface drainage; some of the low areas became permanent wetlands (Mercado-Salas et al., 2021).

Biological samples were collected from 19 different waterbodies – including semi-permanent ponds (locally known as “aguadas”), temporal and permanent wetlands (savannahs). Samples containing the new species were taken at two localities in Calakmul and six in Sian Ka'an Reserves (table I) with a standard plankton net (200  $\mu\text{m}$  filtering mesh) trawled directly across the water body; samples were fixed in 96% ethanol. Several female and male copepods were tentatively recognized as members of the cyclopoid genus *Acanthocyclops*. They were collected from two localities in Calakmul and six in Sian Ka'an (table I).

Morphological observations.— A closer examination of these specimens revealed taxonomically important differences with respect to previously known species, thus motivating a deeper analysis. Specimens were dissected and examined following the techniques described by Williamson & Reid (2001) and Reid (2003). Dissected specimens were mounted in permanent slides with glycerin sealed with paraffin. Drawings were prepared at 100 $\times$  magnification with a drawing tube mounted on a Leica DMR microscope. The new species was described and illus-

TABLE I  
 Sampling localities where *Acanthocyclops fiersi* n. sp. was recorded

Locality	Habitat	Date	Reserve	Latitude (°N)	Longitude (°W)	Collection number
Aguada límite de la Reserva	Aguada	23 Sep. 2014	Sian Ka'an	19.709	87.828	ZMH K-62096
		04 Oct. 2015				ZMH K-62097
Laguna Mosquitero	Lagoon	01 Oct. 2015	Sian Ka'an	19.208	87.538	ZMH K-62098
Savannah 2*	Wetland	21 Sep. 2014*	Sian Ka'an	19.799	87.700	<i>ECO-CH-Z-09328</i>
						<i>ECO-CH-Z-09329</i>
						<i>ECO-CH-Z-09330</i>
						ZMH K-62095
Vigía Chico	Wetland	07 Oct. 2015	Sian Ka'an	19.784	87.610	ZMH K-62099
		20 Sep. 2014				ZMH K-62100
		04 Oct. 2015				ZMH K-62101
Arroyo Aguada Grande	Creek	28 Sep. 2014	Calakmul	18.123	89.815	ZMH K-62102
		20 Sep. 2015				ZMH K-62103
Arroyo Calakmul	Creek	28 Sep. 2014	Calakmul	18.123	89.790	ZMH K-62104
Andrés Quintana Roo	Aguada	28 Sep. 2015	Sian Ka'an	18.060	89.719	ZMH K-64216
Savannah Km 10	Wetland	05 Oct. 2015	Sian Ka'an	19.875	87.717	ZMH K-64217
Savannah camino Playón	Wetland	06 Oct. 2015	Sian Ka'an	19.832	87.542	ZMH K-64218

An asterisk represents the type locality; collection numbers in italics represent type material.

trated following the current standards for the morphological study of the genus (Einsle, 1996; Fiers et al., 2000; Mirabdullayev & Defaye, 2002, 2004; Mercado-Salas et al., 2009; Mercado-Salas & Álvarez-Silva, 2013). Holotype and paratypes were deposited in the Zooplankton Collection held at El Colegio de la Frontera Sur (ECO-CH-Z) in Chetumal, Mexico; additional paratypes were deposited at the Zoological Museum Hamburg, Germany (ZMH K-).

Abbreviations used in the descriptive section are as follows: A1, antennule; A2, antenna; ae, aesthetasc; Cph, cephalothorax; enp, endopod; exp, exopod; Fu, furca; Md, mandible; Mx1, maxillula; Mx2, maxilla; Mxp, maxilliped; P1-P6, legs 1-6; s, seta (setae); sp, spine; Urs, urosomite(s). Furcal setae labelled as follows: II, anterolateral (lateral) caudal seta; III, posterolateral (outermost) caudal seta; IV, outer terminal (terminal median external) caudal seta; V, inner terminal (terminal median internal) caudal seta; VI, terminal accessory (innermost) caudal seta; VII, dorsal seta; general morphologic nomenclature follows Huys & Boxshall (1991). The terms furca and telson are used following Schminke (1976).

Sequencing and genetic analysis.— DNA extractions from 33 specimens were carried out using 40 µl Chelex (InstaGene Matrix, Bio-Rad, Hercules, CA, U.S.A.) according to the protocol and directly used as DNA template for PCR. A 634-bp region of CO1 was amplified using the primers Cyclop-F (5'-GGAACCTTTGTATT

TATTAGCTGGTGC-3') and Cyclop-R (5'-GGTCTCCCCCTCCTCTAGG-3') (Mercado-Salas et al., 2021). Amplifications were performed using AccuStart Gel Track PCR SuperMix (Thermo Fisher Scientific, Waltham, MA, U.S.A.) in a 25  $\mu$ l volume containing 9.5  $\mu$ l H<sub>2</sub>O, 12.5  $\mu$ l PCR Master Mix, 0.5  $\mu$ l of each primer (10 pmol/ $\mu$ l) and 2  $\mu$ l of DNA template. The PCR protocol was 94°C for 3 min, 94°C for 30 s, 45°C for 45 s, and 72°C for 1 min, during 35 cycles and as final elongation 72°C for 2 min. PCR products were checked by electrophoresis and successful products were purified using ExoSap-IT PCR Product Cleanup (Thermo Fisher Scientific). Sequencing was carried out by Macrogen (Amsterdam, The Netherlands). Forward and reverse strands from each specimen and fragment were assembled, edited and checked for the correct amino acid translation frame using Geneious 9.1.7 (created by Biomatters; available online at <http://www.geneious.com>). All sequences were searched in GenBank using BLASTN (Altschul et al., 1990) to discard contaminations: successful sequences of 21 individuals were produced during this study. Sequences of other members of the *A. vernalis-robustus* species-complex were downloaded from the National Center for Biotechnology Information (NCBI) and included in the analysis (see table A1 that can be accessed at 10.6084/m9.figshare.22249456). Genbank accession numbers of sequences obtained during this study are as follows: OQ574608-OQ5746625 and OQ588793-OQ588799 (for more information see table A1 that can be accessed at 10.6084/m9.figshare.22249456).

All DNA sequences were aligned separately using MAFFT v7.017 with the G-INS-i algorithm (Katoh & Toh, 2008), and further edited manually to exclude ambiguous regions. A Best nucleotide fitting model was calculated by jModeltest v.0.1.1 (Posada, 2008) under Java. A Bayesian analysis employing the HKY substitution model was conducted using MrBayes version 3.2.7a (Ronquist & Huelsenbeck, 2003). Posterior probabilities were estimated using 50 000 000 generations with sampling frequency of every 1000 trees through four simultaneous Markov Chains Monte Carlo. MEGA 7 (Kumar et al., 2015) has been used to estimatee the K2P genetic variations of mtDNA *COI* within and between species. The General Mixed Yule Coalescent model (GMYC) (Pons et al., 2006; Monaghan et al., 2009) was used as species delimitation method. The GMYC method was implemented in the “splits” package for R and was applied to the *COI* ultrametric tree obtained with BEAST v1.8.3 (Drummond & Rambaut, 2007).

## RESULTS

A new species of *Acanthocyclops* was detected from samples obtained during this survey: *Acanthocyclops fiersi* n. sp. Its description is based on morphology and

mtDNA *COI* genetic variations and distributional patterns. We include an overview of the Mexican records of the genus and an identification key to the species of the genus distributed in Mexico.

#### SYSTEMATICS

Order CYCLOPOIDA Burmeister, 1834

Family CYCLOPIDAE Dana, 1846

Subfamily Cyclopinae Burmeister, 1834

Genus *Acanthocyclops* Kiefer, 1927

***Acanthocyclops fiersi*** new species (figs. 2-6)

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Material examined.— Holotype: adult ♀, dissected, mounted in glycerin sealed with paraffin (ECO-CH-Z-09328), Savannah 2, Sian Ka'an Biosphere Reserve, Quintana Roo, Mexico (19.799 N; 87.700 W) coll. 21 September 2014 by Nancy F. Mercado-Salas. Allotype: Adult ♂, dissected, semi-permanent slide (ECO-CH-Z-09329), same site, date and collector. Paratypes: Five adult ♀♀, 1 adult ♂ and several undissected copepodites (ECO-CH-Z-09330), same locality and date of collection; 96% ethanol-preserved. Paratypes 5 adult ♀♀, 1 adult ♂ and several undissected copepodites (ZMH K-62095), same locality and date of collection; 96% ethanol-preserved. Additional material in table I.

Type locality.— Savannah 2, Sian Ka'an Biosphere Reserve, Quintana Roo, Mexico (19°47'54.3"N; 87°42'01.8"W).

Etymology.— The species name honours the late Dr. Frank Fiers, for his highly significant contributions to the systematics of the free-living copepods, his interest on the Yucatan Peninsula copepod fauna, and his friendship. It is a noun in the genitive case, the gender is masculine.

Description of adult female.— Body (fig. 2a) cyclopiform. Total body length = 920  $\mu\text{m}$  from anterior end of cephalothorax to posterior margin of furca. Body robust, Cph relatively long, slightly expanded laterally at midlength of cephalosome in dorsal view; lateral margins of pedigers 3 and 4 straight, produced posteriorly. Cph length = 405  $\mu\text{m}$ , representing 44% of total body length. Dorsal surface smooth, ornamented with sensillae as shown in fig. 2a. Urs relatively slender, including fifth pediger plus three succeeding somites and telson. Genital double-somite longest, representing almost 50% of Urs length, moderately expanded at proximal half. Last abdominal somite and telson equally sized.

A1 (fig. 2d).— 17-segmented in all specimens examined; armature per segment as follows: 1(8s), 2(4s), 3(2s), 4(6s), 5(4s), 6(1s + 1sp), 7(2s), 8(1s), 9(1s), 10(0s), 11(1s), 12(1s + 1ae), 13(0s), 14(1s), 15(2s), 16(2s), 17(7s + 1ae). A1 not reaching distal margin of Cph. Dorsal surface of first segment with proximal row of strong spinules.

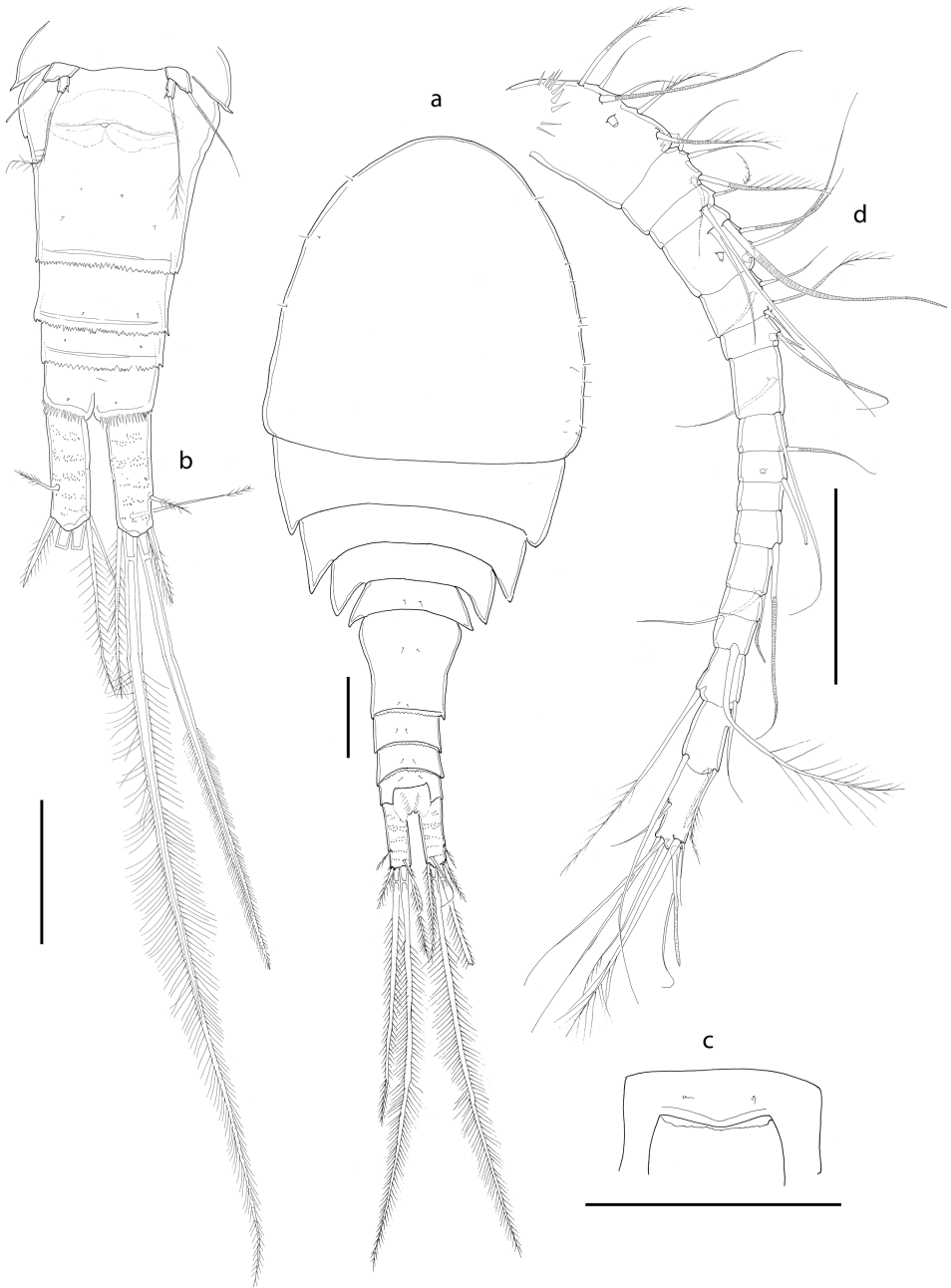


Fig. 2. *Acanthocyclops fiersi* n. sp., female holotype, adult. a, Habitus, dorsal view; b, Urs, ventral view; c, telson, dorsal view; d, A1. Scale bars = 100  $\mu\text{m}$ .



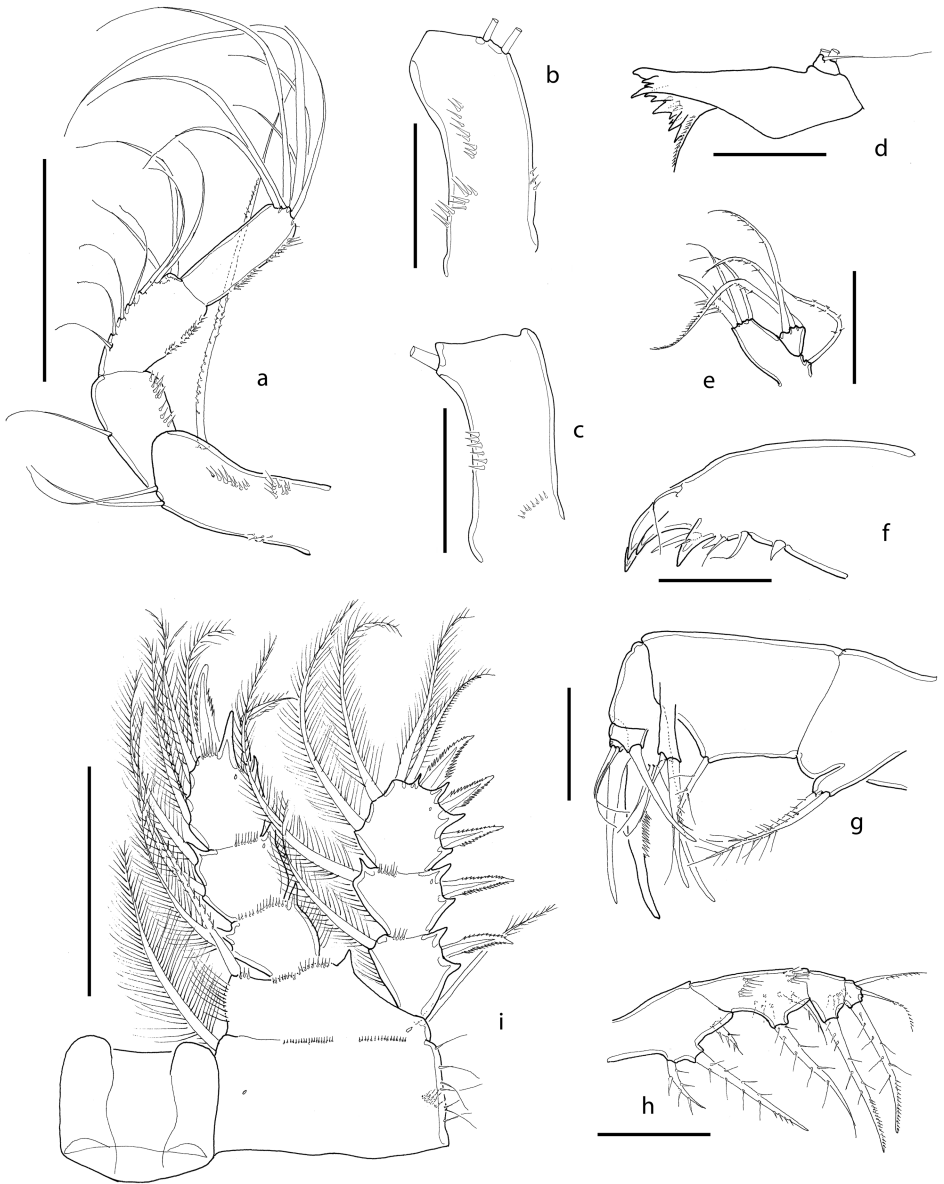


Fig. 3. *Acanthocyclops fiersi* n. sp., female holotype, adult. a, A2; b, A2 basis, caudal view; c, A2 basis, frontal view; d, Md; e, Mx1 palp; f, Mx1; g, Mx2; h, Mxp; i, P1. Scale bars: a, i = 100  $\mu\text{m}$ ; b-g = 50  $\mu\text{m}$ .

A2 (fig. 3a-c).— Four-segmented, basis with three groups of spinules on caudal surface and two groups on frontal surface. Basis with long exopodal seta asymmetrically bipinnate plus two equally long basipodal setae. First enp with single outer seta and inner row of spinules. Second enp segment bearing eight

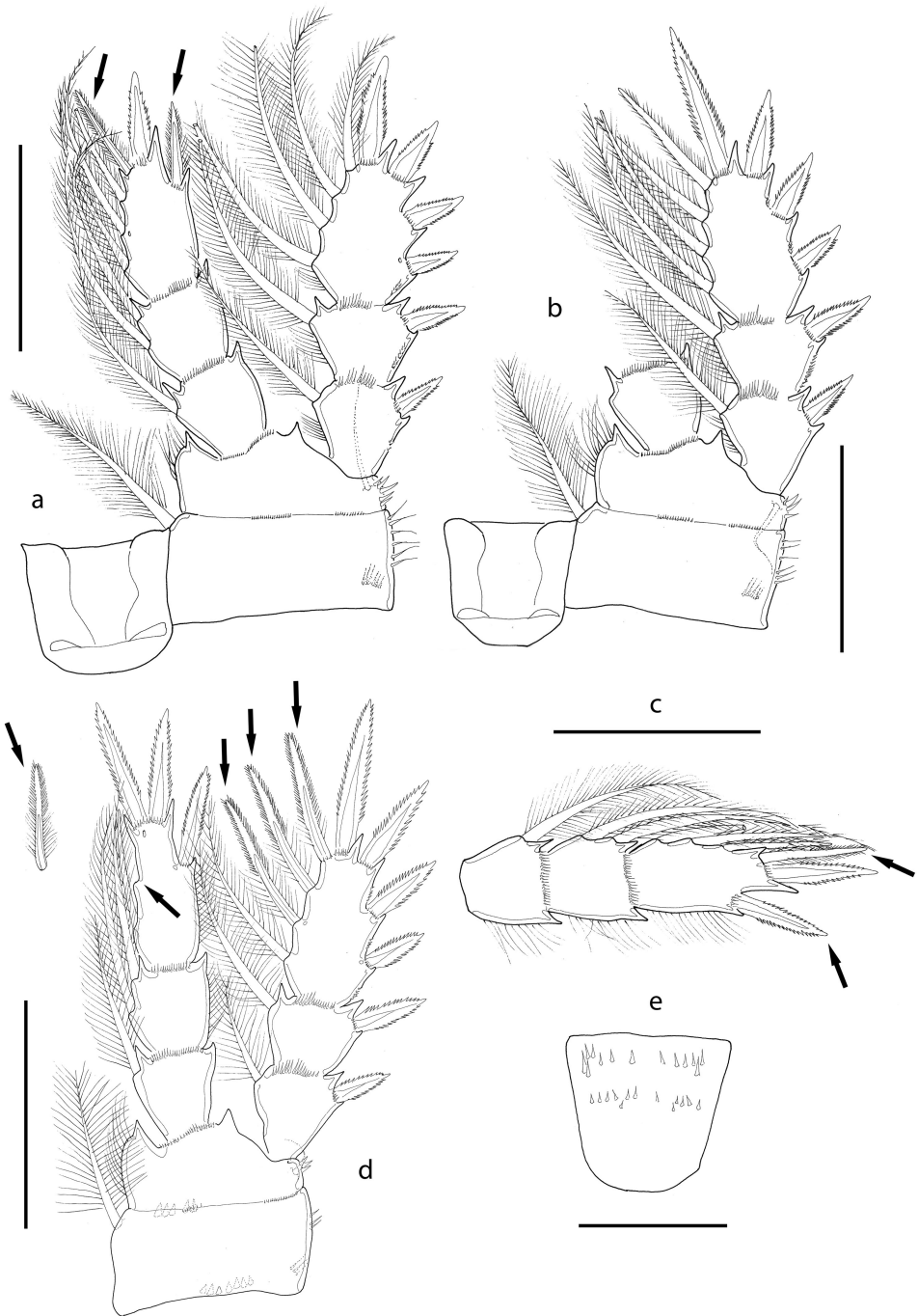


Fig. 4. *Acanthocyclops fiersi* n. sp. female holotype, adult. a, P2; b, P3; c, enp P3; d, P4; e, intercoxal sclerite P4, caudal view. Scale bars: a-d = 100  $\mu$ m; e = 50  $\mu$ m.

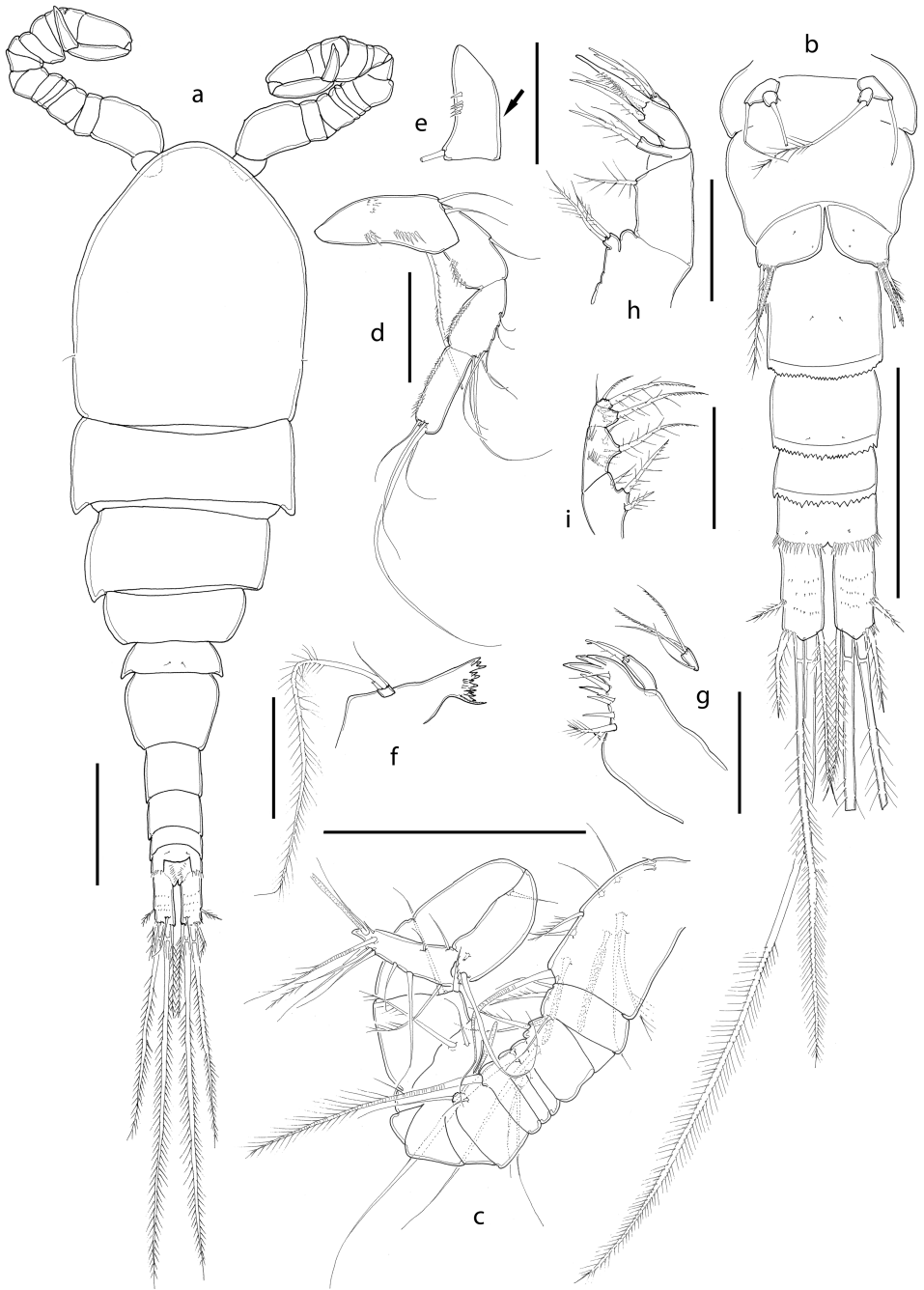


Fig. 5. *Acanthocyclops fiersi* n. sp. male allotype, adult. a, Habitus, dorsal view; b, Urs, ventral view; c, A1; d, A2; e, A2 basis, frontal view; f, Md; g, Mx1; h, Mx2; i, Mxp. Scale bars: a-c = 100  $\mu\text{m}$ ; d-I = 50  $\mu\text{m}$ .

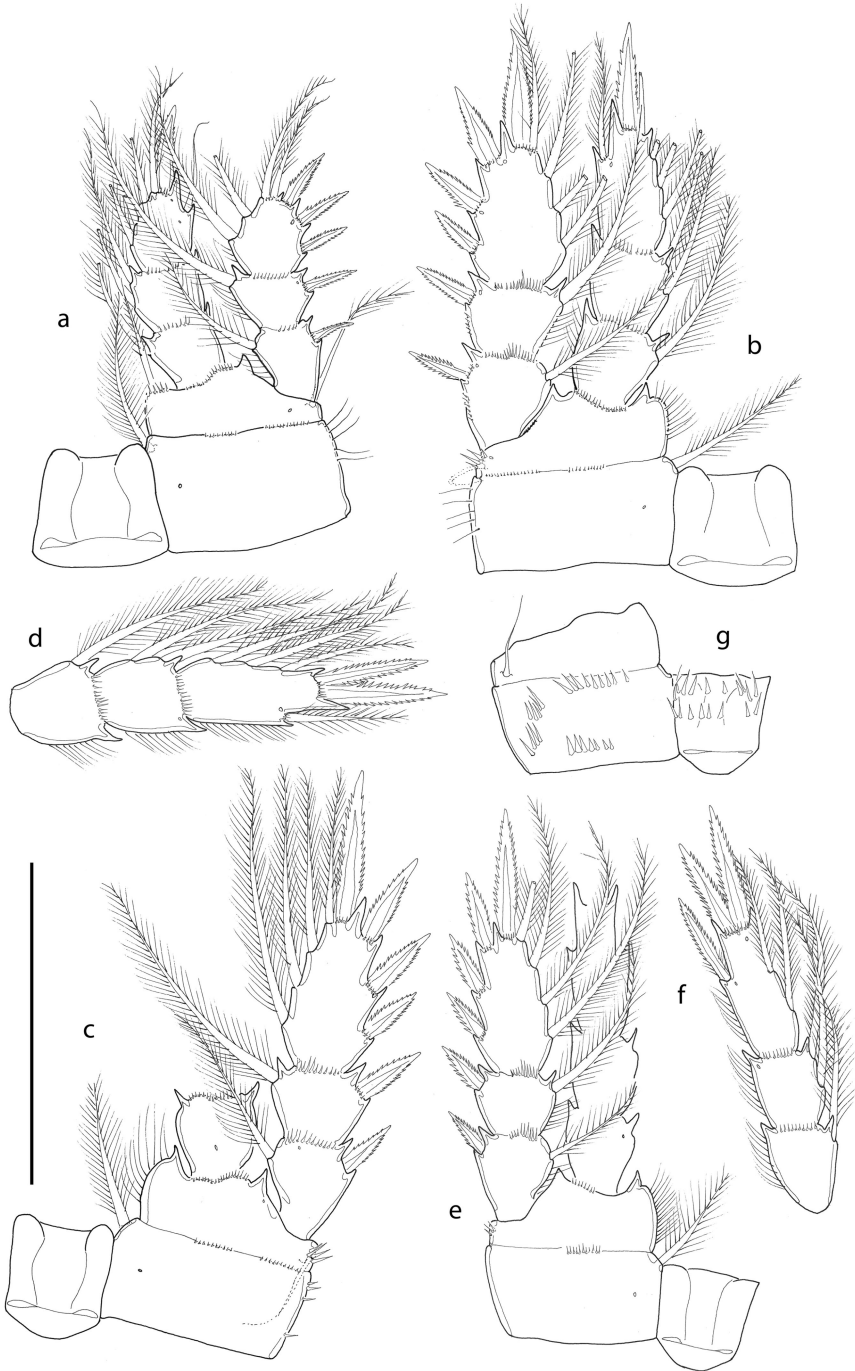


Fig. 6. *Acanthocyclops fiersi* n. sp., male allotype. a, P1; b, P2; c, P3; d, exp P3; e, P4; f, enp P4; g, coxa, basis and intercoxal sclerite P4, caudal view. Scale bars: a-g = 100  $\mu$ m.

setae; inner margin with row of tiny spinules. Enp-3 with seven terminal setae, inner margin with row of spinules.

Md (fig. 3d).— With seven teeth on gnathobase plus short dorsal seta. Row of four strong spinules in middle margin, adjacent to gnathobase. Palp distinct, with usual two long setae (broken off in holotype) and one short, slender seta.

Mx1 (fig. 3e-f).— Precoxal arthrite with smooth surface, with three strong chitinized distal claws and one spiniform setae on frontal side. Basis of palp furnished apically with one asymmetrically bipinnate seta and two simple setae. Enp subquadrate, armed with three subequally long apical setae.

Mx2 (fig. 3g).— Precoxa and coxa not fused; precoxal endite armed with two strong biserially setulated setae. Coxa with single seta on inner margin; coxal caudal surface smooth. Proximal basipodal endite well developed, with two apical setae, one furnished with spinules and noticeably thicker than the other. Claw-like basal endite relatively short, endite with two setae, one large and one small, both inserted near base of claw. Endopod one-segmented, bearing two strong, long spiniform setae plus two short, slender setae.

Mxp (fig. 3h).— Four-segmented. Syncoxa with three spiniform setae along inner margin. Basis with one strong setulated setae and one small unipinnate seta, one longitudinal row of long spinules on inner margin, and two transverse rows of spinules on outer margin. Enp 2-segmented, enp-1 with wide base, stout basal spine asymmetrically spinulated; longitudinal row of spinules close to insertion of seta. Enp-2 armed with three elements: one proximal strong, spiniform asymmetrically spinulated seta plus two shorter, asymmetrically spinulated apical setae.

P1 (fig. 3i).— Intercoxal sclerite smooth, distal margin with two rounded chitinized projections. Coxa with strong biserially setulated inner coxal seta reaching distal margin of end-2; outer margin ornamented with row of short spinules and few setiform elements. Distal margin furnished with minute spinules. Basis with long outer basipodal seta inner margin moderately expanded, ornamented with row of thin setiform elements, armed with strong spiniform seta barely reaching midlength of enp-3; row of small spinules present along insertion of enp. Enp and exp 3-segmented. Armature as in table II.

P2 (fig. 4a).— Intercoxal sclerite smooth, with two weakly developed, slightly rounded chitinized projections. Coxa with row of spinules and few setiform elements along outer margin; coxa furnished with rows of minute spinules along distal margin. Inner coxal seta robust, biserially setulated, not reaching end-2 distal margin. Basis armed with short, slender outer basipodal seta; cluster of robust spinules at insertion of basipodal seta. Basis inner corner and middle distal margin s forming spiniform expansions. Enp and exp 3-segmented. Enp-3 with outer seta and inner apicalmost 1 setae modified (arrowed fig. 4a). Armature as in table II.

TABLE II  
Armature of swimming legs 1-4 of *Acanthocyclops fiersi* n. sp.

	Coxa	Basis	Endopodite	Exopodite
Leg 1	0-1	1-I	0-1; 0-2; 1-I-4	I-1; I-1; II-I-1-3
Leg 2	0-1	1-0	0-1; 0-2; 1-I-1-3	I-1; I-1; III-I-1-3
Leg 3	0-1	1-0	0-1; 0-2; 1-I-1-3	I-1; I-1; III-I-1-3
Leg 4	0-1	1-0	0-1; 0-2; 1-II-2	I-1; I-1; III-I-1-3

Spines in Roman numerals, setae in Arabic. Sequence follows external to internal positions.

P3 (fig. 4b-c).— Intercoxal sclerite smooth, with weakly developed, slightly rounded chitinized distal projections. Coxa armed with biserially setulated inner coxal seta barely reaching beyond enp-1 distal margin; coxa ornamented with row of few short setules on outer margin. Basis armed with slender basipodal outer seta, basis ornamented with robust spinules close to insertion of seta; basis inner corner and middle distal margin forming spiniform expansions. Enp and exp 3-segmented. Row of small spinules along insertion of enp. Exp-3 with two stout, slightly modified subapical inner setae; enp-3 with outer seta spiniform, inner apical seta modified (fig. 4c). Armature as in table II.

P4 (fig. 4d-e).— Intercoxal sclerite with two rows of spinules on caudal surface; distal margin smooth, flat, lacking expansions (fig. 4e). Caudal coxal surface ornamented as in fig. 4d; inner coxal seta strong, biserially setulated, not reaching distal margin of enp-1. Basis with small, slender outer basipodal seta and row of robust spinules at seta insertion; row of small spinules along Enp insertion. Enp and exp 3-segmented, enp slightly longer than exp. Inner enp-3 spine slightly longer than outer spine; outer/inner terminal spines of enp-3 length ratio = 0.90. Enp-3 length/width ratio = 2.4. Outer seta of enp-3 inserted at 70% of enp-3 total length f. Inner endopodal spine/enp-3 length ratio = 0.90. Enp-3 with outer seta transformed into a spine and with most apical seta modified (arrowed in fig. 4d). Exp-3 with three inner apical setae modified (arrowed in fig. 4d). Armature as in table II.

P5 (fig. 2b).— Leg consisting of two free segments, first (proximal) subrectangular, with moderate lateral expansion bearing long apical seta; second (distal) segment twice as long as broad, with long distal seta. Short spiniform element inserted subdistally; spiniform element barely reaching distal margin of segment.

Urs (fig. 2b).— Posterior margins of genital double somite, free urosomites, and telson smooth both dorsally and ventrally, with sensilla and pores as shown in fig. 2b; length ratio of each urosomite and telson as: 52:17:15:16 = 100. Genital double-somite representing 15% of body length (excluding furca). Genital

double-somite smooth on ventral and dorsal surfaces, its anterior half moderately expanded laterally. Ventral surface of telson smooth; distal margin with rows of short, stout spinules along insertion of furcae on ventral surface and ascending along flanks and reaching dorsal margin. Anal operculum weakly coarse (fig. 2c).

Furca (fig. 2b).— Ramus 0.35 times as long as urosome. Length/width ratio = 3.0. Inner margin, ventral and dorsal surfaces ornamented with small spinules arranged in five transverse groups. Lateral seta (II) short, 0.36 times as long as Fu, inserted at about 62% of total Fu length. Outer terminal seta (IV) long, 3.71 times as long as Fu. Inner terminal seta (V) long, 6.2 times as long as Fu. Dorsal seta (VII) slender, as long as Fu. Innermost terminal seta (VI) 1.5 times as long as Fu and 1.5 times as long as dorsal seta (VII). Outermost terminal seta (III) about 0.70 times as long as Fu.

Male.— Habitus (fig. 5a). Total body length = 650  $\mu\text{m}$  from anterior end of cephalothorax to posterior margin of furca. Body slender, cephalothorax relatively long. Cephalothorax length = 239  $\mu\text{m}$ , representing 37% of total body length.

A1 (fig. 5c).— Genuiculate, 16-segmented in all specimens examined; armature as follows: 1(7s + 1ae), 2(4s), 3(2s), 4(2s), 5(1s), 6(1s), 7(2s + 1ae), 8(0), 9(0), 10(0), 11(2s), 12(1s + 1sp), 13(0), 14(1s), 15(2s), 16(10s + 1sp + 1ae). Dorsal surface of first segment with row of spinules.

A2 (fig. 5d-e).— As in female except for lack of longitudinal row of tiny spinules close to outer margin (arrowed in fig. 5e).

Mouthparts (figs. 5f-i).— As in female.

P1 (fig. 6a).— As in female.

P2 (fig. 6b).— As in female except all enp-3 setae unmodified.

P3 (figs. 6c-d).— As in female except all exp-3 setae unmodified; enp-3 only with inner apical seta modified into a spine (arrowed in fig. 6d).

P4 (fig. 6e-g).— As in female except for: 1) intercoxal sclerite ornamentation with stronger spinules; 2) exp-3 setae unmodified; and 3) only enp-3 subapical outer spine modified into a spine (as in female), all other setae are normal.

P5 (fig. 5b).— As in female.

P6 (fig. 5b).— Large low plate at distal corner of genital somite with one strong inner spine, one medial, and one outer seta. Inner spine about 1.4 times longer than medial seta. Outer seta 1.8 times longer than inner spine. Group of strong spinules at inner spine insertion.

Urs (fig. 5a-b).— Genital somite sub-rectangular, length: width ratio = 0.75. Genital somite and succeeding urosomites without ornamentations but with pores and sensilla present (as shown in fig. 5b). Distal dorsal margin of telson with rows of stout spinules.

Furca (fig. 5a-b).— length/width ratio = 2.4, ornamented with spinules as female, the only difference is that in female five groups of spinules can be

distinguished while in male only three groups are present. Lateral seta (II) short and spiniform, 0.43 times as long as Fu, inserted at about 1/3 of total Fu length. Outer terminal seta (IV) long, 4.4 times as long as Fu. Inner terminal seta (V) long, 7.5 times longer than Fu. Dorsal seta (VII) 0.81 times as long as Fu. Innermost terminal seta (VI) longer than Fu (1.83 times) and longer than dorsal seta (VII). Outermost terminal seta (III) about 0.87 times as long as Fu.

Molecular diversity.— A total number of 21 successful sequences of *Acanthocyclops fiersi* n. sp. were produced. The alignment provided includes 21 *COI* sequences generated in this study together with 86 sequences of members of the *A. vernalis-robustus* species-complex downloaded from GenBank. Additionally, four sequences of *Mesocyclops chaci* Fiers (in Fiers, Reid, Iliffe & Suárez-Morales), 1996 were added as outgroup. Table III shows inter- and intraspecific genetic divergences calculated by K2P substitution model for distinct clades of *Acanthocyclops* analyzed. Kimura-two-parameter distance model revealed an average of 0.054% divergence within *A. fiersi* n. sp. and 17.6–22.41% with respect to other *Acanthocyclops* species. The phylogram generated by Bayesian analysis of the *COI* sequences is shown in fig. 7; branches are collapsed into the clade level (the file that can be accessed at 10.6084/m9.figshare.22249456 shows the complete *COI* tree). Eight *Acanthocyclops* species (Clades 1–8) + *Mesocyclops chaci* (outgroup) (Clade 9) were identified with GMYC analyses with a confidence interval of 9–12. *Acanthocyclops fiersi* n. sp. is represented as Clade 8 in fig. 7; records of *A. americanus* from Central and North Mexico clustered together with populations from U.S.A., Spain, France and one individual from Canada identified as *Acanthocyclops* sp. (MG318561.1). Three distinct clades of *A. vernalis* (Clades 1, 3 and 4) were recognized by the Bayesian Analysis and the GMYC species delimitation model. Clade 1 is a single sequence from the U.S.A. (MK329337.1), with an average genetic divergence 22.33–24.67% from all other clades obtained during this analysis. Both Clade 3 and 4 are sister to *A. americanus*; clade 3 includes two

TABLE III

K2P inter- and intraspecific genetic divergence is shown for defined clades of *Acanthocyclops* species

	Clade_8	Clade_2	Clade_3	Clade_4	Clade_6	Clade_5	Clade_7	Clade_9	Clade_1
Clade_8	0.54								
Clade_2	17.60	1.26							
Clade_3	18.93	18.83	0.21						
Clade_4	18.78	17.57	8.62	2.42					
Clade_6	18.05	17.83	19.99	19.64	0.26				
Clade_5	19.08	20.07	21.28	19.37	19.85	0.10			
Clade_7	20.33	22.09	21.22	21.28	18.72	25.01	0.15		
Clade_9	20.83	23.41	24.23	25.17	24.44	24.66	23.85	0.35	
Clade_1	22.41	24.06	23.04	22.44	24.17	24.67	24.04	22.33	n/c



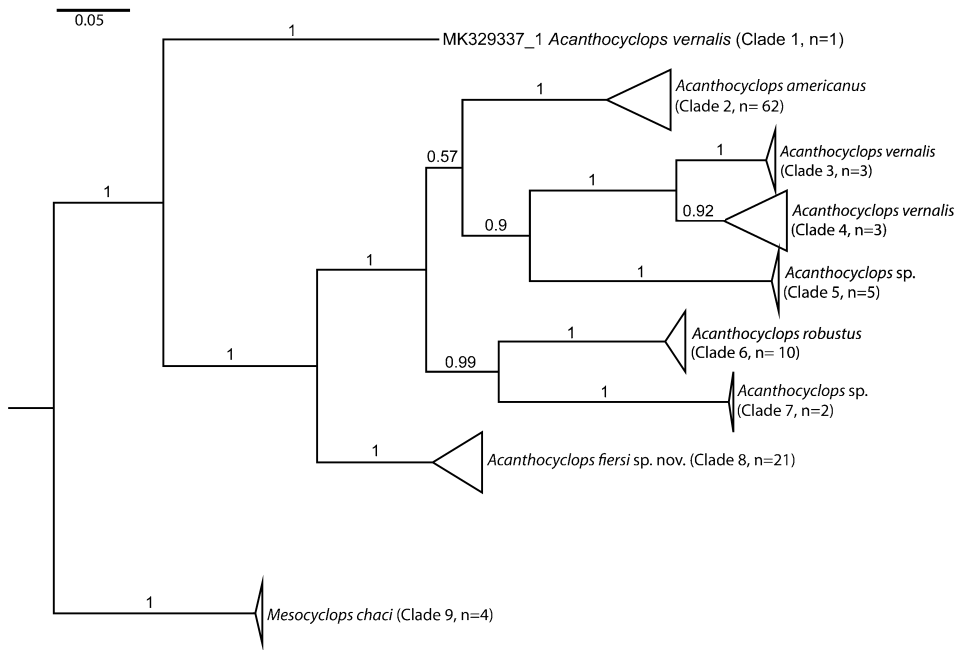


Fig. 7. Bayesian tree of mtDNA COI sequences of *Acanthocyclops* species. Branches are collapsed to clades supported by General Mixed Yule Coalescent model (GMYC). Values on branches are posterior probabilities.

individuals from Russia and one from Canada and, Clade 4 is composed by three sequences from Russia. Eight specimens identified as *A. robustus* and two identified as *A. americanus* clustered together within clade 6. Clades 5 and 7 include five and two sequences — respectively — of yet undetermined *Acanthocyclops* from Canada.

## DISCUSSION

The new species presented here is assignable to the genus *Acanthocyclops* because of its possession of the combination of diagnostic generic characters described by Reid (1985), Einsle (1996), and Dussart & Defaye (2001): (1) the general body shape with the fifth somite broader than the genital double-somite; (2) fifth leg represented by two segments, the distal one is armed with a small sub-apical spine reaching the distal end of the basal segment; (3) 17-segmented female antennules; and (4) 3-segmented rami of all swimming legs.

The new species, *A. fiersi* n. sp., is recognizable as belonging to the *A. vernalis-robustus* species-complex because the combination of the following characters: (1) the number of antennular segments (17); (2) the spine formula of swimming legs

1-4 (usually 3444); (3) the length of the innermost furcal seta; and (4) the shape of the male sixth legs. *Acanthocyclops fiersi* n. sp. can be readily distinguished from *A. vernalis* by: (1) the shape of the female seminal receptacle; (2) the shape of the genital double-somite; (3) its weaker ornamentation pattern on the caudal surface of the antennary basis; (4) the relatively shorter furca with distinctive spinule ornamentation on both ventral and dorsal surfaces; (5) the presence of several modified setae on swimming legs 3 and 4; and (6) the ornamentation pattern on the P4 coxal plate. Both species share the general structure of the male P6, with a strong inner spine clearly longer than the middle seta, and outer seta longer than the inner spine (about 1.8-2.0 times longer in *A. fiersi* n. sp. vs. 1.3-2.3 in *A. vernalis*). Following the characters proposed by Mirabdullayev & Defaye (2002) to distinguish *A. robustus* from its congeners, *A. fiersi* n. sp. can also be easily separated from *A. robustus* by the lack of the group of spinules close to the exopodal seta on the frontal surface of the antennary basis, a relatively shorter furca ornamented with a distinctive pattern of small spinules on both ventral and dorsal surfaces, the shape of the female seminal receptacle, and the ornamentation pattern on P4 coxal sclerite.

In recent years, several species of the *A. vernalis-robustus* species complex have been described or re-described from the Americas, including *A. fiersi* n. sp., *A. marceloi* Mercado-Salas & Suárez-Morales, 2009, *A. caesariatus* Mercado-Salas & Suárez-Morales, 2009, *A. eduardoi* Mercado-Salas & Álvarez-Silva, 2013, *A. trajani*, *A. einslei*, and *A. brevispinosus* (Herrick, 1884), all of them distributed in different geographic areas ranging from central Mexico to Canada. Currently, only three species of *Acanthocyclops* have been reported from the Yucatán Peninsula, the presumably cosmopolitan *A. robustus* and the two known Neotropical members of the genus: *A. smithae* distributed in Mexico, Honduras, and Brazil and *A. rebecae*, known from a single locality in Campeche, Mexico. Together with *A. fiersi* n. sp., both species also belong to the *A. vernalis-robustus* species-complex. These three species were found in similar habitats, including rice ponds (*A. smithae*), ephemeral pools (*A. rebecae*), and flooded plains and pools (*A. fiersi* n. sp.) (Reid & Suárez-Morales, 1999; Fiers et al., 2000). An important character shared by the three Neotropical species that appears to be absent in other congeners is the presence of a second row of strong spinules on the caudal surface of the P4 intercoxal sclerite (in both sexes). This character was previously reported only in males of *A. eduardoi* from central Mexico and was also observed in specimens collected in the state of Puebla (pers. obs., NFM-S). It is probable that this character would allow the distinction between Neotropical and Nearctic species of *Acanthocyclops*. Several works have pointed out the importance of the coxal plates ornamental patterns in the systematics of freshwater cyclopoids (Mercado-Salas et al., 2016 and references therein). In the most recent revision

of the taxonomically complex cyclopoid genus *Eucyclops* the P1-P4 coxal plates ornamentation patterns were highlighted as important characters to distinguish closely related species; these authors emphasized that the presence and type of elements on the coxal plates – not their number – are reliable characters to separate species within the *E. serrulatus* complex (Mercado-Salas et al., 2016). The work by Hołyńska (2000) also explored the role of coxal plates ornamentation in the recognition of different *Mesocyclops* lineages which were morphologically and geographically consistent. The author advanced these patterns as probable signals of evolution in copepods. Karaytug (1999), Karaytug & Boxshall (1998, 1999) and Hołyńska (2000) stated that the ornamental patterns of the P4 coxal plate could represent a key character in sex recognition because these structures are in contact during mating. Accordingly, we propose here the differences on the P4 coxal sclerite ornamentation as a possible character to recognize species with Neotropical affinities from their Nearctic congeners. *Acanthocyclops fiersi* n. sp. and *A. smithae* appear to be more closely related; they can be easily distinguished from *A. rebecca* by the legs 1-4 spine formula: 3444 (vs. 2333 in *A. rebecca*), the presence of modified setae on the terminal segments of P2-P4, the lack of a supplementary spinular row on both faces of the antennal basipodite, the shape of seminal receptacle, the absence of ornamentation on the genital double-somite, and a weakly coarse anal operculum. Another remarkable difference between *A. fiersi* n. sp. and *A. rebecca* is the shape of the rudimentary male sixth leg (males of *A. smithae* remain unknown). *Acanthocyclops rebecca* differs from all other members of the *A. vernalis-robustus* species-complex by possessing a remarkably strong and long P6 inner spine, always being longer than both the medial and outer setae, whereas in *A. fiersi* n. sp., the inner spine is clearly shorter (about a half) than the outer spine, as found in other members of this species complex.

The Neotropical *A. fiersi* n. sp. and *A. smithae* are very closely related but they differ in several aspects including: (1) the shape of the seminal receptacle, with rounded anterior lateral arms and bilobed posterior part, whereas in *A. smithae* both parts are round and simple, none bilobed; (2) a complete egg sac containing 34 eggs in *A. smithae* (Reid & Suárez-Morales, 1999), whereas in *A. fiersi* n. sp. the maximum count of eggs per sac was 8 (in specimens from both reserves); (3) in *A. fiersi* n. sp. the furca is conspicuously ornamented with a distinctive pattern of spinules arranged in five groups on both ventral and dorsal surfaces in females and three groups in males, while in *A. smithae* the furcal surfaces are smooth; (4) the ornamentation on the antennary basis caudal surface is weaker in *A. fiersi* n. sp. than in *A. smithae*; also, in *A. fiersi* n. sp. the antennary enp-2 is armed with eight setae in all specimens examined, whereas *A. smithae* shows 9 such setae. In addition, the position and number of modified setae and setae transformed into spiniform elements differ among species (see fig. 4a-d for *A. fiersi* n. sp. and fig. 3

in Reid & Suárez-Morales, 1999). Finally, in the description of *A. smithae* it is pointed out that this species differs from all its congeners within the *A. vernalis-robustus* species complex by the presence of extremely short, stout terminal spines on P4 enp-3, being clearly shorter than the segmental length, whereas in the new species both elements are as long or slightly longer than segment.

Unfortunately, sequences from *A. smithae* and *A. rebecca* are not available in order to analyse the genetic differences among the Neotropical species of the *A. vernalis-robustus* species-complex. However, *A. fiersi* n. sp. can be clearly distinguished from other members of this species-complex using mtDNA *COI* sequences by an average of 17.6–22.41% divergence. Until recent years, most records of *Acanthocyclops* species in Mexico have been assigned to the “cosmopolitan” *A. robustus* and *A. vernalis*; however, after the works by Miracle et al. (2013) and Alekseev et al. (2021), researchers working on the Mexican freshwater copepod fauna have reported populations of *A. americanus* from central and northern Mexico (Prosser et al., 2013) which are genetically similar to those sequenced from its type locality. *Acanthocyclops fiersi* n. sp. can be distinguished from *A. americanus* by an average genetic divergence of 17.6%. Our results from GMYC delimitation method supported *A. fiersi* n. sp. as an independent lineage within the genus *Acanthocyclops*, sister to a clade enclosing most members of the *A. vernalis-robustus* species complex (except one sequence assigned as *A. vernalis* – Clade 1-). The separation of *A. fiersi* n. sp. from all other sequences available might reflect the separation between the Neotropical and the Nearctic/Palaearctic lineages of this species-complex. However, this hypothesis needs to be tested with a more robust taxa sampling (e.g., including material from *A. smithae* and *A. rebecca*). Furthermore, in the case of *A. vernalis* our analysis revealed three independent lineages (clades 1, 3 and 4), clade 1 comprising only one sequence from specimens collected in the U.S.A. and being sister to all other species included in the analysis. A cluster formed by clades 3 and 4 (assigned to *A. vernalis*) came as sister group to clade 5, which includes specimens from Canada determined as *Acanthocyclops* sp. only. *Acanthocyclops robustus* (clade 6) form well-supported cluster sister to *Acanthocyclops* sp. (clade 7). However, it is important to mention that two sequences identified as *A. americanus* from Russia clustered within the *A. robustus* group (clade 6) in which specimens from the type locality are included. Because we do not have access to the material, we cannot determine if these animals represent a misidentification, however, this should be verified in future investigations.

Most of the known species of *Acanthocyclops* occur mainly in temperate and cold latitudes in Eurasia and North and South America; only *A. smithae*, *A. rebecca* and the new species *A. fiersi* n. sp. could be considered as true Neotropical forms (see Suárez-Morales et al., 2020). The study of the copepod fauna in this area in Mexico and Central America is still limited and further studies are

expected to reveal other undescribed Neotropical forms with presumably restricted distributions (Silva-Briano & Suárez-Morales, 1998; Granados-Ramírez & Suárez-Morales, 2003; Mercado-Salas et al., 2006, 2009; Mercado-Salas, 2009; Mercado-Salas & Suárez-Morales, 2009).

KEY TO SPECIES OF *Acanthocyclops* DISTRIBUTED IN MEXICO

1. Females with less than 17 (commonly 11-12) segments in antennules, terminal spines of enp-3 P4 subequal; caudal seta VI shorter than furca .....  
*A. dodsoni* Mercado, Suárez-Morales & Silva-Briano, 2006  
 Females with 17-segmented antennules ..... 2
2. Genital somite in female wide and with proximal part angular, with tapering lobes .....  
*A. vernalis* (Fischer, 1853)  
 Genital somite in females lacking tapering lobes, proximal part more roundish than angular ..... 3
3. Antennal basis with spinules near to exopodal seta ..... *A. robustus* (Sars G.O., 1863)  
 Antennal basis without spinules near to exopodal seta ..... 4
4. Intercoxal sclerite of P4 with two rows of strong spinules on caudal surface in males and females. Short furca, not more than 3.5 times longer than wide, caudal seta VI particularly long, more than 1.4 times as long as furca ..... 5  
 Intercoxal sclerite of P4 with only one row of strong spinules on caudal surface in females. Furca ratio variable from 2.8-4.0 times longer than wide ..... 7
5. Antennal basis with rows of short, slender spinules on frontal and caudal surfaces; caudal seta VII as long as or longer than furca; modified setae on enp-3 of P2-P4; spine formula 3444. Male P6 with a strong inner spine, middle seta shorter than inner spine; outer seta longer than inner spine and middle seta ..... 6  
 Antennal basis with rows of strong spinules on frontal and caudal surfaces; caudal seta VII slightly shorter than furca; unmodified setae on swimming legs; spine formula 2333. Male P6 with a very strong and long inner spine, longer than outer and middle setae .....  
*A. rebecca* Fiers, Ghene & Suárez-Morales, 2000
6. Antennal enp-2 bearing 8 setae, furca ornamented with small spinules in ventral and dorsal view. Lateral arms of seminal receptacle rounded and posterior part bi-lobulated; spine formula 3444. Terminal spines of enp-3 as long as or longer than segment length .....  
*A. fiersi* n. sp.  
 Antennal enp-2 bearing 9 setae, furca smooth in ventral and dorsal view. Lateral arms of seminal receptacle and posterior part rounded, not divided in lobules. Terminal spines of enp-3 particularly short, shorter than segment length .....  
*A. smithae* Reid & Suárez-Morales, 1999
7. Furca long, more than 4.2 times longer than wide, strongly ornamented with tiny spinules in dorsal and ventral surfaces, inner margin of furca not bearing hairs. Caudal seta VII particularly short (>0.5 times longer than furca). Intercoxal sclerite of male P4 with two rows of strong spinules on caudal surface .....  
*A. eduardoi* Mercado-Salas & Álvarez-Silva, 2013  
 Furca short, less than 3.5 times longer than wide, ornamented with spinules or hairs in dorsal and ventral surfaces, inner margin of furca bearing hairs. Caudal seta VII not particularly short (<0.5 times longer than furca) ..... 8

8. Furca ornamented with tiny spinules in both, dorsal and ventral surfaces; spinules arranged in a particular pattern of groups of spinules. Anal somite ornamented with row of spinules above anal operculum. Spine formula of swimming legs 3443.....  
 ..... *A. marceloi* Mercado-Salas & Suárez-Morales, 2009  
 Furca ornamented with hairs in dorsal, ventral surfaces and inner margin; hairs not arranged in a particular pattern. Anal somite smooth. Spine formula of swimming legs 3444.....  
 ..... *A. caesariatus* Mercado-Salas & Suárez-Morales, 2009

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