Genetic structure and distributional patterns of the genus *Mastigodiaptomus* (Copepoda) in Mexico, with the description of a new species from the Yucatan Peninsula

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Abstract. *Mastigodiaptomus* is the most common diaptomid in the Southern USA, Mexico, Central America and Caribbean freshwaters, nevertheless its distributional patterns and diversity cannot be stablished because of the presence of cryptic species hidden under wide distributed forms. Herein we study the morphological and molecular variation of the calanoid fauna from two Biosphere Reserves in the Yucatan Peninsula and we describe a new species of the genus *Mastigodiaptomus*. Our findings are compared with other lineages previously found in Mexico. *Mastigodiaptomus siankaanensis* sp.n. is closely related to *M. nesus*, from which can be recognized because of the absence of the spinous process in segment 10 of male A1 and the seta formula and ornamentation of female A1. The mitochondrial cytochrome c subunit I gene (COI) revealed a mean of 0–2.77% K2P divergence within *M. siankaanensis* sp.n. and 14.46–22.4% from other *Mastigodiaptomus* species. Within the new species three different populations were detected, two distributed in close localities (sympatric) and the third consistent with allopatric distribution. The General Mixed Yule Coalescence method (GMYC) delimited eight species of *Mastigodiaptomus* distributed in Mexico. The high diversity and endemism of *Mastigodiaptomus* in the Yucatan Peninsula and Antilles suggest a Neotropical origin of the genus.

Key words. Morphology, Biodiversity, sibling species, sympatric speciation, COI mtDNA, biogeography, Caribbean, Neotropical.

1. Introduction

Copepods are an extraordinary diverse group with respect to their morphologies, physiology, life-strategies and habitat preferences (Boxshall & Defaye 2008; Bron et al. 2011). Among freshwater environments the Diaptomidae Baird, 1850 is the largest and dominant family of the order Calanoida Sars, 1903, including more than 450 species widely distributed in Europe, Asia, America, Africa and, two species in Australia (Boxshall & Jaume 2000; Boxshall & Defaye 2008). Most of the Diaptomidae are planktonic, some are benthic and few species inhabit subterranean waters. The family is also characterized by restricted distribution, where around 90% of the species are endemic to a single biogeographic region

(Boxshall & Jaume 2000; Boxshall & Defaye 2008; Barrera-Moreno et al. 2015).

Despite the great diversity of the copepods, their phenotypes tend to be conservative (morphological stasis) (Pesce 1996; Blanco-Bercial et al. 2014) and cryptic speciation seems to be a natural phenomenon, making difficult the separation of species within the group. Numerous efforts have been focused on the development of molecular tools and use of genetic approaches to identify and delimitate copepod species, especially those from marine environments (Lee 2000; Blanco-Bercial et al. 2014). Nevertheless, in continental waters there are few studies dedicated to the evaluation of species boundaries using

Table 1. Sampling localities in Calakmul and Sian ka'an Biosphere Reserves and additional records of *M. siankaanensis* sp.n. (*) indicates the type locality.

Locality	Reserve	Date	Species recorded	Geographic coordinates
Aguada Vigia Chico	Sian ka'an	20-09-2014	*M. siankaanensis* sp.n.	19.784 N, 87.610 W
Savannah 2	Sian ka'an	21-09-2014	*M. siankaanensis* sp.n.	19.799 N, 87.700 W
Aguada limite de la reserva (*)	Sian ka'an	23-09-2014	*M. siankaanensis* sp.n.	19.709 N, 87.828 W
Arroyo Calakmul	Calakmul	28-09-2014	*M. reidae*	18.123 N, 89.790 W
Arroyo Aguada Grande	Calakmul	28-09-2014	*M.reidae*	18.124 N, 89.818 W
Polvora	Sian ka'an	29-09-2015	*M. siankaanensis* sp.n.	19.416 N, 87.899 W
Domin	Sian ka'an	29-09-2015	*M. siankaanensis* sp.n.	19.422 N, 87.937 W
Aguada limite de la reserva	Sian ka'an	03-10-2015	*M. siankaanensis* sp.n.	19.709 N, 87.828 W
Aguada Vigia Chico	Sian ka'an	04-10-2015	*M. siankaanensis* sp.n.	19.784 N, 87.610 W
Savannah Playon	Sian ka'an	06-10-2015	*M. siankaanensis* sp.n.	19.832 N, 87.542 W
Kohunlich	—	31-07-2005	*M. siankaanensis* sp.n.	18.477 N, 88.825 W
Kohunlich	—	31-07-2005	*M. reidae*	18.477 N, 88.825 W
Tres Garantias	—	22-05-2011	*M. siankaanensis* sp.n.	18.369 N, 89.013 W

morphological and genetic approaches (Monchenko 2000; Dodson et al. 2003; Alekseev et al. 2006; Elías-Gutiérrez et al. 2008; Thum & Harrison, 2009; Wyngaard et al. 2009; Karanovic & Krajicek 2012; Marrone et al. 2013; Gutiérrez-Aguirre et al. 2014; Barrera-Moreno et al. 2015; Gutiérrez-Aguirre & Cervantes-Martínez 2016) being the traditional morphological work the most used approach in the description and delimitation of species. It is also known that in some cases these called cryptic species are morphologically distinguishable when traditionally overlooked morphological characters are included in the separation of these "pseudo sibling-species" (Marrone et al. 2013); thus the combination of a well define set of morphological characters, genetic approaches and distributional patterns will lead us to correctly delimitate species within copepods.

In particular, within the Neotropical region the Diaptomidae fauna is highly complex, with many species restricted to small localities such as lakes, reservoirs, wetlands, or particular hydrographic basins. Recent efforts have been made, especially in South America, to clarify not only the taxonomic status of some genera (e.g. *Rhacodiaptomus* Kiefer, 1936, *Argyrodiaptomus* Brehm, 1933, *Notodiaptomus* Kiefer, 1936 and *Diaptomus* Westwood, 1936) but to establish their geographic distribution (Suárez-Morales et al. 2005; Santos-Silva 2008; Perbiche-Neves et al. 2013).

Mastigodiaptomus Light, 1939 is the most common diaptomid genus in Southern USA, Mexico, Central America and the Caribbean, with limited distributions in the most of the species. *Mastigodiaptomus montezumae* (Brehm, 1955), *M. reidae* Suárez-Morales & Elías-Gutiérrez, 2000, *M. maya* Suárez-Morales & Elías-Gutiérrez, 2000, *M. suarezmoralesi* Gutiérrez-Aguirre & Cervantes-Martínez, 2013 and *M. patzcuarensis* (Kiefer, 1938) and the recently described *M. cuneatus* Gutiérrez-Aguirre & Cervantes-Martínez, 2016 are probably endemic to different aquatic systems from Mexico. Other species with restricted distribution are *M. amatitlaensis* (Wilson M.S., 1941) endemic to Lake Amatitlan in Guatemala, *M. purpureus* (Marsh, 1907) from Cuba and recorded in Haiti as well (Reid 1996) and *M. nesus* Bowman, 1986

distributed in Bahamas, Belize and the Yucatan Peninsula. Some other species have wider distributions as *M. texensis* Wilson M.S., 1953 described in Texas but recorded in north and south of Mexico (Elías-Gutiérrez et al. 2008; Elías-Gutiérrez et al. 2008b) and the widely distributed *M. albuquerquensis* (Herrick, 1895) recorded from the Southern USA to Central America, and actually split in two different species (Reid 1997; Suárez-Morales & Elías-Gutiérrez 2000; Suárez-Morales & Reid 2003; Brandorff 2012; Gutiérrez-Aguirre & Cervantes-Martínez 2013; Gutiérrez-Aguirre et al. 2014). The most recent efforts to clarify the taxonomic status of the *Mastigodiaptomus* fauna used modern integrative approach to delimitate the species (Gutiérrez-Aguirre et al. 2014; Gutiérrez-Aguirre & Cervantes-Martínez 2016). This and previous studies have recognized that the diversity of the genus is underestimated because of the presence of cryptic species coexisting not only in the same biogeographic area, sometimes in the same locality (Elías-Gutiérrez et al. 2008). Therefore, the inclusion of integrative approaches in the study of the *Mastigodiaptomus* is needed in order to recognize its real diversity and to understand their biogeographic patterns.

Herein, we include morphological and mtDNA COI genetic analyses of the genus *Mastigodiaptomus* from two Biosphere Reserves from the Yucatan Peninsula in Mexico and the description of a new species of the genus *Mastigodiaptomus*. Our findings are compared with other lineages previously found in Mexico, in order to clarify their biogeographic associations.

2. Materials and methods

2.1. Study area

Two protected areas of Mexico, both under the category of Biosphere Reserve: Sian Ka'an and Calakmul have been sampled for this study (Fig. 1) (collection permit: PPF/DGOPA-003/15 SEMARNAT-CONAPESCA, Mexico). These reserves represent the most extensive ar-
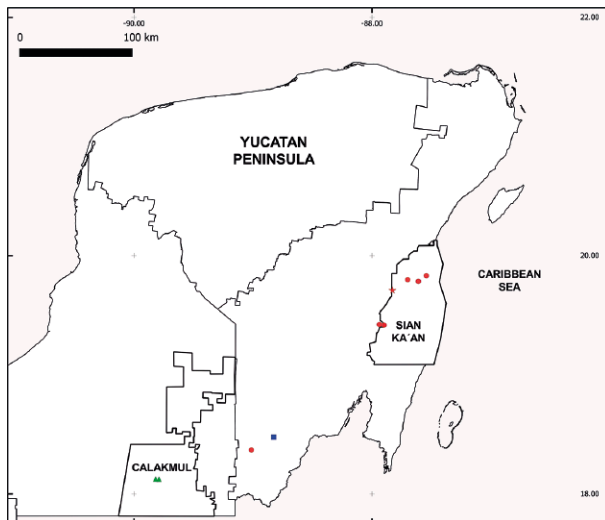
Fig. 1. Sampling localities in Calakmul and Sian ka'an Biosphere Reserves. — **Symbols**: triangles – localities where *Mastigodiaptomus reidae* was found; circles – localities where *Mastigodiaptomus siankaanensis* sp.n. was recorded; star – type locality of *Mastigodiaptomus siankaanensis* sp.n.; square – locality where both species coexist (Kohunlich).

eas of well-preserved tropical forest in Mexico. The Biosphere Reserve Sian Ka'an comprises 528,000 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 flood areas. Within this gradient medium forest, lowlands, flood plains, marshes and mangroves are found. The area has the characteristic hollows and slopes of the limestone substrate and contains variations such as cenotes (sinkholes), hillocks, lagoons, cays and springs. The Biosphere Reserve Calakmul comprises 723,185 ha (terrestrial) it is a combination of high and medium forest with seasonal flooded lowlands and aquatic vegetation. The hydrography of Calakmul surface is determined by the amount and distribution of rainfall; evapo-transpiration, water bodies, soils and surface drainage; some of the low-lying areas are permanent wetlands (CONANP 2016). It hosts 1353 "aguadas" (temporary or permanent pools) and a Paleocene aquifer with a depth to the phreatic zone between 60 and 165 m (GARCÍA-GIL et al. 2002).

Fifteen sampling sites were established in each Reserve, but calanoids were found only in 8 localities (Fig. 1, Table 1). Samples from aguadas, temporal and permanent wetlands (savannahs) were collected using a standard plankton net (200 μm mesh) directly in the water body and then fixed in 96% ethanol. Several males and females of the genus *Mastigodiaptomus* from different sites were sorted from samples and preserved in vials with 96% ethanol at 4°C.

2.2. Morphological observations

Adults of Calanoida were identified to species level following the current standards and techniques for the taxonomic study of diaptomid copepods (SUÁREZ-MORALES et

al. 2005; PERBICHE-NEVES et al. 2013; GUTIÉRREZ-AGUIRRE et al. 2014; GUTIÉRREZ-AGUIRRE & CERVANTES-MARTÍNEZ 2016). For the taxonomic analysis, the appendages of the specimens were dissected and mounted in permanent slides with glycerin and sealed with paraffin. The figures were made with a drawing tube mounted in a Leica DMR microscope at 40 × and 100 × magnifications. 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 Senckenberg am Meer, Dept. DZMB (Wilhelmshaven, Germany).

Four adults of *Mastigodiaptomus siankaanensis* sp.n. were used for Confocal Laser Scanning Microscopy (CLSM) as indicated below. One male and one female were dissected and all structures were stained independently. The complete and the dissected specimens were stained in 1:1 solution of Congo Red and Acid Fuchsin overnight, according to protocol (MICHELS & BÜNTZOW 2010). For ventral and dorsal habitus views, the undissected animals were prepared on slides using Karo® light corn syrup as mounting medium so that the animals were intact and not compressed during the scanning process. Dissected appendages were mounted on individual slides with glycerine. The material was examined using a Leica TCS SP5 equipped with a Leica DM5000 B upright microscope and 3 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 561 nm excitation wave length with 80% acousto-optic tunable filter (AOTF). Series of stacks were obtained, collecting overlapping optical sections throughout the whole preparation with optimal number of sections according to the software. The acquisition resolution was 2048 × 2048. Final images were obtained by maximum projection, and CLSM illustrations were composed and adjusted for contrast and brightness using the software Adobe Photoshop CS4.

Abbreviations used: A1 = antennule, A2 = antenna, Ae = aesthetasc, Cph = cephalothorax, enp = endopod, exp = exopod; Md = mandible, Mx1 = maxillula, Mx2 = maxilla, Mxp = maxilliped, ms = modified seta, P1–P5 = legs 1 to 5, Urs = urosomite(s), vs = vestigial seta. Caudal setae labeled 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; nomenclature follows HUYS & BOXSHALL (1991). The terms furca and telson are used following SCHMINKE (1976) and setae formula modified from PERBICHE-NEVES et al. (2013).

2.3. COI sequencing and genetic analysis

DNA extractions from 33 specimens were carried out using 40 μl Chelex (InstaGene Matrix, Bio-Rad) according to the protocol (ESTOUP et al. 1996) and directly used as DNA template for PCR. A 658 base-pair region of mtDNA COI was amplified using the primers LCO-

1490 (Folmer et al. 1994): GGTCAACAAATCATAAA-GATATTGG and Cop-COI-2198R (Bucklin et al. 2010): GGGTGACCAAAAAATCARAA. The PCR protocol was 94°C for 5 min, 94°C for 45 s, 45°C for 45 s, and 72°C for 50 s, during 38 cycles and as final elongation 72°C for 3 min. PCR was carried using PuReTaq Ready-To-Go PCR Beads (GEHealthcare) in 25 μl volume containing 22 μl nuclease-free water, 0.5 μl of each primer (10 pmol/μl) and 2 μl DNA templates. All PCR products were checked by electrophoresis on a 1% agarose/TAE gel containing 1% GelRed. PCR products were purified using ExoSap-IT PCR Product Cleanup (Affymetrix, Inc) at 37°C followed by an incubation period of 80°C and sequencing were carried out by Macrogen (Amsterdam, Netherlands). Forward and reverse sequences for each specimen were assembled, edited and checked for correct amino acid translation frame using Geneious 9.1.7 (created by Biomatters; available from http://www.geneious.com). All sequences were searched against GenBank nucleotide database using BLASTN (Altschul et al. 1990). Sequences of closely related species were downloaded from NCBI and included in the analyses comprising *M. siankaanensis* sp.n. (4 specimens); *M. montezumae* (36 specimens), *M. reidae* (17 specimens), *M. albuquerquensis* (21 specimens), *M. nesus* (4 specimens), *M. texensis* (3 specimens), *M. patzcuarensis* (22 specimens) and *M. cuneatus* (1 specimen).

Supplementary 1 lists the GenBank accession numbers downloaded from NCBI. Genbank accession numbers of the sequences obtained during this study are as follows:

M. siankaanensis sp.n.:
MK080113, MK080114, MK080115, MK080116, MK080117, MK080118, MK080119, MK080120, MK080121, MK080122, MK080123, MK080124, MK080125, MK080126, MK080127, MK080128, MK080129, MK080130, MK080131, MK080132, MK080133, MK080134, MK080135, MK080136, MK080137, MK080138, MK080139;

M. reidae:
MK080140, MK080141, MK080142, MK080143, MK080144, MK080145.

All DNA sequences including sequences available from this study and downloaded from GenBank were aligned using MAFFT v7.017 under G-INS-i algorithm (Katoh & Toh 1990) and alignment were further edited manually. A Bayesian analysis employing the K2P substitution model were conducted using MrBayes MPI version (Ronquist & Huelsenbeck 2003; Altekar et al. 2004). Posterior probabilities were estimated using 5,000,000 generations with sampling frequency of every 1000 trees through four simultaneous Markov Chains Monte Carlo. The consensus tree with median branch lengths was made, discarding the 1250 first trees. MEGA 7 (Kumar et al. 2015) has been used in order to calculate 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) has been used as species delimitation method in which the simple threshold approach assumes that there is a threshold time, before which all nodes reflect diversification events (inter-specific) and after which all nodes reflect coalescent events (intra-specific). The number of species obtained by this approach is thus estimated by this threshold time. The GMYC method implemented in the "splits" package for R was applied to the COI ultrametrice tree obtained with BEAST v1.8.3 (Drummond et al. 2016). Nucleotide diversity (π) and neutrality test using Tajima's D (Tajima 1989) were calculated with PopART (Bandelt et al. 1999). AMOVA was performed to calculate genetic variations between geographically separated groups. Statistical parsimony method was used to construct a Minimum Spanning haplotype network with PopART (http://popart.otago.ac.nz).

3. Results

Two species of Calanoida were detected from samples obtained during this survey: *M. reidae* and a new species for science, *M. siankaanensis* **sp.n.** Description is based on morphology, mtDNA COI genetic variations and distributional patterns. Additionally, we include an overview of the Mexican records of the genus, their distributional patterns and genetic divergence.

3.1. Molecular diversity and population structure

A total number of 33 specimens have been sequenced in this study comprising 27 sequences of mtDNA COI from *M. siankaanensis* sp.n. from Sian ka'an Biosphere Reserve and six sequences of mtDNA COI of *M. reidae* from Calakmul Biosphere Reserve. The alignment is provided for the 33 COI sequences of this study together with the 85 sequences of all the GenBank COI records available from this genus (supplementary 1). Maximum and minimum length of resulting alignment was 670 and 592 bp, respectively. Table 2 shows minimum and maximum inter- and intra-specific genetic divergence calculated by K2P substitution model for distinct clades of *Mastigodiaptomus* including GenBank available COI sequences of this genus. Kimura-two-parameter distance model revealed the minimum and maximum of 0–2.77% diversity within *M. siankaanensis* sp.n. and 14.46–22.4% from other *Mastigodiaptomus* species. *Mastigodiaptomus reidae* showed 0–2.51% and 17–24.18% minimum and maximum K2P distance within species and compare to other species correspondingly. Figure 2 indicates the phylogram generated by Bayesian analyses of COI sequences from *Mastigodaptomus* species including GenBank sequences in which the branches are collapsed into the clade level (supplementary 2 shows the complete COI tree). The number of eight species (Fig. 2) has been identified with GMYC

Table 2. Percentage of maximum and minimum K2P inter- and intra-specific genetic divergence shown for defined clades of *Mastigodiaptomus* species.

	M.s.(Cl.1)	M.s.(Cl.2)	M.r.	M.t.	M.m.(Cl.1)	M.m.(Cl.2)	M.a.(Cl.1)	M.a.(Cl.2)	M.a.(Cl.3)	M.p.	M.n.	M.c.
M. siankaanensis sp.n. (Clade1)	0–0.68											
M. siankaanensis sp.n. (Clade2)	2.05–2.77	0–1.59										
M. reidae	14.46–17.16	13.87–16.82	0–2.51									
M. texensis	19.26–19.89	17.96–19.26	17.17–19.03	00–0.1								
M. montezumae (Clade1)	17.6–19.21	18.25–18.62	21.5–24.18	19.73–20.70	0–0.9							
M. montezumae (Clade2)	17.1–19	17.98–18.32	23.20–25.64	21.39–21.73	2.77–3.49	0–0.68						
M. albuquerquensis (Clade1)	17.12–19.88	17–19.19	19.59–21.53	23.37–24.07	22–23.01	21.35–22.39	0.22					
M. albuquerquensis (Clade2)	17.11–19.34	16.21–17.11	19.92–21.61	23.2–23.72	20.63–21.61	19.33–20.33	3.26–3.51	0–0.45				
M. albuquerquensis (Clade3)	18.06–20.36	18.09–21.08	20.25–23	23.5–25.51	21.65–23.41	21.35–23.46	3.26–4.99	3.26–4.78	0–2.06			
M. patzcuarensi	17.45–19.99	15.92–20	17.38–20.62	19.76–21.74	21.47–23.97	21.17–22.27	5.21–6.72	5.71–7.24	6.49–9.68	0–2.76		
M. nesus	19.81–20.46	19.83–20.39	20.83–22.46	20.72–23.76	22.74–23.76	23.79–24.15	22.81–23.16	22.46–22.81	22.5–23.86	22.84–24.62	0	
M. cuneatus	21.1–22.4	19.48–20.20	20.72–22.85	21.37	23.27–25.04	21.49–26.48	22.2	22.19	21.89–22.75	19.4–22.47	26.28	0

analyses with confidence interval of 7–15 in which the likelihood of GMYC model (1190.061) was significantly superior to the likelihood of null model (1180.95). Two clades have been defined for *M. siankaanensis* sp.n. in which clade 1 represents the population from Aguada limite de la reserva (sequenced for this study) and clade two represents species from Kohunlich and Tres Garantias (available from GenBank). *Mastigodiaptomus albuquerquensis* has been clustered into three clades; two refer to different genotypes of *M. albuquerquensis* presented in North Mexico, sister to recently validated *M. patzcuarensis*, recorded from Central Mexico (maximum and minimum K2P between species distances: 5.21–9.68%). The newly described species *M. cuneatus* revealed inter-specific COI Kimura-two-parameter genetic differences in the range of 19.4–22.75 % (Table 2). Bayesian analyses identified two distinct clades for *M. montezumae* (with relatively high inter clade genetic distances between 2.77–3.49%; Fig. 2), both distributed in Central Mexico: clade 1 represents haplotypes from Mexico State and clade 2 from both Mexico State and Aguascalientes. Population analyses of *M. siankaanensis* sp.n. revealed 19 polymorphic sites with nucleotide diversity of π = 0.0032 from 10 different haplotypes (Table 3). No haplotype were shared between populations (Fig. 3), and therefore the high significant fixation index (F_{st} = 0.801; P < 0.001). A total number of 38 polymorphic sites have been recognized from the three clades of *M. albuquerquensis* with nucleotide diversity of 0.0073 (13 haplotypes). F_{st} = 0.846 (P < 0.001) was an indication of restricted gene flow between the three clades of *M. albuquerquensis*, in northern Mexico (Table 3). The Minimum spanning network revealed the number of 20 polymorphic sites (π = 0.0122) and 8 different haplotypes for *M. reidae*, in which the F_{st} = 0.2041 (P = 0.002) has been detected among different populations of this species from Quintana Roo, Arroyo Calakmul and Arroyo Aguada Grande (Table 3; Fig. 3). Twenty six polymorphic sites (π = 0.014) and 12 different haplotypes were obtained from *M. montezumae*. Fixation index is lower than other species (F_{st} = 0.042; P = 0.1). There is only a single COI sequence is available in this study from the *M. montezumae* population in Aguascalientes which limits our knowledge from this population, its genetic variabilities and distribution pattern compare to the other population from Mexico State; therefore further attempt is essential to sample this area in future.

3.2. Description

**Order** Calanoida Sars, 1903

Family Diaptomidae Baird, 1850

Genus *Mastigodiaptomus* Light, 1939

### *Mastigodiaptomus siankaanensis* **sp.n.**

Figs. 4–14

Material. *Holotype*: Adult ♀, dissected, mounted in 3 permanent slides sealed with paraffin (ECO-CH-Z-10101), Aguada Límite de la Reserva, Sian ka'an Biosphere Reserve, Quintana Roo, Mexico (19°42′32.6″N 87°49′40.1″W) coll. September 23, 2014 by Nancy F. Mercado-Salas. — *Allotype*: Adult ♂, dissected, mounted in 5 permanent slides sealed with paraffin (ECO-CH-Z-10102), same site, date and collector. — *Paratypes* (ECO-CH-Z-10103): 13 adult ♀♀ and 8 adult ♂♂ undissected, same locality and date of collection; 96% ethanol preserved. Additional paratypes were deposited at the Senckenberg am Meer Collection. — *Additional material*: see Table 1.
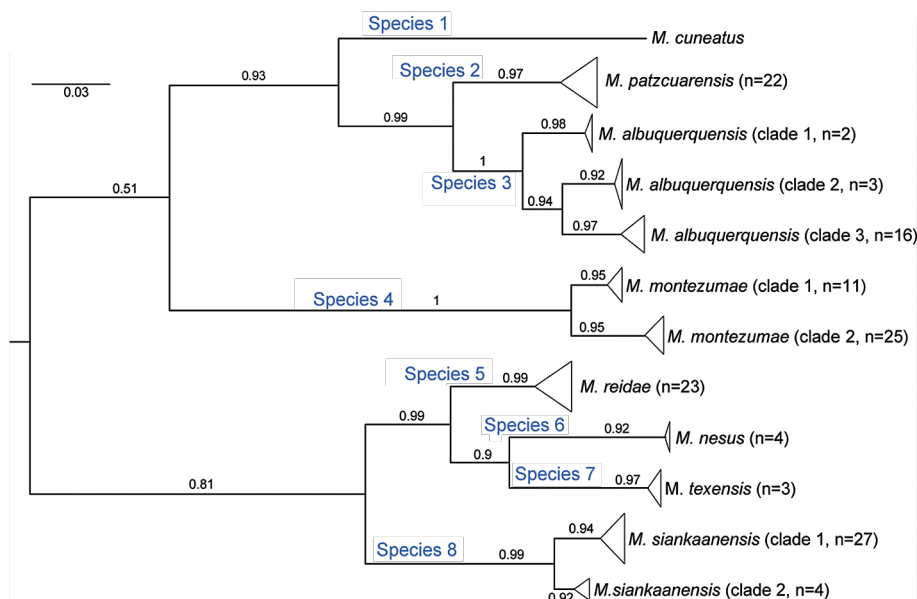
Fig. 2. Bayesian tree of mtDNA COI sequences for 118 specimens (8 species) of *Mastigodiaptomus*. Branches are collapsed to distinct clades. Values on branches are posterior probabilities. The enumeration of species (in blue) represents delimitations supported by General Mixed Yule Coalescent model (GMYC).
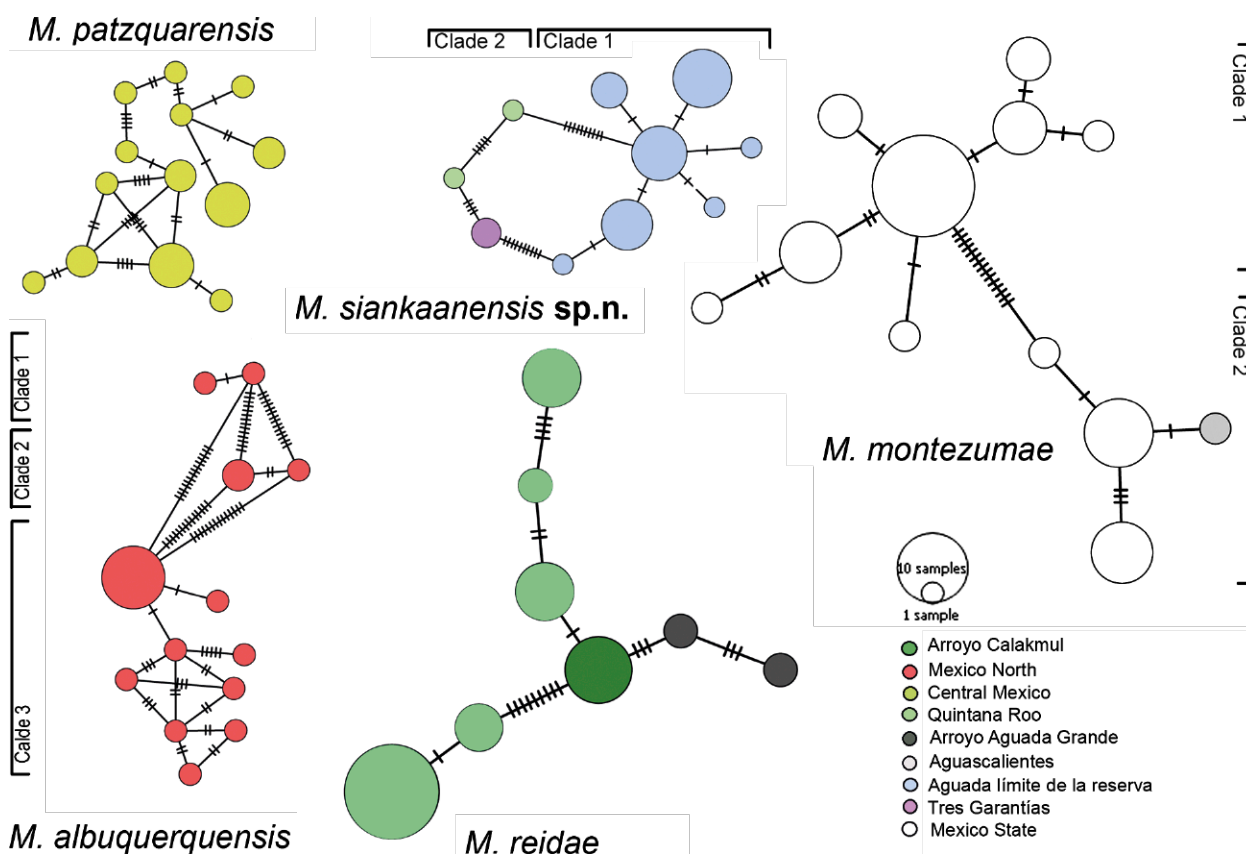
Fig. 3. Haplotype networks of *Mastigodiaptomus* species. Small lines on branches represent mutational steps. The size of circles is proportional to haplotype frequency. Color indicates haplotype location.

Type locality. Aguada Limite de la Reserva, Station 1, Sian ka'an Biosphere Reserve, Quintana Roo, Mexico (19°42′32.6″N 87°49′40.1″W).

Etymology. The name is after the Sian ka'an Biosphere Reserve in which the specimens were collected; Sian Ka'an in Mayan language means "heaven's door" or "place where heaven begins".

Description. *Female*: Total body length 1571 μm (x = 1596.84, n = 13) excluding furca, fifth pediger without dorsal process; Urs 22% of body length. Body symmetrical in dorsal view, prosome slightly wider at distal third (Figs. 4A, 10A). In lateral view (Fig. 4B) the body is arched downwards. Rostrum with long rostral points. Thoracic wings asymmetrical, left longer than right, both bearing 2 strong spinules. Urs 3-segmented;

Table 3. Population analyses based on statistical parsimony method, provided for the COI sequences of four different species of *Mastigodiaptomus*. **: Highly significant; *: Significant.

	No. of Polymorphic Sites	Nucleotide Diversity	No. of Haplotypes	Fixation Index
M. siankaanensis sp.n.	19	0.0032	10	0.801**
M. reidae	20	0.0122	8	0.204*
M. montezumae	26	0.014	12	0.042*
M. albuquerquensis	38	0.0073	13	0.846**
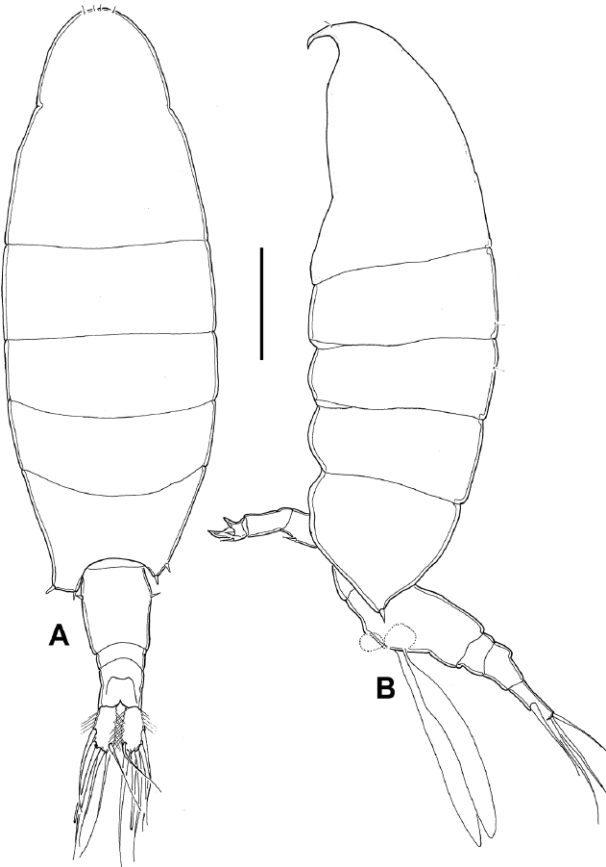
Fig. 4. *Mastigodiaptomus siankaanensis* sp.n. female, holotype. **A**: habitus, dorsal view. **B**: habitus, lateral view. Scale bar = 250 μm.
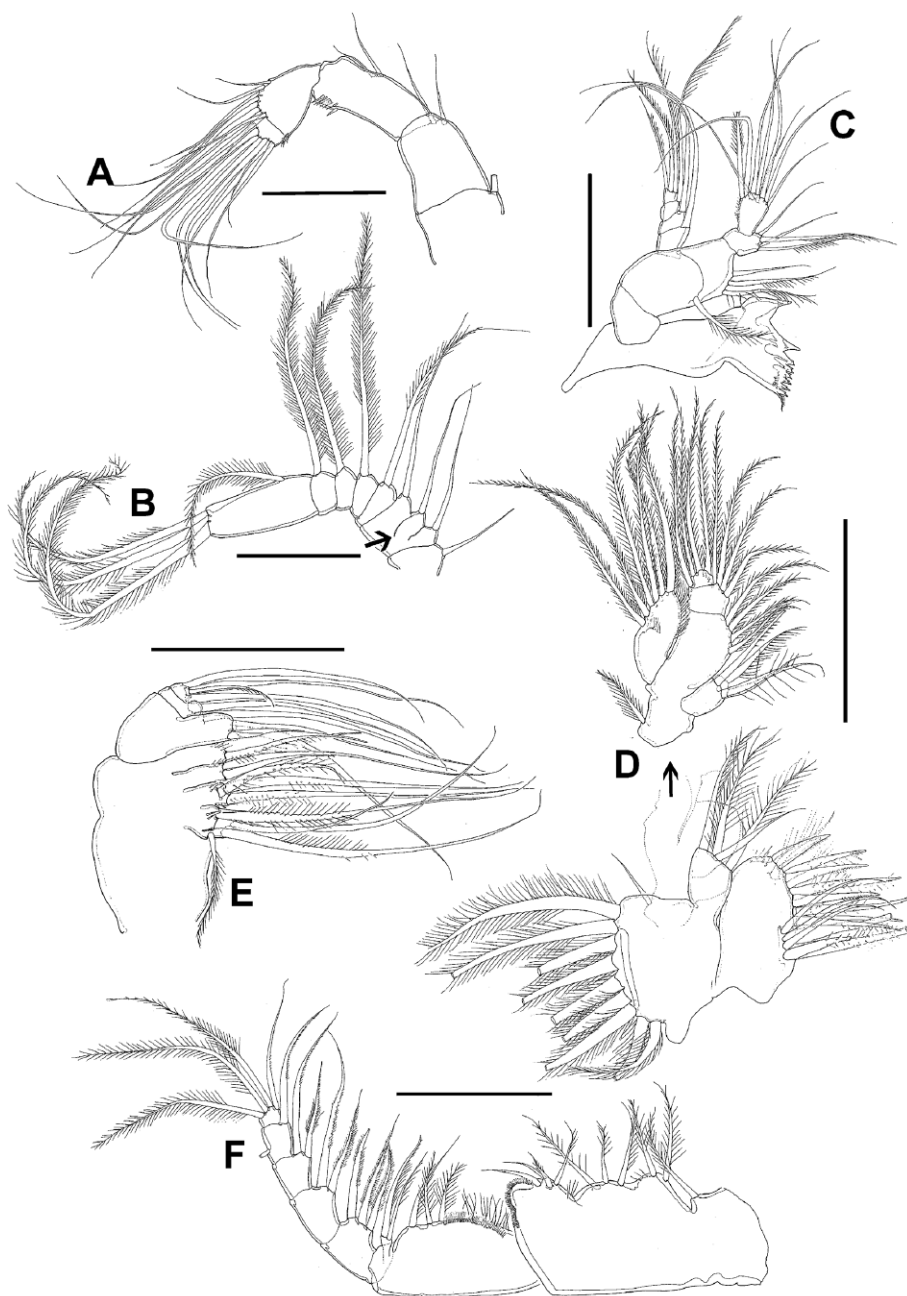
→ **Fig. 5.** *Mastigodiaptomus siankaanensis* sp.n. female, holotype. **A**: Urs, telson, furca, dorsal view. **B**: Urs, telson, lateral view. **C**: A1-right. Scale bars, A,B = 125 μm; C = 100 μm.

genital-double somite bearing 1 large spine on right margin, left margin with a slightly smaller spine (Figs. 5A, 12E). Genital urosome bearing a rounded protuberance on ventral surface (Fig. 5B). Telson about 3.0 × longer than preanal somite and with anal operculum rounded. Furca 2 × longer than wide, bearing hairs on outer and inner margins, caudal setae subequal in length and biserially plumose (Fig. 5A).

A1 (Figs. 5C, 11A,B): 25-segmented; tip of last segment exceeding total length of furca. Armament per segment: 1(1ms), 2(3ms + 1ae), 3(1ms), 4(1ms), 5(1ms + 1ae), 6(1ms), 7(1ms + 1ae), 8(1ms + 1sp), 9(2ms + 1ae), 10(1ms), 11(2ms), 12(1ms + 1sp + 1ae),13(1ms), 14(1ms + 1ae),
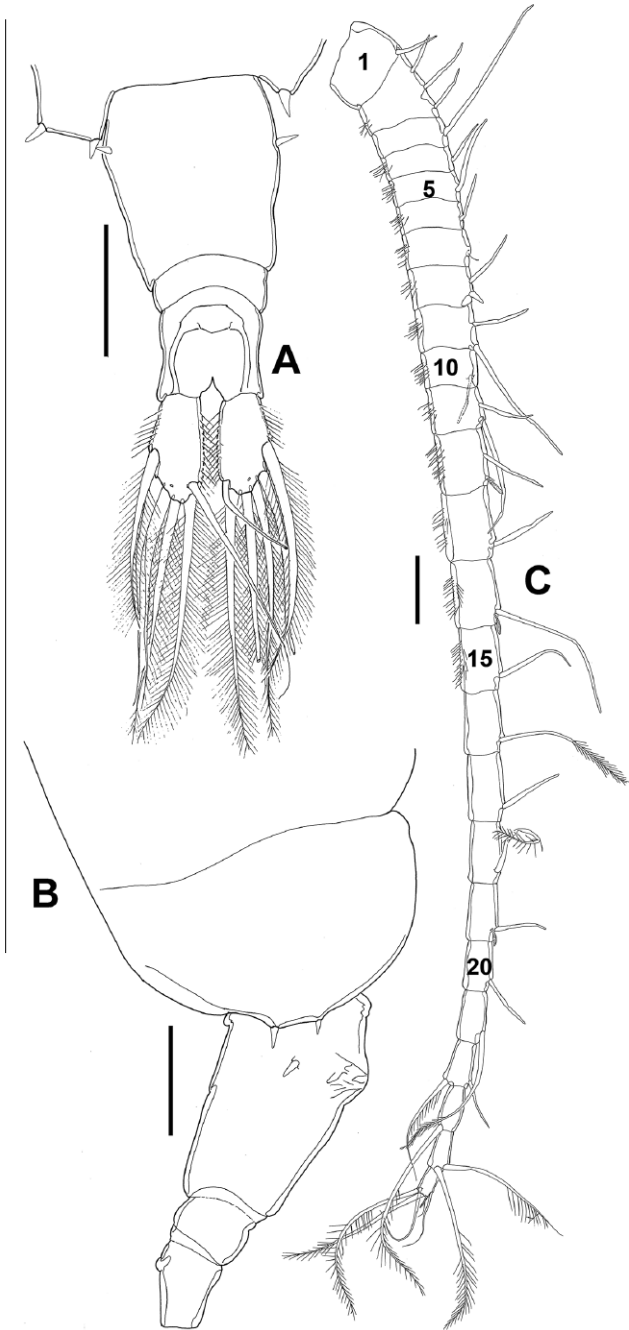
15(1ms), 16(1s + 1ae), 17(1ms), 18(1s), 19(1ms + 1ae), 20(1ms), 21(1s), 22(2s), 23(1s), 24(2s), 25(5s + 1ae). Presence of hairs on inner margin of segments 2, 4 – 15. A1 armament provided represent maximum setation found in holotype and paratypes, Fig. 5C illustrates holotype.

Fig. 6. *Mastigodiaptomus sianka-anensis* sp.n. female, holotype. **A**: A2, coxa, basis, enp. **B**: A2, exp. **C**: Md. **D**: Mx1. **E**: Mx2. **F**: Mxp. Scale bars = 100 μm.

A2 (Figs. 6A,B, 11C): Coxa with 1 strong long seta. Basis slightly elongated with 2 subequal setae on inner margin. 2-segmented enp, enp-1 bearing 2 subequal setae on inner margin and 1 row of strong spinules on outer margin; enp-2 with 2 lobes, outer lobe with tiny spinules on outer margin and bearing 7 setae, inner lobe with 9 setae. Exp 7-segmented, setation pattern: 1, 3, 1, 1, 1, 1, 4. Second segment with 2 pseudosegments (arrow in Fig. 6B).

Md (Figs. 6C, 11D): Gnathobase with 7 rounded teeth, innermost margin with 1 spinulose seta, outermost margin with 1 rounded lateral projection. Coxa bare, basis with 4 setae. Enp 2-segmented; enp-1 bearing 4 setae, enp-2 with row of small spinules on outer margin and with 9 setae on apical margin. 4-segmented exp; setation pattern: 1, 1, 1, 3.

Mx1 (Figs. 6D, 11E): Precoxal arhrite with 15 spini-form setae, coxal epipodite bearing 9 long setae; coxal endite quadrangular with 4 apical setae. Basis with 1 basal endite bearing 4 setae, internal lobe bearing 4 setae and basal exite represented by 1 seta. 2-segmented enp, enp-1 and enp-2 armed with 4 setae each. Exp 1-segmented, bearing 6 setae.

Mx2 (Figs. 6E, 11E): Praecoxa with 2 lobes, first lobe with 5 setae and bearing small basal spinules; second lobe bearing 3 setae and small basal spinules. Coxa with 2 endites both with basal spinules; first endite bearing 2 long setae and second with 3 long setae. Basis with well develop allobasis and 1 distal lobe. Allobasis bearing 4 long setae; distal lobe with 1 long seta. Enp 2-segmented; enp-1 with 1 long seta and enp-2 bearing 4 setae.

Mxp (Figs. 6F, 12A): Praecoxa and coxa fused in 1 long segment. Praecoxal endite bearing 1 seta; coxa with 3 coxal endites, first bearing 2 setae and small basal spinules, second lobe with 3 setae, and third with 4 setae. Coxal distal margin ornamented with small slender spinules. Basis proximal inner margin ornamented with row of small slender spinules and 3 setae on distal margin, distalmost seta longer than others. 6-segmented enp; setation pattern: 2, 3, 2, 2, 1+1, 4.

P1 (Figs. 7A): Coxa with plumose seta on inner margin, reaching proximal end of enp-1. Basis with group of long slender spinules on outer margin (arrow in Fig. 7A). Enp 2-segmented, enp-2 about 1.6 × longer than enp-1. Enp-1 bearing 1 inner seta, enp-2 with 3 inner, 2 apical and 1 outer setae, all plumose. Exp 3-segmented, segments progressively tapering; exp-1 with 1 inner seta and 1 small outer spine, exp-2 with 1 long inner seta and; exp-3 with 2 long inner setae, 3 apical setae (outermost with tiny spinules on outer margin and plumose on inner margin) and, 1 small outer spine.

P2 (Fig. 7B): Outer margin of basis, coxa and enp-1 ornamented with groups of tiny spinules (arrow in Fig. 7B). Coxa with plumose seta on inner margin, exceeding proximal end of enp-1. Enp 3-segmented, segments progressively tapering; enp-1 bearing 1 inner seta, enp-2 with 2 inner setae and, enp-3 with 3 inner, 2 apical and 2 outer setae, all homogeneously plumose, row of small spinules near insertion of 2 apical setae. Exp 3-segmented, exp-1 and exp-3 about the same length, exp-2 slightly shorter (about 0.77 × as long as the other segments). Exp-1 with small spinules on outer margin, with 1 outer spine and 1 inner seta; exp-2 bearing 1 outer spine and 1 long inner seta; exp-3 bearing 1 outer spine, 3 apical long setae (outermost seta with spinules on outer margin and plumose on inner margin) and, 3 long inner setae.

P3 (Fig. 7C): Coxa with plumose seta on inner margin, reaching proximal end of enp-1. Enp 3-segmented, segments progressively tapering; enp-1 bearing 1 inner seta, enp-2 with 2 inner setae and, enp-3 with 2 long inner, 3 long apical and 2 short outer setae, all homogeneously plumose. Exp 3-segmented, exp-1 and exp-3 about the same length, exp-2 slightly shorter (about 0.72 × as long as the other segments). Exp-1 with 1 outer spine and 1 inner seta, exp-2 bearing 1 outer spine and 1 long inner seta, exp-3 bearing 1 outer spine, 3 apical long setae (outermost seta with spinules on outer margin and plumose on inner margin) and, 3 long inner setae.

P4 (Figs. 7D, 12B): Coxa with plumose seta on inner margin, not reaching proximal end of enp-1, basis with small outer seta (arrowed in Fig. 7D). Enp 3-segmented, segments progressively tapering; enp-1 bearing 1 inner seta, enp-2 with 2 long inner setae and, enp-3 with 2 inner, 3 apical and 2 short outer setae, all setae homogeneously plumose. Exp 3-segmented, all about the same size.

P5 (Figs. 7E, 12C,D): Coxa symmetrical, bearing a conical seta on outer margin; basis triangular. 3-segmented exp: exp-1 elongate; exp-2 armed with a strong claw,

ornamented in both margins with strong spinules, outer margin with 1 small strong spine on distal margin (arrow in Fig. 7E); and exp-3 bearing 1 long strong seta and 1 short spine. Enp 2-segmented, total length of enp shorter than exp1; enp2 with 2 long setae plus row of hair-like setae on distal margin, enp1 nude.

Male: Total body length 1375 μm (x = 1387.25, n = 8) excluding furca. Body slender, cph wider at prosomal region in dorsal view (Figs. 13A,B). Complete suture between pedigerous somites 4–5. Left thoracic wing not projected, bearing 1 small spine; right thoracic wing slightly projected, with 1 ventral spine and 1 thin dorsal spine. Right margin of Urs-1 with 1 spine.

A1-right (Figs. 8A, 14B,C): 22 expressed segments, armament per segment: 1(1ms), 2(3ms+1ae), 3(1ms), 4(1ms), 5(1ms), 6(1ms), 7(1ms), 8(1ms+1sp), 9(2ms), 10(1ms+1s), 11(1ms+1sp), 12(1ms+1ae), 13(1s+1sp), 14(1s+1ms+1sp), 15(1s+1ms+1ae+1sp), 16(2s+1ae+1sp), 17(1s), 18(0), 19(1ms+1sp), 20(3s+1sp), 21(1s), 22(6s). Spinous processes on segments 11, 13, 14, 15 and 16 (arrow in Figs. 8B, 14C). Segment 20 with an acute long process distally, reaching proximal margin of segment 21(arrow in Figs. 8B, 13C). Segments 17 and 18 with hyaline process on dorsal margin (arrow in Fig. 14C).

A1-left (Figs. 8B, 14A): 25 expressed segments, armament per segment: 1(3s+1ae), 2(2ms+1ae), 3(1ms+1ae), 4(1ms), 5(1ms+1ae), 6(1ms), 7(1ms+1ae), 8(1ms+1s), 9(1ms+1ae), 10(1s), 11(2ms), 12(1ms+1sp+1ae), 13(1ms), 14(1ms+1ae), 15(1ms), 16(1s+1ae), 17(1s), 18(1s), 19(1ms+1ae), 20(1ms), 21(1s), 22(2s), 23(3s), 24(1s), 25(3s+1ae).

Mouthparts and P1–P4 as in females.

P5-right (Figs. 9E,G, 14D,E): Caudal side. Long distal seta on coxa (arrow in Fig. 9E). Enp longer than exp-1, enp represented by a single segment (Fig. 9G), bearing a row of slender spines on posterior edge. Exp-2 1.6 × longer than wide with one slightly curved hyaline membrane; lateral spine slightly curved, as long as total length of exp-2, bearing small spinules on inner margin. Terminal claw strong, curved and bearing small spinules on inner margin, about 2.8 × longer than exp-2.

P5-left (Figs. 9E,F, 14D,E): Reaching posterior margin of right exp-1. Long distal seta on coxa. Basis slightly elongated, with subterminal lateral seta, reaching distal end of segment. Enp unsegmented, exceeding medial margin of exp-1, and bearing a row of slender spines on posterior edge. Exp-1 longer than wide, 1.7 × longer than exp-2; exp-2 with 1 pad-like process ornamented with long slender setules (arrow in Fig. 9F), exp-2 tapering distally and with 3–4 short chitinous denticles.

Remarks. *Mastigodiaptomus siankaanensis* sp.n. is established within the genus *Mastigodiaptomus* because of the presence of the following characters: (1) A1 segment 11 of females and males bearing 2 setae; (2) coxa of female P5 with long, spatulated seta; (3) 1 outer spine on exp-1 P1 in both females and males; (4) male with P5 right enp longer than exp-1; and (5) male A1 with spiniform process on segments 10, 11, 13–16. Dorsal
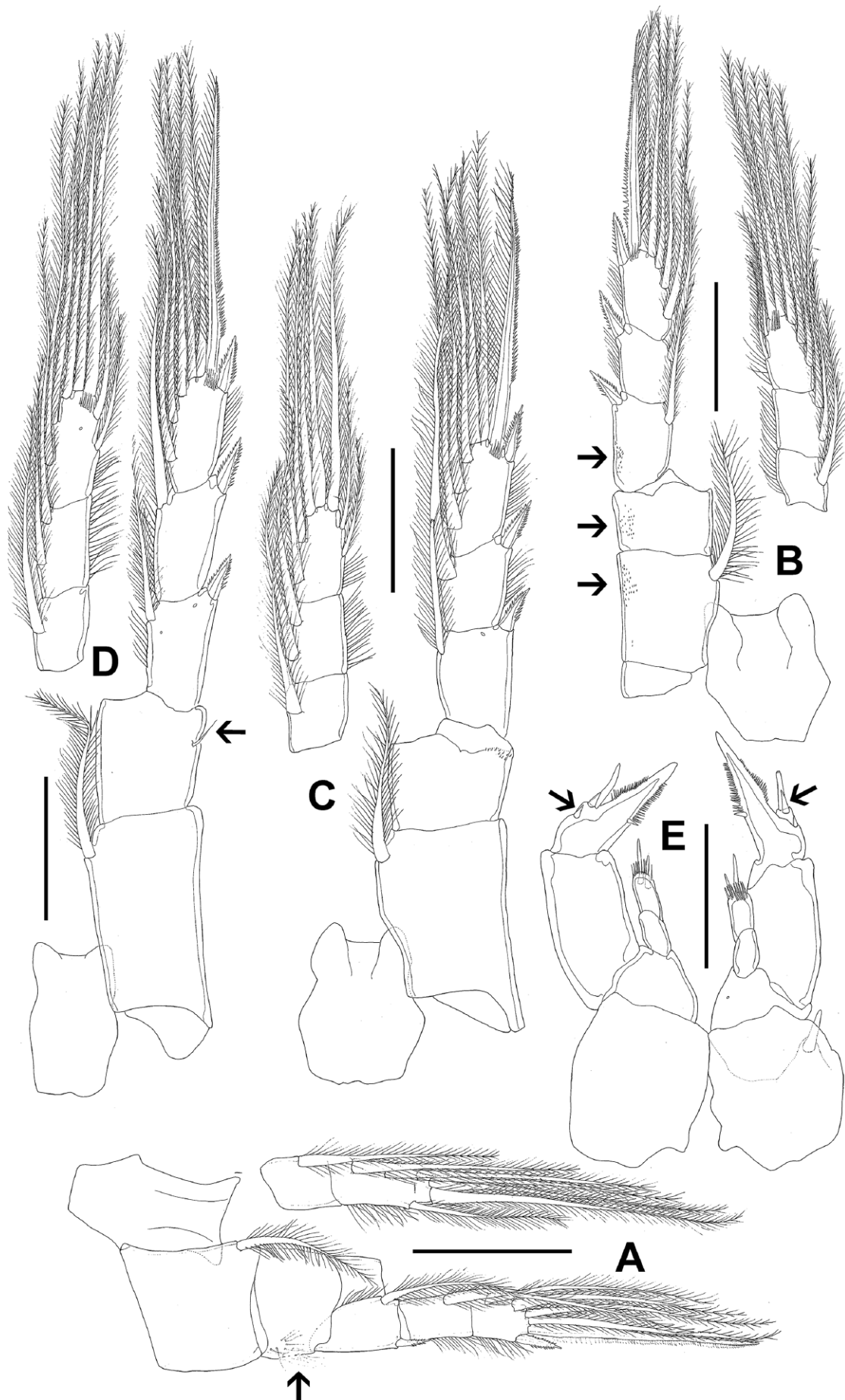
Fig. 7. *Mastigodiaptomus siankaanensis* sp.n. female, holotype. **A**: P1-right. **B**: P2-left. **C**: P3-right. **D**: P4-right. **E**: P5. Scale bars = 100 µm.
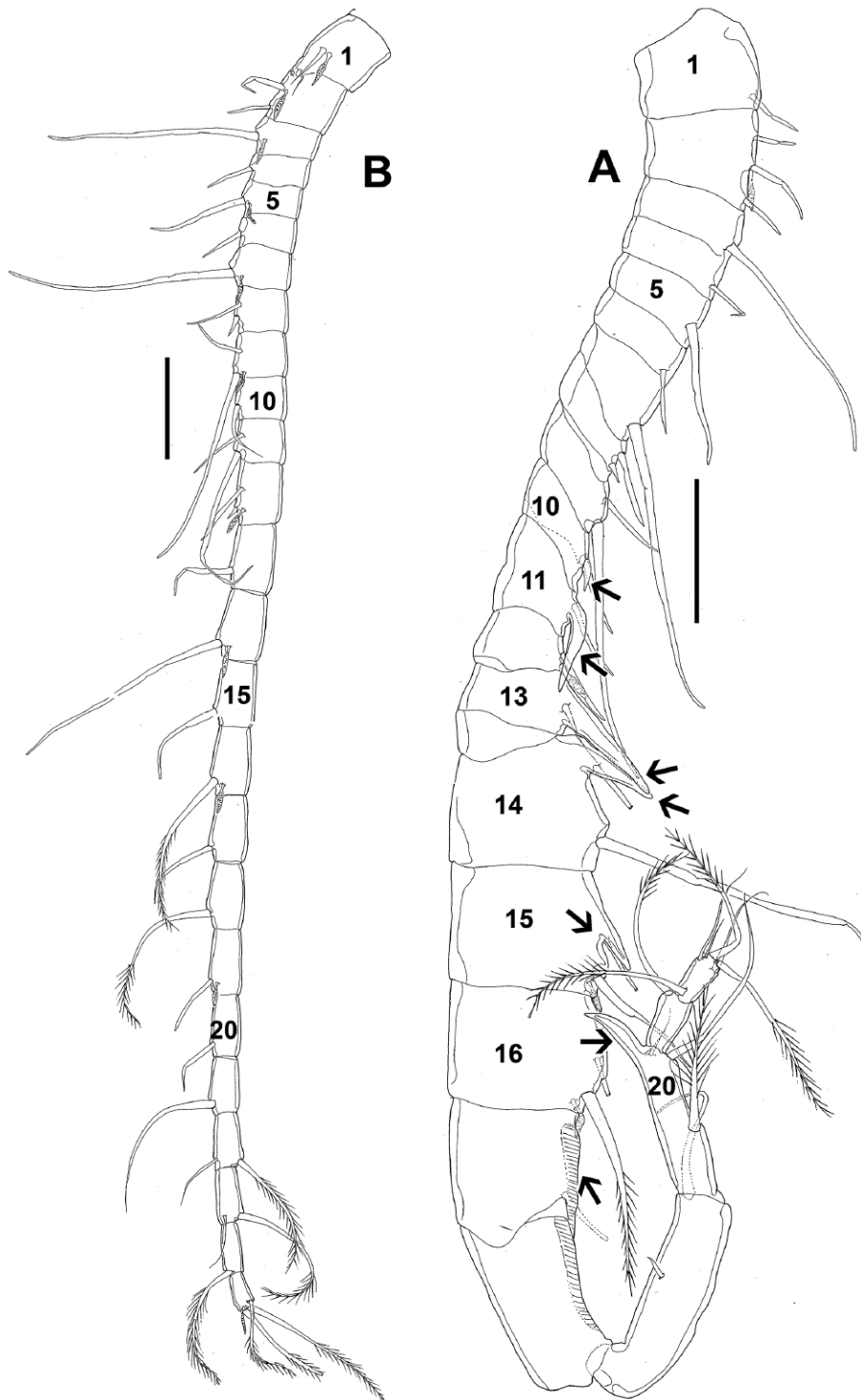
Fig. 8. *Mastigodiaptomus sian-kaanensis* sp.n. male, allotype. **A**: A1-right. **B**: A1-left. Scale bars = 100 μm.

process on the last thoracic segment absent in all specimens analyzed, this character was recognized as a typical morphological character for the genus (SUÁREZ-MORALES et al. 1996). Nevertheless recent studies have shown that its presence is variable in most of the species of *Mastigodiaptomus* with the exception of *M. purpureus* (where is always present), then it should not been used as a key character in the definition of the taxon (GUTIÉRREZ-AGU-IRRE & CERVANTES-MARTÍNEZ 2013).

The distinguishing characters of *M. siankaanensis* sp.n. include the presence of modified and vestigial setae on female A1; inner margin of segments 2, 4–15 in female A1 ornamented with fine hairs; P2 with coxa, basis and enp-1 ornamented with fine spinules; the location of aesthetascs in both right and left males A1; the absence of a spinular process on segment 10 of male right A1 (represented by a small but strong spine) and; the absence of hyaline process on male P5 basis. A detailed morphological comparison among *Mastigodiaptomus* known species is given in Table 4.

Table 4. Morphological comparison of *Mastigodiaptomus* species based on literature and surveyed material (REID 1996; SUÁREZ-MORALES & ELÍAS-GUTIÉRREZ 2000; SANTOS-SILVA 2008; GUTIÉRREZ-AGUIRRE & CERVANTES-MARTÍNEZ 2013; GUTIÉRREZ-AGUIRRE et al. 2014; GUTIÉRREZ-AGUIRRE & CERVANTES-MARTÍNEZ 2016). — **Abbreviations:** *M.s.* = *M. siankaanensis* sp.n., *M.c.* = *M. cuneatus*, *M.r.* = *M. reidae*, *M.m.* = *M. maya*, *M.a.* = *M. albuquerquensis*, *M.mo.* = *M. montezumae*, *M.n.* = *M. nesus*, *M.p.* = *M. patzcuarensis*, *M.t.* = *M. texensis*, *M.am.* = *M. amatitlaensis*, *M.su.* = *M. suarezmoralesi*, *M.pu.* = *M. purpureus*.

Character	M.s.	M.c.	M.r.	M.m.	M.a.	M.mo.	M.n.	M.p.	M.t.	M.am.	M.su.	M.pu.
♀ Total body length (µm)	1514–1680	1500–1700	1470–1600	2300–2470	1375–1825	1360–1720	1440–1540	925–1159	1500–1600	ND	1000–1070	2500
♀ Dorsal hump	Absent	Absent	Absent	Absent	Variable	Variable	Variable	Present	Variable	Variable	Absent	Present
♀ L/W genital-double somite	1.08–1.1	1.82	2.0	1.75	1.0	1.5	1.2	1.23	1.65	1.14	1.16–1.28	1.53
♀ Cph/Urs ornamention	Absent	Absent	Absent	Absent	Present	Absent	Absent	Present	Absent	ND	Absent	ND
♀ L/W Furca	2.0	1.6	1.0	1.0	2.3	2.0	2.0	1.3	1.2	ND	1.7	ND
♀ Hairs on Furca margin	Medial–lateral margin	Medial–lateral margin	Medial margin	Medial–lateral margin	Medial–lateral margin	Medial–lateral margin	Medial–lateral margin	Medial–lateral margin	Medial–lateral margin	Medial–lateral margin	Medial–lateral margin	Medial margin
♀ P5 L.exp-1/ enp	1.5	1.66	1.62	1.38	2.14	2.0	1.9	ND	1.11	2.18	1.12–1.2	1.17
♂ Total body length (µm)	1333–1417	1400–1500	1450–1620	2100–2220	1370–1820	1300–1520	1300–1440	920–1100	1500	ND	910–1000	ND
♂ Right A1, spinal process segment 10	Absent (bearing strong spine)	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
♂ Right A1, spinal process segment 16	Reduced	Reduced	Strongly developed	Reduced	Reduced	Absent	Reduced	Reduced	Reduced	Reduced	Reduced	Absent
♂ Right A1, spinal process segment 20	Acute and long	Acute and short	Acute and medium size	Short, wide knob-like process	Acute and medium size	Acute and short	ND	Acute and medium size	ND	ND	Acute and short	ND
♂ Right A1, aesthetasc	2, 12, 15, 16	1, 2, 3, 5, 7, 9, 12, 13, 14, 15, 16, 19, 22	1, 2, 3, 5, 9, 12	1, 5, 12	ND	1, 2, 3, 5, 7, 9, 12, 16, 22	ND	ND	ND	ND	1, 2, 3, 5, 7, 9, 12, 13, 14, 15, 16, 20, 22	ND
♂ Cph/Urs ornamention	Absent	Absent	Absent	Absent	Present	Absent	Absent	Present	Absent	ND	Present	ND
♂ P5 left enp	Bisegmented	Unsegmented	Unsegmented	Unsegmented	Unsegmented	Unsegmented	Bisegmented	Unsegmented	Unsegmented	Unsegmented	Bisegmented	Unsegmented

4. Discussion

The Neotropical genus *Mastigodiaptomus* has been recognized as the most diverse diaptomid genus in the Yucatan Peninsula with six of the twelve known species distributed in this geographic area (three being endemic) (SUÁREZ-MORALES et al. 2006). *Mastigodiaptomus siankaanensis* sp.n. is recorded after *M. albuquerquensis*, *M. texensis*, *M. nesus*, *M. reidae* and *M. maya*. The new species can be distinguished from its congeners by the combination of morphological characters but also represents a clearly defined species using mtDNA COI sequences. Within *M. siankaanensis* sp.n. 10 different haplotypes were detected, two distributed in close localities (sympatric distribution) and the third being consistent with allopatric distribution (Fig. 3). The first genotype is recorded from a single small pond (Tres Garantias) close to the border between Belize and Mexico; a second genotype inhabiting a single small pond (Kohunlich) around 30 km distance from the last mentioned locality and the third distributed in temporal and permanent water bodies in the Sian Ka'an Biosphere Reserve (about 185 km and 220 km distance from the former localities, respectively).

Closely related species of *Mastigodiaptomus* inhabiting a single small pond in the Yucatan Peninsula were previously recorded (ELÍAS-GUTIÉRREZ et al. 2008). Herein we included the COI mtDNA sequences presented by the above mentioned authors (ELÍAS-GUTIÉRREZ et al. 2008) confirming the presence of three genotypes, two of them blasting with *M. reidae* and the third clustering with *M. siankaanensis* sp.n. in the locality known as Kohunlich (Fig. 1). Tres Garantias also presented sympatric distributions with *M. reidae* and *M. maya*, all of them distributed in the southern part of the Yucatan Peninsula (emerged since the Paleocene). SUÁREZ-MORALES & ELÍAS-GUTIÉRREZ (2000) have suggested that *M. reidae* and *M. maya* (and now *M. siankaanensis* sp.n.) probably have speciated locally more by ecological strengths than as part of isolation process, however these species could also have speciated at different localities and then secondarily met in Kohunlich. Such process could be addressed in posterior works which include more genetic markers and molecular dating of the different linages. *Mastigodiaptomus reidae* is presented in this study by three
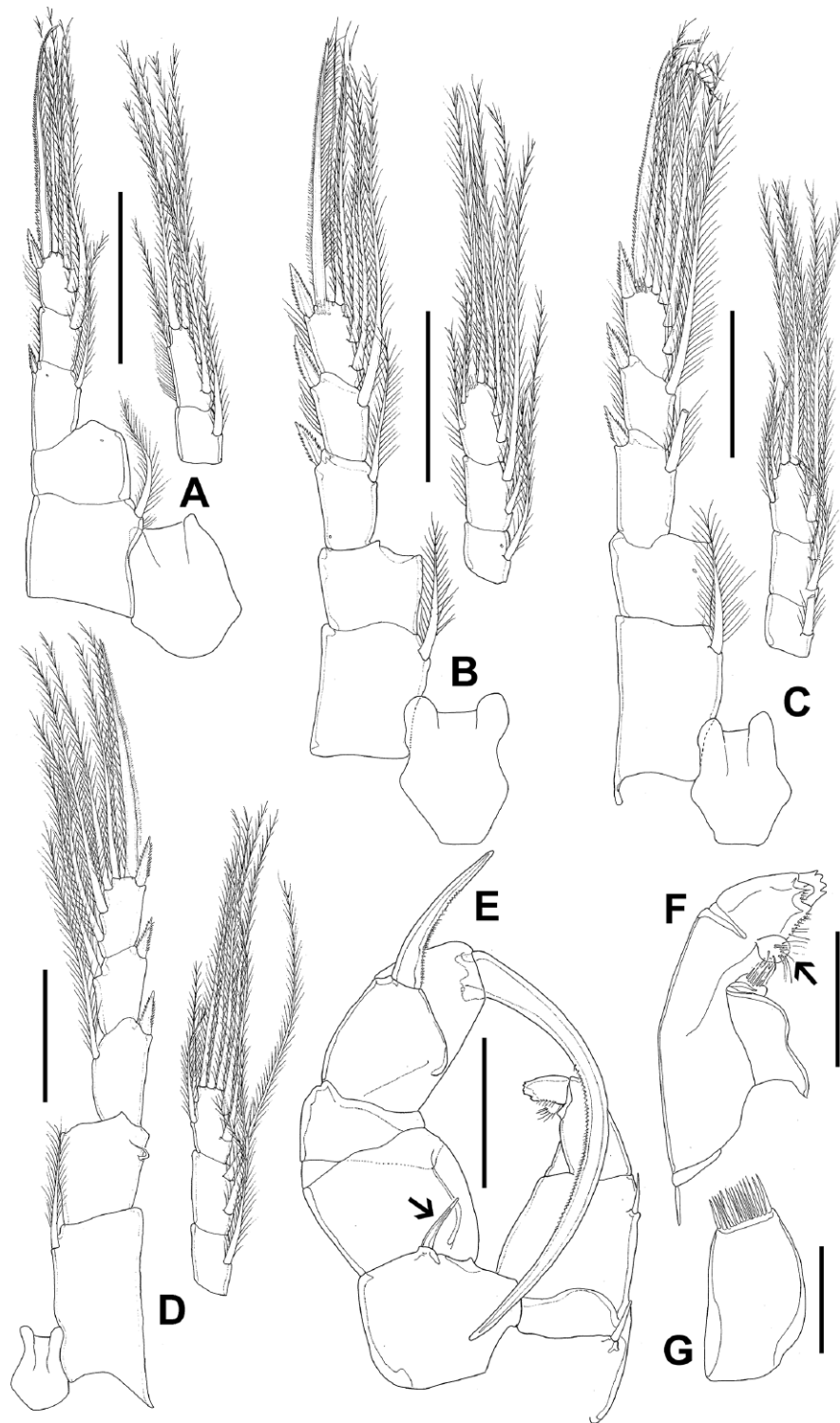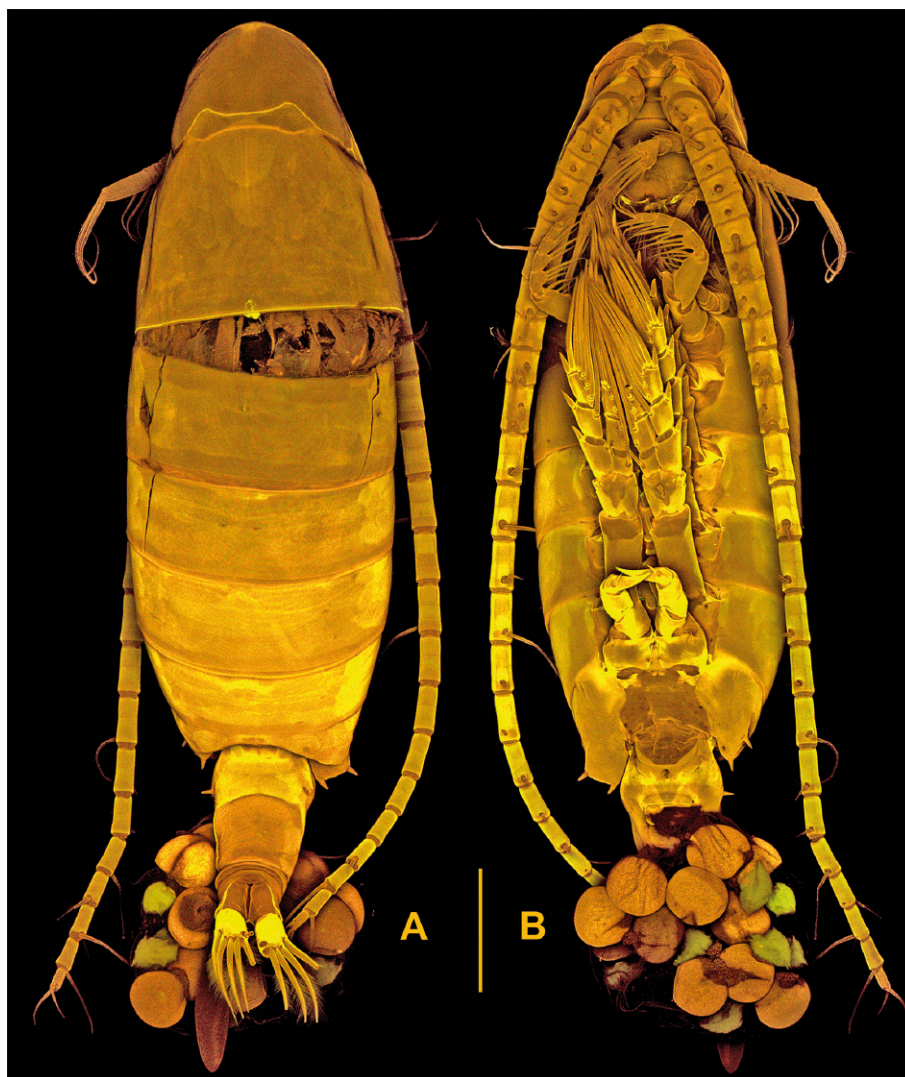
Fig. 9. *Mastigodiaptomus sianka-anensis* sp.n. male, allotype. **A**: P1-right. **B**: P2-right. **C**: P3-right. **D**: P4-left. **E**: P5-right. **F**: P5-left. **G**: P5-right enp. Scale bars, A–F = 100 μm, G = 25 μm.

populations from Quintana Roo, Arroyo Calakmul and Arroyo Aguada Grande with low inter population F_{st} index of 0.204 which confirms their sympatric distribution.

The third haplotype of *M. siankaanensis* sp.n. is distributed in a geologically younger area of the Peninsula where many stages of transgressions and regressions took place during Upper Oligocene, Middle and Upper Miocene and Lower Pliocene. COI mtDNA genetic divergence between three different populations of *M. siankaanensis* sp.n. investigated in this study (2.5–2.77% with high F_{st}

index of 0.801) showed the same pattern as inter-population average distance of lacustrine copepods from Mexico (Barrera-Moreno et al. 2015). Morphologically, the specimens of *M. siankaanensis* sp.n. from the south of Quintana Roo (Tres Garantias and Kohunlich) were similar to those from Sian ka'an Biosphere Reserve, with the exception of the lack of P2 ornamentation. The major difference found among the phenotypes was the size. Southern populations are smaller, with and average total length of 1283 ± 6 μm (n = 3) for females and 1050 ± 27 μm
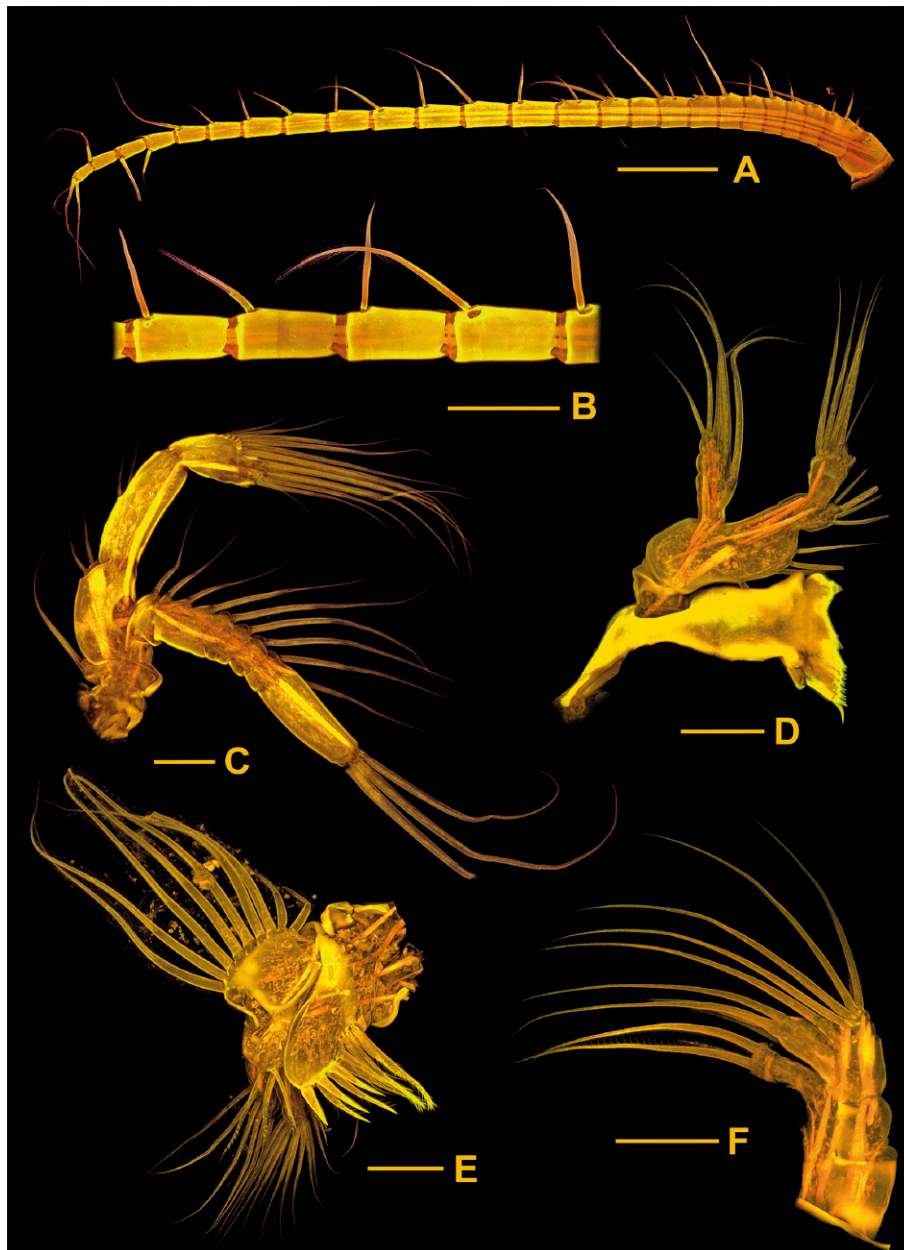
Fig. 10. *Mastigodiaptomus sian-kaanensis* sp.n. female, paratype (CLSM). **A**: habitus, dorsal view. **B**: habitus, ventral view. Scale bar = 200 μm.

(n = 4) for males in Tres Garantias. In Kohunlich they are intermediate in size (1545 and 1260 μm for females and males). These differences can be possibly explained on basis of predation and competence. Tres Garantias is a tropical lake where plenty of fish and invertebrate predators as rotifers, insect larvae and corixids live, whereas Kohunlich pond lacks major predators as fishes, its uniqueness could be associated to the coexistence with two species of the genus: *M. reidae* and another possibly undescribed species which we did not manage to collect for this study. In the latter case, *M. siankaanensis* sp.n. is the smallest one among the three. In congruence with the study of BARRERA-MORENO et al. (2015) about copepod population from neighbor lakes in Mexico, there is no shared COI haplotype from different population of *M. siankaanensis* sp.n. from this study, which further indicates the absence of gene flow and restricted dispersion in this area. The new species is closely related to *M. nesus*, *M. texensis* and *M. reidae* which are distributed in the Yucatan Peninsula. *Mastigodiaptomus nesus* was recorded in several localities near to the distributional area of *M. siankaanensis* sp.n. in the Sian ka'an Biosphere Reserve (especially in "cenotes" or sinkholes) and in the

northwestern area of Yucatan State (SUÁREZ-MORALES et al. 1996). It is important to highlight that the new species was only found in smaller water bodies such as temporal and permanent wetlands and aguadas; it was never found in the cenotes surveyed during this work, this could represent an ecological adaptation very specific for each of the two species. *Mastigodiaptomus siankaanensis* sp.n. can be morphologically distinguished from *M. nesus*, *M. texensis* and *M. reidae* because of the absence of the spinous process in segment 10 of male A1, in the new species this process is represented by a strong but small spine while in the other species the process is strongly developed. Another character that differentiates species is the absence of the hyaline process on male P5 basis in the new species and, a clearly sub-quadrangular process in *M. nesus*. Females of *M. siankaanensis* sp.n. also are easily separated from those of *M. nesus*, *M. texensis* and *M. reidae* by the possession of the A1 strongly ornamented on inner margin in segments 2, 4 – 15. The presence of aesthetascs, modified setae and vestigial setae in both females and males should be included in posterior studies since they could represent useful characters in the differentiation of species within the genus.

Fig. 11. *Mastigodiaptomus siankaanensis* sp.n. female, paratype (CLSM). **A**: A1-left. **B**: A1-left, detail. **C**: A2. **D**: Md. **E**: Mx1. **F**: Mx2. Scale bars, A=200 μm; B = 80 μm; C–F=50 μm.

The origin of the genus *Mastigodiaptomus* has been suggested in the upper Neotropical region as result of the diversification of Neartic diaptomids which dispersed and radiated in and after the Proto-Antilles (70 Mya) (Suárez-Morales & Reid 2003; Suárez-Morales 2003). This process could originate species with restricted distributions in the Caribbean, the Antilles and the Yucatan Peninsula as *M. reidae*, *M. maya*, *M. nesus*, *M. purpureus* and the herein described *M. siankaanensis* sp.n. The dry weather periods during the late Pleistocene and throughout the Holocene could have been an additional factor to limit the recent dispersal of the tropical *Mastigodiaptomus* and could cause the isolation especially of the third haplotype of *M. siankaanensis* sp.n. favoring its disjunctive distributional pattern. Records of *M. albuquerquensis* and *M. texensis* in the eastern coast of the Yucatan Peninsula (Tulum-Playa del Carmen) should be

re-analyzed with the new tools used in this work, in order to clarify their taxonomical identity.

The recently described *M. suarezmoralesi* from Chiapas (Usumacinta Province-Neotropical region) and *M. amatitlaensis* could represent a different radiation process that has been associated with the development of rivers and terraces in the Usumacinta basin during Pleistocene (Gutiérrez-Aguirre & Cervantes-Martínez 2013; Gutiérrez-Aguirre & Suárez-Morales 2001). Their distributional patterns and phylogenetic associations should be addressed by future studies that include new collecting campaigns in Chiapas and Guatemala. The presence of *M. albuquerquensis* (as a complex) and recently redefined *M. patzcuarensis* in Mexico from north to south supports the hypothesis of an, intense radiation of the genus from the Antilles and the Yucatan Peninsula (Suárez-Morales & Reid 1998). Unfortunately,

Fig. 12. *Mastigodiaptomus siankaanensis* sp.n. female, paratype (CLSM). **A**: Mxp. **B**: P4-left. **C**: P5, caudal view. **D**: P5, frontal view. **E**: genital-double somite, ventral view. Scale bars, A,C−D=50 μm; B=100 μm; E= 200 μm.

copepods hardly fossilize giving really few direct records of them, which obscures the historical biogeography and lineage divergence times for this group (Hołyńska et al. 2016), allowing only to conclude mostly based on the actual distribution of the species.

Presumed sister species which are morphologically similar and allopatrically or parapatrically distributed have lead several taxonomist and biogeographers to suggest the Pleistocene as an important time for diaptomid radiation (Gutiérrez-Aguirre & Cervantes-Martínez 2013; Gutiérrez-Aguirre & Suárez-Morales 2001; Stemberger 1995). However more recent studies have tested the hypotheses of Pleistocene divergence and speciation within the genus *Skistodiaptomus* Light, 1939 using mitochondrial (COI, cytochrome b, 16S) and nuclear (ITS) phylogenies and comparing them with molecular clock calibrations available for other crustaceans (Thum

& Harrison 2009). Among the main results, those authors found that DNA sequence divergences among the different species analyzed do not support the hypothesis of Pleistocene speciation inferred from the current parapatric distributions and morphological similarities. *Skistodiaptomus* sequence divergences suggested that speciation within the genus started much earlier, during the Miocene (10−20 Mya) and that Pleistocene play an important role in the divergence within different clades (haplotypes) of some species as *S. pallidus* (Herrick, 1879). This hypothesis seems to be suitable for some species of the genus *Mastigodiaptomus* (*M. maya*, *M. reidae* and *M. siankaanensis* sp.n.) which are distributed in the southern part of the Peninsula (emerged even earlier, since the Paleocene). Probably the third haplotype of *M. siankaanensis* sp.n. evolved later (after Miocene or during Pleistocene) as the inner clades of *Skistodiaptomus pallidus* suggested by
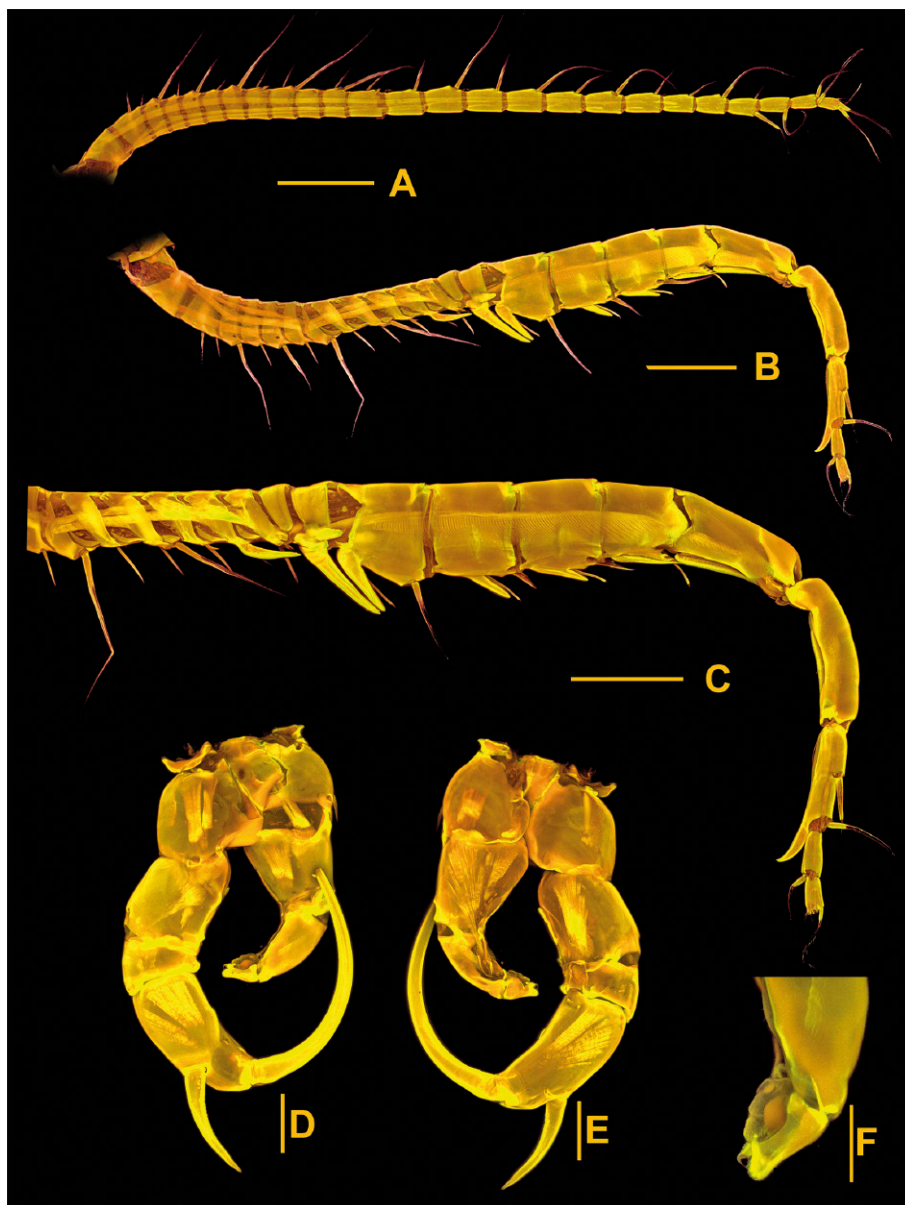
Fig. 13. *Mastigodiaptomus siankaanensis* sp.n. male, paratype (CLSM). **A**: habitus, dorsal view. **B**: habitus, ventral view. Scale bars = 200 μm.

THUM & HARRISON (2009). Genomic investigation in this genus and dating based in copepod material could test the validity of the above mentioned hypothetical distribution patterns, that in the present work are based only on the geographic distribution of the species.

Until recent years, *M. albuquerquensis* was considered the most widely distributed species of the genus ranging from the south of the United States to the Yucatan Peninsula in Mexico nevertheless deeper morphological examination combined with genetic approaches have shown that in fact it is a complex of species probably with restricted distributions (GUTIÉRREZ-AGUIRRE et al. 2014). Our results from GMYC delimitation method have confirmed a single species with three derived clades within this species together with *M. patzcuarensis* as a close separate species (Fig. 2), in congruent with previous study (GUTIÉRREZ-AGUIRRE et al. 2014) with re-

markably high between-species K2P COI divergence (5.21–9.68%). Clade 3 from *M. albuquerquensis* distributed in North of Mexico (Chihuahua and Durango States) and two additional lineages (clades 1 and 2), one distributed in Durango State (northwest side) and the other in Zacatecas State (central east side, more to the south). In the most recent publication about *Mastigodiaptomus* fauna, it was stated that specimens from Zacatecas (clade 2) exhibited morphological differences with respect to clade 3 assigned as *M. albuquerquensis* s.str. by GUTIÉRREZ-AGUIRRE et al. (2014). According to the GMYC result of this study, slightly high mean K2P distances between these two different clades (3.26–4.78%) can be interpreted as intra-species variation. Our result further showed their restricted distribution pattern, as different lineages of *M. albuquerquensis* are distributed in semi-desertic and desertic areas in the North of Mexico

Fig. 14 *Mastigodiaptomus sian-kaanensis* sp.n. male, paratype (CLSM). **A**: A1-left. **B**: A1-right. **C**: A1, detail segments 8–22. **D**: P5, caudal view. **E**: P5, frontal. **F**: P5-left, detail exp2. Scale bars, A,B = 150 μm; C = 100 μm; D,E = 50 μm; F = 25 μm.

and two of them shown sympatric distributions (ELÍAS-GUTIÉRREZ et al. 2008) which can be an indication of high inter population variabilities (F_{st} = 0.846; Table 3).

It was suggested that the closely related species *M. patzcuarensis* which includes records from Guanajuato, Mexico State, Michoacan and Puebla, represents two sibling species, one named as *M. patzcuarensis* and the other as *M.* cf. *albuquerquensis* (GUTIÉRREZ-AGUIRRE et al. 2014); however our result rejected this assumption according to the GMYC delimitation following relatively low COI genetic distances between above mentioned lineages of *M. patzcuarensis* (0–1.08%; not shown here). The COI mtDNA analyses retrieve another *Mastigodiaptomus* species from North Mexico, *M. cuneatus* (GUTIÉRREZ-AGUIRRE & CERVANTES-MARTÍNEZ 2016). The general distribution of *M. albuquerquensis*, *M. patzcuarensis* and *M. cuneatus* further supports allopatric distributions, the *M. albuquerquensis* and *M. cuneatus* including all records from north of Mexico (semi-desertic and

desertic areas) and the *M. patzcuarensis* associated to the Mexican Plateau (including temperate areas).

Another species apparently restricted to the central and north of Mexico is *M. montezumae*, described from Hidalgo State and later recorded in Mexico, Aguascalientes, Guanajuato, Durango and Sinaloa states. This species has been mostly co-existed with other diaptomids as *M. albuquerquensis*, *Leptodiaptomus novamexicanus* (Herrick, 1895) and *L. siciloides* (Lilljeborg in Guerne & Richard, 1889) (DOS SANTOS-SILVA et al. 1996). The mtDNA COI analyses revealed two clades within *M. montezumae* differed by the range of 2.77–3.49% genetic differences with sympatric distributions, coexisting even in the same locality in the central highland plateau (above 1500 m a.s.l.) (Mexico State). Morphological differences between these two clades have not been reported so far and our study further support a single species according to GMYC (Fig. 2). Population structure of this species cannot be discussed here due to only a single haplotype

was available from Aguascalientes State in GenBank (Fig. 3). *Mastigodiaptomus montezumae* is clearly differentiated from its congeners by distinct short projection on segment 20 of male A1, which is long and acute in most of the species and wide knob-like in *M. maya*, and the mammiform projection ending in a blunt spine on P5 right basipod. The recently described *M. cuneatus* morphologically seems to be related with *M. amatitlaensis*, nevertheless these species are distributed in completely different geographic areas, the first in Northern Mexico and the second in Guatemala (GUTIÉRREZ-AGUIRRE & CERVANTES-MARTÍNEZ 2016). Genetically, *M. cuneatus* is closest to *M. albuquerquensis* and *M. patzcuarensis*, however genetic associations are hard to stablish because of the lack of sequences of *M. cuneatus* (only one sequence available), and should be addressed in posterior works.The distribution of microcrustaceans in freshwater ecosystems was explained, until recent years, by the "Cosmopolitanism Paradigm", were scarce morphological differentiation among conspecific populations and the high capacities for passive dispersal allowed them to colonize new areas, keeping an extensive gene flow across their distributional ranges (MARRONE et al. 2013). However, this idea has been challenged by the "Monopolization Hypothesis", where founder effect, rapid local adaptation and resilience against newcomers are combined, restricting the gene flow and restricting the species to smaller areas with specific adaptations (DE MEESTER et al. 2002). The "Monopolization Hypothesis" provided the theoretical basis to explain the distribution and high genetic differences in presumed conspecific freshwater diaptomids of the genus *Occidodiaptomus* Borutsky in Borutsky, Stepanova & Kos, 1991 (MARRONE et al. 2013) and fits to the distribution of the *Mastigodiaptomus* species, where the species radiation seems to be more related to local adaptations than to extreme dispersal capacities.

Mastigodiaptomus is a genus that probably originated in the Neotropics (upper Neotropics) and dispersed and radiated in and after the Proto-Antilles to the Yucatan Peninsula. This hypothesis and the Miocene or Pleistocene radiation of the genus should be further investigated under genomic approaches that will allow us to know the origin and the phylogenetic relationships of the different species within the genus.

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6. References

ALEKSEEV V., DUMONT H.J., PENSAERT J., BARIBWEGURE D., VANFLETEREN J.R. 2006. A redescription of *Eucyclops serrulatus* (Fischer, 1851) (Crustacea: Copepoda: Cyclopoida) and some related taxa, with a phylogeny of the *E. serrulatus*-group. – Zoologica Scripta **35**: 123–147.

ALTEKAR G., DWARKADAS S., HUELSENBECK J.P., RONQUIST F. 2004. Parallel metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. – Journal of Bioinformatics **20**(3): 407–415.

ALTSCHUL S.F., GISH W., MILLER W., MYERS E.W., LIPMAN D.J. 1990. Basic local alignment search tool. – Journal of Molecular Biology **215**: 403–410.

BAIRD W. 1850. The Natural History of the British Entomostraca: I–VIII. – The Ray Society, London.

BANDELT H., FORSTER P., RÖHL A. 1999. Median-joining networks for inferring intraspecific phylogenies. – Molecular Biology and Evolution **16**(1): 37–48.

BARRERA-MORENO O.A., CIROS-PEREZ J., ORTEGA-MAYAGOITIA E., ALCANTARA-RODRIGUEZ J.A., PIEDRA-IBARRA E. 2015. From local adaptation to ecological speciation in copepod populations from Neighbor lakes. – PLoS One doi.org/10.1371/journal.pone.0125524.

BLANCO-BERCIAL L., CORNILS A., COPLEY N., BUCKLIN A. 2014. DNA barcoding of marine copepods: Assessment of analytical approaches to species identification. – PLoS Currents doi:10.1371/currents.tol.cdf8b74881f87e3b01d56b43791626d2.

BORUTSKY E.V., STEPANOVA L.A., KOS M.S. 1991. Opredelitel' Calanoida presnykh vod SSSR. A Handbook of Calanoida from the Freshwaters of the USSR. – Opredeliteli po faune SSSR, Zoological Institute SSSR, Nauka, Leningrad.

BOWMAN T.E. 1986. Freshwater calanoid copepods of the West Indies. In: SCHRIEVER G., SCHMINKE H.K., SHIH C. (eds), Proceedings of the Second International Conference on Copepoda (Ottawa, Canada, 13–17 August, 1984). – Syllogeus **58**: 237–246.

BOXSHALL G.A., DEFAYE D. 2008. Global diversity of copepods (Crustacea:Copepoda) in freshwaters. – Hydrobiologia **595**: 195–207.

BOXSHALL G.A., JAUME D. 2000. Making waves: the repeated colonization of fresh water copepod crustaceans. – Advances in Ecological Research **31**: 61–79.

BRANDORFF G.O. 2012. Distribution of some Calanoida (Crustacea: Copepoda) from the Yucatan Peninsula, Belize and Guatemala. – Revista Biologia Tropical **60**(1): 187–202.

BREHM V. 1933. *Argyrodiaptomus granulosus* nov. spec., ein neuer Diaptomus aus Uruguai. – Zoologischer Anzeiger **103**: 283–287.

BREHM V. 1955. Mexicanische Entomostraken. – Österreichische Zoologische Zeitschrift **6**: 412–420.

BRON J.E., FRISCH D., GOETZE E., JOHNSON S., LEE C.E., WYNGAARD G.A. 2011. Observing copepods through a genomic lens. – Frontiers in Zoology **8**: 22.

BUCKLIN A., ORTMAN B.D., JENNINGS R.M., NIGRO L.M., SWEETMAN C.J., COPLEY N.J., SUTTON T., WIEBE P.H. 2010. A "Rosetta Stone" for metazoan zooplankton: DNAbarcode analysis of species diversity of the Sargasso Sea (Northwest AtlanticOcean). – Deep-Sea Research II **57**: 2234–2247.

CONANP (Comisión Nacional de Áreas Naturales Protegidas, Mexico) 2016 [on line]. http://www.conanp.gob.mx [Consulted: 12 June 2017].

DE MEESTER L., GÓMEZ A., OKAMURA B. SCHWENK K. 2003. The Monopolization Hypothesis and the dispersal-gene flow paradox in aquatic organisms. – Acta Oecologica **23**(3): 121–135.

DODSON S.I., GRISHANIN A.K., GROSS K., WYNGAARD G.A. 2003. Morphological analysis of some cryptic species in the *Acanthocyclops vernalis* species complex from North America. – Hydrobiologia **500**: 131–143.

DOS SANTOS-SILVA E.N., ELÍAS-GUTIÉRREZ M., SILVA-BRIANO M. 1996. Redescription and distribution of *Mastigodiaptomus montezumae* (Copepoda, Calanoida, Diaptomidae) in Mexico. – Hydrobiologia **328**(3): 207–213.

DRUMMOND A.J., RAMBAUT A., SUCHARD M.A. 2016. BEAST: Bayesian evolutionary analysis sampling trees. – Evolutionary Biology **7**: 8.

ELÍAS-GUTIÉRREZ M., MARTÍNEZ-JERÓNIMO F., IVANOVA N.V., VALDEZ-MORENO M., HEBERT P.D.N. 2008. DNA barcodes for Cladocera and Copepoda from Mexico and Guatemala, highlights and new discoveries. – Zootaxa **1839**: 1–42.

ELÍAS-GUTIÉRREZ M., SUÁREZ-MORALES E., GUTIÉRREZ-AGUIRRE M.A., SIVA-BRIANO M., GRANADOS-RAMÍREZ J.G., GARFIAS-ESPEJO T. 2008b. Cladocera y copepoda de las aguas continentales de Mexico. Mexico City. – UNAM-FESI, CONABIO, ECOSUR, CONACYT, SEMARNAT.

ESTOUP A., LARGIADER C.R., PERROT E., CHOURROUT D. 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. – Molecular Marine Biology and Biotechnology **5**(4): 295–298.

FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. – Molecular Marine Biology and Biotechnology **3**: 294–299.

GARCÍA-GIL G., PALACIO-PRIETO J., ORTIZ-PÉREZ M. 2002. Reconocimiento geomorfológico e hidrográfico de la Reserva de la Biósfera Calakmul, México. – Investigaciones Geográficas (UNAM-México) **48**: 7–23.

GUTIÉRREZ-AGUIRRE M.A., CERVANTES-MARTÍNEZ A. 2016. A new species of *Mastigodiaptomus* Light, 1939 from Mexico, with notes of species diversity of the genus (Copepoda, Calanoida, Diaptomidae). – ZooKeys **637**: 61–79.

GUTIÉRREZ-AGUIRRE M.A., CERVANTES-MARTÍNEZ A. 2013. Diversity of freshwater copepods (Maxillopoda: Copepoda: Calanoida, Cyclopoida) from Chiapas, Mexico with a description of *Mastigodiaptomus suarezmoralesi* sp.n. – Journal of Natural History **45**: 5–12.

GUTIÉRREZ-AGUIRRE M.A., SUÁREZ-MORALES E. 2001. Diversity and distribution of freshwater copepods (Crustacea) in Southeastern Mexico. – Biodiversity Conservation **10**: 659–672.

GUTIÉRREZ-AGUIRRE M.A., CERVANTES-MARTÍNEZ A. ELÍAS-GUTIÉRREZ M. 2014. An example of how barcodes can clarify cryptic species: the case of the calanoid copepod *Mastigodiaptomus albuquerquensis* (Herrick). – PLoS One doi:10.1371/journal.pone.0085019.

HERRICK C.L. 1895 *Diaptomus albuquerquensis*. Pp. 67–68 in: Synopsis of the Entomostraca of Minnesota: With Descriptions of Related Species Comprising All Known Forms from the United States Included in the Orders Copepoda, Cladocera, Ostracoda. – Pioneer Press Company.

HOŁYŃSKA M., LEGGITT L., KOTOV A.A. 2016. Miocene cyclopid copepod from a saline paleolake in Mojave, California. – Acta Paleontologica Polonica **61**: 345–361.

HUYS R., BOXSHALL G.A. 1991. Copepod Evolution. – London, the Ray Society.

KARANOVIC T., KRAJICEK M. 2012. First molecular data on the Western Australian *Dicyclops* (Copepoda, Cyclopoida) confirm morpho-species but question size differentiation and monophyly of the *alticola*-group. – Crustaceana **85**(12–13): 1549–1569.

KATOH K., TOH H. 2008. Recent developments in the MAFFT multiple sequence alignment program. – Briefings in Bioinformatics **9**: 286–298.

KIEFER F. 1938. Ruderfubkrebse (Crust. Cop.) aus Mexico. – Zoologischer Anzeiger **115**: 274–279.

KIEFER F. 1936. Uber die Systematik der südamerikanischen Diaptomiden (Crustacea, Copepoda). – American Zoologist **116**: 194–200.

KUMAR S., STECHER G., TAMURA K. 2015. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. – Molecular Biology and Evolution **33**(7): 1870–1874.

LEE C.E. 2000. Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations". – International Journal of Organic Evolution **54**(6): 2014–2027.

LIGHT S.F. 1939. New American subgenera of *Diaptomus* Westwood (Copepoda, Calanoida). – Transactions of the American Microscopical Society **58**: 473–848.

MARRONE F., LO BRUTTO S., HUNDSDOERFER A.K., ARCULEO M. 2013. Overlooked cryptic endemism in copepods: Systematics and natural history of the calanoid subgenus *Occidodiaptomus* Borutzky 1991 (Copepoda, Calanoida, Diaptomidae). – Molecular Phylogenetics and Evolution **66**: 190–202.

MARSH C.D. 1907. A revision of the North American species of *Diaptomus*. – Transactions of the Wisconsin Academy of Sciences, Arts and Letters **15**(2): 381–516.

MICHELS J., BÜNTZOW M. 2010. Assessment of Congo red as a fluorescence marker for the exoskeleton of small crustaceans and the cuticle of polychaetes. – Journal of Microscopy **238**: 95–101.

MONAGHAN M.T., WILD R., ELLIOT M. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. – Systematic Biology **58**: 298–311.

MONCHENKO V.I. 2000. Cryptic species in *Diacyclops bicuspidatus* (Copepoda: Cyclopoida): evidence from crossbreeding studies. – Hydrobiologia **417**: 101–107.

PERBICHE-NEVES G., BOXSHALL G.A., PAGGI J.C., DA ROCHA C.E.F., PREVIATTELLI D., NOGUEIRA M.G. 2013. Two new species of Diaptomidae (Crustacea: Copepoda: Calanoida) from the Neotropical Region (Parana River). – Journal of Natural History **47**(5–12): 449–477.

PESCE G.L. 1996. Towards a revision of the Cyclopinae copepods (Crustacea, Cyclopidae). – Fragmenta Entomologica **28**(2): 189–200.

PONS J., BARRACLOUGH T.G., GOMEZ-ZURITA J. 2006. Sequence based species delimitation for the DNA taxonomy of undescribed insects. – Systematic Biology **55**: 595–609.

REID J.W. *Mastigodiaptomus purpureus*. The IUCN Red List of Threatened Species 1996 [online] http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T12862A3391555.en. [Consulted: 10 May 2017].

REID J.W. 1997. *Argyrodiaptomus nhumirim*, a new species, and *Austrinodiaptomus kleerekoperi*, a new genus and species, with description of *Argyrodiaptomus macrochaetus* Brehm, new rank, from Brazil (Crustacea: Copepoda: Diaptomidae). – Proceedings of the Biological Society of Washington **110**: 581–600.

RONQUIST F., HUELSENBECK J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. – Journal of Bioinformatics **19**(12): 1572–1574.

SANTOS-SILVA E.N. 2008. Calanoid of the families Diaptomidae, Pseudodiaptomidae, and Centropagidae from Brasil. – Biologia Gerale Experimental **8**: 3–67.

SARS G.O. 1903. An account of the Crustacea of Norway, with short descriptions and figures of all the species: IV. Copepoda Calanoida. – Bergens Museum, Bergen.

SCHMINKE H.K. 1976. The ubiquitous telson and deceptive furca. – Crustaceana **30**: 292–300.

Stememberg R.S. 1995. Pleistocene refuge áreas and postglacial dispersal of copepods of the northeastern United States. – Canadian Journal of Fisheries and Aquatic Sciences **52**: 2197–2210.

Suárez-Morales E. 2003. Historical biogeography and distribution of the freshwater calanoid copepods (Crustacea: Copepoda) of the Yucatan Peninsula, Mexico. − Journal of Biogeography **30**: 1851–1859.

Suárez-Morales E., Elías-Gutiérrez M. 2000. Two new *Mastigodiaptomus* (Copepoda, Diaptomidae) from southeastern Mexico, with a key for the identification of the known species of the genus. − Journal of Natural History **34**: 693–708.

Suárez-Morales E., Reid J.W. 2003. An updated checklist of the continental copepod fauna of the Yucatan Peninsula, Mexico, with notes on its regional associations. − Crustaceana **76**: 977–991.

Suárez-Morales E., Reid J.W. 1998. An updated list of free-living freshwater copepods (Crustacea) of Mexico. − The Southwestern Naturalist Journal **43**: 256–265.

Suárez-Morales E., Reid J.W., Elías-Gutiérrez M. 2005. Diversity and distributional patterns of Neotropical freshwater copepods (Calanoida: Diaptomidae). − International Review of Hydrobiology **90**: 71–83.

Suárez-Morales E., Reid J.W., Iliffe T.M., Fiers F. 1996. Catálogo de los Copépodos (Crustacea) continentales de la península de Yucatán, México. – Mexico City: ECOSUR-CONABIO.

Thum R.A., Harrison R.G. 2009. Deep genetic divergences among morphologically similar and parapatric *Skistodiaptomus* (Copepoda:Calanoida:Diaptomidae) challenge the hypothesis of Pleistocene speciation. – Biological Journal of the Linnean Society **96**: 150–156.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. − Genetics **123**(3): 585–595.

Westwood J.O. 1836. *Cyclops*. In: Partington C.F. (ed.), The British Encyclopedia of Natural History, London **2**: 227–228.

Wilson M.S. 1941. New species and distribution records of diaptomid copepods from the Marsh collection in the United States National Museum. − Journal of the Washington Academy of Sciences **31**: 509–515.

Wilson M.S. 1953. New and inadequately known North American species of the copepod genus *Diaptomus*. − Smithsonian Miscellaneous Collections **122**(2): 1–30.

Wyngaard G.A., Hołyńska M., Schulte J.A. 2009. Phylogeny of the freshwater copepod *Mesocyclops* (Crustacea: Cyclopidae) based on combined molecular and morphological data, with notes on biogeography. − Molecular Phylogenetics and Evolution **55**: 753–764.

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Mastigodiaptomus **Light, 1939:** http://zoobank.org/urn:lsid:zoobank.org:act:D068D1C5-AD71-47E7-B1EA-62313E718819

Mastigodiaptomus siankaanensis **Mercado-Salas, 2017:** http://zoobank.org/urn:lsid:zoobank.org:act:20AECE0F-1641-47EA-AEBE-8B2AACE42E7F

Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics

File 1: mercado&al-mastigodiaptomus-asp2018-electronicsupplement-1.xls — GenBank accession numbers of complementary material downloaded from NCBI platform.

File 2: mercado&al-mastigodiaptomus-asp2018-electronicsupplement-2.pdf — COI phylogram of *Mastigodiaptomus* species, 118 specimens (sequenced in this study and downloaded from GenBank) based on Bayesian analyses. Nodal supports indicate Posterior probabilities. Colors show the different localities following the haplotype network in figure 3.