Revista mexicana de biodiversidad
ISSN: 1870-3453
ISSN: 2007-8706
Instituto de Biología

Lagunas-Calvo, Omar; López-Jiménez, Serapio; Oceguera-Figueroa, Alejandro

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Revista mexicana de biodiversidad, vol. 92, e923656, 2021
Instituto de Biología

DOI: https://doi.org/10.22201/ib.20078706e.2021.92.3656

Available in: https://www.redalyc.org/articulo.oa?id=42571635071

Revista Mexicana de Biodiversidad

Revista Mexicana de Biodiversidad 92 (2021): e923656

Taxonomy and systematics

Phylogenetic position of *Dolops bidentata* (Ichthyostraca: Argulidae) based on molecular data: first record of the genus in Mexico

Posición filogenética de Dolops bidentata *(Ichthyostraca: Argulidae) con base en datos moleculares: primer registro del género en México*

Omar Lagunas-Calvo [a, b], Serapio López-Jiménez [c], Alejandro Oceguera-Figueroa [b, *]

[a] *Universidad Nacional Autónoma de México, Posgrado en Ciencias Biológicas, Instituto de Biología, Avenida Universidad 3000, Ciudad Universitaria, 04510 Ciudad de México, Mexico*

[b] *Universidad Nacional Autónoma de México, Instituto de Biología, Departamento de Zoología, Laboratorio de Helmintología, Apartado postal 70-153, 04510 Ciudad de México, Mexico*

[c] *Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Agropecuarias, Carretera Villahermosa-Teapa Km. 25+2, Ranchería la Huasteca 2da. Sección, 86298 Villahermosa Centro, Tabasco, Mexico*

*Corresponding author: aoceguera@ib.unam.mx (A. Oceguera-Figueroa)

Received: 20 July 2020; accepted: 1 March 2021

Abstract

Species included in the genus *Dolops* (Ichthyostraca: Argulidae) have been recorded from Southern and Central Africa, Australia, and mainly from South America, with no records from Central or North America. Specimens of *Dolops bidentata*, previously recorded only in South America, were collected in the state of Tabasco, southern Mexico, parasitizing the common snook, *Centropomus undecimalis*. Here, we provide the first record of the genus and the species in North America (Mexico) and the first molecular characterization of *Dolops bidentata* including 1 mitochondrial and 2 nuclear DNA markers, as well as a morphological description of the specimens. The newly generated molecular data were used to preliminarily investigate the phylogenetic relationships of Branchiura and to include *Dolops bidentata* in a phylogenetic hypothesis. Our results fail to recover the monophyly of *Dolops*; however, more investigations are needed before any taxonomic change is made.

Keywords: Parasitic crustaceans; Branchiura; *Argulus*; *Centropomus undecimalis*; COI; 18S rRNA; 28S rRNA

Resumen

Las especies incluidas en el género *Dolops* (Ichthyostraca: Argulidae) han sido registradas en el sur y centro de África, Australia y principalmente en América del Sur, sin registros de América Central o del Norte. Ejemplares de *Dolops bidentata*, previamente registrados solo en América del Sur, se recolectaron en el estado de Tabasco, al sur de México, parasitando al róbalo *Centropomus undecimalis*. En el presente trabajo proporcionamos el primer

ISSN versión electrónica: 2007-8706; Universidad Nacional Autónoma de México, Instituto de Biología. https://doi.org/10.22201/ib.20078706e.2021.92.3656

registro del género y de la especie en América del Norte (México) y proporcionamos la primera caracterización molecular de *Dolops bidentata* que incluye un marcador de ADN mitocondrial y 2 nucleares, así como una descripción morfológica de los especímenes. La información molecular recién generada se utilizó para investigar, preliminarmente, las relaciones filogenéticas de Branchiura y para ubicar a *Dolops bidentata* en una hipótesis filogenética. Nuestros resultados no logran recuperar la monofilia de *Dolops*, sin embargo, se necesitan más investigaciones antes de realizar cualquier cambio taxonómico.

Palabras clave: Crustáceos parásitos; Branchiura; *Argulus*; *Centropomus undecimalis*; COI; 18S rARN; 28S rARN

Introduction

Branchiura Thorell, 1864, commonly known as fish lice, is a group of ectoparasitic crustaceans mainly associated with freshwater and marine fishes, and less frequently amphibians (Poly, 2008). This group has a worldwide distribution, with the highest species diversity in freshwater habitats in the Afrotropical and Neotropical regions (Fryer, 1964, 1968). Currently, about 140 species arranged in 4 genera are included in the group: *Argulus* Müller, 1785; *Chonopeltis* Thiele, 1900; *Dipteropeltis* Calman, 1912, and *Dolops* Audouin, 1837 (Neethling & Avenant-Oldewage, 2016). *Argulus* has a cosmopolitan distribution and includes around 121 species, *Chonopeltis* has an Afrotropical distribution and includes 13 species, *Dipteropeltis* is restricted to the Neotropics and includes only 2 species, and *Dolops* has a Gondwanan distribution and 13 species, 11 of them restricted to the Neotropics (Fryer, 1968; Lagunas-Calvo et al., 2020; Neethling & Avenant-Oldewage, 2016; Poly, 2008).

Species included in *Dolops* have been recorded from Southern and Central Africa, Australia, and mainly South America (see Neethling and Avenant-Oldewage [2016] for host and locality records). To date, *Dolops geayi* (Bouvier, 1897), parasite of *Andinoacara pulcheri* (Gill, 1858), *Crenicichla geayi* Pellegrin, 1903, and *Hoplias malabaricus* (Bloch, 1794) from Lake Valencia, Venezuela, represents the northern record in America (Pearse, 1920).

Recently, fish lice specimens assigned to the genus *Dolops* were collected in Tabasco, southern Mexico, representing the first record for the genus in Mexico and North America. We provide the morphological description of the specimens and furthermore, we generated partial DNA sequences of both mitochondrial and nuclear genes, to characterize the species for the first time on a molecular basis and to preliminarily investigate the phylogenetic relationships of the group.

Materials and methods

In October 2019, freshwater and estuarine fish specimens of *Centropomus undecimalis* (Bloch, 1792), *Atractosteus tropicus* (Gill, 1863), *Ictalurus meridionalis* (Günther, 1864), and *Oreochromis* sp., captured by local fishermen at "El Chichicastle", San Antonio, Pantanos de Centla, Tabasco (18°14'36.2" N, 92°18'06.5" W), were examined for ectoparasites, with special attention to the skin and gills. Fish lice specimens recovered were fixed in alcohol 80% and transported to the laboratory for further studies.

For morphological studies and measurements, 3 specimens were mounted in temporal slides and observed under a microscope. Microphotographs were taken with a Leica camera (model DFC490) attached to a Leica Z16 APO-A microscope at the Laboratorio Nacional de la Biodiversidad (LANABIO), Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM). For scanning electron microscopy (SEM) procedures, 1 specimen was dehydrated in a gradual series of ethanol from 80% to absolute, CO_2 dried, covered with a Gold-Palladium mixture and observed in a Hitachi SUI510 microscope at LANABIO. A single fish lice specimen was deposited at Colección Nacional de Crustáceos (CNCR-35725), IBUNAM. Taxonomic identification was accomplished following Ringuelet (1948) and Bouvier (1899a, b).

Total DNA extraction of a single specimen was carried out using Chelex 100 (Bio-Rad) following the protocol provided by the manufacturer. Polymerase chain reactions (PCR) used 9.5 µl of DNase-free H_2O, 3 µl of 5× Reaction Buffer, 0.2 µl of each primer, 0.1 µl of My Taq DNA polymerase (Bioline catalogue number BIO-21105), and 2 µl of DNA template (total volume 15 µl) for each sample. For PCR reactions with primer cocktail, the amount of DNase-free H_2O was adjusted to reach a total volume of 15 µl. Mitochondrial cytochrome c oxidase subunit I (COI) DNA sequence was obtained using a primer cocktail with forward NemF1_t1+NemF2_t1+ NemF3_t1 and reverse NemR1_t1+NemR2_t1+ NemR3_t1; including 0.2 µl of each primer. PCR conditions were 94 °C for 2 min, 5 cycles at 94 °C for 40 sec, 45 °C for 40 sec, 72 °C for 1 min, followed by 35 cycles at 94 °C for 40 sec, 51 °C for 40 sec, 72 °C for 1 min, and a final extension at 72 °C for 7 min (modified from Prosser et al. [2013]). For the

nuclear 18S ribosomal RNA (18S rRNA) PCR reactions, the primer cocktail included 1f+18SA2+5R+9R. PCR reactions were performed with initial denaturation at 95 ºC for 2 min followed by 35 cycles with 94 ºC for 1 min, 56 ºC for 45 sec, and 72 ºC for 45 sec, with an additional 10 min extension at 72 ºC. For the nuclear 28S ribosomal RNA (28S rRNA) amplification the primers 28sA+28SBout were used. PCR was performed with initial denaturation at 94 ºC for 5 min, followed by 35 cycles of 95 ºC for 1 min, 52 ºC for 1 min, 70 ºC for 1 min, and final extension at 72 ºC for 7 min (modified from Phillips et al. [2010]). Primer names and sequences are listed in Table 1. Amplification products were purified using CentriSep (Thermo Fisher Scientific) 96 filter plates with Sephadex G-50. Sequencing reactions used 0.4 µl BigDye Terminator ver. 3.1 (Applied Biosystems), 2 µl Buffer 5x, 4 µl ddH2O, 1 µl of primer at 10 µM, and 3 µl purified PCR product (total volume 10 µl). Primers M13F and M13R were used for sequencing the COI fragment while 18SintL and 18SintR were used for 18S. Samples were purified using Sephadex G-50, then 25 µl of 0.5 mM EDTA was added to each sample and finally sequenced in an ABI-PRISM 3100 Applied Biosystems® sequencer at LANABIO. Sequences were reconciled and edited in Geneious ver. 5.1.7 (Biomatters Ltd. Auckland, New Zealand).

The newly generated sequences were compared with the existing sequences available in the non-redundant database of the National Center for Biotechnology Information (NCBI; http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the nucleotide Basic Local Alignment Search Tool (BLASTn; Altschul et al., 1990) to in order to discard the amplification of host DNA.

DNA sequences available in GenBank were selected for the phylogenetic analyses based on BLASTn results and on published literature. Two pentastomids: *Levisunguis subaequalis* Curran, Overstreet, Collins and Benz, 2014 and *Sebekia purdieae* Riley, Spratt and Winch, 1990, were selected as the outgroup based on a previous phylogenetic hypothesis (Zrzavý et al., 1997; Table 2).

The newly generated sequences were aligned together with sequences obtained from GenBank using MAFFT version 7 implemented in the web-version at https://mafft.cbrc.jp/alignment/server/ using the default parameters (Kato et al., 2019). Three matrices were assembled, 1 for each molecular marker. For the COI locus, the final matrix included 17 terminals and 675 aligned characters; this matrix was translated into amino acids using Mesquite ver. 3.61 (Maddison & Maddison, 2007) and included 224 positions; the 18S matrix included 13 terminals and 2085 aligned characters; finally, the 28S dataset included 8 terminals and 821 aligned characters. Phylogenetic analyses were performed for the COI matrix including all characters, then excluding all third positions, and finally, for the amino acid dataset in order to evaluate the occurrence of potential artifacts caused by saturation. Alternatively, based on the results of the analysis of the complete dataset and given an anomalous behavior detected in the grouping of *Dolops ranarum* and *Argulus monodi*, 2 phylogenetic analyses were conducted removing alternatively each of these taxa (Siddall & Whiting, 1999). Analyses of a concatenated dataset is precluded given the lack of common information for some terminals, for example, only outgroup taxa and *D. bidentata* (obtained in this study) have all 3 genetic markers available. In contrast, *A. americanus*, *A. japonicus*, and *A. siamensis* have no information in common.

Phylogenetic analyses were accomplished under the maximum likelihood (ML) criterion in the software RAxMLGUI ver. 2 (Silvestro & Michalak, 2011). For each DNA matrix, the GTR+GAMMA model was implemented as identified by jModelTest ver. 2 (Darriba et al., 2012) and for the analysis of the amino acid dataset, the BLOSUM62 model was implemented as identified by PartitionFinder (Lanfear et al., 2012). Maximum likelihood analyses consisted in 100 replicates using the ML + thorough bootstrap option. Bootstrap branch support values were estimated running 1,000 pseudoreplicates in the same software. The resulting tree was visualized with the software FigTree ver. 1.4.2 (Rambaut, 2012).

Results

In total, 9 specimens of *C. undecimalis*, 3 *A. tropicus*, 6 *I. meridionalis*, and 14 *Oreochromis* sp. collected in Pantanos de Centla, Tabasco, were examined. Only *C. undecimalis* resulted positive for ectoparasites. In total 3 specimens were recovered, 1 male and 2 females. Ectoparasites were found near to the operculum of their host.

Class Ichthyostraca Zrzavý, Hypša and Vlášková, 1997
Subclass Branchiura Thorell, 1864
Order Arguloida Yamaguti, 1963
Family Argulidae Leach, 1819
Dolops Audouin, 1837
Dolops bidentata (Bouvier, 1899a) Bouvier, 1899b
Gyropeltis bidentata Bouvier, 1899a: p. 40; Bouvier, 1899b: p. 63, figs. 2-5; Wilson, 1902: p. 736: plate XXVII: fig. 88; Malta, 1982: p. 524: figs. 4-5; 1984: p. 356; Silva-Souza et al., 2011: p. 147: figs. 1-3; Fontana et al., 2012: p. 655.

Table 1
List of primers used to obtain the DNA sequences for *Dolops bidentata*.

Gene	Primer	Primer sequence	Reference
Mitochondrial			
COI			
	NemF1_t1	5' tgtaaaacgacggccagtCRACWGTWAATCAYAARAATATTGG 3'	Prosser et al. (2013)
	NemF2_t1	5' tgtaaaacgacggccagtARAGATCTAATCATAAAGATATYGG 3'	Prosser et al. (2013)
	NemF3_t1	5' tgtaaaacgacggccagtARAGTTCTAATCATAARGATATTGG 3'	Prosser et al. (2013)
	NemR1_t1	5' caggaaacagctatgactAAACTTCWGGRTGACCAAAAAATCA 3'	Prosser et al. (2013)
	NemR2_t1	5' caggaaacagctatgactAWACYTCWGGRTGMCCAAAAAAYCA 3'	Prosser et al. (2013)
	NemR3_t1	5'caggaaacagctatgactAAACCTCWGGATGACCAAAAAATCA3'	Prosser et al. (2013)
	M13F	5' TGTAAAACGACGGCCAGT 3'	Messing (1993)
	M13R	5' CAGGAAACAGCTATGAC 3'	Messing (1993)
Nuclear			
18S rDNA			
	1f	5' TACCTGGTTGATCCTGCCAGTAG 3'	Møller et al. (2008)
	18SA2	5' ATGGTTGCAAAGCTGAAAC 3'	Tautz et al. (1988)
	5R	5' CTTGGCAAATGCTTTCGC 3'	Møller et al. (2008)
	9R	5' GATCCTTCCGCAGGTTCACCTAC 3'	Møller et al. (2008)
	18SintR	5' GCG GTT AAA AAG CTC GTAG 3'	Møller et al. (2008)
	18SintL	5' TGCAACCATACTTCCCCCGG 3'	Møller et al. (2008)
28S rDNA			
	28sA	5'GACCCGTCTTGAAACACGGA3'	Whiting (2002)
	28SBout	5'CCCACAGCGCCAGTTCTGCTTACC3'	Hovmöller et al. (2002)

Table 2
Parasite taxa, GenBank accession number and hosts, localities, and references included in phylogenetic analyses presented herein.

Parasite species	GenBank accession number			Host (Family)	Locality	References
	COI	18S rRNA	28S rRNA			
Pentastomida						
Levisunguis subaequalis	MN062095	MN065568	MN065508	*Gambusia affinis* (Poeciliidae)	Birmingham, Alabama, USA	Woodyard et al. (2019)
Sebekia purdieae	KU975386	KU975377	KU975381	*Lates calcarifer* (Latidae)	Cleveland Bay, Queensland, Australia	Barton and Morgan (2016)
Branchiura						
Argulus americanus	AY456187	----	----	Not given	Not given	Lavrov et al. (2004)
	----	----	MN688128	*Acipenser oxyrinchus* (Acipenseridae)	Pascagoula River, Mississippi, USA	Andres et al. (2019)
Argulus bicolor	----	----	MN688129	*Acipenser oxyrinchus* (Acipenseridae)	Pascagoula River, Mississippi, USA	Andres et al. (2019)

Table 2. Continued

Parasite species	GenBank accession number			Host (Family)	Locality	References
	COI	18S rRNA	28S rRNA			
Argulus bengalensis	----	KM016968	----	*Labeo rohita* (Cyprinidae)	Bongaon, West Bengal, India	Patra et al. (2016)
	----	KM016969	----		Naihati, West Bengal, India	
	----	KF583878	----		Chakgaria, West Bengal, India	
Argulus flavescens	----	----	MN688125	*Acipenser oxyrinchus* (Acipenseridae)	Pascagoula River, Mississippi, USA	Andres et al. (2019)
Argulus foliaceus	KF713319	----	----	*Carassius auratus* (Cyprinidae)	India	Feroz-Khan et al. (2014)
	----	KF747861	----	Not given	Not given	Direct Submission
Argulus indicus	KF713306	----	----	*Carassius auratus* (Cyprinidae)	India	Feroz-Khan et al. (2014)
	KF723417	----	----	Not given	Not given	Direct Submission
Argulus japonicus	KF713304	----	----	*Carassius auratus* (Cyprinidae)	India	Feroz-Khan et al. (2014)
	KF713314	----	----			
	KF713321	----	----			
	GU937865	----	----	Not given	Rivers, Lattakia, Syria	Wadeh et al. (2010)
	GU937867	----	----	Not given	Rivers, Guangzhou, China	Wadeh et al. (2010)
	GU937869	----	----	Not given	Rivers, Cairo, Egypt	Wadeh et al. (2010)
	----	KF747860	----	Not given	Not given	Direct Submission
	----	----	KF747847	Not given	Not given	Direct Submission
Argulus nobilis	----	M27187	----	*Lepisosteus osseus* (Lepisosteidae)	North America	Abele et al. (1989)
Argulus monody	----	DQ813452	----	*Heterobrachus longifilis* (Clariidae)	Lake Victoria, Tanzania	Mwita and Nkwengulila (2010)
Argulus rhipidiophorus	----	KF747862	----	Not given	Not given	Direct Submission
Argulus siamensis	KF713308	----	----	*Carassius auratus* (Cyprinidae)	India	Feroz-Khan et al. (2014)
	KF713315	----	----			
	KF713318	----	----			
	----	KF583879	----	*Cyprinus carpio* (Cyprinidae)	Chakgaria, West Bengal, India	Patra et al. (2016)
Dolops bidentata	MT582371	MT583738	MT663304	*Centropomus undecimalis* (Centropomidae)	Pantanos de Centla, Tabasco, Mexico	Present study
Dolops ranarum	----	DQ813453	----	*Heterobrachus longifilis* (Clariidae)	Lake Victoria, Tanzania	Mwita and Nkwengulila (2010)
Dolops sp.	DQ889096	----	----	Not given	Not given	Costa et al. (2007)

Description. Specimens are small (3.2-4.1mm length) and dorsoventrally flattened; color white-yellow. Nauplius and compound eyes present, located on dorsal surface. Carapace rounded with lateral lobes reaching the 4th pair of swimming legs. Lobes separated by a rounded sinus at the level of the 1st and 2nd legs. (Fig. 1A). Dorsal surface covered by numerous conspicuous dendriform spots, most of them brown and purple, few spots yellow (Fig. 1A). In ventral view, conspicuous dendriform spots distributed on the margins of the carapace, legs, and on natatory and abdominal lobes (Fig. 1B). Marginal spines over the entire carapace edge; extending from the anterior tip to the posterior end of the respiratory areas (Fig. 1C). Oval antero-lateral depressions, well defined (Fig. 1D). Antennules with thick rounded base and distal claws stout and tipped. Antenna thin, divided into 4 segments, with a group of bristles at the base and a group of apical spines on the terminal segment; without ornamentation at middle segments (Fig. 1D). Mouth without pre-oral sting. Suction disks absent (Fig. 1C, D). First maxillae with 2 distal claws, proximal claw smaller than the terminal (Fig. 1E). Second maxillae divided into 6 segments; first segment with 2 teeth, second segment triangular, segments 4 to 6 with pectinate scales, terminal segment with stout setae (Fig. 1E, F). Thorax formed by 4 segments, each with 1 pair of biramous legs (Fig. 1C). In males, first segment of second leg with 2 triangular projections (Fig. 1C, G). Two fusiform respiratory areas on each side of carapace. Internal respiratory area about half of the size of the external respiratory areas, in close contact to each other (1E).

Based on the lack of suction discs, second maxilla with 2 teeth at the base, and the absence of pre-oral sting, the newly collected specimens were assigned to *Dolops bidentata* (Bouvier, 1899). This record represents the first for the genus *Dolops* in Mexico and North America.

Taxonomic summary

Hosts: Anguilla sp. (Bouvier, 1899a); *Astronotus ocellatus*, *Prochilodus nigricans*, *Rhytiodus microlepis*, and *Piaractus brachypomus* (Malta, 1982. 1984); *Pygocentrus nattereri* (Silva-Souza et al., 2011); *Schizodon fasciatus*, *Serrasalmus maculatus*, and *Serrasalmus marginatus* (Fontana et al., 2012); *Centropomus undecimalis* (present study).

Distribution: French Guyana: Riverie Lummier (Bouvier, 1899a); Brazil: Lago Januacá, Rio Solimões, B (Malta, 1982, 1984); Coqueiro Bay (16º15'12" S, 56º22'12" W), Pirizal district, Poconé Wetland, State of Mato Grosso; and Mexico: El Chichicastle, San Antonio, Pantanos de Centla, Tabasco (18°14'36.2" N, 92°18'06.5" W at sea level).

Phylogenetic results

All phylogenetic trees resulting from the analyses of COI (nucleotides including and excluding third positions, and amino acids), 18S, and 28S rRNA did not recover *Dolops* as monophyletic. All COI trees recovered *Argulus* as monophyletic; *Dolops* sp. appears as the sister taxon of *Argulus* spp. and then *Dolops bidentata* as sister to all samples of Argulidae (Figs. 2, 3). The 18S analysis recovered *Dolops* as polyphyletic while *Argulus* was found to be paraphyletic. In this tree, *D. ranarum* and *A. monodi*, both from Lake Victoria, Tanzania, are sister taxa and show relatively long branches, while *D. bidentata* appears as the sister group of all samples of Argulidae (Fig. 3). Alternative exclusion of *A. monodi* or *D. ranarum* result in changes regarding the polyphyly of *Dolops*. Excluding *A. monodi* from the analyses results in the paraphyly of *Dolops* and monophyly of *Argulus* while the exclusion of *D. ranarum* results in the grouping of *A. monodi* together with *A. bengalensis* well nested within *Argulus*. Finally, the analysis of the 28S recovered *D. bidentata* sister to the monophyletic *Argulus*.

Discussion

Even though only a limited molecular dataset of Argulidae is available for phylogenetic studies, all the analyses presented here with more than 1 representative of *Dolops* recover the genus as either para- or polyphyletic. However, given that only 2 DNA sequences assigned to the genus *Dolops* are available in GenBank, our results should be taken with caution. Our phylogenetic results for *Dolops* disagree with current taxonomic arrangements and phylogenetic analyses based on morphological features (Bouvier, 1899a, b; Møller et al., 2008; Ringuelet, 1948). Morphological traits used to define the genus (i.e., first maxillae segmented and armed with a distal hook and by lack of the pre-oral sting) are, based on our phylogenetic results, plesiomorphic characters that transformed to a suction disk and pre-oral sting in *Argulus*. The transformation of such characters was investigated in previous studies (Møller & Olesen, 2010, 2012; Møller et al., 2008) but these authors concluded that it was impossible, due the lack of molecular information for the genus *Dipteropeltis*, to postulate the plesiomorphic condition for these features.

Møller et al. (2008) suggested that the presence of first maxillae segmented and armed with distal hooks, instead of suction discs, is probably the plesiomorphic condition for the family, based on the ontogenetic development (*sensu* Patterson [1996]). However, these authors recognized that it was equally parsimonious to assume the loss of hooks in *Argulus* and *Chonopeltis* or the loss of suction discs in *Dolops*. Our results suggest that

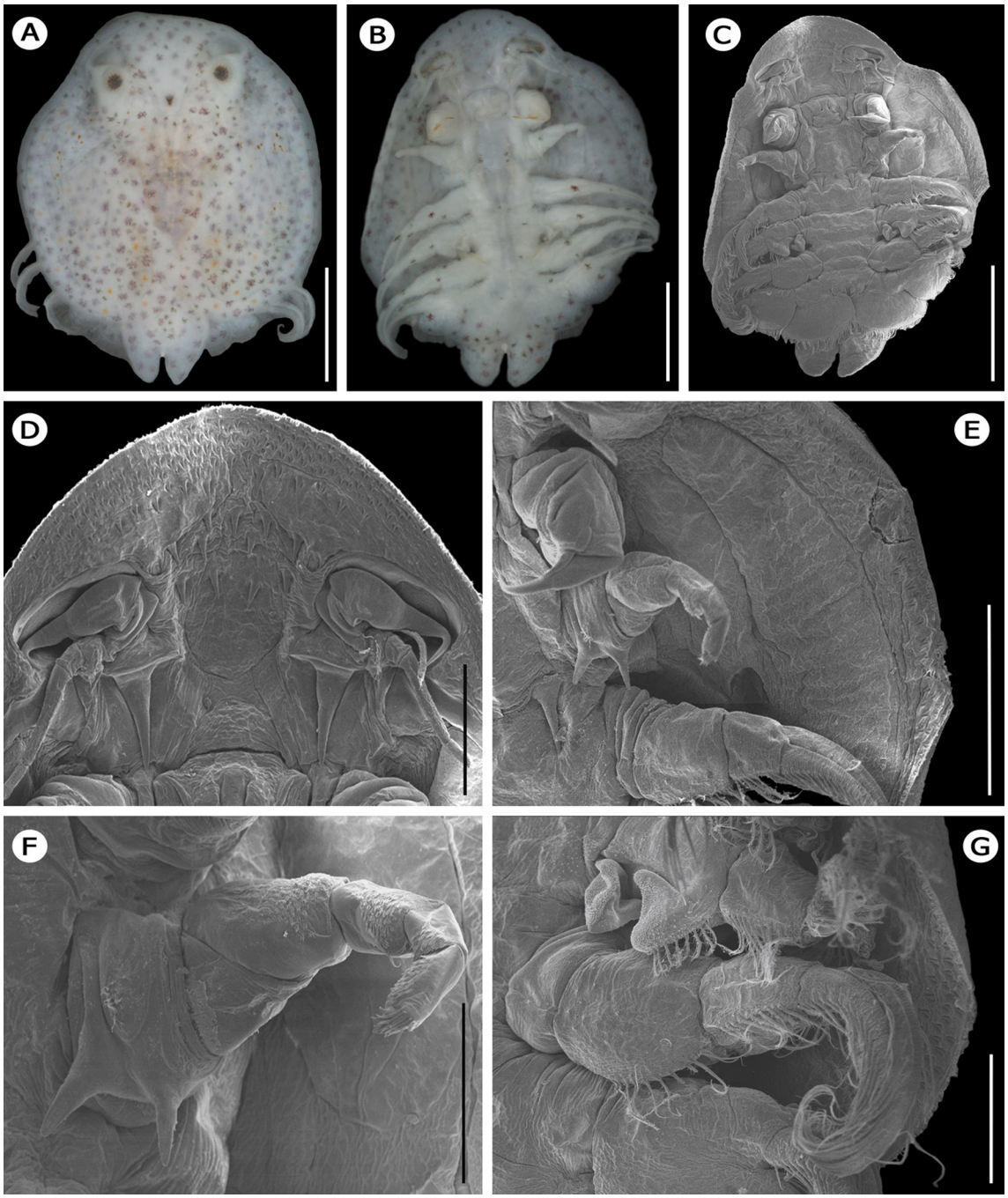

Figure 1. Micrographs of *Dolops bidentata.* A) Dorsal view, female; scale bar = 1 mm; B) ventral view, male; scale bar = 1 mm; C) electron scanning micrograph of male; scale bar = 1 mm; D) detail of spines, antenna, and antennules in antero-lateral depressions and absence of preoral sting; at anterior carapace tip of male; 250 µm; E) detail of anterior and posterior respiratory areas. Distal hook of the first maxillae and second maxillae with 2 teeth at the base are also observed, scale bar = 500 µm; F) detail of the 2 teeth at base of second maxillae characteristic of *Dolops bidentata*, scale bar = 200 µm; G) detail of the triangular projections in second legs of male, 250 µm.

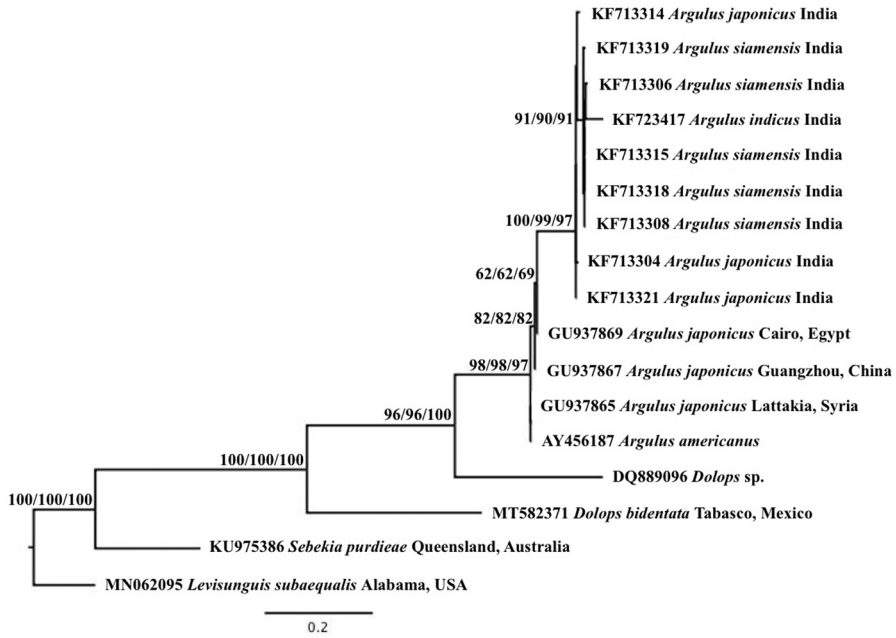

Figure 2. Phylogenetic hypothesis based on maximum likelihood using COI sequences including all positions. Numbers next to nodes indicate bootstrap values for original matrix/excluding third positions/and amino acids. GenBank accession number and localities of each terminal are provided. Scale bar is proportional to the estimated number of nucleotide substitutions per site.

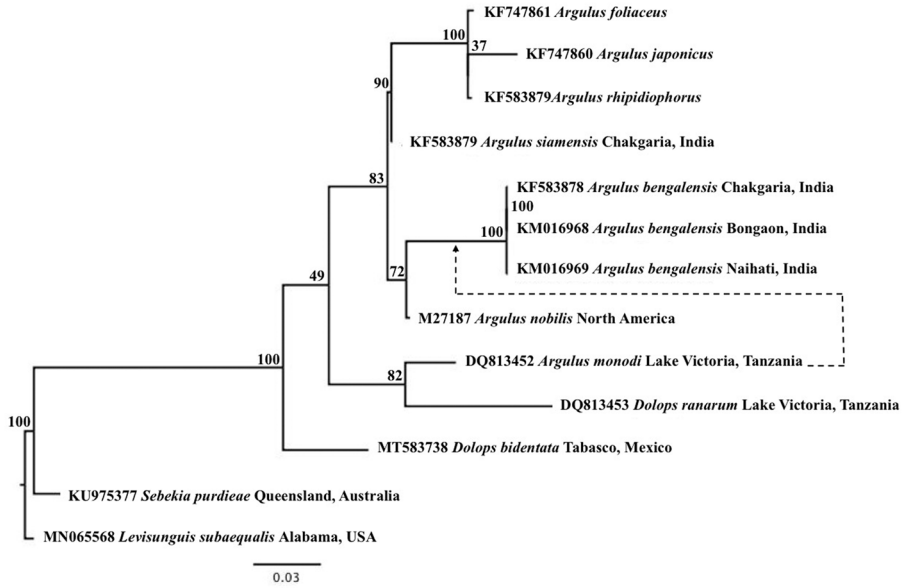

Figure 3. Phylogenetic hypothesis based on maximum likelihood using 18S sequences. Numbers next to nodes indicate bootstrap values. GenBank accession number and localities are provided. The arrow indicates the position of *A. monodi* when *D. ranarum* is excluded from the analysis. Scale bar is proportional to the estimated number of nucleotide substitutions per site.

the first maxillae segmented and armed with distal hooks is the plesiomorphic condition in Branchiura, based on the position of species of *Dolops* in all the phylogenetic analysis performed herein.

Regarding the absence of the pre-oral sting in *Dolops*, our results corroborate the preliminary discussion of Møller and Olesen (2010). These authors proposed that the acquisition of the pre-oral sting probably occurred in the common ancestor of *Dipteropeltis*, *Argulus*, and *Chonopeltis*, and this latter genus lost this character secondarily. Based on the position of species of *Dolops* in our trees and previous studies that show that this structure is absent in newly hatched larvae (stage 1) of *D. ranarum* and *D. carvalhoi* (Avenant-Oldewage et al., 1989; Fryer, 1964; Møller & Olesen, 2012), we propose that the lack of pre-oral sting is the plesiomorphic condition for Branchiura.

The presence of 2 teeth at the base of second maxillae is the distinctive feature of *D. bidentata* and is clearly described in the original description (Bouvier, 1899a), as well as in the expanded description of the species (Bouvier, 1899b). This feature is also present in the specimens studied here collected in Mexico as well as in the specimens collected in the state of Mato Grosso, Brazil, parasitizing the red piranha that were used for the redescription of the species by Silva-Souza et al. (2011). Interestingly, other than this conspicuous feature, specimens form Mato Grosso display morphological characters clearly divergent from those described in the original description and those found on the specimens from Mexico. In both the original description and in the specimens from Mexico, the dorsal surface of the body is covered by numerous dendriform spots not forming regular patterns; most of the spots are brown or purple with few spots in yellow, whereas in the specimens from Mato Grosso, spots are arranged on the borders of carapace and the external borders of abdominal lobes, lateral lines at the middle of lateral lobes, 1 on each side and lines surrounding both eyes, and furthermore, all the spots are black. In addition, the specimens from Mato Grosso lack of a nauplius eye whereas this trait is described in both the original description and in the specimens from Mexico. Furthermore, lateral lobes of the carapace in the specimens from Mato Grosso only reach the 3rd pair of swimming legs, whereas in the Mexican specimens and in the original description of the species, both lobes reach the 4th pair of swimming legs.

One of the main differences between the specimens from Mato Grosso described by Silva-Souza et al. (2011) in comparison with the specimens from Mexico is the shape, size, and arrangement of the respiratory areas. In the specimens from Mato Grosso, internal respiratory areas are small and oval and external respiratory areas large and fusiform with an indentation on the internal side in the posterior part; internal and external areas are well separated from each other. On the contrary, in Mexican specimens, internal and external respiratory areas are fusiform and in close contact to each other. Unfortunately, no information of such characters is available in the original description of the species.

In addition, differences in antennules, male accessory copulatory structures, and abdominal lobes between the original description and the specimens from Mexico in comparison with the specimens from Mato Grosso were found. In specimens described by Silva-Souza et al. (2011: p. 147: fig. 2b), each antennule has a large robust lateral spine and 4 apical spines. In contrast, these spines are not described in Bouvier (1899a, b) and are not present in our material. In both Mexican specimens and in the original description, males have 2 triangular accessory copulatory structures in the second legs, while in the material from Mato Grosso, similar modifications are observed in legs 2 to 4 (Silva-Souza et al., 2011: p: 147: figs. 2g-i). Bouvier (1899b: p. 66: fig. 5c) described 2 laminar projections at the base of third legs similar to those described by Silva-Souza et al. (2011), but such structures were not observed in our material. Abdominal lobes in the specimens from the 3 localities are similar in size, shape, and in the presence of small spines along their margin, but specimens from Mato Grosso have both lobes separated by a broad sinus about half the length of the complete abdomen, while in the Mexican specimens, the sinus is shorter, as in the description of Bouvier (1899b: p. 63: fig. 2a).

Morphologically, the material collected in Mexico resembles the original description of the species by Bouvier (1899a, b), whereas major differences were found in comparison with the specimens described by Silva-Souza et al. (2011). Based on this, we assign our specimens to *D. bidentata*, whereas the material from Brazil probably corresponds to a different species.

Acknowledgments

Andrea Jiménez Marín, Andrea Rebollo Hernández, Laura Márquez Valdelamar, and Nelly López Ortíz (LANABIO, UNAM) assisted in the generation of DNA sequences. Berenit Mendoza Garfias and Susana Guzmán-Gómez assisted in the microphotograph processing. This project was funded by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica IN213520 (PAPIIT-UNAM) to AO-F. Conacyt provided a scholarship to LC-O. We also thank Jose Luis Villalobos and Fernando Álvarez for incorporating the specimens into the Colección Nacional de Crustáceos, Instituto de

Biología, Universidad Nacional Autónoma de México, Mexico City. This paper is part of the first author research in the Posgrado en Ciencias Biológicas-UNAM.

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