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Molecular and morphological characterization of *Andracantha gravida* (Alegret, 1941) (Acanthocephala: Polymorphidae) in piscivorous birds from the Gulf of Mexico

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Research Paper

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Abstract

Adult specimens of *Andracantha gravida* (Alegret, 1941) were recorded from the intestines of the double-crested cormorant *Nannopterum auritus* (Lesson) (type host) and brown pelican *Pelecanus occidentalis* L. in two localities from Mexico: Celestún, Yucatan (south-eastern) and Punta Piedra, Tamaulipas (north-eastern). The specimens of *A. gravida* are morphologically characterized by having a pipe-shaped body without swellings, the absence of small trunk spines between the two fields of spines on the foretrunk and a cylindrical proboscis with 14–16 rows of 10–12 hooks per row. Newly generated partial sequences of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene were generated from adult isolates of *A. gravida* from Mexico and compared with one sequence of *A. gravida* and with sequences of other polymorphid acanthocephalans available in GenBank. Phylogenetic analyses based on maximum likelihood and Bayesian inference methods of the *cox*1 dataset placed all the species of *Andracantha* in a single clade, with weak support. The analyses of the *cox*1 dataset placed *Andracantha sigma* Presswell, García-Varela & Smales, 2018, as sister to the clade formed by *A. gravida*, *Andracantha phalacrocoracis* (Yamaguti, 1939), *Andracantha leucocarboi* Presswell, García-Varela & Smales, 2018 and an unidentified species of *Andracantha* from Japan. The newly generated *cox*1 sequences of *A. gravida* from piscivorous birds of Mexico formed a strongly supported clade with the published sequence of *A. gravida* from the double-crested cormorant from the south-eastern coast of Mexico. The intraspecific genetic divergence among isolates identified as *A. gravida* ranged from 0.0% to 2.2%. A *cox*1 haplotype network inferred with 14 sequences revealed the presence of nine haplotypes, two of which were shared between the populations of piscivorous birds from the north-eastern and south-eastern coasts of Mexico and seven of which were unique. The fixation index between the populations from north-eastern and south-eastern Mexico was low (0.06949), which suggests genetic flow. This can be explained by the migration patterns of the brown pelican and the double-crested cormorant along the coasts of the Gulf of Mexico.

Introduction

Members of the genus *Andracantha* (Schmidt, 1975) are considered to be typical components of helminth fauna of cormorants and shags (Phalacrocoracidae) (see Schmidt, 1975; Presswell *et al.*, 2018). However, some *Andracantha* species have been recorded in diverse hosts, such as the brown pelican *Pelecanus occidentalis* L., the red-breasted merganser *Mergus serrator* L., the American bald eagle *Haliaeetus leucocephalus* L, and the little blue penguin *Eudyptula novaehollandiae* Forster (Schmidt, 1975; Richardson & Cole, 1997; Laskowski *et al.*, 2008; Presswell *et al.*, 2018). Morphologically, species of *Andracantha* are identified by the presence of two fields of spines separated by a bare zone in the anterior part of the trunk, a cylindrical proboscis with a slightly swollen region, a cone-shaped neck, six or eight pyriform cement glands, usually arranged in bilateral pairs and eggs with or without polar protrusion in the middle fertilization membrane (Schmidt, 1975; Presswell *et al.*, 2018). Based on these morphological features, the genus *Andracantha* currently comprises nine species: five of them were described in the Americas: *Andracantha gravida* (Alegret, 1941) (type species), *Andracantha phalacrocoracis* (Yamaguti, 1939), *Andracantha mergi* (Lundström, 1941), *Andracantha baylisi* (Zdzitowiecki, 1986) and *Andracantha tandemtesticulata* (Monteiro *et al.*, 2006); three in Oceania: *Andracantha clavata* (Goss, 1941), *Andracantha sigma* (Presswell, García-Varela & Smales, 2018) and

Andracantha leucocarboi (Presswell, García-Varela & Smales, 2018); and *Andracantha tunitae* (Weiss, 1914), which has been recorded in Africa and Europe (Schmidt, 1975; Zdzitowiecki, 1986; Monteiro *et al.*, 2006; Presswell *et al.*, 2018 2017).

As part of a long-term study on the diversity and distribution of parasites associated with aquatic birds of Mexico, adult specimens of *A. gravida* were collected from the double-crested cormorant *Nannopterum auritus* (Lesson) and the brown pelican *P. occidentalis* from two localities in the Gulf of Mexico: Celestún, Yucatan (south-eastern); and Punta Piedra, Tamaulipas (north-eastern). The aim of the current study was to typify newly collected specimens of *A. gravida* by combining morphological and molecular methods. In addition, we aimed to explore the genetic structure of the populations sampled using partial sequences of the cytochrome *c* oxidase subunit 1 (*cox*1) gene.

Materials and methods

Specimen collection

A total of 14 birds, nine double-crested cormorants and five brown pelicans, were examined. Eight cormorants were collected from Celestún, Yucatán (20°50′53.5″N, 90° 24′22″W), Mexico, in July 2006, while five pelicans and one cormorant were collected from Punta Piedra, Tamaulipas (24° 29′26″N, 97°45′01″W), Mexico, in July 2008. The digestive tract of the birds was dissected and placed in separate Petri dishes containing 0.75% saline and examined using a stereomicroscope. Acanthocephalans were placed in distilled water at 4°C overnight and subsequently preserved in molecular-grade ethanol. Birds were identified following a field guide (Howell & Webb, 1995) and the American Ornithologist Union (1998) guidelines.

Morphological analyses

Selected adult acanthocephalans from brown pelicans and double-crested cormorants were gently punctured with a fine needle in the trunk, stained with Mayer's paracarmine, destained in 70% acid ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Specimens were examined using a Leica DM 1000 light emitting diode (LED) compound microscope equipped with a bright field (Leica, Wetzlar, Germany). Measurements were taken using the Leica Application Suite microscope software and are presented in micrometres (µm). Acanthocephalans collected in the present study were identified following Schmidt (1975) and Presswell *et al.* (2018). For scanning electron microscopy, two adult specimens were dehydrated with an ethanol series, critical point dried, sputter coated with gold and examined with a scanning electron microscope (Hitachi Stereoscan Model S-2469N) operating at 15 kV from the Instituto de Biología, Universidad Nacional Autónoma de México. Voucher specimens were deposited in the Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico (accession number CNHE: 5997, 6834, 11834, 11835).

DNA isolation, amplification and sequencing

Eight adult acanthocephalans obtained from the double-crested cormorant (one from Punta Piedra and seven from Celestún) and five adult acanthocephalans from the brown pelicans from Punta Piedra were used for the molecular analyses. Acanthocephalans were placed individually in tubes and digested overnight at 56°C in a solution containing 10 mM Tris–hydrochloride (pH = 7.6), 20 mM sodium chloride, 100 mM ethylenediaminetetraacetic acid disodium salt (pH = 8.0), 1% sodium lauroyl sarcosinate and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using DNAzol reagent. A fragment partial fragment of the *cox*1 gene was amplified using the forward primer 5′-AGTTCTAATCATAA(R)GATAT(Y)GG-3′ and reverse primer 5′ -TAAACTTCAGGGTGACCAAAAAA TCA-3′ (Folmer *et al.*, 1994). Polymerase chain reaction (PCR) consisted of 10 µl of each primer, 2.5 µl of 10× buffer, 2 mM MgCl2, 2 µl of the genomic DNA (10–20 ng) and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters for the molecular marker consisted of denaturation at 94°C for 1 min, 35 cycles of 94°C for 1 min, 40°C for 1 min and 72°C for 1 min, followed by a postamplification incubation at 72°C for 10 min. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry and using an ABI 3730 capillary DNA sequencer reaction products were separated and detected. Contigs were assembled, and base-calling differences were resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts) and submitted to GenBank (table 1).

Alignments, phylogenetic analysis and haplotype network

Newly generated sequences were aligned with available sequences for other polymorphid acanthocephalans, including a partial sequence for an isolate of *A. gravida* from a double-crested cormorant from Mexico of García-Varela *et al.* (2009) (GenBank EU267822), retrieved from GenBank (see table 1), using Clustal W software (Thompson *et al.*, 1994). A nucleotide substitution model was selected for the dataset using jModelTest version 2.1.7 (Posada, 2008), applying the Akaike criterion. The best nucleotide substitution model was TPM3uf + G + I. Phylogenetic trees were inferred through maximum likelihood (ML) with the program RAxML version 7.0.4 (Stamatakis, 2006). A GTRGAMMAI substitution model was used, and 10,000 bootstrap replicates were run to assess nodal support. We also analysed our data in a Bayesian inference (BI) framework using MrBayes 3.2.2 (Ronquist *et al.*, 2012), with two Markov chain Monte Carlo runs for 10 million generations, sampling every 1000 generations, a heating parameter value of 0.2 and a burn-in of 25%. The resulting trees were visualized using FigTree version 1.4.2 (Rambaut & Drummond, 2007). Finally, uncorrected *p* distances were estimated using MEGA version 11 (Kumar *et al.*, 2016).

To explore whether piscivorous birds from both localities in Mexico, i.e., Celestún (south-eastern) and Punta Piedra (north-eastern), share the same *cox*1 haplotypes, an unrooted statistical network was constructed using PopART (Leigh & Bryant, 2015), with the minimum spanning network option (Bandelt *et al.*, 1999). The degree of genetic differentiation between populations was estimated using the fixation index (F_{st}) (see Hudson *et al.*, 1992) with Arlequin v.3.5 (Excoffier & Lischer, 2010).

Results

Morphological identification (figs 1 and 2).

Acanthocephalans collected from double-crested cormorants and brown pelicans from the Gulf of Mexico were identified as *A.*

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Table 1. List of acanthocephalans used in the phylogenetic analyses, with data on the life-cycle stage, host locality and GenBank accession number for mitochondrial cytochrome c oxidase subunit 1 (*cox1*).

Species	Life-cycle stage	Host	Locality	GenBank number	Source
Andracantha					
Andracantha gravida (Alegret, 1941)	A	*Nannopterum auritus* (Lesson)	Celestún, Yucatán (Mexico)	EU267822	García-Varela *et al.* (2009)
			Celestún, Yucatán (Mexico)	**OQ109040–OQ109043**	Present study
	A	*Nannopterum auritus*	Punta Piedra, Tamaulipas (Mexico)	**OQ109044–OQ109047**	Present study
	A	*Pelecanus occidentalis*	Punta Piedra, Tamaulipas (Mexico)	**OQ109048–OQ109052**	Present study
Andracantha leucocarboi (Presswell *et al.*, 2018)	A	*Leucocarbo chalconotus* (Gray)	Otago Harbour (New Zealand)	MF527023	Presswell *et al.* (2018)
	A	*Phalacrocorax punctatus* (Sparrman)	Otago Harbour (New Zealand)	MF527024	Presswell *et al.* (2018)
Andracantha phalacrocoracis (Yamaguti, 1939)	I	*Zalophus californianus* (Lesson)	Sausalito, California (United States)	MK119254	Lisitsyna *et al.* (2019)
	A	*Phalacrocorax pelagicus* (Pallas)	Hokkaido, Notsuke (Japan)	LC465396-398	Sasaki *et al.* (2019)
	C	*Osmerus dentex* (Steindachner & Kner)	Hokkaido, Nemuro (Japan)	LC465356	Sasaki *et al.* (2019)
	A	*Phalacrocorax capillatus* (Temminck & Schlegel)	Hokkaido, Erimo (Japan)	LC465403	Sasaki *et al.* (2019)
Andracantha sigma (Presswell, García-Varela & Smales, 2018)	A	*Eudyptula minor* (Forster)	Otago (New Zealand)	MF527034	Presswell *et al.* (2018)
	A	*P. punctatus*	Otago (New Zealand)	MF527035	Presswell *et al.* (2018)
Andracantha sp.	C	*Osmerus dentex*	Hokkaido, Nemuro (Japan)	LC465393	Sasaki *et al.* (2019)
	A	*Hypomesus japonicus* (Brevoort)	Hokkaido, Nemuro (Japan)	LC465391	Sasaki *et al.* (2019)
Corynosoma					
Corynosoma australe (Johnston, 1937)	A	*Z. californianus*	La Paz, Baja California Sur (Mexico)	MT676808	García-Varela *et al.* (2021)
	C	*Merluccius hubbsi* (Marini)	San Matías Gulf, northern Patagonia (Argentina)	MT676819	García-Varela *et al.* (2021)
	A	*Otaria flavescens* (Shaw)	Northern Patagonia, Chubut (Argentina)	MF497334	Hernández-Orts *et al.* (2017)
Corynosoma hannae (Zdzitowiecki, 1984)	A	*Phocarctos hookeri* (Gray)	Enderby Island (New Zealand)	KX957715–716	Hernández-Orts *et al.* (2017)

(*Continued*)

Table 1. (*Continued.*)

Species	Life-cycle stage	Host	Locality	GenBank number	Source
Corynosoma strumosum (Rudolphi, 1802)	A	*Pusa hispida botnica* (Gmelin)	Baltic Sea (Finland)	EF467871	García-Varela & Pérez Ponce de León (2008)
	A	*Phoca vitulina* L.	Monterey Bay, California (United States)	EF467870	García-Varela & Pérez Ponce de León (2008)
	A	*Z. californianus*	Sausalito, California (United States)	MK119250	Lisitsyna *et al.* (2019)
Corynosoma magdaleni Montreuil, 1958	A	*P. hispida saimensis* (Nordquist, 1889)	Lake Saimaa (Finland)	EF467872	García-Varela & Pérez Ponce de León (2008)
Corynosoma enhydri (Morozov, 1940)	A	*Enhydra lutris* L.	Monterey Bay, California (United States)	DQ089719	García-Varela & Nadler (2006)
Corynosoma semerme (Forssell, 1904)	A	*Callorhinus ursinus* (L.)	St Paul Island, Alaska (United States)	MK119253	Lisitsyna *et al.* (2019)
	A	*Halichoerus grypus* (Fabricius)	Baltic Sea	MF001277	Waindok *et al.* (2017). Unpublished
	A	*C. ursinus*	St Paul Island, Alaska (United States)	JX442192	García-Varela *et al.* (2013)
Corynosoma villosum (Van Cleave, 1953)	A	*C. ursinus*	St Paul Island, Alaska (United States)	MK119251	Lisitsyna *et al.* (2019)
Corynosoma validum (Van Cleave, 1953)	A	*C. ursinus*	St Paul Island, Alaska (United States)	JX442193, MK119252	García-Varela *et al.* (2013) and Lisitsyna *et al.* (2019)
Bolbosoma					
Bolbosoma turbinella (Diesing, 1851)	A	*Eschrichtius robustus* (Lilljeborg)	Monterey Bay, California (United States)	JX442189	García-Varela *et al.* (2013)
Bolbosoma sp.	I	*C. ursinus*	St Paul Island, Alaska (United States)	JX442190	García-Varela *et al.* (2013)
Pseudocorynosoma					
Pseudocorynosoma anatarium (Van Cleave, 1945)	A	*Bucephala albeola* (L.)	Nueva Ideal, Durango (Mexico)	EU267821	García-Varela *et al.* (2009)
Pseudocorynosoma constrictum (Van Cleave, 1918)	A	*Spatula clypeata* (L.)	State of Mexico (Mexico)	EU267820	García-Varela *et al.* (2009)
Polymorphus					
Polymorphus brevis (Van Cleave, 1916)	A	*Nycticorax nycticorax* (L.)	Michoacán (Mexico)	DQ089717	García-Varela & Nadler (2006)
Hexaglandula					
Hexaglandula corynosoma (Travassos, 1915)	A	*Nyctanassa violacea* (L.)	La Tovara, Nayarit (Mexico)	EU189488	Guillén-Hernández *et al.* (2008)

A, adult; C, cystacanth; I, immature specimens. Newly generated sequences are presented in boldface type.

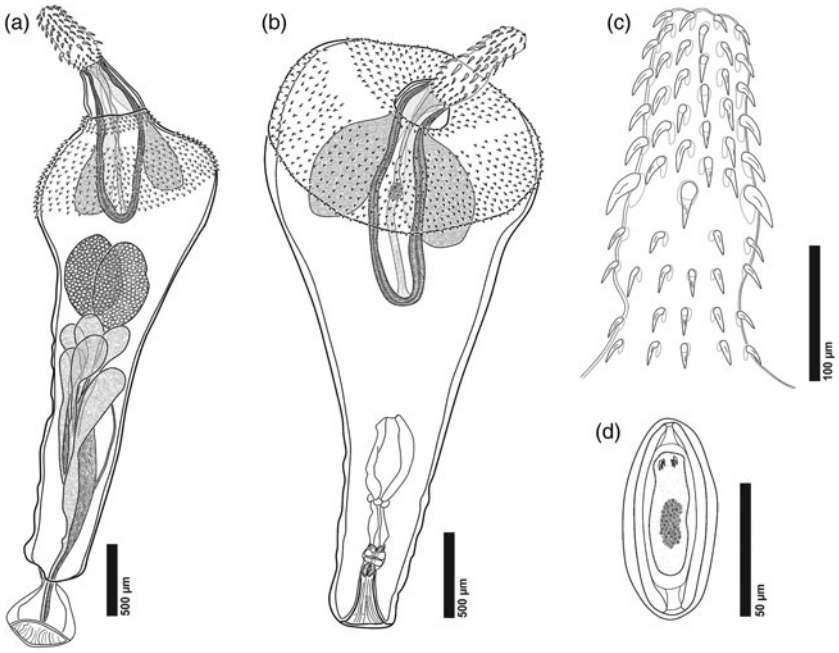

Fig. 1. *Andracantha gravida* from *Nannopterum auritus*. (a) Adult male, total view; (b) adult female total view; (c) proboscis; (d) egg.

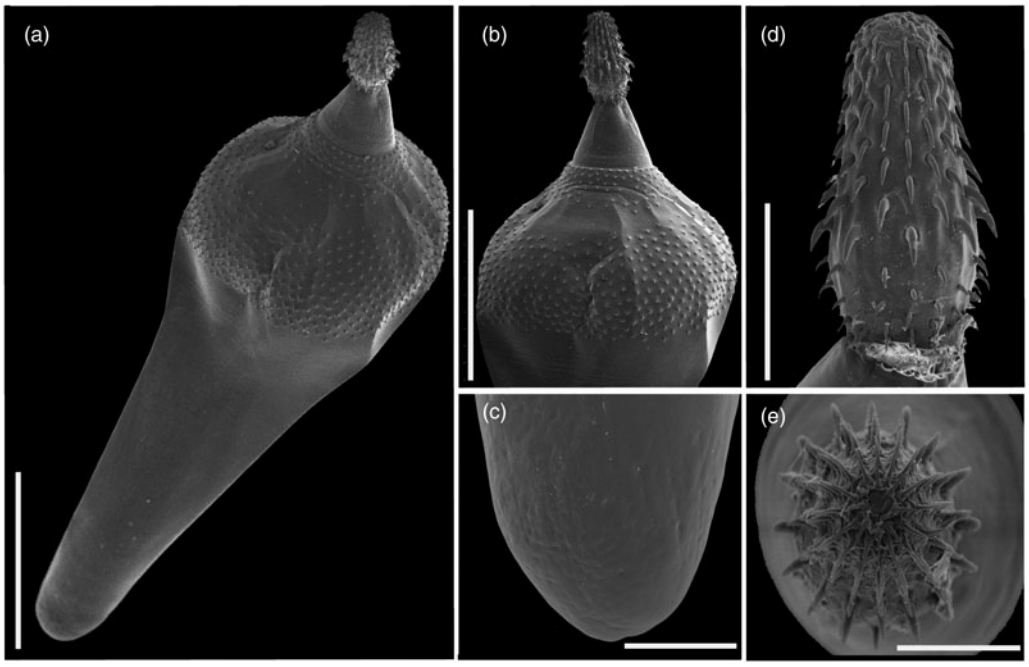

Fig. 2. Scanning electron micrographs of *Andracantha gravida* from *Nannopterum auritus*. (a) Adult male ventral view; (b) adult male anterior trunk; (c) adult male posterior end; (d, e) adult male proboscis. Scale bars: (a, b) 1000 µm; (c) 200 µm; (d) 300 µm; (e) 200 µm.

gravida, following the key for *Andracantha* species of Presswell *et al.* (2018). In addition, the newly collected specimens show matching morphological characteristics with those assigned as *A. gravida* by Alegret (1941) and Schmidt (1975), including: (a) pipe-shaped trunk without swellings; (b) testes parallel; (c) absence of small spines between the two field of spines on the foretrunk; (d) six cement glands; (e) hooks arranged in 15–16 rows with 10–12 hooks per row; (f) absence of genital spines in female; and (g) eggs with polar protrusion in the middle fertilization membrane (figs 1 and 2). In addition, our specimens exhibited variability from those previous descriptions in the following characters: trunk; proboscis; hooks; proboscis receptacle testes; and eggs with those previous descriptions (see table 2).

Phylogenetic analyses and genetic structure

The *cox*1 dataset included 655 characters and 49 sequences. The phylogenies inferred with the ML and BI methods yielded *Andracantha* as a monophyletic group, but with weak bootstrap support and Bayesian posterior probabilities (fig. 3). This result was similar and consistent with previous phylogenetic assessments using the same molecular marker or with nuclear

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Table 2. Comparative metrical data for males and females of *Andracantha gravida*. Measurements in micrometres, unless otherwise indicated.

Reference	This study	This study	Alegret (1942)	Schmidt (1975)
Host	*Phalacrocorax auritus*	*Pelecanus occidentalis*	*Phalacrocorax auritus*	*Phalacrocorax auritus*
General				
No. longitudinal rows of hooks	15–16	15–16	18	16
No. hooks per row	10–12	10–12	11–12	11–12
Male	*n*=7	*n*=2		*n*=10
Trunk length (mm)	1.7–3.5 × 0.7–1.4	2.5–3.1 × 1.0–1.2	3.5– 4.0 × 1.5–1.8	3.2–4.5 × 1.2–1.4
Proboscis	523–600 × 203–263	554–609 × 262–256		528–680 × 240–280
Proboscis receptacle (mm)	0.7–1.2 × 227–354	0.8–1.1 × 316–343	1.60–1.80 × 160	1.04–1.27 × 255–435
Neck length	374–627	304–400		
Apical Hooks length (First four)	29–55	29–56		40–60
Middle hooks length (Fifth-Seventh)	60–73	42–56		54–82
Basal hooks length	20–42	23–37		35–58
Leminisci	467–509	588–609	600	680–800
Testes	571–673 × 345–449	502 × 342	400–470 × 110–140	480–560 × 280–400
Cement gland number	6	6		6
Säfftigen's pouch	388–519 × 343–389	–		–
Females	*n* = 3	*n* = 8		*n* = 10
Trunk length (mm)	3.3–4.4 × 1.41–1.8	3.7–4.1 × 1.18–1.7	4.5–6.0	4.2–6.0 × 1.75–2.0
Proboscis	590 × 272	488–662 × 222–272		640–680 × 265–345
Proboscis receptacle (mm)	1.1 × 335	0.7–1.29 × 310–360		1.3–1.43 × 360–400
Apical hooks length (First four)	27–45	30–45		50–70
Middle hooks length (Fifth–Seventh)	34–69	37–74		70–88
Basal hooks length (Eighth–Sixteenth)	26–36	21–23		40–58
Lemnisci length	900–913	600–842		800–920
Reproductive system (from mouth of uterine bell to genital pore) (mm)	0.7	1.13		1.95
Egg size	60–75 × 23–39	60–71 × 34–43	80–95 × 37–47	68–80 × 28–40

n =number of specimens analysed.

molecular markers (e.g. Presswell *et al.*, 2018; Sasaki *et al.*, 2019; García-Varela *et al.*, 2021; Santoro *et al.*, 2021; Hernández-Orts *et al.*, 2022). Our phylogenetic trees showed that *Andracantha* is divided into five main subclades. The first subclade was formed by two sequences of *A. sigma* (MF527034 and MF527035) from the little blue penguin *E. novaehollandiae* and the spotted shag *Phalacrocorax punctatus* (Sparrman). The second subclade was formed by six sequences of *A. phalacrocoracis*; one sequence for a cystacanth (LC465356) and one for an adult isolate (LC465403) both from the Japanese cormorant *Phalacrocorax capillatus* (Temminck & Schlegel) from Japan; three sequences (LC465396–398) from the pelagic cormorant *Phalacrocorax pelagicus* (Pallas); and one sequence of an immature isolate (MK119254) from a sea lion *Zalophus californianus* (Lesson) from California, United States. The third subclade was formed by two sequences of *A. leucocarboi* (MF527023 and MF527024) from an Otago shag *Leucocarbo chalconotus* (Gray) and a spotted shag *P. punctatus*, respectively. The fourth subclade includes two sequences of cystacanth isolates from fishes (LC465391 and LC465393) of an unidentified species of *Andracantha* from Japan. Finally, the fifth subclade was formed by our 13 newly generated sequences for adult isolates for *A. gravida* and the sequence of an adult isolate identified as *A. gravida* (EU267822) by García-Varela *et al.* (2009) from Yucatan, Mexico (fig. 3). The genetic divergence estimated among the isolates *A. gravida* from Mexico ranged from 0.0% to 2.2%. Finally, the genetic divergence among the four species of *Andracantha* (*A. sigma*, *A. phalacrocoracis*, *A. leucocarboi*, and *A. gravida*) plus an unidentified species of *Andracantha* ranged from 17 to 21%.

The haplotype network, inferred with 14 specimens and 655 characters, represented a total of 9 haplotypes (fig. 4). The most frequent haplotypes, that is, H3 ($n = 4$) and H9 ($n = 2$), were shared by the two bird populations analysed (Punta Piedra, Tamaulipas and Celestún, Yucatán). Haplotypes H1, H4, H7 and H8 were found in Punta Piedra (north-eastern Mexico) parasitizing double-crested cormorants and brown pelicans, while

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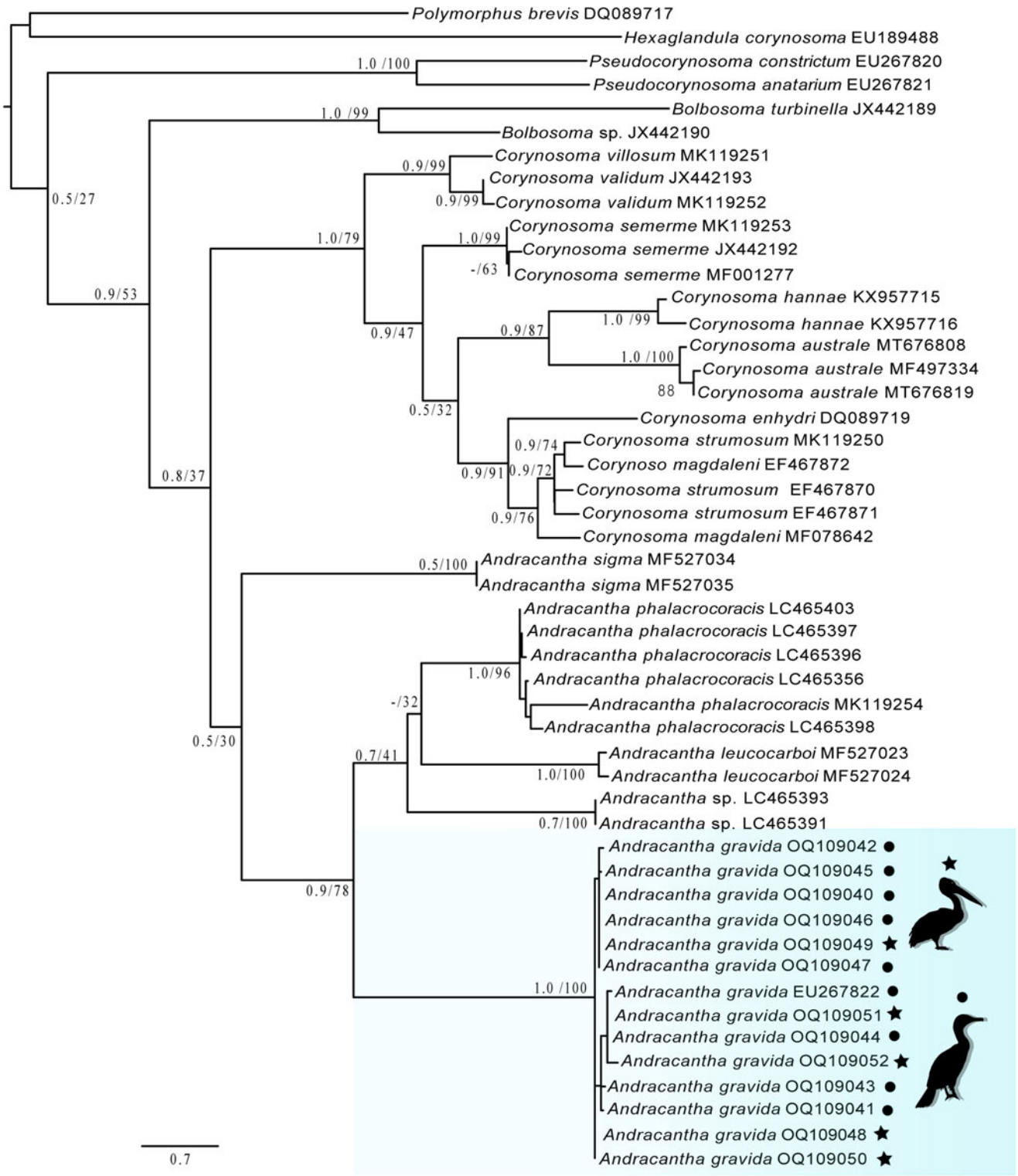

Fig. 3. Phylogenetic tree using maximum likelihood and consensus Bayesian inference for the *cox*1 dataset. Numbers near internal nodes show ML bootstrap percentage (BP) values and Bayesian posterior probabilities (BPP). Scale bars represent the branch length.

haplotypes H2, H5 and H6 were found in Celestún (south-eastern Mexico) parasitizing double-crested cormorants. The level of haplotype diversity (Hd = 0.912) was very high and nucleotide diversity was low (pi = 0.00955) between the two populations. The *Fst* value was low (0.06949) between isolates of *A. gravida* from the two sampled localities in Mexico.

Discussion

Andracantha gravida was described from the double-crested cormorant in Cuba by Alegret (1941). Later, this species was redescribed from the same definitive hosts, and it was recorded in brown pelicans and in the neotropical cormorant *Nannopterum*

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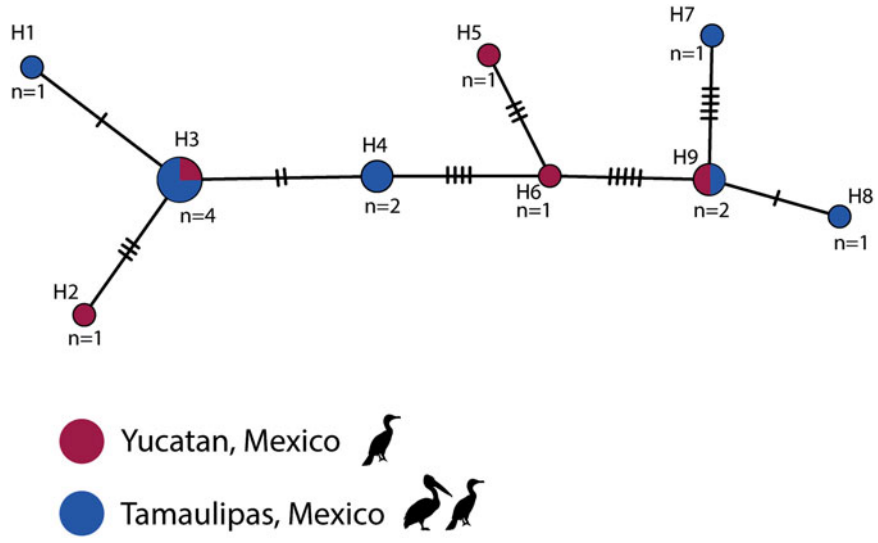

Fig. 4. Median-joining network of samples of *andracantha gravida* built with the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene. Each circle represents a haplotype, with size proportional to the haplotype's frequency in the populations.

brasilianus (Gmelin) in Florida, Texas, and Louisiana in the United States (Schmidt, 1975; Fedynich *et al.*, 1997; Robinson *et al.*, 2008) and in south-eastern Mexico (García-Varela *et al.*, 2013). The current records of *A. gravida* in brown pelicans and double-crested cormorants in north-eastern Mexico represent new locality records of this acanthocephalan species. The morphological characteristics in combination with morphometric data of our specimens fit the descriptions of *A. gravida* provided by Alegret (1941) and Schmidt (1975). However, we found morphometric variation in the acanthocephalans collected mainly regarding the size of the trunk, proboscis, hooks and proboscis receptacle in males and females (table 2). In addition, all the females recovered in both definitive hosts lacked genital spines (see figs 1a–d and 2a–d), and only two males and two females yielded ventral somatic spines in the hindtrunk, which were also described by Schmidt (1975), as genital spines. However, these spines are not genital, because they are displaced to the small field on ventral surface of the hindtrunk. It is well known that the presence or absence of ventral somatic spines in the hindtrunk and genital spines could be due to intraspecific phenotypical variation and represent adaptative features that improve attachment to the host (see Goss, 1940; Johnston & Best, 1942; Zdzitowiecki, 1991; Aznar *et al.*, 2016). Somatic spines have been traditionally used as a diagnostic morphological character to distinguish species of *Andracantha* and *Corynosoma* (Schmidt, 1975; Aznar *et al.*, 2006; Presswell *et al.*, 2018). However, Presswell *et al.* (2018) mentioned that somatic spines should be considered with caution to delineate species in *Andracantha* because it is a variable morphological feature.

Our phylogenetic analyses showed that the genus *Andracantha* is monophyletic, and it was divided into five main subclades, representing four recognized species (*A. sigma*, *A. phalacrocoracis*, *A. leucocarboi* and *A. gravida*) and an undescribed species of *Andracantha*. However, a main subclade was formed with the new 13 sequences of *A. gravida* plus one isolate previously identified as *A. gravida* and available in the GenBank dataset (EU267822) (see fig. 3). The intraspecific genetic divergence among all isolates was very low, ranging from 0.0% to 2.2%. The genetic divergence found was consistent with previous studies. For instance, the genetic divergence detected among ten adults of *A. sigma* recovered from three definitive hosts, the Otago shag *L. chalconotus*, spotted shag *P. punctatus* and little blue penguin *E. novaehollandiae* from New Zealand, ranged from 0.00% to 0.32% (Presswell *et al.*, 2018); that among three adults of *A. leucocarboi* recovered from two definitive hosts, the Otago shag and spotted shag from New Zealand, ranged from 0.00% to 1.38% (Presswell *et al.*, 2018). Finally, the genetic divergence among cystacanths and adults of *A. phalacrocoracis* recovered from the Pacific rainbow smelt *Osmerus dentex* Steindachner & Kner, the Japanese cormorant *P. capillatus* and the pelagic cormorant *P. pelagicus* from Japan was 0.013% (Sasaki *et al.*, 2019).

The haplotype network analysis of *cox*1 sequences inferred with 14 sequences of *A, gravida* revealed the presence of nine haplotypes, two of which (H3 and H9) were shared between the two populations sampled, and seven were unique haplotypes (fig. 4). The F_{st} value was low, suggesting genetic flow between both populations (north-eastern and south-eastern Mexico), which may be explained by the migration of the brown pelican and the double-crested cormorant, which are definitive hosts in the Gulf of Mexico.

Of the nine recognized species of the genus *Andracanatha*, seven species have been recorded in phalacrocoracids (cormorants and shags), representing 77. 7% of the biodiversity of the genus *Andracantha*, suggesting that phalacrocoracid could represent an ancestor host with an independent colonization event to other birds, such as pelicans, mergansers, eagles and penguins (Nickol & Kocan, 1982; Richardson & Cole, 1997; Presswell *et al.*, 2018).

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