

Research Article






Cite this article: García-Varela M, López-Jiménez A, González-García MT, Sereno-Uribe AL, Andrade-Gómez L (2023). Contrasting the population genetic structure of a specialist (*Hexaglandula corynosoma*: Acanthocephala: Polymorphidae) and a generalist parasite (*Southwellina hispida*) distributed sympatrically in Mexico. *Parasitology* **150**, 348–358. <https://doi.org/10.1017/S0031182023000033>

Received: 19 November 2022
 Revised: 31 December 2022
 Accepted: 1 January 2023
 First published online: 12 January 2023

Key words:
 Acanthocephalans; generalist; Mexico;
 Polymorphidae; phylogeography; specialist

Author for correspondence:
 Martín García-Varela,
 E-mail: garcia@ib.unam.mx

Contrasting the population genetic structure of a specialist (*Hexaglandula corynosoma*: Acanthocephala: Polymorphidae) and a generalist parasite (*Southwellina hispida*) distributed sympatrically in Mexico

Martín García-Varela¹ , Alejandra López-Jiménez^{1,2} ,
 Marcelo Tonatiuh González-García^{1,2} , Ana Lucía Sereno-Uribe¹ 
 and Leopoldo Andrade-Gómez³ 

¹Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Avenida Universidad 3000, Ciudad Universitaria, CP 04510 México City, Mexico; ²Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Ciudad Universitaria, CP 04510 México City, Mexico and ³Escuela Nacional de Estudios Superiores Unidad Mérida, Km 4.5 Carretera Mérida-Tetiz, Ucu, Yucatán CP 97357, Mexico

Abstract

Polymorphidae is a monophyletic group of acanthocephalans distributed worldwide. Within this family, *Hexaglandula corynosoma* is a specialist species that uses a single bird species as a definitive host. *Southwellina hispida* is a generalist species that uses a broad spectrum of definitive hosts to complete its life cycle. In the current research, sequences of cytochrome c oxidase subunit 1 (*cox1*) from mitochondrial DNA were generated from 44 specimens of *H. corynosoma* and 76 of *S. hispida* distributed sympatrically in 6 biogeographic provinces of Mexico with the objective of characterizing and comparing the population genetic structure of 2 acanthocephalan species with opposing life strategies. The phylogeographic studies indicated that the populations of both species lacked a phylogeographic structure and exhibited high haplotype diversity, low nucleotide diversity and low F_{st} values among the biogeographic provinces; in combination with negative values on the neutrality test, this suggests that the populations of acanthocephalans are expanding. Paratenic hosts are key for the transmission from intermediate to definitive hosts in the generalist species. However, the inclusion of paratenic hosts does not play a principal role in the population genetic structure of *S. hispida* within its distribution along the coasts of Mexico.

Introduction

Parasitism is a highly successful lifestyle and has evolved independently at least 60 times in different groups of metazoans worldwide (Price, 1980; Poulin and Morand, 2004). Parasites have been traditionally divided into 2 major groups depending on their life cycle: generalists and specialists (Thompson, 1994, 2005). The generalist parasites use a wide range of definitive hosts, whereas the specialist parasites use a single definitive host to complete their life cycle. Under these 2 opposing strategies, generalist parasites infect a broad spectrum of hosts resulting in an optimal or suboptimal level of fitness, whereas specialist parasites prioritize a single optimal host in which fitness is maximized (Rigaud *et al.*, 2010; Lievens *et al.*, 2018). Some studies have suggested that parasite life cycle complexity (generalists vs specialists) could influence population genetic structure (Nadler, 1995; Criscione and Blouin, 2004; Barrett *et al.*, 2008; Archie and Ezenwa, 2011). According to Li *et al.* (2014), a specialist parasite shows significantly less genetic flow; therefore, populations are less connected and are subdivided into smaller populations, leading to strong genetic differentiation. In some cases, the populations might experience bottlenecks, decreasing the effective population size. Moreover, specialist species are more sensitive to stochastic fluctuations that can cause local extinction. In contrast, a generalist parasite shows a high effective population size, high genetic flow and a population that is structured or panmictic. Moreover, a generalist parasite may show greater persistence of populations over the long term because the generalist may be less sensitive to stochastic fluctuations in any given resource as it is able to replace a scarce resource with another (see Sehgal *et al.*, 2001; Brant and Ortí, 2003; Archie and Ezenwa, 2011; Li *et al.*, 2014).

The recent application of molecular markers has helped establishing a more robust classification scheme in acanthocephalans (Near *et al.*, 1998; García-Varela *et al.*, 2000). In particular, cytochrome c oxidase subunit 1 (*cox1*) from mitochondrial DNA is among the most useful molecular markers for defining, recognizing and delineating species and better understanding the population genetic structure in acanthocephalans (Steinauer *et al.*, 2007; Rosas-Valdez *et al.*, 2012, 2020; Alcántar-Escalera *et al.*, 2013; Goulding and Cohen, 2014; Perrot-Minnot

© Universidad Nacional Autónoma de México, 2023. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

et al., 2018; Pinacho-Pinacho *et al.*, 2018; García-Varela *et al.*, 2021; Sereno-Urbe *et al.*, 2022). Polymorphidae (Meyer, 1931) is an emblematic group of obligate endoparasites with complex life cycles that use vertebrates (marine mammals, fish-eating birds and waterfowl) as definitive hosts and invertebrates (amphipods, decapods and euphausiids) as intermediate hosts to complete their life cycle (Schmidt, 1985; Hoberg, 1986; Pichelin *et al.*, 1998; Nickol *et al.*, 1999, 2002; Kennedy, 2006). Currently, the family is classified into 14 genera with approximately 129 species (Schmidt, 1973; Dimitrova and Georgiev, 1994; Nickol *et al.*, 1999, 2002; Aznar *et al.*, 2006; Amin, 2013; García-Varela *et al.*, 2013b; Presswell *et al.*, 2020). Phylogenetic analyses based on multiple molecular markers have suggested that the family is monophyletic as are the genera *Hexaglandula* (Petrochenko, 1950) and *Southwellina* (Witenberg, 1932) (see García-Varela *et al.*, 2011, 2013b).

Members of the Polymorphidae form a monophyletic group that contains generalist and specialist species. Therefore, this family represents an interesting system to explore and compare the population genetic structure of species with 2 opposing life history strategies. *Hexaglandula corynosoma* (Travassos, 1915) is a specialist species that has been recorded as adult only in the intestine of the yellow-crowned night-heron (*Nyctanassa violacea*) (Linnaeus, 1758), and cystacanths have been recorded in 2 decapod species [the fiddler crabs *Leptuca spinicarpa* (Rathbun) and *Minuca rapax* (Smith)], which serve as intermediate hosts in the Americas (Nickol *et al.*, 2002; Guillén-Hernández *et al.*, 2008; García-Prieto *et al.*, 2010). In contrast, the acanthocephalan *Southwellina hispida* (Van Cleave, 1925) Witenberg, 1932 is a generalist species and is considered one of the most abundant species of polymorphids associated with piscivorous birds throughout the world (see Amin *et al.*, 2022). In Mexico, *S. hispida* has been documented in 13 piscivorous bird species (García-Prieto *et al.*, 2010; García-Varela *et al.*, 2012), and the cystacanth has been recorded in the red swamp crayfish *Procambarus clarkii* (Girard) in the USA (Font, 2007), which serves as an intermediate host that is ingested by several vertebrates that act as paratenic hosts such as frogs and freshwater and brackish fishes (Schmidt, 1985). The paratenic hosts must be ingested by piscivorous birds to complete their life cycle (Schmidt, 1985).

In the current study, we examined the sequences of *cox1* from mitochondrial DNA of 2 closely related polymorphid species, a specialist species (*H. corynosoma*) and a generalist species (*S. hispida*), distributed sympatrically in 6 biogeographic provinces (California, Baja California, Pacific Lowlands, Tamaulipas, Veracruz and the Yucatán Peninsula) of Mexico with the objective of characterizing and comparing the population genetic structure of 2 acanthocephalan species with opposing life history strategies.

Materials and methods

Specimen collection

A total of 75 birds, and 36 fishes, were collected between October 2006 and December 2021 in 23 localities across 6 biogeographic provinces (California, Baja California, Pacific Lowlands, Tamaulipas, Veracruz and the Yucatán Peninsula) of Mexico (Table 1; Fig. 1). Birds and fishes were dissected within the following 4 h, and their viscera were placed in separate Petri dishes containing a 0.75% saline solution and examined under a dissecting microscope. The acanthocephalans recovered were washed in 0.75% saline solution and placed overnight in distilled water at 4°C and subsequently preserved in 100% ethanol. Birds and fishes were identified using the field guide of Howell and Webb (1995) and Miller *et al.* (2005), respectively.

Morphological analyses

Selected adult acanthocephalans 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 compound microscope Leica DM 1000 LED equipped with bright field (Leica, Wetzlar, Germany). The acanthocephalans were identified by conventional morphological criteria following Petrochenko (1958). In addition, descriptions of *H. corynosoma* and *S. hispida* were consulted as needed (Schmidt, 1973; Nickol *et al.*, 2002; Amin *et al.*, 2022). For scanning electron microscopy, 2 adult specimens of each species were dehydrated with an ethanol series, critical point dried, sputter coated with gold and examined with a Hitachi Stereoscan Model S-2469N scanning electron microscope operating at 15 kV from the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Adult specimens were deposited in the Colección Nacional de Helmintos, under numbers CNHE: 11823 and 11824, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City

DNA isolation, amplification and sequencing

A total of 97 specimens, 41 identified as *H. corynosoma* and 56 as *S. hispida* were placed individually in tubes and digested overnight at 56°C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM Na₂-EDTA (pH 8.0), 1% Sarkosyl and 0.1 mg mL⁻¹ proteinase K. Following digestion, DNA was extracted from the supernatant using the DNazol reagent (Molecular Research Center, Cincinnati, Ohio, USA) according to the manufacturer's instructions. The *cox1* of the mitochondrial DNA was amplified using the forward primer 5'-AGTTCTAATCATAA(R)GATAT(Y)GG-3' and reverse primer 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.*, 1994). Polymerase chain reactions (PCRs) (25 µL) consisted of 10 µL of each primer, 2.5 µL of 10× buffer, 2 mM MgCl₂ 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 post-amplification incubation at 72°C for 10 min. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts, USA) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts, USA) and submitted to the GenBank dataset (Table 1).

Alignments, population genetic structure and historical demographic

Newly obtained sequences in the current research of *S. hispida* were aligned with 22 other sequences of *S. hispida* (EF467867–868, HM636468–469, FJ824172–189) downloaded from GenBank (Table 1), forming a dataset of 78 sequences with 646 characters, and the new sequences of *H. corynosoma* were aligned with other sequences of *H. corynosoma* (EU189485–486, EU189488 and EF467869), downloaded from GenBank (Table 1), forming a dataset of 46 sequences with 644 characters. Sequences of each dataset were aligned separately using software ClustalW with default parameters implemented in MEGA version 7.0 (Kumar *et al.*, 2016).

To analyse the molecular information in the framework of population genetics, we grouped individuals of *H. corynosoma* and *S. hispida* into populations considering the biogeographic

Table 1. Specimens' information, collection sites (CS), host name; number of host examined/infected (prevalence of infection); number of specimens analysed (*n*); locality, geographical coordinates, GenBank accession number for specimens studied in this study

CS	State	Species	Host	<i>n</i>	Locality	Coordinates		GenBank
						North	West	Cox1
Tamaulipas								
1		<i>Southwellina hispida</i>	<i>Pelecanus occidentalis</i> (5/1)	1	Laguna Madre, Tamaulipas	24°29'00"	97°45'00"	FJ824179
Veracruz								
2		<i>Hexaglandula corynosoma</i>	<i>Nyctanassa violacea</i> (2/2)	5	Rivera, Veracruz	22°06'54.4"	97°46'37.6"	OQ152151-155
		<i>S. hispida</i>	<i>Ardea alba</i> (1/1)	1	Rivera, Veracruz			OQ152190
3		<i>S. hispida</i>	<i>Thalasseus maximus</i> (5/3)	5	Tamiahua, Veracruz	21°15'49"	97°27'41"	OQ152203-207
4		<i>S. hispida</i>	<i>Nycticorax nycticorax</i> (4/3)	1	Los Chivos, Veracruz	18°56'13"	95°58'08"	FJ824173
5		<i>H. corynosoma</i>	<i>N. violacea</i> (8/6)	6	Tlacotalpan, Veracruz	18°36'00"	95°39'00"	EF467869 OQ152124-128
		<i>S. hispida</i>	<i>Tigrisoma mexicanum</i> (1/1)	1	Tlacotalpan, Veracruz			EF467867 OQ152164-166
		<i>S. hispida</i>	<i>A. alba</i> (1/1)	1	Tlacotalpan, Veracruz			HM636468
		<i>S. hispida</i>	<i>Nannopterum brasiliense</i> (7/5)	4	Tlacotalpan, Veracruz			HM636469 OQ152167-169
		<i>S. hispida</i>	<i>Rupornis magnirostris</i> (2/2)	2	Tlacotalpan, Veracruz			OQ152218-219
		<i>S. hispida</i>	<i>Cichlasoma urophthalmum</i> (10/5)	1	Tlacotalpan, Veracruz			FJ824172
6		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	5	Catemaco, Veracruz	18°24'21.6"	95°6'7.2"	OQ152139-143
		<i>S. hispida</i>	<i>N. nycticorax</i> (4/3)	1				FJ824173
Tabasco								
7		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	1	Espino, Tabasco	18°14'47"	92°49'57"	EU189486
		<i>S. hispida</i>	<i>Egretta garzetta</i> (1/1)	1	Espino, Tabasco			EF467868
Campeche								
8		<i>S. hispida</i>	<i>Egretta tricolor</i> (1/1)	1	Laguna de Términos, Campeche	18°44'01"	91°34'50"	FJ824174
9		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	4	Champoton, Campeche	19°16'50"	90°36'51"	OQ152156-159
		<i>S. hispida</i>	<i>Pandion haliaetus</i> (1/1)	1	Champoton, Campeche			OQ152208
10		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	3	Ulumal, Campeche	19°17'50"	90°37'15"	OQ152144-146
Yucatán								
11		<i>S. hispida</i>	<i>A. alba</i> (1/1)	5	Celestún, Yucatán	20° 50' 53.5"	90° 24' 22"	FJ824175 OQ152170-173
		<i>S. hispida</i>	<i>C. urophthalmum</i> (6/4)	1	Celestún, Yucatán			FJ824176
12		<i>H. corynosoma</i>	<i>Leptuca spinicarpa</i>	1	Chuburna, Yucatán	21°13'18"	89°49'44"	EU189485
		<i>S. hispida</i>	<i>Egretta caerulea</i> (1/1)	6	Chuburna, Yucatán			OQ152191-196
13		<i>S. hispida</i>	<i>C. urophthalmum</i> (10/5)	5	Progreso, Yucatán	21°16'24.2"	89°39'34.9"	FJ824178 OQ152186-189
14		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	6	Ría Lagartos, Yucatán	21°35'32"	88°09'48"	EU189487 OQ152129-133
		<i>S. hispida</i>	<i>C. urophthalmum</i> (5/4)	1	Ría Lagartos, Yucatán			FJ824177
Baja California Norte								
15		<i>S. hispida</i>		1		30°24'57"	115°54'72"	FJ824189

(Continued)

Table 1. (Continued.)

CS	State	Species	Host	n	Locality	Coordinates		GenBank
						North	West	Cox1
			<i>Botaurus lentiginosus</i> (1/1)		Bahía de San Quintin, Baja California Norte			
Baja California Sur								
16		<i>S. hispida</i>	<i>Nannopterum auritus</i> (3/1)	3	Guerrero, Negro, Baja California Sur	27°57'32"	114°03'22"	OQ152180-181 FJ824187
		<i>S. hispida</i>	<i>N. nycticorax</i> (1/1)	3	Guerrero, Negro, Baja California Sur			FJ824188 OQ152182-183
Sinaloa								
17		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	4	Caimanero, Sinaloa	22°53'4.65"	106°03'39.15"	OQ152147-150
Nayarit								
18		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	6	La Tovara, Nayarit	21°32'43.66"	105°16'24.12"	EU189488 OQ152134-138
		<i>S. hispida</i>	<i>N. brasiliensis</i> (2/1)	1	La Tovara, Nayarit			FJ824185
		<i>S. hispida</i>	<i>Anhinga anhinga</i> (3/1)	2	La Tovara, Nayarit			OQ152174 FJ824186
		<i>S. hispida</i>	<i>Butorides virescens</i> (2/1)	3	La Tovara, Nayarit			OQ152175-176 FJ824184
		<i>S. hispida</i>	<i>Ardea herodias</i> (2/1)	3	La Tovara, Nayarit			OQ152177-178 FJ824183
		<i>S. hispida</i>	<i>T. mexicanum</i> (1/1)	2	La Tovara, Nayarit			OQ152179 FJ824182
Guerrero								
19		<i>S. hispida</i>	<i>Cichlasoma</i> <i>trimaculatum</i> (5/3)	3	Tres Palos, Guerrero	16°48'00"	99°47'00"	FJ824180 OQ152184-185
		<i>S. hispida</i>	<i>N. brasiliensis</i> (1/1)	1	Tres Palos, Guerrero			FJ824181
20		<i>S. hispida</i>	<i>N. nycticorax</i> (1/1)	1	Marquelia, Guerrero	16°33'19.75"	98°48'38.89"	OQ152217
Oaxaca								
21		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	1	Laguna Manialtepec, Oaxaca	15°56'25"	97°11'40"	OQ152123
		<i>S. hispida</i>	<i>A. alba</i> (1/1)	4	Laguna Manialtepec, Oaxaca			OQ152197-200
		<i>S. hispida</i>	<i>N. brasiliensis</i> (2/1)	2	Laguna Manialtepec, Oaxaca			OQ152201-202
Chiapas								
22		<i>H. corynosoma</i>	<i>N. violacea</i> (1/1)	4	Tonala, Chiapas	15°58'20"	93°51'23"	OQ152160-163
		<i>S. hispida</i>	<i>A. alba</i> (2/2)	5	Tonala, Chiapas			OQ152212-216
23		<i>S. hispida</i>	<i>N. nycticorax</i> (1/1)	3	Pijijapan, Chiapas	15°33'02"	93°15'53"	OQ152209-211

The sample number for each locality corresponds with the same number in Fig. 1. Sequences in bold were generated in the current study.

provinces (California, Baja California, Pacific Lowlands, Tamaulipas, Veracruz and the Yucatán Peninsula). Intrapopulation variation was summarized using standard statistics: number of haplotypes (H), number of segregating sites (S), haplotype diversity (H_d), nucleotide diversity (P_i) and average number of nucleotide differences (K), were all calculated using the program DnaSP v.5.10 (Rozas *et al.*, 2003). To examine haplotype frequency among the populations of *H. corynosoma* and *S. hispida* a statistical network was constructed independently, using the program PopART with the median joining algorithm (Bandelt *et al.*,

1999). The degree of genetic differentiation among the populations was estimated using the fixation indices F_{st} (Hudson *et al.*, 1992), with the program Arlequin v.3.5 (Excoffier and Lischer, 2010). To investigate the genetic variation among populations or within populations, the analysis of molecular variance was performed, considering genetic distance among the haplotypes using Arlequin v.3.5. To investigate the population history and demography, Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) were calculated using DnaSP v.5.10 (Rozas *et al.*, 2003). The values were considered significant when the P values were less than 0.05.

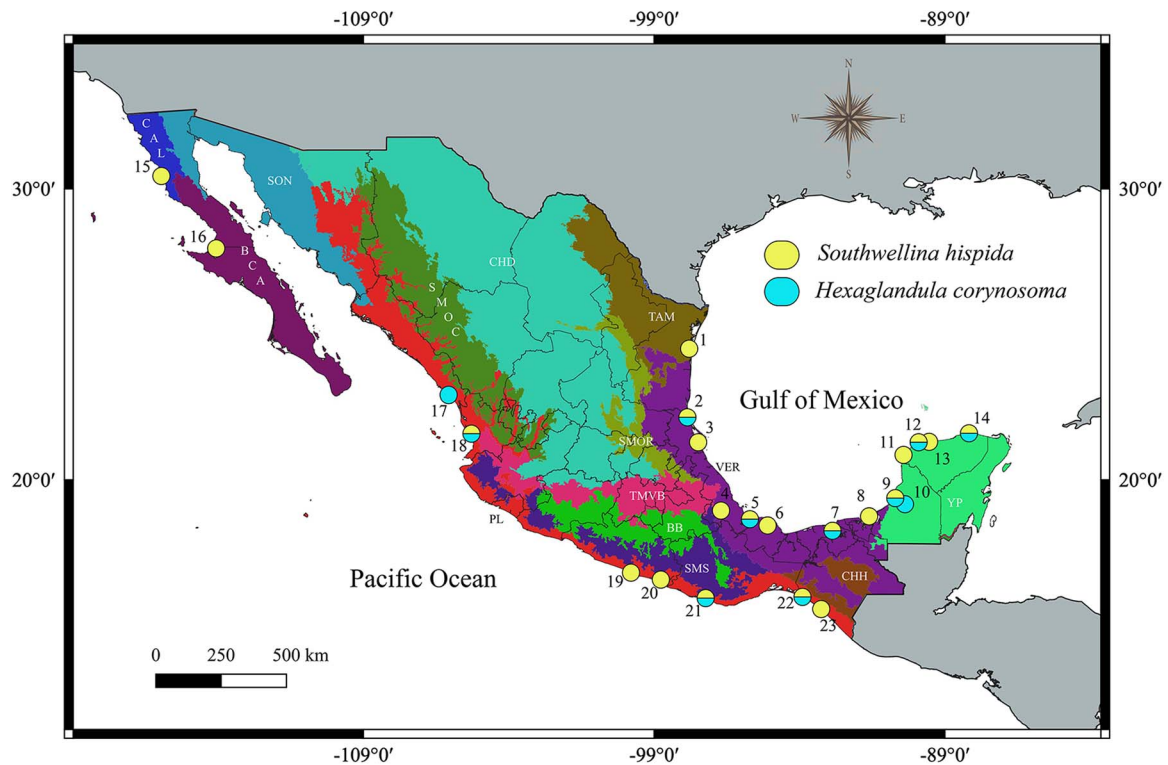


Fig. 1. Map of Mexico showing the sampled sites for the birds. Localities with a circle of yellow and turquoise colour were positive for the infection with *Southwellina hispida* and *Hexaglandula corynosoma*, respectively; localities correspond to those in Table 1.

Results

Morphological identification

The acanthocephalans recovered from diverse definitive hosts such as herons, gulls, cormorants, pelicans and hawks on both coasts of Mexico show similar morphological characteristics compared with those assigned to *S. hispida* by García-Varela *et al.* (2012) and Amin *et al.* (2022), including (i) an elongated cylindrical trunk with 2 fields of somatic spines on the anterior region of the trunk, (ii) a cylindrical proboscis with a swollen region, (iii) proboscis hooks arranged in 16–17 longitudinal rows of 12–15 hooks per row, (iv) a double-walled proboscis receptacle and (v) 4 tubular cement glands in males (Fig. 2A–E). The acanthocephalans recovered from the intestine of the yellow-crowned night-heron (*N. violacea*) in coastal Mexico show morphological characteristics that match those assigned to *H. corynosoma* by Nickol *et al.* (2002), Guillén-Hernández *et al.* (2008) and Amin *et al.* (2022), including (i) an elongate cylindrical trunk, swollen anteriorly with a single field of somatic spines in the anterior region of the trunk; (ii) a cylindrical proboscis; (iii) proboscis hooks arranged in 16 longitudinal rows of 11–12 hooks per row; (iv) a double-walled proboscis receptacle and (v) 6 tubular cement glands in males (Fig. 3A–E).

Population genetic structure and demographic analysis

The mitochondrial marker was successfully amplified for 56 *S. hispida* individuals and 41 *H. corynosoma* individuals. The complete alignment of the *cox1* dataset contained 78 *S. hispida* individuals with a total length of 646 bp, whereas the *cox1* dataset of *H. corynosoma* contained 46 individuals with a total length of 644 bp. No insertions or deletions were detected in any of the sequences, and when the sequences were translated into proteins, no stop codons were found.

The haplotype network built for the generalist species *S. hispida* did not show a phylogeographic structure; in fact, of the

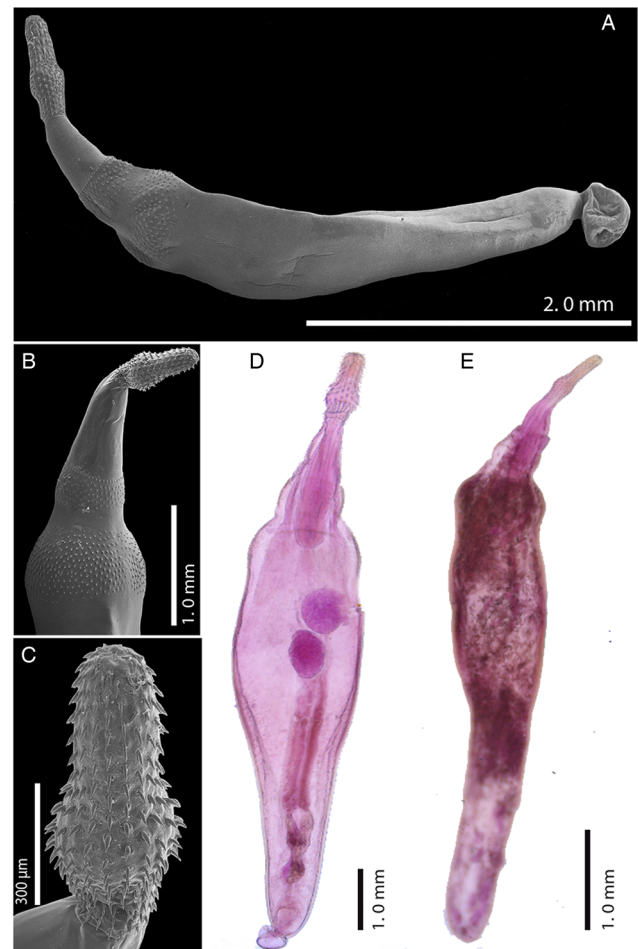


Fig. 2. Scanning electron micrographs and photomicrographs of *S. hispida* from *Ardea alba* from Tonalá, Chiapas, Mexico (locality 22 in Fig. 1 and Table 1): adult male, whole worm (A); male anterior region (B); proboscis (C); adult male, whole worm (D) and adult female, whole worm (E).

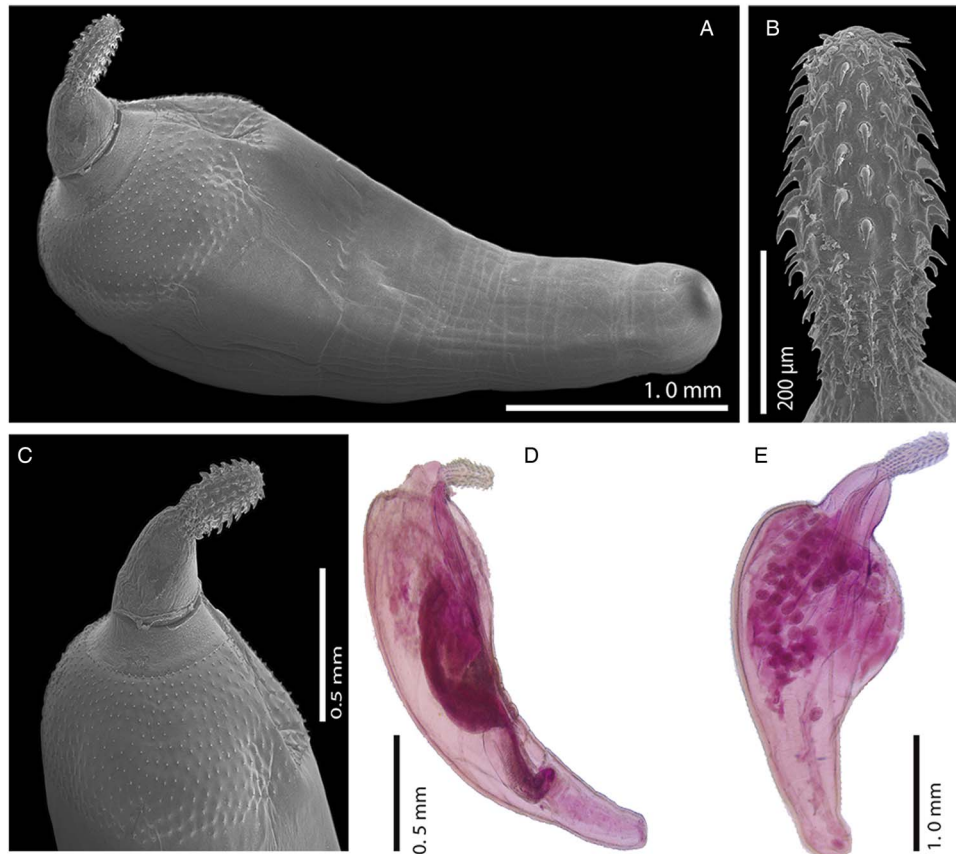


Fig. 3. Scanning electron micrographs and photomicrographs of *H. corynosoma* from *Nyctanassa violacea* from La Tovarita Nayarit, Mexico (locality 18 in Fig. 1 and Table 1): adult male, whole worm (A); proboscis (B); male anterior region (C); adult male, whole worm (D) and adult female, whole worm (E).

55 mtDNA haplotypes detected, 50 were unique (singlets), and 5 others were shared haplotypes (H4, H11, H17, H29 and H30). The most frequent haplotype (H4, $n = 12$) corresponded to specimens from 4 biogeographic provinces (Pacific Lowlands, Baja California, Veracruz and the Yucatán Peninsula). Most of the identified haplotypes were separated from one another by 1, 2, 3 or up to 5 substitutions (see Fig. 4). The haplotype diversity was very high ($H_d = 0.970$) and nucleotide diversity was low ($p_i = 0.00555$) among the populations from the 4 biogeographic provinces sampled (Pacific Lowlands, Baja California, Veracruz and the Yucatán Peninsula) of Mexico. Neutrality tests (Tajima's D , -2.473 and Fu's F_s , -34.252) were negative for all regions (see Table 2), indicating an excess of rare alleles greater than what would be expected under neutrality and suggesting a recent population expansion of *S. hispidus*. The F_{st} values were estimated to assess genetic differentiation among populations from the 4 biogeographic provinces analysed (Pacific Lowlands, Baja California, Veracruz and the Yucatán Peninsula). Despite the large geographic distances, the F_{st} values were low, ranging from -0.007 to 0.058 (Table 3), which indicates that the populations were poorly genetically differentiated from one another. The sampled definitive and paratenic host species of *S. hispidus* were grouped into 8 families (Fig. 5). The host haplotype network did not show a pattern; 30 haplotypes were found in herons scattered throughout the network. In the paratenic hosts sampled (cichlid fishes), 11 haplotypes were detected (H3, H6, H7, H8, H10, H17, H30, H32, H33, H34 and H35), suggesting that the paratenic hosts are able to harbour and transmit diverse haplotypes to definitive hosts (Fig. 5).

The haplotype network of the specialist species *H. corynosoma* did not show a phylogeographic structure for the 42 mtDNA haplotypes detected; 41 were unique (singlets), and only 1 was shared

(H2, $n = 4$) between 2 biogeographic provinces (Veracruz and the Yucatán Peninsula). Most of the identified haplotypes were separated by several substitutions (see Fig. 6). The haplotype diversity ($H_d = 0.993$) was very high and nucleotide diversity was low ($p_i = 0.01166$) among the populations from the 3 biogeographic provinces sampled (Pacific Lowlands, Veracruz and the Yucatán Peninsula). Neutrality tests (Tajima's D , -1.875 and Fu's F_s , -33.964) were negative for all regions (see Table 4), which indicates an excess of rare alleles greater than what would be expected under neutrality, suggesting a recent population expansion of *H. corynosoma*. The F_{st} values were estimated to assess genetic differentiation among the populations from the 3 biogeographic provinces analysed (Pacific Lowlands, Veracruz and the Yucatán Peninsula). Despite the large geographic distances, the F_{st} values were low, ranging from -0.019 to 0.059 (Table 5), which indicates that the populations were poorly genetically differentiated from one another.

Discussion

To the best of our knowledge, *S. hispidus* and *H. corynosoma* are 2 species that use piscivorous birds as definitive hosts and decapods as intermediate hosts, share a common ancestor within Polymorphidae and are distributed sympatrically along the coastline of Mexico (Guillén-Hernández *et al.*, 2008; García-Prieto *et al.*, 2010; García-Varela *et al.*, 2013b). The generalist species *S. hispidus* was described in Japan by Van Cleave in 1925, and since then it has been recorded in the body cavities of fishes and reptiles as a cystacanth (larval stage) and as an adult form in the intestines of diverse piscivorous birds in Asia, Europe and the Americas (see Amin *et al.*, 2022). In Mexico, adults of *S. hispidus* have been recorded in 15 piscivorous bird species

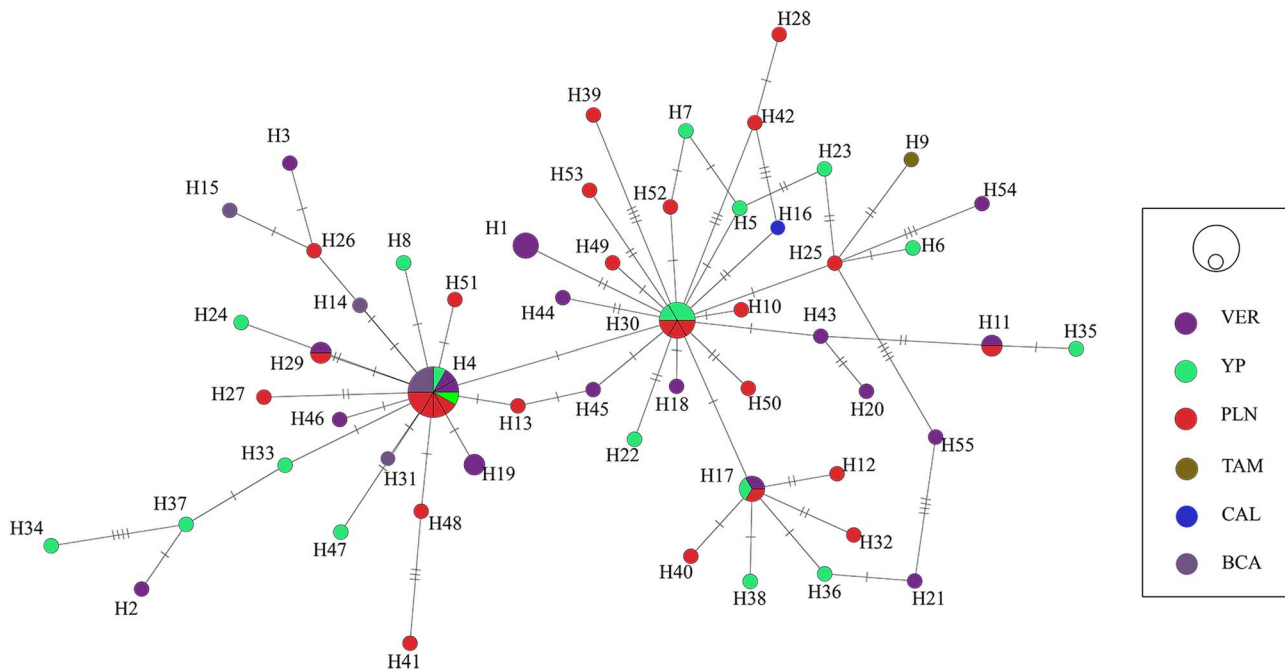


Fig. 4. Haplotype network of samples of *S. hispidia*, built with the gene *cox1* from mitochondrial DNA. Each circle represents a haplotype, with size proportional to the haplotype’s frequency in the populations. Mutational steps are symbolized by dashes. Biogeographic provinces: Veracruz (VER); Yucatán Peninsula (YUC); Pacific Lowlands (PLN); Tamaulipas (TAM); California (CAL) and Baja California (BCA).

Table 2. Molecular diversity indices and neutrality tests calculated for *cox1* datasets among the populations of *S. hispidia* used in this study

Biogeographic provinces	<i>n</i>	<i>S</i>	<i>H</i>	<i>H_d</i> ± s.d.	<i>P_i</i> ± s.d.	<i>K</i>	Tajima’s <i>D</i>	Fu’s <i>F_s</i>
Veracruz	21	29	18	0.981 ± 0.023	0.00663 ± 0.00064	4.285	−1.811	−13.228
Yucatán Peninsula	19	26	17	0.988 ± 0.021	0.00607 ± 0.00083	3.918	−1.859	−13.746
Baja California	7	7	5	0.857 ± 0.137	0.00310 ± 0.00095	2.000	−1.553	−1.548
Pacific Lowlands	31	33	23	0.955 ± 0.026	0.00488 ± 0.00064	3.152	−2.282	−21.079
All	78	67	55	0.970 ± 0.012	0.00555 ± 0.00041	3.587	−2.473	−34.252

n, number of sequences; *H*, number of haplotypes; *S*, number of segregating sites; *H_d*, haplotype diversity; *P_i*, nucleotide diversity; *K*, average number of nucleotide differences.

Table 3. Pairwise *F_{st}* values estimated for *cox1*

	VER	YP	BCAL	PL
VER	–			
YP	−0.007	–		
BCAL	0.088	0.082	–	
PL	0.010	0.005	0.058	–

VER, Veracruz; YP, Yucatan Peninsula; BCAL, Baja California; PL, Pacific Lowlands. *F_{st}* average among 3 biogeographic provinces = 0.03672. Significance level = 0.05.

(García-Prieto *et al.*, 2010; García-Varela *et al.*, 2012). It is well known that helminths with a broad spectrum of definitive hosts show phenotypic plasticity in their morphological traits. Schmidt (1973), García-Varela *et al.* (2012) and Amin *et al.* (2022) documented the phenotypic plasticity of *S. hispidia* recovered from diverse definitive hosts including in traits such as body size, leminisci, proboscis receptacle, testes, cement glands and the reproductive system in females. However, diagnostic characters such as proboscis shape, number of proboscis hooks and the

presence of 2 fields of spines on the anterior trunk region in both sexes did not vary among specimens, including our specimens collected from the coasts of the Gulf of Mexico and the Pacific Ocean slopes (see Fig. 2A–E). In Brazil, the specialist species *H. corynosoma* was described as parasite of the yellow-crowned night heron (*N. violacea*) by Travassos in 1915. Since then, *H. corynosoma* has been recorded in Puerto Rico, the USA and Mexico in the same definitive host species (Cable and Quick, 1954; Nickol *et al.*, 2002; Guillén-Hernández *et al.*, 2008). Our specimens identified as *H. corynosoma* agree morphologically with those previously described by Nickol *et al.* (2002) and Guillén-Hernández *et al.* (2008). For example, an elongated trunk, swollen anteriorly; covered with spines on the anterior region of the trunk; a cylindrical proboscis with 16 longitudinal rows of 11–12 hooks each; a conical neck; ovoid testes located in the swollen portion of the trunk and long, tubular cement glands (see Fig. 3A–E).

The intraspecific genetic divergence estimated in the current study among the 78 isolates of *S. hispidia* and the 46 isolates of *H. corynosoma* ranged from 0.00 to 1.5% and from 0.00 to 2.6%, respectively. These values of intraspecific genetic divergence are similar to those previously reported for isolates of polymorphid species such as *Andracantha sigma* (Presswell *et al.*, 2017)

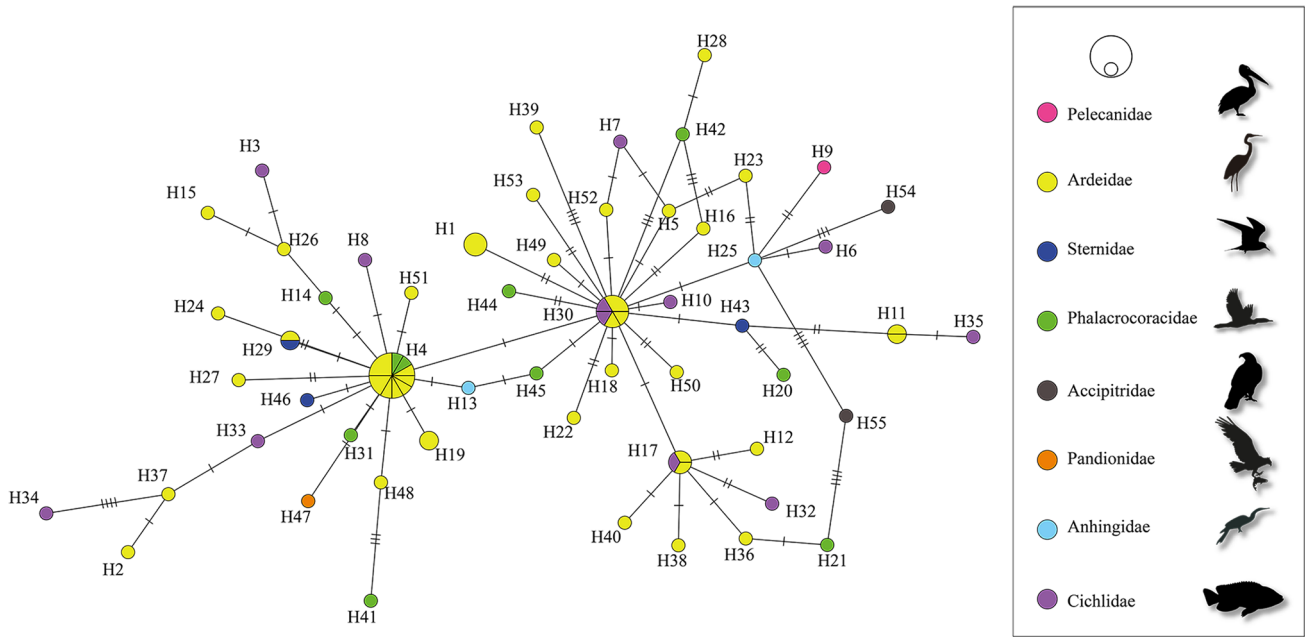


Fig. 5. Host haplotype network of samples of *S. hispidi*, built with the gene *cox1* from mitochondrial DNA. The paratenic and definitive hosts were grouped by families. Each circle represents a haplotype, with size proportional to the haplotype's frequency in the populations. Mutational steps are symbolized by dashes.

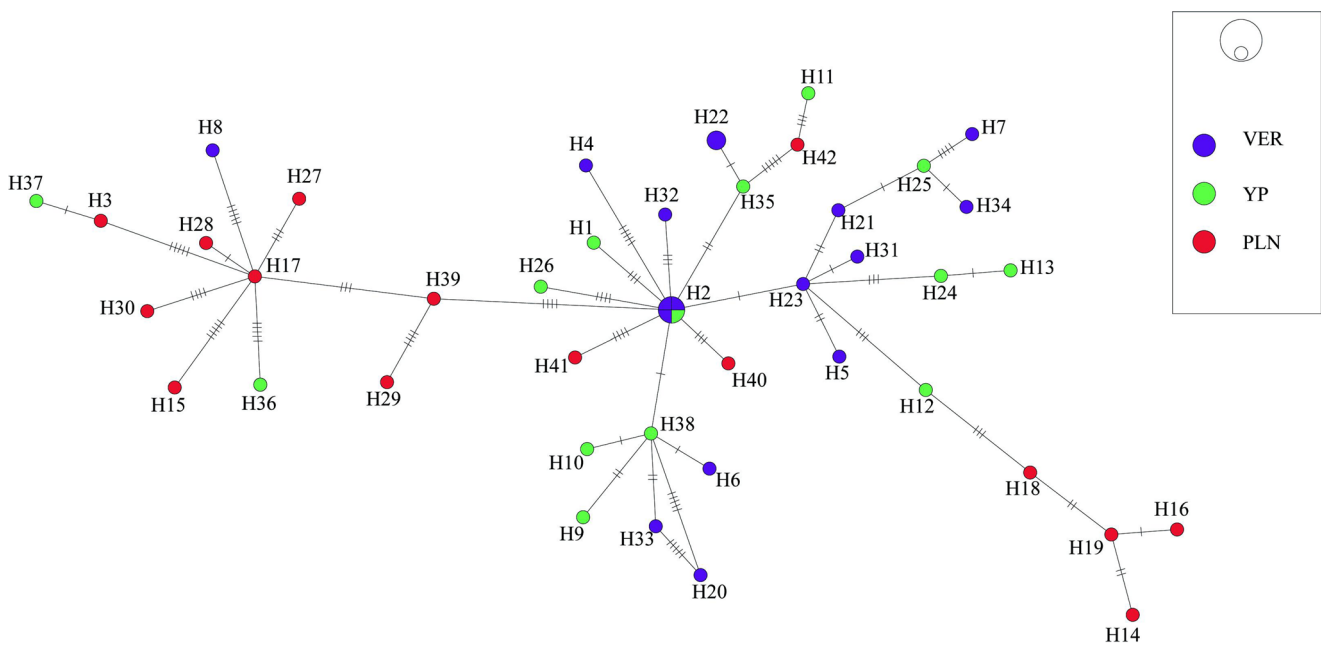


Fig. 6. Haplotype network of samples of *H. corynosoma*, built with the gene *cox1* from mitochondrial DNA. Each circle represents a haplotype, with size proportional to the haplotype's frequency in the populations. Mutational steps are symbolized by dashes. Biogeographic provinces: Veracruz (VER); Yucatán Peninsula (YUC) and Pacific Lowlands (PLN).

Table 4. Molecular diversity indices and neutrality tests calculated for *cox1* datasets among the populations of *H. corynosoma* used in this study

Biogeographic provinces	<i>n</i>	<i>S</i>	<i>H</i>	<i>H_d</i> ± s.d.	<i>P_i</i> ± s.d.	<i>K</i>	Tajima's <i>D</i>	Fu's <i>F_s</i>
Veracruz	17	32	14	0.971 ± 0.032	0.00884 ± 0.00132	5.691	-1.707	-6.056
Yucatán Peninsula	14	38	14	1.0 ± 0.027	0.01165 ± 0.00177	7.505	-1.613	-8.104
Pacific Lowlands	15	35	15	1.0 ± 0.024	0.01344 ± 0.00114	8.657	-0.926	-8.273
All	46	66	42	0.993 ± 0.007	0.01166 ± 0.00083	7.510	-1.875	-33.964

n, number of sequences; *H*, number of haplotypes; *S*, number of segregating sites; *H_d*, haplotype diversity; *P_i*, nucleotide diversity; *K*, average number of nucleotide differences.

Table 5. Pairwise F_{st} values estimated for *cox*

	VER	YP	PL
VER	–		
YP	–0.019	–	
PL	0.112	0.059	–

VER, Veracruz; YP, Yucatan Peninsula; PL, Pacific Lowlands
 F_{st} average among 3 biogeographic provinces = 0.05609.
 Significance level = 0.05.

recovered from 3 definitive hosts, the Otago shag, *Leucocarbo chalconotus* (Gray), spotted shag *Phalacrocorax punctatus* (Sparrman) and Otago little blue penguin, *Eudyptula novaehollandiae* (Forster) from New Zealand, which ranged from 0.00 to 0.32% (Presswell *et al.*, 2017); similar to those among 14 adults, 3 acanthella and 4 cystacanths of *Pseudocorynosoma constrictum* (Van Cleave, 1918) Aznar *et al.* (2006) recovered from 7 wild duck species and the freshwater amphipod *Hyaella azteca* (Saussure) in central Mexico, which ranged from 0.0 to 3.0% (García-Varela *et al.*, 2013a) and similar to those among 19 adults recovered from 3 fish-eating bird species and 33 cystacanths recovered from 19 freshwater fish species identified as *Polymorphus brevis* (Van Cleave, 1916) Travassos, 1926, which ranged from 0.00 to 1.6% (Alcántar-Escalera *et al.*, 2013). Furthermore, the values from this study were also similar to those among adults and cystacanths of *Corynosoma hanna* Zdzitowiecki, 1984 recovered from the New Zealand sea lion (*Phocartos hookeri* Grey), Stewart Island shag (*L. chalconotus* Gray), spotted shags (*P. punctatus* Sparrman), yellow-eyed penguins (*Megadyptes antipodes* Hombrón and Jacquinet), New Zealand brill (*Colistium guntheri* Hutton) and New Zealand sole (*Peltorhamphus novaezeelandiae* Gunther), which ranged from 0.00 to 2.8% (Hernández-Orts *et al.*, 2017) and those among *Corynosoma australe* (Johnston, 1937) recovered from the California sea lion (*Zalophus californianus* Lesson), South American sea lions (*Otaria flavescens* Shaw), South American fur seals (*Arctocephalus australis* Zimmermann), Magellanic penguins (*Spheniscus magellanicus* Forster) and cystacanths recovered from marine fishes in Argentina, which ranged from 1.0 to 1.7% (García-Varela *et al.*, 2021).

The haplotype network genealogy generated in this study based on *cox1* sequences from *S. hispida* (generalist) and *H. corynosoma* (specialist) did not show a phylogeographic structure; therefore, the haplotypes could not be grouped into their own geographic clusters. In fact, the specimens of *S. hispida* were not correlated with their definitive host family (see Figs 4 and 5). The populations analysed for both species of acanthocephalans were classified into biogeographical provinces separated by geographical barriers as follows: mountains, the dry lowlands of the Isthmus of Tehuantepec, the Balsas Depression and the central Trans-Mexicana Volcanic Belt (Barrier *et al.*, 1998; Ferrari *et al.*, 2012; Morrone *et al.*, 2017). Despite the large geographic distances, the F_{st} values estimated among the populations of both species were very low (Tables 3 and 5), indicating that the populations were poorly genetically differentiated from each other; this can be explained by the migration patterns of birds along the coasts of Mexico. Historical events leave signatures in the DNA, and neutrality tests can infer the demographic history of populations. In both species analysed (generalist and specialist), the estimated values of Fu's F_s and Tajima's D among the populations were negative (see Tables 2 and 4). In addition, high haplotype diversity and an excess of low-frequency haplotypes were detected in both networks (Figs 4 and 6), although the haplotypes differed from one another by fewer than 5

nucleotide substitutions. These findings confirmed that both populations of acanthocephalans had experienced rapid population growth in the past.

In this study, we found that 2 species of acanthocephalans with opposing life history strategies (generalist and specialist) showed similar population genetics patterns. This pattern was not consistent with the specialist–generalist variation hypothesis (SGVH), which predicts that: (i) populations of specialists may be less connected and more subdivided into smaller populations than generalists; (ii) specialists are expected to have lower effective population sizes than generalists and to be composed of populations with less gene flow and (iii) generalists will show high effective population sizes, high genetic flow and highly structured populations (see Dennis *et al.*, 2011; Li *et al.*, 2014). According to the SGVH, the populations of *S. hispida* (generalist species) resemble the specialist model. To date, only a few phylogeographic studies of generalist acanthocephalans have been conducted using *cox1* as a molecular marker. For example, *Profilicollis altmani* (Perry, 1942), which has a broad distribution across North and South America and parasitizes multiple species of intermediate hosts and diverse species of marine birds that act as definitive hosts (gulls, ducks, sanderling and common tern), showed a lack of population genetic structure with high haplotype diversity and low nucleotide diversity, suggesting that *P. altmani* have experienced a period of rapid population growth in the past (see Goulding and Cohen, 2014). Similarly, *Profilicollis novaezeelandensis* Brockerhoff and Smales (2002) is a parasite that has been recorded as an adult in gulls (*Larus* spp.) and oystercatchers (*Haematopus* spp.) and as cystacanths in the shore crab *Hemigrapsus crenulatus* (Milne-Edwards) distributed along the east coast of New Zealand's South Island (Brockerhoff and Smales, 2002); population genetic analyses from 50 *P. novaezeelandensis* individuals from 8 localities showed a lack of population genetic structure with high haplotype diversity and low nucleotide diversity (Hay *et al.*, 2018).

The 2 species of acanthocephalans analysed here are considered to be typical components of the helminth fauna of piscivorous birds in the Americas. Of the 19 piscivorous birds recorded as definitive hosts, 11 belong to the family Ardeidae, which represent 58% of the host diversity, suggesting that the ardeids could be the ancestral hosts of *S. hispida* and *H. corynosoma* with secondary and independent colonization events to other piscivorous birds. In particular, *S. hispida* was recorded in diverse piscivorous birds such as pelicans, cormorants, eagles, hawks, anhingas and royal terns with a lower level of infection (prevalence values <20%) than those of ardeid hosts (prevalence values from 80 to 100%), suggesting that these piscivorous birds may act as suboptimal definitive hosts.

The population genetic structures of *S. hispida* and *H. corynosoma* showed similar patterns. However, these species use different strategies to complete their life cycles. The main ecological difference between *S. hispida* and *H. corynosoma* is the inclusion of paratenic hosts in *S. hispida* (fishes from the families Cichlidae, Eleotridae, Characidae, Lutjanidae, Centropomidae, Sparidae, Scianidae and Paralichthyidae) (García-Prieto *et al.*, 2010). The paratenic hosts act as a trophic bridge and facilitate the transmission between the intermediate host and definitive host, but apparently do not play a central role in the population genetic structure of *S. hispida* within its distribution along the coasts of Mexico.

Acknowledgements. We thank Laura Márquez and Nelly López Ortiz from LaNabio for their help during the sequencing of the DNA fragments. We also thank Berenit Mendoza Garfias for her help in obtaining the scanning electron microphotographs. A. L.-J. and M. T. G.-G. acknowledge the support of the Programa de Posgrado en Ciencias Biológicas, UNAM and CONACYT (A. L.-J. CVU No. 706119; M. T. G.-G. CVU No. 956064), for granting a

scholarship to complete her PhD and his Master program, respectively. L. A.-G. acknowledges the Coordinación de la Investigación Científica and Dirección General de Asuntos de Personal Académico (DGAPA-UNAM), Mexico, for granting the Postdoctoral Fellowship.

Author's contributions. M. G.-V. and A. L. S.-U. conceived and designed the study. M. G.-V., A. L.-J. and M. T. G.-G. conducted data gathering. A. L.-J. and M. T. G.-G. performed statistical analyses. A. L. S.-U., L. A.-G. and M. G.-V. wrote and edited the article.

Financial support. This research was supported by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN201122.

Conflict of interest. No conflict of interest exists among the authors.

Ethical standards. The sampling in this work complies with the current laws and animal ethics regulations of México. Specimens were collected under the Cartilla Nacional de Colector Científico (FAUT 0202) issued by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT), to M. G.-V.

References

- Alcántar-Escalera FJ, García-Varela M, Vázquez-Domínguez E and Pérez Ponce de León G (2013) Using DNA barcoding to link cystacanths and adults of the acanthocephalan *Polymorphus brevis* in Central Mexico. *Molecular Ecology Resources* **13**, 1116–1124.
- Amin OM (2013) Classification of the Acanthocephala. *Folia Parasitologica* **60**, 273–305.
- Amin OM, Chaudhary A and Singh HS (2022) Morphological and molecular description of immature *Southwellina hispida* (Van Cleave, 1925) Witenberg, 1932 (Acanthocephala: Polymorphidae) from the body cavity of the paratenic host *Gillichthys mirabilis* Cooper (Gobiidae) in California, with analyses of the chemical composition of hooks and spines. *Acta Parasitologica* **67**, 1107–1125.
- Archie AE and Ezenwa VO (2011) Population genetic structure and history of a generalist parasite infecting multiple sympatric host species. *International Journal for Parasitology* **41**, 89–98.
- Aznar FJ, Pérez Ponce de León G and Raga JA (2006) Status of *Corynosoma* (Acanthocephala: Polymorphidae) based on anatomical, ecological and phylogenetic evidence, with the erection of *Pseudocorynosoma* n. gen. *Journal of Parasitology* **92**, 548–564.
- Bandelt H, Forster P and Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37–48.
- Barrett LG, Thrall PH, Burdon JJ and Linde CC (2008) Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends in Ecology & Evolution* **23**, 678–685.
- Barrier E, Velasquillo L, Chavez M and Gaulon R (1998) Neotectonic evolution of the Isthmus of Tehuantepec (southeastern Mexico). *Tectonophysics* **287**, 77–96.
- Brant SV and Ortí G (2003) Evidence for gene flow in parasitic nematodes between two host species of shrews. *Molecular Ecology* **12**, 2853–2859.
- Brockhoff AM and Smales LR (2002) *Profilicollis novaezelandensis* n. sp. (Polymorphidae) and two other acanthocephalan parasites from shore birds (Haematopodidae and Scolopacidae) in New Zealand, with records of two species in intertidal crabs (Decapoda: Grapsidae and Ocypodidae). *Systematic Parasitology* **52**, 55–65.
- Cable RM and Quick LA (1954) Some acanthocephalan from Puerto Rico with the description of a new genus and three new species. *Transactions of the American Microscopical Society* **73**, 393–400.
- Criscione CD and Blouin MS (2004) Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. *Evolution* **58**, 198–202.
- Dennis RLH, Dapporto L, Fattorini S and Cook LM (2011) The generalism specialism debate: the role of generalists in the life and death of species. *Biological Journal of the Linnean Society* **104**, 725–737.
- Dimitrova ZM and Georgiev BB (1994) *Ardeirhynchus* n. gen. (Palaeacanthocephala: Polymorphida: Polymorphidae), with a redescription of *A. spiralis* (Rudolphi, 1809) n. comb. *Systematic Parasitology* **29**, 149–158.
- Excoffier L and Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567.
- Ferrari L, Orozco-Esquivel T, Manea V and Manea M (2012) The dynamic history of the Trans-Mexican Volcanic Belt and the Mexico subduction zone. *Tectonophysics* **522**, 122–149.
- Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) DNA primers for the amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.
- Font WF (2007) Parasites of Hawaiian stream fishes: sources and impacts. *Bishop Museum Bulletin in Cultural and Environmental Studies* **3**, 157–169.
- Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**, 915–925.
- García-Prieto L, García-Varela M, Mendoza-Garfias B and Pérez-Ponce de León G (2010) Checklist of the Acanthocephala in wildlife vertebrates of Mexico. *Zootaxa* **2419**, 1–50.
- García-Varela M, Pérez-Ponce de León G, de la Torre P, Cummings MP, Sarma SS and Lacleite JP (2000) Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution* **50**, 532–540.
- García-Varela M, Pérez-Ponce de León G, Aznar FJ and Nadler SA (2011) *Ibirhynchus dimorpha* n. gen. (Acanthocephala: Polymorphidae), inferred through morphological, ecological and molecular data. *Journal of Parasitology* **97**, 97–105.
- García-Varela M, Aznar FJ, Rodríguez RP and Pérez-Ponce de León G (2012) Genetic and morphological characterization of *Southwellina hispida* Van Cleave, 1925 (Acanthocephala: Polymorphidae), a parasite of fish-eating birds. *Comparative Parasitology* **79**, 192–201.
- García-Varela M, Pinacho-Pinacho CD, Sereno-Urbe AL and Mendoza-Garfias B (2013a) First record of the intermediate host of *Pseudocorynosoma constrictum* Van Cleave, 1918 (Acanthocephala: Polymorphidae) in Central Mexico. *Comparative Parasitology* **80**, 171–178.
- García-Varela M, Pérez-Ponce de León G, Aznar FJ and Nadler SA (2013b) Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution* **68**, 176–184.
- García-Varela M, Masper A, Crespo EA and Hernández-Orts JS (2021) Genetic diversity and phylogeography of *Corynosoma australe* Johnston, 1937 (Acanthocephala: Polymorphidae), an endoparasite of otariids from the Americas in the Northern and Southern Hemispheres. *Parasitology International* **80**, 102205.
- Goulding CT and Cohen CS (2014) Phylogeography of a marine acanthocephalan: lack of cryptic diversity in a cosmopolitan parasite of mole crab. *Journal of Biogeography* **41**, 965–976.
- Guillén-Hernández S, García-Varela M and Pérez-Ponce de León G (2008) First record of *Hexaglandula corynosoma* (Travassos, 1915) Petrochenko, 1958 (Acanthocephala: Polymorphidae) in intermediate and definitive hosts in Mexico. *Zootaxa* **1873**, 61–68.
- Hay E, Jorge F and Poulin R (2018) The comparative phylogeography of shore crabs and their acanthocephalan parasites. *Marine Biology* **165**, 69.
- Hernández-Orts JS, Brandão M, Georgieva S, Raga JA, Crespo EA, Luque JL and Aznar FJ (2017) From mammals back to birds: host-switch of the acanthocephalan *Corynosoma australe* from pinnipeds to the Magellanic penguin *Spheniscus magellanicus*. *PLoS ONE* **12**, e0183809.
- Hoberg EP (1986) Aspects of ecology and biogeography of Acanthocephala in Antarctic seabirds. *Annales de Parasitologie Humaine et Comparée* **61**, 199–214.
- Howell SNG and Webb S (1995) *A Guide to the Birds of Mexico and Northern Central America*. New York: Oxford University.
- Hudson RR, Boos DD and Kaplan NL (1992) A statistical test for detecting population subdivision. *Molecular Biology and Evolution* **9**, 138–151.
- Kennedy CR (2006) *Ecology of the Acanthocephala*. New York: Cambridge University Press.
- Kumar S, Stecher G and Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.
- Li S, Jovelín R, Yoshiga T, Tanaka R and Cutter AD (2014) Specialist versus generalist life histories and nucleotide diversity in *Caenorhabditis nematodes*. *Proceedings of the Royal Society B* **281**, 20132858.
- Lievens EJP, Perreau J, Agnew P, Michalakakis Y and Lenormand T (2018) Decomposing parasite fitness reveals the basis of specialization in a two-host, two-parasite system. *Evolution Letters* **2**, 390–405.
- Miller RR, Minckley WL and Norris SM (2005) *Freshwater Fishes of Mexico*. Chicago, IL: The University of Chicago Press.

- Morrone JJ, Escalante T and Rodríguez-Tapia G (2017) Mexican biogeographic provinces: map and shapefiles. *Zootaxa* **4277**, 277–279.
- Nadler SA (1995) Microevolution and the genetic structure of parasite populations. *Journal of Parasitology* **81**, 395–403.
- Near TJ, Garey JR and Nadler SA (1998) Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* **10**, 287–298.
- Nickol BB, Crompton DWT and Searle DW (1999) Reintroduction of *Profilicollis* Meyer, 1931, as a genus in Acanthocephala: significance of the intermediate host. *Journal of Parasitology* **85**, 716–718.
- Nickol BB, Heard RW and Smith NF (2002) Acanthocephalans from crabs in the southeastern US, with the first intermediate hosts known for *Arhythmorhynchus frassoni* and *Hexaglandula corynosoma*. *Journal of Parasitology* **88**, 79–83.
- Perrot-Minnot MJ, Špakulová M, Wattier R, Kotlík P, Düsen S, Ayogdu A and Tougard C (2018) Contrasting phylogeography of two Western Palaearctic fish parasites despite similar life cycle. *Journal of Biogeography* **45**, 101–115.
- Petrochenko VI (1958) *Acanthocephala of Domestic and Wild Animals*, Vol. II. Moscow, Russia: Izdatel'stvo Akademii Nauk SSSR, Vsesoyuznoe Obshchestvo Gel'mintologov (in Russian).
- Pichelin S, Kuris AM and Gurney R (1998) Morphological and biological notes on *Polymorphus (Profilicollis) sphaerocephalus* and *Corynosoma stanleyi* (Polymorphidae: Acanthocephala). *Journal of Parasitology* **84**, 798–801.
- Pinacho-Pinacho CD, García-Varela M, Sereno-Uribe AL and Pérez Ponce de León G (2018) A hyper-diverse genus of acanthocephalans revealed by tree-base and non-tree-base species delimitation methods: ten cryptic species of *Neoechinorhynchus* in Middle American freshwater fishes. *Molecular Phylogenetics and Evolution* **127**, 30–45.
- Poulin R and Morand S (2004) *Parasite Biodiversity*. Washington, DC: Smithsonian Institution Press.
- Presswell B, García-Varela M and Smales LR (2017) Morphological and molecular characterization of two new species of *Andracantha* (Acanthocephala: Polymorphidae) from New Zealand shags (Phalacrocoracidae) and penguins (Spheniscidae) within a key to the species. *Journal of Helminthology* **16**, 1–12.
- Presswell B, Bennett JDL and Smales LR (2020) Morphological and molecular characterisation of a new genus and species of acanthocephalan, *Tenuisoma tarapungi* n. g., n. sp. (Acanthocephala: Polymorphidae) infecting red-billed gulls in New Zealand, with a key to the genera of the Polymorphidae Meyer, 1931. *Systematic Parasitology* **97**, 25–39.
- Price PW (1980) *Evolutionary Biology of Parasites*. Princeton, NJ: Princeton University Press.
- Rigaud T, Perrot-Minnot MJ and Brown MJF (2010) Parasites and host assemblages: embracing the reality will improve our knowledge of parasites transmission and virulence. *Proceedings of the Royal Society B* **277**, 3693–3702.
- Rosas-Valdez R, Morrone JJ and García-Varela M (2012) Molecular phylogenetics of *Floridosentis* Ward, 1953 (Acanthocephala: Neoechinorhynchidae) parasites of mullets (Osteichthyes) from Mexico, using 28S rDNA sequences. *Journal of Parasitology* **98**, 855–862.
- Rosas-Valdez R, Morrone JJ, Pinacho-Pinacho CD, Domínguez-Domínguez O and García-Varela M (2020) Genetic diversification of acanthocephalans of the genus *Floridosentis* Ward, 1953 (Acanthocephala: Neoechinorhynchidae), parasites of mullets from the Americas. *Infection, Genetics and Evolution* **85**. doi: 10.1016/j.meegid.2020.104535.
- Rozas J, Sánchez-DelBarrio JC, Messeguer X and Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics (Oxford, England)* **19**, 2496–2497.
- Schmidt GD (1973) Resurrection of *Southwellina* Witenberg, 1932, with a description of *Southwellina dimorpha* sp. n., a key to genera in Polymorphida (Acanthocephala). *Journal of Parasitology* **59**, 299–305.
- Schmidt, GD (1985) Development and life cycles. In Nickol BB and Crompton DWT (eds), *Biology of the Acanthocephala*. Cambridge, UK: Cambridge University Press, pp. 273–286.
- Sehgal RRM, Jones H and Smith TB (2001) Host specificity and incident of *Trypanosoma* in some African rainforest birds: a molecular approach. *Molecular Ecology* **10**, 2319–2327.
- Sereno-Uribe AL, López-Jiménez A, González-García MT, Pinacho-Pinacho CD, Macip Ríos R and García-Varela M (2022) Phenotypic plasticity, genetic structure, and systematic position of *Neoechinorhynchus emyditoides* Fisher, 1960 (Acanthocephala: Neoechinorhynchidae) a parasite of emydid turtles from the Nearctic and Neotropical regions. *Parasitology* **149**, 991–1002.
- Steinauer ML, Nickol BB and Ortí G (2007) Cryptic speciation and patterns of phenotypic variation of variable acanthocephalan parasite. *Molecular Ecology* **16**, 4097–4109.
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595.
- Thompson JN (1994) *The Coevolutionary Process*. Chicago: University of Chicago Press.
- Thompson JN (2005) *The Geographic Mosaic of Coevolution*. Chicago: University of Chicago Press.
- Travassos L (1915) Revisão dos acanthocephalos brasileiros. II. Família Echinorhynchidae Hamann, 1892 (2 nota previa). *Brazil Medico* **48**, 377.
- Van Cleave HJ (1925) Acanthocephala from Japan. *Parasitology* **17**, 149–156.