Phylogeny, genetics, and the partial life cycle of Oncomegas wageneri in the Gulf of Mexico

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Despite the diversity and ecological importance of cestodes, there is a paucity of studies on their life stages (i.e., complete lists of intermediate, paratenic, and definitive hosts) and genetic variation. For example, in the Gulf of Mexico (GoM) 98 species of cestodes have been reported to date; however, data on their intraspecific genetic variation and population genetic studies are lacking. The trypanorhynch cestode, Oncomegas wageneri, is found (among other places) off the American Western Atlantic Coast, including the GoM, and has been reported as an adult from stingrays and from several teleost species in its larval form (as plerocerci). This study represents the first report of 2 previously unregistered definitive hosts for O. wageneri, namely the Atlantic sharpnose shark Rhizoprionodon terraenovae and the southern stingray Hypanus americanus. In this work, partial sequences of the 28S (region D1–D2) ribosomal DNA were analyzed to include O. wageneri within an eutetrarhynchoid phylogenetic framework. All O. wageneri individuals (which included plerocerci and adults) were recovered as monophyletic and Oncomegas celatus was identified as the sister species of O. wageneri. Furthermore, population genetic analyses of O. wageneri from the southern GoM were carried out using DNA sequences of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene, which reflected high genetic variation and a lack of genetic structure among the 9 oceanographic sampling sites. Based on these results, O. wageneri is panmictic in the southern GoM. More extensive sampling along the species entire distribution is necessary to make more accurate inferences of population genetics of O. wageneri.

Key words: barcode, Cyclopsetta chittendeni, population genetics, Hypanus americanus, Oncomegas, Rhizoprionodon terraenovae, 28S rDNA (D1–D2)
Studies of helminth parasite life-cycles and intraspecific genetic variation are surprisingly scarce (Criscone et al. 2005; Criscone 2016; Blasco-Costa and Poulin 2017). More specifically, of the approximately 6,000 known cestode species, of which ca. 17% (i.e., ±1,000 spp.) parasitize elasmobranchs (sharks, rays, and skates) worldwide, the complete life cycles have been established for fewer than 5 species (Caira and Reyda 2003; Caira and Littlewood 2013; Caira and Jensen 2014). Considering this paucity of studies, in regions such as the Gulf of Mexico (GoM) efforts in recent years have been geared toward increasing the knowledge of marine cestode life-cycles by using molecular data (i.e., DNA sequences) to match larval and adult stages. For example, of the 98 species of cestodes reported from the GoM (Jensen 2009), 25 species of adult cestodes (Tetrathyridida and Rhinebothriida) reported particularly from the northern GoM in the USA (n-GoM) from elasmobranchs were linked with larval stages from teleosts, bivalves, gastropods, and shrimps, based on morphological and molecular phylogenetic approaches (Jensen and Bullard 2010). These efforts (i.e., Jensen and Bullard 2010) plus ongoing studies (e.g., Vidal-Martínez VM and Aguirre-Macedo ML, personal communication) contribute to increasing the knowledge of cestode biodiversity in ecosystems where multiple helminth species have been previously identified, based on adult forms only, and where large-scale genetic matching of unidentified helminth juveniles with known adults is the most promising way to resolve multiple life cycles simultaneously (Blasco-Costa and Poulin 2017).

Jensen and Bullard (2010) mentioned that cestode larvae are notoriously difficult to identify based on morphological criteria used for cestode taxonomy, since the determining characters are based on adult morphology and for many cestode orders, the larval stages do not resemble their adult counterparts. Given the difficulties of identifying cestode larvae to species (or even higher taxon level), determining life cycles is almost impossible in many cases. The cestode order Trypanorhyncha is an exception to these difficulties, since their fully armed rhyncheal apparatus allows for species-level identifications of trypanorhynch larvae in intermediate hosts, which include teleosts, molluscs, crustaceans, jellyfishes, and sea cucumbers (Palm 2004; Caira and Jensen 2014). A noteworthy characteristic of the life cycle of trypanorhynch cestodes is that in addition to the larval stages living in one or more intermediate hosts, and the adults living in definitive hosts, the phenomenon of paratenesis often takes place. A paratenic host is not essential for the parasite to complete its life cycle, but within such a host, the larval stages of the parasites survive without developing (Combes 2001; Caira and Reyda 2005). Furthermore, while life cycle studies of cestodes are scarce, studies on population genetics and genetic variation are even fewer. For example, of the 315 Trypanorhynchia species worldwide (Beveridge et al. 2017), only one (Tentacularia coryphaenae Bosc, 1797) has been studied in a context of intraspecific genetic variation (Palm et al. 2007), albeit using a very small sample size (n = 3 individual cestodes).

A Trypanorhynchia species, Oncomegas wageneri (Linton, 1890) Dollfus, 1929 (Trypanorhynchia: Eutetrarhynchoida) (taxonomic classification following the review by Beveridge et al. 2017), has been reported from the n-GoM in its adult stage from elasmobranchs and larval stages from teleosts (Jensen 2009). Oncomegas wageneri has been reported as a plerocercus in the digestive tract of 12 species of marine fishes included in 7 families (for more details, see Online Appendix 1), and as plerocerci in marine plankton (Dollfus 1974; but also see Schaeffner 2018). Adults of this species have been reported from the spiral valve of the elasmobranch Bathytoshia centroura (Mitchell, 1815) (as Dasyatis centroura) from the USA’s Atlantic Ocean (Toth et al. 1992; Palm 2004); however, the life cycle of this parasite has not been formally described and published. In a recent morphological study based on new records of adult specimens of O. wageneri from Hynanus guttatus (Bloch and Schneider, 1801) from the southwestern Atlantic Ocean off Macei, Brazil, Schaeffner (2018) outlined the complicated taxonomic history (e.g., problems and advances) and the necessity to include more representative species of the genera Hispidorhynchus Schaeffner and Beveridge, 2012 and Oncomegas Dollfus, 1929 in a previously inferred molecular phylogenetic framework (Palm et al. 2009; Olson et al. 2010) to support the division of these 2 genera (also see Schaeffner and Beveridge 2012). Schaeffner (2018) also suggests conspecificity and an antitropical distribution for O. wageneri specimens from the northern and southwestern Atlantic Ocean based on the comparison on metric data between adult specimens of O. wageneri and the distribution of their 2 dasytid definitive host species (i.e., B. centroura and H. guttatus). However, other biological and ecological factors (e.g., vagility of the intermediate, paratenic and definitive hosts) which could explain the dispersal of O. wageneri have not been tested to date.

While the marine cestode fauna of the northern and western GoM off the USA has been studied extensively and pioneering works have matched larval and adult forms using genetic tools (Jensen 2009; Jensen and Bullard 2010), the parasite fauna (e.g., marine cestodes) of the southern GoM off Mexico (s-GoM), is particularly poorly studied with the exception of the infracommunities of the flatfish species. The flatfish species from the s-GoM are in fact one of the better studied groups with regards to marine parasites (e.g., Rodriguez-González and Vidal-Martínez 2008; Vidal-Martínez et al. 2014, 2019; Centeno-Chále et al. 2015), having been the focus of several projects during the last 25 years (Vidal-Martínez et al. 2016). Particularly, Centeno-Chále et al. (2015) in a recent study of helminth communities of the flatfish, Cyclopsetta chittendeni Bean, 1895, from s-GoM reported plerocerci of O. wageneri as the most prevalent larval cestode parasite species. Furthermore, Vidal-Martínez et al. (2014) detected a high overall prevalence of plerocerci of O. wageneri in another flatfish species, Syacium gunteri Ginsburg, 1933, suggesting that this marine fish could act as a paratenic host or as a potential third intermediate host.

Given the recent collecting efforts in the s-GoM (Vidal-Martínez et al. 2016) geared at cestodes from different life stages, the aims of this study were to (i) expand host records for O. wageneri, (ii) use genetic (DNA sequence) data to determine sister-group relations of O. wageneri within a phylogenetic framework of the Eutetrarhynchoida and to confirm conspecificity between adults and plerocerci of O. wageneri, and (iii) explore the intraspecific genetic variation of O. wageneri in the s-GoM. Therefore, in this study different biological implications for O. wageneri are explored, that can be inferred from the use of molecular data, be it for analyzing variation in populations, life-stage matching, and/or systematic inferences.

Materials and Methods

Collection of flatfish, shark, stingrays, and cestodes

Plerocerci of O. wageneri used for molecular analysis in this study were collected from the digestive tract of the flatfish C. chittendeni from 8 oceanographic sampling sites (depth range 30–74 m), covering 18,575 km², from the s-GoM. Samples were obtained from August to October 2015. Oceanographic sampling procedures for
the collection of flatfish have been described elsewhere (i.e., Centeno-Chálate et al. 2015; Vidal-Martínez et al. 2015). Adults of *O. wageneri* were collected from the intestines of a male Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Richardson, 1836) (depth range 0–280 m), and 2 stingray females, *Hypopus americanus* (Hildebrand and Schroeder, 1928) (depth range 0–53 m). The Atlantic sharpnose shark and stingrays were caught by artisanal fishermen in Chachalacas, Veracruz, Mexico. This particular locality was assigned as oceanographic sampling site No. 9 (for more details, see Online Appendix 2). Host dissection follows Vidal-Martínez et al. (2015) and Centeno-Chálate et al. (2015); cestode collection and preservation follows Vidal-Martínez et al. (2001, 2015) and Mendez and Vidal-Martínez (2017); preparation of cestodes for morphological study follows Méndez and Vidal-Martínez (2017). The cestode specimens used for molecular analysis were cleaned with 0.7% saline solution and preserved in 100% ethanol. Specimens of an unidentified species of the genus *Rhinebothrium* (Rhinebothriidae: Rhinebothriidae) were also collected from the Atlantic sharpnose shark examined, and specimens of *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 and *Dasyrhynchus giganteus* (Diesing, 1850) Pintner, 1928 (Lacistorhynchoidea: Lacistorhynchiidae) were collected from the intestines of a male Bull shark, *Carcharhinus leucas* (Müller and Henle, 1839), also collected by artisanal fishermen from sampling site No. 9 and preserved for molecular analysis to be used as outgroups for subsequent phylogenetic analysis, based on previous phylogenetic relationships (Palm et al. 2009; Olson et al. 2010; Beveridge et al. 2017; Haseli et al. 2017). Cestode identification follows Palm (1995, 2004). Terminology for the larval stages of cestodes follows Chervy (2002) and Palm (2004). Several plerocerci and adult trypanorhynchs collected for the larval stages of cestodes follows Chervy (2002) and Palm (2004). Termination of the genetic (uncorrected *K*2 distances) was obtained for the 2 runs after discarding the first 5,000 parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) for 20 × 10^6 generations each. Topologies were sampled every 1,000 generations and the average standard deviation of split frequencies was observed until it reached < 0.01, as suggested by Ronquist et al. (2012). A consensus tree with branch lengths was obtained for the 2 runs after discarding the first 5,000 sampled trees as burn-in. The robustness of the cladest was assessed using Bayesian Posterior Probability (PP), where PP > 0.95 was considered strongly supported.

**Molecular data and phylogenetic reconstruction**

To obtain the consensus sequences of *O. wageneri*, *C. gracilis*, *D. giganteus*, and *Rhinebothrium* sp., chromatograms of forward and reverse sequences were assembled and edited using the Geneious Pro v. 5.1.7 platform (Drummond et al. 2010). To determine sister-group relations of *O. wageneri* within a Trypanobatoida phylogenetic framework, the 28S sequence data generated herein were aligned with 28S sequence data for other members of the Trypanobatoida (i.e., members of the Eutetrarhynchoidea and Tentacularioidea *sensu* Beveridge et al. 2017) downloaded from GenBank (see GenBank accession numbers in Figure 1), using an interface available with MAFFT v. 7.263 (Katoh and Standley 2016), an “auto” strategy and a gap-opening penalty of 1.53 with Geneious Pro, and a final edition by eye in the same platform. The genetic (uncorrected) *P* distance, with the bootstrap method (500 replicates) and with a uniform nucleotide substitution (transitions + transversions) rate, was calculated in MEGA v. 7.0 (Kumar et al. 2016). The software ModelTest v. 2.1.3 (Darriba et al. 2012) was used to select a model of evolution through the Bayesian Information Criterion (BIC) (Schwarz 1978). The nucleotide substitution model with the lowest BIC score was GTR + I + G (Tavaré 1986). The Gblocks Web Server (Castresana 2000; Talavera and Castresana 2007) was used to remove ambiguously aligned regions of 28S.

The 28S dataset was analyzed with Bayesian inference (BI) through the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The BI was carried out with MrBayes v. 3.2.1 (Ronquist et al. 2012). The Bayesian phylogenetic tree was reconstructed using 2 parallel analyses of Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) for 20 × 10^6 generations each. Topologies were sampled every 1,000 generations and the average standard deviation of split frequencies was observed until it reached < 0.01, as suggested by Ronquist et al. (2012). A consensus tree with branch lengths was obtained for the 2 runs after discarding the first 5,000 sampled trees as burn-in. The robustness of the clades was assessed using Bayesian Posterior Probability (PP), where PP > 0.95 was considered strongly supported.
Population genetic parameter estimation

Haplotypes for the COI fragment were obtained for 48 plerocerci of *O. wageneri*, collected from 8 oceanographic sampling sites (sampling sites 1–8), and 4 adults of *O. wageneri* collected from Chachalacas (sampling site 9; for more details of sampling site numbers, see Online Appendix 2). To assess the completeness of sampling, a haplotype accumulative curve was obtained (Brown et al. 2012; Coeur d’acier et al. 2014). The genetic variation of the *O.
wageneri samples studied here was calculated based on the number of haplotypes ($h$), haplotype diversity ($H$), and nucleotide diversity ($\pi$) (Nei 1987), using DnaSP v. 6.12.01 (Rozas et al. 2017). To infer their population structure, the individuals from each locality were treated as separate populations and an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was carried out in the program Arlequin v. 3.5 (Excoffier and Lischer 2010) with 10,000 randomizations, to compare the genetic variation within and between the populations. A haplotype network was also constructed using the program popART (Leigh and Bryant 2015). An unrooted network was constructed under the null hypothesis of no genetic differentiation among populations and the spatial population distribution for haplotypes was represented on a map, by running a TCS (Templeton et al. 1992) haplotype network analysis.

**Results**

**Phylogenetic analyses**

In total, 16 bi-directional 28S sequences were obtained from 3 plerocerci and 2 adults of *O. wageneri*, as well as *C. gracilis* (1 adult specimen), *D. giganteus* (1 adult specimen), and *Rhinebothrium* sp. (1 adult specimen) (outgroups). The final lengths (in number of base-pairs) of the 28S sequences are as follows. For the 2 *O. wageneri* adults, 942 base-pairs (bp) and 1,150 bp; for the *O. wageneri* plerocerci, 1 of 1,226 and 1 of 1,193 bp; 1,127 bp for *C. gracilis*, 1,224 bp for *D. giganteus*, and 1,183 bp for *Rhinebothrium* sp. The total alignment length following the Gblocks exclusion was 1,275 bp. The 28S sequences of the 3 plerocerci from *C. chittendeni* were identical, while the sequences of the plerocerci and 2 adults of *O. wageneri* showed a genetic distance of 0.054%. Nucleotide sequence variation in the 28S alignment of the trypanobatoida taxa (excluding the outgroup taxa; i.e., *C. gracilis*, *D. giganteus*, and *Rhinebothrium* sp.) included 621 conserved and 654 variable sites, of which 493 sites were parsimony-informative, and 161 sites represented singletons.

Phylogenetic relationships were inferred using the alignment of 28S, which included 38 sequences from 52 taxa. Figure 1 shows the consensus topology reconstructed from the post-burnin trees of the 2 Bayesian runs in MrBayes. Based on the analysis of the partial 28S alignment, the 3 plerocerci of *O. wageneri* from *C. chittendeni* and the 2 adults of *O. wageneri* from *R. terraenovae* included in the analysis form a well-supported (PP ≥ 0.95) monophyletic group. This clade, representing *O. wageneri*, is sister to the single individual of *Oncomegas celatus* (Beveridge and Campbell, 2005) Schaeffner and Beveridge, 2012, with high nodal support (PP ≥ 0.95).

**Population genetic analyses**

In total, 104 bi-directional COI “barcode” sequences were obtained from 48 plerocerci and 4 adults from *O. wageneri*. The length of all sequence fragments from the plerocerci was 585 bp, except for one sequence that had 511 bp (from sampling site number 1; see Online Appendix 2). Nucleotide sequence variation in the COI alignment was 530 conserved and 55 variable sites, of which 21 sites were parsimony-informative, and 34 sites represented singletons. In total, 44 haplotypes among 52 sequences from *O. wageneri* from the southern GoM were found, and the haplotype accumulation curve has not yet reached the asymptote (Figure 2). The haplotype diversity ($H$) was 0.9864 and the nucleotide diversity ($\pi$) was 0.0086. The AMOVA-based fixation index was $-0.02471 (P = 0.823$) and the among- and within population variances are shown in Table 1. Based on the haplotype distribution across space, there are only 3 haplotypes shared between different oceanographic sample sites, namely haplotype 7 (shared between sites 2, 4, 5, 7, and 8), haplotype 15 (shared between sites 4 and 5), and haplotype 16 (shared between sites 3 and 8) (Figures 3 and 4). The mitochondrial haplotype network displays a star-shaped pattern.

**Discussion**

In this study, new definitive host records are presented for adults of *O. wageneri*, namely *R. terraenovae* and *H. americana*. These new records increase the number of previously registered hosts from 2 elasmobranchs (*B. centrooura* and *H. guttatus*, both rays belonging to the family Dasyatidae) to 4. Comparing these host records to the other 3 species of the genus *Oncomegas* (i.e., *O. celatus*, *O. javensis* Palm, 2004, and *O. trimegacanthus* Schaeffner and Beveridge, 2012), the pattern of host specificity for adult *Oncomegas* with dasytid hosts is consistent, as previously noted (Palm 2004; Palm and Cairn 2008; Schaeffner and Beveridge 2014). The present finding of adult *O. wageneri* from the Carcharhinidae *R. terraenovae* from s-GoM represents the first record of the genus *Oncomegas* for sharks. Because Trypanobatoida occurs primarily in rays (e.g., Olson et al. 2010; Beveridge et al. 2017), the present record from *R. terraenovae* could be considered unusual; however, species of *Dollfusiella* Campbell and Beveridge, 1994 and *Prochristiella* Dollfus, 1946 have been found occurring in sharks (Olson et al. 2010; Beveridge et al. 2017). At the moment, *O. wageneri* and *O. celatus* are the species among their congeners with the highest number of registered elasmobranch hosts (i.e., both 4, respectively).

![Figure 2](image-url) "Haplotype accumulation curve for *Oncomegas wageneri* (Linton, 1890) Dollfus, 1929 in the southern Gulf of Mexico."
On the other hand, O. wageneri is the species among its congeners with the highest number of Actinopterygii intermediate hosts (i.e., 5 orders, 7 families, and 12 spp.), followed by O. javensis (i.e., 4 orders, 4 families, and 6 spp.) (Palm 2004; Schaeffner and Beveridge 2012). Schaeffner (2018) suggests an antitropical distribution for O. wageneri from the north- and southwestern Atlantic Ocean based on the distribution of the 2 dasyatid definitive host species known at the time (i.e., B. centroura and H. guttatus). However, considering the distributions of 3 of the definitive hosts (i.e., H. americanus, H. guttatus, and R. terraenovae) (Toth et al. 1992; Present study) and 4 intermediate and/or paratenic hosts from the GoM (i.e., C. chittendeni, L. campechanus, S. gunteri, and S. papillosum) (Thatcher 1961; Vidal-Martinez et al. 2014, 2019; Centeno-Chale et al. 2015), it is possible to infer that O. wageneri from the American continent’s Western Atlantic Coast could be found along a continuous distributional range between the latitudes 42° N and ~26° S [see Froese and Pauly (2017) for geographic distribution notes of each host species], leading to the rejection of Schaeffner’s (2018) suggestion of an antitropical distribution for O. wageneri.

The DNA sequences generated here represent the first genetic data for >3 specimens of the parasite O. wageneri and proved useful in addressing 3 key points. First of all, through DNA sequences, for the first time the plerocercus and adult stages of O. wageneri were linked, reinforcing the role of flatfishes as potential paratenic or intermediate hosts in the life-cycle of O. wageneri from the southern GoM, as suggested by Vidal-Martinez et al. (2014).

The second issue that was addressed by O. wageneri DNA sequence data deals with taxonomical–systematic uncertainties of the genus Oncomegas. The fact that O. wageneri is resolved as a sister-group of O. celatus in the eutetrarhynchoid phylogenetic framework provides for the first time molecular-based support for the monophyly of 2 members of the genus Oncomegas.

Thirdly, the mitochondrial DNA sequence data generated for O. wageneri individuals from 9 different sampling sites in the southern GoM revealed extremely high intraspecific genetic variation (0.0086 nucleotide diversity). More specifically, the molecular marker used to assess this diversity was the mitochondrial COI “barcode” region, which has recently been used for species delimitation of cestodes (Trevisan et al. 2017; Mello et al. 2018). For trypanorhynch cestodes, the only other study which has made use of the COI marker to assess intraspecific genetic variation found a much lower value (0.01%) for the species T. coryphaenae (Palm et al. 2007). However, the values obtained from Palm et al.’s (2007) study and those obtained here are not directly comparable, not only because there is no overlap in the region of the marker sequenced.
Assessment of the evolutionary forces acting on flow of parasite populations. However, to make a more detailed investigation, the population genetic diversity of trypanorhynch cestode species, and their results are very contrasting. Even in the “genomics era” (Bierne et al. 2016), little is known about the genetic diversity of natural populations of wildlife in biologically complex and resource-rich marine areas such as the GoM. Similar to the PBI, the GoM is starting to be studied in a more systematic and thorough fashion through the collaborative efforts of researchers in the Consorcio de Investigación del Golfo de México (CiGoM; www.cigom.org, last accessed 20 September 2019). Therefore, the population genetic diversity found here for \textit{O. wageneri} from the s-GoM sets a baseline against which future studies can be compared, for cestode parasites of the order Trypanorhyncha, as well as other taxa from the GoM.

Studies on cestode diversity in vertebrate hosts globally have increased during the past 10 years due to international collaborative efforts such as the Planetary Biodiversity Inventories (PBI) (Cairns and Jensen 2017). Even so, including the work presented here, there have only been 2 studies inferring the population genetic diversity of trypanorhynch cestode species, and their results are very contrasting.

In addition to the high genetic variation, the population genetic analyses for \textit{O. wageneri} from the s-GoM revealed a lack of structure among the sampled sites, as can be seen from the fact that there is much higher within than among population variation, based on the AMOVA. Furthermore, the non-significant $P$-value for the fixation index means that in the region studied here, we failed to reject panmixia with the sampling at hand. The high genetic variation and inferred panmixia in the s-GoM could be explained by the parasite’s life history (Detwiler and Criscione 2011; Gorton et al. 2012; Kasl et al. 2015) as well as its wide range of hosts with different vagilities. For example, using genetic data, Richards et al. (2019) recently showed that \textit{H. americanus} undergo coastal migration along the southeastern of USA and Caribbean coasts and can disperse across deep waters for short distances, despite their demersal lifestyle. A host with such vagility may thus contribute to increased gene flow of parasite populations. However, to make a more detailed assessment of the evolutionary forces acting on \textit{O. wageneri} from the s-GoM, the population genetic studies would need to include individuals from many more sites and a wider range of molecular markers. Although there are no comparable studies of trypanorhynch cestode population genetics, studies of other aquatic parasites (both nematodes and platyhelminthes) have shown that host vagilities play a crucial role in determining the parasite’s population structure and gene flow (e.g., Criscione and Blouin 2004; Feis et al. 2015; Sprehn et al. 2015; Gagne et al. 2018).

**Acknowledgments**

The authors thank the staff of the laboratory of Patología Acuática: Arturo Centeno-Chale, Clara Vivas Rodríguez, Gregory Arjona-Torres, Ana L. May-Tec, Francisco Pac Itzá, Nadia Herrera Castillo, Jhonny G. García-Teh, Germán López-Guerra, Daniel Aguirre-Ayala, and Efrain Sarabia from CINVESTAV-IPN, Unidad Mérida, Mexico. They specially thank to Ms. Sc. Arturo Centeno-Chale for providing them with information for this study and discussing a large portion of the information presented in the study. They are grateful to Abril Gamboa and José García Maldonado for their technical assistance in the molecular lab. A.M.-A. thanks Dr Juan G. Tapia (Director of

![Figure 4. Map of the southern Gulf of Mexico showing the geographic distribution of the haplotypes found in each sampled locality, for the barcoding gene COI used for \textit{Oncomegas wageneri} (Linton, 1890) Dollfus, 1929, obtained from the program popART. Each haplotype is represented by a color in the pie charts and by a number (arranged around the pie charts). Haplotypes shared by >1 locality are indicated by colored numbers. The sampling site numbers can be found in the center of each pie chart, within the full white circles (the numbers correspond to the sampling site numbers found in Online Appendix 2). Circle sizes of the pie charts are proportional to the number of haplotypes that were found in each locality.](image-url)
Facultad de Ciencias, UABC) for his valuable support during writing of the present work. The manuscript was greatly improved by the constructive comments and suggestions of 3 anonymous reviewers.

Funding
This work was financially supported from Grant No. A1-S-15134 by the Consejo Nacional de Ciencia y Tecnología (CONACYT) (to F.S.C.). Research funded by the CONACYT (Mexican Ministry of Energy) Hydrocarbon Trust, project 201441. This is a contribution of the GoM Research Consortium (CIGoM).

Supplementary material
Supplementary material can be found at https://academic.oup.com/cz.

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