



Taxonomy and systematics

Haliotrematoides spp. (Monogeneoidea: Dactylogyridae) parasitizing *Lutjanus guttatus* (Lutjanidae) in two localities of the Pacific coast of Mexico, and their phylogenetic position within the Ancyrocephalinae through sequences of the 28S rRNA

Haliotrematoides spp. (Monogeneoidea: Dactylogiridae) parasitando *Lutjanus guttatus* (Lutjanidae) en dos localidades de la costa pacífica de México y su posición filogenética dentro de Ancyrocephalinae a través de secuencias del 28S rRNA

Adriana García-Vásquez^{a,b,*}, Carlos Daniel Pinacho-Pinacho^a, Lilia Catherine Soler-Jiménez^c, Emma Josefina Fajer-Ávila^c, Gerardo Pérez-Ponce de León^a

^a Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado postal 70-153, Coyoacán, 04510 México, D.F., Mexico

^b Red de Biología Evolutiva, Instituto de Ecología, A. C., Km 2.5 Antigua carretera a Coatepec, 91070 Xalapa, Veracruz, Mexico

^c Centro de Investigación en Alimentos y Desarrollo en Acuicultura y Manejo Ambiental, A. C., Unidad Mazatlán, Estero del Yugo s/n, 82000 Mazatlán, Sinaloa, Mexico

Received 20 August 2014; accepted 3 February 2015

Available online 21 May 2015

Abstract

Specimens of wild populations of the spotted rose snapper, *Lutjanus guttatus* (Steindacher) were studied for monogenean parasites in 2 localities along the Mexican Pacific coast (Mazatlán, Sinaloa and Chamela Bay, Jalisco). Five species of dactylogyrids were found on the gills of their hosts: *Haliotrematoides guttati* (García-Vargas, Fajer-Ávila, & Lamothe-Argumedo, 2008), *H. plectridium* Kristky and Mendoza-Franco in Kritsky, Tingbao, & Yuan, 2009, *H. spinatus* Kristky and Mendoza-Franco in Kritsky et al. (2009), *Euryhaliotrema perezponcei* García-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008 and *E. mehen* (Soler-Jiménez, García-Gasca, & Fajer-Avila, 2012). Freshly collected specimens provided an opportunity to study and compare specimens from different localities in further detail and few morphological characters were added to the description of each species. Additionally, a fragment of 856 bp of the 28S ribosomal RNA (D1–D3) was obtained for all the sampled monogeneans, and a phylogenetic analysis along with all available sequences of dactylogyrids was conducted to establish the systematic position of the species within the Ancyrocephalinae. Our results suggest that species of *Haliotrema* might be included in *Haliotrematoides* genus. In addition, the genetic divergence data suggest that *H. guttati* and *H. spinatus* may represent a species complex; however, this asseveration needs additional data. All Rights Reserved © 2015 Universidad Nacional Autónoma de México, Instituto de Biología. This is an open access item distributed under the Creative Commons CC License BY-NC-ND 4.0.

Keywords: *Euryhaliotrema*; 28S rRNA gene; Cryptic species; Mexico; Monogenea; Snapper

Resumen

Se estudiaron los monogéneos que parasitan poblaciones silvestres de “pargos” o “huachinangos”, *Lutjanus guttatus* (Steindacher) en 2 localidades de la costa del Pacífico mexicano (Mazatlán, Sinaloa y Bahía de Chamela, Jalisco). Se recolectaron 5 especies de dactylogíridos de las branquias de sus hospederos: *Haliotrematoides guttati* (García-Vargas et al., 2008), *H. plectridium* Kristky and Mendoza-Franco en Kritsky, Tingbao, and Yuan (2009), *H. spinatus* Kristky and Mendoza-Franco en Kritsky et al. (2009), *Euryhaliotrema perezponcei* García-Vargas,

* Corresponding author.

E-mail address: tocha76@hotmail.com (A. García-Vásquez).

Peer Review under the responsibility of Universidad Nacional Autónoma de México.

Fajer-Ávila & Lamothe-Argumedo, 2008 and *E. mehen* (Soler-Jiménez et al., 2012). El material permitió observar la morfología en mayor detalle y comparar ejemplares de diferentes localidades aportando algunos caracteres morfológicos a la descripción de las especies. Se obtuvo la secuencia de un fragmento de 856 bp del gen ribosomal 28S (dominios D1 a D3), de este modo se estableció la posición sistemática de éstas, en conjunto con las secuencias de otros dactylogíridos, dentro de la filogenia de los Ancyrocephalinae. Este análisis reveló que las especies de *Haliotrema* podrían incluirse en el género *Haliotrematoides*. Asimismo, los datos de divergencia genética sugieren que *H. guttati* y *H. spinatus* podrían representar un complejo de especies, aunque esto requiere ser verificado con datos adicionales.

Derechos Reservados © 2015 Universidad Nacional Autónoma de México, Instituto de Biología. Este es un artículo de acceso abierto distribuido bajo los términos de la Licencia Creative Commons CC BY-NC-ND 4.0.

Palabras clave: *Euryhaliotrema*; 28S rRNA; Especies crípticas; México; Monogenea; Pargo

Introduction

The genus *Lutjanus* (Steindacher) (Lutjanidae), the most species-rich among the family Lutjanidae, contains 68 species distributed around the world (Froese & Pauly, 2014). These fish are commercially important for fisheries, and at least 20 species have been examined for monogeneans, since it is well-known the impact that these parasites may have on fish populations. Species of 4 genera of dactylogyrids have been found on the gills of snappers around the world, *Euryhaliotrema* Kritsky and Boeger, 2002, *Haliotrema* Johnston and Tiegs, 1922, *Haliotrematoides* Kritsky, Tingbao & Yuan, 2009 and *Tetrancistrum* Goto and Kikuchi, 1917; 2 other genera, *Euryhaliotrematoides* Plaisance and Kritsky, 2004, and *Aliatrema* Plaisance and Kritsky, 2004 were recently considered as synonyms of *Euryhaliotrema* based on the morphology of their male copulatory organ (MCO) (Kritsky, 2012). In Mexico, 9 dactylogyrid species have been described from snappers, i.e. *H. cornigerum* (Zhukov, 1976), *H. gracilihamus* (Zhukov, 1976), *H. heteracantha* (Zhukov, 1976), *H. longihamus* (Zhukov, 1976), *H. magnigastrohamus* (Zhukov, 1976), *H. overstreeti* Kritsky and Bullard in Kritsky et al. (2009) from Campeche Bay; *H. guttati* (García-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008), *E. perezponcei* (García-Vargas, Fajer-Ávila & Lamothe-Argumedo, 2008) from Mazatlán, Sinaloa and Cruz de Huanacastle, Nayarit, and *E. mehen* (Soler-Jiménez, García-Gasca & Fajer-Ávila, 2012) from Mazatlán, Sinaloa. The taxonomic status of some of these species of dactylogyrids has been unstable due to the lack of morphological details (mostly in haptor hard parts and copulatory organ) in the original descriptions, although clarification has been gained after further taxonomic work conducted by several authors (e.g., Kritsky et al., 2009).

Recently, specimens of the spotted rose snapper *Lutjanus guttatus* (Steindachner) were collected in Chamela Bay, Jalisco and Mazatlán, Sinaloa, both on the Pacific coast of Mexico, as part of the study of the ectoparasites of fishes with commercial importance. From those fishes, specimens of dactylogyrids were collected from the gills. The objectives of this paper were to report the presence of 3 species of *Haliotrematoides* parasitizing the gills of spotted rose snappers, and to provide additional morphological information for each of them. In addition, we analyze the systematic position of these species (including *E. perezponcei* and *E. mehen* from Mazatlán) within the phylogeny of the Ancyrocephalinae, by using sequences of the 28S rRNA in the context of a wider phylogenetic analysis of dactylogyrids.

Materials and methods

Specimens of adult spotted rose snapper *Lutjanus guttatus* were sampled in 2010 in Mazatlán Bay, Sinaloa (23°18'44" N, 106°29'37" W) and from 2011 through 2012 in Chamela Bay, Jalisco (19°33'15" N, 105°06'45" W), in northwestern Mexico; fish were obtained from local fishermen who set nets in each locality, kept on ice once removed from the nets, and examined for gill parasites a few hours after capture. Gills were extracted, fixed and stored in 96% ethanol; parasites were excised from the host tissue using triangular, surgical, mounted needles (size 16, Barber of Sheffield, U.K.) and were prepared for morphological and molecular evaluation. For morphological comparison, type-specimens deposited at the National Museum of Natural History (NMNH), Smithsonian Institution, Washington, D.C. (formerly U.S. National Parasite Collection – USNPC), USA at the Colección Nacional de Helmintos (CNHE) and at the Colección de Parásitos de Peces del Noroeste de México (CPPNP) were studied as follows: *H. guttati* (USNPC 101370–101371; CNHE, Neotype 8460), *H. plectridium* (USNPC 101367–101369), *H. spinatus* (USNPC 101353–101355; CNHE 6464–6465).

Morphological analysis

The haptor of each specimen was removed using a scalpel and air-dried on a glass slide; the corresponding body was transferred to a labeled Eppendorf tube containing 96% ethanol and stored at –20 °C until required for molecular evaluation. Air-dried haptors were then subjected to a partial digestion using a proteinase K-base method (García-Vásquez, Hansen, Cristison, Bron, & Shinn, 2011). Some specimens were mounted (unstained) in Gray and Wess (Vidal-Martínez, Aguirre-Macedo, Scholz, González-Solis, & Mendoza-Franco, 2001) solution to clear the body tissue and visualize the haptor hooks and copulatory complex. The haptor armature and copulatory complex were studied using an immersion oil objective on an Olympus BX40 compound microscope. For comparisons, specimens were measured following Kritsky et al. (2009). When provided, measurements are presented in micrometers with the average followed by the range in parentheses. Specimens were deposited in the Colección Nacional de Helmintos from Mexico (CNHE) and in the U.S. National Parasite Collection (USNPC).

DNA extraction and sequencing

Parasite bodies were 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% Sakozyl, and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions. Some tissues were extracted using the DNeasy Tissue Kit (Qiagen, Valencia, California). A fragment of the 28S ribosomal gene was amplified using the polymerase chain reaction (PCR). The primers Ancy55F (5'-GAGATTAGCCCATCACCG AAG-3') (Plaisance, Littlewood, Olson, & Morand, 2005) and LSU1200R (5'-GCATAGTTCACCATCTTTCGG-3') (Littlewood, Curini-Galletti, & Herniou, 2000) were used to amplify (PCR) a LSU fragment; also, the internal primers Halio-F (5'-ACCCGCTGAATTTAAGCAT-3') and Halio-R (5'-TGGTCCGTGTTTCAAGAC-3') (Soler-Jiménez et al., 2012). The PCR (25 µl) consisted of 2.5 µl of 1 µl of 10 µM of each primer, 2.5 µl of 10× buffer, 1.5 µl of MgCl₂ 15 mM, 0.5 µl dNTP's 10 mM, 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil), 2 µl of DNA template, and 13.675 µl of water. The PCR cycling protocol included denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, annealing at 50–58 °C depending on dactylogryrid species (*E. perezponcei* 50 °C, *E. mehen* 55 °C, *H. guttati* and *H. spinatus* 58 °C, and *H. plectridium* 55 °C) for 1 min and final extension at 72 °C for 1 min.

The reaction was performed in a MJ Research thermal cycler using a heated lid to reduce refluxing. Sequencing reactions were performed using an ABI Big Dye (Applied Biosystems, Boston, MA) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 310 capillary DNA sequencer. Contigs were assembled and base calling differences resolved using Codoncode Aligner version 3.0.1 (Codoncode Corporation, Dedham, MA). All sequences were deposited in GenBank database.

Alignments and phylogenetic analyses

Sequences obtained in the current study from 28S (D1–D3) were aligned along with other species of Dactylogryridae available in GenBank using Clustal W implemented in Mega 5 (Tamura, Peterson, Peterson, Stecher, & Kumar, 2011) and adjusted manually with the Mesquite 2.75 program (Maddison & Maddison, 2011). Maximum likelihood (ML) and Bayesian Inference (BI) analyses were performed (Huelsenbeck & Ronquist, 2001). The jModelTest software version 0.1.1 (Posada, 2008) was used to select the best model of evolution for our dataset. The model (GTR+I+G) was selected based on the Akaike information criteria. The ML tree was inferred using RAxML 7.0.4, for each dataset (Stamatakis, 2006). Maximum likelihood clade support was assessed by bootstrap resampling with 10,000 replicates. Additionally, Bayesian analyses were performed with the program MrBAYES version 3.1.2 (Huelsenbeck & Ronquist, 2001). The settings were 2 simultaneous runs with 4 Markov chains and 5 million MCMC

generations, sampling every 200 generations, a heating parameter value of 0.2 and a 'burnin' of 10%. Numbers at the interior branches of the consensus tree represent posterior probabilities (PP). Trees were drawn using FigTree program version 1.3.1 (Rambaut, 2006). The genetic divergence among species (*E. perezponcei*, *E. mehen*, *H. plectridium*, *H. guttati* and *H. spinatus*) was estimated using uncorrected "p-distances" method with the program MEGA v. 5 (Tamura et al., 2011).

Results

Three species of *Haliotrematoides* were found parasitizing the gills of *L. guttatus* in Mazatlán, Sinaloa and Chamela Bay, Jalisco (Table 1). The specimens collected in the present study were compared morphologically with type and voucher specimens, mainly in terms of haptor hooks and male copulatory organ (MCO). The following species were found:

Dactylogryridae Bychowsky, 1933

Haliotrematoides Kritsky et al., 2009

Haliotrematoides guttati (García-Vargas et al., 2008)

Taxonomic summary

Site: gills.

Type host and locality: *Lutjanus guttatus* (Steindachner), Pacific Coast off Mazatlán, Sinaloa, Mexico (23°29' N, 106°36' W).

Host and localities: Chamela Bay, Jalisco, Mexico (19°31'49" N, 105°04'55" W). Mazatlán, Sinaloa (23°13'10" N, 106°26'05" W).

Previous records: Cruz de Huanacastle, Nayarit, Mexico (20°44' N, 105°22' W); Taboga Island, Panama (8°49' N, 79°34' W); Perlas Archipelago, Panama (8°22' N, 79°01' W).

Specimens deposited: CPPNP 7293, 5 vouchers (from the type locality), 4 vouchers CNHE 8354 and 1 voucher USNPC 106930 (from Chamela).

DNA reference sequence: the 856 bp ribosomal DNA sequence of the LSU (D1–D3) is deposited in GenBank under accession numbers HQ615993 (from Mazatlán) and KC663673–KC663674 (from Chamela Bay).

Remarks

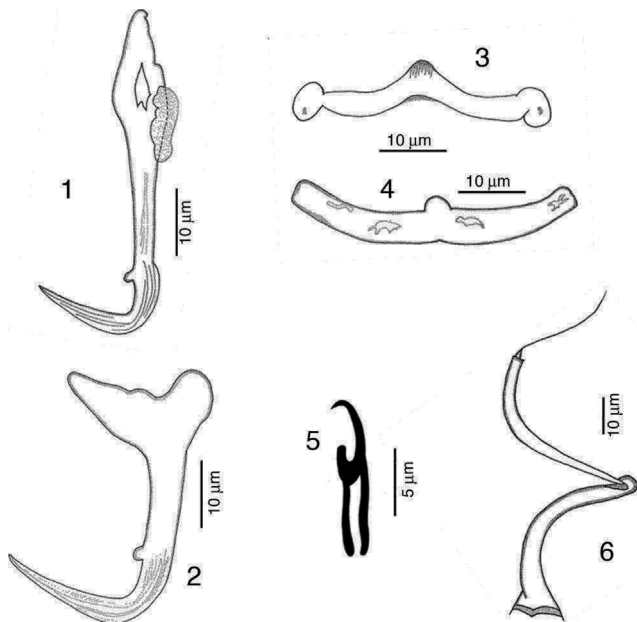
Five specimens of this species were found in Chamela bay, Jalisco and 10 were found in Mazatlán, Sinaloa, Mexico, corresponding with the genus description provided by Kritsky and Mendoza-Franco (Kritsky et al., 2009). This species was originally described as *H. guttati* as a parasite of *L. guttatus* from Mazatlán by García-Vargas et al. (2008). In 2009, Kritsky et al. found 2 specimens from the gills of snappers in Panama, and based on the comparison of their specimens with the original description, and their examination of the paratype and 2 voucher specimens (Harold W. Manter Laboratory, University of Nebraska-Lincoln, HWML, 48570, 48571) transferred the species to the genus *Haliotrematoides*. Following Kritsky et al. (2009) recommendation for the need of a re-description of this species, we studied in further detail our specimens and those

Table 1

Dactylogyrid species reported in the present study from the gills of *Lutjanus guttatus* from Chamela Bay, Jalisco and Mazatlán, Sinaloa, Mexico. CNHE-Coleccion Nacion de Helmintos, UNAM, México. USNPC – United States National Parasite Collection, USA.

Monogenean	Locality	CNHE/USNPC	GenBank
<i>Haliotrematoides guttati</i>	Chamela Bay	8354/106930	KC663673–663674
	Mazatlán	7293/–	HQ615993
<i>Haliotrematoides plectridium</i>	Chamela Bay	8353/106931–106933	–
	Mazatlán	7292/–	HQ615994–KC713622
<i>Haliotrematoides spinatus</i>	Chamela Bay	8355/106934–106935	KC663675–KC663678
	Mazatlán	7291/–	HQ615995

deposited in museum collections (neotype, CNHE, and vouchers from the USNPC from Panama), and herein we provide the following details of some morphological traits: dorsal anchor with a well-developed membrane 12 (11–15) long, showing some striations on the surface of the curvature to the point; in the mid portion of perforation on the anchors base, a small heel is observed (not observed in specimens from Panama), a long inner root, no developed outer root (Fig. 1). Ventral anchor with both roots well developed, inner root 14 (12–16.5) long and ending pointed, longer than the outer root which is 6.5 (6–8) long and rounded (Fig. 2). Dorsal bar delicate, round in the middle, with a small knob, square ends where anchor attaches (Fig. 3). V-shaped ventral bar 41 (38.5–44) wide, but in description made by García-Vargas et al. (2008) is described as “W-shaped”, this was not observed in the type specimens that were studied (Fig. 4). Hooks with terminal depressed thumb directed to point, point opened and beyond thumb (Fig. 5). MCO tubular-shaped forming a spiral, conical base with the anterior part narrower than the base, and bearing a long whip heading to the anterior part of the body (Fig. 6).



Figures 1–6. Drawings of *Haliotrematoides guttati* from *Lutjanus guttatus* found in Chamela Bay, Jalisco and Mazatlán, Sinaloa, Mexico. 1, dorsal anchor; 2, ventral anchor; 3, dorsal bar; 4, ventral bar; 5, hook, male copulatory organ (MCO). All measurements are in micrometers.

Chamela Bay represents a new geographical record for this species.

Haliotrematoides plectridium Kritsky & Mendoza-Franco, 2009

Taxonomic summary

Site: gills.

Type host and locality: *Lutjanus guttatus* (Steindachner), off Taboga Island, Panama (8°49' N, 79°34' W).

Host and localities: *Lutjanus guttatus* (Steindachner), Chamela Bay, Jalisco, Mexico (19°31'49" N, 105°04'55" W), and Mazatlán, Sinaloa, Mexico (23°13'10" N, 106°26'05" W).

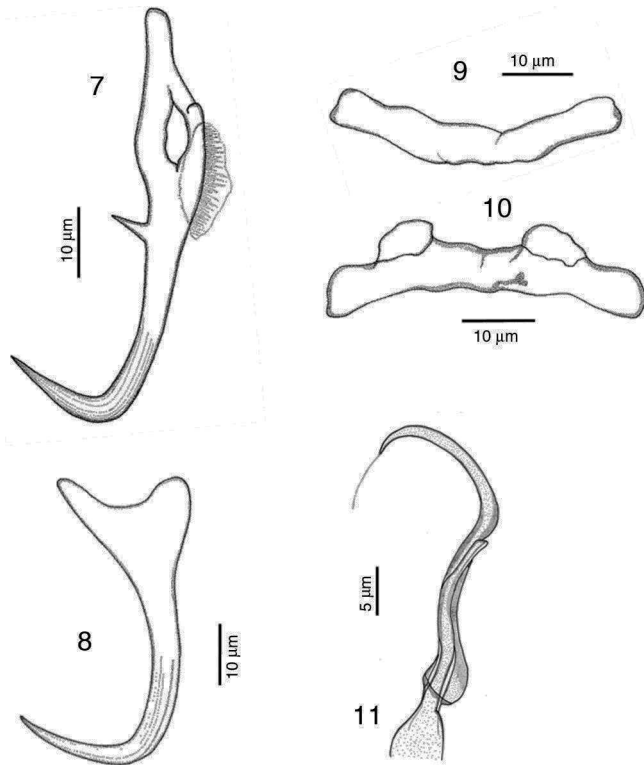
Previous records: Perlas Archipelago, Panama (8°22' N, 79°01' W).

Specimens deposited: CNHE 7292, 2 vouchers (from Mazatlán); CNHE 8353, 4 vouchers (from Chamela Bay).

DNA reference sequence: the 856 bp ribosomal DNA sequence of the LSU (D1–D3) is deposited in GenBank under accession number HQ615994, KC713622 (from Mazatlán). No sequences were obtained from Chamela Bay, Mexico (10 specimens were prepared for molecular analysis but fail to amplify).

Remarks

A total of 10 specimens were found in Mazatlán, Sinaloa and 19 in Chamela Bay, Jalisco. Our specimens are in agreement with the original description of Kritsky and Mendoza-Franco (2009) in Kritsky et al. (2009) from Taboga Island, and from Perlas Archipelago, both in Panama. Records from Mazatlán and Chamela represent new geographical records for this species. We observed in our specimens the following taxonomic traits, some of them slightly different from specimens from Panama: a membrane-like structure in the inner root of dorsal anchor, 8 (6–12.5) long, no outer root developed (Fig. 7). Ventral anchor with a depressed inner root, with the inner root longer than the outer root, 11 (8–17) long (Fig. 8). Dorsal bar 41 (36.5–46) wide, thin and concave while in the Panama specimens the bar is smaller, 35 (31–37) wide (Fig. 9). Ventral bar concave, 43 (40–47) wide, with 2 subterminal pockets in the anterior margin (convex in specimens from Panama) (Fig. 10). The MCO possess a filament at the end, finishing bulged in our specimens (Fig. 11), while this structure was not observed in Panama specimens.



Figures 7–11. Drawings of *Haliotrematoides plectridium* from *Lutjanus guttatus* found in Chamela Bay, Jalisco and Mazatlán, Sinaloa, Mexico. 7, dorsal anchor; 8, ventral anchor; 9, dorsal bar; 10, ventral bar; 11, male copulatory organ. All measurements are in micrometers.

Haliotrematoides spinatus Kritsky & Mendoza-Franco, 2009

Taxonomic summary

Site: gills.

Type host and locality: *Lutjanus guttatus* (Steindachner), off Taboga Island, Panama (8°49' N, 79°34' W)

Host and localities: Chamela Bay, Jalisco, Mexico (19°31'49" N, 105°04'55" W), and Mazatlán, Sinaloa, México (23°29' N, 106°36' W).

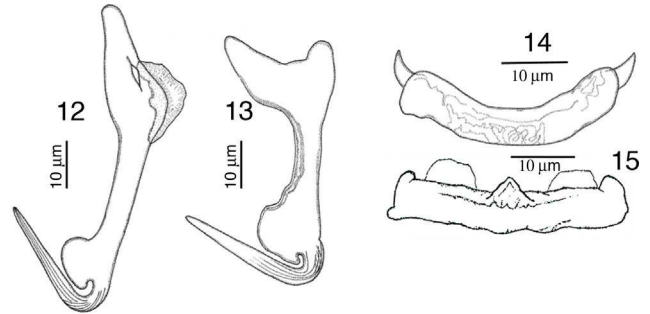
Previous records: Perlas Archipelago, Panama (8°22' N, 79°01' W).

Specimens deposited: CNHE 7291 and CNHE 7546, 4 vouchers (from Mazatlán) and CNHE 8355, 4 vouchers, 2 vouchers USNPC 106934–106935 (from Chamela Bay).

DNA reference sequence: the 856 bp ribosomal DNA sequence of the LSU (D1–D3) is deposited in GenBank under accession numbers HQ615995, KC713623–KC713627 (from Mazatlán) and KC663675–KC663678 (from Chamela Bay).

Remarks

A total of 10 worms of this species were found in Mazatlán, Sinaloa and 11 from Chamela Bay, Jalisco. The specimens collected in the present study, conform to the description provided by Kritsky and Mendoza-Franco (2009) in Kritsky et al. (2009). The finding of this species in *L. guttatus* from Mazatlán and



Figures 12–15. Drawings of *Haliotrematoides spinatus* from *Lutjanus guttatus* found in Chamela Bay, Jalisco and Mazatlán, Sinaloa, Mexico. 12, dorsal anchor; 13, ventral anchor; 14, dorsal bar; 15, ventral bar. All measurements are in micrometers.

Chamela represent new geographical records. Our specimens show a delicate and short membrane in the outer root of the dorsal anchor, possessing a straight and relatively long point 24 (23–26) long, and a straight shaft (Fig. 12), meanwhile in the specimens from Panama the point is 21 long, and shaft is slightly curved, but differences might be due to intraspecific morphological variation. Ventral anchor with inner root small in our specimens, while in those from Panama the inner root is larger (Fig. 13). Dorsal bar slightly curved with short spine-like projections, anteriorly directed on each end, while specimens from Panama have 2 angled base dorsal bar (Fig. 14). The specimens of the present study possess a straight ventral bar, with 2 submedial pockets along the anterior margin and subterminal anterior notches (dorsal view), with a triangular ridge at the mid-portion of the anterior margin (Fig. 15), however in specimens from Panama this was not apparent.

Systematic position and genetic divergence of *Haliotrematoides* spp.

A total of 16 sequences were generated for the 3 species of *Haliotrematoides* recorded in this study: *H. guttati* (3 specimens), *H. plectridium* (2 specimens) and *H. spinatus* (11 specimens); additionally, 13 specimens of other 2 species of dactylogyrids were also sequenced: *E. mehen* (5), and *E. perezponcei* (8). The phylogenetic tree inferred from maximum likelihood analysis is shown in Fig. 16. The maximum likelihood tree ($-\ln$ likelihood = 32,275.20) is presented, as the topology obtained from ML analysis was similar to the consensus tree of the Bayesian analysis. Likewise, the values of posterior probabilities are presented in the ML tree for comparison. The intra and interspecific genetic divergence (uncorrected *P*-distances) of species collected from snappers in Chamela Bay and Mazatlán is shown in Table 2. *Euryhaliotrema* and *Haliotrematoides* are not sister groups, and genetic divergence between genera varied from 20.5 to 24.2. The 3 species of *Haliotrematoides* differ in a range that varied from 4.8% between *H. guttati* and *H. spinatus*, and 17.3% between *H. plectridium* and *H. spinatus*. Intraspecific variation is very low, with the exception of that found among isolates of *H. guttati* (6%) and *H. spinatus* (8%). This intraspecific variation and the tree topology may indicate that these 2 species could represent

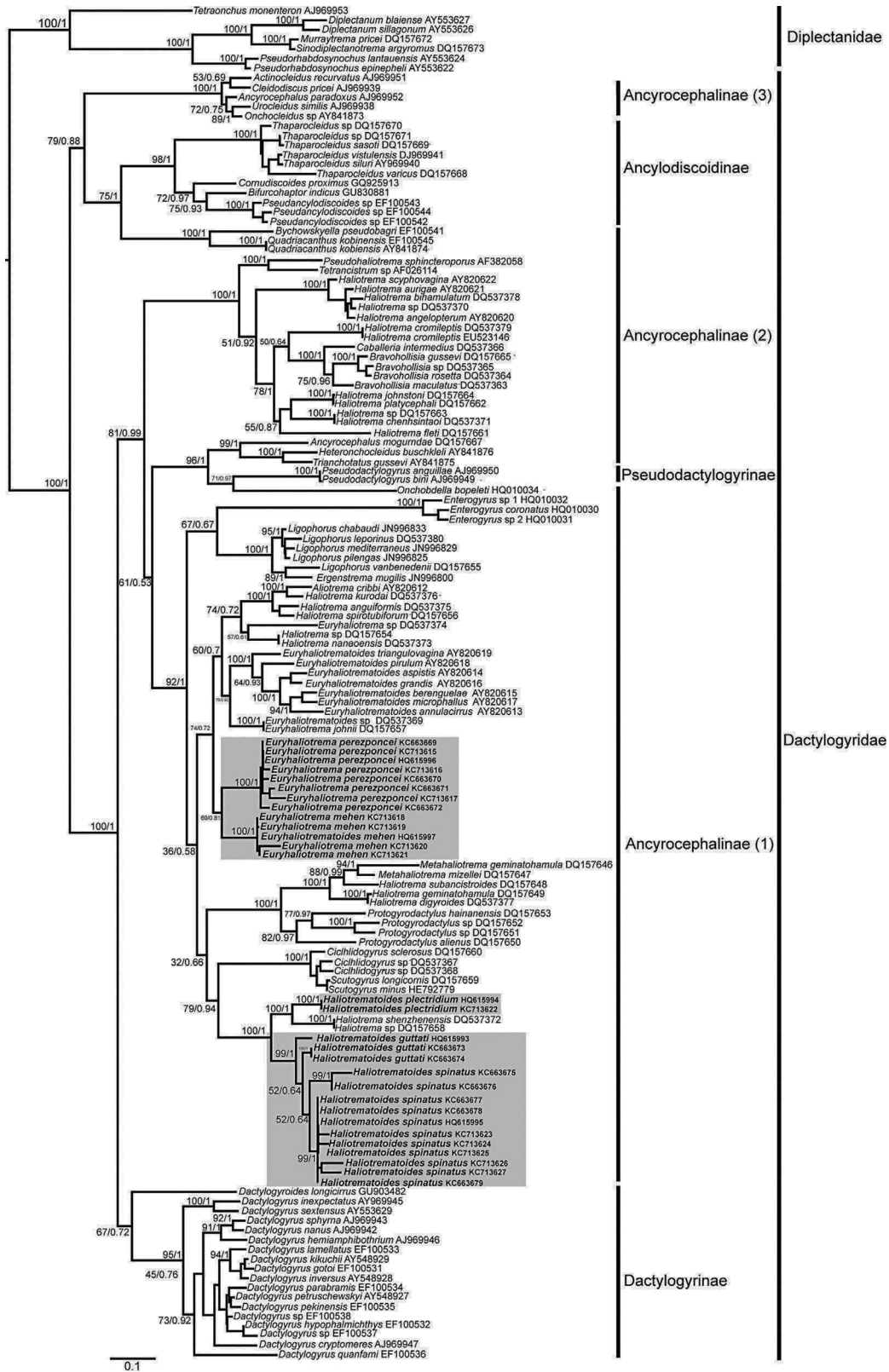


Figure 16. Phylogenetic tree obtained through from maximum likelihood (ML) analysis based on the 28S sequences for selected dactylogyrid species, with some species of diplectanids used as outgroups.

Table 2
Uncorrected pairwise distances of the partial sequences of the 28S rRNA gene among species analyzed from Chamela Bay, Jalisco and Mazatlán, Sinaloa, Mexico. Values are given as a percentage (%).

	<i>E. mehen</i>	<i>E. perezponcei</i>	<i>H. guttati</i>	<i>H. spinatus</i>	<i>H. plectridium</i>
<i>E. mehen</i>	0–0.05	13.6–15.3	20.5–22.8	21.5–24.2	22.25–22.8
<i>E. perezponcei</i>		0–3	20.7–23.9	22.5–25.7	24.3–25.7
<i>H. guttati</i>			0–6	4.8–10.6	14.3–15.5
<i>H. spinatus</i>				0–8	13.6–17.3
<i>H. plectridium</i>					0

a species complex. In the first case, the 3 isolates of *H. guttati* are not monophyletic, although this relationship is supported by low bootstrap and posterior probability values. Instead, the isolates of *H. spinatus* conform a monophyletic clade, although 2 subclades are formed, each with high support values (99/1) (Fig. 16). Even though this could represent a case for the existence of cryptic species, since morphological characters distinguishing those groups are not observed, the inclusion of other molecular markers and a larger sample size is necessary to test the hypothesis of a species complex.

Species of the genus *Haliotrematoides* herein studied are nested within the Ancyrocephalinae sensu lato (Fig. 16). Sequences of 2 species of *Haliotrema* described as parasites of *Sciaenops ocellatus* L. (Sciaenidae) in China (one of them *H. schenzhenensis*, Wang, Lui, & Zhou, 2003), are nested as the sister group of *H. plectridium*, a relationship highly supported by bootstrap and posterior probability values. We were unable to confirm the taxonomic determination of that species since we had no access to museum specimens. This is what the DNA sequence data suggest, however this need to be taken with caution, because it is possible that the species from China may actually belong to the genus *Haliotrematoides*, but this needs to be determined when type-specimens are studied.

Discussion

In this study new geographical records of 3 species of *Haliotrematoides* are presented, and a few details of the morphology of these species are given to complete their taxonomic description. In addition, the specimens collected were sequenced and new data was generated for the 28S rRNA gene, establishing sister-group relationships of the genus within the Ancyrocephalinae sensu lato. Genetic divergence values indicate that the species *H. guttati* and *H. spinatus* potentially represent a species complex. Unfortunately, we found no characters that allowed us to distinguish the specimens on morphological grounds. However, since we used in this study only 1 molecular marker, we took a conservative position and we decided to only recognize the potential existence of 2 cryptic species. These results need to be further corroborated by using at least another molecular marker such as *cox1*, which is more variable and may be more precise in identifying genetic lineages corresponding with separate species, and also it has been proven to be useful in achieving comprehensive species descriptions that facilitate reliable species diagnostics and rapid assessment of biodiversity, as in the case of species in the genus *Gyrodactylus* von Nordmann, 1832 (Hansen, Bakke, & Bachmann, 2007).

A single molecular marker has been used in previous studies on monogenoids when characterizing new species or describing genetic divergence; some studies used either a ribosomal marker (such as internal transcribed spacers) (Huyse & Volckaert, 2002), or a mitochondrial gene such as *cox1* (Hansen, Bachmann, & Bakke, 2003). However, when the potential presence of cryptic species is recognized (Nadler & Pérez-Ponce de León, 2011; Pérez-Ponce de León & Nadler, 2010), the use of several molecular markers in the context of a phylogenetic analysis, and the proper characterization of genetic divergence is highly recommended. This approach has been followed in some species of monogenoids by authors such as Ziętara and Lumme (2003) and Kuusela, Ziętara, and Lumme (2008).

The partial fragment of the 28S rRNA (D1-D3) gene has been widely used to investigate the phylogenetic relationships among some dactylogyrids (Blasco-Costa, Miguez-Lozano, Sarabeev, & Balbuena, 2012; Dang, Levsen, Shander, & Bristow, 2010; Plaisance et al., 2005; Šimková, Matějusková, & Cunningham, 2006; Singh & Chaudhary, 2010, 2011). Our results support the classification scheme proposed by Kritsky and Boeger (1989), who described the polyphyletic and paraphyletic nature of this group of monogenoids. In our study, Ancyrocephalinae was also not recovered as a monophyletic group.

As an additional result of our research, sequences of specimens of other ancyrocephalid monogeneans infecting the spotted rose snapper were obtained, particularly specimens of *E. perezponcei* that were collected from the gills of *L. guttatus*, a species previously described by García-Vargas et al. (2008), as well as specimens of *E. mehen*, a species described by Soler-Jiménez et al. (2012), both from Mazatlán. The taxonomic status of the genus *Euryhaliotrema* has been also unstable. The genus was proposed by Kritsky and Boeger (2002) and, in a recent review of the genus by Kritsky (2012), the author discovered that some specimens collected from snappers possessed a mixture of morphological features, and decided to include the species of *Euryhaliotrematoides* within the genus *Euryhaliotrema*. The species described by Soler-Jiménez et al. (2012) as *Euryhaliotrematoides mehen* infecting *L. guttatus* from Mazatlán was then transferred to *Euryhaliotrema* by Kritsky (2012). The molecular results we obtained herein confirm the proposal by Kritsky (2012) since *E. perezponcei* nests as the sister species of *E. mehen*. The presence of *E. perezponcei* as a parasite of *L. guttatus* in Chamela represents a new geographical record, and the fact that sequences of individuals of this species from Mazatlán and Chamela Bay are nearly identical may indicate that, irrespective

of the geographical distance between localities, populations of the spotted rose snapper move along the Pacific coast of Mexico.

Acknowledgments

We would like to thank the following people for the loan of specimens of *H. guttati*, *H. plectridium* and *H. spinatus*, Eric Hoberg and Patricia Pilitt from United States National Parasite Collection, Luis García Prieto from the CNHE and Rosy Medina from the CPPNP; to fishermen in Chamela Bay, Jalisco, Mazatlán and Sinaloa, for their help to obtain the fish. To Berenit Mendoza Garfias, Rosario Briosio Aguilar, Neptalí Morales Serna, Aline Rojas Sánchez and Rosy Medina for their help during field work. To Martín García Varela for his assistance with molecular analysis. This work was partly supported by PAPIIT IN204514 to GPPL. AGV thanks DGAPA-UNAM for a Postdoctoral scholarship.

References

- Blasco-Costa, I., Miguez-Lozano, R., Sarabeev, V., & Balbuena, J. A. (2012). Molecular phylogeny of *Ligophorus* (Monogenea: Dactylogyridae) and their affinities within the family Dactylogyridae. *Parasitology International*, *61*, 619–627.
- Dang, B. T., Levsen, A., Shander, C., & Bristow, G. A. (2010). Some *Haliotrema* (Monogenea: Dactylogyridae) from cultured grouper (*Epinephelus* spp.) with emphasis on the phylogenetic position of *Haliotrema cromileptis*. *Journal of Parasitology*, *96*(7), 30–39.
- Froese, R., & Pauly, D. (Eds.). (2014). *FishBase. World Wide Web electronic publication*. Retrieved from: <http://www.fishbase.org>
- García-Vargas, F., Fajer-Ávila, E. J., & Lamothe-Argumedo, R. (2008). Two new species of Dactylogyridae (Monogenea) on rose spotted snapper, *Lutjanus guttatus* (Osteichthyes: Lutjanidae), from the coasts of Nayarit, Sinaloa, Mexico. *Zootaxa*, *1729*, 61–68.
- García-Vásquez, A., Hansen, H., Cristison, K. W., Bron, J. E., & Shinn, A. P. (2011). Description of three new species of *Gyrodactylus* vonn Nordmann, 1832 (Monogenea) parasitising *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae). *Acta Parasitologica*, *56*, 20–33.
- Hansen, H., Bachmann, L., & Bakke, T. A. (2003). Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling, and rainbow trout in Norway and Sweden. *International Journal for Parasitology*, *33*, 1471–1478.
- Hansen, H., Bakke, T. A., & Bachmann, L. (2007). DNA taxonomy and barcoding of monogenean parasites: Lessons from *Gyrodactylus*. *Trends in Parasitology*, *23*, 363–367.
- Huelsenbeck, J. P., & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755.
- Huysse, T., & Volckaert, F. A. M. (2002). Identification of a host-associated species complex using molecular and morphometric analyses, with the description of *Gyrodactylus rugienoides* n. sp. (Gyrodactylidae, Monogenea). *International Journal for Parasitology*, *32*, 907–919.
- Kritsky, D. C. (2012). Dactylogyrids (Monogenea: Polyonchinea) parasitizing the gills of snappers (Perciformes: Lutjanidae): Revision of *Euryhaliotrema* with new and previously described species from the Red Sea, Persian Gulf, the eastern and Indo-West Pacific Ocean, and the Gulf of Mexico. *Zoologica*, *29*, 227–276.
- Kritsky, D. C., & Boeger, W. A. (1989). The phylogenetic status of the Ancyrocephalidae Bychowsky, 1937 (Monogenea: Dactylogyroidea). *Journal of Parasitology*, *75*, 207–211.
- Kritsky, D. C., & Boeger, W. A. (2002). Neotropical Monogenea. 41: New and previously described species of Dactylogyridae (Platyhelminthes) from the gills of marine and freshwater perciform fishes (Teleostei) with proposal of a new genus and a hypothesis on phylogeny. *Zoosystema*, *24*, 7–40.
- Kritsky, D. C., Tingbao, Y., & Yuan, S. (2009). Dactylogyrids (Monogenea, Polyonchinea) parasitizing the gills of snappers (Perciformes, Lutjanidae): Proposal of *Haliotrematoides* n. gen. and descriptions of new and previously described species from marine fishes of the Red Sea, the eastern and Indo-west Pacific Ocean, Gulf of Mexico and Caribbean sea. *Zootaxa*, *1970*, 1–51.
- Kuusela, J., Ziętara, M. S., & Lumme, J. (2008). Description of three new European cryptic species of *Gyrodactylus* Nordmann, 1832 supported by nuclear and mitochondrial phylogenetic characterization. *Acta Parasitologica*, *53*, 120–126.
- Littlewood, D. T. J., Curini-Galletti, M., & Herniou, E. A. (2000). The interrelationships of Proseriata (Platyhelminthes: Seriata) flatworms tested with molecules and morphology. *Molecular Phylogeny and Evolution*, *16*, 449–466.
- Maddison, W. P., & Maddison, D. R. (2011). *Mesquite: A modular system for evolutionary analysis. Version 2.75*. Retrieved from: <http://mesquiteproject.org>
- Nadler, S. A., & Pérez-Ponce de León, G. (2011). Integrating molecular and morphological approaches for characterizing parasite cryptic species: Implications for parasitology. *Parasitology*, *138*, 1688–1709.
- Pérez-Ponce de León, G., & Nadler, S. A. (2010). What we don't recognize can hurt us: A plea for awareness about cryptic species. *Journal of Parasitology*, *96*, 453–464.
- Plaisance, L., Littlewood, D. T. J., Olson, P. D., & Morand, S. (2005). Molecular phylogeny of gill Monogeneans (Platyhelminthes, Monogenea, Dactylogyrida) and colonization of Indo-West Pacific butterflyfish hosts (Perciformes, Chaetodontidae). *Zoologica Scripta*, *34*, 425–436.
- Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, *25*, 1253–1256.
- Rambaut, A. (2006). *FigTree v 1.3.1*. Institute of Evolutionary Biology, University of Edinburgh. Retrieved from: <http://www.tree.bio.ed.ac.uk/software/figtree/>
- Šimková, A., Matějsová, I., & Cunningham, C. O. (2006). A molecular phylogeny of the Dactylogyridae sensu Kritsky and Boeger (1989) (Monogenea) based on the D1–D3 domains of large subunit rDNA. *Parasitology*, *133*, 43–53.
- Singh, H. S., & Chaudhary, A. (2010). Genetic characterization of *Dactylogyroides longicirrus* (Tripathi, 1959) Gussev, 1976 by nuclear 28S segment of ribosomal DNA with a morphological redescription. *Scientia Parasitologica*, *11*, 119–127.
- Singh, H. S., & Chaudhary, A. (2011). A new record of *Cornudiscoides proximus* Gussev, 1976, a freshwater Monogenean and its phylogeny, inferred from partial sequence of 28S rDNA. *Pakistan Journal of Zoology*, *4*, 183–189.
- Soler-Jiménez, L. C., García-Gasca, A., & Fajer-Ávila, E. J. (2012). A new species of *Euryhaliotrematoides* Plaisance & Kritsky, 2004 (Monogenea: Dactylogyridae) from the gills of the spotted rose snapper *Lutjanus guttatus* (Steindachner) (Perciformes: Lutjanidae). *Systematic Parasitology*, *82*, 113–119.
- Stamatakis, A. (2006). Raxml-vi-hpc: Maximum likelihood-based phylogenetic with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690.
- Tamura, K. D., Peterson, N., Peterson, G., Stecher, M. N., & Kumar, S. (2011). MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary, distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739.
- Vidal-Martínez, V. M., Aguirre-Macedo, L. M., Scholz, T., González-Solis, D., & Mendoza-Franco, E. F. (2001). *Atlas of helminth parasites of cichlid fish of Mexico*. České Budějovice: Academia.
- Zhukov, E. V. (1976). New species of the genus *Haliotrema* Johnston and Tieg, 1922, from the Gulf of Mexico fishes of the family Lutjanidae in Fauna, systematics and phylogeny of Monogenea. *Proceeding, Institute of Biology and Pedology, Farp East Science Centre. Academy of Sciences of the U.S.S.R., New Series*, *35*, 33–47.
- Ziętara, M. S., & Lumme, J. (2003). The crossroads of molecular, typological and biological species concepts: Two new species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). *Systematic Parasitology*, *55*, 39–52.