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***Mexicana rubra* sp. nov. and *Encotyllabe* cf. *spari* Yamaguti, 1934 (Monogenea) of *Orthopristis ruber* (Cuvier, 1830) from the Brazilian Coast off Rio de Janeiro**A. C. A. CAMARGO<sup>1</sup>, J. L. LUQUE<sup>2</sup>, C. P. SANTOS<sup>1\*</sup>

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**Summary**

*Mexicana rubra* sp. nov. and *Encotyllabe* cf. *spari* are described from the gills of the marine fish *Orthopristis ruber* (Haemulidae) caught off Rio de Janeiro, Brazil. Morphological, ultrastructural and genetic data are provided. The main diagnostic features of this new species of ectoparasite are a posteriorly bipartite testis, a ventral bar with three deep bowed projections and a dorsal bar with two deep, conspicuous, antero-lateral indentations. Genetic data on *Mexicana rubra* sp. nov. are based on the ITS1, 5.8S and partial 18S and 28S rDNA. This is the sixth known species of *Mexicana* Caballero & Bravo-Hollis, 1959, a key to which is also presented. *Encotyllabe* cf. *spari* Yamaguti, 1934 is described from the same host, with new ultrastructural data and new partial 18S and 28S rDNA sequences. A phylogenetic analysis based on partial 18S and 28S sequences is undertaken for both species.

**Keywords:** Monogenea; Dactylogyridae; Capsalidae; Genetic data

**Introduction**

The Haemulidae (Osteichthyes) are marine fish species distributed in the Atlantic, Indian and Pacific Oceans, but may also occur in estuaries (Palazón-Fernández, 2007). In the Atlantic, species of *Orthopristis* Girard, 1858, so-called "grunts" due to their ability to produce sounds when in danger, are found in areas with a stony bottom, sandy beaches, bays and estuarine areas, and are distributed between the Caribbean off Honduras to the coast of southern Brazil (Burkenroad, 1930; Tavoilga & Wodinsky, 1965; Menezes & Figueiredo, 1980).

The parasites of *Orthopristis ruber* (Cuvier, 1830) have been studied by Amato (1982 a, b, c; 1983 a, b), Bashirullah *et al.* (1987), Luque *et al.* (1992; 1993a, b; 1996a) and Paschoal *et al.* (2014), in addition to their citation in the checklists of Paschoal *et al.* (2015). During a survey of the monogeneans of *O. ruber* off Rio de Janeiro, Brazil, a new species of *Mexicana* Caballero & Bravo-Hollis,

1959 parasitizing the gills was found, the morphology and genetic profiles of which are described herein. In addition, specimens identified as *Encotyllabe* cf. *spari* Yamaguti, 1934, also from the gills, are described along with new ultrastructural details and genetic data based on the partial 18S and 28S rDNA genes. The phylogenetic position of both species is discussed.

**Material and Methods**

Specimens of *Orthopristis ruber* were collected off Urca on the Brazilian coast of Rio de Janeiro (22°56'49"N, 43°9'50"W) during 2015 and transferred fresh to the laboratory. The gills were removed and examined in a saline medium under a stereomicroscope. Any parasites recovered were fixed in 70 % or 100 % ethanol. Some specimens were mounted in Berlese medium to improve the visibility of the sclerotised structures. Alternatively, some specimens were stained in Mayer's paracarmine or Gomori's trichrome

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and mounted in Canada balsam. Measurements are presented in micrometers, with the mean followed by the range in parentheses, unless otherwise stated. The measurements of the anchors were based on Vignon and Sasal (2010). Drawings were made with the aid of a drawing tube attached to the Olympus CX31 microscope. Prevalence and intensity data follows Bush *et al.* (1997). Representative specimens were deposited at the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil. For comparative purposes, paratypes (CHIOC 33.109 and 33.110) of *Mexicana atlantica* Luque, Amato & Takemoto, 1992 and micrographs of *Mexicana bychowskyi* Caballero & Bravo-Hollis, 1959 (holotype and paratypes 216-24) and *Mexicana littoralis* Caballero & Bravo-Hollis, 1961 (holotype 218-3) from Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico were studied. For scanning electron microscopy (SEM), specimens were post-fixed for 24 h at room temperature in a solution of 1 % osmium tetroxide and 0.8 % potassium ferrocyanide, dehydrated through a graded alcohol series, critical-point dried and sputter coated with

gold. The samples were examined using a JEOL JSM-6390 LV SEM, from the Plataforma de Microscopia Eletrônica do Instituto Oswaldo Cruz, at an accelerating voltage of 15 kV.

#### Genetic analysis

The DNA extraction was performed using the phenol-chloroform method as described by Billings *et al.* (1998) and a set of primers were used to amplify different regions of the DNA. The rDNA region 28S was amplified by PCR using the primers C1 (5' – ACCCGCTGAATTTAAGCAT – 3') and D2 (5' – TGGTC CGTGT TTCAA GAC – 3') (after Chisholm *et al.*, 2001). The partial 18S, ITS1 and 5.8S were amplified using the primers S1 (5'-TTCCG ATAAC GAACG AGACT -3') and H7 (5'- GCTGC GTTC TTCAT CGATA CTCG -3') (Sinnappah *et al.*, 2001). PCRs were carried out using cycling parameters as previously described by these authors. The PCR products were analysed by electrophoresis in 1.5 % agarose in Tris-borate EDTA gels, stained with SyberGreen (Invitrogen, Eugene, Oregon, USA) and photographed under UV transillumination. Amplified PCR products were purified with Ex-

Table 1. List of the species of Monogenea and outgroups used in the phylogenetic analyses of *Mexicana rubra* sp. nov. and *Encotyllabe* cf. *spari* using sequences of the partial 18S and partial 28S rDNA genes.

Species	Family	18S rDNA	28S rDNA
<i>Benedenia epinepheli</i> (Yamaguti, 1937)	Capsalidae	EU707802	EU707803
<i>Benedenia</i> sp.	Capsalidae	AJ228774	
<i>Benedenia lutjani</i> (Whittington & Kearn, 1993)	Capsalidae		AY033939
<i>Capsala martinieri</i> (Bosc, 1811)	Capsalidae	AJ276423	AF382053
<i>Capsala laevis</i> (Verrill, 1875)	Capsalidae		JN980396
<i>Encotyllabe chironemi</i> (Robinson, 1961)	Capsalidae	AJ228780	AF382054
<i>Encotyllabe</i> cf. <i>spari</i> (Yamaguti, 1934)	Capsalidae	KY553150	KY553149
<i>Encotyllabe caballeroi</i> (Velasquez, 1977)	Capsalidae		AF026112
<i>Encotyllabe caranxi</i> (Lebedev, 1967)	Capsalidae		FJ971990
<i>Bravohollisia rosetta</i> (Lim, 1995)	Dactylogyridae	EF152322	DQ537364
<i>Bravohollisia tecta</i> (Bychowsky & Nagibina, 1970)	Dactylogyridae	KJ571020	KJ571012
<i>Euryhaliotrematoides pirulum</i> (Plaisance & Kritsky, 2004) (= <i>Euryhaliotrema pirulum</i> )	Dactylogyridae	AY820607	
<i>Euryhaliotrematoides triangulovagina</i> (Yamaguti, 1968) (= <i>Euryhaliotrema triangulovagina</i> )	Dactylogyridae	AY820608	
<i>Euryhaliotrematoides microphallus</i> (Yamaguti, 1968) (= <i>Euryhaliotrema microphallus</i> )	Dactylogyridae	AY820606	
<i>Haliotrematoides spinatus</i> (Kritsky & Mendoza-Franco in Kritsky, Yang & Sun, 2009)	Dactylogyridae		KC663678
<i>Haliotrematoides guttati</i> (Garcia-Vargas, Fajera-Ávila & Lamothe-Argumedo, 2008)	Dactylogyridae	JN054406	KC663673
<i>Ligophorus uruguayensis</i> (Failla Siquer & Ostrowski de Núñez, 2009)	Dactylogyridae		KF442630
<i>Ligophorus funnelus</i> (Soo & Lim, 2012)	Dactylogyridae		KM221914
<i>Mexicana rubra</i> sp. nov.	Dactylogyridae	KY553146	KY553148
<i>Microcotyle sebastis</i> (Goto, 1894)	Microcotylidae	AJ287540	AF382051

oSap-IT (USB® Products Affymetrix Inc., Cleveland, Ohio, USA). DNA cycle sequencing reactions were performed using BigDye Terminator v.3.1 (Applied Biosystems, Foster City, CA, USA) and automated sequencing was done using the Sequencing Platform at the Fundação Oswaldo Cruz-PDTIS/FIOCRUZ in Brazil. Sequences of both strands were generated, edited and aligned by using the MEGA version 7.0 software (Kumar *et al.*, 2015). Sequences were compared to others available in the GenBank database using the BLASTN program from the National Center for Biotechnology Information (NCBI) server (<http://www.ncbi.nlm.nih.gov/BLAST>) (Altschul *et al.*, 1990). Taxa, for which sequences

from GenBank were used for the phylogenetic analysis, are listed in Table 1. To examine the phylogenetic relationships, the nucleotide sequences were analysed using the CLUSTAL W algorithm of the MEGA 7.0 package (Thompson *et al.*, 1994). Maximum likelihood (ML) phylogenetic trees (Felsenstein, 1981) were inferred using the MEGA 7.0 using the generalised time-reversible (GTR) and gamma distribution with invariant sites (G + I) for the analysis of 18S rDNA and Kimura2 (K2) with invariant site (I) for partial 28S rDNA. The tree was resampled by 5,000 bootstrap replicates to evaluate the reliability of the groups.

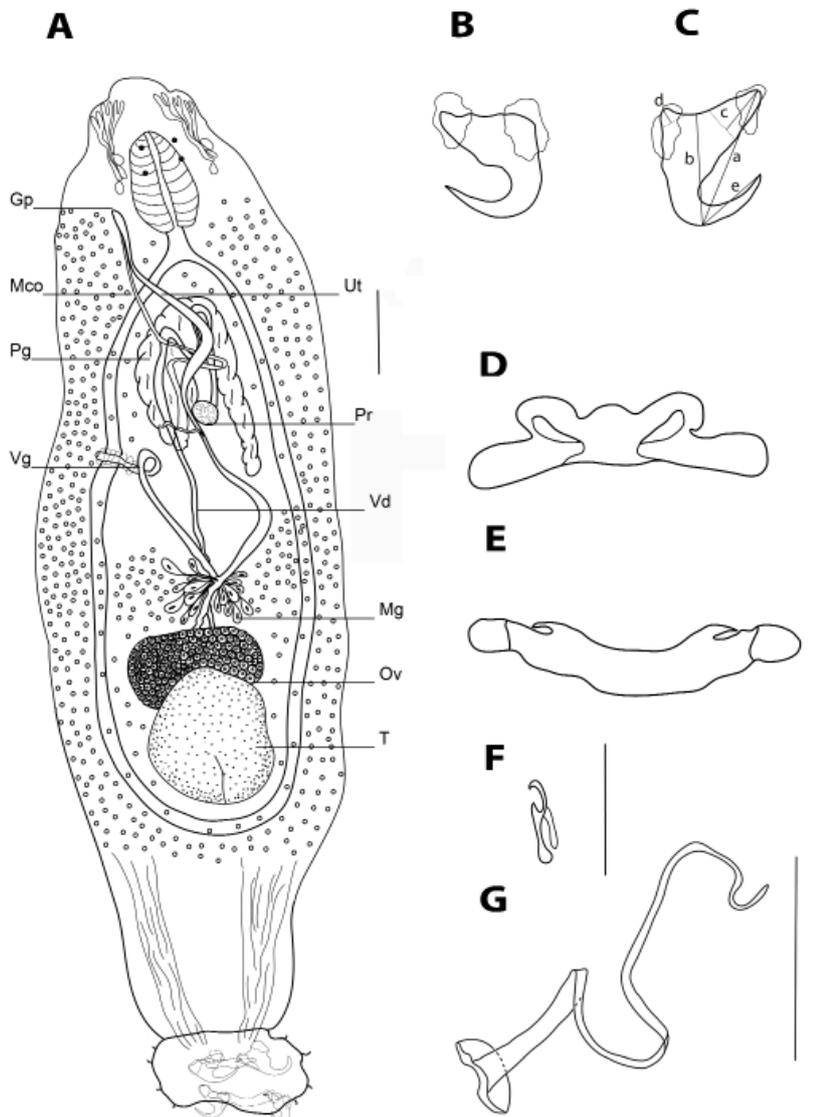


Fig. 1. *Mexicana rubra* sp. nov. (A) Holotype, ventral view; (B) Ventral anchor; (C) Dorsal anchor; (D) Ventral bar; (E) Dorsal bar; (F) Hook; (G) Copulatory organ. a= length of base; b= length of inner root; c= length of outer root; d= length of point; e= total length of marginal hook. The reproductive organ components are abbreviated as follows: T = testis; Ov = ovary; Mg = Mehlis' gland; Vg = vagina; Pr = prostatic reservoir; Pg = prostatic glands; Vd = vas deferens; Mco = male copulatory organ; Ut = uterus; Gp = genital pore. Scale-bars: Figure A = 100mm; B, C, D and E = 50mm; F = 20mm; G = 100mm.

## Results

A total of 101 *Orthopristis ruber* measuring 13.61 (7 – 25) cm long were examined. The monogeneans *Mexicana rubra* sp. nov. and *Encotyllabe* cf. *spari* Yamaguti, 1934 found on the gills are described below.

Dactylogyridae Bychowsky, 1933

*Mexicana* Caballero & Bravo-Hollis, 1959

*Mexicana rubra* sp. nov. (Fig. 1A – G, Table 2)

Type host: *Orthopristis ruber* (Cuvier 1830).

Site of infection: Gills.

Type locality: Coast of Rio de Janeiro, off Urca, Brazil.

Specimens deposited: CHIOC numbers 38466 (Holotype), 38467a–c and 38468 (4 paratypes).

Prevalence and intensity: 2.97 % (3 fish infected/ 101 examined); 1 – 7 parasites per fish.

Etymology: The new species is treated as an adjective and refers to the specific name of the host.

Description: (measurements based on 7 adult specimens): Body elongate, with poorly differentiated haptor. Total length 843 (700 – 950) long and 232 (180 – 360) width at the level of ovary (Fig. 1A). Cephalic region with three lobes pairs and four eyes similar in size. Pharynx ovoid is 74 (62 – 90) long × 56.4 (47 – 70) wide. Haptor 83 (70 – 95) long × 122 (80 – 155) wide; two pairs of dissimilar anchors and 14 marginal hooks. Ventral anchors: a= 27 (26 – 28), b= 16 (16 – 17), c= 5 (3 – 7.5), d= 5 (4 – 6) and e= 25 (25 – 25) (Fig. 1B). Dorsal anchors: a= 27 (23 – 30), b= 22.5 (23 – 23), c= 9 (8 – 10), d= 6 (5 – 8) and e= 21 (15 – 25) (Fig. 1C). Single pair of plates present on either side of anchors. Bars different in size and shape, both with lateral extensions articulated to anchors. Ventral bar is 7 – 10 long × 35 (–) wide, with three deep bowed projections; dorsal bar elongate, 5 – 8 long × 37 – 40 wide (39.54), with two conspicuous antero-lateral indentations (Fig. 2D – E, Table 2). Hooks 14, similar in size and shape, 12 (11 – 13) long, short shank, erect thumb, curved shaft, delicate point, filament (FH) loop almost same length as hook shank (Fig. 1F). Intestinal ceca is confluent forming posteriorly cyclocoel. Testis postovarian is 120 (100 – 145) × 90 (75 – 195), bipartite posteriorly. Vas deferens arises from anterior border of testis, extends and dilates anteriorly to form elongated, inverted U-shaped seminal vesicle in pre-equatorial region. Prostatic reservoir identified at the level of seminal vesicle. Male copulatory organ are sclerotized, tubular, sinuous, 260 (242 – 280) long, with expanded piece at the base, genital pore at the level of pharynx (Fig. 1G). Ovary bilobed 87 (73 – 100) × 50 (48 – 55). Mehlis' gland is well developed, with pedunculate cells. Vagina is muscular, tubular; vaginal pore dextro-ventral, pre-equatorial. Vitellarium follicular; follicles scattered throughout trunk (extending from level of pharynx almost to peduncle) except in region of reproductive organs. Median uterus rises from ootype to genital pore; and eggs not observed.

## Remarks

*Mexicana rubra* sp. nov. is the first species of this genus parasitizing *O. ruber*. Its main diagnostic features are the posteriorly bipartite testis, ventral bar with three deep bowed projections and dorsal bar with a pair of conspicuous, deep, antero-lateral indentations. Congeneric species from the Pacific Ocean, in addition to variation in geographical distribution and host, can be differentiated as follows: in the case of *Mexicana iannaconi* Chero, Cruces, Sáez and Alvariano, 2014 from Peru, by having a completely bipartite testis and larger dorsal and ventral bars; and, in the cases of *M. littoralis* and *M. bychowskyi* from Mexico, although possessing a testis which is bipartite only posteriorly, by having a different size and shape of the bars and anchors. The species occurring in the Atlantic Ocean can be readily differentiated, since *Mexicana anisotremum* Cezar, Paschoal and Luque, 2012 has an entire testis, and the morphologically most similar species, *Mexicana atlantica* Luque, Amato and Takemoto, 1992, which has also a posteriorly bipartite testis, differs mainly in its host and the shape and size of the bars and anchors. A comparative analysis of the bars of these species is presented in Table 2.

## Genetic Analysis

New sequences of the partial 18S rDNA (KY553146) (482bp), partial 18S, ITS and 5.8S rDNA (KY553148) (1064bp) and partial 28S (KY553148) (775bp) have been deposited in the GenBank. The BLAST results for the partial 18S, ITS1 and 5.8S rDNA sequences of 1064 bp indicated a 94 % identity with a species *Euryhaliotrematoides* sp. (DQ537344) (= *Euryhaliotrema* sp.), with a 45 % query cover and maximum score of 732, followed by a 95 % identity, 41 % query cover and maximum score of 693, with both *Dactylogyrus tuba* (KJ605445) and *Dactylogyrus carpathicus* (EF582618). When the ITS1 was analyzed separately, the BLAST result indicated no significant similarity with sequences deposited in GenBank, and, only in the case of the partial 18S, the BLAST indicated a 92 % identity with *Euryhaliotrematoides pirulum* (AY820607) and *E. triangulovagina*, with a 98 % cover and total score 676 and 671, respectively. For the partial 28S rDNA, the BLAST results indicated a 86 % identity, 87 % query cover and a maximum score 699 with *Haliotrematoides spinatus* (KC663678, HQ615995 and KC663677).

Capsalidae Baird, 1853

*Encotyllabe* Diesing, 1850

*Encotyllabe* cf. *spari* Yamaguti, 1934 (Fig. 3A – G)

Type host: *Orthopristis ruber* (Cuvier, 1830).

Site of infection: Gills

Type-locality: Coast of Rio de Janeiro, off Urca, Brazil.

Specimens deposited: CHIOC numbers 38463, 38464a, 38464b and 38465 (vouchers).

Prevalence and intensity: 48.51 % (49 fish infected/ 101 examined); 1 – 6 parasites per fish.

Table 2. Comparative drawings of bars from *Mexicana* species. Scale bars: 20µm.

Species	Ventral Bar	Dorsal Bar
<i>M. iannaconi</i>		
Redrawn from original article		
<i>M. atlantica</i>		
Redrawn from original article		
Redrawn from paratype: CHIOC 33.109		
<i>M. bychowsky</i>		
Redrawn from original article		
Redrawn from photograph of Voucher: CNHE		
<i>M. littoralis</i>		
Redrawn from original article		
<i>M. anisotremum</i>		
Redrawn from original article		
<i>M. rubra</i> sp. nov.		

Description: (measurements based on 10 specimens): Body ellipsoidal, 3.02 (2.3–4.34) mm long and 1130 (780–1.650) mm wide; anterior suckers, 196 (160–280) × 206 (145–280), with projecting crenulate lobes (Fig. 2A). Mouth bordered by digitiform projections on anterior border. Pharynx is muscular, 338 (260–460) × 324 (290–400). Two pairs of eyespots are at level of pharynx. Intestinal ceca branched posteriorly, extending to base of peduncle, not confluent. Peduncle 300–400 (350). Haptor is bell-shaped 529 (470–610) × 532 (375–720); marginal membrane 11–13 (12) wide; one pair of large anchors, one pair of small anchors and 14 marginal hooks. Large anchors 233 (200–280) × 95 (80–110) (Fig. 2B); small anchors 20–25 (Fig. 2C); marginal hooks 10 long (Fig. 2D). Testes two juxtaposed, pre-equatorial, differ in size; left testis 124 (100–210) × 124 (105–170), right testis 126 (100–170) × 128 (100–170). Efferent canal emerges from each testis, uniting to form vas deferens which penetrates bulbous cirrus sac

to become seminal vesicle and ejaculatory duct; cirrus elongate. Prostatic reservoir is inside cirrus sac (Fig. 2E). Ovary noticed pre-testicular, oval, 199 (140–250) × 179 (125–250). Uterus long, thin, passes cirrus sac and ootype to open via genital pore located close to pharynx. Vitelline follicles are dense, extended from level of pharynx almost to the base of peduncle. Vitelline reservoir is pre-ovarian, 137 (80–240) × 124 (80–200). Mehlis' gland surrounds ootype. Vagina found ventrally, at the anterior level of ovary. Eggs are triangular, 90 × 110; anterior filament 70 long (Fig. 2F).

#### Scanning electron microscopy

Body smooth, folded laterally, with distal peduncle and oval haptor (Fig. 3A). Anterior suckers bordered anteriorly by crenulate lobes (Fig. 3B). Mouth ventrally, between anterior suckers, bearing several digitiform projections (Fig. 3B–C). Haptor bordered by small

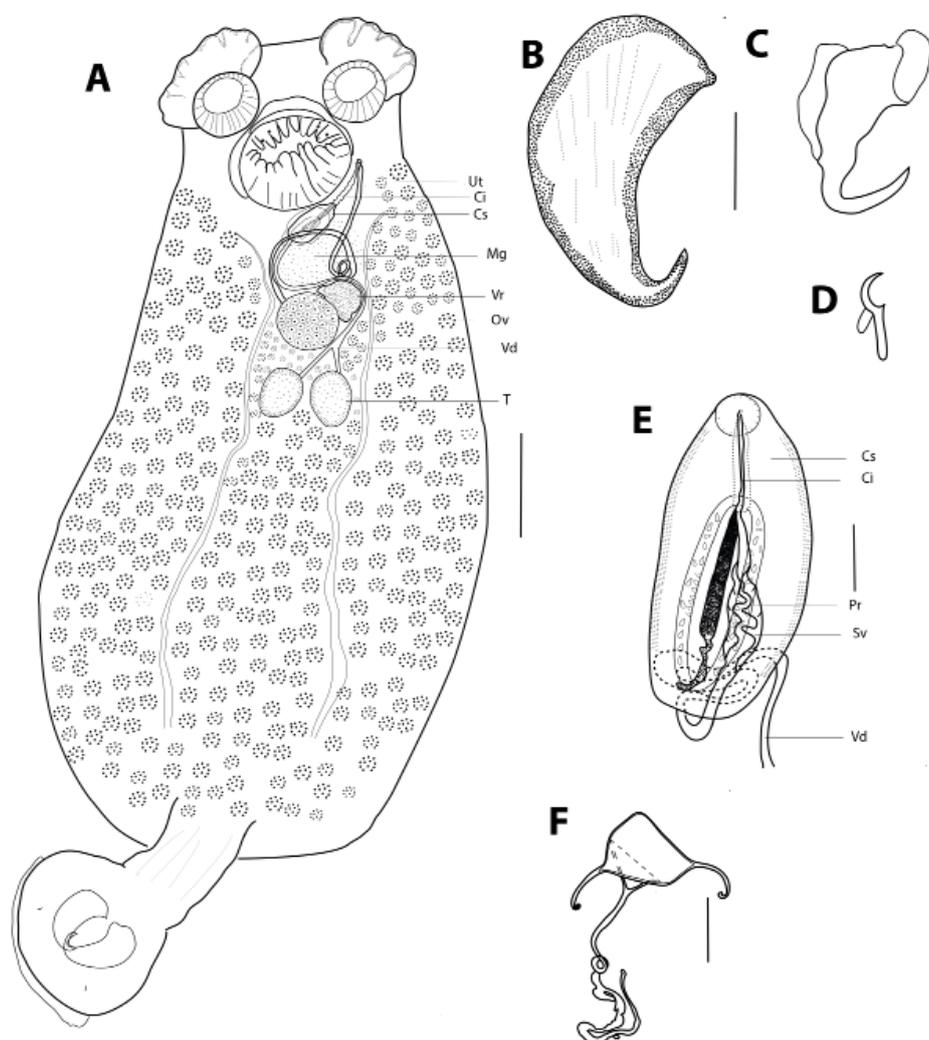


Fig. 2. *Encotyllabe cf. spari*. (A) Body, ventral view; (B) Large anchor; (C) Small anchor; (D) Hook; (E) Copulatory organ, (F) Egg. The reproductive organ components are abbreviated as follows: T = testis; Vd = vas deferens; Vr = vitelline reservoir; Ov = ovary; Mg = Mehlis' gland; Pr = prostatic reservoir; Sv = seminal vesicle; Cs = cirrus sac; Ci = cirrus; Ut = uterus. Scale-bars: A = 500mm; B = 100mm; C, D and E = 50mm; F = 100mm.

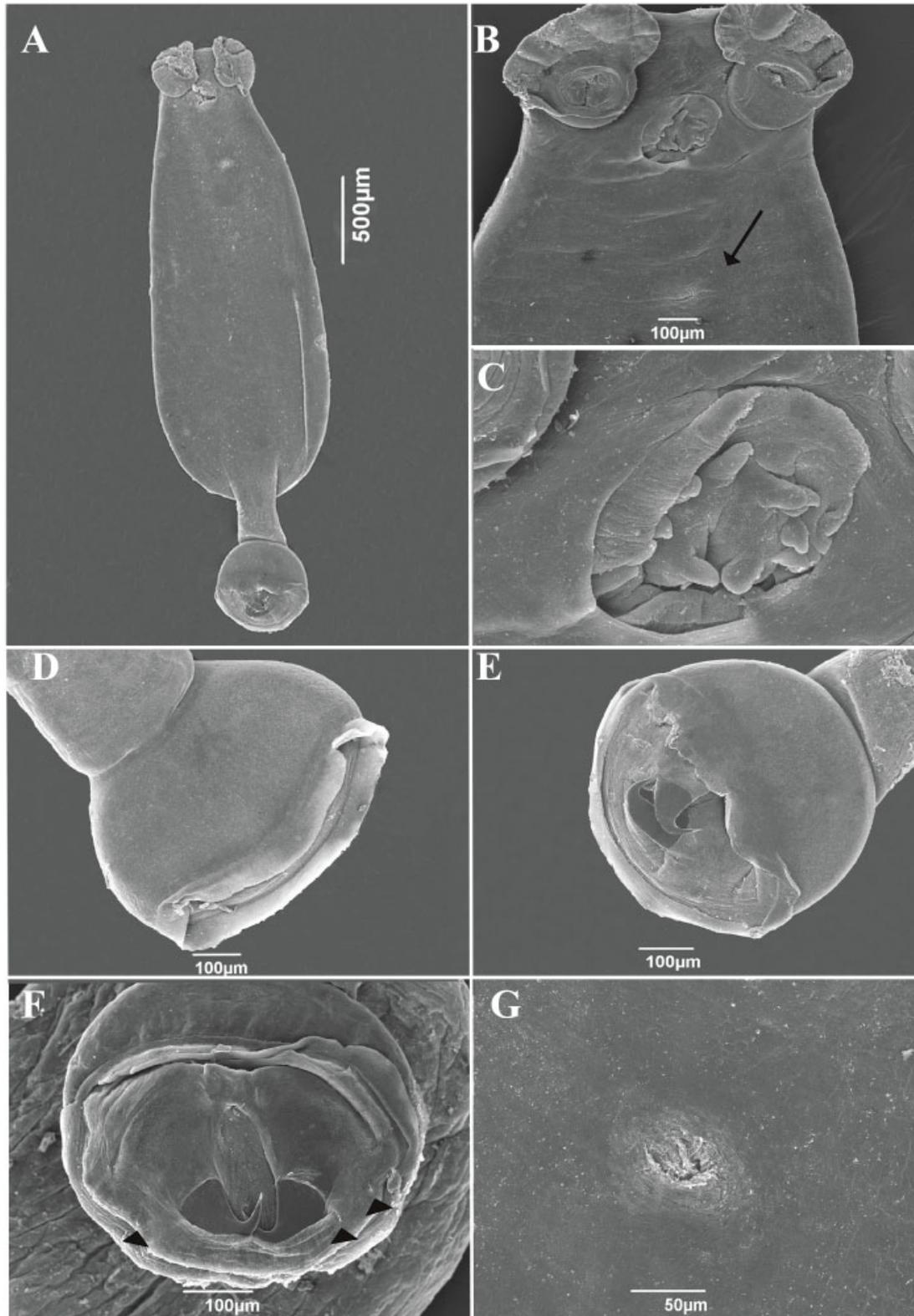


Fig 3. Scanning electron microscopy of *Encotyllabe cf. spari* (A) Adult ventral view; (B) Anterior part of body with oral sucker; (C) Mouth bordered by digitiform projections; (D) Haptor with small membrane ; (E) Haptor with large anchors; (F) Details of large anchors; (G) Vaginal pore.

marginal membrane (Fig. 3D – F). Large anchors with free tips (Fig. 3E – F) bordered by small hooks (arrowhead) (Fig. 3F). Vaginal pore, located mid-ventral and well posterior to the mouth (Fig. 3B – G, arrow).

#### Genetic Analysis

New sequences of *E. cf. spari* obtained in this study were deposited in the GenBank: these are the partial 18S rDNA (KY553150) (499bp) and partial 28S rDNA (KY553149) (796bp). For the partial 18S rDNA, the BLAST result indicated that the present material had a 98 % identity with *Encotyllabe chironemi* (AJ228780), with a 99 % query cover and maximum score of 857, followed by *Megalocotyle marginata* (KU872029), with a 91 % identity, 93 % query cover and maximum score of 632, and *Benedenia epinepheli* (EU707802), with a 89 % identity, 99 % query cover and maximum score of 617. For the partial 28S rDNA, the BLAST indicated *Encotyllabe chironemi* (AF382054) had a 99 % identity, 100 % query cover and a maximum score of 1,435, followed by *Encotyllabe*

and grouped with 100 % statistical support. *Encotyllabe cf. spari* grouped in a clade with *E. chironemi*, with 99 % support, closer than to *Capsala martinieri*, with bootstrap support of 76 % (Fig. 4). The tree topology of the phylogenetic analysis based on the partial 28S rDNA showed that the Dactylogyridae had a high level of support (99 %). A strongly supported (96 %) clade included *Ligophorus uruguayensis* and *L. funellus* (100 %) and two subclades, i.e. *Mexicana rubra* sp. nov., with a 93 % bootstrap value, and *Haliotrematoides spinatus* + *H. guttati* (99 %). The basal clade comprised *Bravohollisia rosetta* and *B. tecta*, with 100 % bootstrap. The Capsalidae, with 100 % statistical support, presented three clades: the Benedeniinae, Encotyllabinae and Capsalinae. The Benedeniinae was represented by *Benedenia epinepheli* and *Benedenia lutjani* with 99 % of bootstrap grouped in a clade closer to Encotyllabinae represented by genera of *Encotyllabe* Diesing, 1850, with 78 % support. Within the Encotyllabinae, three clades were formed; *Encotyllabe caranxi* appeared first, with 100 % support, *E. caballeroi* grouped (87 % support) in a clade containing

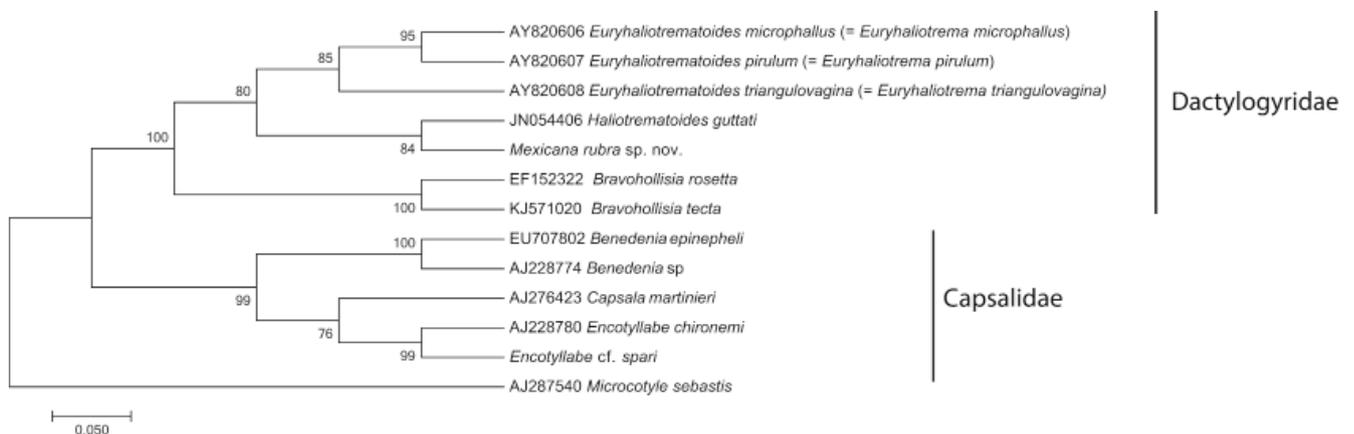


Fig. 4. Maximum-likelihood tree of *Encotyllabe cf. spari* and *Mexicana rubra* sp. nov. based on the 18S region to show their relationships with other species of Monogenea. Numbers (%) on the branches indicate 5,000 bootstrap replicates. GenBank accession numbers are shown and the scale bar indicates the nucleotide mutations per site.

*caballeroi* (AF026112), with 98 % identity, 99 % query cover and maximum score of 1,393, and *Encotyllabe caranxi* (FJ971990) with 98 % identity, 46 % query cover and a maximum score of 647.

*E. cf. spari* + *E. chironemi* (90 % support). *Capsala martinieri* and *Capsala laevis* (Capsalinae) grouped with 100 % of bootstrap in a basal clade (Fig. 5).

#### Phylogenetic analyses

The tree topology of the phylogenetic analysis based on the 18S rDNA of the two species studied here showed two main clades; one was included in the Dactylogyridae with 100 % bootstrap support and the other in the Capsalidae with 99 % support.

The clade formed by the Dactylogyridae showed that *Euryhaliotrematoides microphallus* grouped with *E. pirulum*, with a statistical support of 95 %, and *E. triangulovagina*, with a bootstrap value of 85 %. *Mexicana rubra* sp. nov. grouped with *Haliotrematoides guttati*, with an 84 % bootstrap value and was closest to the *Euryhaliotrematoides* clade, with 80 % support. *Bravohollisia rosetta* and *B. tecta* appeared in a basal clade, with a 100 % bootstrap value. The Capsalidae, included *Benedenia* sp. and *B. epinepheli*,

#### Discussion

Caballero and Bravo-Hollis (1959) proposed *Mexicana* in the Tetraonchidae Bychowsky, 1937, with the type species *M. bychowskyi* described from an unidentified host off the Pacific coast of Mexico. The generic diagnosis included six marginal hooks on the haptor and a postovarian testis. In 1961, these authors (Caballero and Bravo-Hollis, 1961) amended the generic diagnosis, referring eight marginal hooks and a bipartite testis; they also described *M. littoralis* from *Haemulon sexfasciatum* Gill, 1862 from the Gulf of California. According to Bychowsky (1957), members of the Tetraonchidae are characterized by having eight pairs of marginal hooks, two pairs of anchors, one bar and a single intestinal

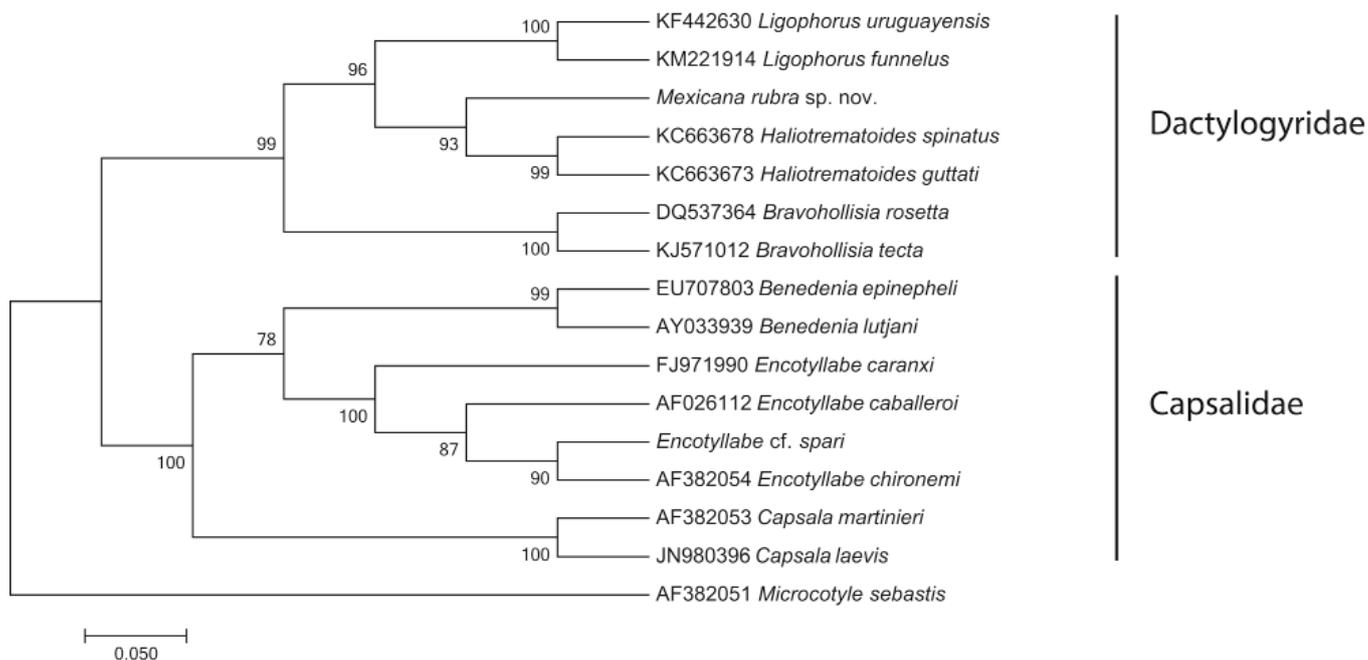


Fig. 5. Phylogenetic tree based on the maximum likelihood of the partial 28S gene to show the relationship of *Encotyllabe* cf. *spari* with other species of the Capsalidae. Numbers (%) on the branches indicate 5,000 bootstrap replicates. GenBank accession numbers are shown and the scale bar indicates the nucleotide mutations per site.

cecum. Consequently, the genus was subsequently allocated to the Dactylogyridae (subfamily Ancyrocephalinae) by Luque *et al.* (1992), with an amended generic diagnosis and the description of *M. atlantica* from *Haemulon steindachneri* (Jordan & Gilbert, 1882) off the coast of Brazil. The main generic characteristics are 14 similar hooks, two pairs of anchors, a dorsal bar with slender lateral ends, a ventral bar with wing-like expansions, intestinal ceca which are confluent posteriorly and a bipartite testis. In the original description of *M. atlantica*, the scale bar for figs 4 (dorsal bar) and 5 (ventral bar) were not in accordance with the measurements given. A re-examination of the paratypes (CHIOC nos. 33.109 and 33.110) showed that measurements given in the description are the correct ones.

*Mexicana anisotremum*, a species with an entire testis, was subsequently described from *Anisotremus virginicus* (Linnaeus, 1758) and *A. surinamensis* (Bloch, 1791) off Rio de Janeiro (Cezar *et al.*, 2012). The most recent species to be described was *M. iannaconi* from *H. steindachneri* off Peru (Chero *et al.*, 2014).

According to Chero *et al.* (2014), the main differential characters separating species of *Mexicana* are the morphology of testis, the sclerotized piece at the base of the copulatory organ and the ventral bar. They also proposed a key to the species, which they separated into two main groups, taking into account the morphology of testis: (1) testis bipartite posteriorly or completely bipartite (*M. atlantica*, *M. bychowskyi*, *M. littoralis* and *M. iannaconi*) and (2) testis entire (*M. anisotremum*). *Mexicana rubra* sp. nov. is included in the first group. We could not find consistent differences in the piece at the base of the copulatory organ, but there are differences in relation to the shape of ventral and dorsal bars. The closest spe-

cies to *M. rubra* sp. nov. is *M. atlantica*, and the main diagnostic features which differentiate the two are the ventral bar (with three deep bowed projections vs rectangular with shallow projections) and dorsal bar (with conspicuous deep indentations vs with lateral extremities enlarged, without indentations) (see the key to species proposed below). The shape of vagina in *M. atlantica* seems to be wider than in *M. rubra* sp. nov. Since it is not sclerotized, this may be a weak difference.

The present sequences for the 18S, ITS1 and 5.8S and 28S of *Mexicana rubra* sp. nov. represent the first available genetic data for the genus. The 18S and 28S rRNA genes are known to be conserved, with lower sequence variation, compared with the ITS1 (Schlötterer *et al.*, 1994). The ML trees using partial 18S and 28S sequences shows that the new species nest as the sister taxa of other dactylogyrid genera, *i.e.* *Euryhaliotrema*, *Haliotrematoides*, *Ligophorus* and *Bravohollisia*, because correlation has a statistical significance. Sequences of ITS1 and 5.8S were not used in the phylogenetic reconstruction due to their low query cover.

The Capsalidae was previously referred as monophyletic by Molaret *et al.* (1997), Whittington (2004) and Perkins *et al.* (2009). Whittington (2004) considered that some capsalid subfamilies and genera are ill-defined and require taxonomic revision. Members of *Encotyllabe* parasitize a wide range of teleost fishes from different geographical areas. Yamaguti (1934) described *E. spari* from the gills of *Sparus macrocephalus* (Basilevsky, 1855), *Plectorynchus pictus* (Thunberg, 1792) and *Epinephalus akaara* (Temminck & Schlegel, 1842) from the Inland Sea of Japan. Kohn *et al.*, (1984) reported this species from *Haemulon sciurus* (Shaw, 1803) and Luque *et al.* (1996) from *O. ruber* off the coast of Rio de Janeiro.

This species was also reported from the gills of *Plectorhynchus cinctus* (Temminck & Schlegel, 1843), *P. pictus* (Thunberg, 1792) and *P. schotaf* (Forsskål, 1775) from the Arabian Gulf (Khalil *et al.*, 1988) and from *Carangoides bajad* (Forsskål, 1775) in Kuwait Bay (Kardousha *et al.*, 2002). Our specimens exhibit small differences compared with Yamaguti's original description and, consequently, we have referred to them as *E. cf. spari*.

According to Sepúlveda *et al.* (2014), the taxonomic status of members of *Encotyllabe* is questionable, the problem being the deficient or incomplete descriptions and/or illustrations of many of the nominal species. The best characteristics for identifying specimens would appear to include body shape, with thin lateral margins which are turned ventrally, the shape and relative position of the testes, the shape of the cirrus, the extent of the vitelline field, caeca which are not confluent, and the size and shape of the anchors (Yamaguti, 1963; Khalil & Abdul-Salam, 1988; Williams & Beverley-Burton, 1989).

Khalil and Abdul-Salam (1988) showed a general ultrastructural view of the body of *E. spari* and we add new information on the mouth with several digitiform projections, detail of the haptor bordered by a narrow membrane, and the shape of the anchors and hooks. Additionally, our sequences of the partial 18S and 28S of *E. cf. spari* show the position of the species within the Capsalidae, together with species of *Benedenia* and *Capsala* with a high statistical support (99 %). In the GenBank, the analysis of the partial 18S rDNA gene included a single sequence of *Encotyllabe* (*E. chironemi* - AJ228780) for comparison with *E. cf. spari* (query cover 99 %, identity 98 %). The presence of only two sequences of *Encotyllabe* influenced the tree topology, grouping *Encotyllabe* with *Capsala martinieri* Bosc, 1811 (AJ276423) with a statistical support of 76 %. The analysis of the partial 28S rDNA, with a greater number of representative species of *Encotyllabe*, showed *Encotyllabe* spp. to be closer to the *Benedenia* clade with low statistical support (78 %) and well separated from *Capsala* clade with 100 % support. Therefore, more new sequences of *Encotyllabe* species are required to augment the genetic profile of the genus.

This work provided the description of a sixth species of *Mexicana*, including new genetic data based on the ITS1, 5.8S and partial 18S and 28S rDNA, and new data on the genetic profile of *E. cf. spari* based on the partial 18S and 28S rDNA genes plus new ultrastructural data. A key to the species of *Mexicana* is proposed below.

Key to the species of *Mexicana*.

1. Parasites of fishes in the Pacific Ocean.....2  
Parasites of fishes in the Atlantic Ocean.....4
2. Testis completely bipartite.....*M. iannaconi*  
Testis bipartite only posteriorly.....3
3. Body length 576 – 736; pharynx length 55 – 66; ventral anchor 40 – 42 long; dorsal anchor 30 – 42 µm long.....*M. bychowskyi*  
Body length 951 – 1057; pharynx length 70 – 74; ventral anchor 20 – 25 long; dorsal anchor 20 – 21 µm long.....*M. littoralis*
4. Testis entire; male copulatory organ tubular, without expanded piece at base.....*M. anisotremum*

- Testis bipartite posteriorly; male copulatory organ tubular, with expanded piece at base.....5
5. Ventral bar rectangular with shallow projections; dorsal bar elongate, with lateral extremities enlarged, lacking deep antero-lateral indentations.....*M. atlantica*  
Ventral bar with three deep bowed projections; dorsal bar elongate with conspicuous deep antero-lateral indentations.....  
.....*M. rubra* sp. nov.

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