Revista Mexicana de Biodiversidad 79: 83S- 88S, 2008
http://dx.doi.org/10.22201/ib.20078706e.2008.001.514

A new species of *Euzetia* (Monogenea: Monocotylidae) on the gills of *Rhinoptera bonasus* (Rhinopteridae) from Ciudad del Carmen, Campeche, México

Una especie nueva de *Euzetia* (Monogenea: Monocotylidae) de las branquias de *Rhinoptera bonasus* (Rhinopteridae) de Ciudad del Carmen, Campeche, México

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Abstract. *Euzetia lamothei* n. sp. (Monogenea: Euzetiinae) is described from the gills of the stingray, *Rhinoptera bonasus*, collected from marine waters near Ciudad del Carmen, Campeche, Mexico. The species is assigned to *Euzetia* Chisholm and Whittington, 2001 because it has a haptor with 10 peripheral loculi and 1 central loculus flanked by 2 lateral loculi. The species described herein can be distinguished from the only known member of the genus, *E. occultum*, by the morphology of the male copulatory organ, the vas deferens sigmoid, and the presence of a vaginal chamber. *Euzetia lamothei* n. sp. was found on 1 of 7 *R. bonasus* (14%). This species is the first record of a member of the genus from the neotropics, the first from Mexico, and the second report of a monocotylid from *Rhinoptera*.

Key words: *Euzetia lamothei* n. sp., *Rhinoptera bonasus*, Euzetiinae, description, Campeche, Mexico.

Resumen. Se describe *Euzetia lamothei* n. sp. (Monogenea: Euzetiinae), parásito de la branquias de la mantarraya *Rhinoptera bonasus*, recolectada en Ciudad del Carmen, Campeche, México. Los ejemplares se asignaron a *Euzetia* Chisholm y Whittington, 2001 porque presentan un haptor con 10 loculi periféricos y 1 loculus central flanqueado por 2 loculi laterales. La especie aquí descrita se distingue de *E. occultum* por la morfología del órgano copulador, el vaso deferente sigmoide y la presencia de una cámara vaginal. *Euzetia lamothei* n. sp. se encontró en 1 de 7 *R. bonasus* (14%). Esta especie representa el primer registro de un miembro del género en el neotrópico, además es el primer registro en México, y el segundo de un monocotílido de *Rhinoptera*.

Palabras clave: *Euzetia lamothei* n. sp., *Rhinoptera bonasus*, Euzetiinae, descripción, Campeche, México.

Introduction

The subfamily Euzetiinae Chisholm and Whittington, 2001 was erected for *Euzetia occultum* Chisholm and Whittington, 2001, an exclusive parasite of *Rhinoptera neglecta* Ogilby, 1912 collected from Moreton Bay, Queensland, Australia (Chisholm and Whittington, 2001). During 2000 to 2004, as part of an ongoing study of helminth parasites of stingrays (Marques et al., 1995; Monks et al., 1996; Pulido-Flores and Monks, 2005) specimens of *R. bonasus* (Mitchill, 1815) were collected in the marine waters off the coasts of the states of Veracruz, Campeche, Yucatán, and Quintana Roo, Mexico. Of those collected near Ciudad del Carmen, Campeche, a single stingray harbored monogeneans on its gills with characteristics that indicated they should be assigned to *Euzetia* Chisholm and Whittington, 2001. These specimens are described herein.

Recibido: 08 julio 2007; aceptado: 13 febrero 2008

Materials and methods

Seven specimens of *Rhinoptera bonasus* were collected from coastal Mexican waters during May, 2000 to January, 2004: 5 specimens were collected at Ciudad del Carmen, Campeche, Mexico (18º 37' 58" N, 91º 49' 57" W), 1 at Champotón, Campeche (19º 21' N, 90º 54' W), 1 at Isla Contoy, Quintana Roo (20º 48' N, 86º 47' W). The external body surface of each stingray was examined using a magnifying glass and each gill arch was excised, placed in a Petri dish with 0.6% sodium chloride saline solution (Pritchard and Kruse, 1982), and examined using a stereomicroscope. Helminths were removed from gill lamellae and transferred to dishes containing 0.6% sodium

chloride saline solution. Helminths were lightly flattened under slight coverslip pressure and fixed with Alcohol-Formalin-Acetic Acid at room temperature. Specimens were stained with Gomori´s trichrome (5 worms), Mayer's carmalum (2 worms), and Delafield's haematoxylin (4 worms). All specimens were dehydrated in an ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Specimens were examined using a compound photomicroscope equipped with bright field and Nomarski optics and drawings were made with the aid of a drawing tube. Measurements of curved organs follow the curve. Measurements were made using an ocular micrometer; all measurements are given in micrometers as the mean followed in parentheses by the range and the number of structures measured. Terminology for structures of the haptor follows that of Chisholm et al. (1995) and Chisholm and Whittington (2001). Specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico; the University of Nebraska State Museum, Harold W. Manter Laboratory (HWML), Division of Parasitology, Lincoln, Nebraska; and the Colección de Helmintos (CHE), Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, Mexico. Three paratypes of *E. occultum* (G 217994-217996) from the Queensland Museum, South Brisbane, Queensland, Australia, were examined.

Description

Euzetia lamothei n. sp. (Figs. 1-3)

Measurements based on 11 lightly flattened specimens. Total body (excluding haptor) 405 (286-498, n=7) long, by 178 (137-259, n=7) wide at level of anterior part of testis. Haptor oval 210 (178-257, n=7) long, 234 (106-369, n=9) wide with 1 central and 10 peripheral loculi, additional loculus present on either side of central loculus (Figs. 1A and 2A). Unsclerotized ridge on septa of loculi; surface of loculi with small hexagonal-shaped structures (Fig. 2A). Left hamulus 62 (40-169, n=8) long with handle 35 (14-56, n=2) long; right hamulus 41 (11-54, n=8) long with handle 36 (24-47, n=2) long, associated with septal pair 1 (Fig. 2B). Marginal membrane present with 14 hooklets, 9 (8-9, n=9) long (Fig. 2C). Anterior glands not observed; 2 ducts from anteromedian gland and a single duct from each anterolateral gland open on anterior margin of head (Fig. 3A). Openings of lateral ducts surrounded by 4-6 papiliform structures (Figs. 1A, 3A). Mouth ventral, subterminal, 39 (22-58, n=5) in diameter at the opening, funnel-like, 52 (34-71, n=4) in diameter at base of oral cavity. Esophagus absent. Pharynx muscular 66 (41-119, n=10) long, 51 (38-81, n=10) wide, with 6 to 8 transversal muscular bands (Figs. 1A-B). Eye-spots in the form of dispersed pigment granules located anterodorsal to pharynx. Intestinal ceca without diverticula, reaching to posterior portion of body proper, not confluent posteriorly. Cecal bifurcation 130 (101-185, n=5) from anterior end of body. Testis single, spherical to oval, 70 (46-86, n=8) long and 80 (56-114, n=8) wide (Fig. 1B). Vas deferens sigmoid, dilated throughout length, arising from right side of testis, loosely coiled and running anteriorly, dorsal to transverse vitelline ducts, curved toward left side of body, crossing to right side posterior to genital pore. Anterior portion of vas deferens widened to form elongate seminal vesicle that enters ejaculatory bulb (Figs. 1B, 3B). Ejaculatory bulb spherical, 22 (17-27, n=6) long and 18 (14-22, n=7) wide, with 2 prominent spherical chambers (Figs. 1B, 3B). Accessory glands associated with ejaculatory bulb not found. Male copulatory organ (Figs. 1A-B, 3B) sclerotized, in form of small funnel, 20 (16-29, n=4) long and 9 (4-13, n=4) wide at junction of ejaculatory bulb, with stem of funnel, distal portion, doubled back over proximal portion (Fig. 3B); accessory piece absent. Common genital pore located at posterior end of pharynx (Fig. 1B). Ovary elongate, 68 (31-119, n=6) long, V-shaped, encircling right intestinal cecum dorsoventrally and narrowing to form oviduct. Oviduct receives duct from seminal receptacle and common vitelline duct and joins oötype. Mehlis' gland not prominent, entering posterior portion of oötype. Oötype 61 (31-117, n=5) long and 44 (17-71, n=5) wide, opening medially at unarmed common genital pore. Vaginal pore located 128 (111-142, n=3) from anterior end of body. Vagina muscular, unsclerotized, in the shape of an elongate sac, 41 (28-67, n=7) long and 11 (6-19, n=7) wide at maximum width. Proximal portion of vagina leading to a small, oval vaginal chamber, unsclerotized, 19 (11-36, n=4) long and 9 (5-12, n=4) wide (Figs. 1B, 3C-D). Narrow duct exiting posterior portion of vaginal chamber, connected to spherical seminal receptacle. Receptacle 28 (15-45, n=11) long and 26 (18-33, n=11) wide (Fig. 1B). Spermatophores not observed. Vitellaria extending from level of mid-portion of pharynx to posterior portion of body proper. Transverse vitelline ducts united slightly to right of midbody, common vitelline duct not observed (Fig. 1B). Egg tetrahedral, side length 73 (54-86, n=3) by 53 (50-56, n=3) (measured from egg within oötype); short appendage present.

Taxonomic summary

Type-host: Rhinoptera bonasus (Mitchill, 1815) (Rhinopteridae).
Type-locality: Ciudad del Carmen, Campeche, Mexico (18º 37' 58" N; 91º 49'57" W).

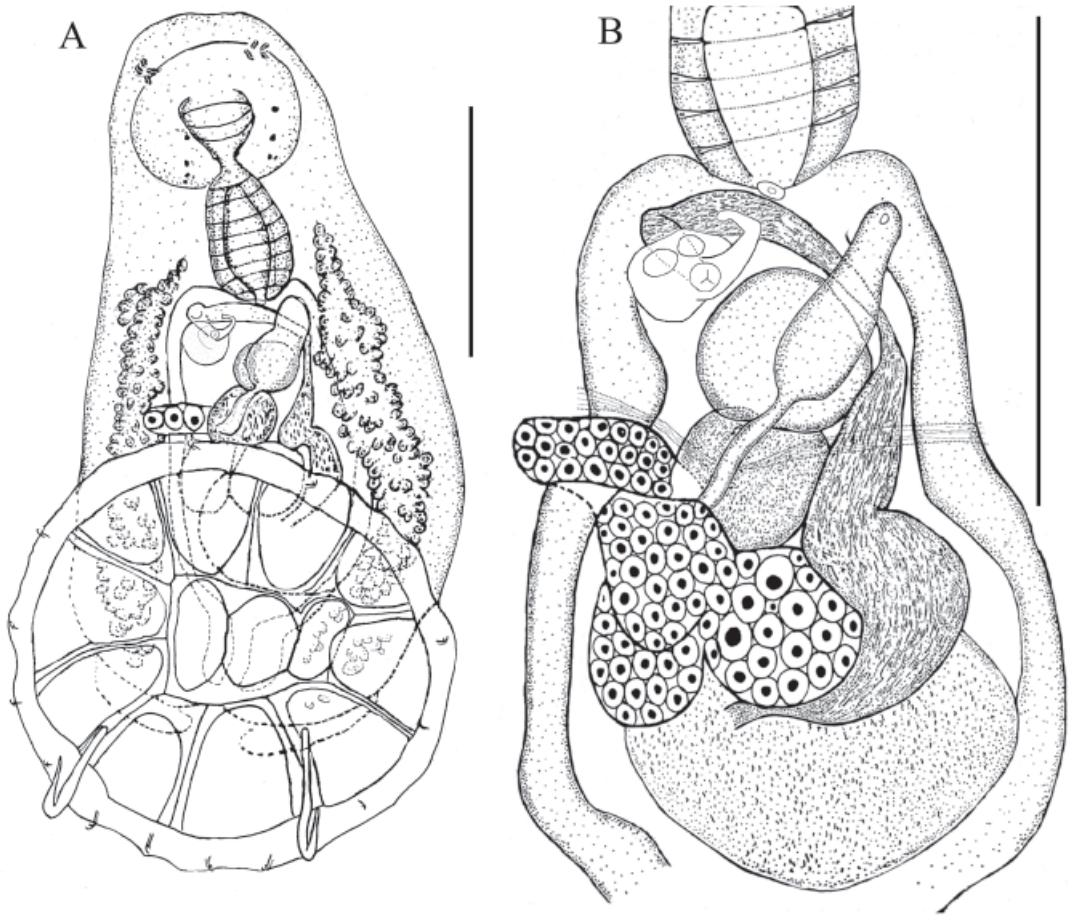

Figure 1. *Euzetia lamothei* n. sp. A, holotype, ventral view. B, detailed ventral view of male and female reproductive system (holotype). Scale bars: A-B = 100 μm.

Site of infection: gills.
Prevalence and intensity: 1 of 1 *R. bonasus* from the type locality, infected with 11 monogeneans, and 1 of 7 (14%) from the Yucatán Peninsula.
Type specimens: holotype CHNE-6067; paratypes CHNE-6068, HWML-48817, 48818, CHE-P-00056.
Etymology: the specific epithet honors Dr. Rafael Lamothe-Argumedo, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico, for his lifelong work and advances in the knowledge of the Monogenea.

Remarks

The subfamily Euzetiinae was erected by Chisholm and Whittington (2001) for *Euzetia,* which to date is the only genus assigned to the subfamily. The haptor of *Euzetia* is similar to that of Decacotylinae in having 1 central and 10 peripheral loculi. However, *Euzetia* can be distinguished from all other Monocotylidae by a unique feature of the haptor, which has an additional loculus on either side of the central loculus. Specimens of *E. lamothei* n. sp., like those of *E. occultum*, have 1 central loculus with 2 flanking loculi and 10 peripheral loculi.

Generally the new species is much smaller than *E. occultum.* The body of *E. lamothei* is smaller than that of *E. occultum* (405 long, 178 wide vs. 1029 long, 674 wide, respectively), as is the haptor (210 long, 234 wide vs. 613 long, 820 wide), pharynx (66 long, 51 wide vs. 152 long, 115 wide), ejaculatory bulb (22 long vs. 34 long), male copulatory organ (20 long vs. 22 long), seminal receptacle (28 long vs. 46 long), and the egg (73 long vs.

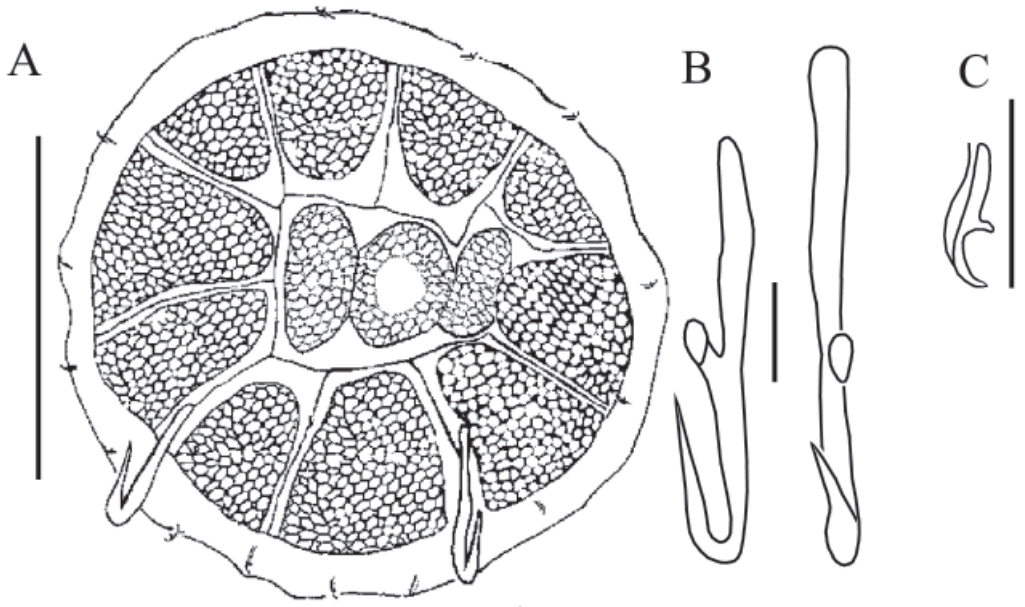

Figure 2. *Euzetia lamothei* n. sp. A, haptor, ventral view. B, hamuli (note: not in profile). C, hooklets. Scale bars: A = 100 µm; B-C = 10 µm.

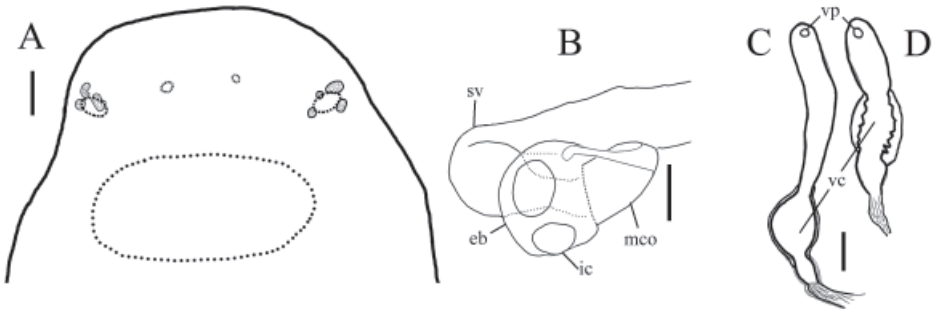

Figure 3. *Euzetia lamothei* n. sp. A, anterior end, ventral view, openings of ducts of anterior glands and papillae surrounding the openings. B, male copulatory complex showing male copulatory organ (mco), ejaculatory bulb (eb) with spherical internal chambers (ic), and seminal vesicle (sv). C, vagina with vaginal pore (vp) and vaginal chamber (vc) (paratype CNHE-6068). D, vagina with vaginal pore (vp) and vaginal chamber (vc) (note: vagina and vaginal chamber contracted) (paratype CHE-P-00056). Scale bars: A-D = 10 µm.

83 long). Finally, *E. lamothei* can be distinguished from *E. occultum*, the only other species currently assigned to the genus, by having the male copulatory organ in the form of a bent cone, the presence of a large sigmoid-shaped vas deferens, and the presence of a vaginal chamber. The copulatory organ of *E. occultum* is a shorter, sclerotized tube that is not bent, the seminal vesicle is small, the vas deferens is an elongated coil having many turns, and the vaginal chamber is absent.

Discussion

At present, only 2 species of *Euzetia* have been described. In addition to the species described by Chisholm and Whittington (2001) (*E. occultum* from *Rhinoptera neglecta* Ogilby, 1912 in Australia), these authors based on an unpublished observation made by L. Euzet, suggested that *Euzetia* may include at least 4 species considering 3 undescribed species: 1 from *Rhinoptera steindachneri* Evermann and Jenkins, 1891 from the Pacific Coast of Baja California Sur, Mexico, 1 from *Rhinoptera marginata* (Geoffroy Saint-Hilaire, 1817) (Geoffroy Saint-Hilaire, 1817) from Senegal, and 1 from *R. bonasus* from Senegal. Three individuals of *R. steindachneri* from Mazatlán, Sinaloa (23º 12' 17.9" N; 106º 24' 36.8" W), located at approximately the same latitude and slightly east of the southern tip of Baja California, were examined using the methods described above but no specimens of *Euzetia* were found.

There are only 2 published records of monocotylids from *Rhinoptera*, although members of that genus have been examined previously during various studies with the intent of recovering monogeneans, including but not limited to those of Hargis (1955) and Chisholm and Whittington (2001). The only other monogenean that has been reported from *R. bonasus* is *Benedeniella posterocolpa* (Hargis, 1955) Yamaguti 1963 (see Pulido-Flores and Monks, 2005). Eight species of Monocotylidae have been reported from Mexico: *Anoplocotyloides papillatus* (Doran, 1953) Young, 1967 and *Spinuris mexicana* Bravo-Hollis, 1969 from *Rhinobatus glaucostigma* Jordan and Gilbert, 1883 (Mazatlán, Sinaloa) (Bravo-Hollis, 1969); *S. zapterygis* Gómez del Prado and Euzet, 1999 from *Zapteryx exasperata* Jordan and Gilbert, 1880 (Puerto Viejo and Bahía Almejas, Baja California Sur) (Gómez del Prado and Euzet, 1999); *Dendromocotyle cortesi* Bravo-Hollis, 1969 from mantarraya gris (Isla Rasa and Golfo de California) (Bravo-Hollis, 1969); *Decacotyle floridana* (Pratt, 1910) Chisholm and Whittington, 1998 (= *Heterocotyle aetobatis* Hargis, 1955) from *Aetobatis narinari* Euphrasen, 1790 (Ciudad del Carmen, Campeche, and Holbox, Quintana Roo) (Lamothe et al., 1997; Pulido-Flores and Monks, 2005); *Calicotyle californiensis* Bullard and Overstreet, 2000 from *Mustelus californicus* Gill, 1894 (Bahía de los Ángeles, Golfo de California) (Bullard and Overstreet, 2000); *C. urobati* Bullard and Overstreet, 2000 from *Urobatis halleri* Cooper, 1863 and *U. maculatus* Garman, 1913 (Santa Rosalía, Puertecitos, San Francisquito, and Bahía de los Ángeles, Golfo de California) (Bullard and Overstreet, 2000); and *Dendromonocotyle octodiscus* Hargis, 1955 from *Dasyatis americana* Hildebrand and Schroeder, 1928 (Blanquizal, Quintana Roo) and *U. jamaicensis* (Cuvier, 1816) McEachran and Fechhelm, 1998) (Xcalak, Isla Contoy, El Paso de los Cedros, Cozumel, Quintana Roo, and Río Lagartos, Yucatán (Pulido-Flores and Monks, 2005). Pulido-Flores (2001) provided a complete list of the monogeneans reported up to that time from Mexican stingrays. *Euzetia lamothei* n. sp. is the first species of *Euzetia* and the ninth species of Monocotylidae to be reported from Mexico.

In their revision of Calicotylinae Monticelli, 1903, Chisholm et al. (1997) defined members of the subfamily as being distinct in having an ejaculatory bulb with 2 spherical internal chambers, a condition identified as apomorphic by Chisholm et al. (1995). Since those works, U-shaped internal chambers have been described in the ejaculatory bulb of *H. mokhtarae* Neifar, Euzet and Ben Hassine, 1999, (Heterocotylinae Chisholm, Wheeler and Beverly-Burton, 1995: see Neifar et al., 1999). The 2 species of *Euzetia* that have been described have an ejaculatory bulb with 2 spherical internal chambers (1 pair of chambers) (Fig. 1B, 3B). It is unclear if this character could alter the phylogenetic hypothesis proposed by Chisholm el al. (1995) for the Monocotylidae.

Acknowledgements

Leslie A. Chisholm supplied numerous copies, reprints, and pre-prints of her work, and was the source of much advice, personal comments, and discussion concerning this fascinating group of monogeneans. The authors thank all those who made possible the examination of specimens, especially Scott L. Gardner of HWML and Skip Sterner, formerly of HWML, and Mary Hanson Pritchard, who provided access to literature of the HWML archive. Part of this manuscript was prepared during a research visit to the HWML, which provided office and laboratory space, access to computers, the reprint collection, and microscopes. Marina Pérez-Romero, CET Mar, Ciudad del Carmen, Campeche, and Wilbert Borges-Ontiveros, ITMAR, Champotón, Campeche, provided use of their laboratory facilities during field collections.

This study was supported by funds from the University of California Institute for Mexico and the United States (UC-MEXUS) and the Consejo Nacional de Ciencia y Tecnología (CONACYT) from Mexico and the Programa de Mejoramiento del Profesorado (PROMEP) to the authors and a doctoral scholarship to GP-F (CONACYT 137558).

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