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Heterobothrium lamothei n. sp. (Monogenea: Diclidophoridae) from the gills of *Sphoeroides testudineus* (Pisces: Tetraodontidae) from the coast of Yucatán, Mexico

Heterobothrium lamothei n. sp. (Monogenea: Diclidophoridae) de las branquias de *Sphoeroides testudineus* (Pisces: Tetraodontidae) de la costa de Yucatán, México

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Abstract. The presence of a member of the genus *Heterobothrium* is described for the first time from the coast of Yucatán Peninsula, southeastern Mexico. *Heterobothrium lamothei* n. sp. is recorded parasitizing the gills of the tetraodontid fish *Sphoeroides testudineus* (Linnaeus, 1758) from 4 coastal lagoons in Yucatán: Celestún (20° 52' N, 90° 24' W), Chelem (21°15'N 89°45'W), Dzilam (21°35'N 88°35'W) and Río Lagartos (21°22'N 87°30'W). The new species differs from the other species described in the genus, by a combination of characters including a copulatory organ armed with 12-15 genital hooks, the distal pair of clamps smaller in comparison with the 3 other pairs and by having 15-26 testes. The infection parameters were for Celestun, 47 % of prevalence, number of fish examined (n) = 47, mean abundance of 1.76 ± 9.62; Chelem, 20 % (n = 30), 0.53 ± 8.48; Dzilam, 2 % (n = 60), 0.02 ± 0.00; Río Lagartos, 25 % (n = 59), 0.34 ± 1.58. *Heterobothrium lamothei* n. sp. may be considered as potentially dangerous species for the aquaculture of *S. testudineus* due to its direct life cycle hat high fish densities would increase the transmission of this monogenean.

Key words: Monogenea, *Heterobothrium*, *Sphoeroides*, Yucatán Peninsula, Gulf of Mexico.

Resumen. Se registra por primera vez la presencia de un miembro del género *Heterobothrium* en la costa de la península de Yucatán, México: *Heterobothrium lamothei* n. sp., parásito de las branquias del pez tetraodóntido *Sphoeroides testudineus* (Linnaeus, 1758) en 4 lagunas costeras de Yucatán: Celestún (20° 52' N, 90° 24' O), Chelem (21°15'N 89°45'O), Dzilam (21°35'N 88°35'O) y Río Lagartos (21°22'N 87°30'O). La especie nueva difiere de otras del género por un conjunto de características que incluyen un órgano copulador con 12 a 15 ganchos genitales, un par distal de pinzas más pequeño que los 3 pares superiores y un número reducido de testículos (15-26). Los parámetros de infección para las 4 localidades fueron: Celestún: 47 % de prevalencia, 47 peces examinados (n = 47), abundancia media de 1.76 ± 9.62; Chelem: 20 % (n = 30), 0.53 ± 8.48; Dzilam: 2 % (n = 60), 0.02 ± 0.00 y Río Lagartos, 25 % (n = 59), 0.34 ± 1.58. *Heterobothrium lamothei* se considera potencialmente peligrosa para el cultivo de *S. testudineus* debido a su ciclo de vida directo y a que las altas densidades de peces en acuacultura propician la transmisión de monogéneos entre hospederos.

Palabras clave: Monogenea, *Heterobothrium*, *Sphoeroides*, península de Yucatán, golfo de México.

Introduction

Heterobothrium Cerfontaine, 1895 includes 11 species of monogeneans infecting the gills of puffer fishes of the family Tetraodontidae (Williams, 1986; Ogawa, 1991). Some of the species in *Heterobothrium,* such as *Heterobothrium okamotoi* Ogawa, 1991, are considered a serious problem for the aquaculture of puffer fish, such as *Takifugu rubripes* Temminck and Schlegel, 1850 in Japan, and extensive research has been undertaken on its biology (Ogawa et al., 2005a, b; Nakane et al., 2005; Kimura et al., 2006). Only 1 species of *Heterobothrium* has been reported from the Pacific coast in Mesoamerica: *Heterobothrium ecuadori* (Meserve, 1938) Sproston, 1946 from the bullseye puffer *Sphoeroides annulatus* (Jenyns, 1842) from Sinaloa and Salina Cruz, Oaxaca in Mexico (Fajer-Ávila et al., 2004; Lamothe-Argumedo, 1967) and Panama (Caballero et al., 1953), and from an unidentified species of *Sphoeroides* Lacepède, 1798 from Costa Rica

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(Caballero and Brenes-Madrigal, 1957). In the Gulf of Mexico, there is only a record of *H. ecuadori* from the checkered puffer *Sphoeroides testudineus* (Linnaeus, 1758) in an unpublished MSc. Thesis in Coral Glabes, Florida (Boucher, 1974).

During studies on the helminth fauna of *S. testudineus* along the coast of Yucatán as part of the POETCY program (Program of Ecological Ordination of the Coastal Territory of Yucatán), a new species of *Heterobothrium* was found infecting the gills of this host. In this paper, the new species is described and data on its infection parameters on the puffer fish and its geographical distribution in coastal lagoons of Yucatán are provided.

Material and methods

Puffers were caught using hook and line and throw nets in 4 coastal lagoons of Yucatán State, Mexico. A total of 196 specimens of *S. testudineus* were sampled in May 2005. Captured fish were transported to the laboratory of Parasitology at Cinvestav-Mérida, kept alive in aquaria, and in all cases were examined within 8 hours. The gills of each host were removed and examined under dissection microscope and the monogeneans obtained were fixed in 4% formalin, labeled and stored in vials for later evaluation. In some cases, entire fish were fixed in 4% formalin for confirmation of its taxonomic identity. Unstained, flattened specimens mounted in Gray and Wess medium or in glycerin ammonium picrate mixture (GAP) were used only to recognize the morphology of sclerotized structures. After evaluation, specimens fixed with GAP were remounted in Canada balsam (Ergens, 1969). All other measurements were obtained from unflattened specimens stained in acid carmine and mounted in Canada balsam (for details on this technique see Vidal-Martínez et al., 2001). Drawings were made with the aid of a drawing tube using an Olympus microscope with Nomarski interference contrast. Average measurements (all in µm) and standard deviation are followed by ranges and the number of specimens or structures measured (*n*) in parentheses. Prevalence and mean abundance concepts were applied following Bush et al. (1997). Type and voucher specimens were deposited in the National Helminthological Collection of Mexico (CNHE), Institute of Biology, National Autonomous University of Mexico, Mexico, the United State National Parasite Collection, Beltsville, Maryland (USNPC), the Helminthological Collection of the Laboratory of Parasitology, at Centre for Research and Advanced Studies, National Polytechnic Institute, Mérida, Yucatán, Mexico (CHCM) and the Helminthological Collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (IPCAS).

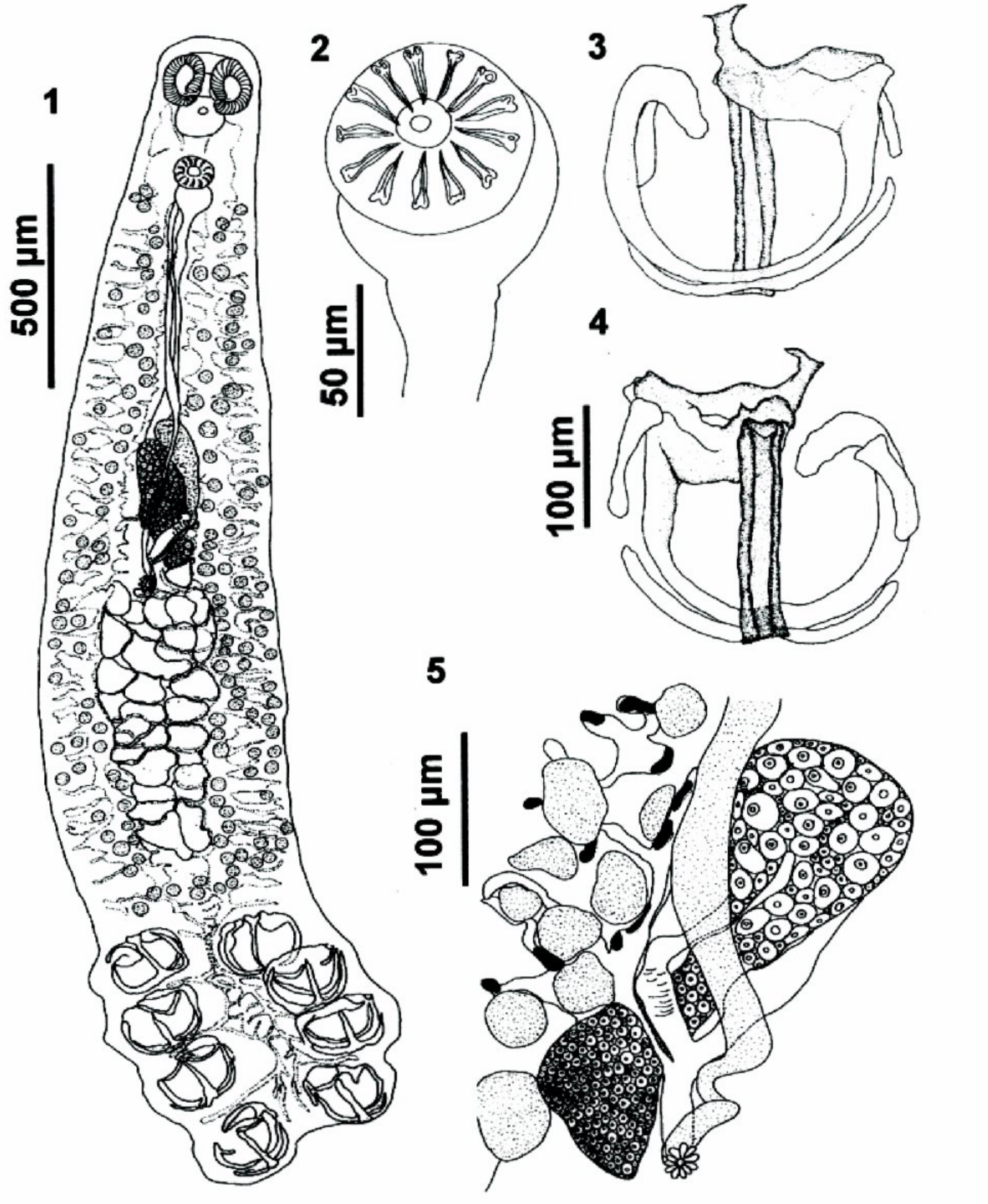
Description

Heterobothrium lamothei n. sp. (Figs. 1-5)

Measurements based on 13 specimens: Body proper elongate, robust, tapering anteriorly. Total length (including haptor) 2433 ± 381 (1880-3030; n = 13). Maximum width at ovarian level, 503 ± 138 (270-780; n = 13). Haptor 601 ± 103 (450-800; n = 9) long, 560 ± 107 (400-800; n = 12) width. Isthmus absent. Paired buccal organs, 90 ± 8 (80-110; n = 12) in diameter. Pharynx diameter 95 ± 15 (72-120; n = 13). Oesophagus with diverticula; bifurcation of intestine at level of gonopore. Caeca with lateral and median diverticula extending to anterior margin of the haptor. Median branches joined. Caeca confluent at anterior margin of the haptor. Haptor rectangular shaped with 4 short non pedunculated clamps arranged symmetrically about midline. Diameters of clamps: anteriormost (first pair, 180° inverted), 188 ± 43 (125-280; n = 19); second pair largest, 187 ± 33 (130-290); third pair, 180 ± 23 (132-230); fourth pair smallest, 164 ± 22 (117-210). Embryonal marginal hooks absent. Clamp comprising 6 sclerites, 5 in posterior fold and 1 in anterior fold as described by Bychowsky et al. (1976) and Williams (1986). Gonopore midventral. Copulatory organ consisting of muscular subspherical cup armed with 12 to 15 genital hooks arranged in a circle, 80 ± 9 (60-95; n = 13) in diameter. Ejaculatory bulb 36 ± 9 (24-50; n = 8) long by 57 ± 16 (38-84; n = 8) width. Testes numerous, irregularly shaped, between caeca, extending from the postovarian region to the anterior margin of opistohaptor. The mean number of testis per individual was 20 ± 4 (15-26). Ovary elongate, inverted U-shaped 328 ± 97 (201-460; n = 5) long by 189 ± 57 (116-296; n = 7) width, situated at the end of first third of body length. Oviduct running left with genito-intestinal canal entering from right intestinal limb, posteriorly connecting with a vitelline duct and followed by ootype. Uterus large. Large vitelline reservoir lying right to the ovary. Vitelline follicles densely scattered from level of gonopore to the anterior margin of the haptor, coextensive with caecal branches; few follicles extending medially in ovarian region. Eggs not observed. Seminal receptacle and vagina absent.

Taxonomic summary

Type-host: checkered puffer fish *Sphoeroides testudineus* (Linnaeus, 1798) (Tetraodontidae).



Figures 1-5. *Heterobothrium lamothei* n. sp. from *Sphoeroides testudineus*. 1, entire worm (Holotype, ventral view). 2, copulatory organ (Paratype, ventral view). 3, clamp (Paratype, dorsal view). 4, clamp (Paratype, ventral view). 5, ovarian complex (Paratype, ventral view).

Site of infection: gills.
Type-locality, date, prevalence (%) (n = number of fish examined) and mean abundance ± standard deviation: Celestún, Yucatán, Mexico (20° 52' N, 90° 24' W) May, 2005, 47 % (n = 47), 1.76 ± 9.62 worms per infected fish.
Other localities: Chelem, Yucatán (21°15'N 89°45'W), May 2005, 20 % (n = 30), 0.53 ± 8.48 worms per infected fish; Dzilam (21°35'N 88°35'W), May 2005, 2 % (n = 60), 0.02 ± 0.00; Río Lagartos (21°22'N 87°30'W), May 2005, 25 % (n = 59), 0.34 ± 1.58.
Type-specimens: holotype (CNHE 5922) and 1 paratype (CNHE 5923); 2 paratypes (USNPC 100508); 2 paratypes (CHCM 505), and 3 paratypes (IPCAS: M-462).
Etymology: this species is dedicated to Dr. Rafael Lamothe Argumedo in recognition to his outstanding contribution to the Mexican helminthology.

Remarks

Heterobothrium lamothei n. sp. most resembles *H. ecuadori* in general appearance, however it differs from this latter species by having a smaller copulatory organ (80-95 *vs*. 114-116 in *H. ecuadori*) armed with 12-15 genital hooks arranged in a circle instead of 14-16 in *H. ecuadori*. Furthermore, *H. lamothei* n. sp. has a rectangular haptor with the distal pairs of clamps smaller in diameter than the 3 previous ones, while *H. ecuadori* has all clamps similar in size (see Table 1 in Williams, 1986). *Heterobothrium lamothei* n. sp. also differs from this latter species in the number of testes (15-26 *vs*. 27-40 in *H. ecuadori*) (see Table 1 in Williams, 1986). It is noteworthy that the number of testes in *H. lamothei* n. sp. is relatively similar to that observed in *Heterobothrium yamagutii* Ogawa, 1991 with 25-30 testes. However, this latter species has a copulatory organ armed with 10 hooks, its 4 pairs of clamps are very similar in size and it is substantially longer in size (7400-14400) than *H. lamothei* n. sp. (1880-3030).

Discussion

Heterobothrium lamothei n. sp. is added to the 11 species currently recognized in *Heterobothrium*: *H. torquigeneri* Williams, 1986, *H. elongatum* Williams, 1986, *H. tetrodonis* (Goto, 1894) Cerfontaine 1895, *H. tonkinensis* Yamaguti, 1958, *H. praeorchis* Bychowsky, Mamaev and Nagibina, 1976, *H. ecuadori* Meserve, 1938, *H. fluviatilis* Euzet and Birgi, 1975, *H. okamotoi* Ogawa, 1991, *H. yamagutii* Ogawa, 1991, *H. shanagawai* Ogawa, 1991 and *H. bychowskyi* Ogawa, 1991. Most of these species have been described from Australian (Williams, 1986) and Japanese (Ogawa, 1991) marine waters. The only species described from America is *H. ecuadori*. This species was originally described as *H. ecuadori* by Meserve (1938) from Galapagos. Later, Sproston (1946) erected the new genus *Tagia* and considered *H. ecuadori* as a member of *Tagia*. *Tagia ecuadori* was described from the gills of *Sphoeroides* sp. in Mata de Limón, Puntarenas, Costa Rica (see Caballero and Brenes-Madrigal, 1957; Lamothe-Argumedo, 1967). However, as Williams (1986) noted, Euzet and Birgi (1975) revised the genus and considered that species to be a synonym of *Heterobothrium* Cerfontaine1895 and *Gempylitrema* Yamaguti, 1968. Thus, by the principle of priority of the ICZN (International Code of Zoological Nomenclature (http://www.iczn.org/iczn/index.jsp), the valid name of the genus is the oldest one, in this case *Heterobothrium*. There are several records of *H. ecuadori* in America, from *Cheilichthys annulatus* Müller, 1841 from Tagus Cove, Albermale, Galapagos Islands and San Francisco (Meserve, 1938). All other geographical records for this monogenean species are those from *Sphoeroides annulatus* from waters of the Pacific Ocean in the Canal zone, Panama (Caballero et al., 1953), Salina Cruz, Oaxaca (Lamothe-Argumedo, 1967) and Sinaloa in Mexico (Fajer-Ávila et al. 2004). In the Atlantic coast, there is only 1 record for *H. ecuadori* parasitizing the gills of *S. testudineus* in Biscayne Bay, Florida in an unpublished MSc. thesis (Boucher, 1974). Boucher (1974) did not provide a formal description of his specimens but, the metric data of the total length (mean = 2200, range 1300-2850, n = 13 specimens) and width (620, 350-850) provided by Boucher are similar to those of *H. lamothei* n. sp. Due to the geographical closeness between Florida and the Peninsula of Yucatán and the fact that the monogenean was parasitizing the same host species (*S. testudineus*), it is possible that the material of Boucher belongs to *H. lamothei* n. sp. If this is true, *H. ecuadori* is restricted to the Pacific Ocean and *H. lamothei* n. sp. to the Atlantic coast, and specifically to the Gulf of Mexico at this point.

The prevalence and mean abundance of *H. lamothei* n. sp. were low for all 4 coastal lagoons that were sampled. The sample size for each of the coastal lagoons was large enough to have a reliable estimation of these infection parameters. However, in the eventual case of aquaculture of this host species, it is predicted that the first helminth species to present numerical increase and a sanitary risk will be *H. lamothei* n. sp. The basis for this, is that transmission would be enhanced due to the direct life cycle of this monogenean and the increased density of host populations typical of aquaculture. Other authors have stressed this potential danger for the aquaculture of the bullseye pufferfish *S. annulatus* in the Mexican Pacific coast (Sinaloa) (Fajer-Ávila et al., 2004). This has been found to be a problem for Japanese aquaculture of the puffer fish *Takifugu* spp., where *H. okamotoi* is a serious sanitary problem. As a consequence, extensive research has been undertaken on its biology to control the infection (see Ogawa et al., 2005a, b; Nakane et al., 2005; Kimura et al., 2006). Thus, further studies on the biology and therapeutic treatment of *H. lamothei* n. sp. on its hosts are strongly recommended.

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