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First record of *Stibarobdella moorei* (Annelida, Hirudinea, Piscicolidae) a marine leech parasitizing *Octopus bimaculatus* (Mollusca: Octopodidae) from the Mexican Pacific coast

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Summary

The occurrence of the parasitic marine leech *Stibarobdella moorei* (Oka, 1910) (Hirudinea: Piscicolidae) along the northwest Mexican Pacific coast is described for the first time. This ectoparasite was collected from the skin of the *Octopus bimaculatus* (Verril, 1983) (Mollusca: Octopodidae). *Stibarobdella loricata* (Hardig, 1924) is synonymized with *S. moorei* as this species resembles other species of the genus based on tubercle patterns and the presence of papillae and a marginal fringe on the oral sucker. The present finding throws new light on the biodiversity and host preference of the ectoparasite and suggests a successful migration to unusual host. The coast of the Pacific Ocean, particularly in the Bay of Los Angeles, Baja California, Mexico is a new geographical distribution area for *S. moorei*, and *O. bimaculatus* is a new host reported for this leech. The morphology of this ectoparasite is briefly described.
Keywords: ectoparasite; Mexico*; Octopus bimaculatus;* Piscicolidae; *Stibarobdella moorei*

Introduction

In several coastal habitats of tropical and subtropical oceans, hirudinean of the family Piscicolidae are well known as marine leeches of sharks and rays (Llewellyn, 1966; Sawyer *et al*., 1975; Williams, 1982; Soto 2000, 2003; Utevsky and tronjelj, 2004; Furiness *et al*., 2007; Alves *et al*., 2014). *Stibarobdella moorei* (Oka, 1910) and *S. macrothela* (Schmarda, 1861) have been found in Brazil (Soto 2000, 2003; Wunderlich *et al*., 2011; Alves *et al*., 2014). There is also evidence of their presence in India (Harding, 1924) and Turkey (Başusta *et al*,. 2015).

In the present study, a brief description of the ectoparasite *S. moorei*'s morphology is presented and its infection intensity on its host *O. bimaculatus* is reported. In Mexico, octopus is one of the most important fishing resources and fishing products exported to Europe (CONAPESCA, 2011; SOFIA, 2012). Octopus fishing activity is concentrated mainly in the Gulf of Mexico and the Caribbean Sea, where the species *Octopus vulgaris* and *Octopus maya* (endemic species of the Yucatan Peninsula) are captured (SEMARNAT, 1999; CONAPESCA, 2011). In addition, three species, *Octopus hubbsorum, O. macropus* and *O. bimaculatus*, are captured in the Pacific Ocean (SEMARNAT, 1999, 2004).

In this work, the occurrence of the marine leech *S. moorei* is reported for the first time parasitizing native octopus *O. bimaculatus* in the Mexican Pacific coast. This record extends the geographical distribution for this hirudinean parasite, and *O. bimaculatus* constitutes a new host for *S. moorei.* This study provides new information and biological data of this hirudinean parasite whose ecology is poorly studied worldwide.

Material and Methods

This study is part of a larger study at the Center for Scientific Research and Higher Education of Ensenada (CICESE) conducted

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Fig. 1. A) Photography of *Stibarobdella moorei* (Oka, 1910) infecting the California two-spot octopus *Octopus bimaculatus* (Molusca: Octopodidae) on the northwest of the Mexican Pacific coast. B – D) Single specimen of the parasitic marine leech *Stibarobdella moorei* (Annelida: Hirudinea: Piscicolidae). They have an anterior oral sucker. No eyes on oral sucker. The caudal sucker is larger and uses it to attach itself to the host.

on the reproductive cycle and feeding habits of *Octopus bimaculatus* under captive conditions. A total of 61 octopuses (210 – 610 mm total length) were sampled from commercial catches at the Bay of Los Angeles, Baja California (29° 02' and 28° 55' N and 113° 32' and 113° 26' W) during four collecting trips; two in 2011 and two in 2012. The samplings were made during the species breeding season from April to August. Octopuses were collected by local fishing cooperative "Buzos de Bahía" using traps with fish as bait. All procedures in studies involving animals were in accordance with the ethical standards consigned to the Mexican laws (NOM-033-ZOO-1995).

Before the octopuses were transported in cool boxes to the CICESE's rearing facilities, they were individually counted, examined *in situ* to detach the ectoparasites from their host, collected in a small container with seawater, and transported to the laboratory for further identification. Subsequently, the ectoparasites were carefully inspected in a Petri dish with seawater using a stereomicroscope (LEICA MZ9.5) and were relaxed with gradual addition of 96 % ethanol followed by fixation in a solution of 70 % ethanol.

To make photographic records of the original live organism's colours, additional *O. bimaculatus* collections were made. The leeches found on each animal were preserved in 70 % ethanol, and photographs were taken using a digital camera SONY attached to a stereomicroscope. Identification of leech species were performed following Soto (2000, 2003), Furiness *et al*. (2007) and Wunderlich *et al*. (2011) descriptions. Specimens of leeches were deposited at the National Helminth Collection (CNHE), in the Institute of Biology of the National Autonomous University of Mexico, catalogue number (6422).

The prevalence of parasites was calculated by dividing the number of octopuses infected with *S. moorei* by the total number of octopuses examined, expressed as a percentage. Intensity of infection (number of parasites by infected hosts) was determined according to Bush *et al*. (1997).

Preparation of specimens for histology analysis

Leeches were fixed for histological analysis in Davidson's solution in a ratio sample-fixative 1:5. They were then dehydrated, embedded in paraffin and, longitudinal cuts in sections of 5 µm were made. In order to contrast tissues, the samples were stained with hematoxylin and eosin (H-E) and Masson´s thricome stains.

Preparation of specimens for scanning electron microscopy (SEM)

Base on Grano-Maldonado (2014), leeches were fixed in 2.5 %

glutaraldehyde in 0.1 M sodium cacodylate buffer and maintained with the fixative for two days at 4 °C. The samples were post-fixed in 1 % osmium tetroxide, dehydrated through a series of ethanol concentration to reach the dry critical point, mounted on aluminium stubs, sputter-coated with gold and then viewed on a Jeol JSM 6460LV scanning electron microscope at an accelerating voltage of 7-10 keV.

Results

Ectoparasites (n=817) were identified as the marine leech *S. moorei* (Hirudinea) (Fig. 1) based on the morphometrical similar features described by Soto (2003), Furiness *et al*. (2007) and Wunderlich *et al*. (2011). The leech has a mean total length of 2.25 ± 0.63 cm, is flattened dorso-ventrally and characterized by a fusiform body narrowing gradually towards the anterior end (Fig. 1). The anterior sucker is cup-shaped attached eccentrically so that the dorsal surface is longer than the ventral. It possesses a marginal border. The diagnostic characters to distinguish *S. moorei* from all other known *Stibarobdella* species are: no eyes; small terminal caudal sucker; large very deeply cupped oral sucker with marginal fringe, and three pairs of small marginal papillae. Octopuses were parasitized all over the skin; mantle, head and arms (prevalence = 92 %; maximum intensity = 130 leeches per host; mean intensity of infection =13.39 leeches per host).

Scanning electron microscopy (SEM)

Scanning electron microscopy provided three-dimensional resolution of *S. moorei* surface morphology structures (Fig. 2). The photographic evidence shows a small terminal caudal sucker and a large very deeply cupped oral sucker. Of particular interest are the prominent arrangements of the oral sucker with marginal fringe.

Histology samples of Stibarobdella moorei (H-E and Masson´s thricome stains) (Figs. 3 – 8)

The longitudinal histological sections of *S. moorei* illustrate the anterior sucker of the leech body (Fig. 3A), the elongated intestinum-caeca (ic) within the crop (C) and a large compartment where ingested blood is stored prior to digestion. Circular muscular fiber (cm) is observed at the edge of the sucker. Minor muscle fibers can also be observed in the area beneath epidermal tissues of the sucker and body joints, which according with Feng *et al*. (2015),

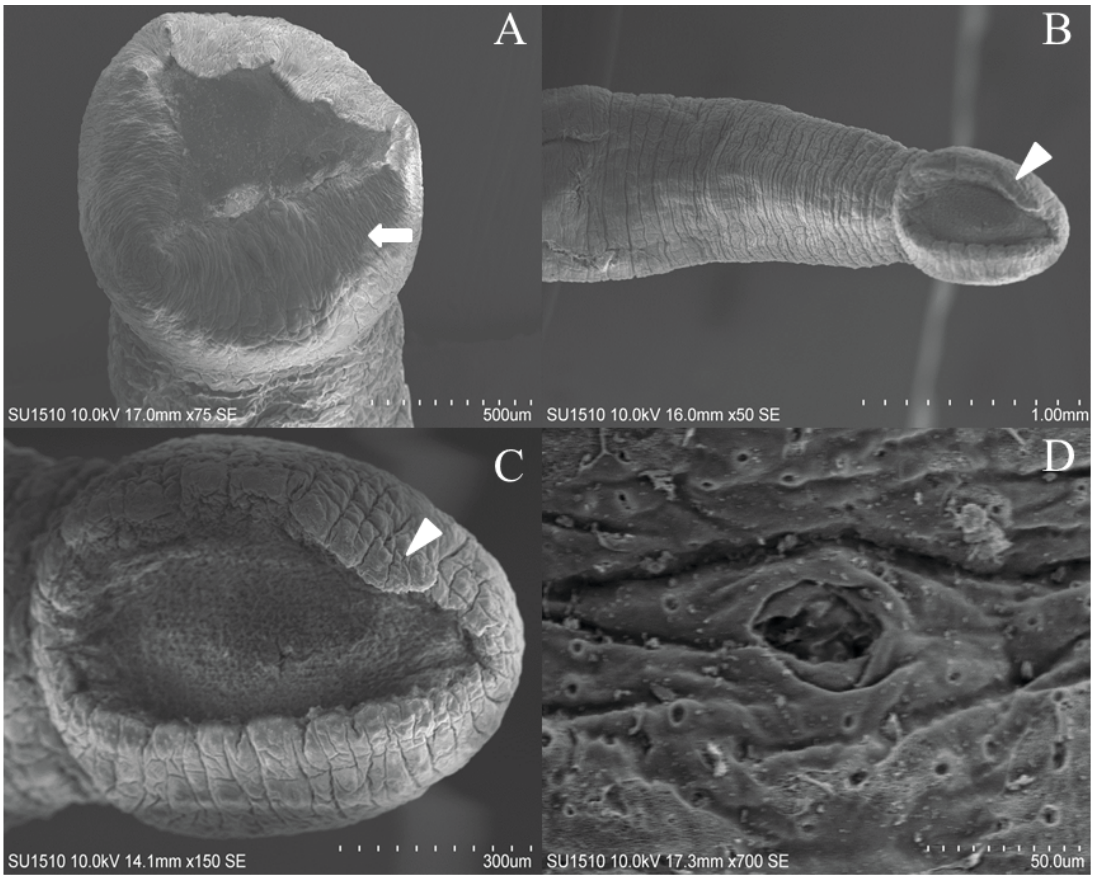

Fig. 2. Scanning electron photomicrographs of *Stibarobdella moorei*. A) Large and deeply oral sucker cup-shaped, eccentrically attached, with obvious marginal fringe (arrow). B – C). Ventral view of the caudal sucker. Posterior sucker small, terminal, not wider than the greatest body width. Caudal sucker and fringe (arrowhead). d) View of the pore.

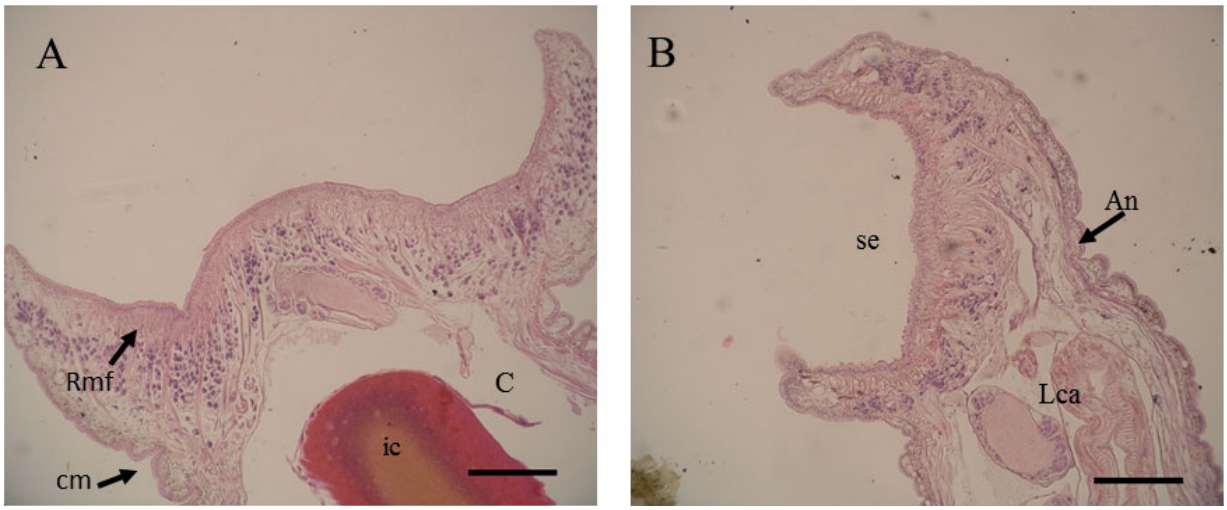

Fig. 3. A) Anterior sucker of the leech *Stibarobdella moorei*. The circular muscular fibre (cm) are observed at the edge of the sucker, minor radial muscular fibre (Rmf) can also be observed in the area beneath epidermal tissues of the sucker and body joints. Elongated intestinum-caeca (ic). B) Posterior caudal sucker. Stratified epithelium (se) and two lateral caecae (Lca) of the crop surrounded by large cells. An) annuli of the body. H-E stain. Scale bar =250 µm.

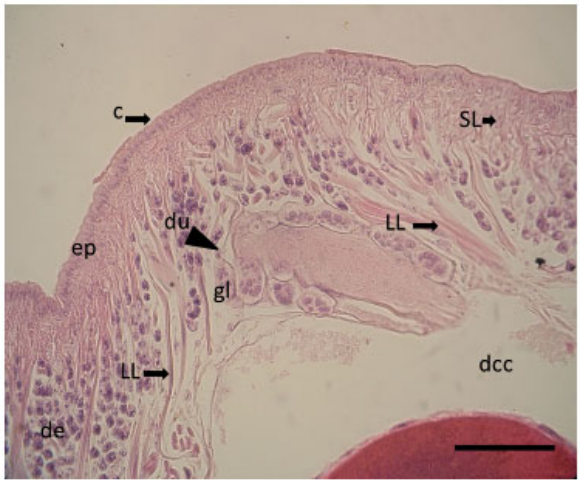

Fig. 4. Photomicrograph of the sucker. Epidermal tissues (ep) and cuticle (c) which is a thin layer with a numerous small or fine projections on the surface; enlarged pear-shape glandular cell (gl) and its duct (du) (arrowhead) reaches the epidermis surface. Long longitudinal muscle fibre (LL); short longitudinal muscle fibre (SL); dorsal coelomic channel (dcc). H-E stain. Scale bar =150 µm.

are considered radial muscular fiber (Rmf). The posterior sucker shows a stratified epithelium and the two lateral caecae of the crop champers surrounded by large cells (Fig. 3B). The dermis of the suckers (Fig. 4) is made of connective tissue with muscle fibers. The musculature below the dermis has a thin layer of longitudinal muscles. All the body muscles were located below the dermis with a relatively thin outer layer of muscle. The posterior sucker and the crop were thinner with diverticula in each side where rounded cell glands arrangements were observed (Fig. 4). These glands localized on the sucker secrete substances to improve smooth attachment and adhesion on the substratum surface as described by Rahemo and Hamad (2013). *S. moorei* has three "jaws", one dorsal and two ventrolateral, and each one has a row of fine "teeth" or denticles on it (Fig. 5).

The cuticle is a thin layer with a numerous small projections on the surface outlining apparent annulations. The epidermis is single layer of hammer-shaped or pear-shaped glands cells. These unitubular epidermal cells are connected with the surface by a duct (mucus secretion nature) which covers the body (Fig. 6). In the connective tissue (dermis), some cells showed granules clearly stained with the haematoxylin-eosin technique (Fig. 7). There is a distinct ring arrangement of these cells lined under the cuticle that likely secretes a cocoon in which after copulation, eggs and sperm are deposited for fertilization and development (Fig. 8).

Discussion

Stibarobdella moorei was identified based on morphometrical characterization in Soto (2000, 2003), Furiness *et al*. (2007) and

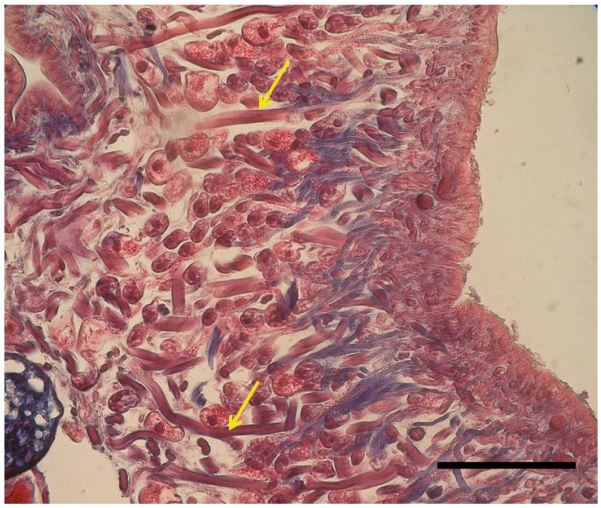

Fig. 5. Photomicrograph of the anterior sucker. Row of fine "teeth" or denticles (arrow). Masson's trichome stain. Scale bar =200 µm.

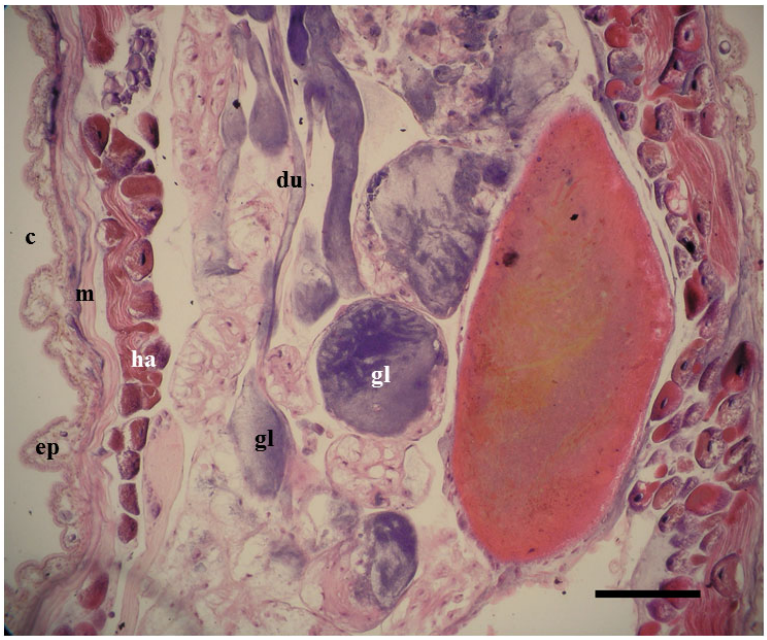

Fig. 6. Section of the body wall showing minor muscle fibers (m) observed under the epidermal tissues (ep); the hammer shape cell (ha) are localized in the loose connective, the large glandular cell (gl) and its duct (du) reaches the epidermis surface and cuticle (c) which is a thin layer with numerous small or fine surface projections with small apparent annulations. H-E stain. Scale bar =250 µm.

Wunderlich *et al*. (2011). The general description of *S. moorei* morphology elaborated by Furiness *et al*. (2007) is the following: no eyes; small, terminal caudal sucker; large, very deeply cupped oral sucker with marginal fringe. Furiness *et al*. (2007) considered *S. moorei* as the valid name and *S. loricata*, as a junior synonym [cited as *S. loricata* (Harding, 1924) previously referred by Soto (2003)].

The genus *Stibarobdella* was described for southern and south-eastern Brazil (Soto, 2000, 2003). Soto (2003) reported *S. loricata* in the southern coast of Brazil, parasitizing on the dorsal region of angel sharks, *Squatina argentina* (Marini, 1930); *S. guggenheim* (Marini, 1936); *S. punctata* (Marini, 1936) and on the head of a sandtiger shark, *Carcharias taurus* (Rafinesque, 1810). Soto (2000) described the marine leech *S. macrothela* in the south coast

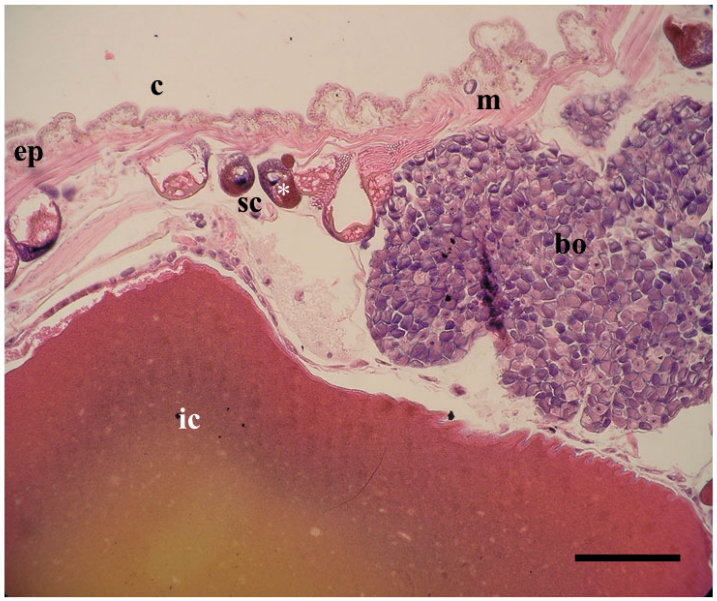

Fig. 7. Section of the body wall showing stained cells (sc) with eosinophilic granules (*) that indicates their secretory nature; minor muscle fibres (m) below epidermal tissues of the body joints are observed; botryoidal 'grape shape' cells (bo) are organized in cords or clusters, localized in the loose connective tissue between the gut and the body wall sac and the elongated intestinum-caeca (ic). H-E stain. Scale bar =250 µm.

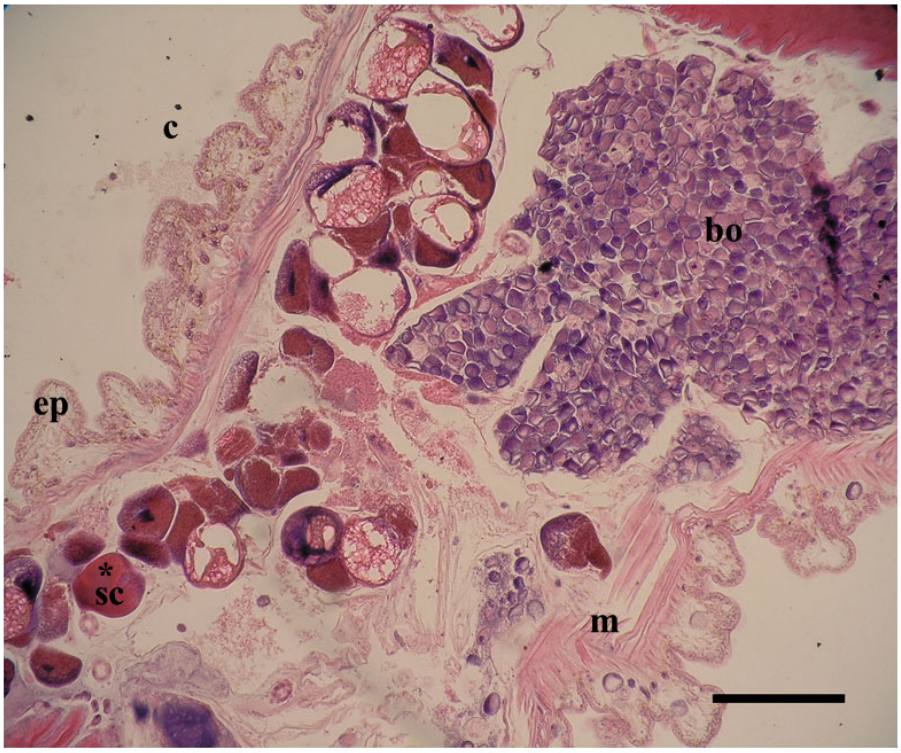

Fig. 8. Section of the body wall showing cuticule (c), epitelial cells (ep), secretional mucus glands (gl) and stained cells (sc) with eosinophilic granules (*) muscle fibres (m), botryoidal 'grape shape' cells (bo). H-E stain. Scale bar =250 µm.

of Brazil, collected from a whaler shark, *Carcharhinus brachyurus* (Günther, 1870). The first report of *S. macrothela* for the north region of Brazil was published by Alves *et al*. (2014).

In Turkey, three species of marine leeches, *Branchellion torpedinis* (Savigny, 1822), *Pontobdella muricata* (Linnaeus, 1758), and *S. moorei* were collected from *Pteromylaeus bovinus* (Geoffroy Saint-Hilaire, 1817), *Dasyatis pastinaca* (Linnaeus, 1758), and *Raja miraletus* (Linnaeus, 1758) (Başusta *et al*., 2015).

Piscicolid marine leeches have been described parasitizing mainly Elasmobranchia (Soto, 2000, 2003; Furiness *et al*., 2007; Wunderlich *et al*., 2011). Species of *Stibarobdella* parasite primarily on sharks (Sawyer, 1986) and rays (Wunderlich *et al*., 2011). As the leeches of the family Piscicolidae commonly parasitize oceanic sharks, the parasite relationship of *S. moorei* with an octopod is unusual. Nevertheless, some marine leeches such as *S. macrothela* commonly parasitize other invertebrates such as the crustacean *Pleoticus muelleri* (Bate, 1888) (Crustacea:Solenoceridae) that inhabits the Argentina seas (Matorelli *et al*., 1994).

Recently, Rahemo and Hamad (2013) described the histology of the body wall of the fish parasitic leech, *Cystibranchus masstacembeli* (Rahemo, 1989) (Piscicolidae) showing that the body wall was similar with some structures found in *S. moorei*. The large pear-shaped cells that were observed beneath the dermis probably secrete "mucus" (Rahemo and Hamad, 2013). In the clitellar region we observed some glands which may be active in the breeding season, however, Rahemo and Hamad (2013) identified on *C. masstacembeli* these albumin glands lying with longitudinal muscles more superficial to the epidermis. The longitudinal section in the posterior sucker of *S. moorei* showed the presence of many mucous secretions in the ventral surface at the posterior end of the leech body. Additionally, in a cross section of the body wall of *S. moorei*, we observed muscular fibers arranged in longitudinal form fibers, analogous to the previous annelids descriptions of Feng *et al*. (2015) consisting in different muscles fibers, long and short longitudinal muscular fibers, circular muscular fibers and radial muscular fibers. Other minor muscle fibers can also be observed in the area below the epidermis of the sucker, and these are different from those of *Whitmania pigra* (Whitman, 1884) (Feng *et al*., 2015). Feng *et al*. (2015) described the adhesion and desorption mechanism achieved by muscle fibers working in different directions: the directional deformation of the dermis interface driven by spatially-distributed muscle fibers facilitates the excretion of fluids in the sucker venter, thus allowing liquid sealing (Feng *et al*., 2015). It appears from above that the histology of the body wall of the present leech *S. moorei* is similar to that of the other leeches such as *C. mastacembeli* (Rahemo and Hama, 2013) and *W. pigra* (Feng *et al*., 2015).

The histology and morphology of cells observed in this study may not be sufficient to provide evidence about their function. Further studies using different histochemical stains may be needed to differentiate the chemical natures of the secretion glands. Additional ultrastructural studies using transmission electron microscopy are

suggested to provide detailed information about the cells functions and physiology of the leech.

The present record of *S. moorei* is the first from México, and the California two-spot *O. bimaculatus* is a new host record for this leech. The present finding, along with previous records of marine leech species in Elasmobranchia throws new light on the biodiversity and host preference of this ectoparasite species and suggests a successful colonization of a previously unknown host. As this study was conducted in the breeding season from April to August, this research highlights the importance of conducting leech samplings in different seasons of the year in order to make a better characterization of the parasite fauna and to determine the environmental factors influencing the population dynamics of host-parasite system. Molecular identification of marine leeches warrants further investigation about taxonomic status and species diversity.

Remarks: The current ectoparasite specie *S. moorei* is a new record paraziting *O. bimaculatus*, and for Mexico.

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References

Alves J.O.P., Monteiro, F.A.C., Matthews-Casconn, H, Cascon, P. (2014): Annelida, Hirudinea, Piscicolidae, *Stibarobdella macrothela* (Schmarda, 1861): First report for northeastern Brazil. *Check List*, 10(5): 1161 – 1163. DOI: 10.15560/10.5.1161

Başusta, N., De Meo, I., Miglietta, C., Mutlu, E., Tunca, M., Şahin, A., Balaban, C., Cengiz M., Usakli, U., Patania, A. (2015): Some marine leeches and first record of *Brachellion torpedinis* Savigny, 1822 (Annelida, Hirudinea, Psicolidae) from elasmobranchs in Turkish waters, with new host records. *Mar. Biodivers.,* 1 – 4. DOI: 10.1007/s12526-015-0411-z

Bush, A. O., Lafferty, K. D, Lotz, J. M., Shostak, W. (1997): Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.*, 83: 575 – 583. DOI: 10.2307/3284227

Conapesca (2011): *Anuario Estadístico de Acuacultura y Pesca* [*Yearbooks of Fisheries and Aquaculture Statistics*]. Secretaria de agricultura, ganadería, desarrollo rural, pesca y alimentación. Gobierno de México.

Feng, H., Chai, N., Dong, W. (2015): Experimental investigation on the morphology and adhesion mechanism of leech posterior Suckers. *PLOS ONE,* 10(11), e0140776. DOI: 10.1371/journal.pone.0140776

Furiness, S., Williams, J.I, Nagasawa, K., Burreson, E.M. (2007): A collection of fish leeches (Hirudinida: Piscicolidae) from Japan and surrounding waters, including redescriptions of three species. *J. Parasitol.*, 93(4): 875 – 883. DOI: 10.1645/GE-979R1.1

Grano-Maldonado, M.I. (2014): *Gyrodactylus gasterostei* a difficult meal to swallow for the 3-spined sticklebacks, *Gasterosteus aculeatus* L. *Scanning,* 36(6): 614 – 621. DOI: 10.1002/sca.21162

Harding, W.A. (1924): Descriptions of some new leeches from India, Burma and Ceylon. *Ann. Mag. Nat. Hist.* (Series 9), 14(82): 489 – 499

Llewellyn, L.C. (1966): Pontobdellinae (Piscicolidae: Hirudinea) in the British Museum (Natural History) with a review of the subfamily. *Bull. Br. Mus. Nat. Hist. Zool.*, 14: 391 – 439

Matorelli, S.R., Etchegoing, J., Mallo, J.C. (1994): Presencia del hirudineo *Stibarobdella macrothela* sobre *Pleoticus muelleri* (Crustacea, Solenoceridae) en Argentina. *Neotrópica,* 40 (103 – 104): 87 – 88

NOM-033-ZOO-1995 Norma Oficial Mexicana 07-16-96 [Official Mexican Standard 07/16/96]. Sacrificio humanitario de los animales domésticos y silvestres.

Oka, A. (1910): Synopsis der japanischen hirudineen, mit diagnosen der neuen species. *Annot. Zool. Jpn.,* 7: 165 – 183

Rahemo, Z.I.F., Hamad, N.R. (2013): Histology of the body wall of the piscine leech, *Cystibranchus mastacembeli* (Hirudinea: Piscicolidae). *J. Agri. Sci. Tech.*, 3: 136 – 143

Sawyer, R.T., Lawler, A.R., Overstreet, R.M. (1975): Marine leeches of the eastern United States and the Gulf of Mexico with a key to the species. *J. Nat. Hist.*, 9: 633 – 667. DOI: 10.1080/00222937500770531

Sawyer, R.T. (1986): *Leech Biology and Behaviour*. Oxford Universitity Press / Claredon Press, Oxford. 1100 pp.

Semarnat (1999): *Estadísticas pesqueras básicas* [*Basic fishing statistic*]. Dir. Gral. de Informática y Reg. Pesq. México. 88 pp.

Semarnat (2004): *Carta Nacional Pesquera* [*National Fishing Charter*]. Comisión Nacional de Acuacultura y Pesca, México.

Sofia (2012): *El estado mundial de la pesca y la acuacultura* [*The state of world fisheries and aquaculture*]. Informe del Departamento de Pesca y Acuacultura de la FAO. Organización de las Naciones Unidas para la Alimentos y la Agricultura. Roma

Soto, J.M.R. (2000): Marine leech, *Stibarobdella macrothela* (Schmarda, 1861) (Hirudinea, Piscicolidae), parasitic on the whaler shark, *Carcharhinus brachyurus* (Günther, 1870) (Chondrichthyes, Carcharhinidae), in southern Brazilian waters. *Rev. Bras. Biol.*, 60(4): 713 – 714. DOI: 10.1590/S0034-71082000000400024

Soto, J.M.R. (2003): The marine leech *Stibarobdella loricata* (Harding, 1924) (Hirudinea, Piscicolidae), parasitic on the angel shark *Squatina* spp. and sandtiger shark *Carcharias taurus* Rafinesque, 1810 (Chondrichthyes: Squatinidae, Carchariidae) in Southern Brazilian waters. *Braz. J. of Biol.,* 63(4): 691 – 694. DOI: 10.1590/S0034-71082000000400024

Utevsky, S.Y., Trontelj, P. (2004): Phylogenetic relationships of fish leeches (Hirudinea Piscicolidae) based on mitochondrial DNA sequences and morphological data. *Zool. Scr.*, 33: 375 – 385. DOI: 10.1111/j.0300-3256.2004.00156.x

Williams, E.H. (1982): Leeches of some marine fishes of Porto Rico and adjacent regions. *Proc. Helminthol. Soc. Wash.*, 49: 323 – 325

Wunderlich, A.C., Fazzano, O.B., Vaske, T., Amaro, M.A. (2011): Annelida, Hirudinida, *Stibarobdella moorei* (Oka, 1910): New distribution and host records. *Check List*, 7(3): 360 – 362. DOI: 10.15560/7.3.360