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Octopus maya parasites off the Yucatán Peninsula, Mexico. II. Salivary gland damage by cestodes

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ABSTRACT: The red octopus *Octopus maya* Voss et Solís-Ramírez, 1966 is an endemic species and one of the most important fishery resources of the Yucatán Peninsula, Mexico. Due to its economic importance and the fact that in recent years interest in farming this species has increased, several initiatives have been implemented to study its biology and requirements for cultivation. Parasites represent an important component of the biology of the red octopus, as they can have an impact on both wild and cultivated populations. A total of 44 *O. maya* specimens were sampled from the fishing ports of Ría Lagartos and Dzilam de Bravo, Yucatán; specimens were measured and subsequently subjected to histological analysis of the buccal masses where cestode larvae (*Prochristianella* sp.) were found in the anterior salivary glands. Results of a chi-squared test showed that specimen size class and infestation levels (parasite abundance) were significantly correlated, with parasite damage levels more pronounced in larger animals. The damage caused to the anterior salivary glands by this parasite could have serious implications for feeding and reproductive success of *O. maya*.

KEY WORDS: *Octopus maya* · Red octopus · Tapeworm · *Prochristianella* sp. · Histology · Anterior salivary gland

INTRODUCTION

The identification of parasite species and the quantification of damage that they can inflict to economically important species are important considerations in the implementation of preventive health measures. It is well documented that parasites are a major cause of losses in farming systems. The octopus fishery in the Yucatán Peninsula (Mexico), represented by *Octopus maya* and *O. vulgaris*, is one of the most important fisheries in this region. Throughout the Gulf of Mexico, the red octopus *O. maya* is the most important species economically, representing up to 80% of the total annual catch of octopus (SAGARPA 2005). The economic income from this activity in the Yucatán Peninsula is approximately 360 million pesos (US$3.6 million) yr^{-1} (SAGARPA 2005). However, a decrease in the catch volume since the early 2000s of this fishery (Salas et al. 2006, Jurado-Molina 2010, Velázquez-Abunader et al. 2013) has motivated research related to nutritional, reproductive and behavioral aspects of *O. maya* aimed at informing the development of aquaculture practices for this species (Santos-Valencia & del Río-Rodríguez 2006, Rosas et al. 2007, 2008, Avila-Poveda et al. 2009, 2016).

Cephalopods play an important ecological role in the life cycle of parasites by acting as intermediate, paratenic and/or definitive hosts (Hochberg 1990, Pascual et al. 1997, Gestal et al. 1998). Recently, comprehensive studies of *O. maya* in the Yucatán Peninsula addressed different features of the biology and exploitation of this species (Avila-Poveda et al. 2009, 2016, Farías et al. 2009, Briceño et al. 2010, Jurado-Molina 2010, Moguel et al. 2010, Velázquez-Abunader

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et al. 2013, Rosas et al. 2014). One of these studies involved the identification of both micro- and macroparasites affecting this octopus as well as the estimation of infection levels (Guillén-Hernández et al. 2018). Results indicated that larvae of a cestode (*Prochristianella* sp.) are particularly abundant and that this parasite has a high prevalence throughout the geographical distribution of *O. maya*. The main microhabitat of this parasite is the buccal mass, particularly the anterior salivary glands (ASGs). Accordingly, here we describe the damage that this parasite causes to the tissue of the salivary glands of *O. maya*. In order to quantify the amount of damage caused by this parasite, descriptions of the buccal masses of uninfected specimens were made and subsequently compared to descriptions from specimens exhibiting varying levels of damage caused by cestode infection.

MATERIALS AND METHODS

Sample collection and processing

Octopus samples were collected from the ports of Ría Lagartos and Dzilam de Bravo in the state of Yucatán, México. In order to detect and quantify the degree of infection at different developmental stages in the life cycle of *Octopus maya*, we conducted a histological examination of the buccal mass of specimens of varying sizes (based on the mantle length). In addition, we examined the buccal mass of healthy (uninfected) individuals grown in captivity at the Unidad Multidisciplinaria de Docencia e Investigación (UMDI), of the Universidad Nacional Autónoma de México (UNAM), in Sisal, Yucatán, México. These descriptions were used to characterize the normal histology of the buccal mass (control) for comparison with that of parasitized specimens. A total of 38 infected specimens belonging to different size classes based on 20 mm intervals, as well as 6 uninfected specimens were examined (see Fig. 3). The buccal mass of each specimen was fixed in 10% formaldehyde or Davidson solution (Lightner 1996) and then transferred to 70% ethyl alcohol for preservation during the next 24–72 h. The preserved material was processed following the histological techniques recommended by Howard & Smith (1983). Tissue sections were placed in an automatic tissue processor (Kedee TS3) where they were dehydrated, cleared and infiltrated with paraffin, to then obtain cross-sections. The cuts were stained using the Harris hematoxylin-eosin technique for structural pathologies, symbionts and parasites, and the samples were then mounted on slides with Canada balsam. Stained sections were examined by light microscopy using an Olympus BX53 at 200× and 400×. Histological damage was related to the number of parasites and was classified as follows: (1) mild (fewer than 5 tapeworms), (2) moderate (between 6 and 50) or (3) severe (>50). Considering that specimen size and age are correlated, we expected that the larger specimens would have a longer history of infection by parasites. A chi-squared test was run to determine if there was a relationship between octopus size and infestation levels.

A parasite may have a negative impact on its host, which implies an impairment that is quantifiable in terms of a reduction in the ecological potential (e.g. survival, growth, reproduction, energy procurement, stress endurance, competition) (Kinne 1980). In order to analyze the relationship between the number of parasites (tapeworms) and the somatic condition of the host (i.e. potential negative effect of parasite), we ran a simple linear regression based on data from 144 octopuses (all *O. maya*) captured at the ports of Celestún and Progreso (Yucatán, México). The non-parasitized individuals measured between 73 and 144 mm, and weighed between 189 and 1358 g, whereas the parasitized specimens measured between 80 and 155 mm and weighed between 154 and 1150 g. We used the relative condition factor K_n (Le Cren 1951), with the formula of Copp (2003): $K_n = (EW_{obs})/(EW_{exp}) \times 100$, where EW_{obs} is the observed eviscerated weight and EW_{exp} is the expected weight which was determined by using the length–weight relationship for *O. maya* specimens without tapeworms (control n = 24). It is worth mentioning that this relationship was estimated by a potential regression model using the formula $EW = 0.0033 \times ML^{2.5782}$ ($R^2 = 0.9534$), where EW is the eviscerated body weight and ML is the mantle length of the specimens of *O. maya*, 0.033 is the intercept (*a*), and 2.5782 is the slope (*b*).

Morphology and normal histology of the buccal mass

The buccal mass is located in the ventral portion of the organism, at the center of the brachial crown, and has a great capacity for rotation and protrusion (Boucaud-Camou & Boucher-Rodini 1983, Salvini-Plawen 1988). It is a complex structure composed of numerous secretory elements and initiators for apprehending and grinding food. Two lips surround the mouth and these are protected by a simple buccal membrane. Two jaws that form the chitin peak delimit the oral cavity and 5 muscles articulate the jaws: 2 lateral muscles, 1 anterior, 1 upper and 1 posterior (Uyeno & Kier 2005). Inserting peaks are lined by epithelium columnar cells. In the ventral side of the mouth, there is a muscular extension similar to a tongue called the salivary papilla or subradular organ (Bidder 1966), above which is the odontophore, an organ that bears the radula. The radula is posteriorly similar to a denticulated tongue, which facilitates the passage of food into the esophagus. The anterior and sublingual glands are also found in the buccal mass. The sublingual glands are fully enclosed within the mouthparts, are unpaired, and run ventrally towards the salivary papilla. The ASGs are arranged in pairs, are located at the base of the oral mouthparts, and partially included therein. These saliva glands are delimited by a thin connective tissue capsule and surrounded by an inner layer of longitudinal muscle fibers and outer transverse muscle fibers. In addition, there are tubulo-acinar glands which are formed by a secretory epithelium separated by connective tissue named trabeculae. Acinar spaces are formed by a line of secreting cells ranging from cuboidal to columnar in shape depending on their size, with mucous material in the cytoplasm (Gennaro et al. 1965) (Fig. 1).

RESULTS AND DISCUSSION

All parasites (identified as *Prochristianella* sp.) were observed in the ASG tissue, and the severity of damage was related to the number of parasites. As observed at the histological level, parasites destroy the epithelial secretory gland cells but also trigger cell host responses that result in proliferation of connective tissue and infiltration of hemocytes to isolate the foreign agent. In cases of mild tapeworm infection, moderate destruction of the glandular tissue around the parasites was observed, but almost no proliferation of connective tissue occurred, such that the gland exhibited its normal appearance (Fig. 2a). In cases of moderate infection, proliferation of connective tissue was observed, as well as thickened trabeculae in some areas with infiltration of hemocytes and destruction of glandular tissue (Fig. 2b,c). Finally, severe infestation was manifested by a marked reduction in the glandular tissue and its replacement by connective tissue, and tapeworm counts were so high that quantifying them was difficult. At this phase, the gland is probably dysfunctional and produces little or no salivary secretions (Fig. 2d).

With regard to the relationship between host size and infection levels, it seems that infection by this parasite occurs at an early age of the host, since small hosts (75–94 mm mantle length) were parasitized (Fig. 3). The infection level in this size class ranged from mild to moderate, and the severity of the infection increased with host size (Fig. 3). At the 95.0% confidence level, the chi-squared test showed that the size classes and infection levels are not independent ($\chi^2 = 14.562$, $p = 0.024$). Therefore, the infection level (mild to severe) is related to host size, which is probably correlated with host age. Accordingly, the longer the amount of time a parasite remains in the salivary glands, the greater the severity of tissue damage. This suggests that para-

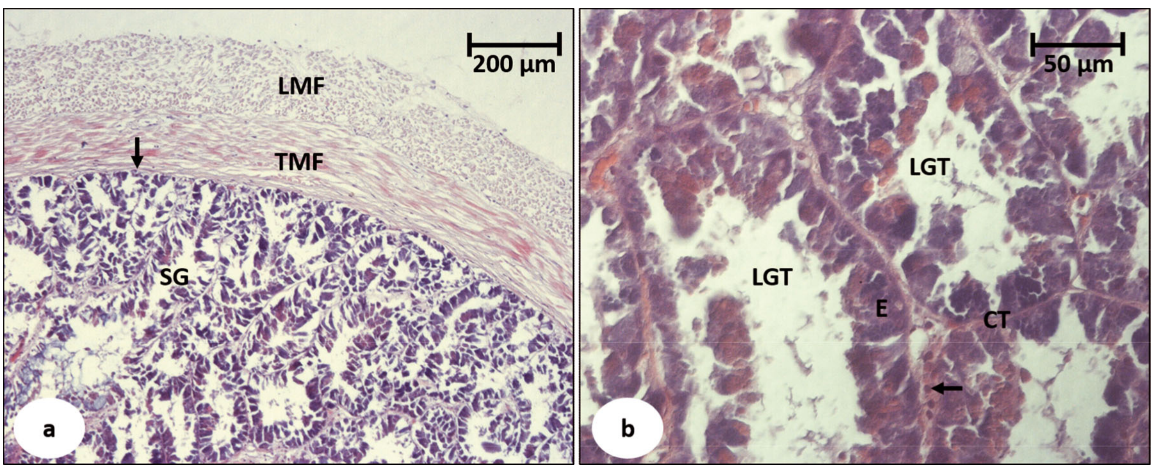

Fig. 1. Histology of the oral mass of uninfected *Octopus maya* (H-E staining). (a) Salivary gland (SG) delimited by a thin connective tissue capsule (arrow) and surrounded by an inner layer of longitudinal muscle fibers (LMF) and outer transverse muscle fibers (TMF). (b) Detail of the salivary gland showing its tubule-acinar structure formed by a columnar epithelium (E) delimited by a thin layer of connective tissue (CT). LGT: lumen of glandular tubules (arrows) in the connective tissue hemocytes

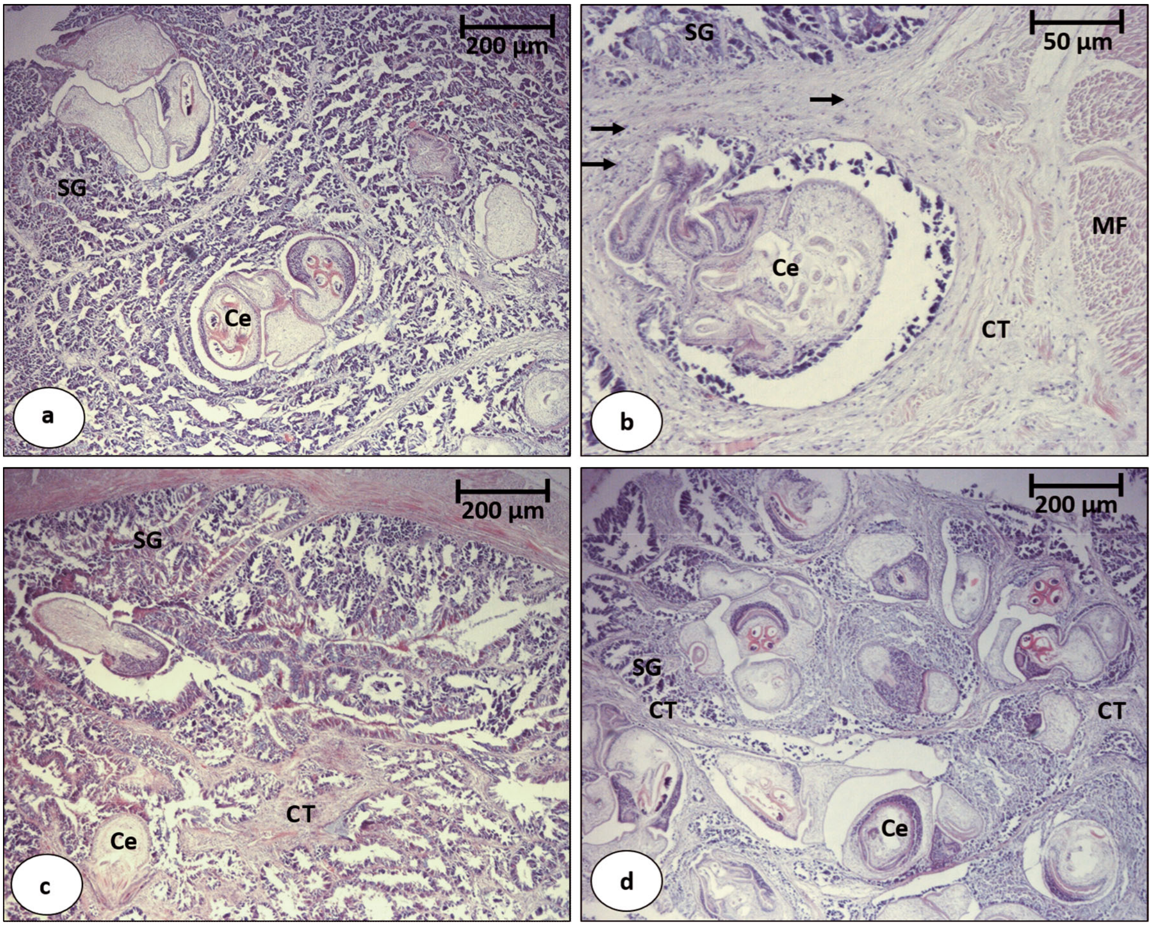

Fig. 2. Histological damage in *Octopus maya* anterior salivary glands (SG) caused by the tapeworm *Prochristianella* sp. (H-E staining). (a) Tapeworm cestodes (Ce) grouped in the center of the SG tissue, in which the proliferation of connective tissue (CT) is mild and glandular tissue appears normal. (b) Detail showing the proliferation of CT around the Ce with infiltration of hemocytes (arrows), and the muscle fibers (MF). (c) SG with moderate numbers of Ce with a slight proliferation of CT. (d) Severe infection of Ce causing intense proliferation of CT, infiltration and destruction of the epithelium hemocytic glands

sites accumulate throughout the lifespan of the octopus and end up causing more damage in older individuals. However, this does not exclude the possibility of high levels of infection and tissue damage during short periods under specific conditions, irrespective of host age. Although the association between relative condition factor (K_n) and the number of parasites was very low ($R^2 = 0.1368$, n = 144, p = 0.0001), there was a significant relationship between these variables at the 95.0% confidence level. The equation of the fitted model was $K_n = 87.3507 - 0.0083 \times \text{Tapeworms}$, and the Pearson correlation coefficient r = –0.370077, both indicating a relatively weak but negative relationship between these variables (Fig. 4). The negative effect of a parasite on its host will depend on both the degree of affectation that it has on the host's health and the host's capacity to counter-attack or neutralize the

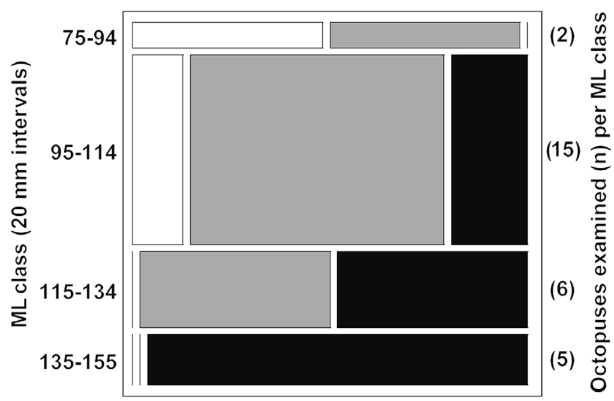

Fig. 3. Mosaic plot showing the number of *Octopus maya* individuals examined in each mantle length (ML) class (20 mm intervals) and the percentage of specimens with different infestation levels: (1) mild (<5 tapeworms) (white bars), (2) moderate (6–50 tapeworms) (grey bars) or (3) severe (>50 tapeworms) (black bars)

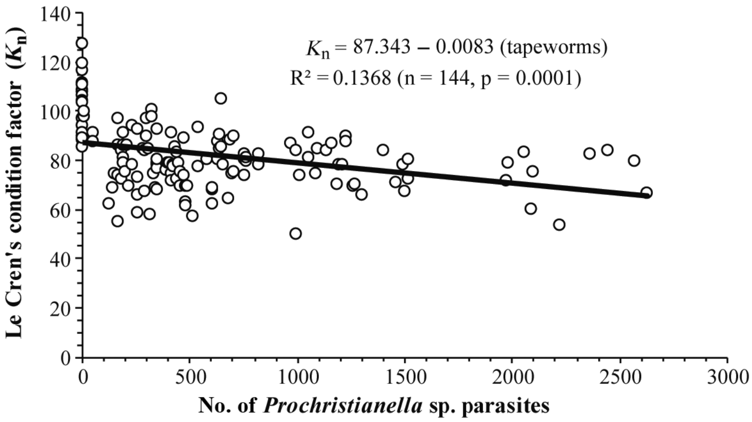

Fig. 4. Linear relationship between the number of tapeworms (*Prochristianella* sp.) infecting individual *Octopus maya* and the condition of *O. maya* specimens based on Le Cren's condition factor estimated as K_n = EW_{obs}/EW_{exp}, where EW_{obs} is the observed eviscerated weight and EW_{exp} is the expected weight. EW_{exp} was calculated from the length–weight relationship in 24 specimens of *O. maya* without tapeworms

infection. Likewise, this effect may be subject to external factors such as environmental conditions (e.g. light, temperature, salinity, oxygen availability, pollution), the host's physiological state (e.g. metamorphosis, nutritional status, reproduction, reduced immunity) or the presence of infections involving more than 1 species of parasite. The fact that the amount of variation explained by this model was relatively low (R^2 = 0.1368) suggests that some of these unaccounted factors come into play in explaining differences in host performance.

There is a lack of information on diseases caused by parasites in cephalopods collected *in situ*, probably because injured or infected individuals are likely to be eliminated more rapidly from the population by predators. Tissue damage caused by *Prochristianella* sp. to the buccal mass could have important physiological effects on the host. In octopuses, a common duct connects both the posterior salivary glands and the ASGs to the oral cavity. The posterior glands in *Octopus maya* are responsible for producing enzymes, specifically proteinaceous compounds that both paralyze and relax their prey (Pech-Puch et al. 2016). ASGs in octopuses are responsible for producing not only neutral glycoproteins with –SH and S–S groups and sialic acid, but also dipeptidase and hyaluridase, which probably help to liquefy the very viscous secretions of the posterior salivary glands (Sawano 1935, Romanini 1952, Wells 1978). Accordingly, the direct effect of *Prochristianella* sp. on ASGs can have major implications in the production of saliva, a secretion that is essential in food acquisition and defense, as well as in minimizing competition among conspecifics (Pech-Puch et al. 2016).

The observed positive association between damage observed and the number of parasites suggests that larger (and potentially older) octopuses are more negatively affected by parasitism, especially considering that these parasites tend to accumulate until the host is consumed by a predator. After oviposition, *O. maya* females do not feed anymore; they stay with the eggs to take care of them by aerating and cleaning them. It has been proposed that saliva contributes to the adhesion of eggs (Gennaro et al. 1965) and probably also plays a role in the cleaning process. Thus, the damage observed in the salivary glands may also have an impact on reproduction and recruitment, which deserves further attention.

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