

ATTACHMENT OF THE NEMERTEAN *MALACOBDELLA ARROKEANA* TO THE MANTLE OF THE GEODUCK *PANOPEA ABBREVIATA* AND SURVIVAL OUTSIDE THE HOST

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ABSTRACT Results of the histopathological study of mantle tissues of the commercial geoduck *Panopea abbreviata* hosting the nemertean *Malacobdella arrokeana* revealed that the normal histology of mantle tissues of the bivalve was not altered by the attachment structure of the nemertean, even when the maximum individuals per clam reached 191 nemerteans. However, the vacuum force generated by the nemertean sucker seems to elicit a slight mechanical stretching of epithelial cells and a negligible infiltration response affecting the connective tissue between inner and outer mantle epithelia beneath the point of attachment. The 99.4% bivalves examined ($n = 657$) hosted at least 1 specimen of *M. arrokeana*. Adult nemerteans were able to survive outside the host for up to 3 mo at 13°C. These results suggest that the relationship between *M. arrokeana* and *P. abbreviata* should be considered as a commensal rather than a parasitic relationship.

KEY WORDS: nemertean, *Malacobdella arrokeana*, *Panopea abbreviata*, entocommensalism, geoduck, *Panopea*

INTRODUCTION

Forty species of nemerteans have been reported as symbionts or commensals on marine invertebrates (Jensen & Sadeghian 2005). The actual nature of these associations is not well known, and is currently characterized as cases of commensalism, parasitism, or specialized egg predation (Berg & Gibson 1996).

The family Malacobdellidae comprises a single genus, *Malacobdella*, with 6 species inhabiting the mantle cavity of bivalves (Jensen & Sadeghian 2005). They are usually considered entocommensals, although Sundet & Jobling (1985) supported their parasitic life style. Members of the genus *Malacobdella* are characterized by a flat body and a terminal simple sucker at the posterior end of the body; the sucker enables the attachment of the nemertean to the host mantle tissues ensheathing the mantle cavity. Entocommensal nemerteans feed by means of a proboscis, presumably ingesting plankton captured by the mucus secretions produced by the bivalve gills. In general, there is only 1 adult specimen per bivalve host (Jensen & Sadeghian 2005). Host specificity of *Malacobdella* is high; 5 of 6 species of the genus have been reported in only 1 bivalve species. Thus, each species exhibits a limited geographical distribution. The exception is *Malacobdella grossa* (Sundberg 1985), found in at least 27 different bivalve species, and is widely distributed in Europe, and the Atlantic and Pacific coasts of North America (Gibson 1994, Jensen & Sadeghian 2005).

Malacobdella arrokeana Ivanov, Bigatti, Penchaszadeh & Norenburg, 2002 (Nemertea: Bdellonemertea) inhabit the mantle cavity of the geoduck *Panopea abbreviata* Valenciennes, 1839 (Ivanov et al. 2002). *M. arrokeana* is the only species of the genus reported for the southwestern Atlantic Ocean. The hiatellid clam *P. abbreviata* is endemic to the southwestern Atlantic and, since 1999, has been a target of incipient artisanal fisheries in northern Patagonia (Ciocco 2000). Martorelli et al. (2003) reported a high prevalence of *M. arrokeana* (86–100%) in Patagonian populations of *P.*

abbreviata. According to Teso et al. (2006), the number of individuals of *M. arrokeana* per host varied between 1 and 60 (mean, 3.7 ± 9.4). When several specimens of *M. arrokeana* are present within the mantle cavity of a single bivalve specimen, only one of them is sexually mature. This fact could be regarded as a sort of intraspecific competition for space (Bush & Lotz 2000). Interference among individuals may result from a chemical substance released by 1 animal, leading to the inhibition of sexual maturity in the others (Teso et al. 2006). A similar situation has been reported for the cysticeroid larvae of *Hymenolepis diminuta* parasitizing the coleopteran *Tribolium confusum* (Wisnivesky 2003).

The aim of this study was to evaluate the possible damage of host mantle tissues elicited by the *M. arrokeana* attachment structure, and to determine the survival of *M. arrokeana* outside its host, to search for evidence about whether this relationship is either commensal or parasitic.

MATERIALS AND METHODS

Specimens of *P. abbreviata* ($n = 657$) were collected from January 2006 to July 2008 at San Matías Gulf (40°40'S, 63°30'W) and San José Gulf (42°20'S, 64°20'W), northern Patagonia, Argentina. Geoducks were collected by scuba diving using a hydro jet to dislodge clams from the sediment. Specimens for histology ($n = 25$) were dissected, and nemerteans and a section of mantle tissue of the attachment area (about 1 cm²) were removed and immediately immersed in Davidson's fixative (Shaw & Battle 1957) for 24 h. After dehydration in ethanol and paraffin embedding, serial sections (5 µm thick) were stained with hematoxylin eosin stain. Nine adult nemerteans were placed in individual flasks containing aerated, nonfiltered seawater and were maintained in darkness at a controlled temperature (13°C). After 3 mo, the water temperature was increased to 21°C until the end of the experiment. Seawater was changed weekly and nemerteans were observed daily for survival. In addition, motility and fixation by the terminal sucker were recorded.

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RESULTS

The nemertean, *M. arrokeana* was found in 99.4% of the geoducks ($n = 657$) studied, varying between 0–191 individuals (adult and young nemerteans) per clam. Adult nemerteans were attached to the mantle at the posterior half of the mantle cavity, near the gills (Fig. 1).

After dislodging the nemerteans from their attachment in the mantle of the geoduck, a circular protrusion corresponding to the place of the sucker remained as a raised flat cup (Figure 1D). Histological study of the mantle area beneath the nemertean attachment disk showed that the normal histology of the mantle epithelium was not altered. Neither hyperplasia nor metaplasia of the inner mantle epithelium was observed, but a distortion of the mantle tissue resulting from mechanical stretching was noticeable (Figs. 1D, 2A–D). A slight hemocytic infiltration was observed in the connective tissue between the outer and inner mantle epithelia, beneath the place where the sucker of the nemertean was in close contact with the mantle of the geoduck. This process was found to be a slight infiltration response as a cellular defense mechanism of the host (Fig. 2C). Secretion was observed between the nemertean sucker and mantle epithelium, apparently derived from the nemertean integument ensheathing the inner surface of the sucker (Fig. 2E).

At 13°C, more than 50% of adult nemerteans were able to survive outside the host for 3 mo ($n = 6$); first deaths ($n = 3$) were observed 7 days after starting the experiment. Three months later, when the temperature was raised to 21°C, 3 surviving nemerteans kept on living attached to the flask for another month.

DISCUSSION

This study is the first report on the histopathological alterations elicited by the attachment of the commensal nemer-

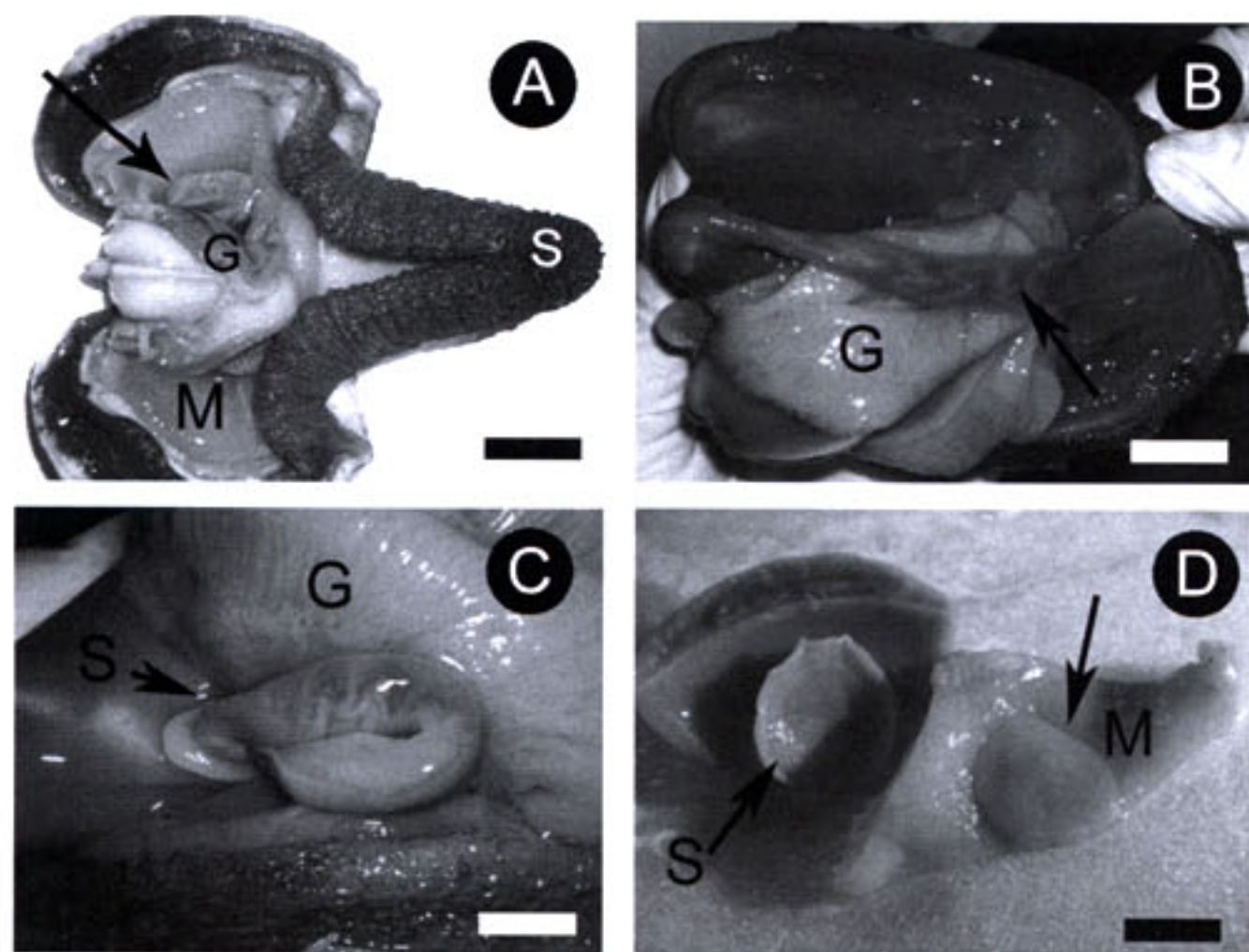


Figure 1. *Malacobdella arrokeana* (Nemertea: Mallacobdellidae). (A) Adult nemertean (arrow) located in the posterior half of the mantle cavity, near the gills. Scale bar: 5 cm. (B) Nemertean attached to mantle epithelium of the geoduck, extending its body (arrow). Scale bar: 2 cm. (C) Detailed view of the sucker of the nemertean (arrow) attached to mantle epithelium of the geoduck. Scale bar: 1 cm. (D) Protrusion in the mantle epithelium of the geoducks associated with the attachment site to which the nemertean was attached. Scale bar: 1 cm. G, gills of the geoduck; M, mantle of the geoduck; S*, siphon of the geoduck; S, sucker of the nemertean.

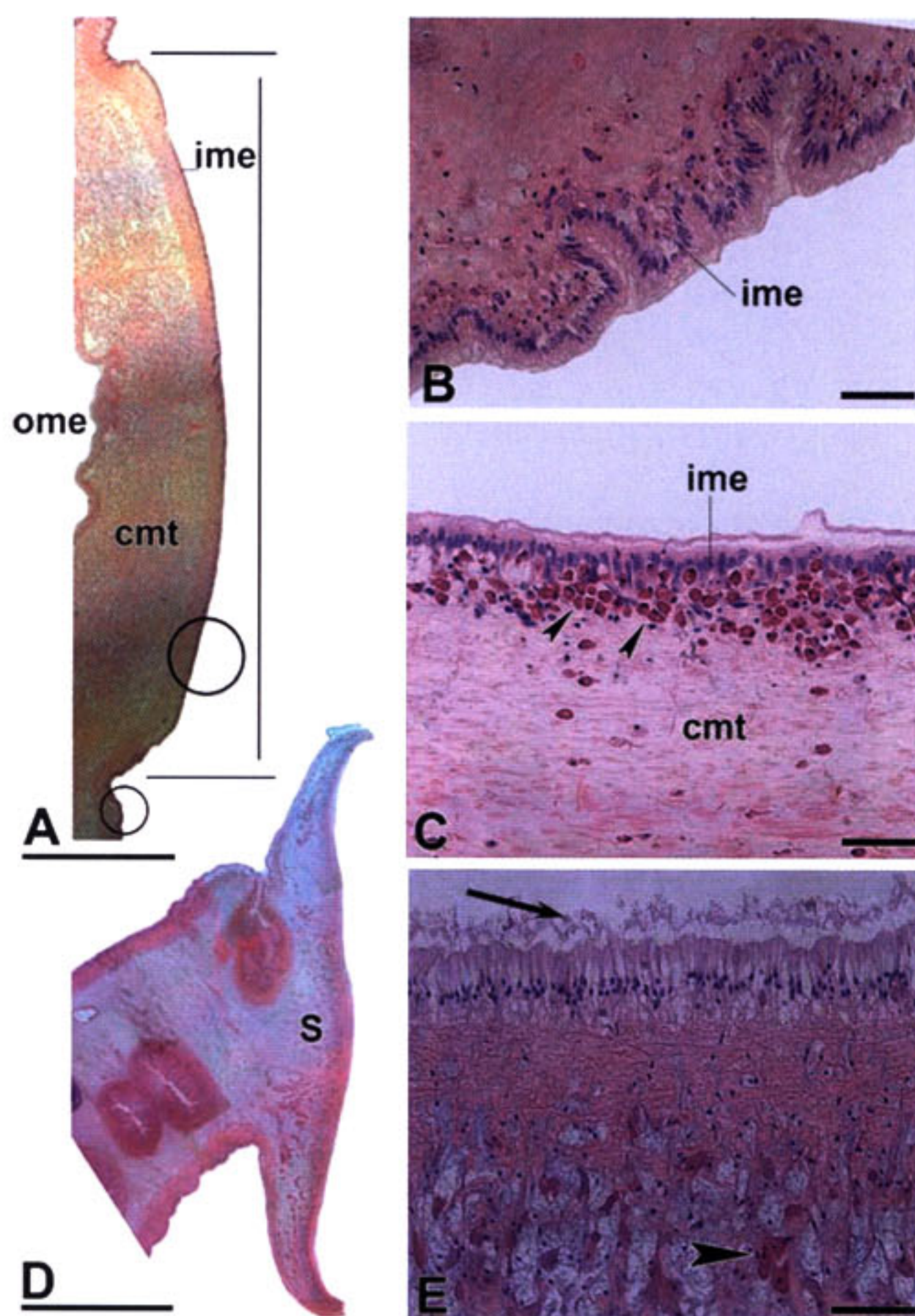


Figure 2. Histological sections (hematoxylin–eosin stain) of the mantle epithelium of *Panopea abbreviata* from Patagonia, Argentina, and the posterior end of a specimen of *Malacobdella arrokeana*. (A) Transverse section of the mantle epithelium of *P. abbreviata* corresponding to the attachment area (lines) of the nemertean. (B) Detail of the normal histology of the inner mantle epithelium corresponding to the small circle in A. (C) Detail of the altered area of the inner mantle epithelium corresponding to the large circle in A, slightly infiltrated by hemocytes (arrows). (D) Sagittal section of the posterior end of *M. arrokeana*. (E) Detail of nemertean tegument, where the secretion (arrow) and sub-epidermal glandules (arrowhead) are shown. Scale bars: A, D = 2 mm; B, C, E = 50 μ m. cmt, connective tissue of mantle epithelium; ime, inner mantle epithelium; ome, outer mantle epithelium; S, sucker of the nemertean.

tean *M. arrokeana* to the bivalve host *P. abbreviata*. The area of the inner mantle epithelium at the point of attachment of the nemertean is mechanically altered by the vacuum force produced by the sucker to secure a commensal attachment to the host within the mantle cavity. From a histological point of view, the absence of hyperplasia and metaplasia of the mantle epithelium is evidence that the presence of the nemertean is innocuous. However, the slight increase in the number of hemocytes in the conjunctive tissues between the inner and outer mantle epithelia adjacent to the nemertean attachment indicates a response elicited by the presence of *M. arrokeana*.

Cellular infiltration is one of the cellular defense mechanisms, constituting an immediate response of tissue to an injury, evidencing the constriction of blood vessels or sinuses as well as

hemocytosis, or the infiltration of hemocytes into the site of injury (Pauley & Sparks 1965, Ruddell 1971).

The concept of parasitism is controversial; however, potential damage to the host and metabolic dependence (mainly nutritional) are generally viewed as the key characters in defining parasitism (Bush et al. 2001). In general, species of *Malacobdella* are considered innocuous for the hosts (Lauckner 1983, Ivanov et al. 2002, Jensen & Sadeghian 2005). Nevertheless, Sundet and Jobling (1985) reported the association between *M. grossa* and *Arctica islandica* as parasitic, because of the slight but significant diminution in the growth rate and a lesser condition index of parasitized bivalves caused by the nemertean. To date, eventual histopathological damage on bivalve tissues caused by entocommensal nemerteans has not been studied.

M. arrokeana was able to survive for 3 mo outside the host under laboratory conditions. This suggests that a commensal, rather than a parasitic, relationship is occurring between these

partners. Combes (2001) concluded that a mutualistic relationship offers 3 advantages: habitat, motility, and energy. Concerning *M. arrokeana*, refuge is the main resource provided by the host, protecting them from predators. We were unable to confirm whether the nemerteans could obtain energy, in terms of food resources, from their host. However, it is probable that *M. arrokeana* feeds on the mucus secretion produced by the gills of the clam to capture plankton and organic particles. This has been reported for the pea crabs (Crustacea: Pinnotheridae) that also inhabit the mantle cavity of bivalves (Bierbaum & Ferson 1986). The high prevalence values, close to 100%, of the nemertean precluded us from obtaining information on the possible diminution in the growth rates of *P. abbreviata*.

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