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Author(s): José E. F. Alfaya, David E. Galván, Annie Machordom, Pablo E. Penchaszadeh and Gregorio Bigatti

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Malacobdella arrokeana: Parasite or Commensal of the Giant Clam *Panopea abbreviata*?

José E. F. Alfaya^{1,2}, David E. Galván³, Annie Machordom⁴,
Pablo E. Penchaszadeh⁵, and Gregorio Bigatti^{1,2*}

¹LARBIM-IBIOMAR, Centro Nacional Patagónico (CENPAT-CONICET). Bvd. Brown 2915, U9120ACV Puerto Madryn, Chubut, Argentina

²Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Bvd. Brown 3100; U9120ACV Puerto Madryn, Chubut, Argentina

³CESIMAR, Centro Nacional Patagónico (CENPAT-CONICET). Bvd. Brown 2915, U9120ACV Puerto Madryn, Chubut, Argentina

⁴Museo Nacional de Ciencias Naturales (MNCN-CSIC). José Gutiérrez Abascal, 2. 28006 Madrid, Spain

⁵Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN-CONICET), Av. A. Gallardo 470 (C1405DJR), Buenos Aires, Argentina

We examined trophic relationship between the nemertean *Malacobdella arrokeana* and its host, the edible geoduck *Panopea abbreviata* by studying the diets of both species by direct (stomach contents) and indirect methods (stable-isotope analysis of C and N). In addition to these methods, the feeding behavior of *M. arrokeana* within the host and the morphology of its feeding organs were examined. The feeding behavior of *M. arrokeana* did not exhibit parasitic characteristics, and the proboscis morphology indicates it is unable to injure host tissues. Analysis of stomach contents revealed a diet consisting mainly of microalgae and diatoms. *Panopea abbreviata* and *M. arrokeana* shared similar trophic levels, presenting no differences in the spread of the isotopic niches and high overlap (SEA_B overlapped 63%). Consistent with this, our results showed no differences in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values between the two species. The combination of direct and indirect approaches revealed that *M. arrokeana* has a diet similar to that of its host, confirming a commensal relationship.

Key words: commensal nemertean, stable isotopes, feeding behavior, diet, Hoplonemertea, proboscis morphology, bivalves

INTRODUCTION

Symbiotic relationships include commensalism, mutualism, and parasitism. Although symbiotic interactions are common in nature (Herre et al., 1999), characterizing the type of interaction can be difficult (Bronstein, 1994). The difficulty arises primarily in attempting to measure the metabolic dependence of one organism on another, and secondarily, in measuring the cost or benefit of that dependence. Several nemertean species are considered true parasites, whereas other species have a relationship with a host (Roe, 1988) but are not considered parasitic. To determine whether a particular nemertean species is parasitic, better knowledge of its biology is needed (Gibson, 1972; Roe, 1988). For instance, it has been shown that nemertean parasites often have reduced feeding structures (Roe, 1988). Species of the genus *Carcinonemertes* have been found on the egg masses of many brachyuran crab species (Wickham

and Kuris, 1985; Kuris, 1993), feeding on the host's yolk, using the acquired energy to develop their own gametes, and laying egg strings among host embryos (Humes, 1942; Kuris, 1978; Roe, 1979; Wickham et al., 1984). This genus is considered parasitic because the high intensity of nemertean populations negatively affects the reproductive output of the host population (killing 50–60% of the host's embryos; Kuris, 1993). In these species, the proboscis is very small and cannot be everted beyond the front of the animal (Wickham, 1978). Species of the genera *Gononemertes* and *Vieitezia* have a shared symbiotic relationship, living inside the atrium of several solitary ascidian species. These nemerteans possess reduced feeding structures (small proboscis) similar to those in *Carcinonemertes* species (Roe, 1988). However, one species of *Gononemertes*, *G. australiensis*, does not feed on its host and has a digestive physiology similar to that of typical free-living nemerteans, and therefore is considered an entozoic nemertean (Gibson and Egan, 1976; Roe, 1988; Junoy et al., 2010). An example of a potentially mutualistic relationship was reported between *Tetrastema fozensis* and the infaunal bivalve *Scorbicularia plana* (Thiel and Francés Zubillaga, 1998). This nemertean

* Corresponding author. Tel. : +54-280-488-3184 ext 1273;
E-mail: gbigatti@cenpat-conicet.gob.ar
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feeds on the parasitic copepods that infest the bivalves, suggesting that *T. fozensis* may be beneficial for its host.

The genus *Malacobdella* consists of six species, all showing symbiotic relationships, typically attached via a terminal sucker to the mantle cavity of marine bivalves (Ivanov et al., 2002). To date, the feeding biology of only one species, *M. grossa* (Müller, 1776), has been studied (Gibson, 1968; Gibson and Jennings, 1969). These authors suggested that *M. grossa* is a filter feeder, presumably ingesting plankton from the host's mantle cavity. The proboscis structure of *M. grossa* differs from other hoplonemertean; its proboscis is covered with sticky papillae and lacks a stylet apparatus.

Malacobdella arrokeana Ivanov et al., 2002 lives in the mantle cavity of the giant geoduck *Panopea abbreviata* Valenciennes, 1839. Both species are endemic to the southwestern Atlantic Ocean coastal zone. Populations of *P. abbreviata* are abundant in northern Patagonian gulfs. Teso et al. (2006), Vázquez et al. (2009), and Alfaya et al. (2013) documented that 99.4–100% of examined *P. abbreviata* hosted at least one mature specimen of *M. arrokeana*. In some cases, more individuals (up to 91 specimens) were recorded within a single host; however, in these cases, all specimens were immature (Teso et al., 2006; Alfaya et al., 2013). Curiously, *M. arrokeana* has never been found in any of the other bivalve species that commonly inhabit northern Patagonian gulfs or in a free living state (Ivanov et al., 2002; Alfaya et al., 2013). The giant geoduck *P. abbreviata* is distributed in shallow waters down to depths of 75 m, from Rio de Janeiro (Brazil) to Nuevo Gulf (Argentina) (Scarabino, 1977; Signorelli and Alfaya, 2014). It inhabits sandy and muddy substrata down to a sediment depth of 40 cm. Morsan and Ciocco (2004) studied the population characteristics and growth of this species and estimated a lifespan of up to 86 years.

The type of symbiotic relationship existing between *M. arrokeana* and *P. abbreviata* is unclear. Vázquez et al. (2009) reported that *M. arrokeana* only produced external tissue damage on the mantle epithelium of *P. abbreviata*, due to the sucker attachment.

Stable-isotope analysis and stomach content have been widely used to characterize symbiotic relationships in several species of invertebrates and fishes (Doucet et al., 1999; Pinnegar et al., 2001; Parmentier and Das, 2004; Zabala et al., 2013; Navarro et al., 2014), providing information on assimilated food (not only ingested food), as well as time-integrated information (Layman et al., 2012). This approach is based on the assumption that stable-isotope ratios of nitrogen (N) and carbon (C) are transformed from dietary sources to consumers in a predictable manner (Layman et al., 2012). The $\delta^{15}\text{N}$ values are indicators of trophic positions, as consumers are predictably enriched in nitrogen isotopes relative to their food (Post, 2002). Carbon isotopes are less indicative of trophic positions, but are useful indicators of the dietary source of carbon (De Niro and Epstein, 1978). Stomach content provides detailed data about food most recently consumed, but may not be representative of the overall diet (Parmentier and Das, 2004). Therefore, the use of stable-isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) provides complementary data to the analysis of stomach content (Pinnegar et al., 2001; Zabala

et al., 2013). Furthermore, analyses of $\delta^{15}\text{N}$ values have successfully provided insight into host–endosymbiont trophic relationships (Deudero et al., 2000; O'Grady and Dearing, 2006). The dynamic equilibrium model proposed by Olive et al. (2003) predicts a ranking of magnitudes in trophic discrimination factors following the sequence: predator > ectoparasite > endoparasite. Predators typically have between 3.0 and 3.4‰ enrichment in ^{15}N relative to their food source (Post, 2002), while endoparasites show no difference with their host or are even depleted relative to the ingested food (Olive et al., 2003). Given that *M. arrokeana* inhabits the mantle cavity of the geoduck, it could be characterized as an ectoparasite. Therefore, one hypothesis is that *M. arrokeana* tissues would be enriched in ^{15}N relative to *P. abbreviata* tissues, indicating it feeds on its host ($\delta^{15}\text{N}_{\text{symbiont}} - \delta^{15}\text{N}_{\text{host}} = \Delta\delta^{15}\text{N}_{\text{s-h}} > 1\text{‰}$). However, if *M. arrokeana* feeds on the plankton from the host's mantle cavity (i.e., the same diet as the host), no difference in ^{15}N levels between host and symbiont would be expected, indicating a commensal relationship ($\Delta\delta^{15}\text{N}_{\text{s-h}} \sim 0\text{‰}$).

The aim of the present study was to determine the trophic relationship of *M. arrokeana* and *P. abbreviata* by studying the diets of both species using direct (stomach contents) and indirect (stable-isotope analysis of C and N) methods. These results, together with studies of the feeding behavior and morphology of feeding structures of *M. arrokeana*, address the question of whether *M. arrokeana* is a parasite or commensal nemertean.

MATERIALS AND METHODS

Sampling

Malacobdella arrokeana specimens were collected during the spring season (October 2010) from geoducks (*Panopea abbreviata*) inhabiting two northern Patagonian gulfs: San Matías (42°00'S, 65°04'W) and San José (42°20'S, 64°10'W). A total of 65 specimens of *Panopea abbreviata* were collected by SCUBA diving. Divers used an onboard water jet pump and a hand-held water jet with a nozzle at the end of the hose (hydro-jet) to help dislodge bivalves, one at a time, from the substratum (Morsan et al., 2010; Alfaya, 2015). Clams were then isolated and transported in plastic bags to the laboratory, where they were opened by severing the anterior

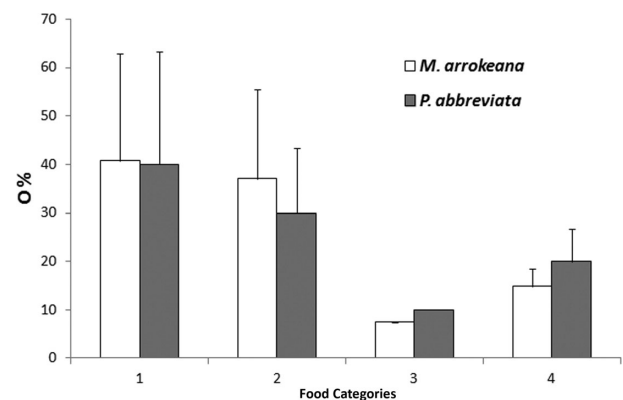


Fig. 1. Frequency of occurrence (O%) of the food categories in the stomachs of *M. arrokeana* and *P. abbreviata*. Data for the two sampling sites, San Matías and San José, were pooled. Food category: 1 diatoms; 2 other microalgae (unspecified); 3 small crustacean exoskeletons; 4 detritus and sediment.

and posterior adductor muscles. Living *M. arrokeana* found within the clams were removed using forceps and immediately fixed in 10% formalin (approximately four hours after clams were collected), then transferred into 70% ethanol.

Stomach contents

Stomach contents of 30 *P. abbreviata* and 30 *M. arrokeana* from each sampling site were analyzed. The clams and nemerteans were opened with a scalpel, and stomach contents were observed under a stereomicroscope. Five *M. arrokeana* proboscises were removed from living specimens, and the pH of the proboscis secretion was measured using pH indicator strips (Merck).

The stomach contents of each species were individually homogenized, preserved in ethanol (70%), and then dyed with Rose Bengal (10%) 24 hours before identification and quantification of the food items. All particles present in the stomach were classified into distinguishable categories. The material identified was photographed with a digital camera mounted on a magnifying glass or microscope, as needed. The frequency of occurrence (O%), defined as the percentage of individuals feeding on each prey category, was calculated for both species (Hyslop, 1980).

Morphology

To study the morphology of the eversible proboscis and pharynx of *M. arrokeana*, histological slides were prepared and images from a stereomicroscope and scanning electron microscopy (SEM) obtained. Specimens of *M. arrokeana* were fixed in 10% buffered formalin in seawater or in Bouin's fluid for standard histological studies. The specimens were embedded in Paraplast (McCormick Scientific, Inc.) following the Peterfi method (Pantin, 1968). Sections (5–6 μ m) were stained using Masson's trichrome method. For SEM of the proboscis and pharynx, samples were fixed in 2.5% glutaraldehyde for 4 h at 4°C, then transferred to 0.1-M phosphate buffer (pH 7.4). The samples were then dehydrated through a graded ethanol series into 100% ethanol, prior to incubation in hexamethyldisilazane (Fluka Instruments) for 5–10 min. The tissues were mounted and coated with gold in a Denton Vacuum Desk IV. Observations were made on a JEOLJSM-6460LV scanning electron microscope.

Feeding behavior

To study the feeding behavior of *M. arrokeana* while within the host, we endoscopically examined five *P. abbreviata* specimens that were kept alive in a recirculating 100-L aquarium (with water pump, UV light, and organic filters) filled with filtered seawater (45 μ m). The clams were buried in sand taken from the sampling location and acclimatized at 10–13°C for one week. No food was provided during this period. Before the examination, the bivalves were anesthetized with a MgCl₂ solution (7.5% in sea water) and crystal menthol for

3–4 h. When the adductor muscles relaxed, an incision (0.5 cm) was made in the anterior opening of the bivalve and a flexible endoscope (9 mm wide, W.I.C. Explorer, Mod. 8849AU) was introduced

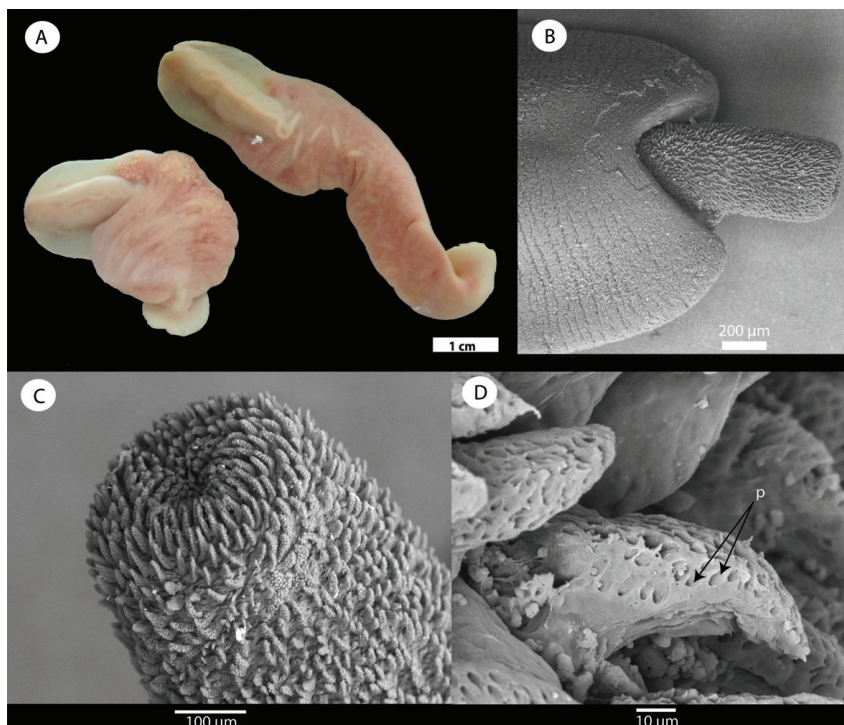


Fig. 2. *Malacobdella arrokeana*. (A) Whole living specimens. (B) Partially everted proboscis, SEM. (C) Distribution of papillae on the proboscis, SEM. (D) Magnification of papillae, showing porous surface.

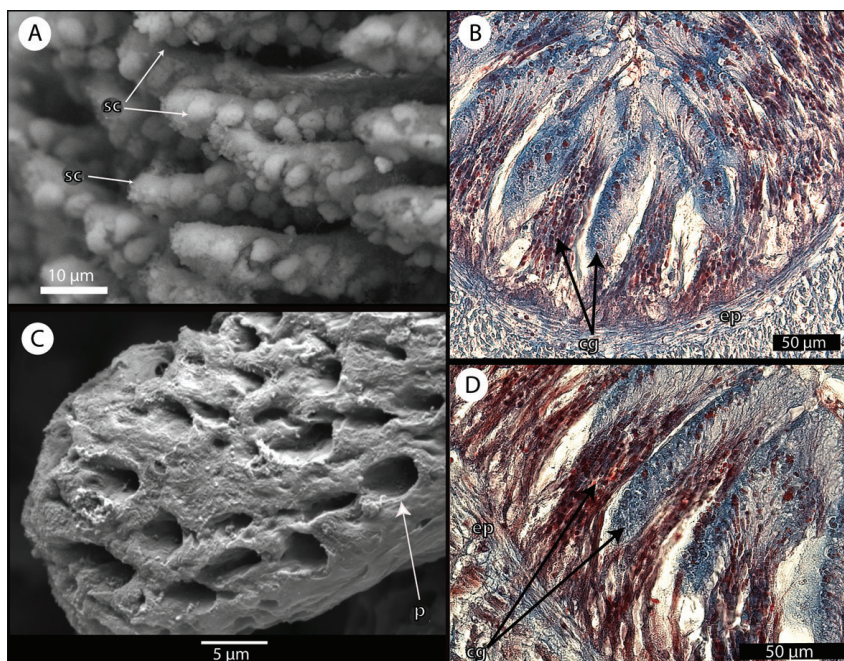


Fig. 3. Proboscis papillae in *Malacobdella arrokeana*; A, C, SEM images; B, D, histological sections (Masson's trichrome stain). (A) Distribution of papillae, showing the surfaces covered with secretions. (B) Glandular cells distributed in the papillae. (C) Details of the surface of the papillae covered with pores. (D) Details of glandular cells. Abbreviations: cg, glandular cell; ep, epithelium; p, pore; sc, secretion.

into the mantle cavity to capture video images of the nemerteans living within the host.

Stable-isotope analysis

Twenty *P. abbreviata* with their respective *M. arrokeana* ($n = 20$) were randomly chosen for each locality (San Matías and San José) from the pool of specimens used for stomach content analyses. A small piece (1 cm^3) of muscle tissue was dissected from each specimen (siphon tissue for bivalves and sucker tissue for nemerteans). Tissue fragments were individually stored in bags at -20°C prior to processing. Samples were cleaned with distilled water and weighed, before drying in an oven at 60°C for 48 h until the weight stabilized. Samples were then ground using a hand mortar.

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable-isotope ratios were determined by mass spectrometry (see Lajtha and Michener 1994 for details) at the Stable Isotope Facility at the Universidad Autónoma de Madrid (Spain). Carbon and nitrogen stable-isotopic ratios were expressed in δ notation according to the following equation:

$$\delta X(\text{‰}) = 1000 [(R_{\text{sam}}/R_{\text{std}}) - 1],$$

where X is ^{13}C or ^{15}N and R_{sam} and R_{std} are the $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$ ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N_2 for nitrogen (Peterson and Fry, 1987). The units are expressed as parts per thousand (‰). Given that lipids have more negative $\delta^{13}\text{C}$ values relative to other major biochemical compounds in animal tissues (Post et al., 2007), $\delta^{13}\text{C}$ values were lipid-normalized following the general mathematical correction for aquatic animals (Post et al., 2007):

$$\delta^{13}\text{C}_n = \delta^{13}\text{C} - 3.32 + 0.99 \times \text{C/N},$$

where $\delta^{13}\text{C}$ is the value determined empirically by mass spectrometry, and C/N is the carbon to nitrogen ratio of the sample.

Data analyses

The frequency of occurrence (O%), and its associated uncertainty were calculated for the entire dataset for each species. Values of 95% CI were generated by nonparametric bootstrap simulations (Tirasin and Jorgensen, 1999). Calculations were done with the R software ver. 2.15.3 (R Development Core Team) using the percentile method implemented in the package Boot (Ripley, 2005), performing 9999 bootstrap replicates.

To analyze stable-isotope data, intraspecific comparisons were first performed to test for possible differences between sampling sites. If the data showed non-significant differences, the data from both gulfs were pooled. Comparisons were done using the nonparametric Wilcoxon rank sum test (Crawley, 2007).

Trophic comparisons between *M. arrokeana* and *P. abbreviata* were made by estimating isotopic niches using multivariate ellipse-based metrics (Jackson et al., 2011). The analysis generates standard ellipse areas (SEA), which are bivariate equivalents to standard deviations in univariate analyses. We used the standard ellipse areas corrected for

small sample size (SEAc) to calculate niche overlap and generate Bayesian estimates of SEA (SEA_B) to test for differences in trophic niches by comparing 95% credible limits (e.g., Jackson et al., 2011; Zabala et al., 2013). Mollusk trophic discrimination of carbon isotopes is higher than the commonly used value of 1 for aquatic animals (e.g., $\Delta^{13}\text{C} = 2$ in Dubois et al., 2007; $\Delta^{13}\text{C} = 2.6$ in McKnight, 2009) and interspecific differences in trophic discrimination could lead to erroneous conclusions. Therefore, we subtracted 1‰ from *P. abbreviata* $\delta^{13}\text{C}$ values to standardize the values. Wilcoxon rank sum tests (W) were used to test for differences in isotopic composition ($\Delta\delta X_{s-h} \neq 0\text{‰}$) between species. Calculations were done with the R software V.2.15.3 (R Development Core Team) and the package SIAR (Jackson et al., 2011).

RESULTS

Stomach contents

Stomach contents from both species were composed mostly of food items commonly found in filter/benthic suspension feeders. Four different food categories could be distinguished: 1) diatoms, 2) other microalgae (unspecified), 3)

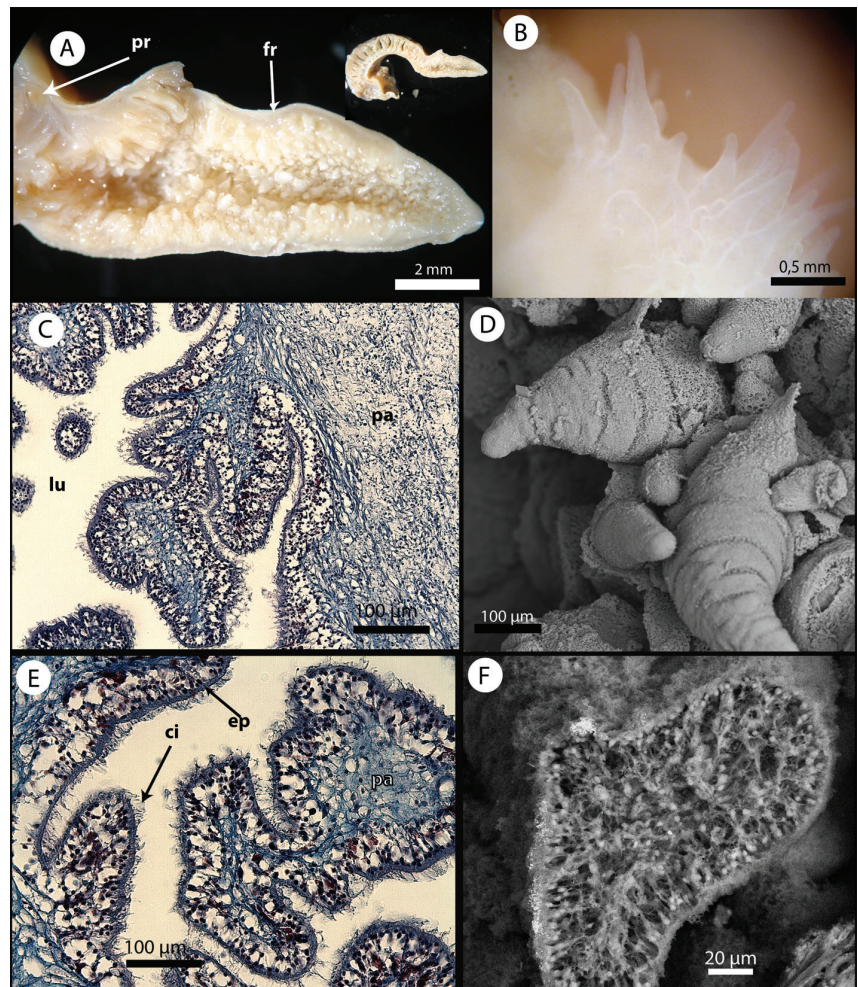


Fig. 4. Pharyngeal morphology of *Malacobdella arrokeana*. (A) Sagittal section of a fixed specimen showing the pharyngeal cavity. (B) Distribution of pharyngeal papillae in a fresh specimen. (C) Histological section (Masson's trichrome) of ciliated papillae, supported by parenchyma (i.e., extracellular matrix). (D) general shape of pharyngeal papillae, SEM. (E) Histological section (Masson's trichrome) of ciliated papillae, showing ciliated epidermis. (F) Cross section of pharynx papillae, SEM. Abbreviations: ci, cilium; ep, epithelium; fr, pharynx; lu, pharyngeal lumen; pa, parenchyma; pr, proboscis.

small crustacean exoskeletons, and 4) detritus and sediment. Food items consumed by each species were similar (Fig. 1). For *P. abbreviata*, the contents consisted of 40% diatoms, 30% other unspecified microalgae, 10% small crustacean exoskeletons, and 20% debris and sand. The contents for *M. arrokeana* consisted of 40% diatoms, 38% other microalgae, 7% small crustacean exoskeletons, and 15% debris and sand.

Morphology

Based on SEM images, the proboscis of *M. arrokeana* is covered with pyramid-shaped papillae that have a porous surface (Figs. 2D, 3A, C). Papillae were distributed in a brush-like pattern along the proboscis length (Fig. 3A). Histological sections showed that the proboscis papillae were composed of glandular cells anchored basally to the epithelium and the cytoplasm projected apically (Fig. 3B, D). The size of the papillae, varying between 20–100 μm in length, increased towards the distal end of the proboscis. Proboscis-cell secretions had a neutral pH ($\text{pH} = 7$), indicating that acidic compounds were not present to aid in the pre-digestion of prey.

The pharynx was well developed, cylindrical in shape and occupying 25% of the total length of the animal. Its length varied between 1 and 7 mm and width between 0.4 and 1.5 mm ($n = 15$) (Fig. 4A). The pharyngeal lumen was covered internally with conical ciliated papillae (Fig. 4B–F). These papillae are parenchymal projections lined externally with a layer of ciliated gland cells (Fig. 4C, E, F), no pores on the surface. The distribution of pharyngeal papillae was uniform, and in living animals, it was observed that papillae were able to move in all directions (Fig. 4B). The size of pharyngeal papillae varied depending on the position of the papillae; anterior ones near the mouth were longer, whereas ones situated on the back near the esophagus were smaller.

Feeding behavior

Endoscopic exploration of *P. abbreviata* showed that adult *M. arrokeana* were usually attached by the terminal sucker to the clam's mantle tissue, behind the gill septum near the gill filaments (Fig. 5A, B, C). *Malacobdella arrokeana* was observed interacting with the gills, examining the surface filaments with its mouth (Fig. 5D, E). We did not observe *M. arrokeana* using its proboscis to feed.

Stable-isotope analysis

The stable-isotope ratios are summarized in Table 1. As no intraspecific differences were found in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between sampling locations, data were pooled (for *P. abbreviata*: $W = 36$, $P = 0.31$ and $W = 49$, $P = 0.97$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively; and for *M. arrokeana*: $W = 22$, $P = 0.77$ and $W = 28$, $P = 0.77$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively).

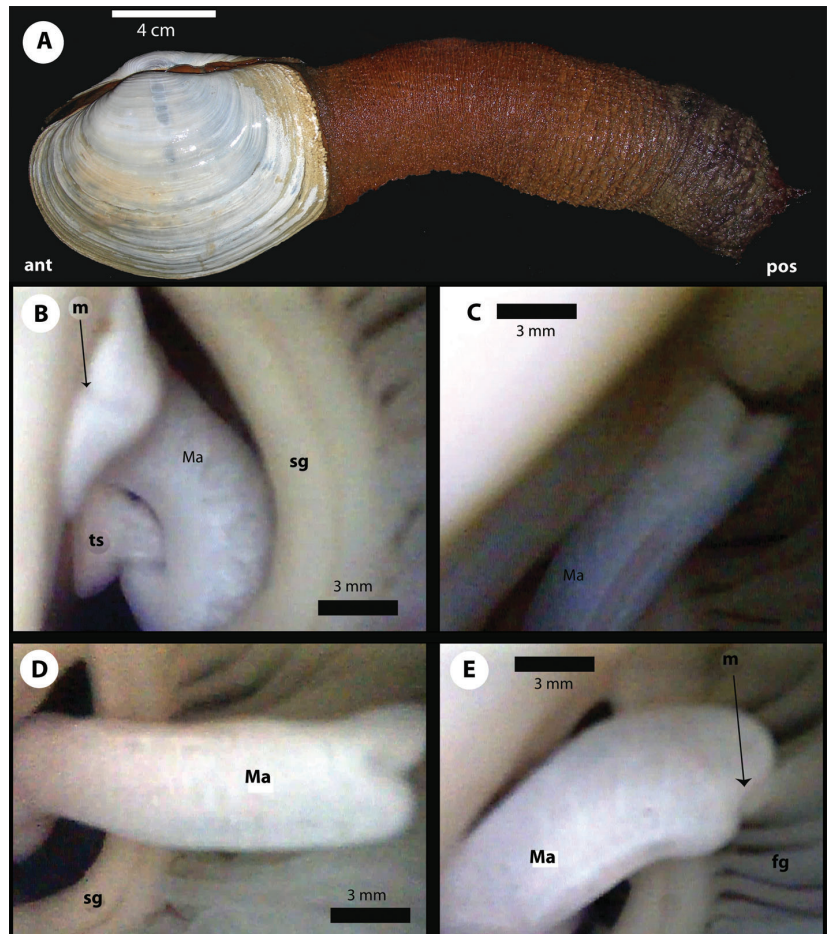


Fig. 5. (A) Fresh specimen of the host bivalve *Panopea abbreviata*, used in endoscopic exploration for *Malacobdella arrokeana*. (B) *Malacobdella arrokeana* attached to the clams mantle tissue, close to the gill septum, endoscopic image. (C–E) Sequences of endoscopic images showing interaction of *M. arrokeana* with bivalve gill filaments. Abbreviations: **ant**, anterior; **fg**, gill filament; **g**, gill septum; **m**, mouth; **Ma** *M. arrokeana*; **pos**, posterior; **ts**, terminal sucker.

Table 1. Mean and standard deviation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopic values and differences in values ($\Delta\delta\text{X}$) between the host *P. abbreviata* (h) and the nemertean *M. arrokeana* (s).

Species	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
San Matías			
<i>P. abbreviata</i>	20	13.815 ± 0.645	-17.315 ± 0.365
<i>M. arrokeana</i>	10	13.485 ± 0.563	-17.297 ± 0.783
$\Delta\delta\text{X}_{s-h}$		-0.330 ± 0.856	0.018 ± 0.863
San José			
<i>P. abbreviata</i>	20	13.797 ± 0.606	-17.600 ± 0.645
<i>M. arrokeana</i>	20	13.565 ± 0.425	-17.209 ± 0.527
$\Delta\delta\text{X}_{s-h}$		-0.232 ± 0.740	0.391 ± 0.832
Total			
<i>P. abbreviata</i>	40	13.806 ± 0.493	-17.454 ± 0.535
<i>M. arrokeana</i>	30	13.539 ± 0.456	-17.238 ± 0.596
$\Delta\delta\text{X}_{s-h}$		-0.267 ± 0.671	0.216 ± 0.800

Panopea abbreviata and *M. arrokeana* showed similar trophic niches. The results did not show differences in the spread of the isotopic niches SEA_B ($P = 0.01$) and showed

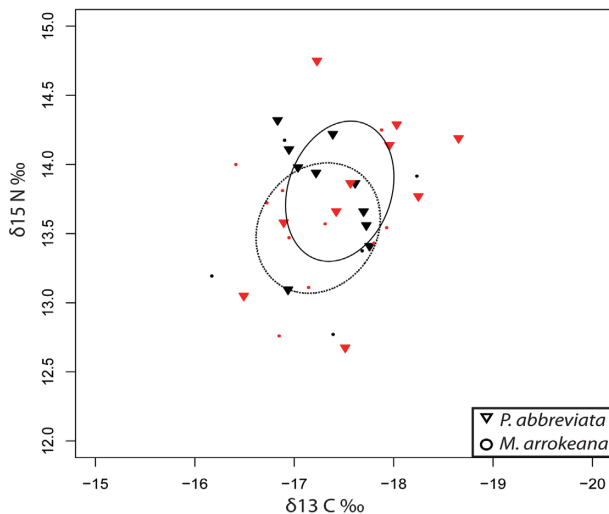


Fig. 6. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *P. abbreviata* and *M. arrokeana* and Bayesian estimates of standard ellipse areas (SEA_B). The dotted line represents the ellipse generated with *M. arrokeana* values and the line represents the ellipse generated with *P. abbreviata* values. Points and triangles represent data for *M. arrokeana* and *P. abbreviata*, respectively; filled and empty symbols represent data from the gulfs of San Matías and San José, respectively.

high overlap (Fig. 6, SEA_B overlapped 63%). Consistent with these findings, there were no differences between species in $\delta^{15}\text{N}$ ($W = 199$, $P = 0.11$, Table 1) or $\delta^{13}\text{C}$ ($W = 118$; $P = 0.30$) values, confirming a commensal relationship. The $\Delta\delta^{15}\text{N}_{s-h}$ values were similar for the two sampling sites (i.e., for San Matías and San José gulfs).

DISCUSSION

Our results using direct (stomach-content observation) and indirect (stable-isotope analysis) approaches revealed that both *M. arrokeana* and *P. abbreviata* have an omnivorous filter feeding diet. *Malacobdella arrokeana* feeds mainly on diatoms and other microalgae, and in a lower proportion, on small crustaceans. *Panopea abbreviata* also mainly consumes diatoms and, in a lower proportion than *M. arrokeana*, other microalgae and small crustaceans. These species showed a similar range of isotopic values, denoting an overlap in diets.

The similarity observed between the $\delta^{15}\text{N}$ values of both species ($\Delta\delta^{15}\text{N}_{s-h} \sim 0$) supports the predictions of the dynamic model proposed to describe trophic-step fractionation (Olive et al., 2003). Under this model's assumptions, if *M. arrokeana* feeds on *P. abbreviata* tissue, the $\Delta\delta^{15}\text{N}_{s-h}$ values would be > 1 , whereas if both species feed on similar items, the $\Delta\delta^{15}\text{N}_{s-h}$ values would be ~ 0 . The high overlap in isotopic niches ($> 60\%$) also confirms that these species share atrophic niche (Jackson et al., 2011). Therefore, as demonstrated for other systems (Deudero et al., 2002; O'Grady and Dearing, 2006; Navarro et al., 2014), stable-isotope data has clarified the host–endosymbiont trophic relationship for *M. arrokeana* and *P. abbreviata*.

The distribution of papillae on the proboscis of *M. arrokeana* provides a large surface area for contact, with secretions from the numerous basal glandular cells in the underlying epithelium likely exiting through the surface

pores. These secretions (mucus) may help to increase the grip of the proboscis on prey. The presence of toxins has been reported in *M. grossa* (Kem, 1985) and may also be present in *M. arrokeana* (Kem, pers. comm.). However, detailed studies of this secretion's properties are needed to confirm this hypothesis. The presence of glandular cells and mucus in the proboscis has also been recorded in *M. grossa* by Gibson and Jennings (1969) and later by Magarlamov and Chernyshev (2010). These authors observed two types of glandular cells, whereas four types of secretory cells have been distinguished in the proboscis of another hoplonemertean (*Paranemertes peregrina*; Stricker and Cloney, 1983). The glandular cells observed in *M. arrokeana* are similar to those observed in *M. grossa* by Magarlamov and Chernyshev (2010), and may be an autapomorphy of the genus *Malacobdella*. Nevertheless, more studies in other species of *Malacobdella* are needed to confirm this hypothesis. The structure of the pharynx and its papillae (ciliated and long in size) likely increases the efficiency of retaining small particles entering the pharyngeal cavity. Water current created by the cilia lining the papillae may be transporting particles into the esophagus. While investigating the feeding behavior of *M. grossa* outside of its host, Gibson and Jennings (1969) observed that this species used its proboscis to catch larger food particles, such as small crustaceans, but not smaller particles. Instead, *M. grossa* used serial muscle contractions of its large pharynx to move small particles into the esophagus. This behavior was also observed in *M. arrokeana* when it was outside of its host, although, in this case, the specimens were not relaxed prior to the observation (Alfaya, 2015).

Unfortunately, we did not observe *M. arrokeana* using its proboscis to feed inside the host. However, the exploration was made in an anesthetized environment (thus also likely relaxing the nemertean) and the flexible endoscopic camera has a built-in light (*M. arrokeana* lives in a dark environment), both of which may have disrupted the normal behavior of the worm. The endoscopic explorations did, however, confirm the position of living *M. arrokeana* within the mantle cavity and document, for the first time, its interaction with the bivalve's gill filaments. Two main feeding strategies are characteristic of representatives of the phylum Nemertea: suctorial and macrophagous feedings (McDermott and Roe, 1985). Both feeding strategies are known to exist in enoplus nemerteans (McDermott and Roe, 1985), particularly in carnivorous ones that feed mostly on polychaetes and small crustaceans (amphipods and copepods, among others). *Malacobdella arrokeana*, like *M. grossa* (Gibson and Jennings, 1969), has a macrophagous feeding strategy, as revealed by the presence of crustacean exoskeletons in the stomach. The genus *Malacobdella* belongs to the Hoplonemertea, but members of this genus lack the stylet apparatus, likely a secondary loss correlated with symbiosis. *Malacobdella* species possess a large pharynx covered with ciliated papillae, which may be an adaptation for filter feeding.

The position of *M. arrokeana* inside the host, the interaction with the gills, and a proboscis covered with sticky papillae suggests that *M. arrokeana* uses its proboscis to capture large food particles from the gills and incurrent mantle cavity water of *P. abbreviata*. *Panopea abbreviata* pres-

ents gill type C (1) sensu Atkins (1937), characterized by outer and inner demibranches with a food groove present in the inner demibranch (Signorelli and Alfaya, 2014). The presence of *M. arrokeana* within *P. abbreviata* does not seem to be detrimental to the host population: nearly 100% of the hosts sampled have at least one nemertean inside, yet its population structure is stable and growing in northern Patagonian gulfs (Morsan et al., 2010). It is likely, however, that *P. abbreviata* has to expend more energy to obtain enough food to meet its metabolic needs.

As stated before, the feeding behavior of *M. arrokeana* does not seem to have parasitic characteristics. The proboscis structure revealed that *M. arrokeana* is unable to damage its host as the soft tissues of the proboscis and the neutral pH (pH = 7) of its secretions suggest that no pre-digestion of prey occurs.

Overall, our results using direct and indirect approaches reveal that *M. arrokeana* is commensal, living in the mantle cavity of *P. abbreviata* and consuming similar prey items as its host.

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