

ORIGINAL ARTICLE

Invasive polychaete *Boccardia proboscidea* Hartman, 1940 (Polychaeta: Spionidae) in sewage-impacted areas of the SW Atlantic coasts: morphological and reproductive patterns

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Keywords

Boccardia proboscidea; morphology; poecilogony; strategy reproductive; SW Atlantic.

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Abstract

Boccardia proboscidea is an alien polychaete species that has been introduced to the Southwestern Atlantic Ocean. This polychaete species has invaded intertidal benthic rocky shores, and builds reefs formed by thousands of individuals per square meter with densities up to 1,500,000 ind. \cdot m⁻² in organically impacted sites. This phenomenon was first recorded in Argentina in 2008. This is the first comprehensive study on the morphology, habitat and reproductive biology of *B. proboscidea* associated with the sewage-induced abrasion platforms on the Argentinean coasts. Adult morphology of *B. proboscidea* from Mar del Plata is described and a comparison with material from other locations is made. Results showed that *B. proboscidea* from Mar del Plata, Argentina share the same diagnostic features as those of the individuals from the original description (California). Moreover, the results also indicated that the reproduction of this species involves a poecilogonous development (production of more than one type of offspring with both kinds of larvae) and implicates adelphophagy (nurse egg ingestion). Patterns of larval development varied both within a single brood and among broods of different females. Continuous reproduction of *B. proboscidea* coupled with a continuous supply of organic matter could explain the possible mechanism that led to the development of reefs.

Introduction

The genus *Boccardia* Carazzi 1893, includes 26 species worldwide (Day 1967; Blake & Woodwick 1971; Rainer 1973; Read 1975; Blake & Kudenov 1978, 1981; Light 1978; Blake 1979, 1981, 1983, 1986; Hutchings & Turvey 1984; Guérin 1990; Martínez *et al.* 2006; Simon *et al.* 2010; Read *et al.* 2013), four of which have been found in the Atlantic Ocean: *Boccardia polybranchia* Haswell 1885 (Blake 1983); *Boccardia natrix* Söderström 1920 (Blake 1983); *Boccardia proboscidea* (Hartman 1940) (Martínez *et al.* 2006; Diez *et al.* 2011; Jaubet *et al.* 2011) and *Boccardia chilensis* Blake & Woodwick 1975; (Read 1975).

Boccardia proboscidea has been recorded with a wide geographical distribution along the Pacific Ocean and to a lesser extent in the Atlantic Ocean. This annelid was originally described from the west coast of North American (California) (Hartman 1940) ranging from British Columbia to Baja California (Berkeley & Berkeley 1950; Hartman & Reish 1950; Imajima & Hartman 1964). Distribution of *B. proboscidea* now includes Japan (Sato-Okoshi 2000), South Australia (Blake & Kudenov 1978; Petch 1995; Leonart 2001; Hewitt *et al.* 2004), South Africa (Robinson *et al.* 2005; Simon *et al.* 2010), Hawaii (Bailey-Brock 2000), New Zealand (Read 2004) and Europe (Martínez *et al.* 2006). In all these sites, it is

considered an introduced species (Kamel *et al.* 2010; Çinar 2013).

Boccardia proboscidea is an opportunistic species in intertidal benthic communities (Johnson 1970) and is considered an indicator of organic (sewage) pollution in Southeast Australia (Petch 1989). *Boccardia proboscidea* bores into calcareous substrates (Sato-Okoshi & Okoshi 1997; Simon *et al.* 2010) as well as forming burrows in a wide variety of substrates, including rock, sand and mud (Petch 1995). It has been reported in both soft bottom and hard substrates: *Zostera* meadows (Mediterranean and North Atlantic), coralline algae (*Corallina elongata*) (Iberian Peninsula) and *Mytilus* banks (Martínez *et al.* 2006). It has also been found boring into, or associated with, the gastropod mollusk *Haliotis midae*, oyster cultures in South Africa (Simon *et al.* 2006, 2010; Simon & Booth 2007); inhabiting mud deposits in shell crevices of living oysters (*Crassostrea gigas*) (Sato-Okoshi 2000); on shells of native gastropods (*Haliotis roei*) in Southwestern Australian waters (Sato-Okoshi *et al.* 2008) and boring into shells of bivalves (*Ostrea edulis*) in Hawaii (Bailey-Brock 2000). Therefore, *B. proboscidea* is considered a pest in different countries of the world for its potential to inflict damage to wild native and cultured mollusks.

The first occurrence of *B. proboscidea* along the Atlantic coast of South America (Argentina) was recorded in November 2008 in the form of large biogenic reefs in intertidal sewage-impacted areas (Jaubet *et al.* 2011). These biogenic structures were associated with the abrasion platforms located close to the output of the wastewater urban effluent of the Mar del Plata city. The reefs were formed by thousands of individuals (on the order of 1,465,000 ind. m⁻²) (Garaffo *et al.* 2012) arranged next to each other vertically. Interestingly, *B. proboscidea* is a non-reef-forming species that was introduced in the Argentine Sea and has invaded a new habitat-building reefs (Jaubet *et al.* 2011).

Growth and reproductive strategies of *B. proboscidea* were described for specimens from California (USA), Australia and South Africa. Reproductive strategy of this species involves poecilogony. Poecilogonous development consists in a type of embryonic development based on the production of more than one type of offspring within a single species (Giard 1905). Females of *B. proboscidea* spawn capsules with eggs within their tubes. Fertilized eggs develop to the larval phase, while eggs that were not fertilized function as nurse eggs. Larval development includes the release of two kinds of larvae: planktotrophic larvae (small larvae that feed on phytoplankton throughout a swimming planktonic phase) and an adelphophagic larvae (offspring that ingest nurse eggs and hatch at an advanced stage) (Gibson 1997). Nurse egg production and larval trophic mode are determining factors in larval

development mode (Gibson 1997; Gibson & Gibson 2004; Oyarzun *et al.* 2011).

Larval development patterns of *B. proboscidea* are also highly variable (Gibson 1997). Several authors have described the larval development mode of *B. proboscidea* from different population and have found females that produce planktotrophic larvae only (Hartman 1941; King 1976; Petch 1989), adelphophagic broods with the release of advanced larvae stage (Woodwick 1977) or mixed broods containing both planktotrophic and adelphophagic offspring (Hartman 1941; Blake & Kudenov 1981; Petch 1989; Gibson 1997; Simon & Booth 2007). Patterns of larval development may vary both within a single brood and among broods of different females (Gibson 1997).

The goals of this work were to describe the morphology of adults of *B. proboscidea* associated to sewage-induced abrasion platforms from Mar del Plata (Argentina) and to compare this species with specimens described from other invaded locations. The study also aims to analyse the habitat and the reproductive variables (number of capsules per brood, eggs and larvae per capsule) in an annual period and describe the larval development patterns (trophic mode and dispersion habitat) of *B. proboscidea* from Mar del Plata, based on the brood characteristics.

Materials and Methods

Study area and data collection

Boccardia proboscidea were collected from intertidal abrasion platforms located in areas adjacent to the sewage discharge of the Mar del Plata city, Argentina. The collection site is situated at 1000–1200 m south of the effluent discharge and is impacted organically (Jaubet *et al.* 2013). The site is almost entirely covered by *B. proboscidea* reefs (Fig. 1). Sample collection was carried out seasonally from June 2008 (winter) to April 2009 (autumn) and monthly during the austral summer seasons of 2008–2009 (December, January and February).

According to previous ecological studies (Sánchez *et al.* 2009), benthic samples were taken randomly on independent intertidal rocks with a corer (16 cm², 20 cm height). The corer was buried into the reef until it contacted the loess substrate (stony rock). Samples were collected using a steel spatula placed between the substrate and the corer and were fixed in a 10% formaldehyde solution with seawater.

Adult morphology

Material was examined with optical equipment (microscope and stereomicroscope) and also by scanning electron



Fig. 1. Photo showing the study area (located 1000–1200 m south of the sewage discharge of Mar del Plata city) covered completely by *Boccardia proboscidea*.

microscope (SEM) (JEOL, JSM–6460 LV). The material for SEM was prepared after fixation for 24 h with glutaraldehyde 3% in buffer of sodium cacodylate 0.1 mol with a pH between 7.2 and 7.4, followed by dehydration in alcohol (50, 70, 80, 90, 95 and 100%). Samples were dried in HMDS (hexamethyldisilazane), mounted on aluminium discs and coated with Au–Pd.

Reference material was deposited in the Invertebrate Collection of the *Museo de Ciencias Naturales de La Plata* (MLP, Argentina).

Habitat

Samples of abrasion platforms located in the organically impacted site were collected to determine the habitat of *B. proboscidea*. Different independent rocks with developed reefs were selected randomly and sampled. The samples were collected using a corer and stainless steel spatulas.

Reproductive variables and larval development pattern

To assess the reproductive biology of *B. proboscidea*, 20 intertidal benthic samples (replicates) were taken at random each month. In the laboratory, samples were sieved through a 0.5-mm mesh and the polychaetes retained with their tubes were removed and placed in Petri dishes. Tubes were opened carefully and egg capsules were removed from the maternal tube and placed in Petri dishes (all egg capsules found in one tube were placed on one Petri dish).

The reproductive period of *B. proboscidea* was analysed by quantifying the number of egg capsules per brood by

season. The number of eggs and larvae per capsules were also quantified for season. Data obtained in each replicate were averaged.

Broods were classified into three reproductive types, according to the presence of the kind of larvae per capsule (planktotrophic or adelphophagic). Thus, broods were assigned to each reproductive type following the studies of Blake & Kudenov (1981) and Gibson (1997). These authors defined three reproductive patterns: broods with capsules containing only planktotrophic larvae (Type I); broods with capsules containing only adelphophagic larvae (Type II); and broods with eggs and both kinds of larvae – planktotrophic and adelphophagic (Type III). The frequency of each reproductive type (I, II, III) and the dominance of each kind of larva (planktonic or benthic) were quantified for each month, according to the brood characteristics, the size and the larval morphology.

Data analysis

Differences in reproductive variables (number of capsules per brood, eggs and larvae per capsule) were tested against seasons with a one-way ANOVA test. Homogeneity of variances and normality were assessed using Cochran and Shapiro–Wilks tests, respectively. A Scheffé test was performed for post-hoc comparisons (Zar 1999).

A contingency table with two classification variables (reproductive type and month) was constructed to assess which reproductive pattern was more frequent. In addition, another frequency table with two classification variables (dispersion habitat and month) was constructed to evaluate the dominance of each kind of larva (planktonic or benthic) for each month. In both cases, differences were tested by a chi-squared test (Zar 1999).

Samples collected in November were not used in the analysis of frequency (of reproductive types and kind of larva) due to logistical constraints.

Results

SYSTEMATIC

Family Spionidae Grube 1850

Genus *Boccardia* Carazzi 1893

Species *Boccardia proboscidea* Hartman 1940

Adult morphology of specimens from Mar del Plata, Argentina

Largest complete specimen 29 mm long, 1.10 mm wide at chaetigers 5, with 103 chaetigers. Black pigment along edges of prostomium and a dark line along feeding groove of palps and along margins of prostomium and caruncle.

Prostomium rounded on anterior margin, with two to three pairs of eyes in trapezoidal arrangement, caruncle extending to posterior margin of chaetigers 3 (Fig. 2a), with nuchal organs consisting of paired ciliary bands along caruncle (Fig. 2b). First chaetiger reduced but having notopodial lobe with short notochaetae. Anterior chaetigers (2, 3, 4 and 6) with notochaetae with short tapering ends and some needle-like chaetae. Neurochaetae in these chaetigers are short and numerous with tapered ends (Fig. 2c and d).

Fifth chaetiger with reduced parapodial lobes, having two types of modified spines arranged in two rows: dorsal

row with two heavy curved spines (falcate) and ventral row with three blunt, bristle-topped spines. Only a fascicle of short ventral inferior chaetae present in chaetigers 5 (Fig. 2e).

From chaetigers 7 to posterior region of body, neuropodia with six to seven bidentate hooded hooks and notopodia with some needle-like notochaetae (Fig. 2f; Fig. 3a and b). Branchiae filiform, emerging from notopodial lobes of chaetigers 2–4 to near posterior end of body (except for chaetigers 5) (Fig. 2a and g). Branchiae longer on chaetigers 7–9 and decreasing in length towards end of body (Fig. 2g).

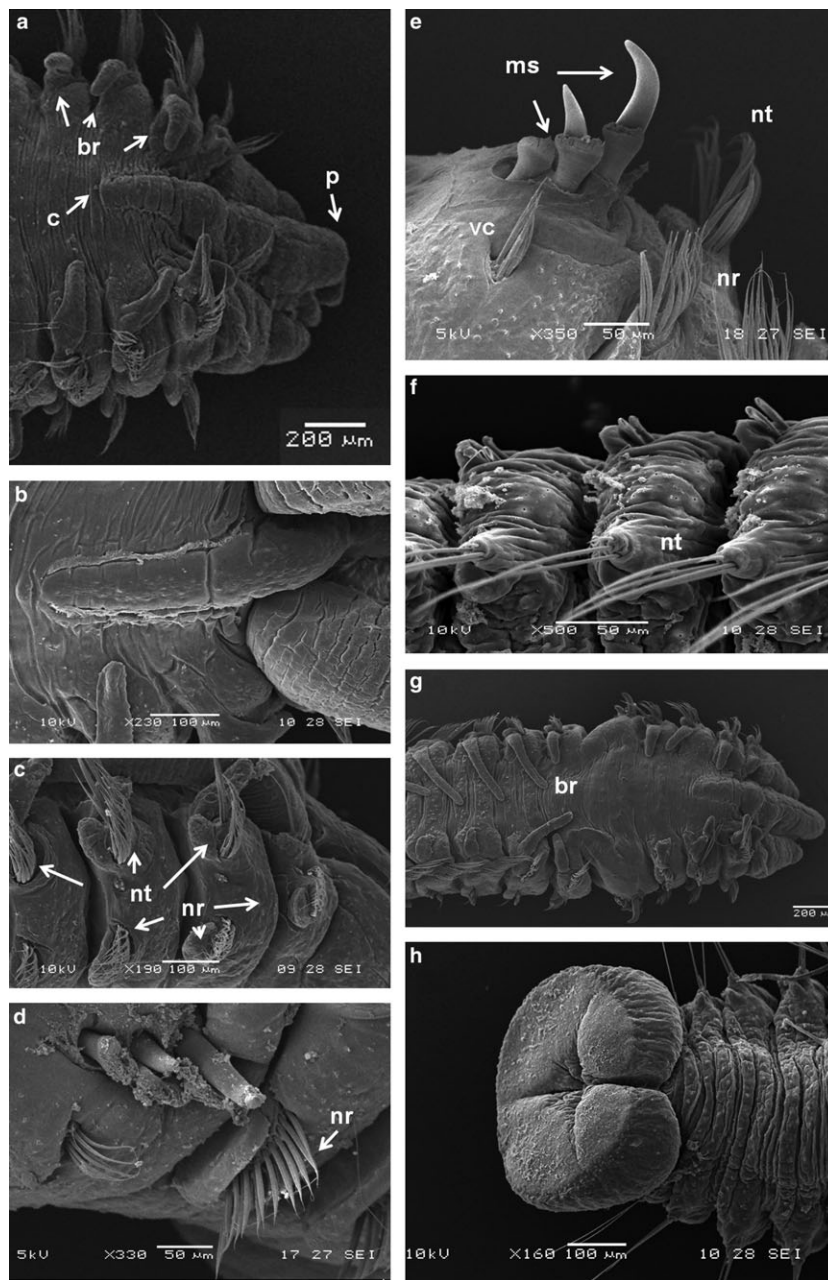


Fig. 2. Photos of *Boccardia proboscidea* obtained by scanning electronic microscope (SEM): (a) Dorsal anterior end showing prostomium (p) rounded on anterior margin, caruncle (c) length and branchiae (br) on chaetigers 2,3,4; (b) ciliated bands of nuchal organs (no) on both sides of the caruncle; (c) dorso-lateral view of the anterior end; showing notochaetae (nt) and neurochaetae (nr) of the parapodium; (d) lateral view of the chaetigers 4 and 5; showing neurochaetae (nr) of the parapodium; (e) modified spines (ms) and ventral inferior capillary chaetae (vc) on chaetiger 5; (f) lateral view of chaetigers 7, 8 y 9; showing notopodia with some needle-like notochaetae (nt); (g) dorsal view of the anterior end showing caruncle length and branchiae; (h) dorsal view of the pygidium with the four well-developed lobes.

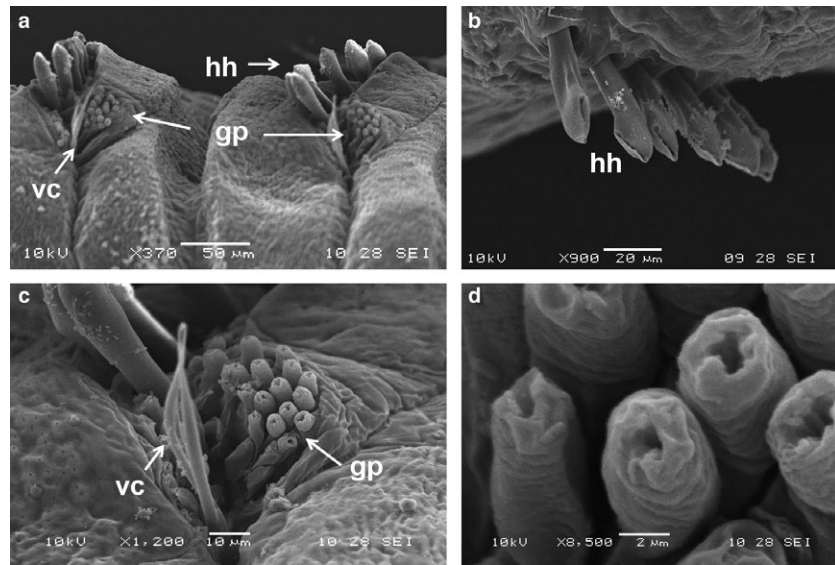


Fig. 3. Photos of *Boccardia proboscidea* obtained by scanning electronic microscope (SEM): (a) hooded hooks (hh) with external openings of glandular pouches (gp) and ventral inferior chaetae (vc) on chaetiger 7 and 8; (b) detail of the hooded hooks (hh); (c) openings of the glandular pouches (gp) visible at base of hooded hooks; (d) detail of the external opening of the glandular pouches.

Chaetigers 7, 8 and 9 with small glandular pouches (Fig. 3a and c). Openings of these glandular pouches visible at base of hooded hooks (Fig. 3d).

Pygidium with a fleshy disk having a dorsal groove and four lobes; the dorsal lobes pair often are smaller than the ventral ones (Fig. 2h).

Habitat

On the intertidal abrasion platform of Mar del Plata, Argentina, *B. proboscidea* was found on both horizontal (platforms) and vertical surfaces (lateral sides of rocks). Its habitat is both endolithic, excavating galleries in the abrasion platform, as well as epilithic or epifaunal building tubes bonded with sediment particles from the environment. Thus, on the coasts of the SW Atlantic Ocean, this species is both a borer and a tube-dwelling polychaete (Fig. 4). In organically impacted sites, *B. proboscidea* builds massive reefs of polychaete tubes over an abrasion platform. However, this species may also form large patches or small reefs in non-impacted sites. It was also found as companion fauna in the benthic community dominated by *Brachidontes rodriguezii*, an ecosystem engineer. It is also common to find their tubes trapped between the valves of local mussels.

Reproduction

Boccardia proboscidea reproduces throughout the year. Broods consist of eggs capsules contained within the maternal tube. Egg capsules contain fertilized eggs, nurse eggs and larvae.

Egg capsules of *B. proboscidea* are transparent and oval and are deposited in a row individually within maternal

tubes. Each capsule is attached to the wall of the tube by a transparent double stalk. The mean capsule height was 1.3 mm and the width was 1 mm. Up to 25 capsules were observed in a single tube, but 2–10 capsules were most commonly observed (annual average 5.5 ± 3.07). Eggs contained in the capsules are spherical and white in colour (Fig. 5a). The mean diameter of the eggs was between 0.12 and 0.14 mm and up to 176 eggs may occur in a single capsule (annual average 65 ± 21.83). The maximum number of larvae developing inside a capsule was 29, but the range commonly observed was three to eight larvae per capsule (annual average 2.08 larvae per capsule, $n = 930 \pm 2.75$).

Egg capsules, eggs and larvae were observed during the entire sampling period. However, the number of capsules, eggs and larvae showed significant differences among seasons. The number of capsules was higher during spring and summer ($F_{3,60} 8.871$; $P = 0.0000$) (Fig. 6a) as was egg production per capsule ($F_{3,60} 5.5005$; $P = 0.0020$) (Fig. 6b). The number of larvae per capsule also reached high values during the same seasons (spring and summer) ($F_{3,60} 3.86158$; $P = 0.0135$) (Fig. 6c). Post-hoc analysis showed that the spring and summer seasons differed significantly from the winter and autumn seasons ($P < 0.05$) for the three variables analysed.

Larval development pattern

Four different reproductive types (Type I, II, III and Undetermined Type) were observed from *B. proboscidea* females collected in the intertidal abrasion platform of Mar del Plata.

Each reproductive type was defined based on brood characteristics, in particular on the observation of the

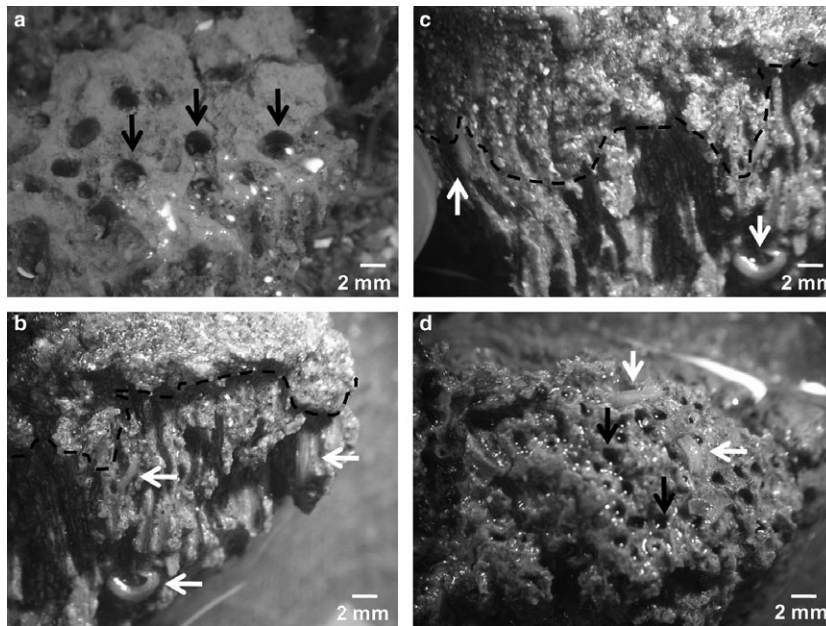


Fig. 4. Photos showing: (a,d) horizontal surface of the abrasion platform bored by *Boccardia proboscidea*; (b,c) lateral view of an abrasion platform sample where the platform-sediment interface is shown by the dotted line. Some tube openings bored by the worms within the platform are indicated by the black arrows and individuals of *B. proboscidea* (inside or outside of the tubes) are indicated by the white arrows.

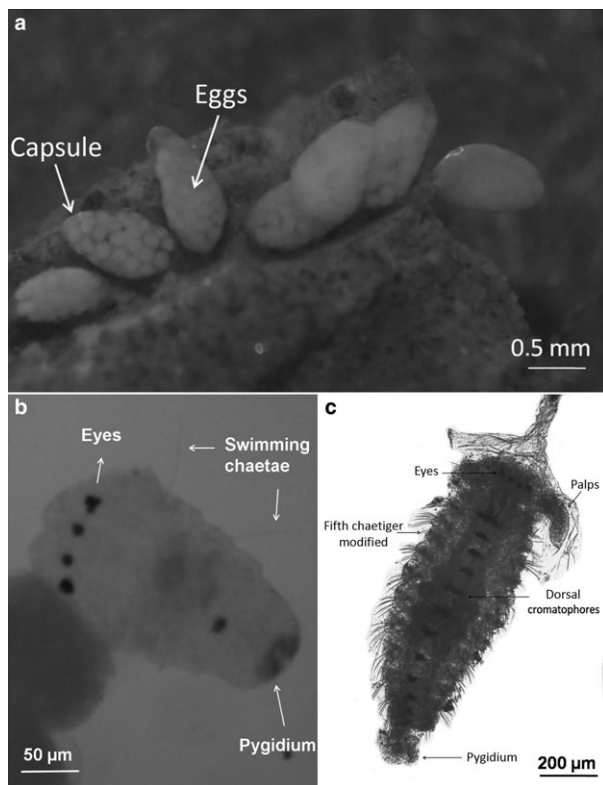


Fig. 5. (a) Part of a brood of *Boccardia proboscidea* showing seven egg capsules; (b) planktotrophic larvae of *B. proboscidea* and (c) adelphophagic larvae of *B. proboscidea* that will hatch as benthic juveniles.

degree of development of larvae within the capsule and egg production. Thus, broods were classified as: Type I: capsules with eggs and planktotrophic larvae; Type II:

capsules with eggs and adelphophagic larvae; Type III: capsules with eggs and both kind of larvae (planktotrophic and adelphophagic); Undetermined Type: capsules with only eggs.

Planktotrophic larvae were small with up to three chaetigers (some exceptions with six chaetigers). While rare, two pairs of eyes located in a transverse row were observed. The lateral eyes were the largest (Fig. 5b). Adelphophagic larvae were larger than planktotrophic larvae. These larvae had between 11 and 12 chaetigers and two to three pairs of eyes. From the third chaetiger to the posterior region of the body, a medium-dorsal pattern of chromatophores was observed. The pygidium was commonly observed (Fig. 5c).

The occurrence frequency of the different reproductive types (Type I, II, III, and Undetermined Type) within a single brood of *B. proboscidea*, showed significant differences among the summer months ($\chi^2 = 45.185$, $P < 0.001$).

Broods of Type I (only planktotrophic larvae) and Type III (both kinds of larvae) were dominant in all summer months (December, 43.87% and 17.43%; January, 41.96% and 26.67%; and February, 33.33% and 33.33%, respectively), whereas very low percentages of Type II broods (only adelphophagic larvae) were observed in the 3 months analysed (December, 1.72%; January, 5.69%; February, 2.95%). High percentages of broods of Undetermined Type (only capsules with eggs, without larvae) were observed throughout this season (December, 36.97%; January, 25.69%; February, 30.38%) (Fig. 7a).

During the summer season, a total of 18,353 larvae were found and classified according to their dispersal

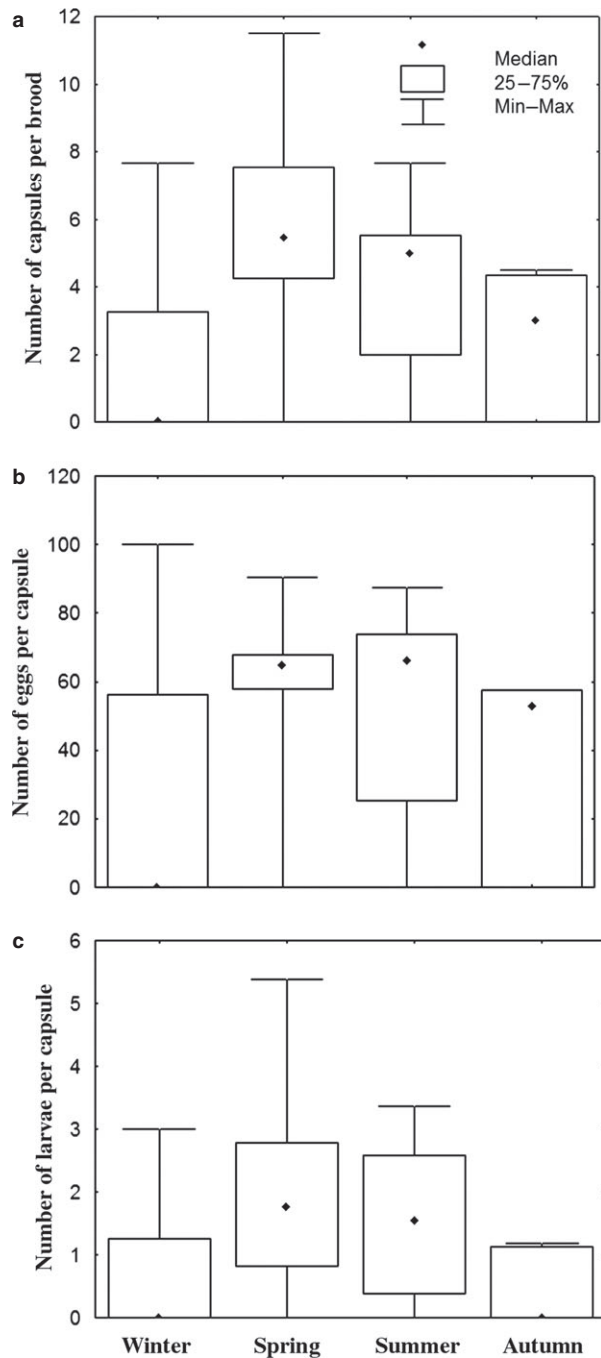


Fig. 6. (a) Number of egg capsules; (b) number of eggs per capsule and (c) number of larvae per capsule produced by brood of *Boccardia proboscidea* during the entire sampling period.

habitat. With regard to the dominance of the kind of larvae during this season, a total of 15,032 (80.45%) larvae were planktonic and only 3652 (19.54%) were benthic (Fig. 7b). The mean number of planktonic larvae per capsule during this season was 2.11 (max: 22 ± 3.077) and the mean number of benthic larvae per capsule was

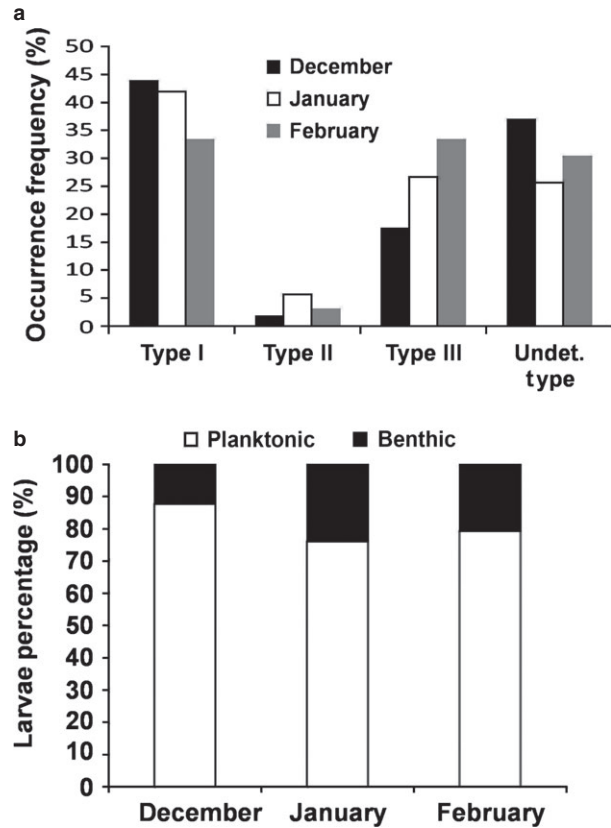


Fig. 7. (a) Occurrence frequency of the different reproductive types of *Boccardia proboscidea* during December 2008, January and February 2009. Type I: broods with capsules containing eggs and only planktotropic larvae; Type II: broods with capsules containing eggs and only adelphophagic larvae; Type III: broods with capsules containing eggs and both kinds of larvae (planktotropic and adelphophagic); Undetermined Type: broods with capsules containing only eggs. (b) Percentage of planktonic and benthic larvae found within the capsules of the *B. proboscidea* broods during the summer months.

0.52 (max: 12 ± 1.492). These differences were statistically significant ($\chi^2 = 307.6$, $P < 0.001$).

Discussion

Boccardia proboscidea collected in Mar del Plata (Argentina), as the specimen type, has short notochaetae in the first chaetigers; prostomium rounded prolonged anteriorly so as to extend beyond the peristomium; a caruncle extended posteriorly to the posterior margin of the third chaetiger; two kinds of the major heavy spines in the fifth (modified) segment: curved spines (falcate) and bristle-topped spines; branchiae on setigers 2, 3, 4, 6 and to near end. Therefore, *Boccardia proboscidea* found in Mar del Plata shares diagnostic features with specimens described originally.

Boccardia proboscidea is a native species from the west coast of North America (California). However, due to its opportunistic traits, it has dispersed to almost of the all world's oceans. Several variations in external morphology related to their geographical distribution have been noted. Different morphological features have been detected among specimens described in South Africa (Simon *et al.* 2010) and Spain (Martínez *et al.* 2006) compared with specimens found in Argentina (this study).

One difference found was the number of hooded hooks on posterior chaetigers (from the 7th chaetiger). South African specimens have seven to eight hooded hooks per ramus, whereas Argentine specimens have six to seven. The number of modified spines (of each type: simple or brushed) on the 5th chaetiger also varied between specimens living in South Africa (Simon *et al.* 2010) and Argentina (this study) with respect to the inhabitants of the Iberian Peninsula (Martínez *et al.* 2006). Worms described in Europe have three simple spines (dorsal row) and three to four bristle-topped spines (ventral row) (Martínez *et al.* 2006), whereas in the specimens from Argentina spines of the same type (simples and brushed) but two heavy curved spines (falcate) in the dorsal row and three blunt, bristle-topped spines in the ventral row were observed. Differences in the pygidium shape were also observed among these specimens. In all specimens examined from Argentina, like those described in South Africa, the pygidium consisted of four lobes with inequality between dorsal and ventral lobes, whereas in the worms described in Spain the pygidium consisted of a disc divided into two or four lobes interchangeably, with some specimens with four equal lobes and some individuals whose dorsal lobes were smaller than the ventral ones (Martínez *et al.* 2006). These phenotypic differences between specimens from Spain, South Africa and from Argentina suggest that this could be a species complex. Future molecular studies of this species will be necessary to determine whether or not they belong to the same species.

Boccardia proboscidea inhabiting the intertidal coast of Mar del Plata was found in colonies that had bored into coastal abrasion platforms and in massive aggregations of tubes built up over the platforms. In this region, it is considered primarily a boring species and secondarily a tube-dwelling species. The ability to build tubes and form reefs appears to be directly related to environmental contaminates from the sewage discharge of the city. The high rate of sedimentation of organic and inorganic particles leads these polychaetes to build tubes to avoid being buried. *Boccardia proboscidea* from Argentina was cited as one of the greatest threats to the biodiversity of the area (Jaubet *et al.* 2013). The percent cover of this reef-forming polychaetes reached almost 100% in sewage-impacted

sites. This invading polychaete seems to outcompete local intertidal species, and displaces the ecosystem engineering mussel *Brachidontes rodriguezii* as a structuring species within the intertidal due to competitive exclusion for space. Thus, the intertidal benthic community structure was modified by the massive settlement of *B. proboscidea* among mussel beds (Jaubet *et al.* 2013). In the southern coasts of Argentine (Province of Chubut), especially in the coastal city of Puerto Madryn (42° S, 65° W), *B. proboscidea* was found boring abrasion platforms on concrete piles (Port) (Diez *et al.* 2011) and as well as building tubes in sandy beaches (V. Radashevsky, pers. comm.).

Populations of *B. proboscidea* have been reported from different habitats including mudflats, sandy harbours, seagrass beds, among barnacles, in coralline algae, sandstone or sedimentary rocks, limestone reefs, sewage outfalls and gastropod shells inhabited by hermit crabs (Hartman 1940; Woodwick 1963; Imajima & Hartman 1964; Petch 1989; Gibson 1997). Populations from South Africa are considered pests building their tubes on the shells of important commercial species (*Haliotis midae*), oysters and other bivalves, causing blistering and fracturing of the shell (Simon & Booth 2007; Simon *et al.* 2010). It also has been considered a species potentially harmful to oyster culture systems in Hawaii (Bailey-Brock 2000), Australia (Leonart 2001) and New Zealand (Read 2004).

The development pattern and reproductive strategy of *B. proboscidea* have been described by several authors, each examining a different population geographically and most reporting a different development mode (Gibson 1997; Blake & Arnofsky 1999; Gibson *et al.* 1999).

Poecilogony in the population of *B. proboscidea* associated with the sewage-induced abrasion platforms surrounding the effluent from the city of Mar del Plata varies among broods produced by different females and also within a single brood (Fig. 8). This strategy involves the development of both larval trophic modes (planktotrophic and adelphophagic) and the release of both stages at the time of hatching (planktonic larvae and benthic juvenile).

Reproductive traits analysed in the current study (capsule number per brood, number of eggs per capsule and number of larvae per capsule) show no significant differences with the populations described in California (Hartman 1941), Australia (Blake & Kudenov 1981) or South Africa (Simon *et al.* 2010). However, the population of *B. proboscidea* from South Africa reproduces throughout the year, increasing their number by the end of winter and early summer (Simon & Booth 2007). In addition, females produce capsules containing the three reproductive patterns, but the brood of the Type III (planktotrophic

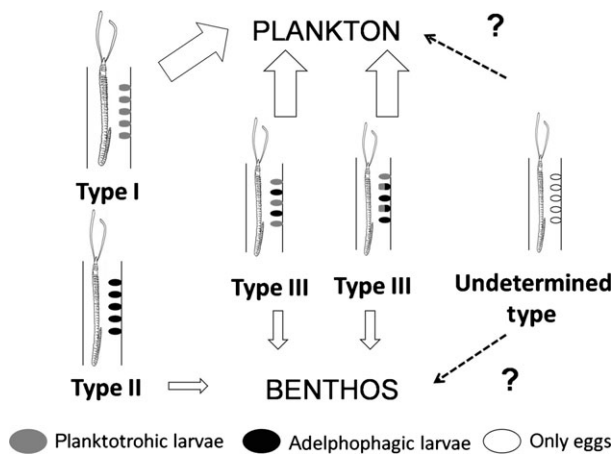


Fig. 8. Scheme representing the reproductive types produced by females of a *Boccardia proboscidea* population associated with the intertidal sewage-induced abrasion platforms of Mar del Plata, Argentina. Type I: Capsules of eggs with only planktotrophic larvae; Type II: capsules of eggs with only adelphophagic larvae; Type III: capsules of eggs with both kinds of larvae. These larvae were found sharing the same egg capsule or in different capsules within the same brood; Undetermined Type: capsules with only eggs. Sizes of the arrows represent the degree of the incorporation of each kind of larvae to the plankton or benthos.

and adelphophagic larvae in the same capsule) were dominant (50–80%) (Simon *et al.* 2010).

Like the California population described by Hartman (1941), the population of *B. proboscidea* associated with the sewage-associated abrasion platforms in the Argentinian coasts (Province of Buenos Aires) reproduces throughout the year, reaching their reproductive peak during the austral summer. This population includes females producing four different reproductive types (Type I, II, III and Undetermined Type). Broods of Type I (only planktotrophic larvae) and Type III (presence of both kind of larvae) were dominant in the three summer months (December, January and February), whereas only very low percentages of the broods of Type II (only adelphophagic larvae) were found. Broods of Undetermined Type (capsules with only eggs, without larvae) were also observed in a high percentages in every month.

Capsules may contain three types of eggs: (i) nurse eggs, (ii) eggs that will develop embryos that will hatch planktotrophic larvae and (iii) eggs that will develop adelphophagic embryos that will hatch as juveniles. Working with living material, the types of eggs can be identified according to their coloration. Nurse eggs are distinguished by a yellow colour and the fertilized eggs by an orange colour (Blake & Kudenov 1981). It is important to note that in this study a fourth reproductive type (Undetermined Type) was found. This may be due to work on fixed material,

which does not make it possible to identify whether the eggs in the capsules were fertilized.

Other species of the genus, such as *Boccardia polybranchia*, also exhibit poecilogony and produce capsules containing only eggs. These eggs do not develop and are food for the larvae in neighbouring capsules. The absence of larval development in some capsules could be due to problems during the fertilization process or spermato-phore transfer process during pseudocopulation (Duchêne 2000). Results of the present study suggests that the most eggs from this type of brood (Undetermined Type), begin with cleavage and only a few eggs develop into nurse eggs, as the Type I and III reproductive types were dominant and therefore the planktotrophic larval abundance was higher.

Planktotrophic larval development has been well described by King (1976). The larvae are small and do not exceed eight chaetigers (usually three to five) at hatching. They are released after 6 days of incubation and remain feeding on phytoplankton for a period of 30 days before settling. In this paper, these larvae did not exceed three chaetigers and rarely could be observed by eye. Adelphophagic development was described by Hartman (1941), Blake & Kudenov (1981) and Petch (1989). These larvae are larger than the planktotrophic larvae (9–16 chaetigers). In this work, these larvae reached between 11 and 12 chaetigers, two to three pairs of eyes, dorsal chromatophores from the third chaetiger and the pygidium.

The determining factor for the kind of larva that a female will produce is still little known. For example, in opisthobranch molluscs, the nutritional status of the female can be correlated with the kind of larvae produced. Thus, well-nurtured females would produce more yolk and lecithotrophic larvae, whereas undernourished females would produce planktotrophic larvae (Krug 1998). This does not seem to occur with spionid *Streblospio benedicti* populations, where environmental factors (organic enrichment and temperature) and intrinsic factors (age) can affect the reproductive traits such as egg number, number of larvae per brood, and brood size. However, the diameter of the eggs, fertility, planktonic larval period and some aspects of larval morphology (natatory chaetae) are also controlled by genetic factors (Levin *et al.* 1991; Levin & Bridges 1994).

Rasmussen (1973) found that differences in the development pattern of *Pygospio elegans* Claparède were related to environmental factors and were not genetically inherent in a population. In populations of *Polydora cornuta* complex, the variability in larval size results from the limitations concerning the level of stored sperm in seminal receptacles of the female, rather than being a genetically determined reproductive strategy (Rice & Rice 2009).

To date, there are no studies on the population of *B. proboscidea* from the Southwest Atlantic determining whether the kind of larva produced is related to extrinsic or intrinsic factors in the population. Probably, like the *S. benedicti* population, environmental factors (food and space) have a major role in the development of the population of *B. proboscidea* due to the combination of continuous supply of organic matter from the effluent discharge and the high rate of colonization of this species. Therefore, females would not have nutritional problems, favouring lecithotrophy. However, planktotrophic larvae production was higher than that of non-planktotrophic larvae. Probably, the organic particles are converted into somatic tissue rapidly and are invested in the offspring with the aim of increasing the larval production, as occurs with the *S. benedicti* spionid population (Levin 1984).

Another possible explanation for development variability of the *B. proboscidea* could be that its reproductive strategy varies according to season. Certain polychaetes species, such as *Pygospio elegans* Claparede, 1863, *Spio martinensis* Mesnil, 1896, and *Spio setosa* Verrill, 1873, can vary their mode of reproduction and development pattern throughout their geographic range or from one season of the year to the next (Thorson 1950; Blake & Kudenov 1981). Reproduction includes a long-lived pelagic larvae for one season of the year and a long period of protection of the offspring (involving adelphophagy) for another season (Blake & Kudenov 1981).

In this study, it was found that *B. proboscidea* from Argentina develops planktotrophy mainly during summer seasons. However, more detailed studies and long-term studies will be needed to determine whether the reproductive strategy of this species has seasonal limits, and whether its variability in the larval development pattern is related to environmental factors. The pattern of development may be determined genetically or environmentally. Future studies will be necessary to determine the various aspects which still remain unknown.

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Conflict of interest

None of the authors has any potential conflicts of interest.

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