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Shell Infestation of the Farmed Pacific Oyster *Magallana gigas* by the Endolith Bivalve *Rocellaria dubia*

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Abstract: Oyster shells are substratum for different epibiontic and endobiontic organisms, including pests and parasites. *Rocellaria dubia* is endolithic and facultative tube-dwelling bivalve, boring in different calcareous substrates, including the shells of bivalves. In 2020, *R. dubia* was found as endolithic in the shells of the Pacific oyster *Magalana gigas*, from an oyster farm off the Sacca di Goro lagoon (Emilia-Romagna region, Northern Adriatic Sea, Italy). The purpose of this study was to describe this newly recorded association. Altogether, 136 specimens of *R. dubia* were found in 15 oysters, photographed under a stereoscope, and their length was measured. Heavily infested oysters hosted tens of *R. dubia* borers, which were perforating the whole thickness of the oyster valves. The flesh of these oysters was heavily damaged, suggesting parasitic association. *R. dubia* specimens were categorized into three age classes (0–1, 1–2, and 2–3 years old). *M. gigas*/*R. dubia* might be a widespread association, overlooked due to the very scarce research on macrofauna associated with *M. gigas*. Considering the negative effects of *R. dubia* endobiosis on oyster fitness, and possible impacts on oyster aquaculture, further research should be conducted in order to elucidate the distribution and ecological characteristics of this parasitic association.

Keywords: endobiont; borer; parasite; Mediterranean; Adriatic; flask shells; Gastrochaenidae; *Crassostrea*; aquaculture

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1. Introduction

Rocellaria dubia (Pennant, 1777) is an endolithic and facultative tube-dwelling bivalve from the family Gastrochaenidae. It bores in a wide variety of natural and artificial non-biogenic and biogenic calcareous substrates, from shallow subtidal habitats down to 200 m depth [1–3]. It is an important bioeroder [4,5], and it can also be found as a fossil in deposits of marine origin [6–8]. *R. dubia* is distributed in the Mediterranean Sea, Black Sea, British islands, and various locations in the eastern Atlantic Ocean, including Morocco, Angola, West Africa, the Canaries, Cape Verde Islands, and Island St Helena [2,3,9–13]. The species is very common and widely distributed in the Adriatic Sea, boring in infralittoral rocks of carted limestone, coralligenous biogenic outcrops, and other biogenic structures, as well as artificial concrete blocks [2,14–17]. *R. dubia* perforates skeletons and shells of different dead and live marine organisms, such as stone corals [5,17], coralline algae [5], echinoderms [6], gastropods [18], and bivalves [2]. Some studies suggest a negative impact of this borer on host bivalves [19].

The shells of oysters act as a support for an array of epibiontic and endobiontic organisms [20–24]. Many of them are pests and parasites and damage the oyster aquaculture industry [24–26]. Despite its importance, the knowledge on the fauna associated with natural or farmed oysters in the Adriatic and the Mediterranean Sea is very scarce.

The Pacific oyster *Magallana gigas* (Thunberg, 1793), native to Japan and south-east Asia, was introduced across Europe and the Mediterranean Sea for aquaculture purposes in the second half of the 20th century. It replaced the farming of the European flat oyster *Ostrea edulis* Linnaeus, 1758, whose production decreased due to the high mortalities caused by viral and parasitic diseases [27–29]. *M. gigas* has also been introduced for farming in many other parts of the world in order to support and develop oyster aquaculture. While initially it was limited to farming sites, it soon spread outside farming sites, establishing wild populations. Nowadays, the Pacific oyster is the most commonly farmed oyster with worldwide distribution [30]. Although in Italy, Pacific oyster farming is limited and less important than the aquaculture of other mollusks, such as the Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) and Manila clam (*Ruditapes philippinarum* (A. Adams and Reeve, 1850)), it is continuously developing [31–33]. In Italy, most of the *M. gigas* farms are in Sicily, Liguria, Apulia, Sardinia, and Emilia-Romagna regions [34], Prioli, *pers. comm*.

In the frame of a project investigating the fauna associated with Pacific oysters *M. gigas* along the Emilia-Romagna coast (Italy, northern Adriatic Sea), *R. dubia* boring in oyster shells were observed in the aquaculture facility off the Sacca di Goro lagoon. Careful analyses of the literature revealed that *R. dubia* was never reported in association with *M. gigas* neither in wild, nor in farmed oysters. Moreover, information on the effects of *R. dubia* infestation on oyster hosts are very limited.

The aims of this research were to report for the first time the association between *M. gigas* and *R. dubia*, and to describe the morphological characteristics of the oyster hosts and the borers.

2. Materials and Methods

Sampling was performed in the frame of the project "Monitoraggio delle specie aliene negli impianti di molluschicoltura dell'Emilia Romagna: impatti e misure di mitigazione". Pacific oysters (*M. gigas*) were sampled in September 2020 from the oyster farm off the Sacca di Goro lagoon (Northern Adriatic Sea, Italy), managed by Cooperativa Sant'Antonio di Gorino (Figure 1). Oysters are grown in tubular baskets of about 3 to 5 m depth attached on a long line system, which extends along about 3 nautical miles.

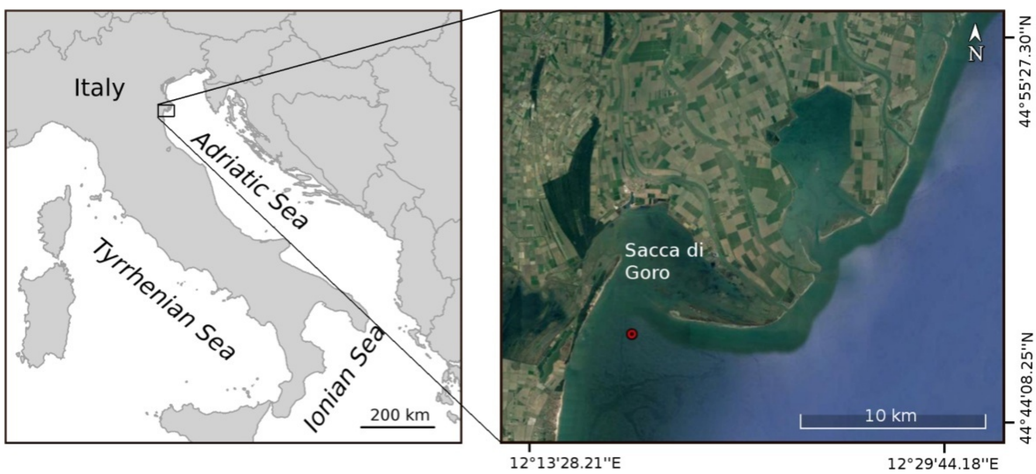


Figure 1. Map of sampling site. Sampling site marked with a red dot.

Three samples, of five oysters each, were collected manually from three random sites along the long line and placed in plastic containers filled with sea water. In the laboratory, oysters were first rinsed with running water on a 0.5 mm mesh size sieve, in order to separate vagile epibiontic organisms for later taxonomic analyses, and then transferred to 90% ethanol. Each oyster was opened, the soft flesh was removed, and the length and width of both the upper and lower valves were measured with a caliper following Harding et al. [35]. Shells were then carefully broken with pliers in order to avoid damage to the observed bivalve *R. dubia* and other associated invertebrates. Fragments of shells were analyzed under a stereoscope (Leica Wild M3B), and all specimens were extracted and preserved in 90% ethanol for later taxonomic determination. The abundance of *R. dubia* was determined cumulatively for each sample containing 5 oysters. Photographs of the outer and inner sides of the shell were taken only of the most infested oysters, and specimens of *R. dubia* were counted separately.

Each specimen of *R. dubia* was observed under a stereoscope (Nikon SMZ1500) with mounted DS-5M-U1 Digital Photomicrographic Camera System with 5Mpixels sensor. The shell length (maximum distance along the anterior posterior axis) and width (maximum lateral axis) of each specimen were measured following Ricci et al. [4], Schiaparelli et al. [36], and Carter et al. [37], using the computer program Motic Images Plus 3.0.

3. Results

The smallest oyster had a 71.4 mm long and 50.3 mm wide upper valve and a 60.02 mm long and 37.4 mm wide lower valve (Table S1). The largest oyster had a 204 mm long and 120.6 mm wide upper valve and a 192.0 mm long and 89.8 mm wide lower valve.

On the external sides of some oyster shells, characteristic "8"-shaped siphons of the boring bivalve *R. dubia* were visible (Figure 2a. *R. dubia* siphons were also perforating other epibionts covering the oysters, such as barnacles and serpulid polychaetes (Figure 2a). One oyster from the first sample, with a 79.3 mm long and 48.8 mm wide upper valve and a 67.8 mm long and 42.9 mm wide lower valve (Table S1), was infested by 32 *R. dubia* individuals. In this, and other heavily infested oysters, *R. dubia* was perforating the whole thickness of the valves, and the holes were internally visible. Some of these holes were partly or completely covered by the internal callus produced by the host oyster in order to protect itself (Figure 2b). The flesh inside heavily infested oysters was reduced to approximately one-third of the flesh size found in undamaged oysters of similar size (Figure 3). Moreover, the flesh color was grey, instead of the normal white-grey-yellowish color, and it had a soggy structure. The white-yellow greasy muscular part of the flesh seemed completely absent.

Altogether, 136 specimens of *R. dubia* were found in 15 oysters: 68 specimens in Sample 1 (including 32 from the most infested oyster), 49 in Sample 2, and 19 in Sample 3 (Figure 4). The shell length and width of 122 specimens were measured, while the shells of the remaining 14 specimens were damaged during extraction (Table S2). The smallest *R. dubia* was 1.58 mm long and 0.83 mm wide, while the largest one was 12.86 mm long and 6.18 mm wide (Table S2). In the most heavily infested oyster, the smallest *R. dubia* was 4.69 mm long and 1.98 mm wide, while the largest one was 9.67 mm long and 4.97 mm wide. Overall, most *R. dubia* were 7–8 mm long (Figure 5a). A similar size distribution of the *R. dubia* specimens is also reported in the most infested oyster (Figure 5b).

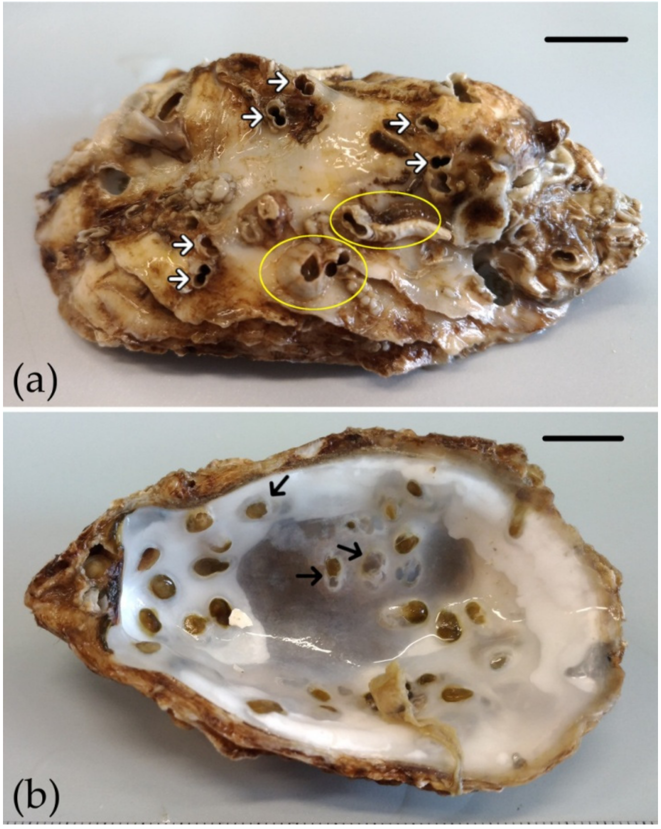


Figure 2. Upper valve of the oyster *Magallana gigas* infested by *Rocellaria dubia* (the most infested oyster from research): (**a**) external view: white arrows indicate "8"-shaped siphon openings of *Rocellaria dubia*, yellow circles indicate siphons penetrating through barnacle and polychaete tube; (**b**) internal view: black arrows indicate holes made by *Rocellaria dubia*, covered by the callus produced by the oyster. Scale bars—10 mm.

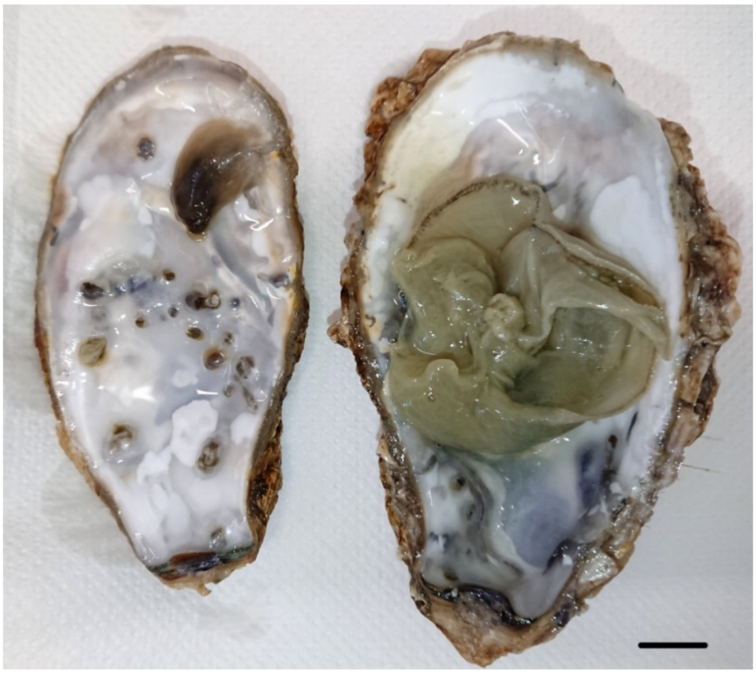


Figure 3. Lower (left) and upper (right) valve of the oyster *Magallana gigas* infested by *Rocellaria dubia*. Scale bar—10 mm.

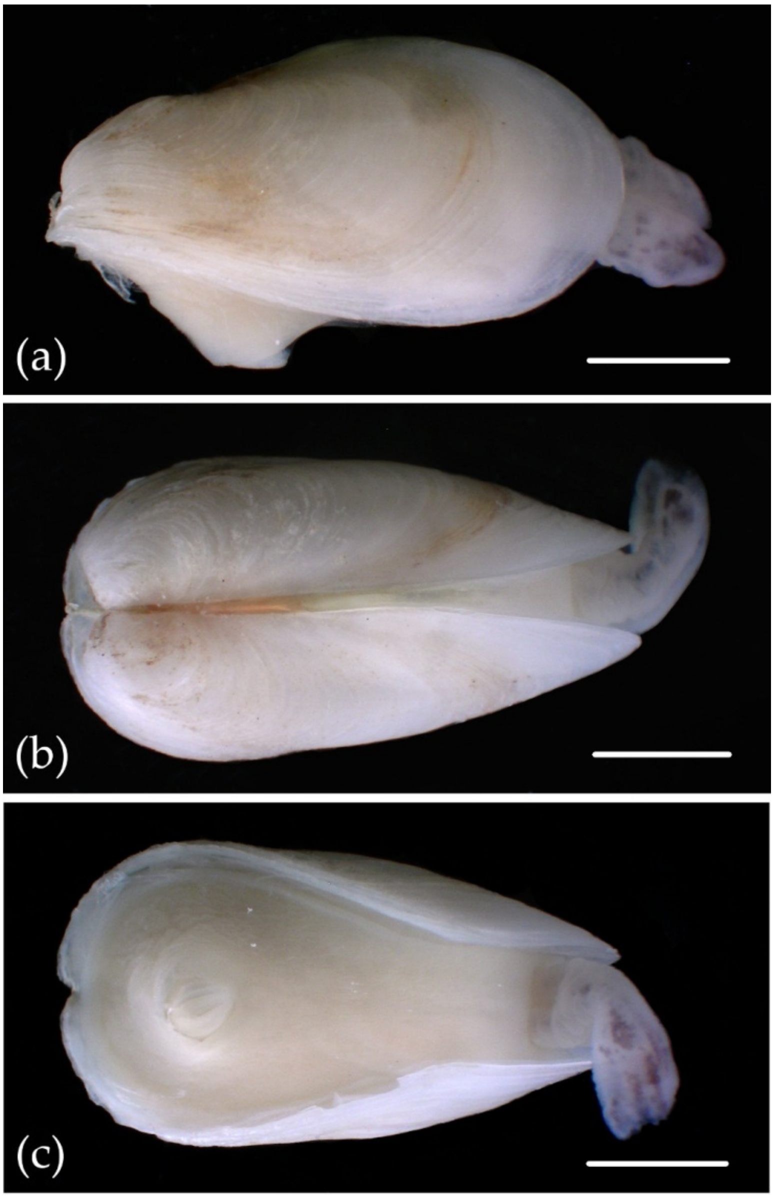
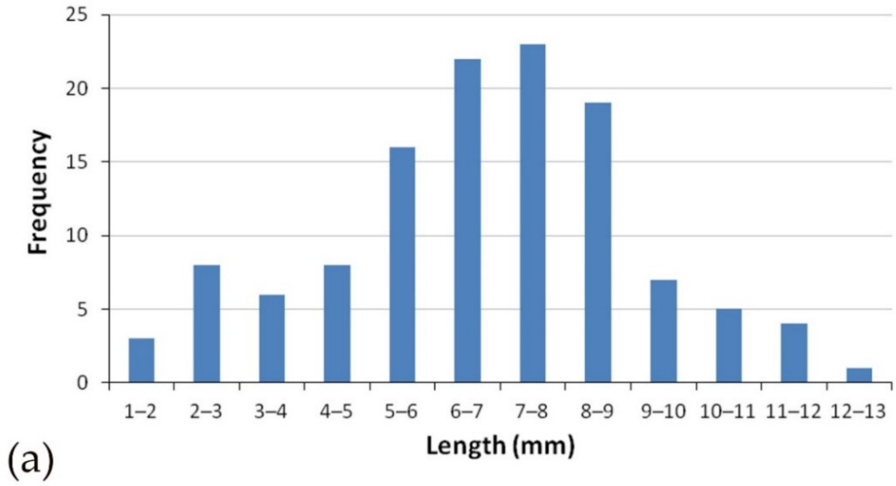


Figure 4. *Rocellaria dubia* extracted from the oyster *Magallana gigas*: (**a**) lateral view, right valve; (**b**) dorsal view; (**c**) ventral view. Scale bars—2 mm.



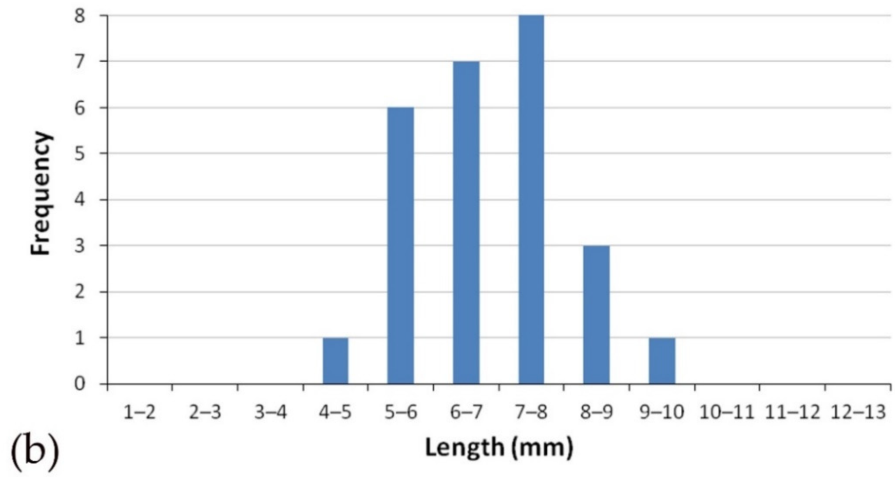


Figure 5. Size distribution of *Rocellaria dubia* shell length: (**a**) 122 individuals from all samples; (**b**) 26 individuals from the most infested oyster.

4. Discussion

The endolitic bivalve *R. dubia* was found for the first time boring into the shells of Pacific oyster *M. gigas*. Until now, it has been reported as a borer in the shells of bivalves *O. edulis* [2,3,10,38,39], *Spondylus sp.* [3], *Pinna nobilis* Linnaeus, 1758 [2,3,38], *Venus verrucosa* Linnaeus, 1758 [2,19], *Glycymeris nummaria* (Linnaeus, 1758) [2], *Arca noae* Linnaeus, 1758 [17], *Modiolus barbatus* (Linneaeus, 1758) [14,17], *M. galloprovincialis* Lamarck, 1819 [40,41], *Pseudochama gryphina* (Lamarck, 1819) [17], *Mimachlamys varia* (Linneaeus, 1758) [17], and gastropode *Stramonita haemastoma* (Linnaeus, 1767) [18]. Oysters are well known to host different epibiontic and endobiontic invertebrates [20–26]. They have friable, multilayered shells suitable for colonization by *R. dubia* [2]. Indeed, *R. dubia* was reported in wild and farmed populations of the European flat oyster, *O. edulis*. In 1865, Jeffreys found a cluster of a dozen *R. dubia* in a single oyster shell from Britain [10], while in 1906, Clark recorded *R. dubia* from oysters in England [39]. Rodríguez and Sánchez reported in 1997 *R. dubia* from oysters in the Canaries [3]. In the Mediterranean, in 1961, Parenzan outlined that *R. dubia* often infests larger oysters in Taranto Gulf (Italy) [38], while in 2011, Morton and co-authors found that almost all *O. edulis* specimens in an oyster farm at Mali Ston Bay (south Adriatic Sea) were infested by this endolith [2].

Considering the ability of *R. dubia* to bore in a variety of non-biogenic and biogenic calcareous substrates, it is surprising that it has never been reported in association with a widely distributed and commercially important species such as *M. gigas*. The absence of *R. dubia* in farmed oysters might be partly due to its ecological requirements, since it is considered to be a species very sensitive to disturbance [42]. Aquaculture facilities are usually located in shallow areas in proximity to the coast, lagoons and estuaries. These habitats show a high variability of environmental parameters, siltation rate, and organic matter load, which might be unfavorable for the development of *R. dubia*. In the frame of our project, aimed at investigating the macrofauna associated with natural and farmed *M. gigas*, oyster samples were collected at four locations in the Northern Adriatic Sea. Farmed oysters were sampled from two aquaculture facilities, one referring to this study, located off the Sacca di Goro lagoon, and one inside the Sacca di Goro lagoon. Wild oysters were sampled inside the Sacca di Goro lagoon and in the river Bevano estuary. However, *R. dubia* was recorded only from the farmed oysters off the Sacca di Goro lagoon. Both Sacca di Goro lagoon and river Bevano estuary are characterized by high concentrations of organic matter, reduced water exchange with the open sea, and high variability of environmental parameters, which might negatively affect the survival of *R. dubia*. The influence of environmental conditions on endolith infestation in farmed mollusks was reported previously [14]. Further research should be performed to understand the relationship among environmental conditions, farming method, and the incidence of *R. dubia* infestation in oysters.

On the other hand, the lack of reports of *R. dubia* on farmed *M. gigas* might also be derived from the lack of research on fauna associated with Pacific oysters, particularly in the Mediterranean Sea. There are only three papers reporting species associated with farmed *M. gigas* in the Mediterranean Sea [43–45], while no papers refer to the fauna associated with natural populations of Pacific oysters (Mikac et al., in prep.). Moreover, comprehensive investigation of the literature dealing with fauna associated with farmed Pacific oysters outside the Mediterranean (Mikac et al., in prep.), suggests that there are no studies covering regions in which *R. dubia* occurs. Considering the high level of infestation of *M. gigas* we found, we suppose that the association between *R. dubia* and *M. gigas* could be a much more widespread phenomenon than currently known, and it could be largely overlooked.

Heavily infested oysters hosted tens of *R. dubia* specimens, some of which were completely penetrating the shell of the host. The holes of *R. dubia* on the inner side of the oyster valves were completely or partially covered by the internal callus, an energetically costly material produced by the oyster in order to prevent complete perforation of the shell. This phenomenon is also well documented in *O. edulis* and other bivalves infested by *R. dubia* [2,19]. Moreover, heavily infested oysters had highly reduced flesh inside. Formerly, Trigui El-Menif and co-authors reported that the occurrence of *R. dubia* had negative effects on shell thickness, dry weight, and flesh biomass of *V. verrucosa*, leading to the death of the occupied host [19]. These authors classified the interaction between the *R. dubia* endobiont and the *V. verrucosa* host depending on the level of infestation. As long as *R. dubia* does not completely perforate the host shell, the association can be regarded as commensalism, while progressive internal perforation of the clam shell can disturb the host, and lead to a parasitic association. The endobiont can weaken the valve structure of the host by multiplying the number of galleries and their size, and can also prevent the host from hermetically closing its valves, making the host more vulnerable to further attacks from pathogenic agents and predators [19]. The magnitude of infestation and the state of infested oysters in our research suggest a parasitic relationship between *R. dubia* and *M. gigas*.

Based on available data on shell length/age correlation in Pacific oysters, the collected specimens range from 1 to 4 years [35,46,47]. However, defining age classes based on shell length seems to not be very accurate, since the literature data differ for wild and farmed oyster populations [47]. Measurements of *R. dubia* shells showed that analyzed oysters hosted individuals of different sizes, probably having different ages. The growth rate of *R. dubia* was investigated on vertical substrata in the Marine Protected Area (MPA) of Baiae

(Tyrrhenian Sea, Naples, Italy) [1]. Based on that study, the following size–age relationship was proposed: 0–5 mm length (age up to one year), 5–9 mm length (age 1–2 years), and 9–13 mm length (age 2–3 years). In another study on horizontal substrata conducted in the same area, a higher growth rate than what was observed on vertical experimental panels for the same age class was found [1,4]. It is well known that *R. dubia* prefers horizontal or slightly inclined substrates [5,48], so horizontal position might positively influence its growth. Unfortunately, there are no other studies on the settlement and growth of *R. dubia*. Based on previous data [1], the size distribution of *R. dubia* shell length in our research shows that specimens might appertain to three age classes, 0–1, 1–2, and 2–3 years. However, the favorable horizontal position of the studied substrate (i.e., *M. gigas* shells positioned more or less horizontally in the aquaculture chests) might have favored a higher growth rate. In general, despite its wide distribution and importance as a borer, the ecology of *R. dubia* is studied very little. More research regarding the growth rate and age of *R. dubia* in relation to environmental conditions (such as substrate type, inclination, morphology of the rocky bottom, and depth) should be performed.

As suggested by our observations, heavy infestation of *R. dubia* has negative impacts on the oyster host and can be considered parasitic. Considering the importance of oyster aquaculture and damages that endolithic organisms could provoke in this industry, efforts should be made to better understand the settlement and growth process of *R. dubia* on *M. gigas*, and the effect of this endobiont on the oyster host should be clarified. Moreover, extensive research should be conducted in order to obtain information on the geographic distribution of this newly reported association, both in the Mediterranean Sea and worldwide.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13110526/s1, Table S1: Length and width measures of *Magallana gigas* from each sample. Table S2: Length and width measures of *Rocellaria dubia* found in oysters from each sample.

Author Contributions: Conceptualization, B.M. and F.C.; methodology, B.M., F.C. and M.A.C.; validation, B.M.; formal analysis, B.M.; investigation, B.M. and A.T.; resources, B.M., A.T., M.A.C., M.A. and F.C.; data curation, B.M.; writing: original draft preparation, B.M.; writing: review and editing, B.M., A.T., M.A.C., M.A. and F.C.; visualization, B.M.; supervision, B.M. and F.C.; project administration, F.C.; funding acquisition, M.A. and F.C. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, since it included animals that are normally harvested for human consumption.

Data Availability Statement: Data supporting reported results are presented in Table S1 of the Supplementary Material.

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