

The symbiotic hesionid *Parasyllidea humesi* Pettibone, 1961 (Annelida: Polychaeta) hosted by *Scrobicularia plana* (da Costa, 1778) (Mollusca: Bivalvia: Semelidade) in European waters

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Abstract Heretofore, the hesionid polychaete *Parasyllidea humesi* was only known from its original description, living in association with the bivalve *Tellina nymphalis* in mangrove swamps north of Pointe-Noire (Republic of Congo, West Africa). The discovery of a stable population in Río San Pedro (Gulf of Cádiz, southern Atlantic coast of Iberian Peninsula) thus represents the second report for this species worldwide, and the first for European waters. Furthermore, the new population is associated with another bivalve, *Scrobicularia plana*. The host-symbiont relationship is characterized by a high host-specificity (the symbiont was absent from *Ruditapes decussatus* and

Cerastoderma glaucum collected in the same habitat and location), regular distribution (one, exceptionally two symbionts per host and then being male and female), and prevalence ranging from 0.22% (in Caño Sancti Petri) to 4.74% (Río San Pedro). The symbionts seem to affect the metabolism of their hosts and, thus, their normal growth, so this association may tentatively be considered as close to parasitism. *Parasyllidea humesi* seems to be restricted to salt marsh areas with stable marine salinities all over the year. As there is no evidence that the presence of *P. humesi* in the Gulf of Cádiz results from an introduction, we strongly suggest that it may be better considered as native to the region, with our finding representing the northernmost known geographical limit of its distribution.

Keywords: Hesionidae; Symbiosis; Bivalvia; NE Atlantic; Iberian Peninsula.

Running head: New symbiotic hesionid for the European Atlantic coasts

Introduction

The Hesionidae (Annelida: Polychaeta) are relatively small polychaetes usually less than 50 mm long, which are most often considered as active carnivores. They occur widely from both hard and soft subtidal habitats to the deep sea, where they are frequent but rarely dominant numerically, and also on intertidal habitats, most often as interstitial forms.

Like many other polychaete families, some species have developed symbiotic modes of life. Among them, *Parasyllidea humesi* Pettibone, 1961 was originally described as new genus and species from specimens living inside the mantle cavity of the bivalve *Tellina nymphalis* Lamarck, 1818 occurring in shallow waters in the vicinity of mangrove swamps at Luango, North to Pointe Noire, Middle Congo (Republic of Congo, West Africa) (Pettibone 1961). Since then, the species has never been reported again. Only two species have been later included within the genus: *P. australiensis* Hartmann-Schröder, 1980, based in a single record (Hartmann-Schröder 1980) and *P. blacki* (Knox 1960), an eyeless species from deep water originally described as

Nereimyra blacki and later transferred to *Parasyllidea* by Pleijel (1998). None of them have been reported as symbionts.

During the study of the association between the pinnotherid crab *Afropinnotheres monodi* Manning, 1993, and the peppery furrow shell *Scrobicularia plana* (Da Costa, 1778), the grooved carpet shell *Ruditapes decussatus* (Linnaeus, 1767), and the lagoon cockle *Cerastoderma glaucum* (Bruguière, 1789) in the Gulf of Cádiz (Atlantic coast of southern Iberian Peninsula) (Subida et al. 2011), a stable population of *Parasyllidea humesi* was discovered living also in association with *S. plana*.

In this article, we report this finding, the second one for *P. humesi* worldwide, and the first for European waters. The characteristics of the association with the host bivalve, the second known for the species, are also described in terms of infestation rates, host/symbiont size relationships, and type of association.

Material and methods

Specimens of the host bivalve *Scrobicularia plana* were collected in February 2009 by hand digging at low tide in Río San Pedro and Caño Sancti Petri (Gulf of Cádiz, southern Atlantic coast of the Iberian Peninsula), as well as in other estuaries: Guadalquivir, Salado, Barbate, and Palmones (in Spain) and Ria Formosa (in Portugal) (Fig. 1). Additional specimens were collected in March, April, July, and August 2011 at Río San Pedro to confirm the presence of the species and to obtain enough material for a more detailed study of the association. Once collected, the bivalves were kept alive and transported to the laboratory, where they were counted, measured (length, width and height, in mm), opened to check for the presence of the symbiont, and weighed (as dry weight: 80 °C, 48 h, without shell).

All obtained worms were counted, relaxed in isotonic magnesium chloride (7.2 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in 100 ml of distilled water) and preserved in different preservatives for further studies (i.e., 95% alcohol, in 4% seawater/formalin solution and in a filtered seawater/osmium tetroxide solution). Prior to preservation, some selected worms were photographed in detail with a Canon EOS 5D Mark II, equipped with Canon MP-E65/2.8 1-5x macro lens. As the worms may be easily damaged during handling, the relationships width/length and width/number of setigers were estimated to allow further comparisons between the hosts and their respective symbionts. Twenty entire worms

were selected to estimate the width (at the tenth setiger, parapodia included, in mm) vs. length (in mm) and number of segments relationships.

The worm/host size relationships were assessed by linear regression. The relationships between shell width, length, height, and biomass in infested and non-infested hosts were assessed using regression and covariance (ANCOVA) analyses. The corresponding relationships between width, length, and height were based on a subset of *Scrobicularia plana* specimens collected in June and July 2011 (72 specimens), while those between length and height were assessed later based on a different subset collected in July and August 2011 (41 specimens). All these analyses were carried out by means of the software XLSTAT version 2008.4.02, Copyright Addinsoft 1995-2008.

DNA was extracted using DNAeasy Tissue Kit (Qiagen) following the protocol supplied by the manufacturer. We used the primers 16SarL and 16SbrH for 16S rDNA (Palumbi 1996). PCR mixtures contained 21 µl ddH₂O, 1 µl of each primer (10 µM), 2 µl of DNA template, and puReTaq Ready-To-Go PCR beads (Amersham Biosciences). The temperature profile was as follows: 96°C/240s–(94°C/30s–48–58°C/30s–72°C/60s)*45 cycles –72°C/480s. PCR products were purified with a 5 µl mixture of exonuclease I and FastAP thermostable alkaline phosphatase (Fermentas) (Werle et al. 1994). Sequencing was performed at Macrogen Inc. facilities (Seoul, Korea). Overlapping sequence fragments were merged into consensus sequences using Geneious 5.1.7 (Drummond et al. 2010).

The obtained 16S rDNA sequence and a voucher paragenophore (Pleijel et al. 2008) have been deposited in the GenBank and the “Museo Nacional de Ciencias Naturales” (MNCR) of Madrid, Spain, respectively.

Results

The *Parasyllidea humesi* specimens from the Gulf of Cádiz are relatively large hesionids, measuring around 30 mm in length for about 70 setigers (Figs. 2a, b). The species is characterized by having a prostomium with two lateral antennae, one minute median antenna (which seems to be lacking in some specimens), a clearly marked median antennal furrow (present in all specimens, independently of the presence of the median antenna), and two biarticulate palps. The species has six pairs of tentacular cirri (three pairs on each side), sub-biramous parapodia, and indistinct notopodia,

represented by one acicula inside the elongate cirrophores of the dorsal cirri, and lacking capillary notosetae. Neurosetae are present from segment 4. The neuropodia are conical, without extra lobes, and have compound heterogomph neurosetae with blades of varying length. The proboscis is unarmed and without papillae, and shows a ring of fine cilia around the opening. These characters placed the genus within the Ophiodrominae clade, as previously reported by Ruta et al. (2007). Accordingly, the best hit from a blast search of the 16S rDNA sequence of *P. humesi* (GenBank accession no. JQ691830, MNCM 16.01/14309) is *Ophiodromus pallidus* (Claparède, 1864) (GenBank accession no. DQ442579) with a 76.9% identity for the entire sequence length.

With the exception of a single specimen collected in Caño Sancti Petri from 461 *Scrobicularia plana* specimens, all *P. humesi* were found in Río San Pedro, and always in association with the same host species, *S. plana* (10 specimens from 269 clams in February 2009, 17/1065, 26/548, 11/339 and 10/378 in March, April, July, and August 2011, respectively). The corresponding prevalence was 0.22 in Caño Sancti Petri and 3.80, 2.60, 4.74, 3.2, and 2.6 in the Río San Pedro surveys, respectively. Neither *Ruditapes decussatus* (n = 185) nor *Cerastoderma glaucum* (n = 627) from Caño Sancti Petri, nor *S. plana* from the Guadalquivir (n = 308), Salado (n=24), Barbate (n = 717), Palmones (n = 125) and Ria Formosa (n = 139) estuaries hosted the symbiotic hesionid. In turn, the intensity of the infestation was always a single worm per host, except for a single case in June 2011, where two worms (one male and one female) co-inhabited the same *S. plana* specimen.

There were positive relationships between width (W) and length (L) of the worms, with higher significance when expressed in mm (Fig. 3a) rather than in the number of setigers (Fig. 3b):

- L (in mm) = $-8.70981 + 13.8992 * W$ ($t = 13.481$, $R^2 = 0.910$, $p < 0.0001$)
- L (num. set.) = $21.19995 + 17.12758 * W$ ($t = 4.976$, $R^2 = 0.579$, $p < 0.0001$)

The worms were located in the mantle cavity of the hosts, always in specimens measuring more than 20 mm in length, and were more frequent in intermediate-sized shells, i.e., from > 26 cm to about 36 cm long, which, in turn, were the most frequent shell lengths (Fig. 4a). As the symbionts were absent from the smallest hosts, there was a non-significant relationship (i.e., $R^2 = 0.021$) between worm and shell lengths (Fig. 4b).

The relationships of shell length vs. width, height and biomass were always significant, independently of the fact that *S. plana* shells were infested or not (Figs. 5a-5c):

- Length (L) vs. Width (W). Non-infested: $W = 0.7579L - 0.1622$ ($R^2 = 0.927$, $t=20.705$, $p < 0.0001$); infested: $W = 0.7428L - 0.46$ ($R^2 = 0.949$, $t = 25.128$, $p < 0.0001$).
- Length (L) vs. Height (H). Non-infested: $H = 0.3134L - 0.4281$ ($R^2 = 0.910$, $t = 13.826$, $p < 0.0001$); infested: $H = 0.3277L - 0.9462$ ($R^2 = 0.886$, $t = 11.814$, $p < 0.0001$).
- Length (L) vs. Biomass (B). Non-infested: $B = 0.0126L - 0.2433$ ($R^2 = 0.645$, $t=7.857$, $p < 0.0001$). Infested: $B = 0.0095L - 0.1862$ ($R^2 = 0.616$, $t = 7.3869$, $p < 0.0001$).

When comparing L vs. W and L vs. H, there were non-significant differences between infested and non-infested *S. plana* shells (ANCOVA, $F = 0.8434$, $p = 0.362$ and $F = 0.2142$, $p = 0.646$, respectively) (Figs. 5a, 5b). Conversely, the infested shells had significantly lower biomasses for the same length than non-infested ones (ANCOVA $F = 12.7461$, $p < 0.001$) (Fig. 5c).

Discussion

The presence of *Parasyllidea humesi* in the Bay of Cádiz represents the second report of the species worldwide, and the first one in European waters. A stable European population of *P. humesi* was found only in Río San Pedro, an estuary characterized by having stable salinities of around 37.5‰ on average during the whole year (ranging from 31 to 40‰, depending on the season) but, particularly, by lacking the strong fluctuations in the water salinity that typically occur in the remaining surveyed estuaries during rainfall periods. These estuaries often show fluctuations of more than 10‰ in a single rainfall episode (Subida et al. 2011). Accordingly, the presence of *P. humesi* seems to be restricted to salt marsh areas with stable marine salinities all year round. The same occurs with the pea-crab *Afropinnotheres monodi* found in the same habitat and inside the same host species (but never sharing the same clam), whose distribution also includes the Atlantic coasts of West Africa. Additionally, the dispersal of the species eastwards from the Bay of Cádiz may be limited by the ocean circulation from

the African shelf towards the Gulf of Cádiz (Hagen 2001), as also reported for *A. monodi* (Subida et al. 2011). In turn, the coastal counter-current flowing westwards along the Gulf of Cádiz coast may form a cyclonic cell in its eastern part. Under certain conditions, this cell may reach the western region of the Gulf, and then progress towards the north under the influence of the Iberian Poleward Current (or Portugal Coastal Counter-current), which flows mostly in fall, winter, and beginning of spring (García-Lafuente et al. 2006). This environmental pattern allows us to postulate that the finding of *P. humesi* in the Gulf of Cádiz may be found in more northern areas. However, our finding currently represents the northern known limit for its distribution, and the species may certainly be native of the Gulf. In turn, the amount of *Scrobicularia plana* has not been examined in other Spanish regions often enough to fully discard the presence of the symbiont (e.g., less than 30 in Salado Estuary or less than 130 in Palmones Estuary), and the same occurs for the Portuguese coasts and the single surveyed estuary checked, Ria Formosa (i.e., about 140 bivalves). Moreover, in this estuary, the environmental conditions are adequate to allow the presence of the symbiont. Also, the fact that its presence may also be the result of a punctual and relatively recent introduction cannot be discarded, despite that there are no data supporting such a possibility. As the species has only been found twice, none of these hypotheses can be supported over the other, although we still consider the former as more reliable and parsimonious than the latter.

The original population of *P. humesi* from Congo was reported by Pettibone (1961) as living in association with *Tellina nymphalis*, a species that seems to have been cited mainly from the Western coasts of Africa. *Tellina nymphalis* was briefly and vaguely described by Lamarck (1818: 533), without supporting drawings or indication of type locality or habitat, and the name has been posteriorly applied, quite probably wrongly, to materials collected at estuarine shallow water habitats of Western Africa, which may belong to other species of Bivalvia. The current state of *T. nymphalis* is not clear, and the species is not cited in main recent works on the Mollusca from West Africa (e.g. Ardonini and Cossignani 2004) nor included (either as valid or synonymized) in WoRMS (Gofas 2011), so it may be an indeterminable or invalid species. In turn, the sparse graphic information found on *T. nymphalis* from West Africa, such as in the Natural History Museum of Rotterdam, catalogue no. NMR993000018393, <http://nlbif.eti.uva.nl/nmr/detail.php?taal=uk&naam>, or in Nicklès (1950, fig. 432) shows a species closely resembling the genus *Macoma*, such as *M. cf.*

innominata sensu Ardvini and Cossignani (2004), so a misidentification of the original host cannot be fully discarded.

In the Gulf of Cádiz, the association of *P. humesi* with *S. plana* appears as highly specific. Neither *Ruditapes decussatus* nor *Cerastoderma glaucum*, very abundant at the same location, hosted the polychaete. Also, there are no previous records of *P. humesi* (or a similar hesionid that could be misidentified by a non-specialist) as free-living, nor in the studied areas (Carvalho et al. 2011; Arias and Drake 1999; Drake et al. 1997), nor in previous faunistic checklists (Parapar et al. 2004). The association is regular (sensu Martin and Britayev 1998), with only one symbiont per host in all cases except for a single one in which one male and one female were found living inside the same host clam.

Currently, there are 11 known commensal hesionid species (Table 1), representing about 2.9% of the known commensal polychaetes (and around 7.5% of the known hesionid species). Commensalism in hesionids seems to be restricted to the clade Ophiodrominae, which also includes *Parasyllidea*, but certainly is a feature with multiple origins within hesionids. In fact, *Ophiodromus* and *Gyptis*, two of the most representative Ophiodrominae genera including commensal species (Table 1), are not closely related (Ruta et al. 2007). Moreover, both are species-rich genera, and we today have no evidence on whether commensalism within each of them has arisen once or several times.

Commensal hesionids are involved in about 36 different types of associations. Except for the polyxenous species of *Ophiodromus* (i.e., *O. obscurus*, *O. flexuosus*, and *O. pugettensis*), which are associated with many different hosts (i.e., 3, 10, and 12, respectively), most symbiotic hesionids are monoxenous, occurring in only one (6 species) or two (2 species) hosts. The most common hosts are echinoderms (particularly starfishes), followed by other Polychaeta (Table 1). A peculiar case is that of *Anoploneurus hermanni*, a species described by Giard (1882) as associated to two species of *Balanoglossus*. According to Pleijel (1998), the original description agrees with *Ophiodromus*, a well-known genus including several commensal species, but it is poorly known and there is no type material. Thus, it must at present be considered a *nomen dubium*.

In addition to *P. humesi* there is only one more hesionid living in association with a bivalve, and this is *Ophiodromus pugettensis*, which seems to be able to detect at a certain distance the presence of at least two of its host starfishes, *Patiria miniata* and

Luidia foliolata (Davenport et al. 1960). Also there are some indications of mutualistic behavior in their relationships, with one of its echinoid hosts, the sand dollar *Clypeaster humilis* (Storch and Nigemann 1967). During sample handling, all isolated specimens of *P. humesi* that were maintained in small aquaria containing specimens of *S. plana* were always found in close contact with them, either below or crawling on the shell. Although this seems to indicate that the symbiont may be attracted by its host from a certain distance, further studies are required to experimentally demonstrate either the presence of a host-factor (similar to that in the association between *O. pugettensis* and its host starfish) or the apparent specificity of its association with *S. plana* (e.g., by allowing the worm to be in contact with other bivalves).

However, contrary to *O. pugettensis*, our results point to a negative effect of the presence of *P. humesi* inside *S. plana*, as the infested hosts showed a significantly reduced body mass compared to the non-infested ones (Fig. 5c). The fact that the presence of symbionts may affect the metabolism of their host bivalves, and thus their normal growth (Bierbaum and Ferson 1986), has been previously reported for other polychaete species living in the mantle chamber of bivalves, such as *Branchipolynoe seepensis* living inside *Bathymodiolus* spp. (Britayev et al. 2007). Accordingly, this could be considered as an indication that the association between *P. humesi* and *S. plana* is closer to parasitism than to commensalism or mutualism. However, contrary to *B. seepensis*, no damages in the tissues of *S. plana* have been observed in the Gulf of Cádiz population. Thus, further studies are again required to assess the exact nature of the association.

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Fig. 1 *Parasyllidea humesi* Pettibone, 1961.
Geographic location of the two known populations in the Republic of Congo and in the Gulf of Cádiz (Caño Sancti Petri and Río San Pedro) and location of the remaining studied estuaries: (1) Ria Formosa; (2) Guadalquivir; (3) Salado; (4) Barbate; (5) Palmones.

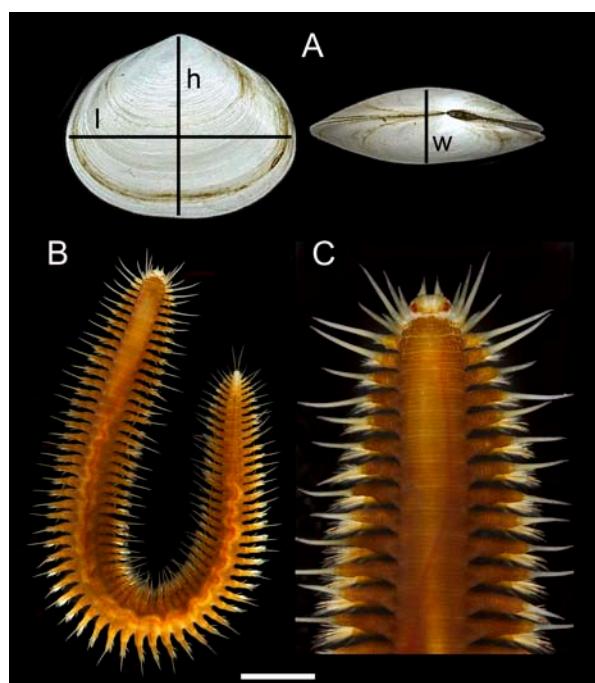


Fig. 2 *Scrobicularia plana* (Da Costa, 1778). **a** Measurements taken on shells: length(l), width(w) and height (h). *Parasyllidea humesi* Pettibone, 1961. **b** Whole body, dorsal view. **c** Detail of anterior end, dorsal view. Scale bar: 3 mm, **b** and 1mm, **c**.

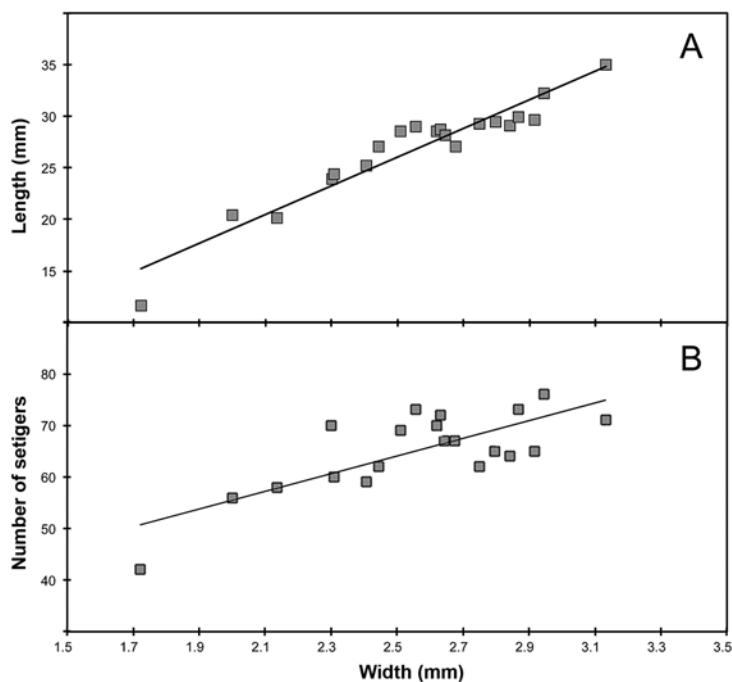


Fig. 3 *Parasyllidea humesi* Pettibone, 1961. Relationships between width and length. **a** Length in mm. **b** Length as number of setigers.

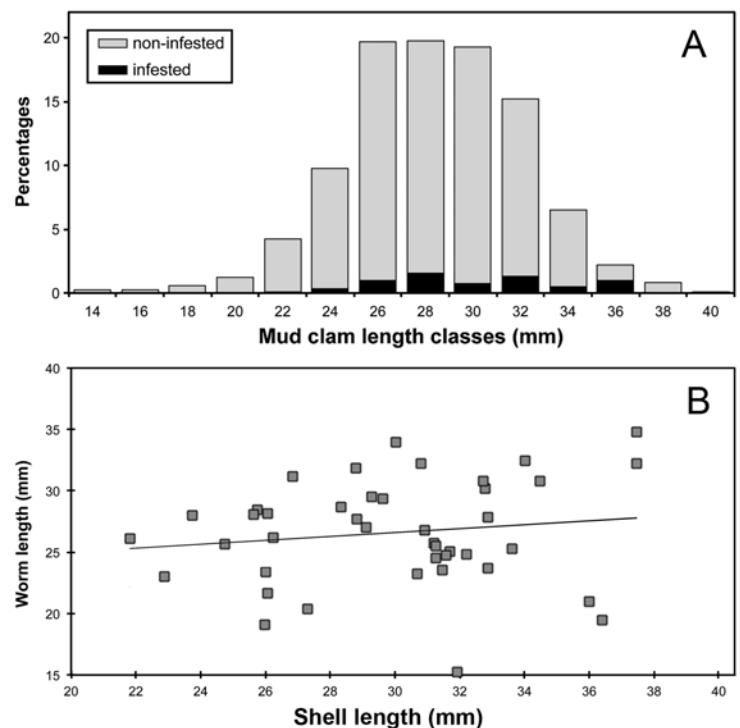


Fig. 4 *Scrobicularia plana* (Da Costa, 1778). **a** Percentages of infested and non-infested specimens according to the size classes (shell length in mm) ($n = 2,373$). **b** Relationships between length of infested shells and length of *Parasyllidea humesi*.

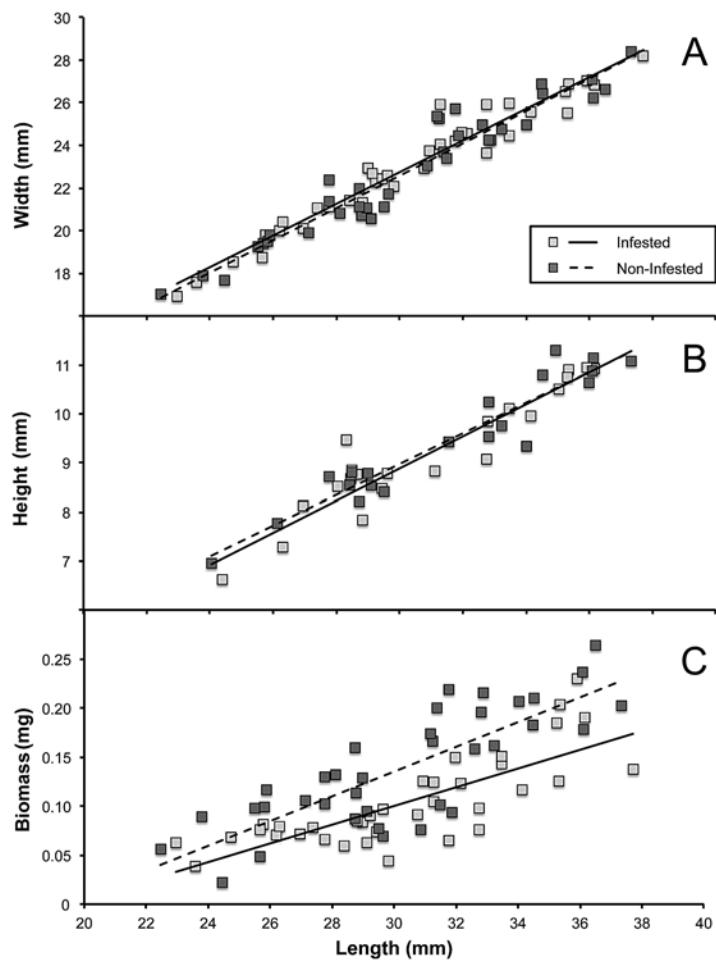


Fig. 5 *Scrobicularia plana* (Da Costa, 1778). Relationships of length vs. width (**a**), height (**b**), and biomass (**c**) in infested and non-infested specimens.

Table 1. List of known symbiotic species of Hesionidae, updated from Martin and Britayev (1998).

SPECIES	AUTHORITY	HOST		SOURCE
		Group	Species	
<i>Anoplonereis herrmanni</i>	Giard, 1882	Entereopneust	<i>Balanoglossus robinii.</i>	(Giard 1882)
		Entereopneust	<i>Balanoglossus salmonetus</i>	(Giard 1882)
<i>Gyptis ophiocomae</i>	Storch & Niggemann, 1967	Ophiuroid	<i>Ophiocoma scolopendrina</i>	(Storch and Niggemann 1967)
<i>Gyptis vittata</i>	Webster & Benedict, 1887	Polychaete	<i>Notomastus lobatus</i>	(Gardiner 1976)
<i>Podarkeopsis brevipalpus</i>	(Hartmann-Schröder, 1959)	Polychaete	<i>Glycera robusta</i>	(Gardiner 1976)
		Holothuroid	<i>Leptosynapta tenuis</i>	(Gardiner 1976)
<i>Parahesione luteola</i>	(Webster, 1879)	Decapod	<i>Upogebia affinis</i>	(Pettibone 1956)
<i>Parasyllidea humesi</i>	Pettibone, 1961	Bivalve	<i>Tellina nymphalis</i>	(Pettibone 1961)
		Bivalve	<i>Scrobicularia plana</i>	This paper
<i>Ophiodromus angustifrons</i>	(Grube, 1878)	Asteroid	<i>Pentaceros hedemanni</i>	(Jones 1964)
<i>Ophiodromus flexuosus</i>	(Delle Chiaje, 1827)	Asteroid	<i>Astropecten aranciacus</i>	(Barel and Kramers 1977)
		Asteroid	<i>Astropecten bispinosus</i>	(Barel and Kramers 1977)
		Asteroid	<i>Astropecten platyacanthus</i>	(Barel and Kramers 1977)
		Asteroid	<i>Astropecten irregularis</i>	(Barel and Kramers 1977)
		Asteroid	<i>Luidia ciliaris</i>	(Barel and Kramers 1977)
<i>Ophiodromus obscurus</i>	(Verrill, 1874)	Hemichordat	<i>Balanoglossus</i> sp.	(Clark 1956)
		a	<i>Maxmuelleria lankesteri</i>	(Anker et al. 2005)
		Holothuroid	<i>Leptosynapta</i> sp.	(Barel and Kramers 1977)
		Polychaete	<i>Amphitrite edwardsi</i>	(Barel and Kramers 1977)
		Polychaete	<i>Euclymene lumbricoides</i>	(Barel and Kramers 1977)
<i>Ophiodromus pallidus</i>	(Claparède, 1864)	Polychaete	<i>Lysilla alba</i>	(Pettibone 1963)
		Echinoid	<i>Lytechinus</i> sp.	(Hartman 1951)
		Holothuroid	<i>Thyone</i> sp.	(Pettibone 1963)
<i>Ophiodromus pugettensis</i>	(Johnson, 1901)	Echiuroid	<i>Lissomyema exilia</i>	(Anker et al. 2005)
		Asteroid	<i>Patiria miniata</i>	(Barel and Davenport 1956; Davenport et al. 1960; Lande and Reish 1968; Ricketts et al. 1985)
		Asteroid	<i>Luidia foliolata</i>	(Davenport et al. 1960; Stewart 1970; Hilbig 1994)

	Asteroid	<i>Luidia magnifica</i>	(Storch and Rosito 1981)
	Asteroid	<i>Pteraster tesselatus</i>	(Storch and Niggemann 1967)
	Asteroid	<i>Oreaster occidentalis</i>	(Steinbeck and Ricketts 1941)
	Asteroid	<i>Pisaster ochraceus</i>	(Davenport and Hickok 1957; Hickok and Davenport 1957)
	Holothuroid	<i>Protanthyra bidentata</i>	(Okuda 1936)
	Decapod	<i>Eupagurus</i> sp.	(Hickok and Davenport 1957)
	Gastropod	<i>Aletes</i> sp.	(Storch and Niggemann 1967)
	Bivalve	<i>Chama</i> sp.	(Storch and Niggemann 1967)
	Echinoid	<i>Clypeaster humilis</i>	(Storch and Niggemann 1967)
Unidentified hesionid	Echinoid	<i>Linopneustes longispinus</i>	(Miller and Wolf 2008)
