

Spermatophore and Gonopore Morphology of the Southwestern-Atlantic Hermit Crab *Pagurus exilis* (Benedict, 1892) (Anomura, Paguridae)

Marcelo A. Scelzo¹, Marina Z. Fantucci², and Fernando L. Mantelatto^{2,*}

¹Departamento de Ciencias Marinas, FCEyN, Universidad Nacional de Mar del Plata/CONICET. Funes 3350, (AYL7600B), Mar del Plata, Argentina. E-mail:mascelzo@gmail.com

²Laboratory of Bioecology and Crustacean Systematics, Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP), University of São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP CEP 14040-901, Brazil E-mail marinzaf@gmail.com

(Accepted September 23, 2009)

Marcelo A. Scelzo, Marina Z. Fantucci, and Fernando L. Mantelatto (2010) Spermatophore and gonopore morphology of the southwestern-Atlantic hermit crab *Pagurus exilis* (Benedict, 1892) (Anomura, Paguridae). *Zoological Studies* 49(3): 421-433. The form and function of the spermatophore have been used as a complementary tool in studies of the reproductive biology and systematics of hermit crabs. In this context, we describe the spermatophore and gonopore morphology of *Pagurus exilis*. The spermatophores were extracted from the distal part of the vas deferens of specimens collected in Argentina and Brazil. The spermatophores were composed of 3 major regions: a main ampulla (with a sperm capsule inside and an accessory ampulla at the base), a stalk, and a pedestal. Each spermatophore had a distinct dorsolateral suture line around the ampulla, where the rupture occurs to release the sperm. The spermatophore total length was 1.5 times the main ampulla length. The main ampulla was oval and slightly flattened. A triangular accessory ampulla extended from the main ampulla base to the pedestal on 1 side, and contained no to several sperm. The stalk is short and flattened, and as wide as the main ampulla. One to 3 spermatophores were found attached to each pedestal, which was almost oblong in shape. The dimensions of the spermatophore and its component parts were directly influenced by the size of the hermit crab. Gonopores of males were covered by long pappose setae, while female gonopores bore a few short cuspidate setae. Specimens from Brazil and Argentina had the same spermatophore morphology, corroborating the previously observed absence of genetic differences between the both populations. The spermatophore morphology of this species has similarities with the broad general pattern of the Paguridae, being most similar to one of the (at least) 3 patterns of spermatophore morphology described for *Pagurus*. <http://zoolstud.sinica.edu.tw/Journals/49.3/421.pdf>

Key words: Decapoda, Reproductive morphology, Testes.

The superfamily Paguroidea, encompassing common hermit crabs, currently consists of the families Coenobitidae, Diogenidae, Paguridae, Parapaguridae, Pylochelidae, and Pylojacquesidae (McLaughlin et al. 2007). The family Paguridae, in which “right-handed” hermit crabs are included, is considered to be a plesiomorphic, monophyletic group (Forest 1995, McLaughlin et al. 2007), but its monophyly has been questioned (Tudge

1995 1997). It is a morphologically diverse taxon, currently including 74 genera (Patsy McLaughlin pers. commun.).

The family Paguridae is represented by 11 genera and 22 species on the Brazilian coast (Melo 1999, Nucci and Melo 2003). In Argentinean waters, it is represented by 2 genera and 5 species (Boschi et al. 1992, McLaughlin and Saint Laurent 1998). The genus *Pagurus* Fabricius, 1775 is

*To whom correspondence and reprint requests should be addressed. E-mail:flmantel@usp.br

the most speciose in both areas, with 8 (Brazil) and 4 (Argentina) recognized species, 2 of which (*P. exilis* and *P. criniticornis*) are recorded in overlapping waters.

Pagurus exilis (Benedict, 1892) is endemic to the southwestern Atlantic Ocean. It is one of the most common hermit crabs inhabiting shallow littoral waters of southern Brazil, Uruguay, and Argentina, down to a depth of 35 m (Meireles et al. 2006). Although data about the entire life cycle of this species are still unclear, knowledge about some biological features has accumulated in recent years. There are reports on its postembryonic development in laboratory conditions (Scelzo and Boschi 1969), biogeographic aspects (Melo 1999), spatial and seasonal distributions (Meireles et al. 2006), shell occupation (Terossi et al. 2006), experimental shell selection under laboratory conditions (Mantelatto et al. 2007a), population biology (Mantelatto et al. 2007b), and reproductive aspects in Argentinean and Brazilian waters (Terossi et al. in press).

Most commonly, studies on the reproductive aspects of Anomuran were devoted to female mechanisms such as gonad development, embryo carrying, and fecundity, while the male reproductive characteristics, equally essential to understanding reproductive processes, have received less attention. Males produce spermatophores, structures that serve as packaging and protection for non-motile spermatozoa during transmission, and may also provide an energy-rich substrate for prolonged sperm storage in females (Subramoniam 1991). These roles in sperm transfer and storage are especially notable in anomuran hermit crabs, which have developed a broad range of adaptations to successfully reproduce and survive in different habitats using gastropod shells and other types of shelter (Mantelatto et al. 2009a).

Since the earliest research on reproduction and mainly with detailed descriptions of decapod spermatophores, such as those of Mouchet (1930 1931), the typical, pedunculate anomuran/hermit crab spermatophore, composed of 3 major regions (a sperm-filled ampulla, stalk, and foot or pedestal) is recognized as being variable among members of different families (Tudge 1991 1999, Scelzo et al. 2004). Members of the Paguridae are distinctive in possessing a large, elongate main ampulla, with an accessory ampulla at its base, and a pseudo-stalk analogous to the true stalk of the Coenobitidae and Diogenidae (Tudge 1991). Due to this wide variation, the form and function of the spermatophore are used as complementary

information to elucidate the reproductive biology and systematics of hermit crabs.

Considering the enormous abundance and variety of the genus *Pagurus*, and the fact that few studies have provided complete and integrated descriptions of the reproductive system in hermit crabs, we herein describe, illustrate, and provide some biometric information on the gonopore morphology in both sexes, the male reproductive apparatus, and the spermatophore morphology of the endemic southwestern-Atlantic hermit crab *P. exilis*, in order to compare it with other members of the family Paguridae. This contribution is part of a long-term effort to understand reproductive strategies, as well as the spermiotaxonomy, of southwestern-Atlantic hermit crab species.

MATERIALS AND METHODS

Hermit crabs were obtained in the area of Mar del Plata and Mar Chiquita (Buenos Aires Province, Argentina, 38°S) and Ubatuba (São Paulo, Brazil, 23°S) from 1998 through 2009, at depths of 5-25 m by trawl nets in both areas. Each individual was measured in relation to the cephalothoracic shield length (SL) from the tip of the rostrum to the V-shaped groove at the posterior edge of the dorsal shield, with a caliper rule (with an accuracy of 0.01 mm) or a stereomicroscope fitted with a graduated eyepiece. The spermatophore morphology and biometry of 40 specimens of hermit crabs (20 from each region) were analyzed. Additional specimens were used for gonopore morphology and photographic documentation.

Coxae from the 3rd pereopods (P3) in females and from the 5th pereopods (P5) in males were isolated and submerged for at least 24 h in lactic acid to clear the cuticle. Because the gonopores are oval, measurements of maximum (MGD) and minimum (mGD) diameters for males and females were obtained with a scale mounted on a light microscope or through scanning electron microscopic (SEM) images, for at least 2 individuals from each 0.5 mm class of SL for the range of sizes analyzed. Gonopore diameters of males and females were compared in relation to the SL. Standard setae terminology of gonopores, according to Watling (1989), was used.

The male reproductive system was dissected from fresh or fixed specimens. The thin cuticle of the dorsal abdomen was cut to obtain the testis and vas deferens (VD). The distal part of the VD which joins the basal part of P5 was

specifically used for the spermatophore analysis. Spermatophores obtained from the proximal part of the VD, near the testis, were also observed for comparison with mature spermatophores from the distal region. The dissected materials were preserved in 3% glutaraldehyde or 10% formalin (Tudge 1999).

Five to 10 spermatophores from each individual were measured. No clear division or “neck” was found between the ampulla and peduncle, making specific measurements of these structures difficult or inexact, which drove us to establish a pattern to achieve more confidence in the measurements. The following measurements were taken (Fig. 1): the total length of the spermatophore (TLSp) was the maximum length of the spermatophore from the ampulla to the base or pedestal; peduncle length (PL) was from the base or pedestal to the base of the lateral ridge; peduncle width (PW) was the size across the peduncle; ampulla height (AH) was the maximum length from the anteroposterior axis of the ampulla; ampulla width (AW) was the maximum external width across the ampulla; pedestal length (PeL) was the maximum length of the spermatophore pedestal; and pedestal width (PeW) was the maximum measurement across the spermatophore pedestal. A few measurements of ampulla depth (AD) and of the accessory ampulla length (AAL)

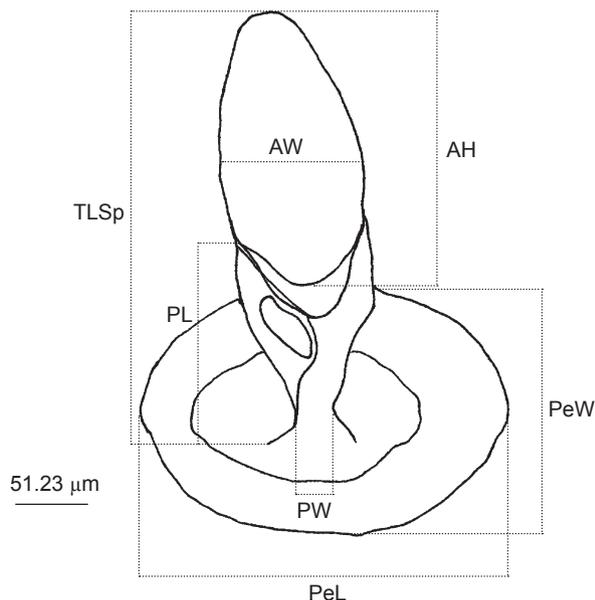


Fig. 1. *Pagurus exilis*. Diagrammatic representation of the spermatophore, indicating the measurements taken. TLSp, total length of spermatophore; AH, ampulla height; AW, ampulla width; PL, peduncle length; PW, peduncle width; PeL, pedestal length; PeW, pedestal width.

were taken.

A regression analysis was performed to compare dimensions of the spermatophores and the size of the crabs. The mean, standard deviation (SD), number of individuals sampled (n), and coefficient of determination (r^2) in the specific case of regressions (Sokal and Rohlf 1981) are indicated. Voucher specimens were deposited in the Crustacean Collection of the Biology Department (CCDB/FFCLRP), University of São Paulo, Brazil under catalogue number CCDB 2551.

RESULTS

There were no differences in spermatophore external morphology between the analyzed specimens from the both regions (Argentina and Brazil). Therefore, the general data are presented without reference to the origin of the material. The male hermit crabs analyzed ranged 3.1-7.1 (5.14 ± 0.88) mm SL and 0.19-2.08 (1.14 ± 0.60) g in weight (Table 1).

Reproductive system

Pagurus exilis is a gonochoric species. Male and female gonopores are rounded to ovoid, and the aperture is closed by a gonopore cover. Male gonopores are surrounded by long pappose setae with a few setulae. Those setae are longer than the gonopore diameter (Figs. 2A, B). Female gonopores have a few short cuspidate setae (Figs. 2C, D). No correlation was found between the number of setae and hermit-crab size.

Female gonopores were larger and more-oval than male gonopores. Gonopores of males measured 334 ± 108 (range, 170-545) μm MGD and 198 ± 106 (range, 57-386) μm mGD for crabs measuring 3.3-7.1 mm SL. Gonopores of females measured 424 ± 112 (range, 289-605) μm MGD and 183 ± 48 (range, 125-261) μm mGD for crabs measuring 3.5-5.3 mm SL. A direct relationship was found between gonopore diameter and SL in males ($\text{MGD} = 0.0666 \times \text{SL} - 0.01$; $r^2 = 0.815$; $n = 17$) and females ($\text{MGD} = 0.103 \times \text{SL} - 0.0309$; $r^2 = 0.377$; $n = 11$).

The male reproductive system was composed of testes and vasa deferentia, located in the pleon over the hepatopancreas. The testes were membranous lobate structures connected to the proximal part of the vasa deferentia, which had a coiled portion near the testis. This portion was thinner than the distal one, which was nearly

straight (Fig. 3A). The latter was visible in a dorsal position at the left side of the proximal region of the pleon, and open in the gonopores located at the base of P5. Many tripartite spermatophores, which

were completely formed, were randomly arranged within the entire vasa deferentia (Fig. 3B). In some male individuals, we also found that they adhered externally to the ventral (sternite) region of the crab

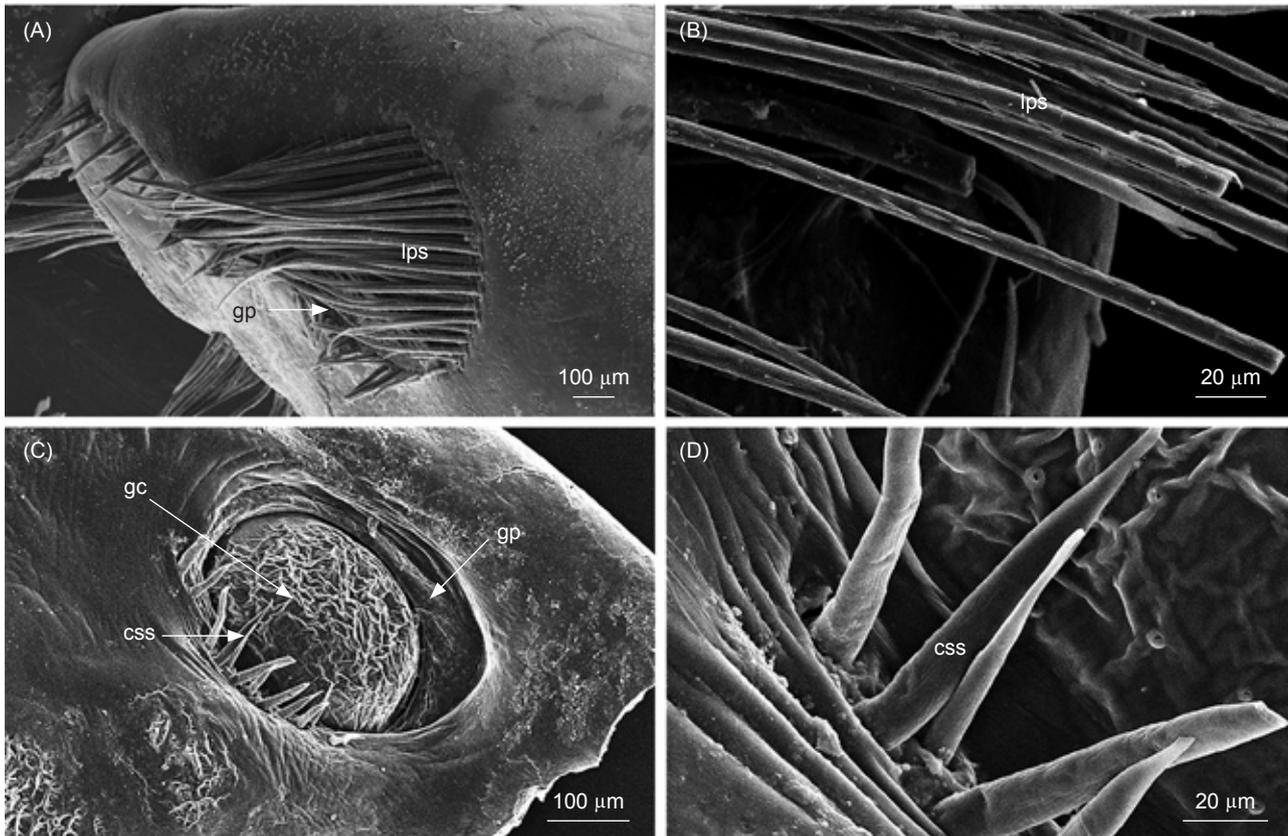


Fig. 2. *Pagurus exilis*. Scanning electron photomicrographs of the gonopore. (A, B) Male: coxae of the 5th pereopods with gonopores (gp) totally surrounded (A) by long pappose setae (lps) (B). (C, D), Female: gonopore cover (gc), and a few short cuspidate setae (css).

Table 1. *Pagurus exilis*. Male and spermatophore measurements. SL, shield length; TLSp, total length of spermatophore; AH, ampulla height; AW, ampulla external width; PL, peduncle length; PW, peduncle width; PeL, maximum pedestal length; PeW, maximum pedestal width; AAL, accessory ampulla length; *n*, number of measurements

Variable	Mean	SD	Min	Max	<i>n</i>
SL (mm)	5.14	0.88	3.10	7.10	40
TLSp (μm)	162	31.03	80	243	298
AH (μm)	117	27.22	37	188	296
AW (μm)	50	14.50	17	103	281
PL (μm)	88	23.03	45	163	231
PW (μm)	33	12.84	6	63	231
PeL (μm)	145	38.30	38	229	219
PeW (μm)	88	24.90	25	160	219
AAL (μm)	64	15	49	89	7

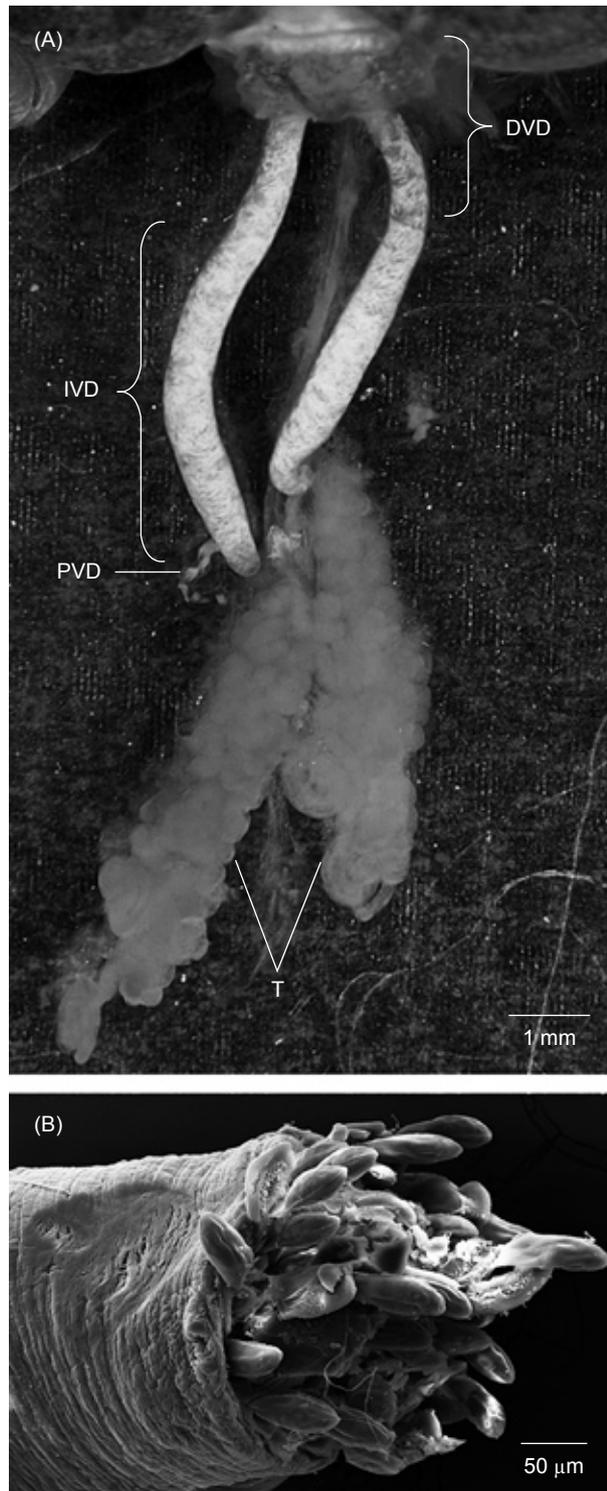


Fig. 3. *Pagurus exilis*. Male reproductive apparatus. (A) General view of the reproductive system; (B) details of the distal region of the vas deferens, showing the numerous, randomly arranged spermatophores (scanning electron photomicrograph). DVD, distal vas deferens; IVD, intermediate vas deferens; PVD, proximal vas deferens; T, testis.

near P5 or to the distal portion of P5.

Spermatophore morphology

Spermatophores had 3 distinct regions: a sperm-filled, elongated ovoid head or main ampulla; a short stalk or peduncle; and a foot, base, or pedestal (Figs. 4, 5). Spermatophores ranged 80-243 μm TLSp (Table 1), and their length was positively correlated with the size of the crab (Fig. 6).

The main ampulla was elongated (AH: AW of 1.3: 1-4.6: 1), oval, and slightly flattened anteroposteriorly, with the distal end almost flat, sometimes rounded, or pointed. A distinct dorsolateral ridge or suture was present on each spermatophore, and extended as an obvious line around almost the entire lateral border of the main ampulla, to allow the release of sperm. Usually the ampulla was full of spermatozooids, but in some cases, it was empty or with only a few spermatozoa inside, and the 2 valves appeared open.

The main ampulla measured $117 \pm 27 \mu\text{m}$ AH and $50 \pm 14 \mu\text{m}$ AW, with a close correlation between the AH and TLSp ($r^2 = 0.70$) (Fig. 6). An accessory ampulla, which contained no to several sperm cells (Figs. 4, 5), was found with each spermatophore, and was $64 \pm 15 \mu\text{m}$ long (Table 1). It was a wide triangular tab that was attached to the base of the main ampulla and extended through the entire peduncle (Fig. 5).

The peduncle is the portion of the spermatophore that joins the main ampulla to a pedestal. In the spermatophores produced by *P. exilis*, the peduncle was short (PL: AH of 0.75: 1), obviously flattened, and wide in relation to the size of the main ampulla (PW: AW of 0.66: 1) (Fig. 4). The pedestal was large, oval, and variable in size, and its main axis was more than 1.5 times greater than the short axis (PeL: PeW of 1.65: 1) (Table 1). A large external ring and central core comprised the pedestal where 1 or more spermatophores were attached. In the same individual, pedestals with 1 or 2 and rarely 3 spermatophores were present. A small spermatophore associated with a larger one on the same pedestal was infrequently observed (Fig. 5).

DISCUSSION

Our observations of *P. exilis* spermatophores are part of a long-term effort to carry out a

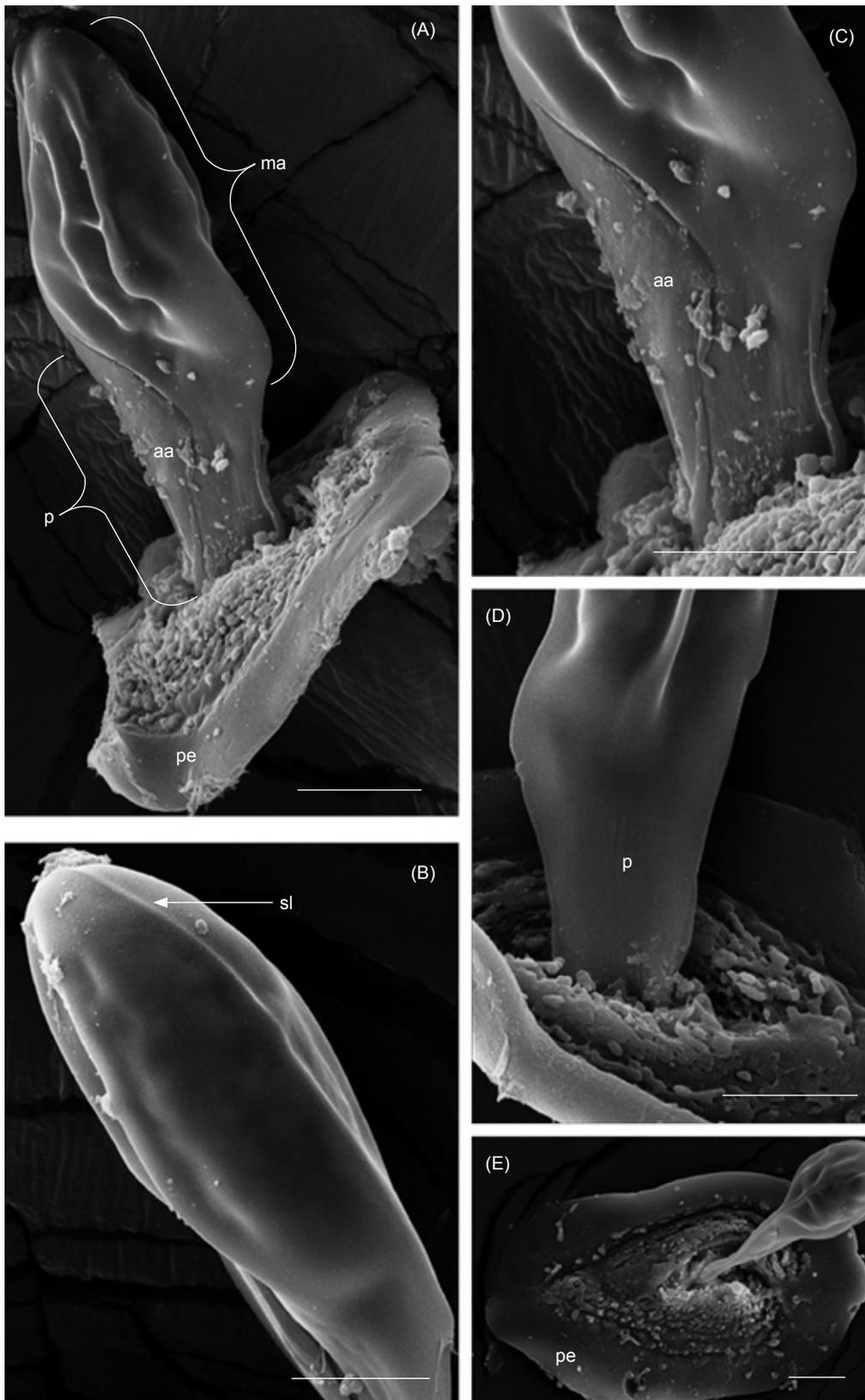


Fig. 4. *Pagurus exilis*. Spermatophores. (A) General view of a spermatophore; (B) details of the suture line; (C) details of the accessory ampulla; (D) general view of the peduncle; (E) general view of the pedestal (scanning electron photomicrographs). Scale bar = 20 μm . ma, main ampulla; aa, accessory ampulla; p, peduncle; pe, pedestal; sl, suture line.

spermiotaxonomic study of hermit crabs inhabiting different areas of the southwestern Atlantic. With these results, knowledge of spermatophore morphology of pagurid hermit crabs covers 19 species of the genus *Pagurus*, including the 1st description of the spermatophore morphology and morphometry of a member of this genus from the southwestern Atlantic (Table 2).

The large number of long setae surrounding the male gonopores is a characteristic that differentiates *P. exilis* from some species of the Diogenidae recently investigated, such as *Clibanarius vitattus*, *Cli. erythropus*, *Isocheles sawayai*, and *Calcinus tibicen* (Hess and Bauer 2002, Tirelli et al. 2007, Mantelatto et al. 2009a,

Amadio and Mantelatto 2009), all of which have short setae. The male gonopores of the diogenid *Diogenes pugilator* and the pagurid *Micropagurus acantholepsis* have a setae pattern similar to that found in *P. exilis*. In these species, the setae are thought to have a sensory function during the mating process (Manjón-Cabeza and García Raso 2000, Tudge and Lemaitre 2004). In addition, these setae may be related to copulation by providing better adhesion to female gonopores and acting as sensors during the transfer of spermatophores to females (Manjón-Cabeza and García Raso 2000, Tirelli et al. 2007, Amadio and Mantelatto 2009, Mantelatto et al. 2009a). All of these functions are probably also performed by

Table 2. List of hermit crab species of the family Paguridae with a description of the spermatophore

Species	References
<i>Anapagurus chiroacanthus</i> (Lilljeborg) (as <i>A. brevicarpus</i>)	Mouchet 1931
<i>Anapagurus hyndmanni</i> (Bell)	Pérez 1930, Mouchet 1930 1931
<i>Anapagurus laevis</i> (Bell)	Mouchet 1931
<i>Cestopagurus timidus</i> (Roux) (as <i>Eupagurus</i>)	Mouchet 1931
<i>Micropagurus acantholepis</i> (Stimpson)	Tudge and Lemaitre 2004
<i>Nematopagurus</i> sp.	Tudge 1999
<i>Pagurus anachoretus</i> (Risso) (as <i>Eupagurus</i>)	Mouchet 1931
<i>Pagurus bernhardus</i> Linnaeus (as <i>Eupagurus</i>)	Pérez 1930, Mouchet 1931, Chevallier 1970, Fretter and Graham 1976, Subramoniam 1993
<i>Pagurus bernhardus</i> Linnaeus	Tudge 1995 1999
<i>Pagurus brevidactylus</i> (Stimpson)	Fantucci and Mantelatto unpubl. data
<i>Pagurus chevreuxi</i> (Bouvier)	Tudge 1995 1999
<i>Pagurus criniticornis</i> (Dana)	Fantucci and Mantelatto unpubl. data
<i>Pagurus cuanensis</i> (Bell) (as <i>Eupagurus</i>)	Mouchet 1931
<i>Pagurus excavatus</i> (Herbst) (as <i>Eupagurus</i>)	Mouchet 1931
<i>Pagurus excavatus</i> (as <i>Pagurus meticulosus</i>)	Schaller 1979
<i>Pagurus excavatus</i> (Herbst)	Tudge 1999
<i>Pagurus exilis</i> (Benedict)	Present study
<i>Pagurus forbesi</i> Bell (as <i>Eupagurus</i>)	Mouchet 1931
<i>Pagurus hirtimanus</i> Miers, 1880	Tudge 1991 1995 1999
<i>Pagurus longicarpus</i> Say	Tudge 1999
<i>Pagurus macclaughlinae</i> García-Gomez	Tudge 1999
<i>Pagurus novizealandiae</i> (Dana) (as <i>P. novae-zealandiae</i>)	Greenwood 1972, Subramoniam 1993
<i>Pagurus pollicaris</i> Say	Tudge 1999
<i>Pagurus prideauxi</i> Leach (as <i>Eupagurus</i>)	Mouchet 1931, Bennati-Mouchet 1934, Hamon 1937 1939a b
<i>Pagurus prideauxi</i>	Tudge 1995
<i>Pagurus setosus</i> (Benedict)	Fasten 1917
<i>Pagurus stimpsoni</i> (A. Milne-Edwards and Bouvier) (as <i>P. bonairensis</i>)	Brown 1966
<i>Pagurus sculptimanus</i> (Lucas) (as <i>Eupagurus</i>)	Mouchet 1931
<i>Pagurus</i> sp.	Tudge 1999
<i>Porcellanopagurus</i> sp.	Tudge 1995
<i>Solitariopagurus tuerkayi</i> Forest	Tudge 1999
<i>Xilopagurus caledonicus</i> Forest	Tudge 1995 1999

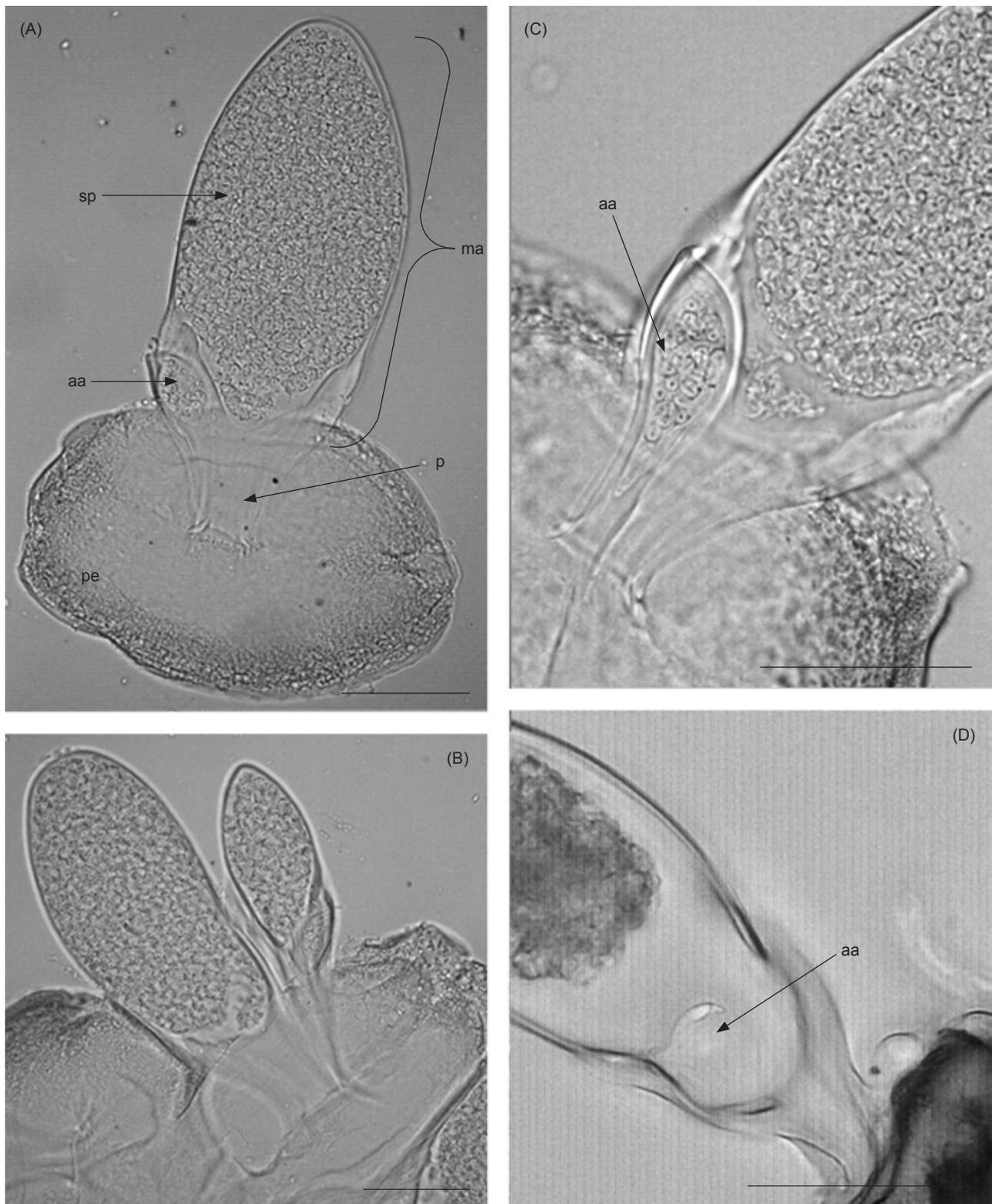


Fig. 5. *Pagurus exilis*. Spermatophores. (A) General view of a spermatophore; (B) a pedestal supporting a normal spermatophore and a smaller one; (C) details of the accessory ampulla filled with sperm cells; (D) details of an empty accessory ampulla (light-microscopic photomicrographs). Scale bar = 50 μm . ma, main ampulla; aa, accessory ampulla; p, peduncle; pe, pedestal; sp, sperm cells.

setae of *P. exilis*. The large number of long setae on the male gonopores of *P. exilis* possibly indicate that this species uses particular strategies for copulation. Unfortunately, the few descriptions of female gonopores in the literature are restricted to the recent descriptions of the diogenid *I. sawayai* and *C. tibicen* by Mantelatto et al. (2009a) and Amadio and Mantelatto (2009), respectively, and the pagurid species studied here. Such sparse information and descriptions are insufficient to support inferences on the role of the setae, and further studies are needed to investigate their possible function in reproductive behavior.

The external morphology of the reproductive system of *P. exilis* is similar to that of other hermit crabs, in terms of components and the position of the pleon (McLaughlin 1980 1983). The most conspicuous difference found relates to the external morphology of the vasa deferentia, which are relatively shorter and straighter in this species than in recently described members of the Diogenidae such as *Loxopagurus loxochelis*, *Cal. tubularis*, *Cli. erythropus*, *D. pugilator*, *I. sawayai*, and *Cal. tibicen*, in which the vasa deferentia are proportionally longer and mostly helicoidal (Scelzo et al. 2004, Tirelli et al. 2006 2007 2008, Amadio and Mantelatto 2009, Mantelatto et al. 2009a). Unfortunately, we found a lack of information about detailed descriptions of the morphology of the vasa deferentia, which prevented us from making comparisons among members of the genus. The relatively shorter vasa deferentia of *P. exilis* are probably a consequence of the fact that in species of the Paguridae, 2 regions of this male structure, responsible for secretion and elongation of the spermatophore peduncle, are absent (Matthews 1957, Greenwood 1972), but are present in hermit crabs of other families (Mouchet 1931).

The spermatophore morphology of *P. exilis* is consistent with the pedunculate spermatophore type that is common in species of the Paguroidea (Tudge 1991 1999, Tudge and Jamieson 1991). Until now, most spermatophore descriptions of hermit crabs were made on members of the families Diogenidae and Paguridae. Studies on members of the family Paguridae showed a greater diversity of spermatophore forms than in other hermit-crab families, within the limits of the characteristic familial morphology. In pagurids, the spermatophores are composed of an elongated main ampulla, an accessory ampulla (lacking in some species), a short stalk or peduncle, and a pedestal or base. Most members of the Paguridae studied have a homogeneously granular

spermatophore wall structure (Tudge 1999).

In recorded species of the Paguridae, the spermatophores have a total length of 150-615 μm (Tudge 1999). The majority of species fall in a range of 150-200 μm , and much of the variation in spermatophore shape stems from differences in stalk length and width. The spermatophore of *P. exilis* had a total length of 80-243 μm , and was directly correlated with the size of the hermit crab. However, these data should be used with caution as an additional criterion to distinguish congeneric species, because in some cases, as described by Amadio and Mantelatto (2009), there is wide variation in the spermatophore size along the vasa deferentia (i.e., spermatophores in different stages of maturation), as demonstrated in *L. loxochelis*

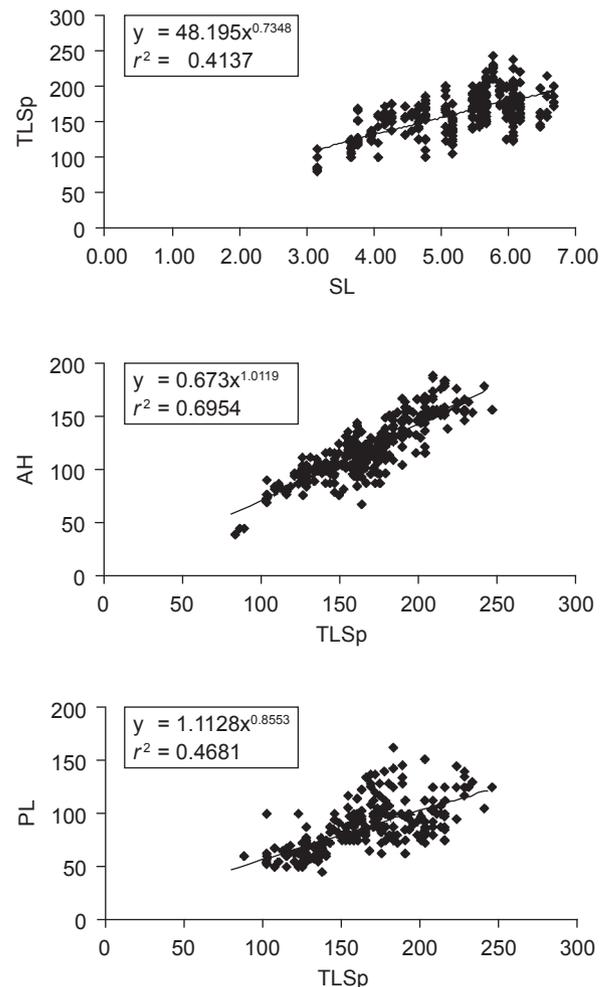


Fig. 6. *Pagurus exilis*. Diagrams of dispersion of empirical points for the relationships between hermit crabs (mm) and spermatophore (μm) dimensions. TLSp, total length of spermatophore; SL, shield length; AH, ampulla height; PL, peduncle length.

(Scelzo et al. 2004), *Birgus latro* (Sato et al. 2008), and *Cal. tibicen* (Amadio and Mantelatto 2009). In *P. exilis*, there is wide variation in spermatophore size, as a function of the size of the crabs. Therefore, we agree with Amadio and Mantelatto (2009) who recommended measuring the size of spermatophores from crabs of different sizes.

The ampulla is composed of 2 halves or valves that meet at a conspicuous lateral ridge. It is mostly filled with many spermatozoa; however, empty ampullae were found. We believe that this is a natural condition, but we cannot reject the possibility that it was an effect of sample preparation. This structure was recorded in most of the pagurid hermit crabs studied. In representatives of the family Paguridae, this capsule is called the main ampulla, and was recorded as being augmented by a secondary smaller one, named the accessory ampulla (Tudge 1999). Four species of pagurids lack an accessory ampulla: *Cestopagurus timidus* (Mouchet 1931), *Solitariopagurus tuerkayi*, *Xylopagurus caledonicus*, and *P. chevreuxi* (Tudge 1999). There is a single accessory ampulla for each spermatophore, and it is attached to the main ampulla in its lower portion, near the stalk. The main ampulla of the pedunculate spermatophore is a distally situated capsule containing most of the spermatozoa (Tudge 1999), whereas the accessory ampulla is smaller and contains from no to only a few spermatozoa.

The shape of the main ampulla in the genus *Pagurus* appears to primarily be elongate and cylindrical. However, the spermatophores produced by *P. exilis* do not have a main ampulla with this shape. In this species, the main ampulla is an ovoid and slightly flattened capsule, as in *P. pollicaris*, *P. longicarpus*, and *P. hirtimanus* (Tudge 1999). We thus considered that at least 2 patterns of spermatophore morphology are present in the family Paguridae. The accessory ampullae of *P. exilis*, *P. pollicaris*, and *P. longicarpus* (Tudge 1999) are more similar to each other than to other pagurid species. The 1st 2 species have a large wide accessory ampulla that extends along the entire length of the peduncle, and is larger than those in the spermatophores of most known hermit-crab species. Usually, only the presence of the accessory ampulla is recorded, with no reference to its shape or relative size; however, the shape and size may be informative and should receive specific attention.

There is no single pattern for the spermatophore peduncle, a structure that shows very wide

variation in members of the Paguridae, as thoroughly reviewed and compared by Tudge (1999). This structure can be of variable lengths and shapes: apparently non-existent (*P. excavatus* and *P. hirtimanus*); long and thin (*P. chevreuxi*), when the peduncle is equal to or longer than the main ampulla; short and thin (*P. maclaughlinae* and *X. caledonicus*), when the peduncle is smaller than the main ampulla; or short and wide (*P. pollicaris*, *P. longicarpus*, and *P. exilis* reported in the present study) (Tudge 1999). Another peculiar characteristic of the spermatophore stalk of *P. exilis* and other pagurid species is the absence of a clear transition between the main ampulla and the stalk, similar to the "neck" present in spermatophores of the Diogenidae and Coenobitidae (Tudge 1991, Scelzo et al. 2004, Tirelli et al. 2007 2008, Amadio and Mantelatto 2009, Mantelatto et al. 2009a). The peculiarity of the pagurid spermatophore peduncle is attributed to the previously mentioned lack of 2 regions of its vas deferens, which secrete and elongate the peduncle (Matthews 1957, Greenwood 1972). This absence resulted in pagurid spermatophores being considered non-stalked (Matthews 1957). However, a peduncle is gradually formed, being molded from the ampulla sheath (Greenwood 1972), and consequently there is no evident transition between these 2 parts of the spermatophore.

The base or pedestal is the point of attachment for the spermatophore when it is applied to the external surface of a female crab during mating. The commonest arrangement is for each spermatophore to be attached to a single pedestal; but in many cases, more than 1 spermatophore is attached to the same pedestal. One, 2, and seldom 3 spermatophores for each pedestal were found in *P. exilis* (present study). The pedestal is most often oval, but a shape approximately triangular or rectangular was seen in *P. exilis*. In this species, as in others of the family such as *P. novaezelandiae*, *P. maclaughlinae*, *P. longicarpus*, and *Micropagurus acantholepis* (Greenwood 1972, Tudge 1999, Tudge and Lemaitre 2004), the pedestal is an isolated unit instead of the long cord with many jointed spermatophores that is present in other hermit-crab families (e.g., the Diogenidae and Parapaguridae).

Tudge (1991) presented a practical branching key to classify hermit crabs into their respective families based on spermatophore morphology. Our findings agree with that author, in which light-microscope observations of spermatophores

were successfully used to distinguish hermit-crab families of the Paguroidea, especially the Diogenidae, due to the diversified patterns found for the Paguridae. The spermatophore morphology in the numerous members of Paguridae was described for only 8 genera and 31 species, including the present study (Table 2), and additional contradictions will likely appear when spermatophore studies widen to include more species. The branching key developed by Tudge (1991) that indicated the characteristic of the spermatophore with the “presence of main ampulla and accessory ampulla” for the Paguridae is not very practical, because some pagurid species do not appear to have an accessory ampulla (see above). Perhaps it is more convenient to characterize the pagurid spermatophore as having the “presence of main ampulla mostly with accessory ampulla and attached to a non-continuous base or pedestal”. The addition of more characteristics in the descriptions, especially of morphological details, might be a useful tool to avoid this kind of confusion as knowledge of spermatophores increases.

The Paguridae is a highly diverse family, and includes the polyphyletic genus *Pagurus*. This genus has an increasing number of described species, with more than 170 species currently assigned worldwide. Their affinities and phylogenetic inferences are, as yet, poorly defined (Mantelatto et al. 2009b), and the number of sister groups is rapidly increasing (Tudge 1997, Lemaitre and Cruz Castaño 2004). Some of these species are closely allied, and can be inserted in informal groups of the *Pagurus* that were proposed by various carcinologists based on morphological similarities (Lemaitre and Cruz Castaño 2004). One such group, known as the *Group exilis*, is composed of 7 species, including *P. exilis* (Forest and Saint Laurent 1968, McLaughlin 1974, Lemaitre et al. 1982). *Pagurus longicarpus* is the only other species of this group for which the spermatophore morphology is known (Tudge 1999), and it is very similar to the pattern of *P. exilis* described here.

Spermatophore descriptions may be an important tool in determining phylogenetic relationships. Recently Tirelli et al. (2008) carried out a phylogenetic analysis of some diogenid species based on the ultrastructural morphology of the spermatophore and sperm, and the results agree with most current morphologically based taxonomy. Obviously, other taxonomic criteria used in combination with spermiotaxonomy may

help elucidate the phylogeny and evolution among the Paguridae, as well as within the Anomura. There still remain many genera and species of Paguridae for which spermatophore/sperm morphology is unknown, and their description and comparison should be topics for future spermiotaxonomic studies.

Acknowledgments: This study was supported in part by the following grants: PIP CONICET 5135/06 and Universidad Nacional de Mar del Plata, EXA 357/06 (Mar del Plata, Argentina). We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasilia, Brazil) during the International Cooperative Project - CNPq/Prosul Program (grants 490340/2004-0 and 490122/2006-0), which provided financial support to FLM and MAS during the Brazil-Argentina visiting program. MZF and FLM are also respectively indebted to CAPES (Brasilia, Brazil) and CNPq for research fellowships. Special thanks go to those who collaborated during the course of this study, especially to Emerson C. Mossolin of the Laboratory of Bioecology and Crustacean Systematics of FFCLRP/USP (Ribeirão Preto, Brazil) for his help during field work, to the Postgraduate Program in Comparative Biology of FFCLRP/USP and the Centro de Biologia Marinha (CEBIMar/USP, São Sebastião, Brazil) for support, and to anonymous reviewers for suggestions and contributions which improved this paper. This research is dedicated to the memory of the fisherman José Luis Ungarelli, in recognition and appreciation of his great help during field work in Argentina. Fernando A. Scelzo (Mar del Plata, Argentina) and Janet W. Reid (JWR Associates, Martinsville, Virginia, USA) revised the English text.

REFERENCES

- Amadio LM, FL Mantelatto. 2009. Description of the male reproductive system of the hermit crab *Calcinus tibicen* (Decapoda: Anomura: Diogenidae). *J. Crustacean Biol.* **29**: 466-475.
- Bennati-Mouchet S. 1934. Castration parasitaire de l'*Eupagurus prideauxi* par le *Peltogaster curvatus*. *Trav. Stat. Biol. Roscoff* **12**: 9-19.
- Boschi EE, CE Fischbach, MI Iorio. 1992. Catálogo ilustrado de los crustáceos Estomatópodos y Decápodos marinos de Argentina. *Frente Marit.* **10**: 1-94.
- Brown GG. 1966. Ultrastructural studies on Crustacea spermatozoa and fertilization. PhD thesis, Univ. of Miami, Miami, FL.
- Chevallier P. 1970. Recherches sur la structure et les

- constituants chimiques des cellules germinales males de Crustacés Décapodes. PhD dissertation, Univ. of Rennes, Rennes, France.
- Fasten N. 1917. Male reproductive organs of Decapoda, with special reference to Puget Sound forms. Puget Sound Mar. Stat. Publ. **1**: 285-307.
- Forest J. 1995. Crustacea Decapoda Anomura: revision du genre *Trizopagurus* Forest, 1952 (Diogenidae), avec l'établissement de deux genres nouveaux. In A Croisnier, ed. Résultats des Campagnes MUSORSTOM. Mém. Mus. natl. d'Hist. nat. **163**: 9-149.
- Forest J, M Saint-Laurent. 1968. Campagne de la Calypso au large de côtes atlantiques de l'Amérique du Sud (1961-1962). 6. Crustacés Décapodes: Pagurides. Ann. Inst. Oceanogr. **45**: 47-169.
- Fretter V, A Graham. 1976. A functional anatomy of invertebrates. London: Academic Press, 589 pp.
- Greenwood JG. 1972. The male reproductive system and spermatophore formation in *Pagurus novae-zelandiae* (Dana) (Anomura: Paguridae). J. Nat. Hist. **6**: 561-574.
- Hamon M. 1937. Les mécanismes produisant de la déhiscence des spermatophores d'*Eupagurus prideauxi* Leach. C. R. Acad. Sci. **204**: 1504-1506.
- Hamon M. 1939a. La constitution chimique des spermatophores de Crustacés supérieurs du groupe des Pagurides. C. R. Seances Soc. Biol. **130**: 1312-1315.
- Hamon M. 1939b. Détermination de la présence de certains acides aminés dans les spermatophores de pagurides. C. R. Seances Soc. Biol. **131**: 109-110.
- Hess GS, R Bauer. 2002. Spermatophore transfer in the hermit crab *Clibanarius vittatus* (Crustacea, Anomura, Diogenidae). J. Morphol. **253**: 166-175.
- Lemaitre R, N Cruz Castaño. 2004. A new species of *Pagurus* Fabricius, 1775 from the Pacific coast of Colombia, with a checklist of eastern Pacific species of the genus. Nauplius **12**: 71-82.
- Lemaitre R, PA McLaughlin, J García-Gómez. 1982. The provenzanoi group of hermit crabs (Crustacea, Decapoda, Paguridae) in the western Atlantic. Part IV. A review of the group, with notes on variations and abnormalities. Bull. Mar. Sci. **3**: 670-701.
- Manjón-Cabeza ME, JE García Raso. 2000. Morphological reproductive aspects of males of *Diogenes pugilator* (Roux, 1829) (Crustacea, Decapoda, Anomura) from southern Spain. Sarsia **85**: 195-202.
- Mantelatto FL, R Biagi, AL Meireles, MA Scelzo. 2007a. Shell preference of the hermit crab *Pagurus exilis* (Anomura: Paguridae) from Brazil and Argentina: a comparative study. Rev. Biol. Trop. **55**: 153-162.
- Mantelatto FL, DLA Espósito, M Terossi, R Biagi, AL Meireles. 2007b. Population features of the western Atlantic hermit crab *Pagurus exilis* (Anomura, Paguridae) in Brazil. Atlántica **29**: 107-114.
- Mantelatto FL, LM Pardo, LG Pileggi, DL Felder. 2009b. Taxonomic re-examination of the hermit crab species *Pagurus forceps* and *Pagurus comptus* (Decapoda: Paguridae) by molecular analysis. Zootaxa **2133**: 20-32.
- Mantelatto FL, MA Scelzo, CC Tudge. 2009a. Morphological and morphometric appraisal of the spermatophore of the southern hermit crab *Isocheles sawayai* Forest and Saint-Laurent, 1968 (Anomura: Diogenidae) with comments on gonopores in both sexes. Zool. Anz. **248**: 1-8.
- Matthews DC. 1957. Further evidences of anomuran non-pedunculate spermatophores. Pac. Sci. **11**: 380-385.
- McLaughlin PA. 1974. The hermit crabs (Crustacea, Decapoda, Paguridae) of northwestern North America. Zool. Verh. **130**: 1-396.
- McLaughlin PA. 1980. Comparative morphology of the recent Crustacea. San Francisco, CA: WH Freeman, 177 pp.
- McLaughlin PA. 1983. Internal anatomy. In LH Mantel, ed. The biology of Crustacea. London: Academic Press, Vol. 5, pp. 1-52.
- McLaughlin PA, R Lemaitre, U Sorhannus. 2007. Hermit crab phylogeny: a reappraisal and its "fall-out". J. Crustacean Biol. **27**: 97-115.
- McLaughlin PA, M Saint Laurent. 1998. A new genus for four species of hermit crabs formerly assigned to the genus *Pagurus* Fabricius (Decapoda: Anomura: Paguridae). Proc. Biol. Soc. Wash. **111**: 158-187.
- Meireles AL, M Terossi, R Biagi, FL Mantelatto. 2006. Spatial and seasonal distribution of the hermit crab *Pagurus exilis* (Benedict, 1892) (Decapoda: Paguridae) in the Southwestern coast of Brazil. Rev. Biol. Mar. Oceanogr. **14**: 87-95.
- Melo GAS. 1999. Manual de identificação dos Crustacea Decapoda do litoral brasileiro: Anomura, Thalassinidea, Palinuridea e Astacidea. São Paulo: Plêiade Editora, 551 pp.
- Mouchet S. 1930. Mode de formation des spermatophores chez quelques Pagures. C. R. Acad. Sci. **190**: 691-692.
- Mouchet S. 1931. Spermatophores des Crustacés Décapodes Anomures et Brachyours et castration parasitaire chez quelques Pagures. Bull. Stat. Oceanogr. Salammbô **6**: 1-203.
- Nucci PR, GAS Melo. 2003. A new species of *Pagurus* (Decapoda: Anomura: Paguridae) from Brazil. J. Mar. Biol. Assoc. UK **83**: 351-353.
- Pérez C. 1930. Asymétrie viscerale et dimorphisme des spermatophores chez quelques Pagures. C. R. Acad. Sci. **190**: 393-396.
- Sato T, K Yoseda, O Abe, T Shibuno. 2008. Male maturity, number of sperm, and spermatophore size relationships in the coconut crab *Birgus latro* on Hatoma Island, southern Japan. J. Crustacean Biol. **28**: 663-668.
- Scelzo MA, EE Boschi. 1969. Desarrollo larval del cangrejo ermitaño *Pagurus exilis* (Benedict) en laboratorio. Physis **29**: 165-184.
- Scelzo MA, FL Mantelatto, CC Tudge. 2004. Spermatophore morphology of the endemic hermit crab *Loxopagurus loxochelis* (Anomura, Diogenidae) from the southwestern Atlantic - Brazil and Argentina. Invertebr. Reprod. Develop. **46**: 1-9.
- Schaller F. 1979. Significance of sperm transfer and formation of spermatophores in arthropod phylogeny. In AP Gupta, ed. Arthropod phylogeny. New York: Van Nostrand Reinhold, pp. 587-608.
- Sokal RR, FJ Rohlf. 1981. Biometry. New York: WH Freeman, 1-859 pp.
- Subramoniam T. 1991. Chemical composition of spermatophores in decapod crustaceans. In RT Bauer, JW Martin, eds. Crustacean sexual biology. New York: Columbia Univ. Press, pp. 308-321.
- Subramoniam T. 1993. Spermatophore and sperm transfer in marine crustaceans. Adv. Mar. Biol. **29**: 129-214.
- Terossi M, DLA Espósito, AL Meireles, R Biagi, FL Mantelatto. 2006. Pattern of shell occupation by the hermit crab *Pagurus exilis* (Anomura, Paguridae) on the northern coast of São Paulo State, Brazil. J. Nat. Hist. **40**: 77-87.

- Terossi M, LS Torati, I Miranda, MA Scelzo, FL Mantelatto. 2010. Comparative reproductive biology of two southwestern Atlantic populations of the hermit crab *Pagurus exilis* (Crustacea: Anomura: Paguridae). *Marine Ecology*. (in press)
- Tirelli T, E Campantico, D Pessani, C Tudge. 2006. Description of male reproductive apparatus of the hermit crab *Calcinus tubularis* (Decapoda: Anomura: Diogenidae). *Crust. Res.* **6**: 13-21.
- Tirelli T, E Campantico, D Pessani, C Tudge. 2007. Reproductive biology of Mediterranean hermit crabs: male reproductive apparatus of *Clibanarius erythropus* (Decapoda: Anomura: Diogenidae). *J. Crustacean Biol.* **27**: 404-410.
- Tirelli T, D Pessani, D Silvestro, C Tudge. 2008. Reproductive biology of Mediterranean hermit crabs: fine structure of spermatophores and spermatozoa of *Diogenes pugilator* (Decapoda: Anomura) and its bearing on a sperm phylogeny of Diogenidae. *J. Crustacean Biol.* **28**: 534-542.
- Tudge CC. 1991. Spermatophore diversity within and among three hermit crab families, Coenobitidae, Diogenidae, and Paguridae (Paguroidea, Anomura, Decapoda). *Biol. Bull.* **181**: 238-247.
- Tudge CC. 1995. Ultrastructure and phylogeny of the spermatozoa of the infraorders Thalassinidea and Anomura (Decapoda, Crustacea). In Jamieson BGM, JL Justine, eds. *Advances in spermatozoal phylogeny and taxonomy*. *Mem. Mus. Natl. Hist. Nat.* **166**: 251-263.
- Tudge CC. 1997. Phylogeny of the Anomura (Decapoda: Crustacea): spermatozoa and spermatophore morphological evidence. *Contrib. Zool.* **67**: 125-141.
- Tudge CC. 1999. Spermatophore morphology in the hermit crab families Paguridae and Parapaguridae (Paguroidea, Anomura, Decapoda). *Invertebr. Reprod. Dev.* **35**: 203-214.
- Tudge CC, BGM Jamieson. 1991. Ultrastructure of the mature spermatozoa of the coconut crab *Birgus latro* (L.) (Coenobitidae, Paguroidea, Decapoda). *Mar. Biol.* **108**: 395-402.
- Tudge CC, R Lemaitre. 2004. Studies of male sexual tubes in hermit crabs (Crustacea, Decapoda, Anomura, Paguroidea). I. Morphology of the sexual tube in *Micropagurus acantholepis* (Stimpson, 1858), with comments on function and evolution. *J. Morphol.* **259**: 106-118.
- Watling I. 1989. A classification system for crustacean setae based on the homology concept. In BE Felgenbauer, I Watling, AB Thisle, eds. *Functional morphology of feeding and grooming in Crustacea*. Rotterdam: AA Balkema, *Crust. Issues* **6**: 15-26.