Morphology of the vasa deferentia of *Parastacus defossus* and *P. varicosus* and comparison within the Parastacidae

Clarissa K. Noro; Daiana da Silva-Castiglioni; Laura López-Greco;
Ludwig Buckup; Georgina Bond-Buckup

(CKN, DS-C, LB, GB-B) Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul. Av. Bento Gonçalves 9500, prédio 432435, 9501970, Porto Alegre, RS, Brazil (clarissanoro@hotmail.com). (LL-G) Laboratory of Comparative Animal Physiology, Department of Biodiversity and Experimental Biology, FCEyN, University of Buenos Aires, Argentina.

Abstract

In decapod crustaceans, spermatophores are specialized structures used for the transfer of mature spermatozoa from male to female during the mating process. With the purpose to characterize the vas deferens and spermatophore formation, two Brazilian species of crayfishes were investigated: *Parastacus defossus* and *P. varicosus*. Members of the freshwater crayfish family Parastacidae have two patterns of spermatophores: in the genus *Cherax,* found in Oceania, the spermatophore is composed of two layers, and each vas deferens is highly convoluted and has three macroscopically distinguishable parts; in Brazilian species *P. defossus* and *P. varicosus,* the vas deferens is short and straight, and no macroscopically different parts can be identified. The spermatophore is formed by only one layer, which consists of a PAS-positive fluid where spermatozoa are embedded. This PAS-positive matrix corresponds to the secondary layer found in the spermatophore of the Australian species of *Cherax*. The spermatophore structure in *Parastacus* is, therefore, simpler than in the studied Astacidae and than in the *Cherax* species.

Key words: Morphology, Parastacidae, *Parastacus,* spermatophore, vas deferens.

Introduction

In decapod crustaceans the male reproductive system is composed of testis and vasa deferentia leading to the external gonopore. The vas deferens functions to pack the spermatozoa into spermatophores which protect them against desiccation and microbial infection, among other functions. Upon exit from the testis into the vas deferens, the spermatozoa are surrounded by epithelial secretions that consolidate the sperm mass and develop the noncellular spermatophore wall layers (Dudenhausen and Talbot, 1983; Bauer, 1986; Subramoniam, 1991; Krol *et al.,* 1992; Vogt, 2002).

The spermatophores are stored in the distal vas deferens and during copulation, they are extruded through the gonopores and transferred to the female. In decapods there is considerable morphological variation and three general types of spermatophores are recognised. The simplest type is found in brachyuran crabs, where the spermatophore is spherical or ellipsoidal and consists of a sperm mass surrounded by a thin, noncellular wall, which is suspended in a seminal fluid in the vas deferens. The second type, produced by the (Galatheidea, Penaeidea, Caridea, Astacidea, Palinura, and Thalassinidea, is tubular and consists of a sperm mass covered by several investing layers of variable number and thickness. The third type is the pedunculate form present in most anomurans, which is composed of sperm-filled ampullae elevated on stalks which are attached to a common gelatinous base or pedestal (except for species of the family Hippidae) (see Kooda-Cisco and Tal-

bot, 1982; Dudenhausen and Talbot, 1983; Subramoniam, 1984; Bauer, 1986; Tudge, 1991, 1997, 1999).

In the infraorder Astacidea, the spermatophore is of the tubular type. The spermatophore ultrastructure is well known in the marine lobster *H. americanus* H. Milne Eduards, 1837 (Kooda-Cisco and Talbot, 1982) but in freshwater crayfishes it has been examined in only a few species. In the Astacidae, there is information for *P. leniusculus* (Dana, 1852) (Dudenhausen and Talbot, 1983; Vogt, 2002) and in the Parastacidae, for *C. tenuimanus* Smith, 1912, *C. albidus* Clark, 1936 (Beach and Talbot, 1987; Talbot and Beach, 1989), and *C. quadricarinatus* Von Martens, 1868 (López Greco *et al.,* 2007).

There is no information available on the spermatophore structure in Brazilian parastacid species. In addition, another important feature about the South American species of the genus *Parastacus,* is that the presence of male and female gonopores in the same individual is a common characteristic (Rudolph 1995a). Although intersexuality is common in parastacids and is a strong indication of the occurrence of hermaphrodism, functional hermaphrodism is rare among crayfishes and is of the protandric type (Rudolph 1995b). The first case of hermaphrodism in the genus, was described by Rudolph (1995b) for the Chilean species *P. nicoleti* (Philippi, 1882). Hermaphrodism was later reported for *P. brasiliensis* and *Samastacus spinifrons* (Philippi, 1882)*,* by Almeida and Buckup (2000) and Rudolph (1999, 2002), respectively.

Due to this lack of information and with the purpose to characterize the vas deferens and spermatophore formation, two Brazilian species of crayfishes were investigated: *Parastacus defossus* is a fossorial species found only in Rio Grande do Sul (Brazil) and Uruguay (Buckup and Rossi, 1980), and *Parastacus varicosus,* a species found in freshwater environments in Uruguay, Argentina and Brazil (Buckup, 1999).

Materials and Methods

Ten male specimens of *Parastacus defossus* were collected with a partial-vacuum pump in the Lami region, Porto Alegre Municipality, Rio Grande do Sul State, Brazil, and ten male specimens of *P. varicosus* were collected with traps at Cova do Touro, Gravataí, Porto Alegre Municipality, Rio Grande do Sul State, Brazil.

In the laboratory, the carapace length of the animals was measured with a digital caliper, then they were anesthetized in a -4 degrees C freezer for 20 min and finally dissected. The testis and vas deferens were removed and fixed in Bouin's solution for 4-6 hours. After they were dehydrated through increasing concentrations of ethanol (from 70% to 100%) (for 20 min) and then embedded in paraffin. The vas deferens were sectioned to 7 μm thick (with a LEICA RM 2145 ultramicrotome) and were stained with haematoxylin-eosin and Periodic Acid Schiff (PAS) (modified from Behmer *et al.* 1976).

The sections were analyzed under a estereomicroscope (Olympus CX31) and photographs of the vas deferens sections were taken.

Results

The vas deferens histological analysis was made in males with a cephalothoracic length between 27.40 and 31.42 mm. Only male fase specimens were used for analysis, which could be confirmed by the observations of the gonads in the moment of the dissection.

In *P. varicosus* and in *P. defossus,* both males and females possess two pairs of genital ducts, the oviducts extend toward the female openings on the coxae of the third pereiopods and the vas deferens extends to the coxae of the fifth pereiopods, where the male genital openings are located. In the male fase specimens, the oviducts are very thin and translucent, and together with the condition of obstruction of the female gonopore indicate the lack of functionality of the female function. The vas deferens is short and straight, and no different parts can be macroscopically identified (figure 1A). The mean length of the vas deferens of both species analyzed was 13.02 mm (SE = ± 0.61 mm).

By means of the histological analysis, the vas deferens wall consists of an external layer of connective tissue, a central muscular layer and internal epithelial tissue (Fig. 1C-F). It was possible to microscopically distinguish three regions: proxi-

mal, medium and distal vas deferens (Figure 1A). The proximal vas deferens has a thin diameter (mean = 0.15 mm, SE = ± 0.012 mm) and a single-layered epithelium. Above this epithelium, a thin muscular layer is seen. The middle vas deferens also has a thin epithelium and a medium muscular layer and is the thickest portion of the vas deferens (mean = 0,53 mm, SE = ± 0.034 mm). The andro-

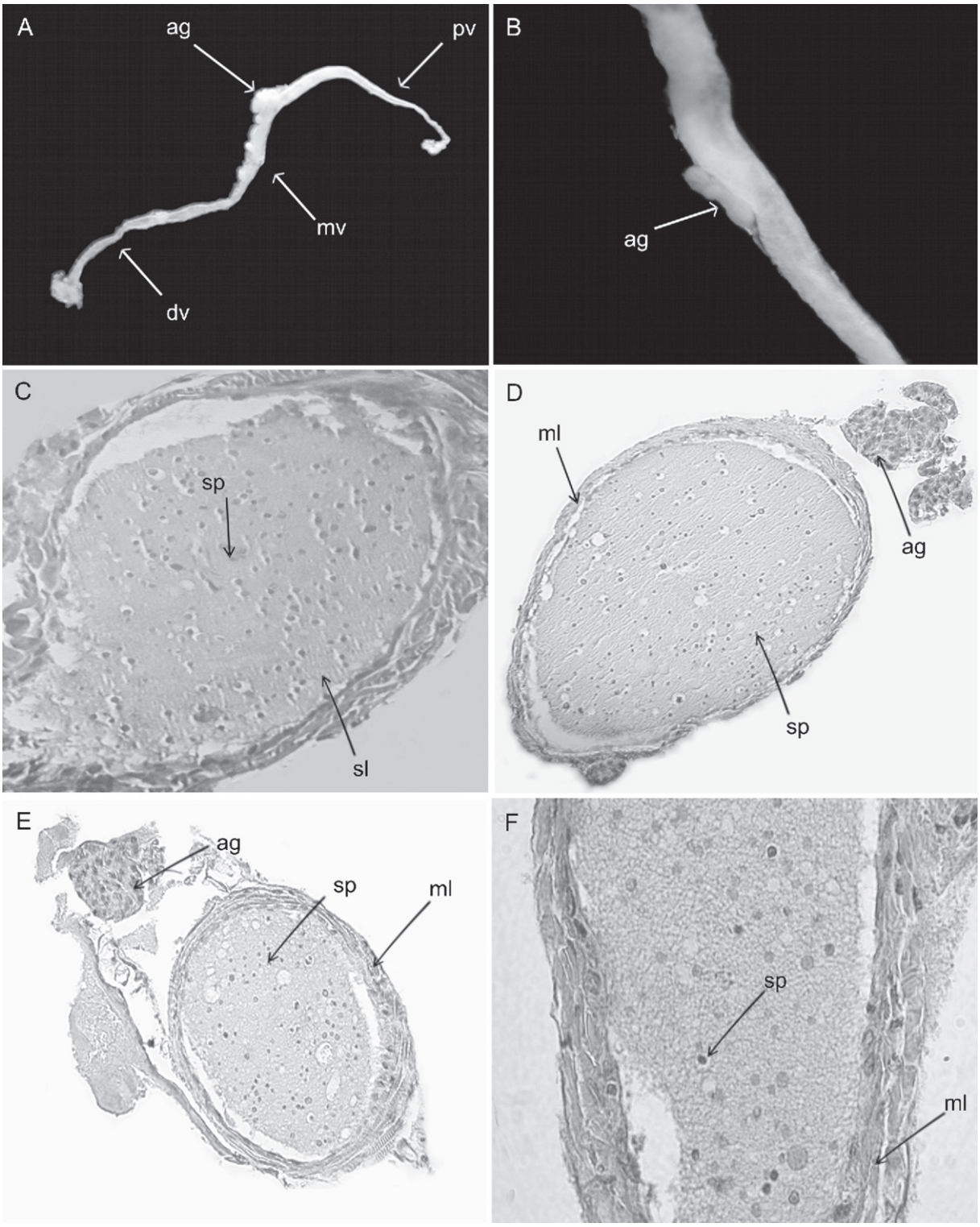

Figure 1. A. *Parastacus defossus,* proximal, middle and distal vas deferens. B. Detail of the androgenic gland. C. *Parastacus varicosus,* histology of middle vas deferens. D-E. *P. defossus,* histology of middle vas deferens. F. *P. defossus,* histology of distal vas deferens. Ag: androgenic gland; pv: proximal vas deferens; mv: middle vas deferens; dv: distal vas deferens; Sp: spermatozoa; sl: secondary layer of spermatophore; ml: muscular layer.

genic gland occurs in this region (Figure 1B). The distal vas deferens has a thick muscular layer and has a diameter of 0.20 mm (SE = ± 0.012 mm) becoming narrower as approximates to the gonopore in the fifith pereiopod coxae.

In the lumen of the proximal, middle, and distal vas deferens, PAS-positive material was observed surrounding the mass of spermatozoa (Figure 1C-F). Our histological observations on *P. defossus* and *P. varicosus* indicate that the spermatophore is formed by only one layer, which consists of a PAS-positive fluid in which spermatozoa are embedded.

Discussion

In freshwater crayfishes, the spermatophore structure has been examined in only a few species. In the North American astacid crayfish *Pacifastacus leniusculus,* the spermatophore consists of two main parts, a sperm mass composed of spermatozoa embedded in a matrix, and a noncellular wall formed from secretions produced in the vas deferens. Each individual sperm cell is surrounded by a thin capsule. The wall of the spermatophore is composed of three concentric layers, a thin primary layer which directly surrounds the sperm mass, a thick middle layer, and a thick outer globular layer (Dudenhausen and Talbot, 1983). On extrusion, the sticky outer layer transforms into a fibrillar thickened ridge anchoring the oval end of the spermatophore to the female body and the middle layer is responsible for the hardening of the spermatophore for prolonged external storage (Dudenhausen and Talbot, 1983; Subramoniam, 1991).

In the Parastacidae, the spermatophore structure differs from the three concentric layers found in the Astacidae. In parastacid species found in Oceania *(Cherax albidus, C. destructor,* Clark, 1936, and *C. quadricarinatus),* the spermatophore is composed of two layers, and the vasa deferentia are highly convoluted and have three macroscopically distinguishable portions: proximal, middle, and distal vas deferens (Talbot and Beach, 1989; Jerry, 2001; López Greco *et al.,* 2007). In *C. quadricarinatus,* the proximal vas deferens has a single-layered epithelium composed of tall cylindrical cells. There is also a single layer of muscle cells and connective tissue internal to the epithelium. In the vas deferens lumen, a PAS-positive material surrounds the mass of spermatozoa (primary layer of the spermatophore). In the distal vas deferens a thick muscle layer can be seen and the contraction of this muscle tissue is responsible for the evacuation of the spermatophore from the gonopore (López Greco *et al.,* 2007). In *C. albidus* and *C. tenuimanus,* the spermatozoa entering the proximal vas deferens, from the testis, are supported in a matrix which becomes surrounded by the primary spermatophore layer during their transit through the proximal segment. The secondary layer begins to be secreted in the middle vas deferens, but the final synthesis occurs in the distal vas deferens, which also functions to store the mature spermatophore (Beach and Talbot, 1987; Talbot and Beach, 1989).

Histologically, the organization of the vas deferens of *P. defossus* and *P. varicosus* is similar to that of *Cherax* species studied by Talbot and Beach (1989) and by López Greco *et al.,* (2007). The vas deferens structure of *P. defossus* and *P. varicosus,* is similar to that of *P. brasiliensis* (see Almeida and Buckup, 1997; 2000) and in the Chilean *P. nicoleti* (see Rudolph, 1995b; Rudolph *et al.,* 2001) but differs from the Chilean species *S. spinifrons* where some kind of macroscopically visible regionalization is observed (Rudolph, 2002); although no histological studies have been done to elucidate microscopic aspects.

For the South American Parastacidae, there are no detailed studies of the spermatophore structure. However, the two-layered spermatophore found in *Cherax* species was not observed in *P. defossus* and *P. varicossus.* Our histological observations on *P. defossus* and *P. varicosus* showed that the spermatophore is formed by only one layer, which consists of a PAS-positive fluid where spermatozoa are embedded. This PAS-positive matrix corresponds to the secondary layer found in the spermatophore of the Australian species of *Cherax* (Talbot and Beach, 1989; López Greco *et al.,* 2007).

Second Subramonian (1993), lobsters and crayfish spermatophores are generally complex masses consisting mainly of spermatophoric tubes embedded in a protective gelatinous matrix.

However, in *Parastacus,* the spermatophoric tubes are absent and this overall spermatophore structure implies that sperm would be evacuated from the testis to the vas deferens without any capsule surrounding each spermatozoa (as in the astacid *Pacifastacus*) and without the primary acellular layer present in *Cherax* species. The spermatophore structure in *Parastacus* is, therefore, simpler than in the studied Astacidae and than in the *Cherax* species.

Another interesting fact is the size and position of the androgenic gland. In *P. defossus* and *P. varicosus* the androgenic gland could be seen in the middle vas deferens while in *C. destructor* and *C. quadricarinatus,* this gland is located in the distal part of the vas deferens (Fowler and Leonard, 1999), as is also usual in other studied Decapoda (Charniaux-Cotton and Payen, 1985).

Spermatophores of several astacids are known to harden on exposure to water. The mechanism of hardening has long been controversial, mainly because of a lack of information about the chemical nature of the spermatophore layers (Subramoniam, 1991). Recent studies on lobster and crayfish have shed some light on this question, by examining the structural layers of the spermatophore (Kooda-Cisco and Talbot 1982, 1986; Dudenhausen and Talbot, 1983; Beach and Talbot, 1987; Talbot and Beach, 1989; Subramoniam, 1991; López Greco *et al.,* 2007).

Currently, no information is available about the mechanisms involved in spermatophore transfer, hardening, and dehiscence in South American Parastacidae, and we have never observed females with spermatophores of *P. defossus* and *P. varicosus* neither in nature nor in the laboratory.

Some aspects of the external morphology of the Parastacidae are different from the freshwater crayfishes of the Northern Hemisphere (families Cambaridae and Astacidae) and may be related to different mating strategies. In the Cambaridae e Astacidae, the main external character of sexual differentiation is the form of the pleopods, especially of the first abdominal somite that in males, are modified for spermatophore transfer (Holdich 2002). Secondary sexual characteristics of sexually active cambarid females include a prominent cornified seminal receptacle *(annulus ventralis)* located between the bases of the walking legs where the spermatophore are deposited (Huner and Barr, 1991; Vogt, 2002). In the Parastacidae, the first abdominal somite bears no pleopods in either males or females, and the simultaneous and constant presence of both pairs of genital pores in individuals of *Parastacus* renders identification of the sex in these animals difficult (Holdich, 2002). The exception is the Chilean species *P. nicoleti,* which shows wide variability of sexual forms (Rudolph 1995b).

The structural modification in spermatophores of the South American genera *Samastacus* and *Parastacus* compared to *Cherax* and Astacidae is most likely related to different mating strategies (external versus internal), morphology of mating structures (gonopores and pleopods), habitats, life histories or phylogenetic trends that need to be studied.

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