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OCCURRENCE OF PROTANDRIC HERMAPHRODITISM IN A POPULATION OF THE NEOTROPICAL FRESHWATER CRAYFISH *PARASTACUS BRASILIENSIS* (PARASTACIDAE)

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ABSTRACT

Preliminary studies have shown that males and females of the freshwater crayfish *Parastacus brasiliensis* (von Martens, 1869) have an intersexed internal genitalia characterized by the existence of genital ducts of both sexes that connect to a gonad with male or female components, according to the sex. To determine the type of sexuality of this species, specimens ($n = 92$) from 11.7 to 40.0 mm carapace length were collected at Mariana Pimentel municipality, Rio Grande do Sul, Brazil (30°20′39″S, 51°22′39″W). Anatomical and histological analysis of the gonads disclosed the existence of three sexual forms: (1) intersexed males ($n = 36$), (2) transitionals between male and female sex ($n = 8$), and (3) intersexed females ($n = 48$). The transitionals present the following common morphological features: both genital apertures are present, but the female apertures are closed, as is usual in males; coexistence of oocytes and testicular acini in the same gonad (ootestis); and a longitudinal collecting tubule, large in diameter in the testicular region. The ovarian component of the gonad includes previtellogenic and primary vitellogenic oocytes. Of the 44 individuals classified as males by the analysis of the aspect of the genital apertures, 18.2% were transitionals, suggesting the existence of primary males that never change sex and a sexual system that is known as partial protandry. The existence of small females suggests that some females never go through a male phase. The histology of the gonads and genital ducts is described, and the germ cells are identified in each sex. The possible reasons for the existence of hermaphroditism in the studied population are discussed based on theoretical models.

Intersexuality, i.e., the existence of both male and female sexual characteristics in an individual, has been documented in gonochoric (nonfunctional hermaphroditism) and hermaphrodite species (functional hermaphroditism) (Khalaila and Sagi, 1997). According to those authors, intersexuality can either be limited to external morphology or reach differentiation of the gonads. Hermaphroditism, i.e., the capacity a set of genes has to enable the simultaneous or sequential formation of gametes of both sexes, is a particularly interesting strategy due to the diversity of forms occurring within the Crustacea.

Sequential hermaphroditism is characterized by the presence of gonads of both sexes in the same individuals at different times in their lives. According to Warner (1975), when the first sex is female, the condition is called protoginy, and when the first sex is male, the condition is called protandry. In Crustacea, the change of sex is mainly protandric, for 82% of the sequential hermaphrodite species change from male to female (Brook *et al.*, 1994).

Intersexuality has been well documented in the Parastacidae, such as in species of Australian *Cherax* Erichson, 1846 (see Sokol, 1988; Medley and Rouse, 1993; Sagi *et al.*, 1996), *Engaeus* Erichson, 1846, and *Engaewa* Riek, 1867 (see Horwitz, 1988).

In the neotropical genus *Parastacus* Huxley, 1879, that occurs in southern South America, intersexuality, characterized by the existence of gonopores and genital ducts of both sexes in the same individuals, has been documented since the last century (von Martens, 1869; Faxon, 1898; Lönnberg, 1898). In *P. nicoleti* (Philippi, 1882) from Chile, the phenomenon is due to the existence of partial protandric hermaphroditism (Rudolph, 1995). The same system seems to occur in *Samastacus spinifrons* (Philippi, 1882), another Chilean parastacid (Rudolph, 1999). In *P. pugnax* (Poeppig, 1835) (see Rudolph, 1997), *P. varicosus* Faxon, 1898, and *P. pilimanus* (von Martens, 1869) (Rudolph, personal communication), intersexuality is not associated with the existence of hermaphroditism.

Almeida and Buckup (1997) studied the reproductive system of *P. brasiliensis* (von Martens, 1869). The existence of two pairs of genital ducts in testes and ovaries was verified: an anterior pair (oviducts) that connect to the female gonopores located in the coxopodites of the third pair of pereiopods, and a posterior pair (vasa deferentia) that connect to the male gonopores located in the coxopodites of the fifth pair of pereiopods. The authors did not find any gonad in transition between the two sexes.

The purpose of this study was to determine the type of sexuality of *P. brasiliensis*, a freshwater crayfish species which burrows in the humid banks of lotic systems in Rio Grande do Sul, the southernmost state of Brazil. The investigation included the histology of the gonads, the male vas deferens and female oviduct, and the identification of germinative cells of both sexes.

MATERIALS AND METHODS

Specimens of *P. brasiliensis* were collected between September 1997 and December 1998 in the municipality of Mariana Pimentel (30°20′39″S, 51°22′39″W), in the State of Rio Grande do Sul, Brazil. Specially designed traps were used, made of PVC pipes of 50 mm diameter and 20 cm length (see Fontoura and Buckup, 1989a).

A total of 92 animals were obtained. In the laboratory, emphasis was given to the identification of sexual forms. Hence, the animals were separated by sex, measured, and dissected. Carapace length (CL) was measured from the anterior tip of the rostrum to the posterior margin of the carapace. The sex of the animals was determined following Almeida and Buckup (1997).

The animals were dissected according to the technique suggested by Kükenthal *et al.* (1969). To identify the sex in the smaller specimens, slides with fresh gonads were prepared for study under an optical microscope. A note was made about the state of the gonads and genital ducts at the time of dissection. The anatomic parts which were removed for histological examination were fixed in Bouin's solution for 24 h and later preserved in 70% alcohol for at least 24 h, followed by dehydration, diaphanization, and embedding in paraffin. The gonads were longitudinally sectioned, whereas the genital ducts were transversely sectioned. Sections 5 and 7 µm thick were stained with H-E.

The identification of the histological and cytological components of the reproductive systems followed Word and Hobbs (1958), Wielgus-Serafinska (1976), Adiyodi and Subramoniam (1983), and Krol *et al.* (1992).

RESULTS

A total of 92 animals were collected—36 males, 48 females, and 8 with ootestis. Carapace lengths (CL) were 13.0 mm for the smallest male and 39.8 mm for the largest; 11.7 mm for the smallest female and 40.0 mm for the largest. The smallest mature female, i.e., with completely unobstructed female apertures, was 27.9 mm CL.

Sexual Forms

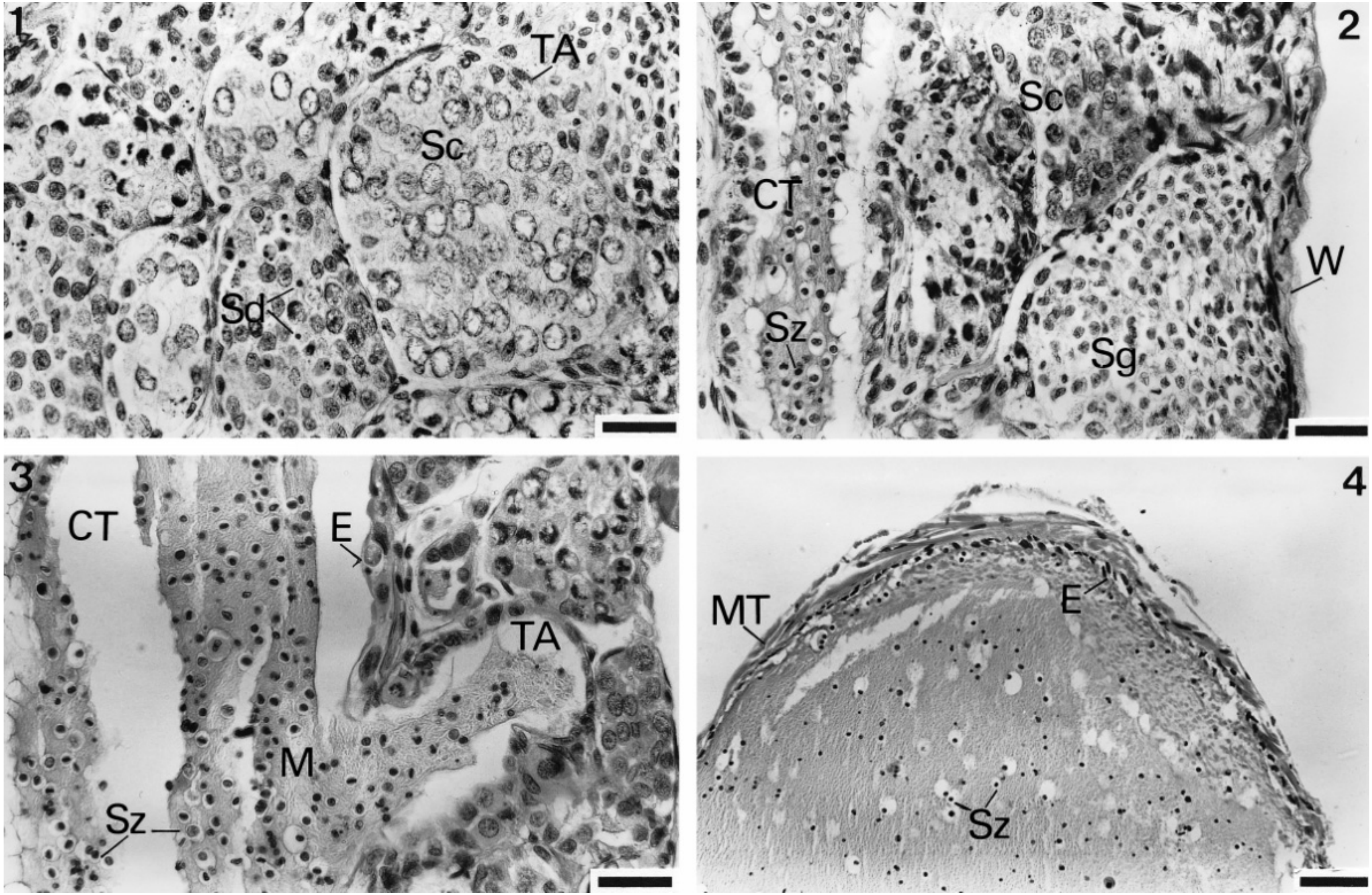
Anatomical and histological analysis of the gonads of *P. brasiliensis* indicated the existence of three sexual forms: (1) intersexed males, (2) transitionals, and (3) intersexed females. As mentioned earlier, males and females are intersexed, with two pairs of genital ducts (oviducts and vasa deferentia) that connect to the respective gonopores. The anatomical and functional aspects of the reproductive system of the intersexed males and females was described by Almeida and Buckup (1997). Transitionals, which also possess two pairs of genital ducts, display ootestis, where germ cells of both sexes can be found. The presence of this sexual form indicates that these animals change sex.

Histology and Cytology of Gonads and Genital Ducts

Intersexed Male Form.—The main unit of the testis is the testicular acinus (Figs. 1–3), inside of which a sequence of transformations and cell divisions take place leading to the formation of spermatozoa.

The male gonad is covered with connective tissue that penetrates the gonad at some points (Fig. 2). Inside the testis there is the remarkable presence of epithelial tissue, which is responsible for the formation of the walls of both the acini and the collecting tubules, in the form of a simple cubic epithelium. These tubules stretch longitudinally, from the front to the back area of the testis, and their function is to lead the spermatozoa from the acini to the vas deferens (Figs. 2, 3).

The histological preparations showed acini with spermatozoa inside releasing its content directly into the collecting tubule (Fig. 3). The acinus may degenerate after the spermatozoa are formed, as occurs with other species of crayfish (Word and Hobbs, 1958; Wielgus-Serafinska, 1976). Thus, the acini located adjacent to the tubule are possibly the first to release their spermatozoa, followed by the acini located in the periphery of the gonad. This is probably the reason why this tubule has an increased diameter at the end of the



Figs. 1–4. *Parastacus brasiliensis*. Sections of the reproductive system of the male intersexed form. Figs. 1–3. Longitudinal sections of the testis. Scales = 100 µm. Fig. 4. Transversal section of the male vas deferens. Scale = 125 µm. (CT) collecting tubule; (E) epithelium; (M) matrix; (MT) muscular tissue; (Sc) spermatocytes; (Sd) spermatids; (Sg) spermatogonia; (Sz) spermatozoa; (TA) testicular acini; (W) testicular wall.

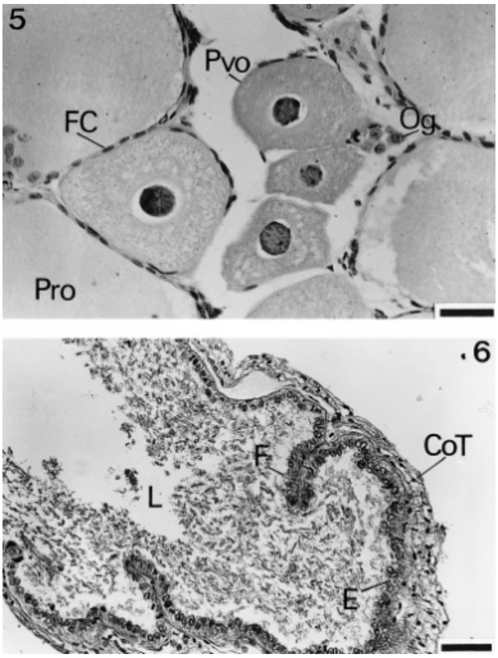
reproductive period and during the period of reproductive inactivity, because this diameter is smaller during the reproductive period. While inside the tubule, the spermatozoa seem involved in a matrix (Figs. 2, 3).

Spermatogonia are cells with scarce cytoplasm (Fig. 2) when compared with the cytoplasm of spermatocytes (Figs. 1, 2), which are cells with a slightly larger nucleus. Spermatids are cells with eosinophilic cytoplasm and small nucleus (Fig. 1), central at first but eccentric later on, which suffer a decrease in their cytoplasm during spermiogenesis.

Although the vas deferens is made up of three distinct parts (proximal, medial, and distal), the histology of the male vas deferens displays little variation throughout its length. The wall of the vas deferens consists of a thin external layer of connective tissue, adjoining the testis; a central muscular layer, especially developed in the medial and distal regions where the ejaculatory duct is located; and a reduced layer of epithelial tissue in the inside (Fig. 4), different from what is described for other species of crayfish whose epithelium is columnar (Johnson, 1960; Delong and Payne, 1987).

Intersexed Female Form.—The fundamental unit of the ovary is the ovarian follicle. The germinal zones, which contain the germinal epithelium where oogonia and follicular cells originate (Krol *et al.*, 1992), are located in the central region of the gonad. The oogonia are more frequently seen in immature gonads. The area where oocytes grow lies between the germinal zone and the ovary wall, which is made up of an external connective layer; it is there that oocytes are covered with follicular cells, from the beginning of vitellogenesis, making up the follicle (Fig. 5).

The female oviducts also have a wall made up of a thick external layer of connective tissue and an internal layer formed by a cubic epithelium. The wall of the oviduct is folded (Fig. 6), which may be related to the necessity to withstand the pressure of the oocytes during egg-laying.



Figs. 5, 6. *Parastacus brasiliensis*. Sections of the reproductive system of the female intersexed form. Fig. 5. Longitudinal section of the ovary. Scale = 100 µm. Fig. 6. Transversal section of the female oviduct. Scale = 125 µm. (CoT) connective tissue; (E) epithelium; (F) fold; (FC) follicular cells; (L) lumen; (Og) oogonia; (Pro) primary vitellogenic oocyte; (Pvo) previtellogenic oocyte.

Description of the Individuals with Ootestis

The size of transitionals varied between 27.1 and 35.5 mm CL. Of the eight individuals belonging to this sexual form, four were dissected in March 1998, and they measured 28.0, 28.3, 29.7, and 30.5 mm CL. Two individuals were dissected in July 1998, and they measured 27.1 and 33.9 mm CL. One individual was dissected each month during October 1997 and April 1998, and they measured 31.7 and 35.5 mm CL, respectively. The specimens with ootestis displayed some common morphological features. On the outside, the female gonopores were always closed as in males or in females prior to sexual maturity, while the male gonopores had medium and large projections, as in males. On the inside, male and female germ cells coexisted in the same gonad, oocytes were in previtellogenesis and primary vitellogenesis and surrounded by follicular cells, and a longitudinal collecting tubule of large diameter was in the testicular region (Figs. 7, 8). The existence of spermatogenetic activity inside the acini indicates that these animals can fertilize females even at the beginning of feminization, thus maximizing its reproductive success. When the oocytes were in the front region of the ootestis, there was always a

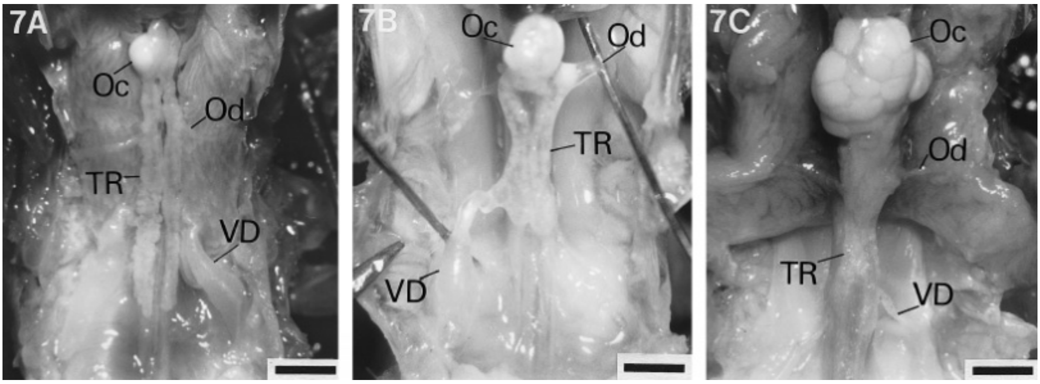


Fig. 7. *Parastacus brasiliensis*. Dorsal views of three ootestes, showing the gradient of feminization; 7A, few oocytes differentiated in the anterior end of the gonad; 7B and 7C, oocytes occupying the whole of the anterior region of the gonad. Scales = 2 mm. (Oc) oocytes; (Od) oviduct; (TR) testicular region; (VD) vas deferens.

large number of them. Oocytes in medial and posterior regions were always isolated among testicular acini. The vasa deferentia were enlarged, as in males, but the oviducts were sometimes widened, as in females (see Almeida and Buckup, 1997).

Discussion

The external analysis of the gonads of the 44 specimens considered males by the analysis of the aspect of the gonopores showed that eight of them (18.2%) displayed structures that resembled female germ cells found inside their testis, similar to what is seen in protandrous hermaphrodites of *P. nicoleti* in male phase (see Rudolph, 1995). The histological sections of these gonads, which were supposedly in a transitional phase, showed that the structures were actually female sexual cells characterizing the ootestis. In sequential hermaphroditic decapods, ootestes are a characteristic feature of the transitional period, where the gonad is in a redifferentiation phase. Differently from *P. nicoleti*, where all individuals in the male phase have male gonads with oocytes in previtellogenesis, *P. brasiliensis* males have only acini in their testes. So this species perhaps occupies an intermediary position between the two Chilean species, because some specimens have ootestes, like *P. nicoleti,* whereas others are intersexed males and females with two pairs of apertures and genital ducts, like *P. pugnax.*

The existence of varying numbers of oocytes inside the ootestis, from small amounts to full occupancy of the regions (Fig. 7), reveals a gradient of feminization such as observed by Gherardi and Calloni (1993) in *Athanas indicus* (Coutière, 1903) (Caridea: Alpheidae) and Rudolph (1999) in *S. spinifrons*. These oocytes stay chiefly in primary vitellogenesis, which may be explained, according to Charniaux-Cotton and Payen (1985), by the inhibiting action of the androgenic hormone on the secondary vitellogenesis. Recently, Khalaila and Sagi (1997) demonstrated that the ablation of the androgenic gland (responsible for the production of androgenic hormone) in intersexed specimens of the parastacid *Cherax quadricarinatus* (von Martens, 1868) results in an increase of the gonadosomatic index of the ovarian component.

The existence of large males suggests the existence of primary males that never change sex, which is known as partial protandry, the sexual system that was first described by Bauer (1986) in *Thor manningi* Chace, 1972 (Caridea: Hippolytidae) and also verified in *A. indicus* (see Gherardi and Calloni, 1993) and in *P. nicoleti* (see Rudolph, 1995). The existence of small females suggests that some of them never go through a male phase and are, therefore, primary intersexed females.

Rudolph (1997) comments that the coexistence of oviducts and vasa deferentia in males and females of *P. pugnax* challenges the control that the androgenic gland exerts over sex differentiation in Malacostraca. Many of the results obtained in relation to sexual differentiation of decapods have con-

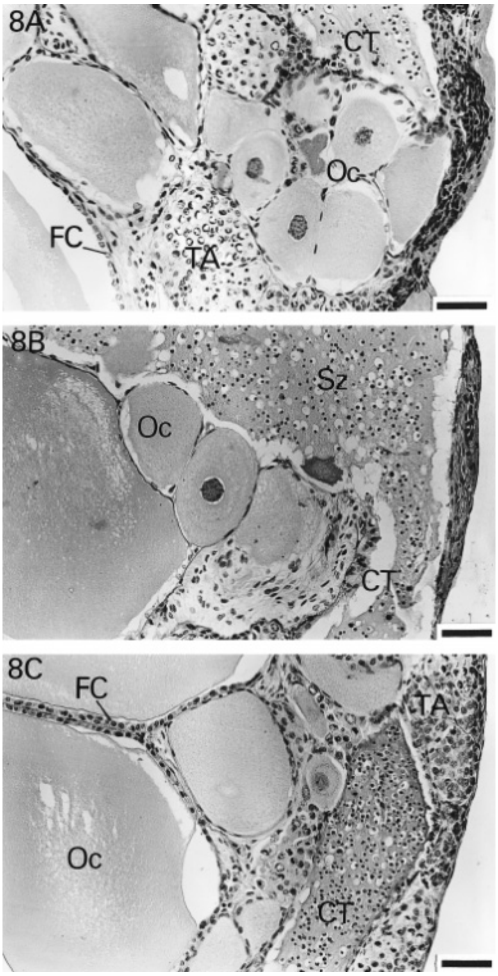


Fig. 8. *Parastacus brasiliensis*. Longitudinal sections of three ootestes. 8A and 8C, oocytes rounded by testicular acini with spermatogenetic activity. 8B, oocytes near to a calibrous collecting tubule, full of spermatozoa. Scales = 125 μm. (CT) collecting tubule; (FC) follicular cells; (Oc) oocytes; (Sz) spermatozoa; (TA) testicular acini.

firmed those in relation to the Peracarida (see Sagi *et al.*, 1997), which suggest that the mechanism of sexual differentiation must be common to all the Malacostraca (see Hasegawa *et al.*, 1993). Implants of the androgenic gland after the fourth postembryonic molt in females of *Armadillidium vulgare* (Latreille, 1804) do not inhibit the development of oviducts (Hasegawa *et al.*, 1993). Hence, a late development of the androgenic gland could explain the development of oviducts in males of *P. brasiliensis*.

Once the existence of hermaphroditism in a population has been detected, a natural question arises: which factor(s) is (are) responsible for the selection of hermaphroditism in this population? Two theoretical models were proposed by Ghiselin (1969) to account for the evolution of sequential hermaphroditism. They are the *size advantage model* and the *gene dispersal model.*

The *size advantage model* is based on the fact that the reproductive success of a sex is enhanced in individuals of a particular size (Ghiselin, 1969). In *P. brasiliensis*, it is estimated that the females reach sexual maturity at the age of three years when they are an average of 31.37 mm CL, based on a correlation between the size of the female and fecundity (Fontoura and Buckup, 1989b). Anatomical and histological analyses showed that males with CL smaller than these produce spermatozoa. Therefore, if a specimen, while small-sized, is potentially able to perform during reproduction as a male and, as it enlarges, acquires the sex that is more favorable and, principally, is capable of behaving as a male during the transitional phase, this animal would have its reproductive success notably augmented. Males with an ootestis are from 27.1 to 35.5 mm CL, and this is very close to the size of sexually mature females.

The *gene dispersed model* is based on the fact that the limitations over population dispersion can affect the populational structure, due to the reduction of gene flow and, consequently, genetic variability (Ghiselin, 1969). Factors such as environmental heterogeneity, absence of the larval stage, and dependence on humidity undoubtedly restrict the dispersion of *P. brasiliensis*. Therefore, individuals might not move too far away from their birth places, and hermaphroditism would decrease the probability of inbreeding.

The situation described above indicates that there is partial protandric sequential hermaphroditism in some individuals of the population of *P. brasiliensis* studied. Studies of the life history of *Parastacus* populations must be made, together with studies of the role of the androgenic gland in the sexual differentiation of the species of this genus.

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