

INTERSEXUALITY IN THE BURROWING CRAYFISH, *PARASTACUS PILIMANUS* (VON MARTENS, 1869) (DECAPODA, PARASTACIDAE)

BY

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ABSTRACT

Various authors have reported the occurrence of supernumerary gonopores in many species of *Parastacus*, which has generated discussion about their sexual system. In this study, we examine the external and internal sexual characters of 102 specimens of the burrowing crayfish, *Parastacus pilimanus*, captured in the province of Maldonado in southeast Uruguay, with the aim of clarifying its sexual system. In this study, we show that in all the specimens analysed, female and male gonopores co-existed externally, and we verified the presence of only one type of gonad internally: either ovary (intersex females) or testis (intersex males), from which one pair of oviducts and another of sperm ducts originated, connecting the gonad to the respective gonopores. We also verified that all specimens smaller than 27.2 mm cephalothorax length (CL) (N = 73) had the same external morphology. Nevertheless, dissection revealed that their gonads were already differentiated. We established that 54.8% of these specimens were intersex females, while the remaining percentage corresponded to intersex males. Twenty-nine specimens were found with a CL equal to, or above, 27.2 mm. Of these, 34.5% (N = 10) were intersex males with the same external morphological traits that characterize all specimens smaller than 27.2 mm CL. The remaining 19 specimens (65.5%) were intersex females, 15 of which presented secondary sexual characters, probably associated with egg incubation. The evidence gathered suggests that *Parastacus pilimanus* has permanent intersexuality, similar to *Parastacus pugnax* and *Parastacus varicosus* in Uruguay. We conclude by identifying some topics that require further study, in order to understand the adaptive value of this type of intersexuality.

RESUMEN

Varios autores han reportado la ocurrencia de gonoporos supernumerarios en muchas especies de *Parastacus*, lo que ha generado discusión acerca del sistema sexual de ellas. En este trabajo nosotros examinamos los caracteres sexuales externos e internos de 102 especímenes del camarón excavador *Parastacus pilimanus* capturados en el Departamento de Maldonado, sureste de Uruguay,

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con el objetivo de clarificar su sistema sexual. Aquí nosotros demostramos que todos los especímenes analizados presentaron externamente coexistencia de gonoporos de hembra y de macho, e internamente una gónada única — ya sea ovario (hembras intersexo) o testículo (machos intersexo) — desde la cual se originan un par de oviductos y otro de espermiductos que la conectan a los respectivos gonoporos. También comprobamos que todos los especímenes de tallas menores a 27,2 mm de longitud cefalotorácica (LCT) ($N = 73$), tienen la misma morfología externa. No obstante, las disecciones demostraron que sus gónadas ya estaban diferenciadas. En ellos comprobamos que el 54,8% eran hembras intersexo y el porcentaje restante machos intersexo. De tallas iguales o superiores a 27,2 mm de LCT, encontramos 29 especímenes. De ellos, el 34,5% ($N = 10$) eran machos intersexo los que presentaron los mismos rasgos morfológicos externos que caracterizan a todos los especímenes de tallas inferiores a 27,2 mm de LCT. Los 19 especímenes restantes (65,5%) eran hembras intersexo, en 15 de las cuales comprobamos la presencia de caracteres sexuales secundarios, probablemente asociados a la incubación de los huevos. Las evidencias reunidas sugieren que *Parastacus pilimanus*, al igual que *Parastacus pugnax* y *Parastacus varicosus* de Uruguay, presenta intersexualidad permanente. Nosotros concluimos sugiriendo algunos tópicos que necesitan de mayores estudios para comprender el valor adaptativo de este tipo de intersexualidad.

INTRODUCTION

Intersexuality is characterized by the occurrence of sexual characters of both sexes in the same individual. It can be limited to the external morphology, or extend to gonadal differentiation, and can be present in both gonochoristic and hermaphroditic species (Khalaila & Sagi, 1997). Its origin is attributed to different causes, e.g., parasitary or viral infections (Ginsburger-Vogel, 1991; Juchault et al., 1991), genetic or hormonal anomalies (Zou & Fingerman, 2000; Parnes et al., 2003; Weeks et al., 2006), and to the effects of contaminating chemicals present in the environment (Ford et al., 2004; Jungmann et al., 2004). Over the last few years, numerous cases of intersexuality have been reported in Malacostraca, especially in Amphipoda (cf. Dunn et al., 1994; Ladewig et al., 2003; McCurdy et al., 2004; Ford & Fernandes, 2005), Isopoda (cf. Korczynski, 1988; Rigaud & Juchault, 1998), and Decapoda (cf. Galil & Tom, 1990; Laurent, 1991; Micheli, 1991; Zou & Fingerman, 2000; Hanamura & Ohtsuka, 2003; Turra, 2004; Tang et al., 2005). In freshwater crayfishes of the family Parastacidae, intersexed species have been reported in the genera *Engaeus*, *Engaewa*, *Cherax*, *Euastacus*, *Parastacus*, *Samastacus*, and *Virilastacus* (cf. Horwitz, 1988; Medley & Rouse, 1993; Honan & Mitchell, 1995; Rudolph & Almeida, 2000; Rudolph et al., 2007).

Ever since Von Martens (1869) and Faxon (1898) established that *Parastacus* species are characterized by the occurrence of supernumerary gonopores, their sexuality has been the subject of discussion. The functional significance of the supernumerary gonopores in this genus has been poorly documented for many years, mainly due to the small number of specimens analysed (Von Ihéring, 1893; Lönnberg, 1898; Hay, 1905; Turner, 1935; Thompson, 1982). While Lönnberg

(1898) and Hay (1905) postulated a probable hermaphroditism, the other authors cited discarded that alternative. Nevertheless, from the 1990s onwards, studies on the sexual system of this species have notably increased. Evidence gathered in these recent studies permits to distinguish two sexual systems in *Parastacus*: partial protandric hermaphroditism in *Parastacus nicoleti* (Philippi, 1882), *Parastacus brasiliensis* (Von Martens, 1869), and *Parastacus defossus* Faxon, 1898 (cf. Rudolph, 1995; Almeida & Buckup, 2000; Noro et al., 2008), and permanent intersexuality in *Parastacus pugnax* (Poeppig, 1835) (cf. Rudolph, 1997). The sexual system of *Parastacus varicosus* Faxon, 1898, has been studied by Rudolph et al. (2001) and by Silva-Castiglioni et al. (2008) in Uruguayan and Brazilian populations, respectively. Nevertheless, this species can not be classified into any of the aforementioned sexual systems, because the results obtained are contradictory. According to Rudolph et al. (2001), the species presents permanent intersexuality, whereas Silva-Castiglioni et al. (2008) classify it as a protandric hermaphrodite.

Parastacus pilimanus (Von Martens, 1869) is a burrowing species that constructs shallow galleries (maximum 30 cm) with numerous entrances (3 to 7), that converge in a large central chamber (Buckup & Rossi, 1980). Its geographic distribution includes the southeast of the Río Grande do Sul state in Brazil, northeast Argentina, and southeast Uruguay (Amestoy, 1982; Morrone & Lopretto, 1994). Only some aspects of the biology of this species are known, related to its burrowing behaviour (Amestoy, 1982) and early embryonic and postembryonic development under laboratory conditions (Amestoy, 1983). Thompson (1982) analysed the anatomy and gonadal histology of 100 *Parastacus pilimanus* and *Parastacus varicosus* females, concluding that they have gonopores of both sexes, connected to the ovary through a double set of gonoducts. From then on, no further contributions have been made to the knowledge on the sexuality of *Parastacus pilimanus*.

This article describes the external and internal sexual characters of *Parastacus pilimanus*, aimed at clarifying the sexual system of this South American parastacid.

MATERIAL AND METHODS

One-hundred-and-two specimens of *Parastacus pilimanus* were collected in July 1998, using a partial vacuum pump, in the province of Maldonado (34°54'09''S 54°56'55''W), in southeast Uruguay. Immediately after capture, the specimens were fixed in Bouin's solution for 24 hours and then conserved in 70% ethanol. In the laboratory, the descriptions and keys of Riek (1971) and Hobbs (1974) were used to identify the species. Morphological characteristics of each specimen were examined under a stereomicroscope, equipped with a drawing

chamber. The morphometric data, recorded using callipers to 0.01 mm accuracy, were: cephalothorax length (CL), length of abdominal somites, width of abdominal somites 2 to 6, height of the pleura on abdominal somites 2 to 5, length of the pleuron on abdominal somite 2, length and width of the telson, and length and width of the uropodal endopodite and exopodite. The relationship between the cephalothorax length and each of these morphometric characters was verified, in the male and female intersexes, through regression analysis. In each regression a Student's *t*-test was applied, to establish differences between the respective slopes (Zar, 1999). The sex ratio was compared with a hypothetical 1 : 1 relationship, using the chi-square test with Yates correction. Width of the gonoducts was measured using an ocular micrometer. Some specimens (N = 78) of different sizes were also selected to analyse gonad and gonoduct anatomy. Subsequently, these structures were removed for histological study that began with dehydration in a series of alcohols, followed by diaphanization and subsequent embedding in paraffin. Sections, 7 μ m thick, were stained with hematoxylin-eosin. In order to identify and characterize gonadal histology, the following descriptions and photomicrographs were used: Hobbs et al. (2007) in Astacidea, Payen (1973) in *Astacus leptodactylus* (Eschscholtz, 1823), Rudolph (1995, 1997) in *Parastacus nicoleti* and *Parastacus pugnax*, respectively, and Rudolph et al. (2001) in *Parastacus varicosus*. The terminology proposed by Hobbs et al. (2007) was adopted to designate the structures of the male genitalia. A Wild equipment was used for photomicrographic recording of the external and internal sexual characters. Finally, the photographs were manipulated in Adobe Photoshop 6.0.

RESULTS

External sexual characters

Coexistence of female and male gonopores was observed in all specimens of *Parastacus pilimanus* collected (fig. 1). Analysis of the morphological characteristics of their gonopores disclosed two sexual forms:

Intersex 1. — Specimens (N = 87) with female gonopores, internal border more prominent than external border, giving them a semi-ellipsoidal aspect, in the coxae of the third pair of pereopods (P₃). No setae were observed on either of these borders, or close to them. The cuticle that covered them was slightly convex, calcified, with no trace of an opening (fig. 2A). These specimens also showed a small, fixed, calcified phallic papilla, close to the internal border of the ventral surface of the coxae of the fifth pair of pereopods (P₅) in the form of a truncated cone, and a male gonopore that opens onto its apical end. Both P₅ coxae show male

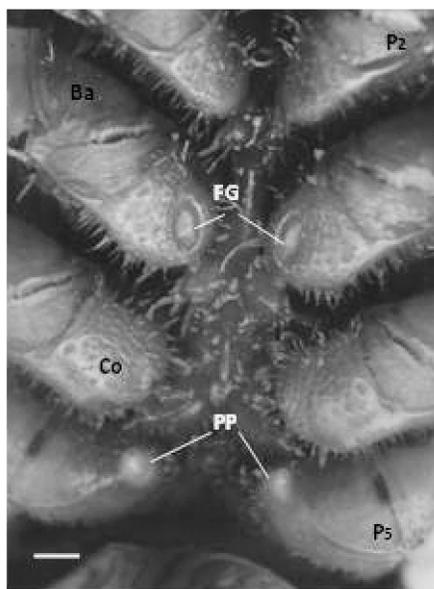


Fig. 1. *Parastacus pilimanus* (Von Martens, 1869). Sternal region and coxae of pereopods 2 through 5, showing the coexistence of male and female gonopores. FG, female gonopore; PP, phallic papillae; Co, coxa; Ba, basis; P₂, second pair of pereopods; P₅, fifth pair of pereopods. Scale bar = 2.0 mm.

cuticle partition (fig. 2B). The size of these intersex specimens ranged from 10.8 to 44.5 mm CL.

Intersex 2.— Specimens (N = 15) with ellipsoidal female gonopores, external borders as prominent as internal borders. With many, relatively long setae on the surface of the coxa close to its external borders. Furthermore, these gonopores were covered with a non-calcified, semi-transparent, slightly convex membrane, or, alternatively, partially calcified, flat, and non-transparent. In both cases, these membranes presented a semi-ellipsoidal opening in the zone closest to the internal border of the gonopores (fig. 2C). The phallic papillae were morphologically similar to the papillae described in intersex 1. However, unlike the latter, they presented many setae around the base of the cone (fig. 2D). Both P₅ coxae also showed a male cuticle partition. The size of these intersexes ranged from 27.2 to 45.3 mm CL.

Anatomy of gonads and gonoducts

The gonad of *Parastacus pilimanus* is formed by a pair of longitudinal lobes, in close proximity to each other in the intersex females, with oocytes in advanced vitellogenesis and slightly more separated in the intersex males and females, with ovaries in previtellogenesis or incipient vitellogenesis. Nevertheless, no connecting transversal commissure was observed. Each of these lobes comprised an anterior

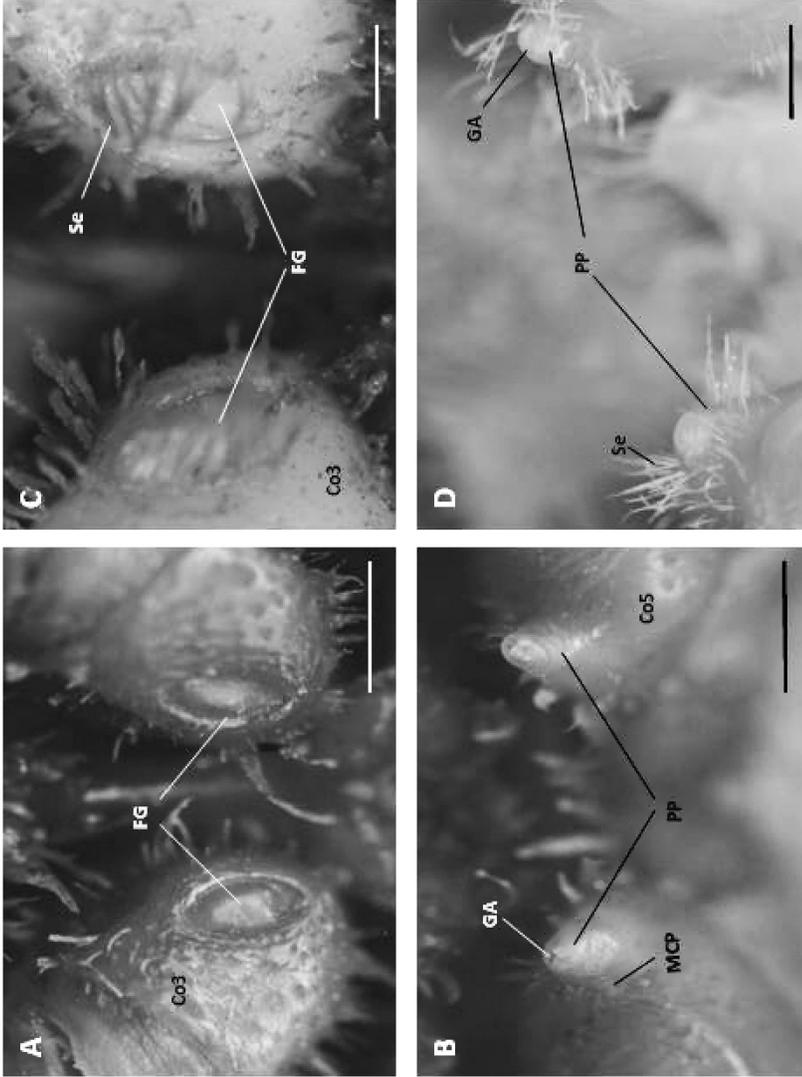


Fig. 2. *Parastacus pilimanus* (Von Martens, 1869). Gonopore morphology of the intersexes 1 and 2. A, B, female and male gonopores of intersexes 1, respectively; C, D, female and male gonopores of intersexes 2, respectively; Co3, coxopodite of P₃; Se, setae; FG, female gonopore; Co5, coxopodite of P₅; PP, phallic papillae; GA, genital atrium; MCP, male cuticle partition. Scale bars = 1.0 mm.

zone, dorsoventrally adjacent to the anterior hepatopancreas, and another, larger, posterior zone, located under the heart, that extends posterolaterally over the posterior hepatopancreas and/or the flexor and transverse thoracic muscles, without invading the abdomen. The gonoducts of both sexes originate from this last portion. The oviducts emerge close to the base of the anterior regions of the gonadic lobes. From there, they descend directly towards the P_3 coxae. Furthermore, they were flat, of a similar width all along, and without a seminal receptacle. The sperm ducts originated from the middle part of the posterior portion of each gonadic lobe, and from there they ran towards the P_5 coxae. All specimens presented gonoducts of both sexes, that connected one entirely female or entirely male gonad with the respective gonopores.

When the gonad was a testis, the oviducts were thin and translucent ($\varnothing = 0.27$ mm; $SD = \pm 0.057$; $N = 12$). In contrast, the sperm ducts were then cylindrical and it was possible to distinguish a short, straight, thin proximal portion; another wider medial portion ($\varnothing = 0.75$ mm; $SD = \pm 0.122$; $N = 12$), curved ventrally; and finally a distal portion, thinner ($\varnothing = 0.39$ mm; ± 0.151 ; $N = 12$) but longer than the anterior part. This distal part penetrated the musculature of the P_5 coxae and was observed to be connected to the ejaculatory zone of the sperm duct (fig. 3A). This latter opens to the exterior through the genital atrium, located at the end of a small, truncated, cone-shaped phallic papilla. In larger specimens, it was possible to distinguish the androgenic gland adjacent to the terminal third of the sperm duct's distal portion.

When the gonad was an ovary, the oviducts were wide ($\varnothing = 0.63$ mm; $SD = \pm 0.15$; $N = 11$). The sperm ducts, on the other hand, then were thin ($\varnothing = 0.29$ mm; $SD = \pm 0.014$; $N = 11$) and flat. Due to the aforementioned, it was not possible to distinguish the various sections observed in the sperm ducts of the specimens with testes (fig. 3B). Neither was it possible to distinguish the androgenic gland in this type of sperm duct.

All specimens smaller than 27.2 mm CL ($N = 73$) had supernumerary gonopores with the morphological characteristics described for intersex 1. Nevertheless, once dissected, it was observed that the gonad of each of these specimens was either an ovary, or a testis. Thus, it was established that 40 individuals (54.8%) were intersex females and 33 (45.2%), intersex males. Twenty-nine specimens were found to be of sizes equal to or larger than 27.2 mm CL. According to the morphology of the female gonopores, it was found that 14 of these latter specimens were intersex 1 and the remaining 15 were intersex 2. However, dissection of these 29 specimens revealed that 19 (65.5%) were intersex females, because their gonads were an ovary, and the remaining 10 (34.4%), whose gonads were a testis, intersex males. This showed that at sizes equal to or above 27.2 mm CL, 28.6% ($N = 4$) of the intersex 1 specimens were intersex females, whose external morphology did not yet exhibit the female condition.

Gonadal histology

Independent of the size of the specimens analysed, the testes of *Parastacus pili-manus* were whitish. They consisted of testicular acini and collecting tubules, surrounded by connective tissue. Each acinus contained cells in a similar spermatogenic stage, thus some were observed with spermatogonia, others with spermatocytes, or spermatids, or spermatozoa. The lumen of the collecting tubules can either be empty or be filled with varying quantities of spermatozoa (fig. 3C).

The size and colour of *Parastacus pilimanus* ovaries not only depended on the size of the females, but also on the vitellogenetic stage of the oocytes. However, in general, in small, intersex females (<27.2 mm CL) the oocytes were in previtellogenesis, thus both the oocytes and the ovaries were small and whitish. In intersex females larger than 27.2 mm CL, oocytes were in primary or secondary vitellogenesis; consequently, both the oocytes and the ovaries were larger and their colour fluctuated between yellow and orange. The ovaries were surrounded by an external muscular sheath. Oocytes surrounded by follicular cells were observed in their interior, immersed in the ovarian stroma (fig. 3D). Coexistence of the ovarian and testicular tissue in the same gonad was not observed in any of the histological sections.

Sex ratio

After dissecting the 102 specimens captured, 43 were established to be intersex males and 59 were intersex females, representing a sex ratio of 0.7 : 1 in favour of females. This value did not differ significantly from a 1 : 1 sex ratio ($\chi^2 = 3.84$, $P > 0.05$). When analysing sex ratio per size class, it was found that all classes contained a larger number of females (table I). Under 29.9 mm CL, there were 33 intersex males and 40 intersex females (0.8 : 1), above 29.9 mm, we found 10 intersex males and 19 intersex females (0.5 : 1).

Secondary sexual characters

No significant differences were found between the abdomens of intersex males and females smaller than 27.2 mm CL. However, when comparing the abdomens of the 15 intersex 2 females with those of the 10 intersex 1 males measuring over 27.2 mm in cephalothoracic length, significant differences ($t_{0.05(2),21} = 2.080$; $P \leq 0.05$) were found in 15 out of the 16 pairs of slopes analysed (table II). These females had significantly broader abdomens and higher pleura than the male intersex abdomens. Furthermore, the telson was wider and the uropodal endo- and exopodites were longer and wider.

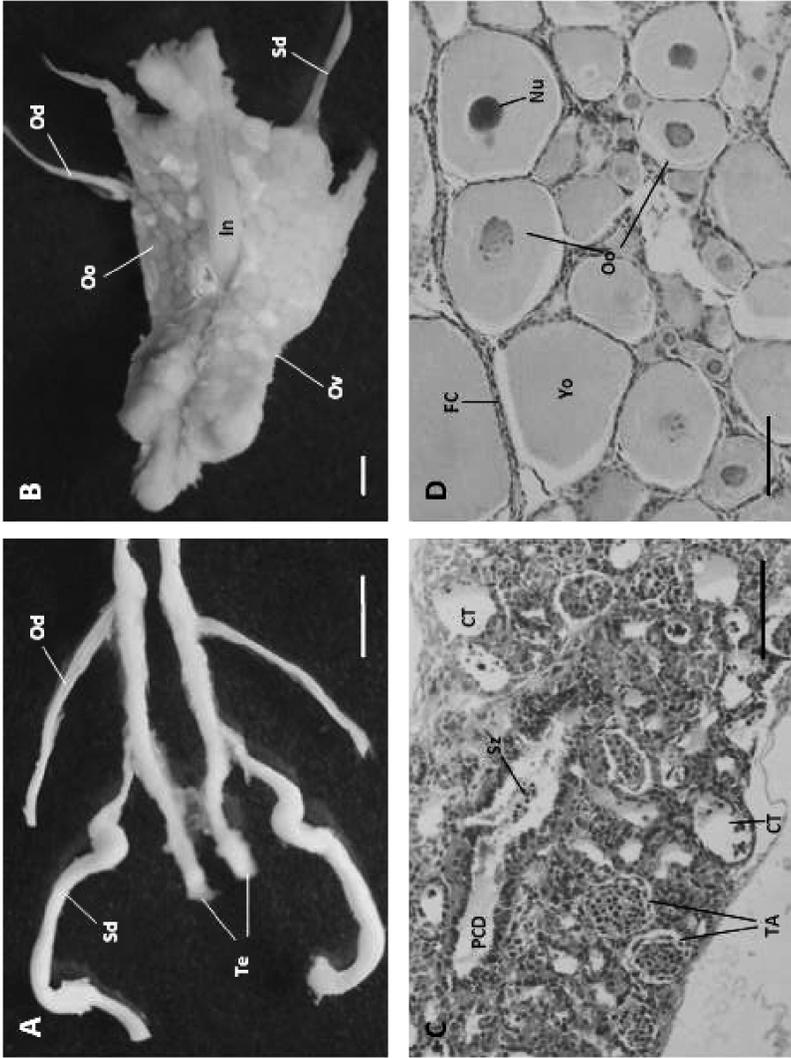


Fig. 3. *Parastacus pilimanus* (Von Martens, 1869). Anatomical and histological characteristics of the genitalia of intersexes 1 and 2. A, B, anatomy of the genitalia of the intersexes 1 and 2, respectively; C, D, histology of the gonads of intersexes 1 and 2, respectively; Sd, sperm duct; Od, oviduct; Te, testis; Oo, oocytes; Ov, ovary; PCD, primary collecting duct; CT, collecting tubule; TA, testicular acini; Sz, spermatozoa; Nu, nuclei; Yo, yolk. Scale bars = A, 3.0 mm; B, 2.0 mm; C, 120 μ m; D, 130 μ m.

TABLE I

Sex ratio by size range of *Parastacus pilimanus* (Von Martens, 1869), captured in the Province of Maldonado, Uruguay

Size ranges (CL mm)	Males		Females		Total	Rate
	N	%	N	%		
10-14.9	2	26.8	5	71.4	7	0.4 : 1
15-19.9	12	46.2	14	53.8	26	0.9 : 1
20-24.9	14	48.3	15	51.7	29	0.9 : 1
25-29.9	5	45.5	6	54.5	11	0.8 : 1
30-34.9	4	36.4	7	63.6	11	0.6 : 1
35-39.9	3	30.0	7	70.0	10	0.4 : 1
40-44.9	3	42.9	4	57.1	7	0.8 : 1
45-49.9	0	0.0	1	100.0	1	—
Total	43	42.2	59	57.8	102	0.7 : 1

TABLE II

Results of comparisons between the cephalothorax length slopes and each one of the characters measured in *Parastacus pilimanus* (Von Martens, 1869) intersex males and females, of a size greater than 27.2 mm CL

Relation	Slopes		Student's <i>t</i> -test ($t_{0.05(2),21} = 2.080$)
	Males intersex (N = 10)	Females intersex (N = 15)	
CL × Abdominal somites L	0.897	0.869	3.429*
CL × Somite 2 W	0.358	0.798	3.055*
CL × Somite 3 W	0.320	0.908	3.022*
CL × Somite 4 W	0.268	0.866	3.175*
CL × Somite 5 W	0.208	0.930	2.875*
CL × Somite 6 W	0.175	0.648	3.250*
CL × Telson W	0.238	0.134	2.399*
CL × Telson L	0.298	0.249	1.479
CL × Uropodal endopodite L	0.297	0.330	6.430*
CL × Uropodal exopodite L	0.390	0.300	4.810*
CL × Uropodal endopodite W	0.216	0.267	4.810*
CL × Uropodal exopodite W	0.270	0.366	3.330*
CL × Somite 3 pleuron H	0.095	0.091	7.240*
CL × Somite 4 pleuron H	0.094	0.100	6.250*
CL × Somite 5 pleuron H	0.098	0.110	6.320*
CL × Somite 2 pleuron L	0.205	0.162	6.890*

CL, cephalothorax length; L, length; W, width; H, height.

All *t* statistics indicated with *, correspond to $P \leq 0.05$.

DISCUSSION

Female and male gonopores coexisted in all specimens of *Parastacus pilimanus* analysed (N = 102), which was consistent with previous descriptions of supernumerary gonopores in this species by Von Martens (1869), Thompson (1982), and Rudolph & Almeida (2000). Furthermore, sufficient records exist to qualify this coexistence as constant in all species of *Parastacus*, with the exception of *Parastacus nicoleti*. In this latter species, only a fraction of the populations present the coexistence of male and female gonopores in the same individual (cf. Rudolph, 1995). As a consequence, with respect to this character, *Parastacus pilimanus* conforms to the diagnosis of *Parastacus* offered by Riek (1971) and Hobbs (1974, 1991). Nevertheless, many of the 102 specimens (N = 87; 85.3%) had sealed female gonopores with a semi-ellipsoidal aspect (intersex 1); while 14.7% (N = 15) had ellipsoidal female gonopores, open to the exterior (intersex 2). This diversity in form and sealing of the female gonopore also exists in other species of *Parastacus* with supernumerary gonopores. Thus, in *Parastacus pugnax*, 57.8% of the specimens analysed by Rudolph (1997) (N = 538) had female gonopores sealed by a highly calcified cuticle. In a Uruguayan population of *Parastacus varicosus*, 93.7% of the individuals under study (N = 142) also had sealed female gonopores (Rudolph et al., 2001). This diversity has also been described in *Parastacus saffordi* Faxon, 1898, *Parastacus brasiliensis*, and *Parastacus defossus*, by Faxon (1898), Almeida & Buckup (1997), and Noro et al. (2008), respectively.

Evidence gathered from anatomical dissections and histological sectioning disclosed that, despite having coexisting male and female gonopores, specimens of *Parastacus pilimanus*, have a male or female gonad from which gonoducts of both sexes emerge, connecting it with the respective gonopores. In the ovaries, it could be verified that their histology was uniquely female throughout, with no traces of testicular tissue, and that oviducts were well developed, but sperm ducts were rudimentary. On the contrary, the histology of the testes was entirely male in the whole gland, with no trace of ovarian tissue, and sperm ducts were well developed, while oviducts were rudimentary. This type of genitalia was already observed in the first dissections of the reproductive system carried out in some species of *Parastacus* (cf. Von Ihéring, 1892; Lönnberg, 1898; Turner, 1935), and confirmed in more recent descriptions (Almeida & Buckup, 1997; Rudolph, 1997; Rudolph et al., 2001). However, in the case of *Parastacus pilimanus*, this had only been documented for the females of this species (Thompson, 1982).

The data provided by morphometric analyses of the abdomen and their comparison with evidence gathered from anatomical dissections, enables us to postulate that in individuals under 27.2 mm CL, there is only one type of external morphology (intersex 1), making it very difficult to distinguish the sex of these

specimens externally. However, their gonads are already differentiated (54.8% intersex females and 45.2% intersex males). The changes observed in the external morphology of specimens over 27.2 mm CL, suggest that the gonadic females around this size would undergo the puberty moult. This would enable the female gonopores to complete their ellipsoidal form, develop setae on the external margin, and open to the outside (intersex 2), facilitating the extrusion of oocytes and their posterior displacement towards the abdomen. Furthermore, the abdomen acquires the appropriate morphology for egg incubation, i.e., greater relative width, higher pleura, and a larger tail fan. According to our observations, as well as those of Almeida & Buckup (2000) and Noro et al. (2008), these changes reflect the maturing of the gonadic females. Each one of these morphological traits, taken separately, and particularly together, permit the sex of specimens over 27.2 mm CL to be distinguished externally.

Although the number of females is greater in all size classes where the sex ratio was analysed, this difference could not be attributed to an eventual sex change, given the presence of males in all of them, except between 45.9 and 49.9 mm CL. This is not compatible with a protandric-type sex change unless this were partial, which is not supported by our results, since no evidence of an ootestis was found in any of the gonads analysed. The results of this study, together with previous records of the permanent occurrence of gonopores and gonoducts of both sexes in the same individual of *Parastacus pilimanus* (cf. Von Martens, 1869; Thompson, 1982; Rudolph & Almeida, 2000), suggest that this species presents permanent intersexuality, similar to *Parastacus pugnax* and *Parastacus varicosus* in Uruguay.

Sex differentiation in the Malacostraca is controlled by the presence or absence of the androgenic hormone. In the presence of this hormone, the gonadal primordia are differentiated into male genitalia. In the absence of this hormone, ovarian auto-differentiation occurs with female morphogenesis (Fingerman, 1987; Charniaux-Cotton & Payen, 1988; Hasegawa et al., 1993; Bauer, 2000). In *Parastacus pilimanus*, the permanent occurrence of oviducts and female gonopores in gonadal males, and sperm ducts and phallic papillae in gonadal females, does not coincide completely with the previously mentioned hormonal mechanisms. We have no knowledge of the sex differentiation processes in *Parastacus pilimanus* and the size or juvenile stage at which these occur. Neither have we identified the factor/factors responsible for maintaining gonoducts and gonopores of one sex in individuals whose gonad is of the opposite sex. We only know that these can be of different types, because among other causes, the androgenic gland and its hormone are controlled by a neuroendocrine gland: the X-organ-sinus-gland complex (Van Herp & Payen, 1991). Similarly, we are unable to provide an answer with respect to the adaptive value of a sexual system of this type. In order to provide such information, and also to fill the previously mentioned gaps in our knowledge, we must await

new studies that include larger samples from a greater number of populations, in which, in addition to undertaking anatomical and histological analyses of the sexual characters, the social organization and some physicochemical parameters of the habitat are also studied, not only in this species, but in all species of *Parastacus* that present permanent intersexuality.

ACKNOWLEDGEMENTS

The collaboration of the Research Department of the Universidad de Los Lagos, for partially financing this study, and that of Susan Angus, for translating the manuscript, are gratefully acknowledged.

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First received 15 September 2008.

Final version accepted 2 April 2009.