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TESE DE DOUTORADO

REPRODUÇÃO EM PENAEOIDEA: MORFOLOGIA E TENDÊNCIAS EVOLUTIVAS

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Tese apresentada ao curso de Pós-Graduação em Ciências Biológicas – Instituto de Biociências da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP – campus de Botucatu, como parte dos requisitos para obtenção do título de doutor em Ciências Biológicas (Zoologia).

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"Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana seja apenas outra alma humana".

Carl G. Jung

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Ideia-chave do estudo

Os camarões da superfamília Penaeoidea Rafinesque-Schmaltz, 1815 apresentam uma grande variabilidade morfológica do télico e do petasma (Burkenroad, 1934; Kubo, 1949; Pérez-Farfante, 1969; Bauer, 1991). Bauer (1991) observou ainda uma tendência evolutiva na constituição morfológica destas estruturas.

Télico é a área de contato genital da fêmea com o macho durante a cópula e transferência do espermatóforo, sendo esta estrutura oriunda de modificações dos esternitos torácicos 6 (em alguns casos), 7 e 8. Em alguns peneóideos, as fêmeas apresentam, além do télico, invaginações que adentram o cefalotórax com capacidade de armazenamento do espermatóforo, a esta estrutura dá-se o nome de receptáculo seminal ou espermateca (Bauer, 1994).

Segundo Bauer (1991), é possível identificar uma progressiva internalização do espermatóforo dentre os peneóideos associada à modificações estruturais do petasma e do télico. Espécies de télico aberto possuem espermatóforo estruturalmente complexo (várias asas, placas e material adesivo) e completamente externo ao corpo da fêmea, sendo que os machos da mesma espécie apresentam petasma aberto, flexível e estruturalmente simples, como no caso das espécies da família Solenoceridae Wood-Mason, 1891. Já em espécies de télico fechado, observa-se a alocação dos espermatóforos em câmaras localizadas atrás do télico, não havendo, porém, invaginação na cavidade cefalotorácica. O espermatóforo é bem menos complexo, constituído por pouco material acessório, além de um petasma comprimido e menos flexível.

O gênero *Rimapenaeus* (como *Trachypenaeus* em Bauer 1991) é dado como o próximo passo no processo de internalização do espermatóforo, constituído por duas substâncias: o material seminal e o plug espermático. O petasma é mais fechado, mais rígido e mais complexo em relação aos demais peneóideos. Por fim, os membros da família Sicyoniidae Ortmann, 1898 apresentam uma tendência extrema de internalização de um espermatóforo simples associada a um petasma fechado, sem flexibilidade com presença de projeções e espinhos que o torna mais complexo (Bauer, 1991).

Vários pesquisadores vêm propondo como condição primitiva espécies de espermatóforo externo e complexo, associado a um télico aberto e petasma aberto e flexível. Em contrapartida, uma massa simples de espermatóforo invaginada em uma espermateca atrás de um télico fechado e machos com petasma complexo, fechado e pouco flexível representam condições derivadas (Burkenroad, 1934, Burkenroad, 1936; Kubo, 1949; Bauer, 1991).

Objetivos gerais e organização do estudo

Este estudo procura associar a tendência evolutiva das genitálias de camarões peneóideos observada por Bauer (1996) à hipótese chave-fechadura (nunca estudada antes para crustáceos) postulada por Dufour (1844). Acredita-se que ocorrerá chave-fechadura em espécies de camarões consideradas mais derivada na escala evolutiva postulada por Bauer (1996), uma vez que possuem télico fechado e petasma intromitente, ou seja, uma possível chave e uma possível fechadura.

Esta tese foi organizada em dois capítulos em forma de artigos científicos, sendo o primeiro um compilado de estudos produzidos desde o século XIX até escritos mais atuais, acerca da morfologia genital de camarões peneóideos. Este capítulo foi escrito com o objetivo de organizar o rico material acerca de morfologias genitais de camarões peneóideos existentes na literatura, além de analisar ultraestruturalmente as genitálias dos camarões e propor novas nomenclaturas para a organização da diversidade de petasmas e télicos. Já o segundo capítulo trata-se de um estudo alométrico, com o intuito de testar a hipótese chave-fechadura em nove espécies de camarões peneóideos que ocorrem no Atlântico ocidental.

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Capítulo 1

Genital morphology of Penaeoidean shrimps: an overview

Abstract

The aim of this research was gather the largest number of studies on genital morphology in penaeoidean shrimps and organizes evolution of this theme along the years, besides, describe thelycum and petasma of five different penaeoidean shrimps and determine some degree of complexity of the petasma along the evolution trends. The five species considered (*Litopenaeus schmitti*, *Pleoticus muelleri*, *Rimapenaeus constrictus*, *Farfantepenaeus brasiliensis* and *Sicyonia typica*) were sampled in the coast of São Paulo and Santa Catarina states during 2006 and from 2011 to 2016 using a shrimp boat. The individuals were identified and classified according to the sex. Petasma and thelycum were dissected and fixed in Karnovsky and it were conducted to the ultrastructure procedures in the Scanning Electron Microscopy (SEM). All the genital structures were compared in the literature. All the species have symmetrical petasma with cincinulli belt and ventral costae evident (except *S. typica*). *Litopenaeus schmitti* was characterized by the presence of many spines on the dorsolateral lobe of the petasma, whereas in *R. constrictus* was possible to observe hornlike projections and channels. In *S. typica* was also possible to evidence channels and spoutlike structures. The thelycum of the species were composed by plates, lips, flaps and median protuberances. We found forty-one researches about genital morphology of penaeoidean shrimps, composing identification keys, or study of the morphology/ function of the genitalia. The studies on genitalia shrimps are ancient and rich of information. The petasma is the most variable structure which passed for many classifications along the scientific scenario; we proposed divided the male genitalia into three classifications and the female genitalia, into two classifications. It was not possible to evidence an increasing of complexity of the petasma in the species studied, but it was possible to identify the presence of channels and hornlike/ spoutlike structures as a derivate character among the penaeoidean shrimps.

Keywords: genitalia evolution, ultrastructure, petasma and thelycum morphology.

Introduction

The genital morphology in the animal kingdom has been target of studies since nineteen century (Dufour, 1844; Bate, 1881; Smith, 1885) and reveals the genitalia can evolves in response to different kinds of environmental pressures as coevolution, sexual selection and selections to avoid cross-specific copulations (Eberhard, 1992; Brown and Eady, 2001; Brennan et al., 2007). The animals most studied about genital evolution are the insects, which frequently have small and hidden genitalia (Eberhard, 1998), as the majority of birds, spiders and milipeds described in the literature (Brennan et al., 2007; Shapiro and Porter, 1989; Sota and Kubota, 1998; Tanabe and Sota, 2008). On the other hand, in many species of shrimps is possible to observe exposed, large and complex male genitalia, used to describe some species (Burkenroad, 1936; Pérez-Farfante, 1988).

Male shrimps are characterized by the presence of petasma, a tubular structure originated by the joining of cincinulli of the modified endopods of the first pair of pleopods. This structure is usually related to the function of deliver the spermatophores to the female genitalia; however petasma has also been described as the structure that assists the correct position during copulation (Bauer, 1996). While females have the thelycum, which consists of modifications of the posterior two or sometimes three thoracic sternites, constituting a structure to receive the spermatophores deposited by the males. The spermatophores can be storage in a seminal receptacle (=spermatheca), constituting a closed thelycum or temporarily attached on the thelycum, what characterize an open thelycum (Bauer, 1994; Pérez-Farfante and Kensley, 1997).

The first study documented about genital morphology in shrimps date from 1881 by Bate and the expressive studies of Burkenroad (1934), Bauer (1991) and Pérez-Farfante and Kensley (1997) contribute to determine and classify the thelycum and petasma morphology in the penaeoidean shrimps. By this scenario, the aims of this study were (1) determine the complexity of male genitalia, besides understand the most applicable terms to define the petasma in five Penaeoidean shrimps, and (2) review the literature that describe petasma and thelycum morphology

Material and methods

Sampling

Five species of Penaeoidea Rafinesque-Schmaltz, 1815 shrimps were used in this study: *Litopenaeus schmitti* (Burkenroad 1936), *Pleoticus muelleri* (Spence Bate

1888), *Rimapenaeus constrictus* (Stimpson 1871), *Farfantepenaeus brasiliensis* (Latreille 1817) and *Sicyonia typica* (Boeck 1864). The species were collected by a shrimp fishery-boat equipped with double-rig nets, during 2006 and from 2011 to 2016 at the coast of São Paulo State (Ubatuba - 23°26'10"S, 45°01'36"W, São Vicente - 24°03'59"S, 46°16'57"W and Cananéia - 25°07'02"S, 47°50'48"W) and Santa Catarina State, Brazil (São Francisco do Sul and Itapoá - 26°06′40″S, 48°34′62″W). The species were identified according to Costa et al. (2003) and the sex assessment performed by the observation of the presence of thelycum (females) and petasma (males).

Ultrastructure of petasma and thelycum

Petasma and thelycum were dissected from five individuals of each species and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.6) during 24 - 48 hours, according to Electron Microscopy Laboratory procedure. Bellow, the structures were dehydrated during 20 minutes into each crescent series of ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100%) and it were conducted to the Scanning Electron Microscopy (SEM) and the procedures were conducted, as follow: the dehydration was completed in a critical-point-dried with liquid CO_2, the genital structures were putted in the stabs and sputtered-coated with gold. Samples were analyzed and photographed under Zeiss EVO MA-10 (10-20 Kw).

Literature review

A computer search based on citations of previous researches (starting with Pérez-Farfante and Kensley, 1997) was realized to accumulate works about petasma and thelycum morphology (search methodology based on Eberhard, 2008).

Results

Petasma description

Pleoticus muelleri: Petasma symmetrical and ventral costae (*vc*) with protuberances and extensions. The lateral lobes are flexible and short dorsal lobe joined by cincinulli (*c*) (Figures 1 A, B, C).

Litopenaeus schmitti: Petasma symmetrical with ventral costae (*vc*) and presence of many spines (*sp*) in the dorsolateral lobe. Long dorsal lobe joined by cincinulli (*c*) (Figures 1 D, E, F).

Farfantepenaeus brasiliensis: Petasma symmetrical with ventral costae (*vc*) curved in the distal region. Long dorsal lobe joined by cincinulli (*c*) (Figures 1 G, H, I).

Rimapenaeus constrictus: Petasma symmetrical with long ventral costae (*vc*) characterized by the presence of hornlike projections (*hp*) in the distal portion, characterized by the presence of deeply channels (*ch*), which connects with the inside of the tube formed by the lateral lobes. The dorsal lobe is joined by a cincinulli (*c*) (Figures 1 J, K. L).

Sicyonia typica: Petasma symmetrical with a distal projection in the ventrolateral lobe and a grooved distal projection in the dorsolateral lobe, forming spoutlike (*sl*) structures and the presence of not deeply channels (*ch*). The dorsal lobe is joined by a cincinulli (*c*).

Thelycum description:

Pleoticus muelleri: Thelycum open with sternites XIV forming a long, smooth and plan structure for receiving spermatophores, called lips (*l*) and another portion called prominence (*p*) (Figures 2A).

Litopenaeus schmitti: Thelycum open with sternites XIV bearing ridges and depressions, forming a unique and extensive lip (*l*) (Figure 2B).

Farfantepenaeus brasiliensis: Thelycum closed, with the lateral plates emerging from the sternite XIV and forming mesial lips (*l*) on a vertical position (Figure 2C).

Rimapenaeus constrictus: Thelycum closed, with a median protuberance (*mp*) and lateral plates emerging from the sternites XIV forming two lips (*l*) mesially fused and opened above on horizontal position. The *mp* is a uniform and rounded plate on sternite XIII (Figure 2D).

Sicyonia dorsalis: Thelycum closed, with a median protuberance (*mp*) and lateral plates emerging from the sternites XIV forming two lips (*l*) mesially fused and opened above on horizontal position as *R. constrictus*. The *mp* is rounded plate with a long spike in the distal position. It is possible to observe many setae (*s*) on the thelycum (Figure 2E).
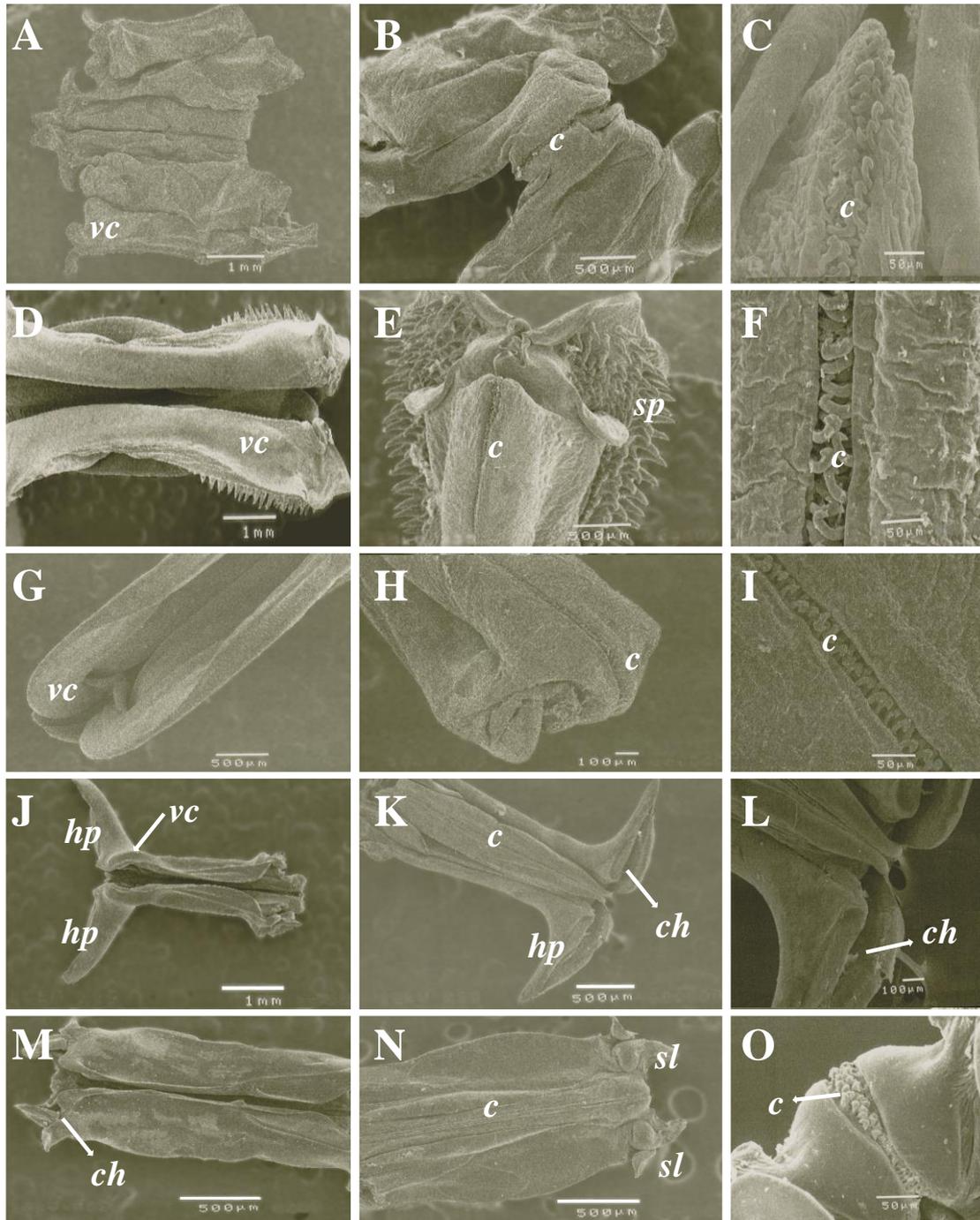
Figure 1. Scanning Electron Microscopy (SEM) of petasma of five penaeoidean shrimps. *Pleoticus muelleri:* (A) dorsal view, (B) ventral view and (C) cincinulli detail; *Litopenaeus schmitti:* (D) dorsal view, (E) ventral view and (F) cincinulli detail; *Farfantepenaeus brasiliensis:* (G) dorsal view, (H) ventral view and (I) cincinulli detail; *Rimapenaeus constrictus*: (J) dorsal view, (K) ventral view and (L) cincinulli detail; *Sicyonia typica*: (M) dorsal view, (N) ventral view and (O) cincinulli detail. *ch*: channel; *c*: cincinulli; *hp*: hornlike projection; *sl*: poutlike projection; *sp*: spine; *vc*: ventral costae.
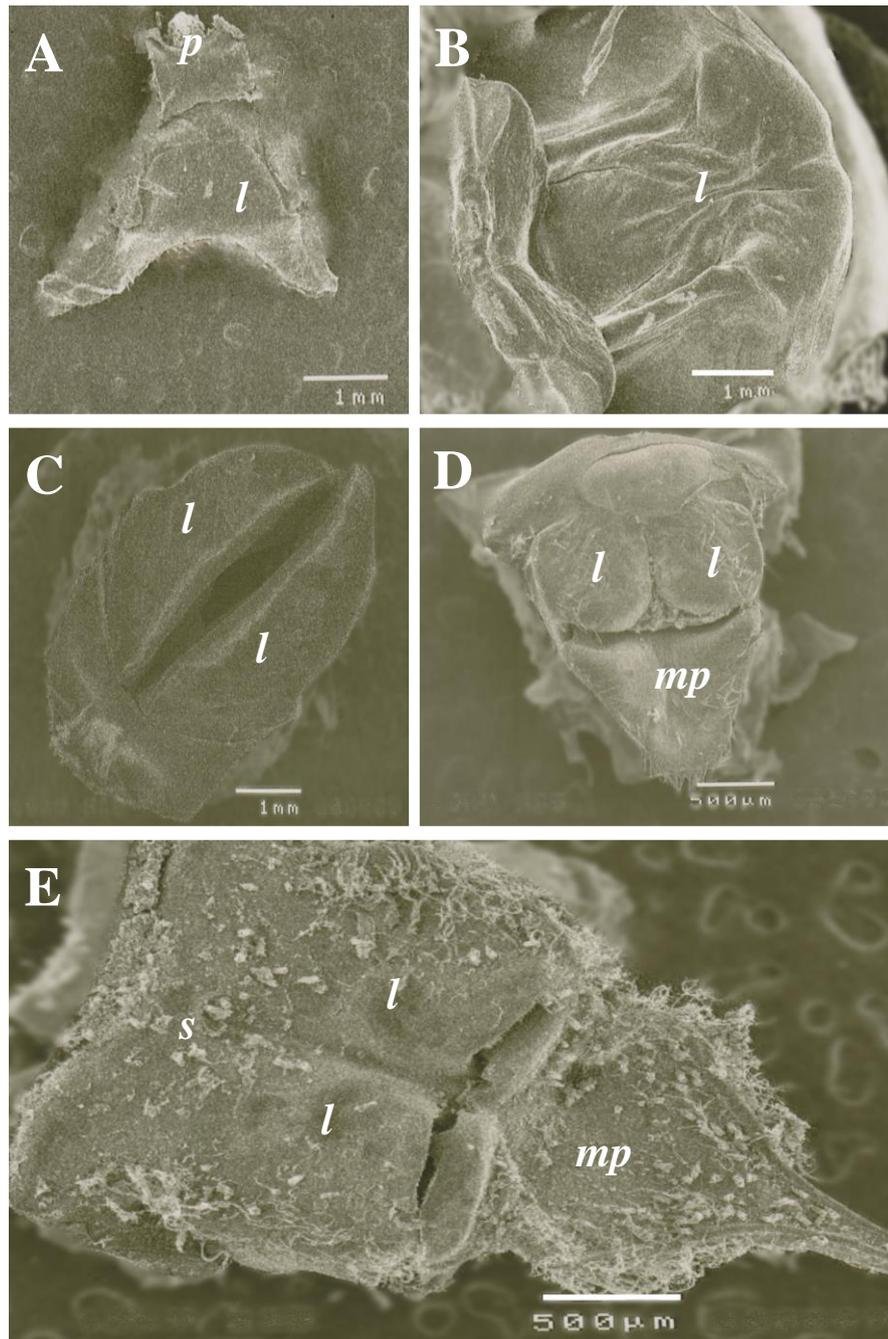
Figure 2. Scanning Electron Microscopy (SEM) of thelycum of five penaeoidean shrimps. (A) *Pleoticus muelleri;* (B) *Litopenaeus schmitti,* (C) *Farfantepenaeus brasiliensis;* (D) *Rimapenaeus constrictus*; (E) *Sicyonia typica*. *l*: lip; *mp*: median protuberance, *p*: prominence; *s*: setae.

Petasma and thelycum morphology review

Forty-one researches about genital morphology of penaeoidean shrimps were analyzed in the present study. These studies, ten considered a discussion about the genital function (both briefly or in details), besides the morphological description. The most ancient study date from 1881 and the most recent date from 2012 (Table 1).

Table 1. Summary of third six literatures about the genital morphology/ function of penaeoidean shrimps in chronological order.

Title	Author, year	Genital morphology	Genital function
On the Penaeidea	Bate, 1881	Petasma and vulvae	No
On some genera and species of Penaeidae, mostly from recent dredgings of the United States Fish Commission	Smith, 1885	Petasma	No
Report on the Crustacea Macrura collected by the H.M.S. Challenger during the years 1873-76	Bate, 1888	Thelycum	No
A descriptive catalogue of the Indian deep-sea Crustacea Decapoda Macrura and Anomala, in the Indian Museum. Being a revised account of the deep-sea species collected by the Royal Indian marine survey ship "Investigator".	Alcock, 1901	Petasma and thelycum	No
A revision of the "Genus" *Peneus*, with diagnoses of some new species and varieties	Alcock, 1905	Petasma and thelycum	No

Sperm transfer in certain decapods	Andrews, 1911	Petasma and thelycum	Yes
The Percy Sladen Trust Expedition to the Indian Ocean in 1905 under the leadership of Mr. J. Stanley Gardiner. Volume 5, no. 5. Pelagic Crustacea Decapoda of the Percy Sladen Expedition in H.M.S. "Sealark".	Kemp, 1913	Petasma and thelycum	No
Penaeid crustaceans with the symmetrical petasma.	Kishinouye, 1929	Petasma and thelycum	No
Anomuran, macruran crustacea from Panama and canal zone.	Boone, 1931	Petasma and thelycum	No
The Penaeidea of Louisiana with a discussion of their world relationships	Burkenroad, 1934	Petasma and thelycum	Yes
The Aristeinae, Solenocerinae and Pelagic Penaeinae of the Bingham Oceanographic Collection	Burkenroad, 1936	Petasma and thelycum	Yes
Status of the name *Sicyonia* H.M.E., with a note on *S. typica* (Boeck) and descriptions of two new species.	Burkenroad, 1946	Petasma	No
Some macrurous decapod crustacea found in Japanese waters, with descriptions of four new species	Kubo, 1951	Petasma and thelycum	No
A study of some developmental stages of the thelycum and its relation to the spermatophores in the prawn *Penaeus japonicus* Bate	Tirmizi, 1958	Thelycum	Yes
Penaeid prawns of Ceylon (Crustacea, Decapoda,	Bruin, 1965	Petasma and thelycum	No

Penaeidae).			
A new species and two new subspecies of shrimp of the genus *Penaeus* from the western Atlantic	Pérez-Farfante, 1967	Petasma and thelycum	No
A description of the development of primary and secondary sexual characters in the banana prawn, *Penaeus merguiensis* de Man (Crustacea: Decapoda: Penaeinae).	Tuma, 1967	Petasma and thelycum	Yes
Observations on the development of the external genitalia in some Indian penaeid prawns	George and Rao, 1968	Petasma and thelycum	No
On the structure and some developmental stages of genitalia in the prawn *Parapenaeopsis stylifera* (H. Milne Edwards) (Decapoda, Penaeidea).	Tirmizi, 1968	Petasma and thelycum	No
Western Atlantic shrimps of the genus *Penaeus*	Pérez-Farfante, 1969	Petasma and thelycum	No
A new species of *Sicyonia* (Decapoda: Penaeidae) from the western Atlantic with notes on *S. stimpsoni* Bouvier	Cobb, 1971	Petasma and thelycum	No
A key to the American Pacific shrimps of the genus *Trachypenaeus* (Decapoda, Penaeidae), with the description of a new species	Pérez-Farfante, 1971a	Petasma and thelycum	No
Western Atlantic shrimps of the genus *Metapenaeopsis* (Crustacea, Decapoda, Penaeidae), with descriptions of	Pérez-Farfante, 1971b	Petasma and thelycum	Yes

three new species			
New records of Penaeid prawns from the east coast of Southern Africa with notes on *Penaeus marginatus* Randall and a new species of *Metapenaeopsis*.	Champion, 1973	Petasma and thelycum	No
Morphological study of diagnostic characters in western Atlantic *Hepomadus* (Crustacea, Decapoda, Penaeidae)	Pérez-Farfante, 1973	Petasma and thelycum	No
Penaeid shrimps (Decapoda: Penaeidae) collected off East Africa by the fishing vessel "Van Gogh", 1. *Solenocera ramadani* sp. nov., and commercial species of the genera *Penaeus* and *Metapenaeus*.	Ivanov and Hassan, 1976	Petasma and thelycum	No
Study of juveniles of *Metapenaeus stebbingi* Nobili (Decapoda: Penaeidae) with particular reference to the structure and development of the genitalia.	Tirmizi and Javed, 1976	Petasma and thelycum	No
American solenocerid shrimps of the genera *Hymenopenaeus, Haliporoides, Pleoticus, Hadropenaeus* new genus, and *Mesopenaeus* new genus	Pérez-Farfante, 1977	Petasma	No
Families Hippolytidae. Palaemonidae (Caridea), and Penaeidae, Sicyoniidae and Solenoceridae (Penaeoidea).— *In:* W. Fisher, ed,, FAO species identification sheets for fisheries purposes, Western Central Atlantic (Fishing Area 31), Vol. VI (unpaginated).	Pérez-Farfante, 1978	Petasma and thelycum	No

A new species of *Solenocera* (Crustacea: Decapoda: Solenoceridae) from Northern Australia	Pérez-Farfante and Grey, 1980	Petasma and thelycum	No
The Penaeoidea of southeast Africa. V. The family Sicyoniidae.	Freitas, 1984	Petasma and thelycum	No
Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida	Williams, 1984	Petasma and thelycum	No
The rock shrimp genus *Sicyonia* (Crustacea: Decapoda: Penaeoidea) in the eastern Pacific	Pérez-Farfante, 1985	Petasma and thelycum	No
Deepwater decapod Crustacea from eastern Australia (Penaeidea and Caridea).	Kensley et al., 1987	Petasma and thelycum	No
Illustrated key to the penaeoid shrimps of commerce in the Americas	Pérez-Farfante, 1988	Petasma	No
Phylogenetic trends in sperm transfer and storage complexity in Decapod crustaceans	Bauer, 1986	Petasma and thelycum	Yes
Transfer and storage structures in Penaeoid shrimps: a functional and phylogenetic perspective	Bauer, 1991	Petasma and thelycum	Yes
Ovarian development in relation to changes in the external genitalia in captive *Penaeus monodon*	Quinitio et al., 1993	Thelycum	Yes
Role of the petasma and the appendices masculinae during copulation and insemination in the penaeoid shrimps, *Sicyonia dorsalis* (Crustacea: Decapoda: Dendrobranchiatta)	Bauer, 1996	Petasma and thelycum	Yes

Penaeoid and Sergestoid shrimps and prawns of the world: keys and diagnoses for the families and genera	Pérez-Farfante and Kensley, 1997	Petasma and thelycum	No
A new genus of deep-sea Solenocerid shrimp (Decapoda: Penaeoidea) from Papua New Guinea.	Chan, 2012	Petasma and thelycum	No

Discussion

The perspectives for the genital morphology in the five shrimp species studied

The Alcock's (1905) description of thelycum dividing it into distinct parts is interesting, because none of the penaeoidean shrimps, observed in the present study, showed this richness of division. Alcock (1905) defined the male genitalia of *Metapenaeus stridulans* Alcock, 1905 [= *Metapenaeopsis stridulans* (Alcock, 1905)] as follow: abdominal appendages were moderate length and the exopodite was longer than the endopodite. In the first pair of males' pleopods, the endopodite is a structure more or less rigid, longitudinally pleated or convoluted plates known as "petasma" or "andricum", which together form a canal or tube. This general description is shared by all the penaeoidean shrimps as the Bate's (1881) description.

In addition, the thelycum of *M. stridulans* was characterized as a structure containing three parts: (1) between the fifth pair of legs there is a transverse lamina more or less distinctly divided into three lobes, and the outer of which are dentiform; (2) between the fourth pair of legs, there is a broad transverse plate of which the anterior part is large, smooth and somewhat oval facet; (3) in the interval between the fourth and fifth legs, there is a narrow transverse bar, sinuous and shaped like a W.

Considering the description of this author, we could observe two divisions into the thelycum of the five species present in this study: (1) between the fifth pair of legs there is a lip more or less distinctly divided into two lobes and (2) between the fourth pair of legs there are prominences (*Farfantepenaeus brasiliensis* and *Pleoticus muelleri*), median protuberance (*Sicyonia typica* and *Rimapenaeus constrictus*) or simply the continuing of the lobes (*Litopenaeus schmitti*). Our thelycum division converged with the genus *Parapenaeopsis* also described by Alcock (1905).

From 1934, Burkenroad brought more details about the petasma morphology and divided this structure into three possible designs: (1) Open petasma, the simplest form, that occurs in the subfamilies Aristaeinae, Solenocerinae and some Penaeinae; (2) Semitubular petasma with laterodistal spoutlike corners deeply channeled and no posterodistal projection occurs in *Trachypeneus* series of subfamily Penaeinae and (3) Semitubular petasma with two pairs of distal spines (lateral and posterior) and anteromedian distal lobes. The distolateral spines form a shallowly channeled spoutlike injection apparatus. This kind of petasma occurs in the subfamily Eusicyoninae and some species of Penaeinae.

So, according to Burkenroad (1934) research, it is possible to infer the species *L. schmitti* and *P. muelleri* have an open petasma, whereas *R. constrictus* has a semitubular petasma with laterodistal spoutlike corners deeply channeled and *S. typica* with a semitubular without deeply channel in the spoutlike injection apparatus. It is not possible to be sure about the design of the petasma of *F. brasiliensis*.

Bauer (1991) has an ample study about transfer and storage structures in penaeoidean shrimps, in which, he studied the morphology of spermatophores and petasma. This author found an evolutionary sequence: the families Aristeidae, Solenoceridae and the genus *Litopenaeus* present structurally complex external spermatophores, whereas Sicyoniidae possess highly internalized simple spermatophoric masses. In relation to the petasma morphology, he considered three characteristics as the most important to define it: the degree of openness and flexibility, besides the presence/ absence, or degree of development of distal horns or spouts.

Considering this, he found (1) open petasma, what means the ventral costae (= a ridge that extends along the ventromesial margin of the ventrolateral lobe of the petasma) is not turned ventrally and petasma is flexible (families Aristeidae and Solenoceridae); (2) closed petasma, when the ventral costae nearly meet at the midline and the petasma is also flexible [*Litopenaeus setiferus* (Linnaeus, 1767)]; (3) the next stage of petasma closure was found in *Thachypenaeus*, with hardened ventrolateral lobules reflexed back dorsally against of the rest of the petasma and (4) the extreme petasma closure was defined in *Sicyonia*, in which ventrolateral lobules are very rigid and almost meet under the rest of the petasma.

Bauer (1991) also found petasma with complex folding distally in the genus *Solenocera* and some penaeid shrimps, but horns and spoutlikes were observed only in the genus *Trachypenaeus* (changed name to *Rimapenaeus*) and *Sicyonia*. The first species has ventrolateral lobules produced into long horns distally with dorsal channels and *Sicyonia* presents both dorsolateral and ventrolateral lobules terminating in projections, and the dorsolateral has grooves or channels, resulting in spoutlike structures.

Pérez-Farfante and Kensley (1997) were other researchers that deeply studied the petasma morphology and classified it into four categories based on the Bauer's (1991) study: (1) Open petasma: the lateral lobes are flexible, partially or entirely extended laterally and the ventral costae is not or barely turned ventrally; (2) Semi-open petasma: the lateral lobes are flexible and folded and the ventral costae is turned

ventromesially, forming an ample space extending from proximal to distal ends; (3) Semi-closed petasma: the lateral lobes are rather flexible and marked folded with strong ribs and the ventral costae approaching closely, delimiting a moderately large space, narrowly open distally with well-developed distomedian projections and (4) Closed petasma: the lateral lobes are heavily sclerotized with lateral spouts or horns in the distal region and the ventral costae is situated ventromesially, delimiting a small space. In this study, the authors identified channels on the petasma only in the genus *Artemesia*, *Parapenaeopsis* and *Rimapenaeus*, confirming what Burkenroad (1934) has observed.

Burkenroad (1934) and Pérez-Farfante and Kensley (1997) defined almost the same petasma morphology. However, the first author was not clear to define the morphology of the three designs proposed, but it is possible to suggest his designs were based on the degree of aperture of each male genitalia. Bauer (1991) and Pérez-Farfante and Kensley (1997) observed this degree of aperture was defined by the ventral costae and they added another characteristics to its morphology, such as the degree of flexibility of the lateral lobes.

Considering Pérez-Farfante and Kensley (1997) research, it is possible to infer the species *F. brasiliensis* and *R. constrictus* have semi-closed petasma design, whereas *L. schmitti* and *P. muelleri* have semi-open petasma, and *S. typica* is an example of closed petasma. The degree of flexibility of the lateral lobes seems constitutes a characteristic highly variable and according to these authors, *P. muelleri* and *L. schmitti* have the same degree of flexibility of the lateral lobes, but the first species present higher mobility than *L. schmitti* in this structure. In addition, *F. brasiliensis* and *R. constrictus* would have lateral lobes moderate flexible as *L. schmitti*. So, we believe the degree of flexibility of lateral lobes and the degree of the petasma aperture need to be carefully considered to do some generalization.

Therefore, we propose the following terms: (1) open petasma: lateral lobes are flexible, and the ventral costae is not or barely turned ventrally delimitating an ample space; (2) semi-closed/ semitubular petasma: the ventral costae is situated ventromesially delimitating an ample or moderate space that turn the male genitalia a structure similar to a tube, with lateral lobes flexible or weakly flexible; the petasma could have complex folding distally or the ventrolateral lobules can be produced into long horns distally and (3) closed petasma: the ventral costae delimitate an small space and the lateral lobes are highly sclerotized and has virtually no flexibility, the lateral lobules terminate in spoutlike structure with the presence of channels.

The inclusion of "weakly flexibility" in the "semi-closed petasma" we solved the question about the flexibility of the lateral lobes in the petasma of *L. schmitti*. Considering this proposition, the species studied in this research would be classified as in the follow: the species, *P. muelleri*, *L. schmitti*, *R. constrictus* and *F. brasiliensis* would be examples of semi-closed/ semitubular petasma and *S. typica*, would have an closed petasma. According to the presence of channels on the petasma, it was possible to observe them in the species *R. constrictus* and *S. typica*. We could not identify the presence of channels in any open thelycum species studied in the present study, what corroborate what Burkenroad (1934), Bauer (1991) and Pérez-Farfante and Kensley (1997) found.

Another final question to be considered is about the evolutionary scenario. Ma et al. (2009) found a relation among penaeid and sicyoniid genera. This is an interesting scenario, because we could infer the spoutlike projections found in the sicyoniid genera are a sinapomorphic characteristic, result of the regression of the hornlike projections described in *R. constrictus* in this research and also in *Xiphopenaeus kroyeri* (Heller, 1862) described in many other researches (Bauer, 1991; Pérez-Farfante and Kensley, 1997)

The evolution of the study on genital morphology in penaeoidean shrimps

Bate (1881) considered the sexual characters of both penaeoidean males and females as peculiar. In this research, the author proposed for the first time call the male genitalia as "petasma" and characterized it as follow: a large curtain-like membrane attached on the base of each first pleopods. These membranes were joined each other by the presence of small hook-like processes, Sars called these processes as cincinnuli for the first time on a research no referenced. Whereas, the female genitalia were still called vulvae by Bate (1881): a structure situated at the extremity of open projections on the third pereiopods. It is a ventral plate of varying form and appearance, according to the species. Only in 1888, Bate named the vulvae as "thelycum" for the first time for the female *Penaeus*. The Bate's research of 1888 was one of the results of the voyage of H.M.S. Challenger occurred during 1870s, considered the first true oceanographic expedition because of the wealth information about the marine environment that brought to the scientific community (Bishop et al., 2003).

Other two expressive expeditions, as the H.M.S. Challenger, were realized in the history, which originate important researches about taxonomy and identification of new

species, including genital analysis in crustaceans. The expeditions were known as (1) Percy Sladen Expedition in the years 1980's which originated the Kemp's (1913) study with species of the families Sergestidae, Penaeidae and Hoplophoridae and the (2) expedition realized by the commercial vessel "Van Gogh" in April-June 1966 around the east coast of Africa, which originated the Ivanov and Hassan's (1976) research, which described *Solenocera ramadani* Ivanov and Hassan, 1976 and other species of the genus *Penaeus* and *Metapenaeus*.

After Bate (1881, 1888), many other researchers characterized the sexual characters of penaeoidean shrimps. Some authors have initiated to associate the function of the thelycum to its definition, as Kubo (1949): "the thelycum consists of a series of prominences, depressions or grooves, plates and sacs raised from sternites of the sixth to eighth thoracic segments", and other authors that observed sternal modifications of the seventh and eighth thoracic segments (which corresponds to the body segments XIII and XIV) to form a median genital area where sperm-free male products are deposited (Andrews, 1911; Burkenroad, 1934; Pérez-Farfante, 1969; Bauer and Min, 1993). In other words, the thelycum has been considered as a "peculiar receptacle" found a certain prawn of Penaeidae (Andrews, 1911). Burkenroad (1934) defined the female genitalia as an open sperm receptacle in most of Aristaeinae, Solenocerinae and some species of *Penaeus* series of Penaeinae; where a large pair of more or less complex spermatophores was attached. According to this author, in other Penaeidae, a depressed anterior belt of sternite XIV was invaginated to produce unpaired median or paired lateral enclosed sperm receptacles.

Pérez-Farfante (1978) defended the term "thelycum" was used to define not only the sperm-storing sternal invaginations, but also the sternal protuberances and grooves where external spermatophores attach. And Bauer (1986) defined open thelycum as a structure completely open ventrally and closed thelycum as the structure that contains a noninvaginated chamber in which sperm substances are deposited, "cover a space which leads to spermathecal openings, or form an external shield over the internal spermatheca". For Bauer (1986), the spermathecae is an invagination of the exoskeleton in which sperm material is stored after copulation and it is a synonymous with receptacle, but not with thelycum, what differs of the first ideas of Andrews (1911) and Burkenroad (1934).

From 1934, Burkenroad brought more details about the petasma morphology and divided this structure into three possible designs, originating the terms: open petasma,

semitubular petasma with laterodistal spoutlike corners deeply channeled and semitubular petasma with two pairs of distal spines (lateral and posterior). This author identify many morphologies that corroborates the petasma is essentially a transfer organ, as the petasma of *Litopenaeus setiferus* (Linnaeus, 1767) which is strong laterally compressed what allow a perfect adaptation to entering on the slit of the thelycum of the same species. Another example is some species of *Parapenaeopsis*, in which the petasma is a tube completely sealed by fusion of its anterior and posterior lips, forming a perfect injection apparatus.

Despite these characteristics that strongly suggest petasma is a structure of transfer, Bauer (1996) found some evidences that could change this view for the species *Sicyonia dorsalis* Kingsley, 1878, what becomes the study of petasma morphology more intriguing. According to Bauer (1996), the petasma and appendices masculinae of this species would serve to adjust the position of the male genital papillae to the aperture of spermathecae and temporarily connect the male and female genitalia during the copulation. Bauer (1991) and Pérez-Farfante and Kensley (1997) contribute extensively for the genital morphology, including structures as ventral costae and flexibility as important attributes to define the petasma morphology.

Some other researches were designed considering the genital morphology (both petasma and thelycum) according to the degree of gonadal development of the individuals (Tirmizi, 1968; Tirmizi and Javed, 1976; Tuma, 1967; Quinitio, 1993) and, concluding interesting topics, as the presence of the extra thickening in the petasma of the *Penaeus merguiensis* de Man, 1888 [= *Fenneropenaeus merguiensis* (de Man, 1888)] was considered as a more efficient morphology for the identification of the petasmal adult form than the union of the endopods of the first pair of pleopods (Tuma, 1967). These works also discuss briefly about the genitalia function.

More ancient studies focused only on the morphological description of the petasma (Smith, 1885; Burkenroad, 1946). The research of Quinitio (1993) deserve attention, in this sense, because it was one of the few works to deal with the thelycum morphology in details, and finding interesting development of the lateral plates in order to accommodate the spermatophores in adult females. Noteworthy another two researches that also studied the thelycum in details: Tirmizi (1958) considering *Penaeus japonicus* Spence Bate, 1888 [= *Marsupenaeus japonicus* (Spence Bate, 1888)] as target species and Chan (2012), studying species of Solenoceridae from Papua New Guinea.

The last author was one of the most recent studies about genital morphology in penaeoidean shrimps.

There are many other works about taxonomic descriptions and identification keys of an a diversity of species in a diversity of localities of the earth, which include a morphological analysis of petasma and thelycum, but not about these functions, as Williams (1984), Pérez-Farfante and Grey (1980), Pérez-Farfante (1967, 1971ab, 1973, 1985, 1988), Kubo (1951), Kishinouye (1929), Kensley (1987), George and Rao (1968), Freitas (1984), Cobb (1971), Champion (1973), Burkenroad (1946), Bruin (1965), Boone (1931) and Alcock (1901).

Conclusions

The study of genital morphology of penaeoidean shrimps is very ancient and rich of information. The most complete studies about this theme were Burkenroad (1934), Bauer (1991) and Pérez-Farfante and Kensley (1997). The petasma is the most intriguing apparatus because, at the same time, it is possible to infer this structure is obviously related to the spermatophores transfer, in other moment this relation is not so evident. It is difficult to categorize the petasma morphology once the species have much particularity, and sometimes we assume the risk to be very generalist in our classifications. But, the classifications are important when the theme of study is so diverse as the genital morphology of penaeoidean shrimps. The petasma and thelycum morphology showed them as much diverse and the researchers found important particularities in the male and female genitalia along the history. We believe we could not say the petasma increase in complexity along the evolutionary scale, but we can observe the change of certain characters, as the presence of channels found only in the genus *Rimapenaeus* and *Sicyonia*. So, the channels can be certainly considered a derivate character on the evolutionary scale proposed by Bauer (1991), as the reduction of the hornlike projections in spoutlike projections.

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Capítulo 2

Evidence for lock-and-key hypothesis in penaeoideans shrimps

Abstract

Sexual selection and lock-and-key model supported the arthropod's genital evolution, mainly on insects; but this approach has never been tested on decapod crustaceans. The aim of this investigation was identify sexual selection patterns and test lock-and-key model using different species of penaeoideans shrimps. In addition, we identified how different the species are with respect to their genital morphology view. Two open thelycum species (*Litopenaeus schmitti* and *Pleoticus muelleri*) and seven closed thelycum species (*Xiphopenaeus kroyeri, Artemesia longinaris, Rimapenaeus constrictus, Farfantepenaeus brasiliensis, F. paulensis, Sicyonia dorsalis* and *S. typica*) were selected. The individuals were collected with a fishery shrimp boat equipped with double-rig nets in the Southeastern and Southern Brazilian coasts. The sex of each studied individuals was achieved, and some parts of their genitalia were measured. Data was analysed by means simple linear regressions and discriminant analysis . The lock and key model was identified in six species (*L. schmitti, P. muelleri, X. kroyeri, A. longinaris, R. constrictus* and *F. brasiliensis*) evidencing a sexually antagonistic coevolution of the genitalia between males and females. It was possible to infer the old lock-and-key is related to a diversity of allometric patterns and two species (*S. dorsalis* and *S. typica*) are still under sexual selection by cryptic female choice.

Keywords: species isolation, crustaceans, genital morphometry, static allometry.

Introduction

The first ideas for genital evolution in the arthropods species were developed by Dufour's (1844) using insects as a model. Insect male genitalia evolve in order to adapt to the female genitalia, as a manner to guarantee the legitimacy of the species, what originated the lock and key hypothesis (Shapiro and Porter, 1989). Darwin (1871) also proposed an important role in the genital evolution studies distinguishing two mechanisms which occurred by female's dispute among males (male-male battles and female choice), clarifying posterior idea that male's competition leads to a sexual selection. However, many news ideas emerged since then complementing the Dufour (1844) and Darwin's (1871) propositions (Eberhard 1985, 2009).

The lock and key hypothesis was corroborated in some studies which observed injuries on the female genitalia, due to the incompatible genital morphologies, after interspecific copulation in beetle and millipede species (Sota and Kubota 1998, Tanabe and Sota 2008). Additionally, an interesting case in species of flies in which females had sclerotized cavities in the genitalia to receive the sclerotized spikes of the aedeagus of males from a same species, confirming a perfect mechanical fit (Lachaise et al. 2000). However, many other researchers criticized this hypothesis due to the lack of evidence that morphological differences in genitalia really avoid the copulation process in closely related species (Eberhard 1985, Shapiro and Porter 1989, Mutanen et al. 2006). From that, other ideas stood-out to complement this hypothesis like the sensory lock and key that predict female genitalia have sensory neurons capable to recognize and avoid cross-specific males (DeWilde 1964, Masly 2012). In these cases, the interspecific copulation is interrupted as soon as the female recognize that male is not of the same species (Eberhard 1992). By this scenario, the structural lock and key hypothesis became more an exception than a general rule in the animal kingdom, as proposed by Shapiro and Porter (1989).

Nevertheless, many additional explanations have been raised about sexual selection since the genital evolution proposed by Darwin (1871). Darwin thought about sexual selection only until the copulation act preceded by male-male fights. Actually, the sexual selection can occurs even after the males initiate the copulation because not all copulations result in insemination neither all insemination leads to the oocyte fertilization (Eberhard 2009). The term "cryptic female choice" was introduced to designate all female choice occurring just after the beginning of the copulation. This

choice can be modulated by several mechanisms such as contraction of the internal female genitalia to avoid/ allow the male genitalia penetration in an optimum place to release the sperm; digestion of the gametes; or even modulation of the ovulation and embryo maturation (Eberhard 2010).

A common tool to study genital evolution is the static allometry, which is a proportional measure of certain body parts of a specimen from a population with individuals in a range of sizes (Eberhard 2008). Historically, the genital compatibility has been studied using the males as reference and it is believed the genital compatibility is favoured by the negative allometry (Eberhard 2008). The rationale is as follow: the best size for the male genitalia is the one that fits the greatest number of conspecific female genitalia (considering normal distribution for females). This optimal male genital size is the same for all bodies' sizes (small, medium and large). Thus the expectation is that male genital size will vary little even when body size is variable and this is a negative allometry. The sexual selection was frequently associated with positive allometry traits, especially when the genitalia was used as weapons or ornaments (Kodric-Brown et al. 2006, Eberhard 2008), however, this scenario has been changing and currently, neither all positive allometry can be associated with sexual selection since the evolution of positive allometry is a result of narrower selective conditions that cannot be inferred only by slopes (Bonduriansky and Day 2003).

Despite of all scientific debate, most of genital morphology studies are concentrated only on insects, thus, we noticed a lack of information on crustaceans. The male genitalia of penaeoidean shrimps is composed by petasma (modified endopods of the first pair of pleopods) and appendices masculinae (a process formed by the second pair of pleopods) (Bauer, 1991; 1996).The female genitalia is formed by thelycum (open or closed) and seminal receptacle (in closed thelycum species), which are thoracic sternal structures associated, respectively, with the attachment and storage of the spermatophores, i.e., a complex structure which enclose the sperm (Bauer 1991, 1996, Bauer and Min 1993, Becker et al. 2013, Krol 1992). We have reasons to expect different patterns between crustaceans and insects, since these crustaceans have exposed and relatively large genitalia, whereas the insects frequently have small and hidden genitalia (Eberhard et al. 1998). Nine penaeoidean shrimp species were selected and classified in two groups to investigate the morphometry of their genital trait. The first group being two open thelycum species (spermatophores is externally deposited over the thelycum); and the second one, seven closed thelycum species (the sternal plates

enclose the seminal receptacle). The first purpose was identifying if the lock-and-key hypothesis is acting in these species; we expected that this hypothesis would be confirmed in closed thelycum species because the spermatophores need to be introduced inside the female genitalia, and not confirmed in open thelycum species. . The second purpose was investigate the relative growth (static allometry) of genital traits (it was utilized the size of petasma and appendices masculinae of all nine species). And the last purpose was separate the species according to the morphological genital differences among than.

Material and methods

Sampling

We classified the nine species of Penaeoidea Rafinesque-Schmaltz, 1815 used in this study were classified in the following groups: 1) the two open thelycum species are *Litopenaeus schmitti* (Burkenroad 1936) and *Pleoticus muelleri* (Spence Bate 1888); 2) the seven closed thelycum species are *Rimapenaeus constrictus* (Stimpson 1871), *Artemesia longinaris* Spence Bate, 1888; *Farfantepenaeus paulensis* (Pérez Farfante 1967); *Farfantepenaeus brasiliensis* (Latreille 1817); *Xiphopenaeus kroyeri* (Heller 1862); *Sicyonia dorsalis* (Kingsley 1878) and *Sicyonia typica* (Boeck 1864). The species were collected by trawling, using a shrimp fishery-boat equipped with double-rig nets. The samplings were carried outin 2006 and from 2011 to 2016 at the coast of São Paulo State (municipalities of Ubatuba - 23°26'10"S, 45°01'36"W, São Vicente - 24°03'59"S, 46°16'57"W and Cananéia - 25°07'02"S, 47°50'48"W) and Santa Catarina State, Brazil (municipality of São Francisco do Sul and Itapoá - 26°06′40″S, 48°34′62″W). The species were identified according to Costa et al. (2003) and the sex assessment performed by the observation of the presence of thelycum (females) and petasma (males). In this study, we considered only adult males and females as follows: adult males when the endopods of the first abdominal appendage are joined by coupling hooks (cinccinnuli) forming the petasma (Bauer 1996) and adult females when the ovaries were green (reproductive females) or white (spent females) and extended from the carapace to the abdomen (Pérez-Farfante and Kensley 1997). More details of macroscopically observation of female gonads we adopted studies on reproduction of shrimps (Costa and Fransozo 2004, Castilho et al. 2012, 2015, Garcia et al. 2016).

Lock-and-key hypothesis

In order to test the lock-and-key hypothesis, we choose the male genital traits which were the morphological correspondent of the female genital trait in the same species (standard-measures). For the closed thelycum species, the standard-measures were defined considering the petasma aperture (= petasma width (PW)), the local of spermatophores exit, and the thelycum aperture (= thelycum length (TL): when the aperture is vertical or thelycum width (TW): when the aperture is horizontal), the local of receiving spermatophores. In the open thelycum species, the female genitalia there is no aperture, therefore we considered all possible female genitalia traits (*L. schmitti*: maximum thelycum width (MaxTW), minimum thelycum width (MinTW), maximum thelycum length (MaxTL) and minimum thelycum length (MinTL); *P. muelleri:* thelycum width (TW), thelycum length (TL), thelycum total extension (TTExt)), which could combine with the petasma aperture (PW) and it was applied a simple linear regression to determine the standard-measure (the smaller p value possible) (Table 1 and figures 1 and 2). We applied simple linear regressions in the standard-measures determined and it was considered the species presented a lock and a key when the correlation was evidenced ($p < 0.05$).

Table 1. Standard-measures (genital traits of males and females morphologically correspondents) of nine species of Penaeoidea shrimps.

Species	Standard-measures Male vs Female
L. schmitti	PW vs MaxTW
P. muelleri	PW vs TTExt
R. constrictus	PW vs TW
A. longinaris	PW vs TW
F. paulensis	PW vs TL
F. brasiliensis	PW vs TL
X. kroyeri	PW vs TW
S. dorsalis	PW vs TW
S. typica	PW vs TW

PW, petasma width; MaxTW, thelycum maximum width; TW, thelycum width; TL, thelycum length.
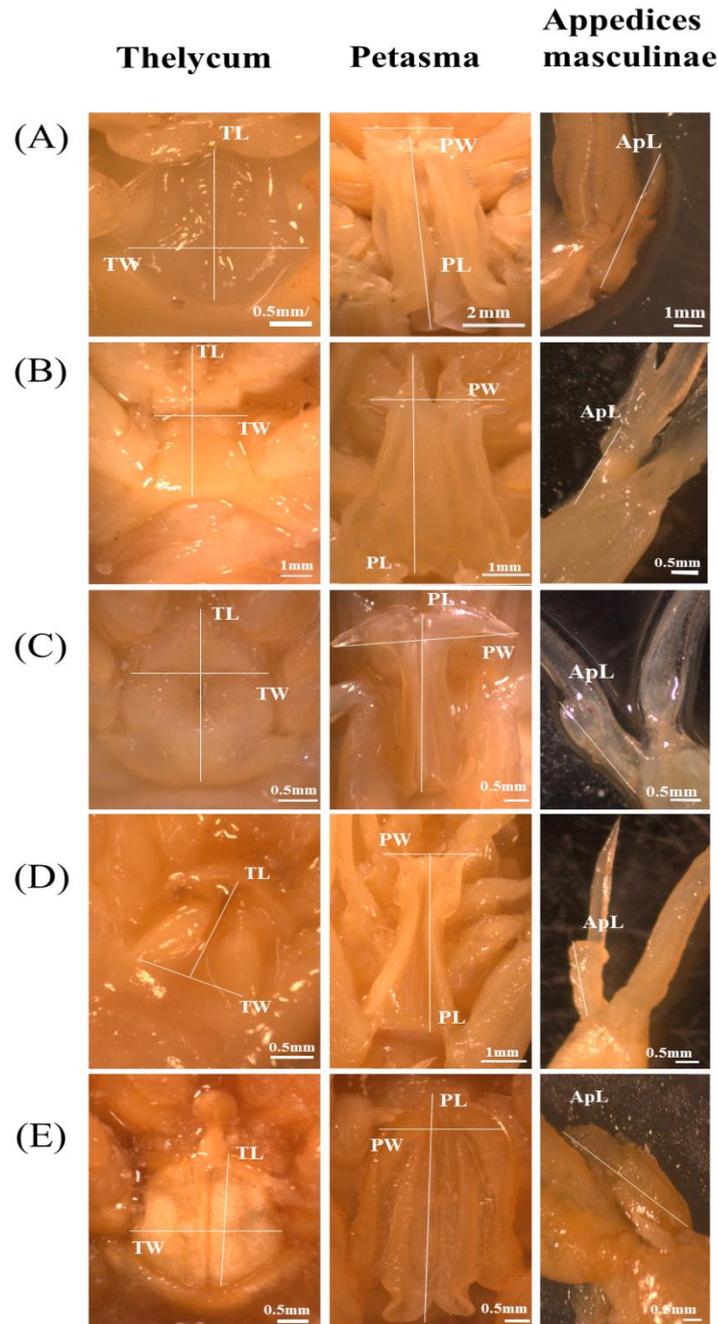
Figure 1. Measures of thelycum, petasma and appendices masculinae of five species of penaeoidean shrimps: (A) *L. schmitti*, (B) *P. muelleri*, (C) *R. constrictus*, (D) *A. longinaris* and (E) *F. paulensis.* **Female** measurements: TL, thelycum length; MaxTL, maximum thelycum length; MinTL, minimum thelycum length; TW, thelycum width; MaxTW, maximum thelycum width; MinTW, minimum thelycum width; TTExt, thelycum total extension; CW, cap width; TA, thelycum aperture; TLExp, thelycum length with expansion. **Male** measurements: PL, petasma length; PW, petasma width; ApL, appendices length.
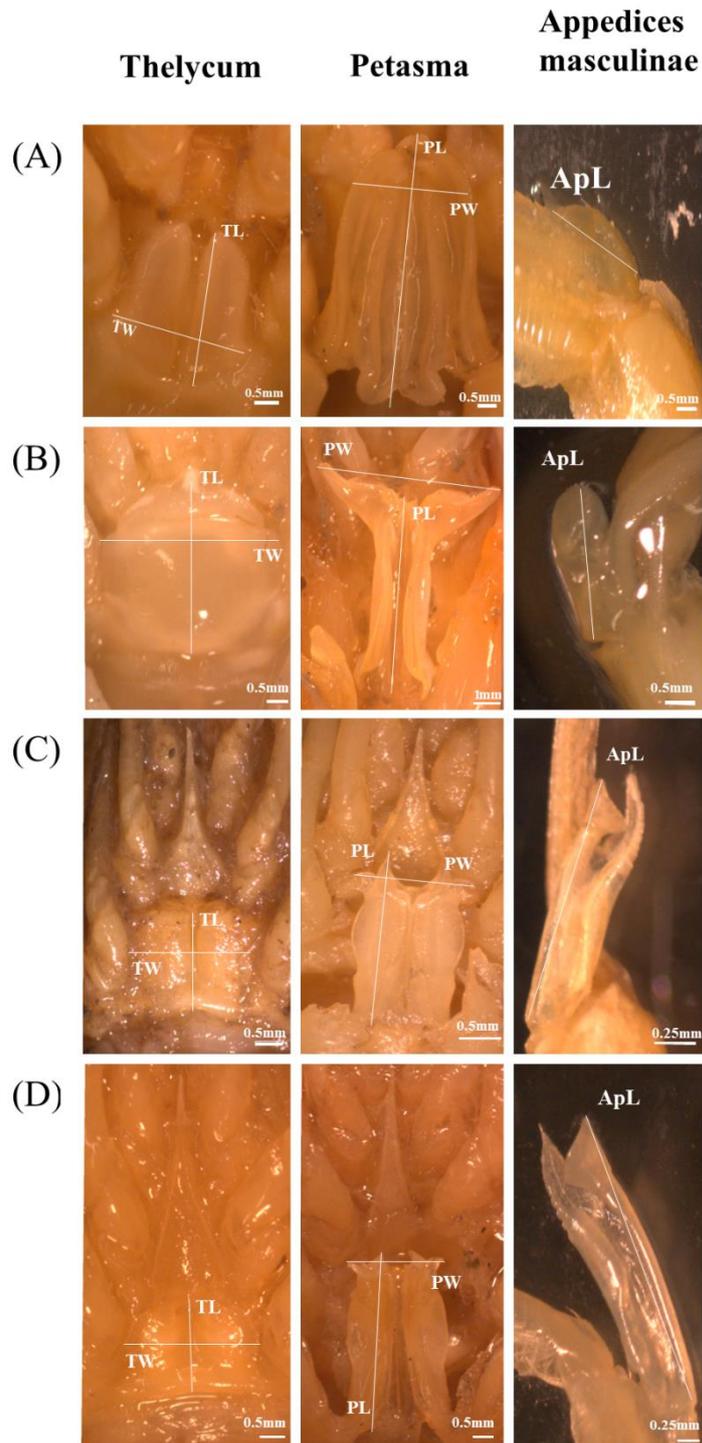
Figure 2. Measures of thelycum, petasma and appendices masculinae of four species of penaeoidean shrimps: (A) *F. brasiliensis*, (B) *X. kroyeri*, (C) *S. dorsalis* and (D) *S. typica.* **Female** measurements: TL, thelycum length; TW, thelycum width; TLExp, thelycum length with expansion; **Male** measurements: PL, petasma length; PW, petasma width; ApL, appendices length.

Relative growth

The relative growth was analysed by simple linear regressions between a non-genital trait (carapace length) and a genital trait for both sexes in all species. Data were log-transformed to fit a linear model and the allometry was verified by Student's t-test ($\alpha = 0.05$). The possible results from the allometry are: 1 (isometry – the analysed structure has the same proportional size in individuals of different body sizes), > 1 (positive allometry – larger individuals have disproportionately larger structure in comparison with small individuals) and < 1 (negative allometry – the structure is disproportionately larger in small individuals than in the larger individuals) (Eberhard 2008).

The non-genital trait considered was the carapace length (CL), which has been chosen as an indicator of overall body size in penaeoideans shrimps (Bauer and Lin 1994, Castilho et al. 2008, Grabowski et al. 2015, Garcia et al. 2016), and the genital traits used for males were: PL, ApL, PW for all species and the genital traits considered for females were: TL and TW for all species. To analyze the relative size of appendices masculinae among the species, we determined the difference between PL and ApL for each species and we applied it in the Student's t-test single sample with the reference constant equal zero (a test of means against reference constant) to confirm the existence or not of difference between the variables for some species.

Morphological genital differences

To compare the genital traits among the species, the traits considered were: ApL, PL and PW for males and TL and TW for females. By means of the allometric equation $Y = aX^b$ it was possible to eliminate the CL size effect on the genital traits and standardize the features according to the equation: $Y_i^* = Y_i [X/X_i]^b$, where Y_i^* is the standardized size of the desired feature. Y_i is the size of the characteristic that will be standardized. X is the mean of the CL of the sample, X_i is the CL of the individual and b is the angular coefficient (Tzeng 2004). After standardization, we applied the measurements chosen for both sexes in a discriminant analysis, where the first two linear discriminant scores (LD1 and LD2) were plotted (the ellipses around the data set of a group of species represent the morphological proximity of them and do not represent the confidence level).

Results

Six of the nine species studied showed a mechanical fit due to the significance observed in the standard-measures chosen, what suggests a genital evolution with reference to the lock-and-key hypothesis. The species were the closed thelycum *R. constrictus*, *A. longinaris*, *F. brasiliensis*, *X. kroyeri* and the open thelycum *L. schmitti* and *P. muelleri*. The species *R. constrictus* and *X. kroyeri* presented the highest values of the determination coefficient (r^2) and the last three closed thelycum species do not fit in the hypothesis (*F. paulensis*, *S. dorsalis* and *S. typica*) (Table 2 and Figure 3).

Table 2. Observed relations in nine species of Penaeoid shrimps testing the lock-and-key hypothesis.

Species	N	Observed relations	p	r^2_{adj}
L. schmitti	Adults ♂ 95 ♀ 89	PW vs MaxTW**	0.006*	0.062
		PW vs MinTW	0.246	0.003
	Juveniles ♂ 11 ♀ 18	PW vs MaxTL	0.239	0.004
		PW vs MinTL	0.202	0.006
P. muelleri	Adults ♂ 99 ♀ 99	PW vs TW	4.2.E-8*	0.193
		PW vs TTExt**	6.4.E-11*	0.268
	Juveniles ♂ 39 ♀ 39	PW vs TL	1.7.E-5*	0.124
R. constrictus	Adults ♂ 100 ♀ 100	PW vs TW**	5.5.E-10*	0.281
	Juveniles ♂ 16 ♀ 16			
A. longinaris	Adults ♂ 101 ♀ 50	PW vs TW**	0.034*	0.035
	Juveniles ♂ 0 ♀ 51			
F. paulensis	Adults ♂ 45 ♀ 69	PW vs TL**	0.776	-0.013

	Juveniles ♂ 26 ♀ 2			
F. brasiliensis	Adults ♂ 27 ♀ 91	PW vs TL**	2.4.E-05*	0.163
	Juveniles ♂ 70 ♀ 06			
X. kroyeri	Adults ♂ 100 ♀ 100	PW vs TW**	2.2.E-16*	0.552
	Juveniles ♂ 29 ♀ 29			
S. dorsalis	Adults ♂ 95 ♀ 47	PW vs TW**	0.878	-0.010
	Juveniles ♂ 0 ♀ 48			
S typica	Adults ♂ 27 ♀ 53	PW vs TW**	0.195	0.029
	Juveniles ♂ 0 ♀ 0			

N, number of individuals; p, significance of α=0.05 detached by (*); r^2_{adj}, adjusted determination coefficient; (**) indicates the standard-measures; **males** measurements: PW, petasma width; **females** measurements: TL, thelycum length; MaxTL, maximum thelycum length; MinTL, minimum thelycum length; TW, thelycum width; MaxTW, maximum thelycum width; MinTW, minimum thelycum width; TTExt, thelycum total extension.
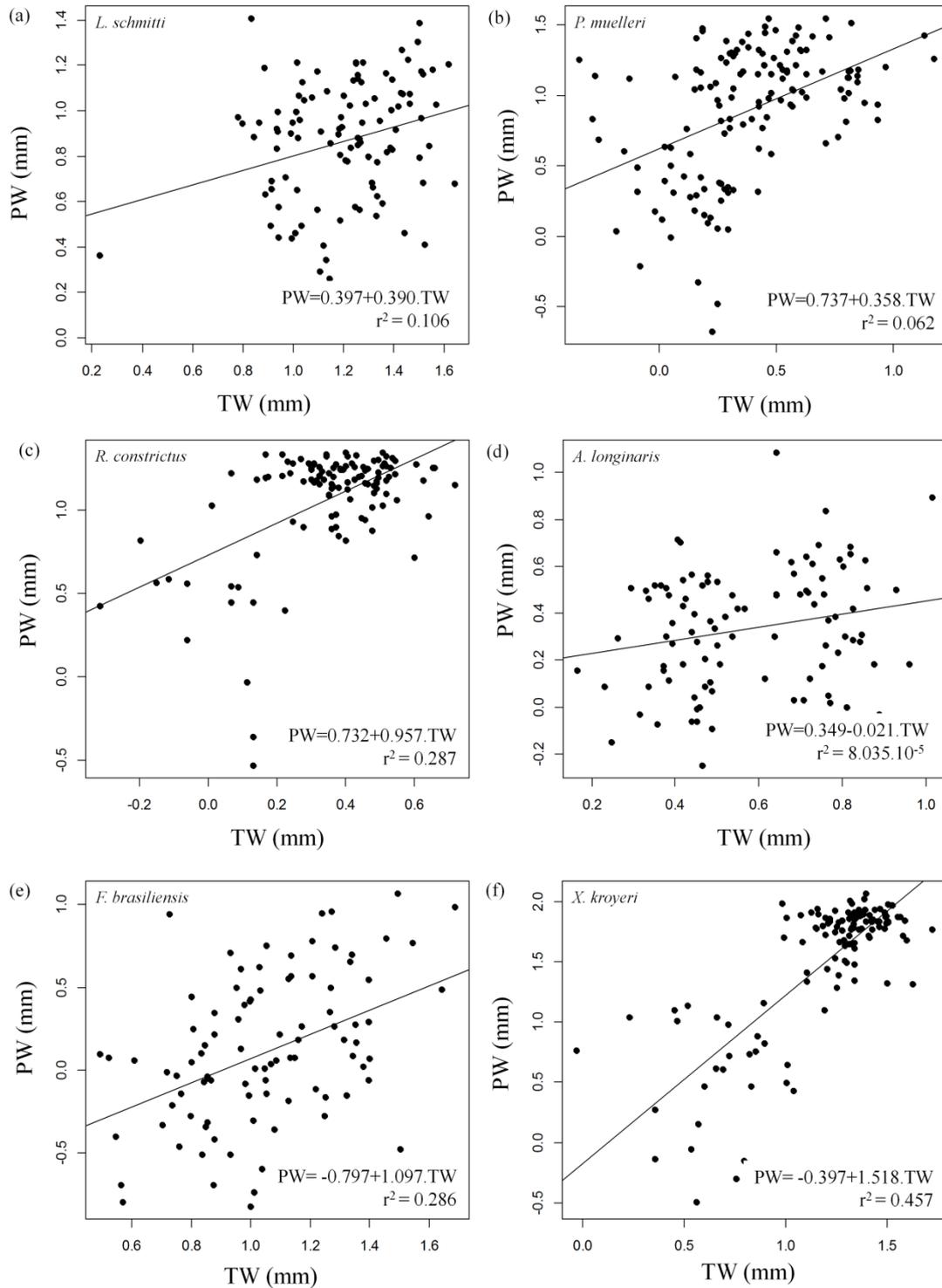
Figure 3. Simple linear regression between standard-measures for lock-and-key hypothesis tested in the six species that showed positive correlation (p < 0.05).

The genital traits showed positive allometry with the body size for the majority of species with a high determination coefficient. The exceptions were the open thelycum *L. schmitti* and the closed thelycum *S. dorsalis* and *S. typica* with negative allometry for males and females in some traits analysed here. Nevertheless, we found the most interesting results were found in the species showing mechanical fit such as: *R. constrictus* had positive allometry for males and negative for females; *A. longinaris* had isometry for males and negative allometry for females; and *X. kroyeri* and *F. brasiliensis* had positive allometry for both of sexes. In other words, none of the species had the known allometric pattern for maintenance of the genital evolution with reference to the model lock and key (negative allometry for both of sexes), despite of these species have compatible genital morphologies between males and females (Table 3).

All mean differences between PL and ApL of the nine species differed from zero (t-Student, single sample $p < 0.05$). However, we observed a slightly difference between these structures in the representatives of the Sicyoniidae family, what could suggest a different function for the ApL considering the other studied species (Table 4).

Table 3. Relative growth analysis for nine species of Penaeoid shrimps, evidencing angular coefficient (b), determination coefficient (r^2) and standard error of b (SEb).

		L. schmitti	*P. muelleri*	*R. constrictus*	*A. longinaris*	*F. paulensis*	*F. brasiliensis*	*X. kroyeri*	*S. dorsalis*	*S. typica*
Males										
CL vs PL	b	0.35	**1.21***	1.60	**1.03***	1.77	2.45	1.61	0.80	0.84
r^2		0.18	0.30	0.91	0.93	0.86	0.93	0.87	0.77	0.93
SEb		0.07	0.16	0.05	0.03	0.08	0.07	0.05	0.05	0.05
CL vs PW	b	0.37	**1.03***	2.14	**1.16***	1.53	1.87	1.10	0.83	0.86
r^2		0.15	0.49	0.90	0.66	0.50	0.51	0.88	0.80	0.94
SEb		0.08	0.09	0.07	0.08	0.16	0.19	0.04	0.05	0.05

CL vs ApL	b	0.40	0.74	**1.24***	**1.03***	1.24	1.44	1.26	0.84	**0.97***
	r^2	0.26	0.49	0.58	0.86	0.81	0.90	0.73	0.72	0.97
	SEb	0.06	0.06	0.10	0.04	0.07	0.05	0.07	0.06	0.04
Females										
CL vs TL	b	0.49	0.81	**0.95***	0.73	**1.03***	1.30	0.94	NS	**0.98***
	r^2	0.19	0.89	0.69	0.79	0.84	0.94	0.85		0.79
	SEb	0.10	0.02	0.04	0.03	0.05	0.03	0.03		0.07
CL vs TW	b	0.44	1.05	0.86	0.61	**0.95***	1.11	1.06	0.74	**0.91***
	r^2	0.15	0.90	0.65	0.80	0.77	0.88	0.86	0.71	0.78
	SEb	0.10	0.02	0.04	0.02	0.06	0.04	0.03	0.03	0.07

b, angular coefficient; r^2, determination coefficient; SEb, standard error of b; CL, carapace length; PL, petasma length; PW, petasma width; ApL, appendices length; TL, thelycum length; TW, thelycum width; (*) indicates the isometry; NS, non-significant.

Table 4. Petasma length (PL) related to the appendices masculinae length (ApL) in nine species of Penaeoid shrimps.

Species	N	Mean difference between PL and ApL (mm)
Litopenaeus schmitti	112	3.14*
Pleoticus muelleri	138	1.74*
Rimapenaeus constrictus	116	1.48*
Artemesia longinaris	101	1.88*
Farfantepenaeus paulensis	88	1.42*
Farfantepenaeus brasiliensis	97	1.23*
Xiphopenaeus kroyeri	130	2.38*
Sicyonia dorsalis	95	0.40*
Sicyonia typica	27	0.24*

PL, mean of petasma length; ApL, mean of appendices masculinae length; (*) indicates significant values ($p < 0.05$).

The discriminant analysis evidenced three isolated species for males: (1) *L. schmitti*, (2) *S. typica*, (3) *P. muelleri* and other three groups of closest species: (4) *R. constrictus* and *S. dorsalis*, (5) *X. kroyeri* and *A. longinaris* and (6) *F. paulensis* and *F. brasiliensis.* And for females, we could define one isolated species (1) *P. muelleri* and three groups of closest species: (2) *A. longinaris*, *F. paulensis*, *F. brasiliensis* and *R. constrictus*, (3) *X. kroyeri* and *L. schmitti* and (4) *S. typica* and *S. dorsalis* (Figure 4).
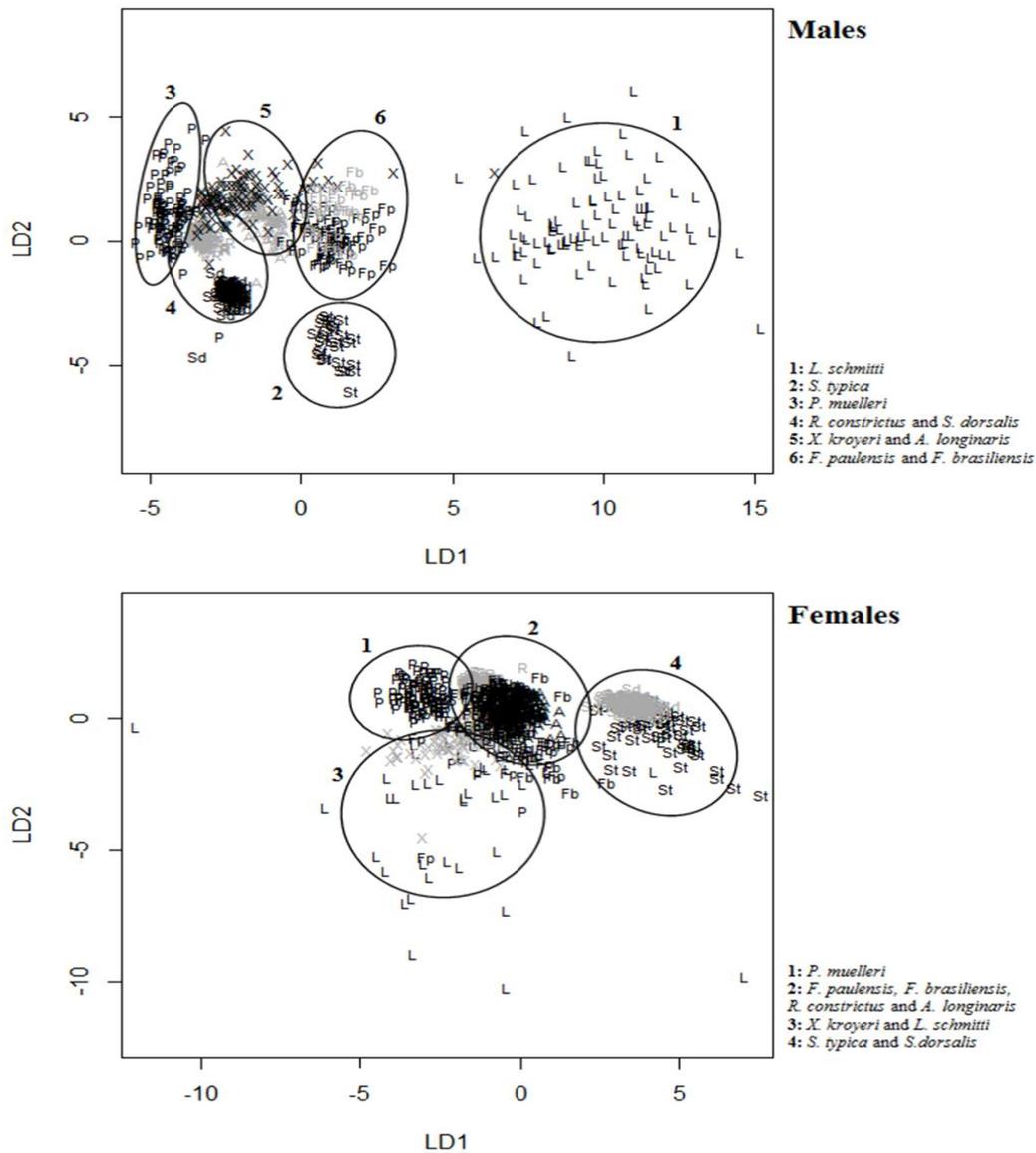
Figure 4. Differentiation of males (a) and females (b) of nine species of penaeoidean shrimps grouped according to their genital traits.

Discussion

        The fact of the lock and key hypothesis has been associated with negative allometry in the animal kingdom (old lock and key) (Eberhard 2008) means there is a single type of "key" for the greatest number of "locks" in the nature allowing a perfect mechanical fit. However, in this study the mechanical fit occurred in two species with positive allometry of standard-measures for males and females (*Xiphopenaeus kroyeri* and *Farfantepenaeus brasiliensis*). Considering that both sexes showed the same

allometric pattern, it is possible to justify this uncommon scenario by the size-assortative mating model, when the mating occur in standard sizes presuming large males copulate with large females and small males copulate with small females (Elwood et al. 1987, Eberhard et al. 1998). It is known penaeoid females are usually larger than males (Garcia et al., 2016), however, considering the normal distribution of the carapace length of the species, when males are much smaller than females, they represent a few percentage of the total males, allowing the majority of the species participate the reproductive activity.

The most difficult scenario found in this study occurred with *Rimapenaeus constrictus*. The fact of this species shows positive allometry for males and negative for females, means there are many types of "keys" for a unique type of "lock". Usually, the penaeid males exhibit higher growth rates than females and *R. constrictus* is not an exception (Petriella and Boschi 1997, Garcia et al. 2016). Thus, we propose that in the early stages of adult life, when petasma is small the males do not copulate. Moreover, while the males are growing fast it is possible to a size in which the petasma been compatible with the most thelycum sizes, considering normal distribution for females. These characteristics found in penaeid males probably compensate the difference of allometric patterns between males and females to maintain the lock and key in this species. This same situation probably also occurring to *Artemesia longinaris* that showed isometry for males and negative allometry for females. In short, it is possible to have many types of "keys" for a unique type of "lock", being the diversity of "keys" for *A. longinaris* lower than to *R. constrictus* probably due to the isometry.

Other intriguing finding of this study occurred with both species *Farfantepenaeus paulensis* and *F. brasiliensis*. The first species do not show mechanical fit and the second had, despite of they been close related species, demonstrated through both sperm ultrastructure and molecular (Camargo et al. 2017). The question emerged from this found is: how is it possible to occur considering these two species are very similar in genital morphology to both males and females? The species presented different allometric patterns. *Farfantepenaeus brasiliensis* was positive allometric for both sexes, what is in accordance with the size assortative mating for maintenance of the presence of a "lock" and a "key".

On the other hand, *F. paulensis* was positive allometric for males and isometric for females what means the variance of the size thelycum is high what become impossible the growth rate of the male compensate the different allometric patterns

between males and females as observed for *R. constrictus*, and at the same time, the isometry produce a lower variance than the observed in positive allometry and the size assortative mating is also not possible as observed for its congener species and *X. kroyeri*, so because of this combination of allometries, the mechanical fit is not maintained in this species.

All of our find, indicate the maintenance of a mechanical fit seems to be associated with a diversity of allometric patterns: when both sexes are positive allometric (the mechanical fit is maintained by the size assortative mating) and when males present positive allometry or isometry combined with a female which are negative allometric (the mechanical fit is maintained by the higher male growth rate). Besides the research of Mutanen et al. (2006) which discovered a lock and key associated with male negative allometry.

For the species with higher complementarity between the genital traits (*R. constrictus* and *X. kroyeri*, see figure I in the attachment section in the final of the thesis), the lock and key hypothesis would predict that changes in the female genital morphology are closely related to the morphological changes in the male genitalia in a process of "sexually antagonistic co-evolution" of males and females. This co-evolution leads each sex evolving new morphology to counteract the recent advances by the other sex. This process can occur simultaneously or right after the cryptic female choice, leading to conspecific pattern of gametes and ultimately to speciation (Arnqvist et al. 2000, Brown and Eady 2001, Arnqvist and Rowe 2002, Eady 2001, Simmons 2014).

The gametic isolation represents an important role in the reproductive isolation for free-spawning marine invertebrates, and usually because of interactions among sympatric species (Eady 2001). Camargo et al. (2016) found an interesting scenario of morphological differences in the sperm ultrastructure of *Sicyonia dorsalis* and *Sicyonia typica,* and a possible gametic isolation is acting in this genus. According to these authors, *S. typica* shared more sperm traits with allopatric species, as *Sicyonia carinata* (Brünnich 1768) and *Sicyonia brevirostris* Stimpson, 1871, than with the sympatric species *S. dorsalis*.

These results corroborate our finding, since we demonstrate distance between males of *S. typica* and *S. dorsalis* by morphological analysis of the petasma and appendices masculinae. Thus, it is reasonable to suppose the morphological differences in the gametes are produced by a cryptic female choice and not by the mechanical fit, since both Sicyoniidae do not show "key" neither "lock" as showed in this study. Ma et

al. (2009) and Camargo et al. (2016, 2017) using molecular and sperm ultrastructure indicated that Sicyoniidae is closer to Trachypenaeini (include the genus like *Xiphopenaeus* and *Rimapenaeus*) than Penaeini tribe (include genus like *Litopenaeus*) because, by many reasons, the shape of the genitalia in SICYONIIDAE is similar to many genus of representatives of the Trachypenaeini tribe. Our own results indicate that males of *S. dorsalis* are closer to *R. constrictus* concerning to the genital morphology that possibly means an adaptive convergence is occurring.

Closed thelycum species, like Sicyoniidae representatives, without mechanical fit seems to be no biologically coherent, as species with no intromitting petasma (open thelycum) with mechanical fit, like *L. schmitti* and *P. muelleri*. However, the adjust observed in open thelycum species is probably because of males and females have the same pattern of relative growth or close and not necessarily means the genitalia are co-evolving. Thus, the lock and key is probably dispensable in open thelycum species. The absence of lock and key in the closed thelycum *S. dorsalis* and *S. typica* could be justified by the evidence found by Bauer (1996) that petasma is not a spermatophore transfer structure in *S. dorsalis* and the Sycioniidae sperm is transferred free in the seminal fluid to the spermatheca (Bauer 1991).

Considering the Sicyoniidae species, we have found negative allometry in males of *S. dorsalis* and *S. typica*. We propose this situation is related to the "One-size-fits-all" hypothesis proposed by Eberhard et al. (1998); according to the authors, when the cohort involves contact between male and female, there is a tendency of females chose males in a standard size, in other words, an appropriated size for the most numbers of females. This scenario results in low allometric values (negative allometry) because the petasma is used only as a delivery structure of tactile stimulation and we have found evidences in the literature to support this proposition.

Bauer (1996) found a membranous tissue covering the thelycum of *S. dorsalis*, what could function as a sensory area for receiving male stimulation and support the sensory lock and key hypothesis of recognition and avoidance of cross-specific males (DeWilde 1964, Masly 2012). Masly (2012) proposed that the appendices masculinae and petasma in this species would be a structure of stimulation or a structure to assist the temporarily connection between male and female genitalia, whereas the transfer of the spermatophores would be completed by the male genital papilla. Our results seems to corroborate this proposition, once the appendices masculinae of Sicyoniidae representatives were almost the same size of petasma, differing among the other species,

and both structures would facilitate the holding function during copulation; on the similar morphology, we believe *S. typica* presents the same functions described for *S. dorsalis*. This difference observed could occur as Sicyoniidae and the other studied species are in separated clades based on molecular analysis, besides they are very different in the sperm ultrastructure (Camargo et al. 2016). Probably, the other species have the primary function of transferring spermatophores by petasma (and probably appendices masculinae) as early proposed by Bauer (1991).

In addition, the negative allometry to *L. schmitti* males is not related to stimulation or holding during the copula as proposed to Sicyoniidae representatives, once the male of *L. schmitti* was observed checking the thelycal region using the rostrum and after the spermatophore be attached, maybe in an attempt to assist the release of the sperm of the spermatophore attached against the thelycum (Bueno 1990). Thus, what could generate this allometry? Actually, an important factor described for open thelycum species is about the complexity of the spermatophore, demonstrated by the complex ampoule at the ejaculatory duct (Fransozo et al. 2016). It is feasible that males allocate more energy to produce a seminal fluid and adhesive spermatophores (showing wings, flanges and plates) to warrant the viability of this exposed structure (Bauer 1991, Fransozo et al. 2016) than allocating energy to the petasma growth, leading to an absence of positive allometry in males *L. schmitti* and *P. muelleri* (both open thelycum).

Conclusions

The genitalia evolution in penaeoideans shrimps seems to be involved with both mechanical fit (lock and key hypothesis), sexual selection by cryptic female choice and sexually antagonistic coevolution. Furthermore, we have found some evidences for the mating behaviors associated with "Size-assortative mating", "One-size-fits-all" and "Sensory lock and key". It is possible to suppose that the male-female fit occurs in a diversity of allometric patterns and it is not necessarily related to the fitness neither with body design of the individuals, since the lock and key hypothesis was not verified for all species, but occurring to species showing both open and closed thelycum species. The presence of a "key" and a "lock" in the genital morphology of penaeoideans shrimps seems to represent a mechanism of reproductive isolation for some species (*R. constrictus* and *X. kroyeri*), as the cryptic female choice for others (*S. typica* and *S. dorsalis*) and is not related to the phylogenetic relationship.

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Considerações finais

Considerações finais

Neste estudo, pudemos observar que a ciência da morfologia genital de camarões peneóideos é bastante antiga, porém, vem se modificando com o tempo e as definições de télico e petasma tornam-se, cada vez mais complexas. Burkenroad (1934) contribuiu de maneira expressiva para essa ciência, sendo o primeiro estudo a definir com minúcia a morfologia das genitálias dos camarões peneóideos; após, podemos destacar o estudo de Bauer (1991) que incluiu em suas definições, características nunca antes consideradas, como a flexibilidade dos lobos laterais do petasma e a presença da costa ventral. Já Pérez-Farfante e Kensley (1997) vieram tornar mais amplo e minucioso o trabalho apresentado por Bauer (1991).

Há dificuldades em se propor generalizações, embora sejam importantes para a compreensão da morfologia e evolução genital. Observamos que Pérez-Farfante e Kensley (1997) unem em um mesmo tipo de petasma, as espécies *P. muelleri* e *L. schmitti*. A partir disso, propomos a união dos termos petasma semi-aberto e semi-fechado, no que denominamos de petasma semitubular ou semi-fechado, tornando menos generalista a classificação do petasma quanto à flexibilidade dos lobos laterais. Pudemos ainda observar a presença de canais no petasma de espécies consideradas como mais derivadas na escala proposta por Bauer (1991). Esses canais foram observados no presente estudo e no amplo estudo de Pérez-Farfante e Kensley (1997).

Já quanto ao télico, é importante destacar que a sua morfologia parece bem mais difícil de generalizar como foi feito com a morfologia do petasma tantas vezes ao longo da História. Neste estudo, pudemos identificar a divisão do télico em apenas duas áreas comuns dentre as cinco espécies analisadas no primeiro capítulo desta tese.

Observamos uma grande diversidade de padrões alométricos entre as genitálias dos camarões estudados e a pergunta a ser respondida seria: é possível haver chave-fechadura em uma ampla diversidade de padrões alométricos? Foi a partir desta questão que formulamos hipóteses para propor a manutenção deste processo chave-fechadura em camarões peneóideos, são elas: (1) "Size assortative mating", quando observamos ambos os sexos com alometria positiva da genitália e (2) "Higher male growth rate", quando machos apresentam alometria positiva ou isometria e as fêmeas, alometria negativa. Além do "Old lock and key", que considera que ambos os sexos possuem

alometria negativa e o qual foi considerado como a única possibilidade de manutenção da chave-fechadura até agora.

Dessa forma, não corroboramos a hipótese de que a chave-fechadura ocorreria nas espécies consideradas mais derivadas na escala evolutiva proposta por Bauer (1991), já que encontramos tal diversidade alométrica que nos permitiu observar ajuste mecânico em espécies primitivas, assim como, ausência de chave-fechadura em espécies consideradas como mais derivadas.

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Anexo

Anexo
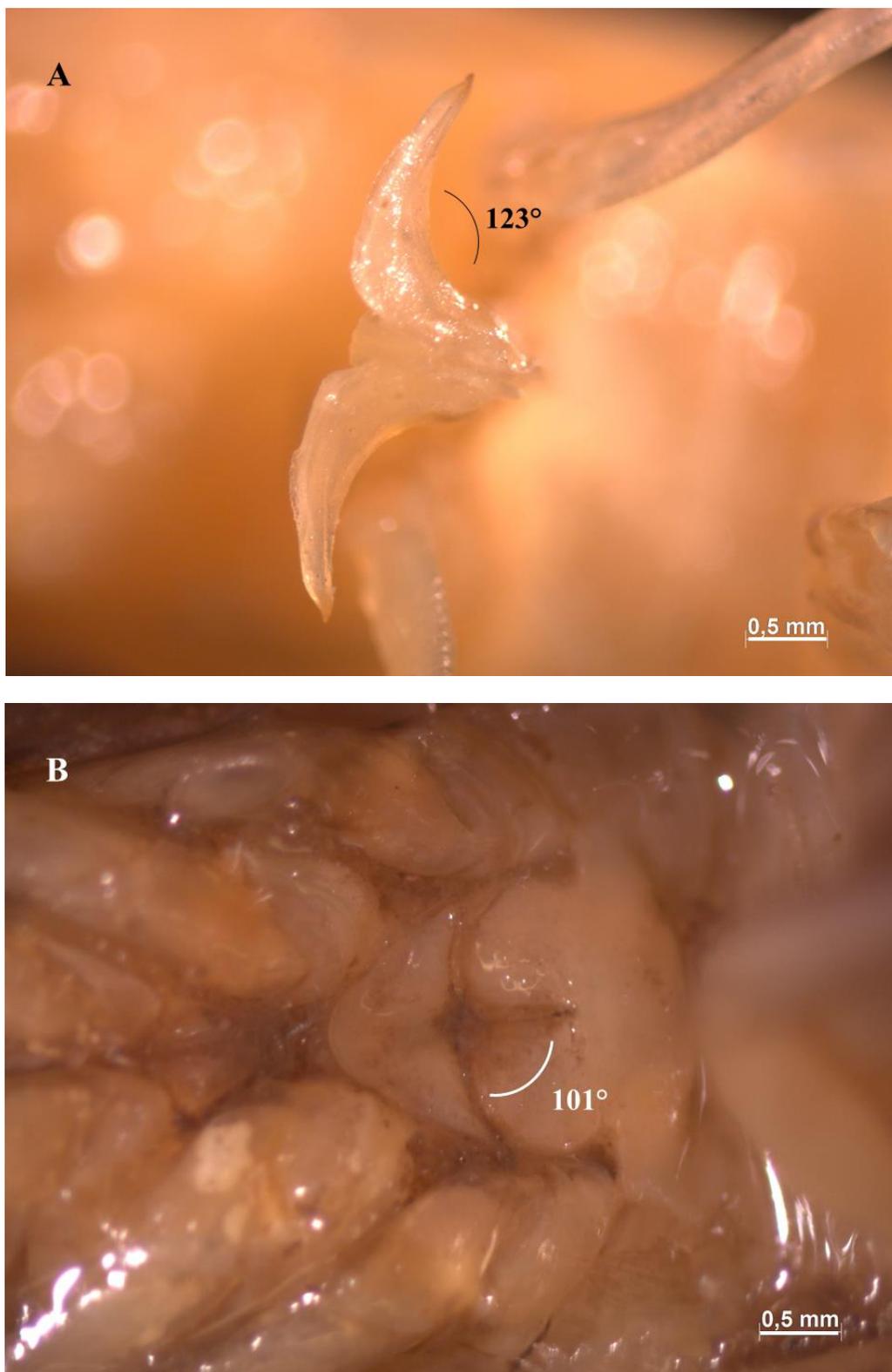
Figura I. *Rimapenaeus constrictus*. (A) Extremidade do petasma, evidenciando uma curvatura de 123° das projeções laterais e (B) télico da espécie evidenciando curvatura de 101° nos "*flaps*".