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MORFOLOGIA DO SISTEMA REPRODUTOR MASCULINO DO CAMARÃO PENEÍDEO *Penaeus schmitti* (BURKENROAD, 1936) CAPTURADO NO NORDESTE DO BRASIL

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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

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Resumo

O camarão marinho *Penaeus schmitti* tem um potencial considerável para a aquicultura, considerando que a produção comercial já ocorreu em países onde a espécie é nativa. Estudos sobre reprodução são cruciais para o desenvolvimento de programas de melhoramento que facilitem a produção de larvas e o cultivo desta espécie. Portanto, este estudo utilizou técnicas histológicas para descrever a morfologia do sistema reprodutivo masculino e o processo de desenvolvimento das células germinativas de P. schmitti maduros e imaturos. Machos adultos de *P. schmitti* foram capturados na costa nordeste do Brasil e classificados como maduros e imaturos. A característica sexual externa, o petasma, estava fundida em machos maduros e não fundida em machos imaturos. Animais maduros apresentavam maior comprimento total, comprimento do cefalotórax, peso total, comprimento do petasma e peso da ampola terminal quando comparados aos imaturos. Macroscopicamente, o sistema reprodutivo de *P. schmitti* mostrou simetria bilateral, com um testículo dividido em 8 a 10 lobos, um vaso deferente dividido em três regiões: proximal, média e distal, e uma ampola terminal. Essas características estavam presentes tanto em animais maduros quanto imaturos, diferindo apenas em tamanho, turgidez e coloração observados após a dissecação do sistema reprodutivo. Microscopicamente, todas as células germinativas estavam presentes em animais maduros: espermatogônias, espermatócitos (I e II), espermátides e espermatozoides foram encontrados nos túbulos seminíferos do testículo, onde ocorre a espermatogênese, além da presença de células acessórias, as células de Sertoli. O vaso deferente tinha características de transporte de esperma, pois continha apenas espermatozoides e fluido seminal. A ampola terminal era a região mais complexa do sistema reprodutivo e serve como estrutura para armazenamento de espermatozoides até a cópula. Espermatozoides e fluido seminal foram observados no lúmen desta região. A ampola terminal continha células secretoras responsáveis por produzir a massa adesiva à qual os espermatóforos se fixam ao télio da fêmea. Em animais imaturos, apenas espermatogônias e células germinativas de espermatócitos I foram encontradas no testículo, juntamente com células de Sertoli. Além disso, a ampola terminal não apresentava secreção, fluido seminal ou células germinativas nesses animais. Isso provavelmente indica que a maturidade morfológica está sincronizada com a maturidade fisiológica em *P. schmitti*. Portanto, os resultados deste estudo fornecem informações úteis sobre o sistema reprodutivo masculino de peneídeos e o desenvolvimento das células germinativas, bem como para melhorar a reprodução controlada de *P. schmitti* na aquicultura.

Palavras-chave: Aquicultura; reprodutores; histologia; maturação.

Abstract

The marine shrimp *Penaeus schmitti* has a considerable potential for aquaculture, considering that commercial production has already taken place in countries where the species is native. Studies on reproduction are crucial for the development of breeding programs to facilitate larval production and farming of this species. Therefore, this study used histological techniques to describe the morphology of the male reproductive system and germ cell development process of mature and immature P. schmitti. Adult males of P. schmitti were captured in the northeast coast of Brazil and classified as mature and immature. The external sexual characteristic, the petasma, was fused in mature and unfused in immature males. Mature animals had greater total length, cephalothorax length, total weight, petasma length and terminal ampulla weight when compared to immature ones. Macroscopically, the reproductive system of *P. schmitti* showed bilateral symmetry, with a testis divided into 8 to 10 lobes, a vas deferens divided into three regions: proximal, middle and distal, and a terminal ampulla. These features were present in both mature and immature animals, differing only in size, turgidity and coloration observed after dissecting their reproductive system. Microscopically, all the germ cells were present in mature animals: spermatogonia, spermatocytes (I and II), spermatid and spermatozoa were found in the seminiferous tubules of the testis, where spermatogenesis occurs, in addition to the presence of accessory cells, the Sertoli cells. The vas deferens had the characteristics of sperm transport, as it contained only spermatozoa and seminal fluid. The terminal ampulla was the most complex region of the reproductive system and serves as a structure for the storage of spermatozoa until the copulation. Spermatozoa and seminal fluid were observed in the lumen of this region. The terminal ampulla contained secretory cells that are responsible for producing the adhesive mass to which the spermatophores attach to the female thelycum. In immature animals, only spermatogonia and spermatocyte I germ cells were found in the testis, along with Sertoli cells. Furthermore, the terminal ampulla showed no secretion, seminal fluid or germ cells in these animals. This probably indicates that morphological maturity is synchronized with physiological maturity for P. schmitti. Therefore, results from this study provide useful information on penaeid male reproduction system and germ cells development, as well as to improve controlled breeding of *P. schmitti* in aquaculture.

Keywords: Aquaculture; broodstock; histology; maturation.

Lista de figuras

Lista de figuras Capítulo 1

Lista de tabelas

Página

Sumário

1. Introdução

A crescente demanda de alimento de alto valor proteico tem acelerado o crescimento de criação de espécies que apresentam alto valor agregado, tanto nutricional quanto econômico (FAO, 2022). Uma das atividades responsáveis por promover alimentos com essas características é a aquicultura. A aquicultura é uma importante atividade econômica e se destaca por ser reconhecida mundialmente pela geração de emprego, renda e segurança alimentar (FAO, 2022). Dentro da aquicultura, nos últimos anos, a carcinicultura marinha tem apresentado grande destaque. Em 2020, a produção total de crustáceos mundial foi de 11,3 milhões de toneladas, sendo os camarões peneídeos um dos principais grupos, representados principalmente pelas espécies *Penaeus vannamei* (52,9%) e *Penaeus monodon* (8%) por apresentar um alto valor econômico e nutricional (FAO, 2022).

No Brasil, o desenvolvimento da carcinicultura iniciou na década de 70, por meio do "projeto camarão" com estudos para adaptação da espécie exótica *P. japonicus* em salinas da região Nordeste (LARA et al., 1974; NATORI et al., 2011). Entretanto, a espécie não se adaptou às condições ambientais do local, seguindo para a segunda fase de desenvolvimento do projeto, com estudos de viabilidade de produção com espécies nativas *Penaues. Paulensis* e *Penaeus. schmitti* a partir da implantação do laboratório de larvicultura na Universidade Federal de Santa Catarina (ANDREATTA et al., 1985; ALFONSO et al., 1997; NATORI et al., 2011; VINATEA e ANDREATTA, 1997). Embora os estudos tenham obtido resultados satisfatórios em relação a reprodução e produção de pós-larvas em cativeiro, eles foram interrompidos no final da década de 80 com a introdução do *P. vannamei,* uma exótica do oceano Pacífico, por apresentar excelente desempenho zootécnico e alta adaptabilidade ao clima brasileiro e pacote tecnológico estabelecido (NATORI et al., 2011, TAIN, DAMACENO e ARAÚJO, 2019).

Atualmente, o Brasil é o 10º país em produção mundial de crustáceos, com a espécie *P. vannamei,* atingindo uma produção de 113,3 mil toneladas em 2022, representando um aumento de 18.14% em relação ao ano de 2020 (FAO, 2022; IBGE, 2023). No entanto, as fugas de espécies exóticas dos sistemas de criação podem causar diversos impactos ecológicos, econômicos e sanitários, entre eles, a alteração na população selvagem, de habitats e introdução de doenças (BARBIERI et al., 2014; COOK et al., 2008; MMA; 2019). No país existem registros das espécies exóticas *P. vannamei* e *P. monodon* em

ambientes naturais, sendo capturados tanto maturos quanto imaturos, indicando que estes animais já estão completando seu ciclo reprodutivo (SANTOS e COELHO, 2002; SILVA et al., 2022).

Diante deste cenário, é importante que haja o desenvolvimento de tecnologias que permitam a produção de espécies nativas, contribuindo assim para a sustentabilidade da atividade, uma vez que o desenvolvimento de reprodutores em cativeiro, independentes de espécies selvagens, possibilitam trabalhos para seleção e melhoramento genético (RAMOS et al., 2019). Além disso, a produção de espécies nativas apresentam vantagens como uma melhor tolerância às condições ambientais locais, disponibilidade de reprodutores e possibilitam a criação em sistemas alternativos em ambientes marinhos como cercados e tanques-rede (SANDIFER et al., 1993, POERSCH e WASIELESKY, 2016).

Apesar das vantagens da produção de espécies nativas, atualmente não há produção comercial de nenhuma espécie da costa brasileira. Espécies nativas de camarões peneídeos com grande importância econômica têm sido estudadas ao redor do mundo com o foco no desenvolvimento da aquicultura, como o *Penaeus. setiferus* nos Estados Unidos (SAMOCHA et al., 1998; SANDIFER et al., 1993; VALENZUELA-JIMÉNEZ et al., 2020), e *P. paulensis* (PEIXOTO et al., 2004; KRUMMENAUER et al., 2006; VINATEA e ANDREATTA, 1997; WASIELESKY et al., 2006) e *P. schmitti* (ALFONSO et al., 1997; RAMOS et al.; 2019) no Brasil.

Dentre as espécies de camarões marinho distribuídas na costa brasileira, o *P. schmitti* (Fig. 1), é uma das espécies nativas mais promissoras para a aquicultura no país por alcançarem tamanhos maiores, além da excelente aceitação no mercado consumidor (BOCHINI, 2014). O *P. schmitti* pertence à família Penaeidae, tem distribuição do Atlântico Ocidental, nas Antilhas, até a região sul do Brasil. A espécie habita regiões marinhas, desde pequenas profundidades até 30 metros, apresentando registros de ocorrência em até 50 metros. Além disso, apresentam ciclo de vida em dois ecossistemas, quando estão na fase de pós-larvas, derivam em correntes oceânicas até atingirem o estuário, onde permanecem durante toda a fase jovem, migrando de volta para o oceano, onde termina seu desenvolvimento e se tornam adultos (DIAS-NETO, 2011; SANTOS, PEREIRA e IVO, 2004).

Assim como os demais peneídeos, o *P. schmitti* é dioico, e apresenta reprodução sexuada com fertilização externa, que ocorre em mar aberto (DIAS-NETO, 2011). Dente

os diversos grupos de decápodes, a morfologia do sistema reprodutor pode variar (DÌAZ et al., 2002). Entretanto, os machos possuem uma estrutura morfológica externa, denominada de petasma, que auxilia na transferência da massa espermática para o télico das fêmeas, que é do tipo “aberto” em *P. schmitti*.

A morfologia interna do sistema reprodutor dos camarões, de forma geral, é dividida em testículo, vaso deferentes e ampola terminal (BAUER, 1991; CHOW et al., 1991). Dentre os estudos de biologia reprodutiva, o conhecimento da morfologia do trato reprodutivo é um dos aspectos mais importantes para o desenvolvimento da criação de espécies que apresentam interesse econômico, como manutenção de plantel de reprodutores e emprego de biotecnologias (ANRADE et al. 2015; DÍAZ et al., 2002; PAPA, 2007; ROCHA, 2010).

Figura 1: *Penaeus schmitti* (Burkenroad, 1936)

Nos últimos anos, os estudos com *P. schmitti* tiveram como enfoque aspectos relacionados à biologia pesqueira, abordando dinâmica reprodutiva e descrição do sistema reprodutor, principalmente de fêmeas (PEIXOTO et al., 2018; CRAVEIRO et al., 2018); e dinâmica populacional (CAPPARELLI et al., 2012; BOCHINI et al., 2014; BARIOTO et al., 2017, SILVA et al., 2018, BARROS et al., 2021; DE CARVALHO et al., 2021).

Em relação aos machos, os estudos são mais escassos, uma vez que o potencial reprodutivo é medido pela fecundidade das fêmeas (VAN ENGEL, 1990). Além disso, os machos apresentam um sistema reprodutor mais complexo e recebem menos atenção em relação a estudos voltados a morfologia, fisiologia e endocrinologia (ALFARO-MONTOYA, 2010; BROWDY, 1992; BROWDY, 1998).

No Brasil, estudos voltados para a aquicultura da espécie nativa *P. schmitti* foram realizados com o enfoque em parâmetros zootécnicos em condições laboratoriais utilizando sistema multitrófico (FRAGA et al., 2002; MELO et al., 2018; MÁRQUEZ et al. 2018), e estudos com foco exclusivamente em machos da espécie focaram em criopreservação de sêmen e viabilidade espermática (CHAVES et al., 2014; FERNANDES et al., 2014) e estudos preliminares sobre a morfologia funcional do sistema reprodutor de machos maturos (FANSOZO, 2016). Apesar dos desenvolvimentos desses estudos, um dos entraves na produção de espécies nativas pode estar relacionado à falta de bioensaios para avaliar o desempenho reprodutivo de machos de peneídeos através das células germinativas masculinas, sendo utilizadas informações obtidas a partir do conhecimento advindo do *P. vannamei* (PARNES et al., 2004) ignorando as particularidades que podem existir no *P. schmitti.*

Além das poucas informações sobre o desenvolvimento das células germinativas em *P. schmitti*, estudos voltados para a reprodução em cativeiro de outras espécies nativas também encontraram alguns problemas. Alfaro-Montoya (2010), através de uma revisão bibliográfica, verificou três principais problemas relacionados ao sistema reprodutivo de machos do gênero Penaeus em cativeiro, sendo eles síndrome degenerativa do trato reprodutivo masculino, melanização do sistema reprodutor masculino e deterioração dos espermatóforos.

Diante dos problemas apontados e visando as melhorias nas tecnologias para o desenvolvimento de espécies nativas em cativeiro, sobretudo para os machos de *P. schmitti*, o conhecimento detalhado da morfologia do sistema reprodutor de machos e formação dos espermatóforos, assim como das substâncias associadas ao desenvolvimento das células germinativas, são essenciais para a compreensão dos mecanismos de inseminações artificiais, formação e a liberação dos espermatozoides durante a fertilização e a cópula (BAUER, 1991; BAUER; LIN, 1993).

Neste contexto, o presente estudo descreve a morfologia do sistema reprodutor de machos maturos e imaturos do camarão marinho *P. schmitti*, utilizando análise histológica.

2. Objetivos

2.1 Objetivo geral

O presente estudo teve como objetivo descrever a morfologia do sistema reprodutor de machos maturos e imaturos do camarão *P. schmitti* capturados no litoral norte da Paraíba, Brasil, visando contribuir para o desenvolvimento do potencial reprodutivo de espécies nativas em cativeiro.

2.2 Objetivos específicos

Para alcançar o objetivo geral, os seguintes objetivos específicos foram definidos:

- Descrever morfologicamente aspectos macroscópicos do sistema reprodutor masculino de indivíduos imaturos e maturos do camarão *P schmitti*;

- Descrever microscopicamente a morfologia do trato reprodutivo, células acessórias e células germinativas de indivíduos imaturos e maturos do camarão *P. schmitti*;

- Comparar o sistema reprodutivo masculino de indivíduos imaturos e maturos do *P schmitti*.

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CAPÍTULO I

Artigo científico a ser submetido ao periódico Aquaculture. Todas as normas de redação e citação deste capítulo atendem às estabelecidas pelo periódico (em anexo)

Male reproductive system morphology of penaeid shrimp: a new approach on the reproduction and development of germ cells in *Penaeus schmitti* using histological techniques.

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Abstract

The marine shrimp *Penaeus schmitti* has a considerable potential for aquaculture, considering that commercial production has already taken place in countries where the species is native. Studies on reproduction are crucial for the development of breeding programs to facilitate larval production and farming of this species. Therefore, this study used histological techniques to describe the morphology of the male reproductive system and germ cell development process of mature and immature *P. schmitti*. Adult males of *P. schmitti* were captured in the northeast coast of Brazil and classified as mature and immature. The external sexual characteristic, the petasma, was fused in mature and unfused in immature males. Mature animals had greater total length, cephalothorax length, total weight, petasma length and terminal ampulla weight when compared to immature ones. Macroscopically, the reproductive system of *P. schmitti* showed bilateral symmetry, with a testis divided into 8 to 10 lobes, a vas deferens divided into three regions: proximal, middle and distal, and a terminal ampulla. These features were present in both mature and immature animals, differing only in size, turgidity and coloration observed after dissecting their reproductive system. Microscopically, all the germ cells were present in mature animals: spermatogonia, spermatocytes (I and II), spermatid and spermatozoa were found in the seminiferous tubules of the testis, where spermatogenesis occurs, in addition to the presence of accessory cells, the Sertoli cells. The vas deferens had the characteristics of sperm transport, as it contained only spermatozoa and seminal fluid. The terminal ampulla was the most complex region of the reproductive system and serves as a structure for the storage of spermatozoa until the copulation. Spermatozoa and seminal fluid were observed in the lumen of this region. The terminal ampulla contained secretory cells that are responsible for producing the adhesive mass to which the spermatophores attach to the female thelycum. In immature animals, only spermatogonia and spermatocyte I germ cells were found in the testis, along with Sertoli cells. Furthermore, the terminal ampulla showed no secretion, seminal fluid or germ cells in these animals. This probably indicates that morphological maturity is synchronized with physiological maturity for *P. schmitti*. Therefore, results from this study provide useful information on penaeid male reproduction system and germ cells development, as well as to improve controlled breeding of *P. schmitti* in aquaculture.

Keywords: Aquaculture; broodstock; histology; maturation.

1. Introduction

The marine shrimp *Penaeus schmitti* is considered one of most economically important native penaeid species in Brazil, as well as one of the most promising for shrimp farming due to its large body size and weight (Moss and Moss, 2009; Bochini, 2012). Commercial production of *P. schmitti* occurred in several countries where the species is native, such as Cuba and Brazil, where preliminary studies and farming initiatives were interrupted by the introduction of the exotic species *Penaeus vannamei* (Nascimento et al. 1991, Bezerra and Ribeiro-Alves 1995, De Paiva et al. 1995; Fonseca and Fernández de Alaiza 2003). Given its potential for aquaculture, an understanding on the morphology of the reproductive system and development of germ cells are of paramount importance for breeding programs of this species (Díaz et al., 2002). This knowledge is beneficial not only in maintaining and selecting breeding stocks, but also in applying biotechnologies and comprehending the processes of artificial insemination and mating (Bauer, 1991; Bauer and Min, 1993; Medina, 1995; Díaz et al., 2002; Fernandes et al., 2014).

The morphology of the reproductive system varies within the diverse groups of decapods (Díaz et al., 2002). However, the reproductive system morphology of male shrimp is typically divided into three main components: the testis, vas deferens, and terminal ampulla (Dall, 1990; Bauer, 1991; Chow et al., 1991). Furthermore, penaeid males present an external morphological structure, the petasma, which facilitates the transfer of sperm mass to the open thelycum of females, such as *P. schmitti* (Dall, 1990). The functional morphology of the reproductive system and external sexual characteristics of penaeid males have been studied in species of interest for aquaculture, including *Penaeus setiferus*, *Penaeus duorarum*, and *Penaeus aztecus* (Bauer, 1991); *P. vannamei* (Dougherty and Dougherty, 1989; Peralta Martínez et al., 2013; Alfaro-Montoya, 2013); *Xiphopenaeus kroyeri* (Fransozo et al., 2011; Andrioli et al., 2024); *P. schmitti* (Fransozo et al., 2016); and *Penaeus monodon* (Feng, 2017; Feng, 2018).

Despite the current knowledge on penaeid reproduction, there is limited information on the morphology of the male reproductive system, spermatogenesis, and spermiogenesis for most species, especially for *P. schmitti* which has only one study (Fransozo et al., 2016). This gap is primarily because breeding selection criteria have historically focused on females (Lawrence and Huner, 1987; Diaz et al., 2002; Peralta Martínez et al., 2013). Therefore, this study aims to describe and compare the morphology of the reproductive system in mature and immature males of *P. schmitti* using macroscopical and histological characterization.

2. Materials and Methods

2.1 Shrimp sampling

Adults of *P. schmitti* were captured in the northeast coast of Brazil (06° 53′ 50″ S; 034° 51′ 01″ W) using a beach seine net a depth of 6 meters. Males of this species were identified using a guide for penaeid shrimp identification according to the method described by Costa et al., (2003). The animals were then preserved in ice and transported to the Aquaculture Technology Laboratory at the Federal Rural University of Pernambuco for further analysis.

2.2 Biological measurements

In the laboratory, the animals were separated into two categories, mature and immature males, based on the degree of fusion of the petasma, either fully fused or unfused, respectively. A total of 45 males were measured for total length (TL - cm,

measured from the tip of the rostrum to the tip of the telson), cephalothorax length (CL - cm, measured from the base of the rostrum to the end of the cephalothorax), petasma length (PL - cm), total weight (TW - g), and weight of the terminal ampulla (TAW - g) using a digital caliper (0.01 cm) and digital balance (0.0001 g), respectively. The statistical differences between these variables, for mature and immature individuals, were evaluated by student's t-test, after testing for normality (Shapiro-Wilk, $p < 0.05$) and homogeneity (Levene, $p < 0.05$). Statistical analysis were performed using R software, version 4.3 for Windows (R Core Team, 2022).

2.3 Gross analysis of the reproductive system

The macroscopic analysis of terminal ampullas and petasma was conducted using fresh mature and immature animals. The reproductive system was dissected and washed in running water to remove any possible impurities, before being arranged on a Petri dish. Photos for description were taken using a stereo microscope (Leica - EZ4, Leica Microsystems).

2.4 Microscopic analysis of the reproductive system

After the macroscopic analysis, the reproductive system was fixed in Davidson's solution for 24 hours and subsequently preserved in 70% alcohol. Each portion of the reproductive system of the specimens (testes, proximal/middle/distal vas deferens, and terminal ampulla) was identified and preserved separately in 0.5 ml Eppendorf tubes.

The fragments of each structure were dehydrated in an ethanol gradient (80% - 100%), cleared in xylene and embedded in liquid paraffin (57°C). Slides of glass were prepared with 5 μm thick sections using a rotary microtome (Leica RM2145, Leica Microsystems). The sections were stained with hematoxylin and eosin-phloxine (H-E/P) (Junqueira and Junqueira, 1983) for the general description of the morphology of the male reproductive system, as well as for the description of germ cells and accessory cells according to histological characteristics (Bell and Lightner, 1988). Slides were observed under a light microscope (Leica DM500) equipped with a digital camera (Leica ICC50HD). Photomicrographs of the slides were captured using Leica LAS EZ 3.4 software (Leica Microsystems).

2.5 Germ cell histomorphometry

Histomorphometric analysis of germ cells were carried out using photomicrographs with ImageJ software version 1.5 (National Institutes of Health, Bethesda, Maryland, USA). The mean total diameter of cells (CD), mean nucleus diameter (ND), and nucleus diameter/cell diameter ratio (ND/CD) were calculated at different stages of germ cells development. A total of 30 cells were measured at each stage of spermatogenic development (spermatogonia, spermatocyte I, spermatocyte II, and spermatid). Spermatozoa diameter was analyzed at different locations within the reproductive system (testis, proximal/middle/distal vas deferens, and terminal ampulla) by measuring the head region of the spermatozoa.

For statistical analysis, histomorphometric data were first subjected to normality (Shapiro-Wilk, $p < 0.05$) and homogeneity (Levene, $p < 0.05$) tests. After meeting these assumptions, one-way ANOVA was employed and, in case of significant differences, Tukey's test was used to identify which cells differed statistically among each stage of

spermatogenic development with a significance level of 0.05 (Zar, 2010). The tests were conducted using R software, version 4.3.1 for Windows (R Core Team, 2022).

3. Results

3.1 Morphological measurements of mature and immature animals

A total of 37 mature and 8 immature males of *P. schmitti* (n = 45) were captured and analyzed. All measurements for mature animals were either larger or heavier when compared to immature ones (Table 1). Mature males exhibited a mean TL of 13.55 cm and TW of 20.64 g, while immature ones showed a mean TL of 10.08 cm and TW of 8.0 g (Table 1).

Table 1: Mean (±SD), minimum (Min), and maximum (Max) total length (TL - cm), cephalothorax length (CL - cm), total weight (TW - g), petasma length (PL - cm), and terminal ampulla weight (TAW - g) of mature and immature *P. schmitti*.

		TL	**CL**	**TW**	**PL**	**TAW**
Mature	Mean	13.55±0.99[a]	2.85±0.28[a]	20.67±4.64[a]	0.93±0.19[a]	0.098±0.05[a]
	Min	10.70	2.11	10.35	0.43	0.0089
	Max	15.00	3.25	27.48	1.15	0.1774
Immature	Mean	10.08±1.14[b]	2.00±0.26[b]	8.28±2.83[b]	0.40±0.1[b]	0.008±0.0001[b]
	Min	9.00	1.72	5.21	0.24	0.0024
	Max	12.10	2.54	13.87	0.54	0.0194

* Different letters in the same column indicate a significant difference ($p < 0.05$)

3.2 Gross analysis of the reproductive system

The reproductive system of *P. schmitti* males is located entirely in the cephalothorax region, below the heart and on the dorsal portion of the hepatopancreas (Figure 1 A), with the most terminal portion reaching the 5th pair of pereopods where the gonopods are located (Figure 1 B). The reproductive system shows bilateral symmetry, and it is composed of three regions: testis, vas deferens (proximal - PVD, middle – MVD and distal - DVD), and terminal ampulla (TA). This configuration is presented for both mature (Figure 2 A) and immature (Figure 2 B) animals. However, a translucent coloration was observed in the testis and whitish in the vas deferens of mature animals, while immature animals showed a translucent coloration throughout the reproductive system.

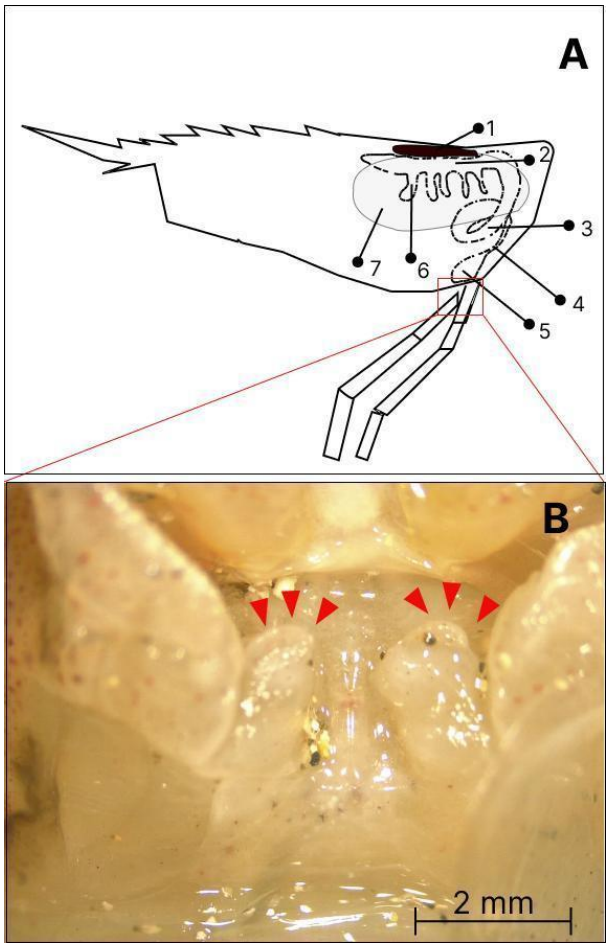

Figure 1: (A) Representation of the location of the reproductive system of *P. schmitti.* (1) heart, (2) proximal vas deferens, (3) middle vas deferens, (4) distal vas deferens, (5) terminal ampulla, (lobes of the testis), (7) hepatopancreas; (B) Location of the pair of gonopods at the base of the fifth pair of pereopods (red arrows).

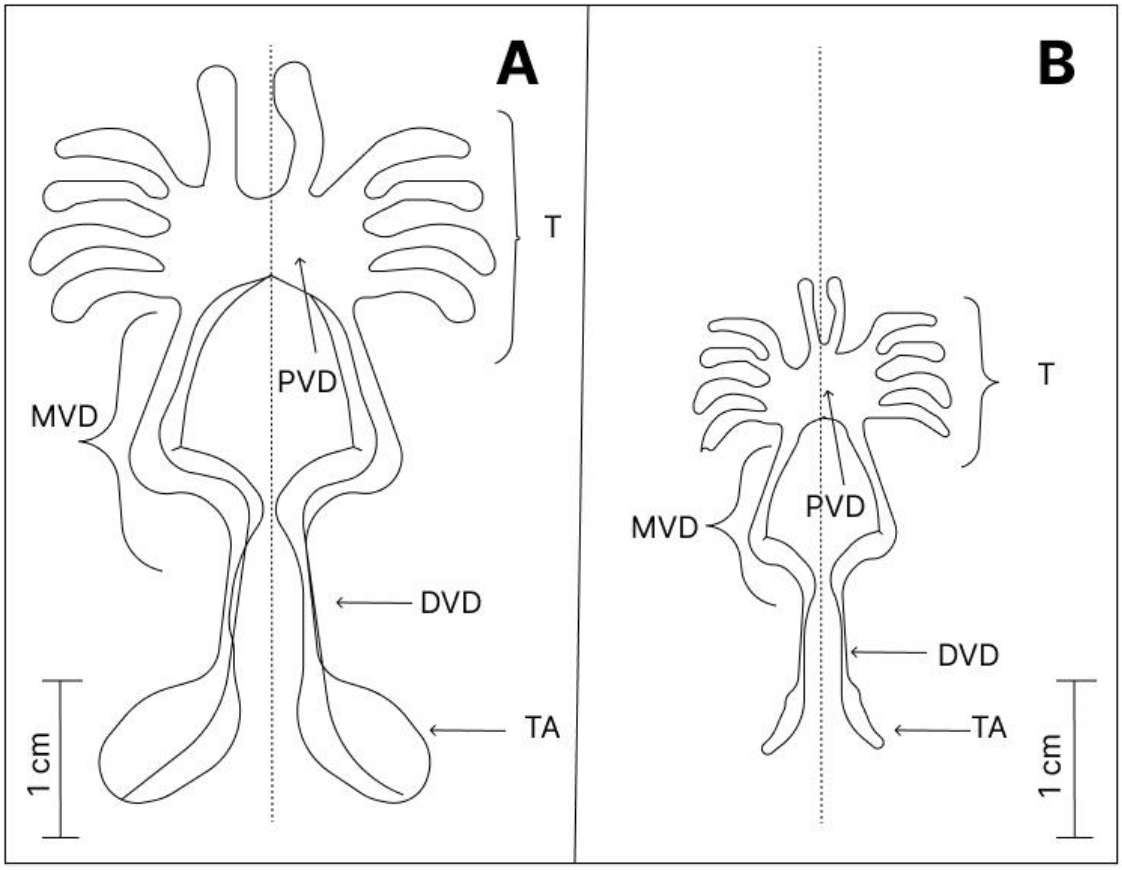

Figure 2: General morphology of the male reproductive system of *P. schmitti*.(A) Male reproductive system of mature shrimp divided into testis (T), proximal vas deferens (PVD), middle vas deferens (MVD), distal vas deferens (DVD), and terminal ampulla (TA); (B) Male reproductive system of immature shrimp divided into testis (T), proximal vas deferens (PVD), middle vas deferens (MVD), distal vas deferens (DVD), and terminal ampulla (TA).

The testis region consists of 8 to 10 lobes in total (Figure 3 A,B). The proximal vas deferens (PVD) is the portion of the vas deferens connected to the testis (Figure 3 A,B). The middle vas deferens (MVD) forms the longest and larger portion of the vas deferens (Figure 3 C,D). The distal vas deferens (DVD) is the portion of the reproductive system with a smaller diameter and it connects the vas deferens to the terminal ampulla (TA), located in the base of the fifth pair of pereopods (Figure 3 E, F).

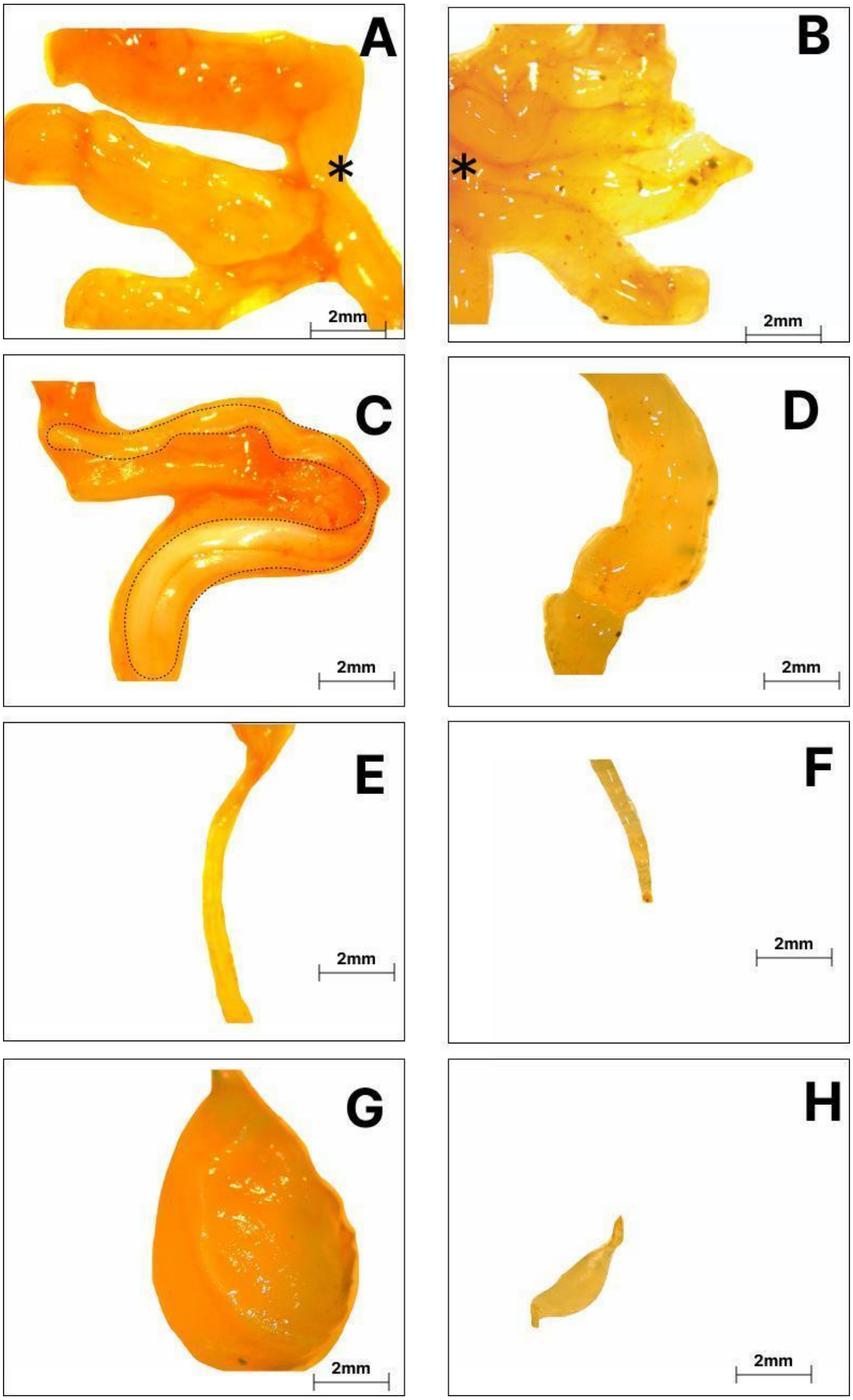
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Figure 3: Gross morphology of the male reproductive system of *P. schmitti* .(A) Testis of mature shrimp (*proximal vas deferens); (B) Testis of immature shrimp (*proximal vas deferens); (C) Middle vas deferens of mature shrimp (dotted black line, color change in the vas deferens); (D) Middle vas deferens of immature shrimp; (E) Distal vas deferens of mature shrimp; (F) Distal vas deferens of immature shrimp; (G) Terminal ampulla of mature shrimp; (H) Terminal ampulla of immature shrimp (Magnification 8X).

Regarding dimorphism, mature and immature animals exhibit morphological differences in their reproductive system, evidenced by the observation of terminal ampulla through the exoskeleton at the base of the fifth pair of pereopods. While in immature animals these structures are not visible (Figure 4 A), in mature animals they are visible and present a whitish coloration (Figure 4 B).

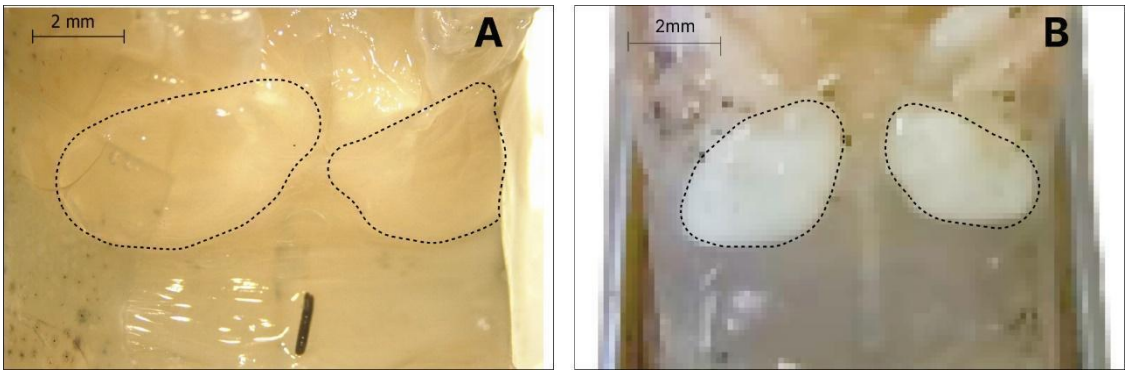

Figure 4: Location of the terminal ampulla of *P. schmitti***.** (A) Immature shrimp, terminal ampulla not externally apparent (black line, indication of the terminal ampulla); (B) Mature shrimp, terminal ampulla apparent with whitish coloration through the exoskeleton (black line, indication of the terminal ampulla).

The presence of petasma is an external sexual characteristics of *P. schmitti* males, located between the first pair of pleopods, which is a modification of their endopodite (Figure 5 A). This structure is fused in mature animals (Figure 5 B) and unfused in immature shrimp (Figure 5 C), representing another significant morphological difference between these two groups.

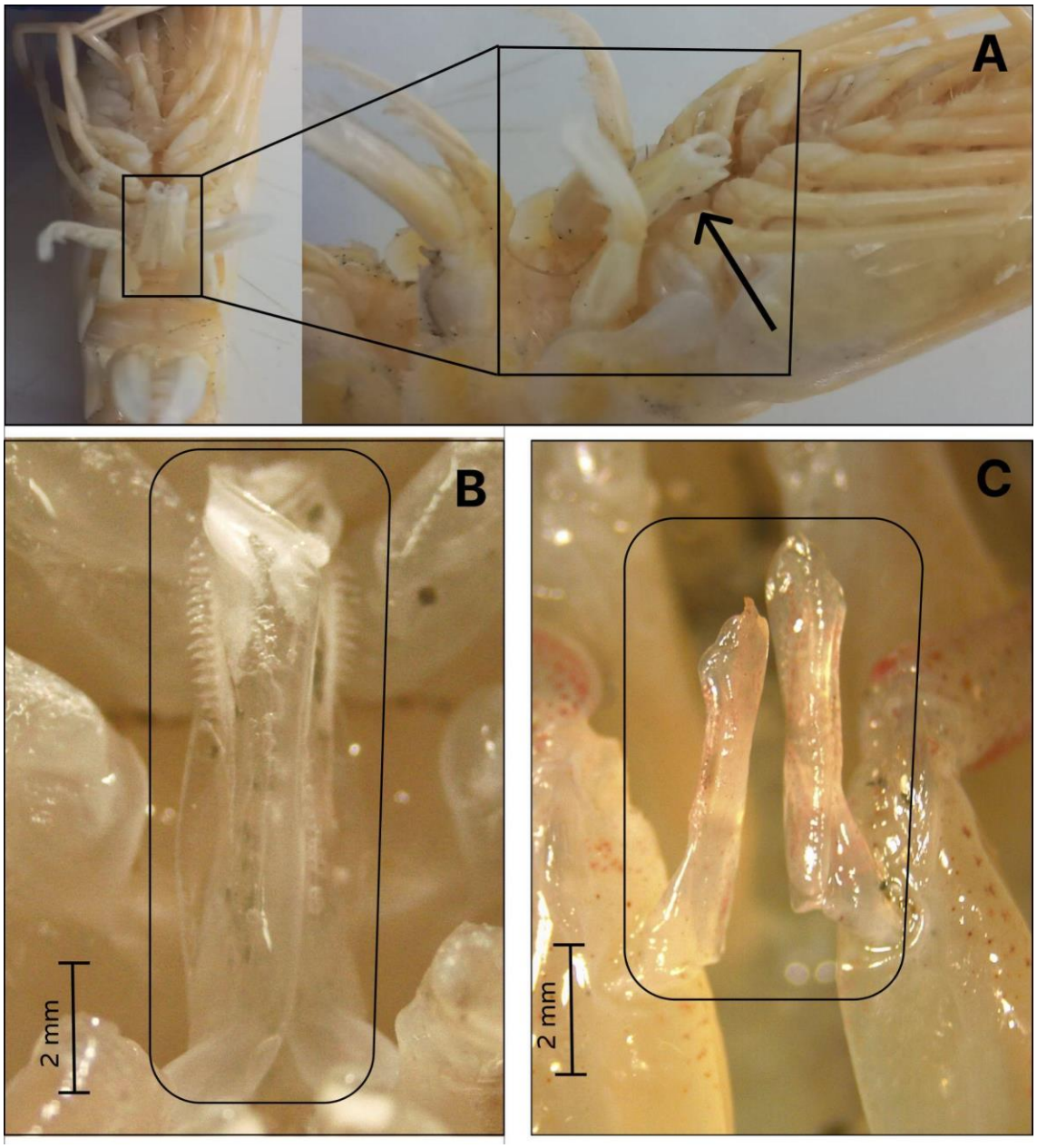

Figure 5: External sexual characteristics of *P. schmitti.* (A) Location of the petasma (black arrow) on the ventral region of the shrimp, between the first pair of pleopods; (B) Fused petasma, a characteristic for identifying a mature shrimp; (C) Unfused petasma, a characteristic for identifying an immature shrimp.

3.3 Microscopic description of the germline cells, accessory cells, and tissues found in the testis

The following germline cells were found in the testis of mature animals: spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, and immature spermatozoa, which do not exhibit developed acrosomes and spikes (Figure 6 A,E). The five germline cells found in the seminiferous tubules of the testis exhibit the following characteristics:

Spermatogonia (SG): they are the largest germline cells present in the testis. Cells at this stage are located at the periphery of the seminiferous tubules, forming the germinal zone. They are rounded cells with a large granular nucleus featuring dispersed chromatin and scant cytoplasm. They exhibit an average nucleus diameter of 6.49 ± 0.73 μm. These cells undergo mitosis to differentiate into spermatocytes (Figure 6 A; Table 2).

Primary Spermatocyte (Sp I): they exhibit a nucleus with a circular shape and condensed chromatin, a vesicle near the nucleus and an evident cytoplasm. They are smaller than spermatogonia and have a more reduced nucleus. They have an average nucleus diameter of 4.85 ± 0.73 μm (Figure 6 B; Table 2).

Secondary Spermatocyte (Sp II): they have a smaller nucleus than the primary spermatocyte, with the chromatin exhibiting a granular appearance. They show an average nucleus diameter of 3.59 ± 0.46 (Figure 6 B; Table. 2).

Spermatid (Spt): they show an irregular nucleus, which is smaller than the nucleus of secondary spermatocytes. In this phase, nuclei with irregular shapes may be found. It has a nucleus diameter of 3.75 ± 0.38 μm (Figure 6 C; Table. 2).

Spermatozoa (Spz): their nucleus is reduced compared to previous stages. They are the smallest germline cells found. They exhibit an acrosome and undeveloped spike when located in the testis, as they have not yet completed the maturation process (Figure 6 D).

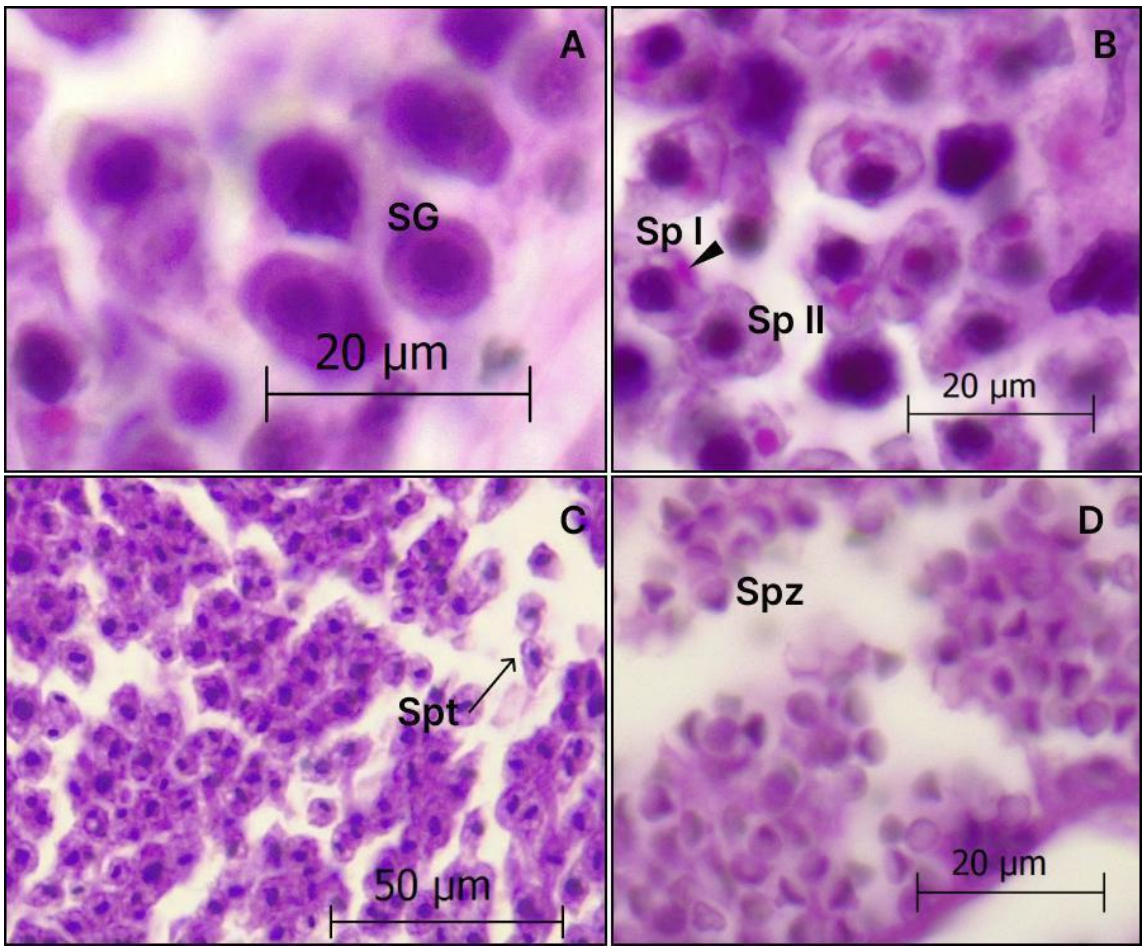

Figure 6: Photomicrograph of germline cells present in the testis of *P. schmitti* at different stages of development, stained with Hematoxylin-Eosin/phloxine. (A) Spermatogonia (SG); (B) Primary spermatocyte (Sp I) with pro-acrosomal vesicles (black arrow) and Secondary spermatocyte (Sp II); (D) Spermatid (Spt); (D) Spermatozoa (Spz).

Regarding the diameter of the germline cells, a reduction in the nucleus diameter relative to the total cell diameter was observed throughout the spermatogenesis process (Table 2). The average nucleus diameter was significantly larger for spermatogonia and followed by spematocyte I, but did not differ between spermatocyte II and spermatid which showed significantly smaller diameters. (Table 2). However, the mean diameter of these cells did not change during their development (Table 2).

Table 2: Mean (±SD) cell diameter (CD) and nucleus diameter (ND) in micrometers (µm), and nucleus diameter/cell diameter ratio (ND/CD) of spermatogonia, spermatocyte I and II, and spermatid of the mature *P. schmitti*.

Cell type	DC	DN	DN/DC
[1]Spermatogonia	**	6.49±0.73[a]	100%
[1]Spermatocyte I	11.29±0,73[a]	4.85±0,73[b]	43%
[2]Spermatocyte II	10.64±0,90[a]	3.59±0,46[c]	34%
[2]Spermatid	11.41±2,50[a]	3.75±0,38[c]	33%

Different letters in the same column indicate significant differences ($p < 0.05$)
** Not measured.
[1] Mature and immature
[2] Mature

In mature shrimp, it was possible to observe the process of sperm maturation through morphological changes in different regions of the reproductive system, such as the size of the sperm head and the development of the spike (Figure 7 A,E). In the testicular region, the spermatozoa exhibits a smaller diameter of the head region compared to other regions of the reproductive system. In the distal vas deferens region, no spermatozoa were observed in the analyzed animals. (Table 3).

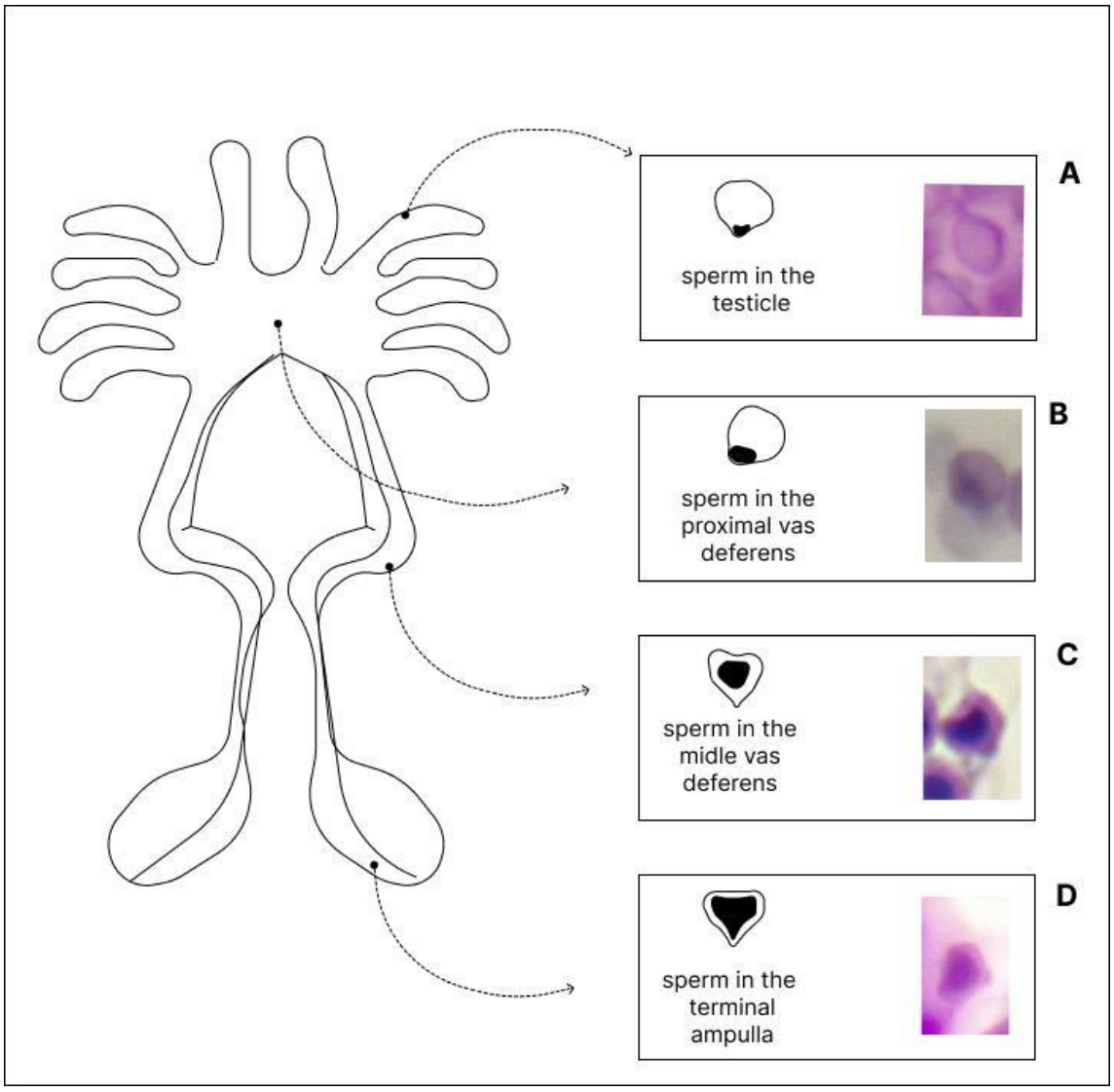

Figure 7: Schematic representation of the sperm maturation process of *P. schmitti*. (A) Sperm located in the testicular region, (B) Sperm located in the proximal vas deferens (PVD) region, (C) Sperma located in the medial vas deferens region, (D) Sperm located in the terminal ampulla region.

Table 3: Mean (±SD) of spermatozoa diameter (µm) in different location of the reproductive systems of *P. schmitti*.

Location of the spermatozoa	Diamater
Testicle	3.653 ± 0.304^{a}
Proximal vas deferens	3.897 ± 0.305^{ab}
Middle vas deferens	3.880 ± 0.434^{ab}
Terminal ampulla	3.908 ± 0.433^{b}

Different letters in the same column indicate significant differences ($p < 0.05$)

The testis of both mature and immature shrimp is composed of lobules, and these lobules have a network of interconnected seminiferous tubules, forming a duct network throughout the reticular region. These tubules are surrounded by a thin layer of lining tissue (Figure 8 A and B). At the periphery of the seminiferous tubules, a germinal zone can be observed, composed of primary cells, at the spermatogonia stage (Figure 8 C). Hemal sinuses are arranged between the networks of seminiferous tubules (Figure 8 D).

In the inner part of the basal membrane of the seminiferous tubule network, a set of flattened-shaped accessory cells called Sertoli cells can be observed (Figure 8 D). Within the tubule network of the testis, the entire process of spermatogenesis occurs. It is possible to observe germline cells in five stages of development in mature animals: spermatogonia, primary and secondary spermatocytes, spermatids (Figure 8 F), and immature spermatozoa without developed spikes (Figure 8 G and H). The less developed germline cells were found at the periphery of the seminiferous tubules and, as they undergo the process of cell division, they move toward the center of the seminiferous tubules.

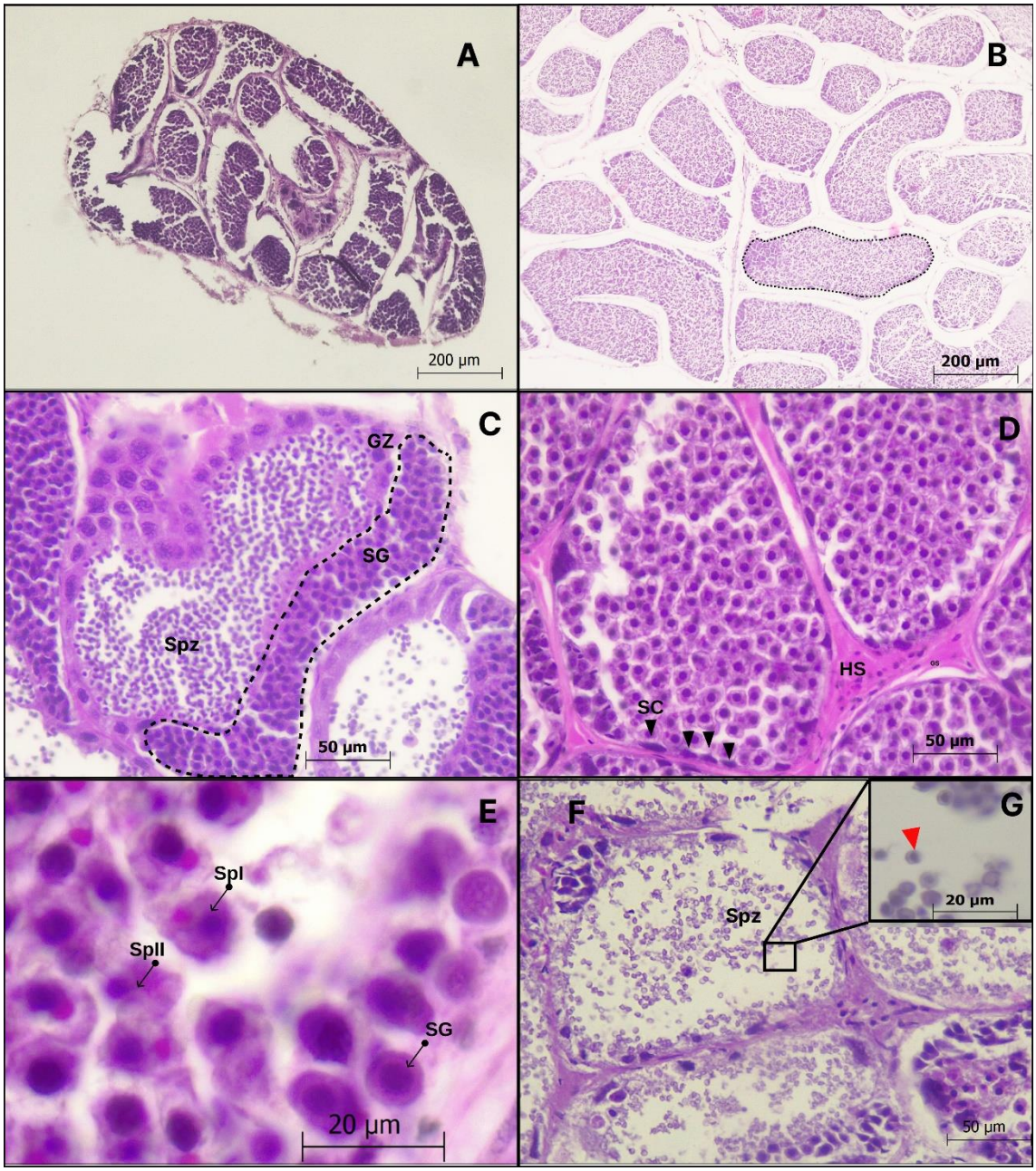

Figure 8: Photomicrograph of the mature testis of the *P. schmitti* stained with Hematoxylin/Eosin - phloxine.(A) Overall view of the testicular lobule; (B) Boundary of the seminiferous tubule region within the testicular lobule (dotted line); (C) Different regions in the seminiferous tubule, germinal zone (GZ - black dotted line) filled with spermatogonia (SG) (Black arrow) and spermatozoa (Spz); (D) Hemal sinuses (HS) between the tubules and Sertoli cells (SC) at the periphery of the tubules (black arrow); (E) Different germline cells in the seminiferous tubule, spermatogonia (SG), primary spermatocyte (SpI), secondary spermatocyte (SpII), and immature spermatid, without formed spike (Spt); (F) Immature spermatozoa,

without formed spike (Spz) in the center of the seminiferous tubules; (G) 100x zoom showing a spermatozoon (Red arrow).

Immature animals also exhibit testicular lobules formed by a network of seminiferous tubules (Figure 9 A). Within these tubules, two types of germinal cells can be found, representing the initial stage of spermatogenesis, namely spermatogonia and primary spermatocytes (Figure 9 B). Sertoli cells are located at the periphery of the tubules, while hemal sinuses are interspersed between the tubules (Figure 8 C).

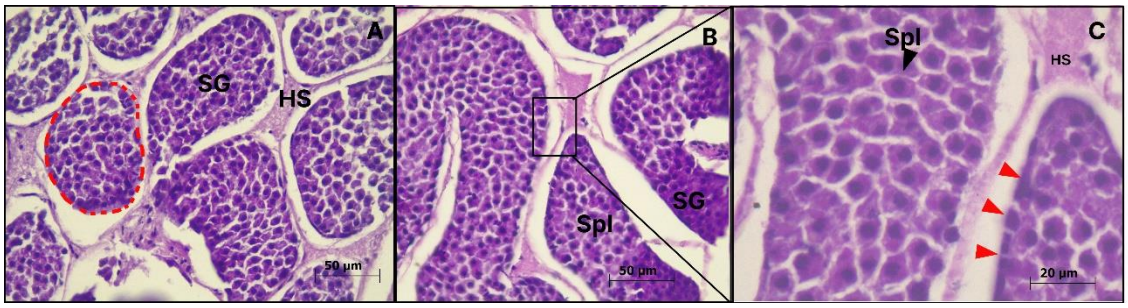

Figure 9: Photomicrograph of the immature testis of the *P. schmitti* stained with Hematoxylin/Eosin-phloxine. (A) Seminiferous tubules (black dotted line) filled with spermatogonia (SG) and hemal sinuses (HS) between the tubules;(B) Germinal cells within the seminiferous tubules, spermatogonia (SG), and primary spermatocyte (SpI);(C) Sertoli cells (red arrow).

3.4 Microscopic description of the germinal cells, accessory cells, and tissues found in the vas deferens.

The proximal vas deferens (PVD) is connected to the region of the testis. The outermost part is lined with connective tissue and the inner layer is composed of epithelial tissue. In this region, the presence of partially developed free spermatozoa can be observed (Figure 10 A). The region of the proximal vas deferens (PVD) is externally lined by a layer of connective tissue and internally by a layer of muscle (Figure 10 A)

The region of the middle vas deferens (MVD) is characterized by being the longest and widest part of the entire vas deferens (Figure 10 B). This region has the same tissues as the proximal vas deferens. The outermost part is lined with connective tissue and the inner layer is composed of epithelial tissue. It is possible to observe spermatozoa and non-cellular substances, the seminal fluid, in this region. Here, the spermatozoa undergo spermiogenesis, the process of sperm maturation, during which the development of the acrosomal region and the formation of the spike occur (Figure 10 C).

The region of the distal vas deferens (DVD) is the part with the smallest diameter. It consists of a layer of connective tissue, and the vas deferens canal is separated by a longitudinal fold called a typhlosole, without the presence of spermatozoa (Figure 10 D).

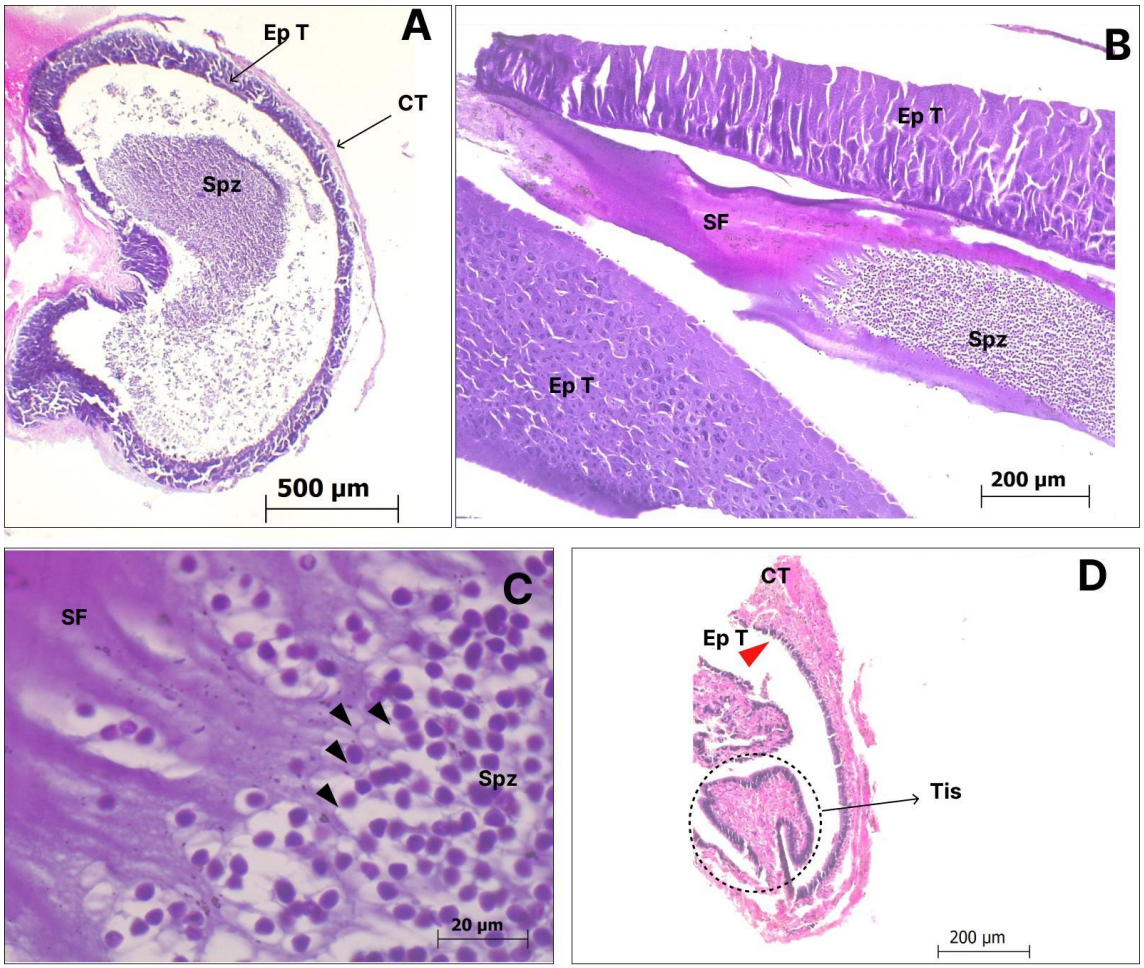

Figure 10: Photomicrograph of the different portions of the vas deferens of the mature *P. schmitti* shrimp captured off the coast of Lucena, PB, stained with Hematoxylin-Eosin- phloxine. (A) Proximal vas deferens showing the external layer of connective tissue (CT), internal layer of epithelial tissue (Ep T), and free spermatozoa in the center of the lumen (Spz);(B), spermatozoa (Spz), and seminal fluid (SF) and internal layer of epithelial tissue (Ep T);(C) Magnification of the middle vas deferens highlighting spermatozoa (Spz) and seminal fluid (SF);(D) Distal vas deferens without the presence of spermatozoa, showing the layer of connective tissue (CT), epithelial tissue (Ep t, red arrow) and typhlosole (Tis, dotted circle black arrow).

3.5 Microscopic description of the germinal cells, accessory cells, and tissues found in the terminal ampulla.

The terminal ampulla (TA) is a highly modified region of the deferent canal, characterized by a thick layer of connective tissue and an interior lumen divided by a longitudinal fold, the typhlosole, as shown in the longitudinal section (Figure 11 A). At the periphery of the terminal ampulla, internally, secretory chambers can be observed, which do not contain cellular material but rather an acidophilic secretion in mature animals (Figure 11 B and C).

Mature animals exhibit a sperm mass in the lumen, with developed spermatozoa featuring spikes and a developed acrosome (Figure 11 D and F). In the terminal ampulla, two types of secretions surrounding the spermatozoa can be observed: one more basophilic, staining more strongly with hematoxylin, and another less basophilic, staining weeklies with hematoxylin (Figure 11 E). However, in immature animals, these chambers are smaller and do not contain any spermatozoa in the lumen (Figure. 11 G) nor secretion in the secretory glands (Figure 11 H).

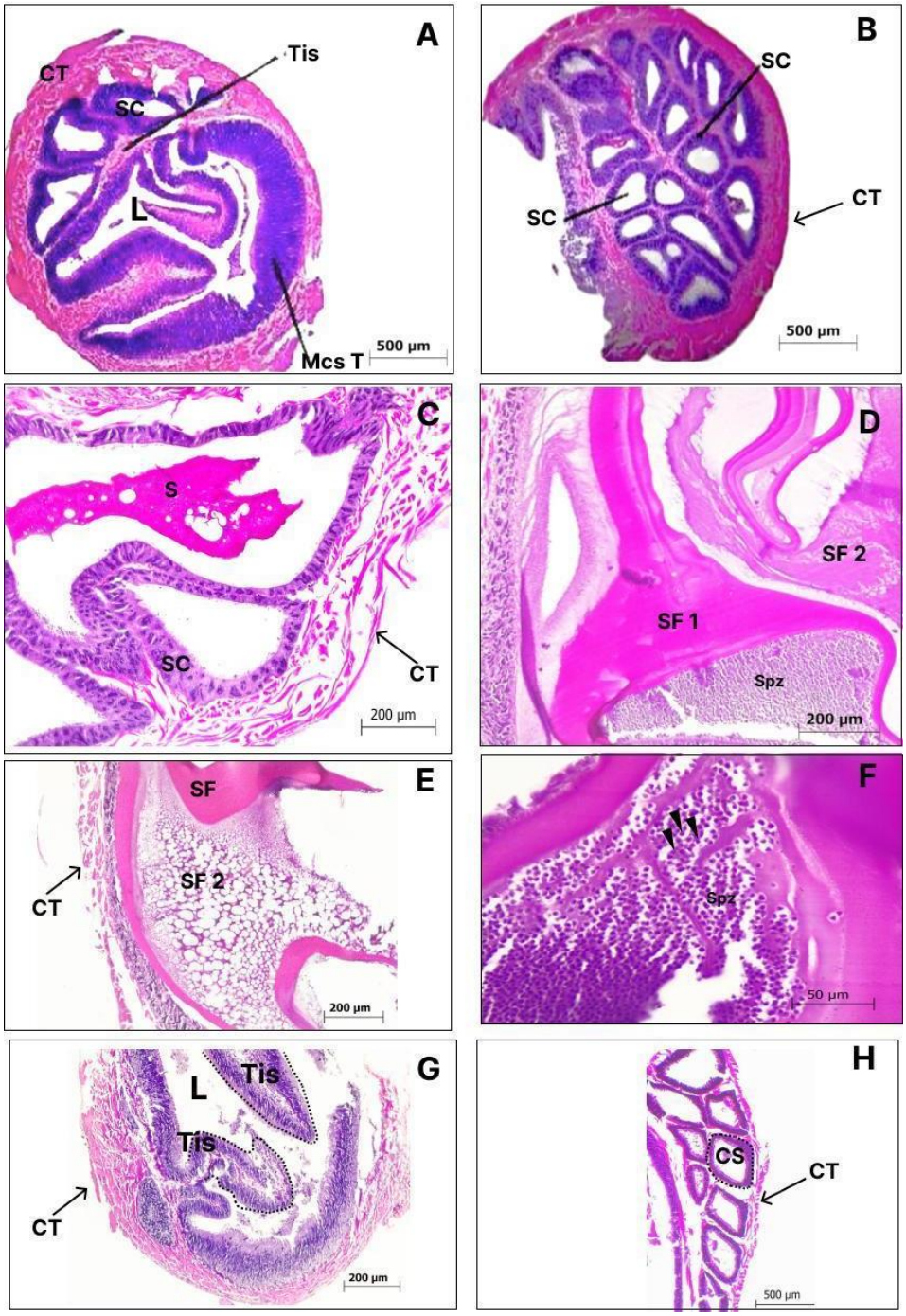

Figure 11: Photomicrograph of the terminal ampulla of the *P. schmitti* stained with Hematoxylin-Eosin-phloxine. (A) Cross-section of the terminal ampulla showing the lumen (L), secretory cells (SC), and typhlosole (Tis);(B) Longitudinal section of the terminal ampulla showing secretory cells (GS);(C) Secretory cell with remnants of secretion (S);(D) Sperm mass (Spz) surrounded by seminal fluid stained purple, showing acidophilic character within the lumen;(E) Difference between seminal fluids in the lumen of the terminal ampulla, basophilic in purple (FS2) and acidophilic in pink (FS1);(F) Spermatozoa (black arrow) in the lumen of the terminal ampulla;(G) Terminal ampulla of an immature animal, highlighting the typhlosole (Tis) and lumen (L);(H) Secretory cells (CS) of the terminal ampulla from an immature animal.

4. Discussion

The results of this study provide new insights into the synchrony between morphological and physiological maturity, correlating them with alterations in the germ cells of the male reproductive system of both mature and immature marine shrimp *P. schmitti*, being the first study to carry out this comparison for the species. Mature individuals showed all body measurements larger or heavier when compared to immature ones. Although the number of immature animals analyzed macro- and microscopically was relatively smaller than that of mature ones, it was possible to observe relevant characteristics of the maturation process.

As expected, the location of the male reproductive system of *P. schmitti*, as well as its macroscopic morphology, followed the same pattern as other penaeids. The reproductive system is entirely located in the cephalothorax region and is divided into three parts: the testis, vas deferens, and terminal ampulla (Tuma 1967; Dall et al. 1990; Chow et al. 1991; Bauer and Min 1993; Fransozo et al., 2016; Andrili et al., 2024; Chang et al., 2024). These morphological characteristics were found in both mature and immature *P. schmitti*. Therefore, it is not possible to determine the maturation stages based on internal macroscopical changes in shape, size and coloration of the reproductive system through the exoskeleton, as usually performed in penaeid females (Peixoto et al, 2003; Silva et al., 2016; Craveiro et al; 2019; Craveiro et al., 2022; Bernabé et al., 2022). The only way to observe the internal changes in turgidity and coloration is by dissecting the animals (Chang et al., 2024). However, even when dissecting the reproductive system, the only indication of color change for mature *P. schmitti* can be observed at the region of the vas deferens and the terminal ampulla (Fransozo et al., 2016; Andrili et al., 2024; Chang et al.,2024), while immature animals do not show any color alterations in these structures. Such differences in coloration pattern of the male reproductive system between mature and immature *P. schmitti* may be related to the presence of spermatozoa along the vas deferens and seminal fluid in mature animals, while absent in immature ones.

In the present study for *P. schmittti*, and most penaeid males, macroscopic maturation is primarily associated with changes in the external sexual structure, the petasma, which is fused in mature animals and unfused immature ones, as well as by the degree of development of the terminal ampulla (Tirmizi and Javed, 1976; Ceballos-Vázquez et al., 2003; Ceballos-Vázquez et al., 2010; Andrioli et al., 2024; Chang et al.,2024). Conversely, Chang et al. (2024) studying the reproductive system morphology of *P. vannamei* males, divided its gonadal development into four phases based on changes in turgidity and development of the terminal ampulla. The principal function of the petasma is associated with insemination, as it is responsible for transferring the sperm mass to the thelycum of the female, which is an open depression located between the fifth pair of pereiopods (Tuma, 1967; Bauer, 1986, 1991; Subramoniam, 1995; Farfante and Kensley, 1997). The function performed by this structure may explain why mature animals exhibit a fused and longer petasma, while immature ones have an unfused and shorter petasma, as observed in the present study. Furthermore, it was possible to observe morphological alterations of the terminal ampulla through the exoskeleton, with this structure being visible (i.e. whitish color) in mature animals and not visible in immature *P. schmitti*. Nevertheless, for controlled reproduction purposes in aquaculture, these external characteristics alone should not be considered to determine the maturation stages for penaeids, since sperm quality is also related to size and age of the animals (Ceballos-Vázquez et al., 2003; Ceballos-Vázquez et al., 2010; Crocos and Coman, 1997). However, this information on the morphological characteristics could be useful as a first criteria for capturing and selecting wild broodstock of *P. schmitti*.

The confirmation of physiological maturity in penaeids (i.e., presence of spermatozoa in the reproductive system) can only be determined through microscopic analysis, by examining the histological changes of germ cells occurring in the testes and terminal ampullas of deferent ducts (Ro et al., 1990; Fransozo, 2011; Andrioli et al., 2024). This study suggests that there is synchronicity in morphological and physiological maturity, based on the analyses of *P. schmitti* with fused and unfused petasma. However, Andrioli et al. (2024) found that the morphological maturity precedes physiological aspects by analyzing different fusion degrees (united, semi-united and disunited) of *X. kroyeri* petasma.

When analyzed microscopically, the morphology of the testis of the shrimp *P. schmitti* can be described as tubular, composed of a network of seminiferous tubules throughout all lobes for both mature and immature animals. A similar configuration is presented in other penaeid species (Dall, 1990). Additionally, it was possible to observe accessory cells, namely Sertoli cells, in the testicular lobes of mature and immature animals. These cells play a role in supporting the development of germ cells, being responsible for differentiation during the spermatogenesis process. In addition to controlling the maturation of germ cells, they are responsible for the migration process of cells toward the center of the seminiferous tubules (França and Garcia, 2005; Hai et al., 2014). The initial germ cells in the process of spermatogenesis (i.e., spermatogonia) found in the testis of *P. schmitti*, were located at the periphery of the seminiferous tubules. These cells form the germinal zone and are the germ cells with the largest nucleus in mature and immature animals. In lobular-type testes, each testicular lobe presents germ cells at the same stage or successive stages of meiosis, differentiating into spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, and spermatozoa in mature animals (Simeó et al., 2009; Zara et al., 2012; Nascimento and Zara, 2013). However, some authors found that the testes of immature penaeids only contain germ cells at the spermatogonia and primary spermatocyte stages (Bell and Lightner 1988; Andrioli et al., 2024; Chang et al.,2024), which is consistent with the findings of the present study.

The differentiation of germ cells in the testis of *P. schmitti* was observed through changes in morphological characteristics and the proportion of nucleus diameter to germ cell diameter. Additionally, in the primary spermatocyte, the second cell stage of the spermatogenesis process, the presence of a pro-acrosomal vesicle was observed. This vesicle emerging in the primary spermatocyte is formed by the rearrangement of cytoplasmic organelles, and it is present in other shrimp species such as *P. vannamei* (Alfaro Montoya et al., 2016) and *P. monodon* (Feng et al., 2017). During the maturation process of germ cells in the testis of *P. schmitti*, a cellular division process occurs, which is the probable reason for the reduction in nucleus size, resulting in a smaller nucleus-to-cell ratio from the second stage of development onwards. A similar pattern was also observed in other decapod crustaceans (Andrioli, 2018; Fansozo et al., 2016; Feng et al., 2017; Simeó et al., 2009; Tiseo et al., 2014). Furthermore, this cellular division is part of spermatogenesis, which occurs continuously without interruption once the shrimp reaches maturity (King, 1948).

In the testis of *P. schmitti*, it was possible to observe a region near the proximal vas deferens filled with spermatozoa, ready to be transported to the proximal vas deferens. The proximal and medial vas deferens contain spermatozoa, and both are lined with a thick layer of connective tissue and inner epithelial tissue. According to Diáz et al. (2002), the epithelium of the vas deferens serves various functions such as transportation, maintenance of germ cells and production of the protective layer of the spermatophore. Additionally, the epithelium of the vas deferent may explain the presence of seminal fluid due to the nature secretory of this tissue (King, 1948).

In the region of the proximal and medial vas deferens of *P. schmitti*, it is possible to observe germ cells at the stage of immature spermatozoa, as they do not exhibit developed spikes. In mature shrimp, spermatozoa appear fully matured within the seminiferous tubules, with increased spike size and acrosome development (Camargo et al., 2017). However, it has been reported that sperm maturation in penaeids occurs along the vas deferens or even after transferring the spermatophore to the female's thelycum, with sperm maturation assisted by seminal fluid (Alfaro et al.,2007; Alfaro-Montoya, 2010; Fransozo et al.,2016). In this context, the absence of seminal fluid in the proximal vas deferens in *P. schmitti* may explain the presence of immature spermatozoa in the testis and their different shapes along the vas deferens of the analyzed animals.

The terminal ampulla of penaeid constitutes the most complex region of the male reproductive system (Alfaro-Montoya, 2010). In this region, a sperm mass can be observed within the lumen, along with seminal fluid exhibiting both acidophilic and basophilic characteristics. According to Feng et al. (2018), this seminal fluid found in the terminal ampulla originates from the vas deferens and serves not only in sperm maturation, but also in spermatophore formation. The spermatophore is responsible for protecting the sperm until fertilization occurs (Erkan et al., 2009; Subramoniam, 2016). At the periphery of the terminal ampulla, there is a region known as the secretion chamber, responsible for producing mucus and adhesive substances to facilitate the attachment of the spermatophore in open thelycum females, as these females lack a structure for storing the sperm mass (Wang et al., 1995; Diaz et al., 2002; Harlioğlua, Farhadia, and Gür, 2018). In the present study, remnants of this secretion were observed in the terminal ampulla of *P. schmitti*, consistent with previous findings for other penaeids in the literature.

In immature *P. schmitti*, the terminal ampulla exhibits a lower weight than in mature individuals. Microscopically, the terminal ampulla did not show any secretion in the secretory glands, although they are present. Additionally, germ cells are not observable, nor the seminal fluid produced in the vas deferens, as found in the terminal ampulla of mature animals. The absence of these substances and germ cells may be due to the lack of hormonal stimuli that have not yet being developed in immature animals. Therefore, a greater action of inhibitory hormones produced by the neurosecretory complex of the X-gland organ was probably occurring in these animals, as well as more energy investment in growth (Chang et al., 2024; Andrioli et al., 2024).

4. Conclusion

Macroscopically, *P. schmitti* can be classified as mature or immature based on the visualization external morphology of the terminal ampulla on the fifth pair of pereopods, as well as the fusion degree of their petasma. Regarding the internal morphology of the reproductive system, mature and immature shrimp exhibited similar characteristics. Microscopically, mature animals had germ cells at different stages of development, while immature ones showed germ cells only in the initial development stage. Only animals with an apparent terminal ampulla and fused petasma had spermatozoa in the reproductive system. By correlating the fusion degree of the petasma, visualization of the terminal ampulla, and histological analysis, it can be suggested that morphological and physiological maturity is synchronous for this species.

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5. Considerações finais

Analisando a morfologia do sistema reprodutor masculino do camarão *P. schmitti* pode-se concluir que apresenta similaridade com a morfologia de outros camarões do gênero Penaeus, sendo a morfologia de animais maturos e imaturos igual.

- Camarões maturos e imaturos apresentam diferença morfológica do sistema reprodutor apenas em dimensões e coloração., onde maturos possui uma coloração translucida e esbranquiçada enquanto os animais imaturos têm uma coloração translucida por todo sistema reprodutor.

- Os caracteres sexuais secundários, o pestasma, em animais maturos aparecem fundidos enquanto animais imaturos o petasma aparece não fundido.

- Microscopicamente foi possível observar todas as células germinativas em todos os estágios de desenvolvimento em animais maturos. Já animais imaturos possuem apenas células germinativas nos estágios espermatogônia e espermatócito I.

-Pode-se usar as características macroscópicas e microscópicas para relacionar a maturidade morfológica com a maturidade fisiológica, concluindo que enquanto os camarões *P. schmitti* estiverem com petasma desunido eles não apresentam capacidade reprodutiva, pois não possuem espermatozoide no sistema reprodutor.

- Estudos futuros utilizando histoquímica podem ser realizados para obter informações sobre a o fluxo de nutrientes no sistema reprodutivo do *P. schmitti* tanto de animais maturos quanto em animais imaturos.

ANEXO I

NORMA DO PERIÓDICO AQUACULTURE

The aim of Aquaculture is to publish and make available the highest quality international scientific contributions concerning to aquaculture. The Journal publishes disciplinary, interdisciplinary and transdisciplinary aquaculture research related to the science of aquaculture. The scope of Aquaculture includes the traditional priorities of its sections, but also includes papers from non-traditional scientific areas such as sustainability science, social-ecological systems, as well as aquaculture of various species for ornamental, conservation and restoration purposes.

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Review Articles can cover either narrow disciplinary subjects or broad issues requiring interdisciplinary discussion. They should provide objective critical evaluation of a defined subject. Reviews should not consist solely of a summary of published data. Evaluation of the quality of existing data, the status of knowledge, and the research required to advance knowledge of the subject are essential.

Short Communications are used to communicate results which represent a major breakthrough or startling new discovery and which should therefore be published quickly. They should not be used for preliminary results. Papers must contain sufficient data to establish that the research has achieved reliable and significant results.

Technical Papers should present new methods and procedures for either research methodology or culture-related techniques.

The Letters to the Editor section is intended to provide a forum for discussion of aquacultural science emanating from material published in the journal.

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