A reprodução sexuada em espécies de águas-vivas (Scyphozoa, Cnidaria) do litoral sudeste brasileiro

Sexual reproduction in jellyfish species (Scyphozoa, Cnidaria) from the southeastern Brazilian coast

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Sumário

1. Introdução Geral

O filo Cnidaria é formado por um grupo diverso de animais relativamente simples definidos pela presença da cnida (Odorico and Miller 1997; Collins 2002; Daly et al. 2007; Reft and Daly 2012; Morandini et al. 2016a). O filo é composto por dois grandes clados monofiléticos: Anthozoa e Medusozoa (Collins 2002; Collins et al. 2006) e tem cerca de 11.000 espécies descritas (Appeltans et al. 2012; Zapata et al. 2015) não se considerando os Myxozoa. Dentro de Medusozoa, a classe Scyphozoa é uma das quatro que compõem o subfilo (Collins 2002; Marques and Collins 2004; Collins et al. 2006) possuindo 241 morfoespécies descritas (Mianzan and Cornelius 1999; Daly et al. 2007; Jarms & Morandini, 2019). Seus representantes são comumente chamados de "águas-vivas verdadeiras" (Mianzan and Cornelius 1999) e encontram-se divididos em duas subclasses: Coronamedusae e Discomedusae (Dawson 2004; Collins et al. 2006; Calder 2009).

Coronatae é a única ordem da subclasse Coronamedusae (para detalhes ver Tabela 1). Seus representantes encontram-se divididos em seis famílias (Morandini 1999; Daly et al. 2007) que compartilham três sinapomorfias: tubo peridérmico envolvendo o corpo do cifístoma, a presença do sulco coronal nas medusas (Kramp 1961; Jarms 1991; Morandini 1999; Morandini and Silveira 2001) e ovócitos que se desenvolvem sem células acessórias (Eckelbarger 1994; Marques & Collins, 2004).

A subclasse Discomedusae é divida em duas ordens: Semaeostomeae e Rhizostomeae (Calder 2009). A ordem Semaeostomeae possui cinco famílias (Dawson 2004; Daly et al. 2007) e seus representantes distinguem-se por uma única abertura oral central (Kramp 1961), quatro braços orais não fusionados e a presença de tentáculos, em geral, na margem da umbrela (Cornelius 1997). Já a ordem Rhizostomeae possui 10 famílias divididas em duas subordens (Subordens Kolpophorae e Dactyliophorae, Tabela

1) sendo as espécies caracterizadas pela presença de oito braços orais fusionados com microbocas e ausência de tentáculos marginais na umbrela (Kramp 1961; Cornelius 1997; Mianzan and Cornelius 1999; Daly et al. 2007).

Tabela 1: Organização hierárquica da classe Scyphozoa.

Classe Scyphozoa			
Subclasse Coronamedusae	**Subclasse Discomedusae**		
Ordem Coronatae	**Ordem Semaeostomeae**	**Ordem Rhizostomeae**	
		Subordem Kolpophorae	**Subordem Dactyliophorae**
Família Atollidae	Família Cyaneidae	Família Cassiopeidae	Família Lychnorhizidae
Família Atorellidae	Família Drymonematidae	Família Cepheidae	Família Catostylidae
Família Linuchidae	Família Pelagiidae	Família Mastigiidae	Família Lobonemidae
Família Nausithoidae	Família Phacellophoridae	Família Thysanostomatidae	Família Rhizostomatidae
Família Paraphyllinidae	Família Ulmaridae	Família Versurigidae	Família Stomolophidae
Família Periphyllidae			

1.1.Ciclo de Vida, Reprodução sexuada, descrição da gônada e gametogênese

O ciclo de vida metagenético é característico de todas as classes de Medusozoa (Bridge et al. 1995; Odorico and Miller 1997; Marques and Collins 2004; Morandini et al. 2016b). Em Scyphozoa o pólipo (cifístoma) pode reproduzir-se assexuadamente originando outros pólipos (brotamento ou formação de cistos) ou originar uma ou mais medusas jovens através de um mecanismo de reprodução assexuado chamado estrobilização. A estrobilização pode ser polidisco – com a formação de várias medusas –, oligodisco – com a formação de poucas éfiras (2-10) (Fuentes et al. 2011) – ou monodisco – somente uma éfira formada por vez (Bigelow 1900; Holst et al. 2007; Schiariti et al. 2008). As ordens Coronatae e Semaeostomeae apresentam um padrão

polidisco e Rhizostomeae padrão oligodisco ou monodisco (Werner 1973; Fuentes et al. 2011). Uma vez que a éfira é liberada na coluna d'água, ela cresce em tamanho até chegar à fase adulta. É na fase adulta que ocorre o desenvolvimento e crescimento da gônada. Os gametas podem ser liberados na água – fecundação externa – ou mantidos na cavidade gastrovascular da fêmea – fecundação interna – (Widersten 1965; Morandini et al. 2004; Tiemann and Jarms 2010; Ikeda et al. 2011; Schiariti et al. 2012; Tiseo 2016). Os ovos fertilizados se desenvolvem em uma larva plânula que assenta em substrato e diferencia-se em pólipo (Kikinger 1992; Morandini et al. 2004; Calder 2009; Jarms 2010; Schiariti et al. 2012).

Das 241 espécies de cifozoários descritas, 49 tem seu ciclo de vida conhecido e destes, 47 são metagenéticos (Jarms 2010). O ciclo de vida de *Nausithoe maculata* (Da Silveira and Morandini 1997), *Chrysaora lactea* (Morandini et al. 2004), *Aurelia coerulea* (Scorrano et al. 2016), *Lychnorhiza lucerna* (Schiariti et al. 2008), *Cassiopea andromeda* - espécime do Rio de Janeiro (Bigelow 1900) e *Stomolophus* cf. *meleagris* (Calder 1982) seguem o padrão para Scyphozoa (Morandini et al. 2016b). A estrobilização polidisco produz até 89 éfiras em *N. maculata*, 10 éfiras em *C. lactea* e 17 éfiras em *A. coerulea*. A espécie *Cassiopea andromeda* – espécime do Rio de Janeiro – apresenta estrobilização monodisco e a estrobilização oligodisco produz 3 éfiras em *L. lucerna* e de 2 a 3 éfiras em *Stomolophus* cf. *meleagris* (Bigelow 1900; Calder 1982; Da Silveira and Morandini 1997; Lucas 2001; Morandini et al. 2004; Schiariti et al. 2008; Gambill and Jarms 2014). Werner (1973, 1974) descreve o ciclo de vida de *Nausithoe eumedusoides* evidenciando que, nesta espécie, o pólipo passa pelas fases iniciais comuns de estrobilização, porém os medusóides (de 5 a 8) permanecem conectados ao estróbilo, sofrendo a maturação e se reproduzindo junto à cadeia de estrobilização. Por fim, para a espécie *C. plocamia* não há registro de trabalhos descrevendo o ciclo de vida, mas Riascos

et al. (2013) apresenta experimentos com os pólipos e isso é indicativo de ciclo metagenético. De maneira complementar Morandini (comunicação pessoal) afirma que a espécie apresenta estrobilização polidisco produzindo de 7 a 10 éfiras. As espécies *Peripphylla periphylla* e *Pelagia noctiluca* são exceções ao padrão de ciclo de vida metagenético tendo um ciclo de vida holopelágico (Jarms et al., 1999; Delap, 1907).

Em sua maioria, os cifozoários apresentam gonocorismo (Berrill 1949). No entanto, existem espécies de cifomedusas como *Chrysaora hysoscella* que apresentam claramente hermafroditismo, e há relatos de que exemplares do gênero *Cassiopea* também possam ser hermafroditas (Berrill 1949; Hofmann and Hadfield 2002). Para Scyphozoa, a gônada é descrita como um espaço preenchido com células germinativas em desenvolvimento que migraram da endoderme para a mesogléia (Miller 1983). De modo geral, a estrutura gonadal (Figura 1) apresenta um formato circular (Morandini and Silveira 2001) ou alongado (Tiemann and Jarms 2010) em Coronatae, semicírculo em Semaeostomeae (Morandini and Marques 2010) e em forma de cruz em Rhizostomae (Kikinger 1992; Schiariti et al. 2012). Histologicamente, a gônada é dividida em três camadas de tecido: uma externa – camada endodermal cilíndrica, ciliada – uma interna (epitélio genital) e entre elas encontram-se as células germinativas imersas na mesogléia (Widersten 1965; Miller 1983; Tiseo 2016). Para os representantes de Scyphozoa, a ovogênese e a espermatogênese são relativamente bem documentadas (Smith 1936; Widersten 1965; Eckelbarger and Larson 1988; Kikinger 1992; Morandini and Silveira 2001; Ikeda et al. 2011; Tiseo 2016), porém são poucos os trabalhos descrevendo o processo de formação (Widersten 1965), a morfologia macroscópica da gônada, sua conformação e organização dentro da cavidade gastrovascular (Hyman 1940; Eckelbarger and Larson 1988; Kikinger 1992; Ohtsu et al. 2007; Schiariti et al. 2012).

Cronologicamente, os dados sobre a reprodução sexuada em Scyphozoa se resumem a descobertas sobre a origem das células germinativas (Widersten 1965; Campbell 1974; Miller 1983), conformação e organização epitelial da gônada quando maduras (Haeckel 1882; Hyman 1940; Werner 1973; Lesh-Laurie and Suchy 1991; Arai 1997; Tiseo 2016), e descrição da gametogênese de algumas espécies (Gohar and Eisawy 1960; Beams and Kessel 1983; Eckelbarger and Larson 1988; Kikinger 1992; Morandini and Silveira 2001; Tiemann and Jarms 2010; Schiariti et al. 2012; Tiseo 2016). Dentro deste contexto, alguns trabalhos descrevem a morfologia gonadal macroscópica, mas não em uma abordagem evolutiva sendo ainda poucos os trabalhos que abordam temas como a organização histológica da gônada, estratégias de liberação de gametas, comportamento de corte, tipos de fecundação (se externa e interna), incubação de gametas e semelparidade.

2. Estrutura da Tese

Esta tese encontra-se dividida em 5 capítulos independentes (no formato de artigos) para serem submetidos para publicação. Optamos por esse estilo para que os resultados já fossem apresentados e discutidos em seu formato final. No **Capítulo 1: "The evolution of gonads in coronate medusae (Scyphozoa, Coronatae)"** reviso todo o conteúdo relacionado a reprodução sexuada de Coronatae e descrevo de forma comparada a morfologia gonadal macroscópica e histológica para os representantes da ordem dentro de um recorte evolutivo. No **Capítulo 2: "Seminiferous tubule in lower metazoans, insights from scyphozoan Discomedusae (Cnidaria)"** descrevo de forma comparada a conformação da gônada, espermatogênese e ultraestrutura do espermatozoide das espécies *Chrysaora lactea*, *Lychnorhiza lucerna* e *Cassiopea maremetens*. Adicionalmente, discutimos sobre a semelhança da organização da gônada masculina em

cifozoários e as estratégias de liberação de gametas em um enfoque morfológico-evolutivo. No C**apítulo 3: "The role of trophocytes in the vitellogenesis of true jellyfishes (Scyphozoa, Cnidaria)"** abordo o tema do papel dos trofócitos nos processos de vitelogênese e coriogênese de *Chrysaora lactea* e discutimos a presença de uma membrana vitelínica semelhante à zona pelúcida de mamíferos produzida pelo próprio ovócito. No **Capítulo 4: "Sexual reproduction in three upside-down jellyfish (*Cassiopea* spp.): gonochorism, hermaphroditism and internal fertilization"** faço o detalhamento com análises histológicas e ultraestruturais. do processo de gametogênese comparado para três espécies do gênero *Cassiopea* e registro a fertilização interna e hermafroditismo sequencial para *Cassiopea frondosa*. No **Capítulo 5: "A evolução da estrutura gonadal em Scyphozoa"** é apresentada uma revisão bibliográfica da morfologia gonadal macroscópica e histológica da gônada dos representantes de Scyphozoa abordando a conformação e organização da gônada, gametogênese, estratégias de liberação de gametas, comportamento de corte, tipos de fecundação, incubação e semelparidade juntamente com novos dados. Ao final o **Capítulo 6** apresenta as considerações finais desta tese e faz uma síntese dos resultados obtidos nos capítulos anteriores.

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Capítulo 1

The evolution of gonads in coronate medusae (Scyphozoa, Coronatae)

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Abstract

The first coronate jellyfish description appeared 234 years ago. Since that time, no study reviewed the sexual reproduction considering the different gonadal morphologies, reproductive strategies, types of life cycle and embryonic development. Here we summarize the state of the art on coronates sexual reproduction based on the literature and some morphological and histological new observations. Our review provides a comparative evolutionary scenario about sexual reproduction in this group.

Key-words: Reproduction, Gametogenesis, Cnidaria, Jellyfish.

1. Introduction

The coronates occur in all oceans (Hingston et al., 2007) and are known as deep-sea jellyfishes because several species from the families Atollidae, Periphyllidae and some Nausithoidae are found below 500m depth; but several other species from the families Linuchidae and other Nausithoidae are found in shallow waters (Silveira & Morandini, 1997, 1998; Jarms et al., 1999; Jarms et al., 2002; Jarms & Morandini, 2019; Swartz, 1788; Tiemann & Jarms, 2010). The order Coronatae is considered the ancestral monophyletic group of Scyphozoa (Collins, 2002, 2009; Kayal et al., 2018; Marques & Collins, 2004) and comprise 57 species included in six families: Atollidae, Atorellidae, Linhuchidae, Nausithoidae, Paraphyllidae and Periphyllidae (Daly et al., 2007; Jarms & Morandini, 2019). There are two contrasting hypotheses of phylogenetic relationship between all Coronatae families. Molinari (2019) hypothesis shows the monophyly of all families with Nausithoidae as the sister group of a clade defined by the other 5 families. Dawson (2004) hypothesis shows the polyphyly of Nausithoidae and a polytomy with Periphyllidae, other Nausithoidae and Linuchidae (note that no species of Atollidae, Atorellidae and Paraphyllidae was used in this analysis).

As occurs in all scyphozoans, the general life cycle pattern for coronates is assumed to be metagenetic. Conversely, there is much variation in life cycle patterns among the order Coronatae (Jarms, 2010). There are species with only the polyp stage (holobenthic) – as the nausithoids *Nausithoe racemosa*, *N. planulophora*, *N. eumedusoides* and *Thecoscyphus zibrowii* (Komai, 1935; Sötje & Jarms, 1999; Werner, 1971, 1973) – and others only with the medusa stage (holoplanktonic) – as it is suggested for the atollids and paraphyllinids, and confirmed in *Periphylla periphylla* (Jarms et al., 1999, 2002). Some Nausithoidae species are known to have reduced the medusa stage (Jarms & Morandini, 2019; Sötje & Jarms, 2009; Werner, 1971, 1974). There are data about the sexual reproduction for 19 coronate species (Broch, 1913; Silveira & Morandini, 1997; Jarms, 1990, 2001; Jarms et al., 1999, 2002; Sötje & Jarms, 1999). From these, only 11 species have histological features of the gonads or gametogenesis described (Bigelow, 1909; Eckelbarger & Larson, 1992; Lucas & Reed, 2010; Maas, 1897; Morandini & Silveira, 2001; Russell, 1956; Sötje & Jarms, 2009; Tiemann & Jarms, 2010; Vanhöffen, 1902). Concerning the reproduction in coronates, Sötje & Jarms (1999) proposed two evolutionary trends: i. sexual reproduction is gradually reduced, as most nausithoids like *Nausithoe punctata* and *Nausithoe maculata* express sexual reproduction and both life cycle stages (polyp and medusa) contrasting with a few species such as *Nausithoe planulophora* who is apogamous and possess only the polyp stage of life cycle; ii. suppression of the male sex in Nausithoidae, as species like *Nausithoe racemosa* (the medusae are reduced to eumedusoids), *Nausithoe eumedusoides* (the hermaphrodite medusoids do not detach from the polyp), who starts the development of their eggs in the gastric longitudinal septae and *Thecoscyphus zibrowii* which is parthenogenetic.

Even after 234 years of the first coronate species described – *Linuche unguiculata* (Swartz, 1788) – there are no updated study reviewing the sexual reproduction of this

scyphozoan order while comparing and contrasting the different gonad morphologies, reproductive strategies, types of fertilization (internal or external), type of life cycle (metagenetic or with reduced medusae) and development (direct or indirect). Within this framework this paper summarizes the present state of knowledge on the coronates sexual reproduction based on a review from the data available in the literature with some morphological and histological new observations. We then focus on relevant comparative evolutionary considerations about sexual reproduction features in a systematic framework.

2. Material and Methods

2.1. Literature review and analysis

Search on old descriptions of coronataes species was performed in the Biodiversity Heritage Library and other museums online libraries. Additionally, search of actual descriptions and original studies on Coronatae reproductive biology were made in online search platforms, containing information on at least one of the following items, were examined: (1) the macroscopical gonad morphology; (2) the histological gonad morphology and gametogenesis; (3) the size of released eggs; (4) types of reproductive strategies as hermaphroditic, gonochoric or parthenogenetic; (5) the type of development as direct or indirect and (6) the life cycle as holopelagic or metagenetic. We summarized all data in Table 1.

2.2. Cultivation of polyps, Museum specimens, and Microscopy techniques

Polyps of *Nausithoe eumedusoides* were reared in the laboratory for more than a year to obtain five medusoids. The polyps were fed once a week with *Artemia* sp. following the methods described in Jarms et al. (2002). The medusoids released from the

strobila were preserved in 4% paraformaldehyde solution buffered with a saline solution of 0.2 M sodium phosphate (pH 7.2) for at least 24 hours.

Samples of five specimens of *Periphylla periphylla* and one of *Atolla vanhoeffeni* from the authors' private collection were preserved in formalin 4%. The tissue samples were dehydrated in ethanol series (from 30% to 95%) and embedded in Leica® methacrylate historesin. Slides were made with serial sections 3-5 μm thick cut on a rotating microtome. For histological and histochemical description, the slides were stained with hematoxylin and eosin (HE), toluidine blue to acids components Audino et al., 2015), mercuric bromophenol blue (Pearse, 1960) and ponceau xylidine (Mello & Vidal, 1980) for proteins and PAS technique used to visualize neutral polysaccharides (Junqueira & Junqueira, 1983). The Mallory's and Gomori trichomic stains were used to visualize collagen and mucus, respectively (Bancroft & Stevens, 1982; Humason, 1962).

For measurements, slides stained with HE was qualitatively and quantitatively analyzed with respect to germ cells at different stages. Measurements were taken for a total of 20 nuclei (randomly sampled) from each preparation, corresponding to each of the four spermatogenic cell types (spermatogonia, spermatocyte, spermatid, and sperm) or ovogenic cells types (oocyte I, oocyte II and oocyte III), when present. The nuclear measurements were made using the ImageJ software, using the appropriate scale calibrations. Minimum and maximum measurements were presented (minimum-maximum).

3. Results and Discussion

State of the art

(1) Overview of the gonadal morphology

In coronates the gonads may be equidistant adradially, or paired near the interradii, being formed as an evagination of the floor of the gastrovascular cavity, proximate to the coronal groove and peripheral with the gastric filaments within the central stomach (Eckelbarger & Larson, 1992; Jarms & Morandini, 2019; Lucas & Reed, 2010; Morandini & Silveira, 2001; Thiel, 1936; Tiemann & Jarms, 2010). Komai (1935) stated that in *Nausithoe* the first primordia of the gonads start in the ephyra; and although they are hardly recognizable in living specimens they may be found in histological sections. Most species have eight adradial gonads, except some species of the genera *Atorella*, *Linantha*, *Palephyra*, *Periphyllopsis* and *Nausithoe* which may have four or six gonads (Bigelow, 1909; Komai, 1935; Maas, 1897; Russell, 1956; Thiel, 1936; Vanhöffen, 1902; Werner, 1974). See Table 1 for details. Thiel (1936) pointed that in some cases – as seen in *Palephyra pelagica* – the 4 crescent-shaped gonads can be easily understood as the typical 8 adradial gonads of other coronates. In other species – as in *Atorella* – the gonad may have suffered a fusion process but more developmental studies are needed to make further suggestions. In the case of *Nausithoe racemosa* and *Nausithoe eumedusoides* the presence of 4 gonads seems to be related with the polyp tetraradial symmetry and with the distinct type of development of the species.

Based on literature evidences and our own observations, 15 macroscopical gonad shapes are recognized (Figs 1-2). Atollidae usually has oval (Fig. 1C), bean-shaped (Figs 1A, 2C-E) but elongated (Fig. 1B) (in *Atolla chuni*) and oblong shapes (Fig. 1E) (in *Atolla verrili*) are also seen (Fewkes, 1886; Haeckel, 1880; Maas, 1897; Repelin, 1962; Vanhöffen, 1902). Atorellidae is the family in which we found various shapes of gonads: oval, bean-shaped, irregular (Fig. 1F) or fusiform (Fig. 1G) (Bigelow, 1909; Kawaguti & Yoshimoto, 1973; Leloup, 1937; Mills et al., 1987; Vanhöffen, 1902). The species *Linuche unguiculata* and *Linantha lunulata* have gonads crescent-shaped (Fig. 1H) while

Linuche aquila and *Linuche draco* have horseshoe-shaped gonads (Fig. 1I) (Haeckel, 1882; Swartz, 1788). The family Nausithoidae have one species with no gonads (*N. planulophora*) and eight different gonad shapes: oblong, round (Fig. 1D; 2A-B), bean-shaped, oval, crescent-shaped, horseshoe-shaped, heart-shaped (Fig. 1J) and triangular faint (Fig. 1K) (Agassiz & Mayer, 1902; Broch, 1913; Silveira & Morandini, 1997; Gegenbaur, 1856; Haeckel, 1880; Hartlaub, 1905; Jarms, 1990, 2001; Kirkpatrick, 1890; Kölliker, 1853; Komai, 1935; Maas, 1897; Molinari, 2019; Vanhöffen, 1892, 1902, 1910; Werner, 1971). The W-shaped (Fig. 1L) is a form exclusive to the Paraphyllinidae but *Paraphyllina intermedia* have bean-shaped gonads (Maas, 1903; Neppi, 1915; Russell, 1956). Seven gonad forms are seen in the family Periphyllidae: *Pericolpa campana*, *Pericolpa quadrigata* and *Pericolpa tetralina* have oval gonads, *Periphyllopsis braueri* have the exclusive C-shaped gonad (Fig. 1O), *Periphyllopsis galatheae* horseshoe-shaped and *Periphylla periphylla* have the exclusive J or U-shaped gonads (Figs 1M-N; 2F-G) (Haeckel, 1880; Lucas & Reed, 2010; Tiemann & Jarms, 2010; Vanhöffen, 1902). The gonad can also be distinguished due to its color (c.f. dark red, chestnut brown, cream-orange, yellowish brown whitish, dark purple, carmine or transparent, details in Table 1). Color may vary from species to species (Agassiz & Mayer, 1902; Broch, 1913; Silveira & Morandini, 1997; Fewkes, 1886; Jarms, 1990; Lucas & Reed, 2010; Maas, 1897, 1903; Neppi, 1915; Russell, 1956; Tiemann & Jarms, 2010; Vanhöffen, 1902) due to the presence of sperm follicles (Fig. 2D-E) or oocytes (Fig. 2F-G). Occasionally, the gametes can be seen thought the gonad by the naked eye (*Atolla vanhoeffeni*, *Nausithoe maculata*, *Nausithoe werneri* and *Periphylla periphylla*, pers. observ.).

(2) **The histological gonad morphology and gametogenesis**

In comparison with macroscopic gonadal data, detailed description of the histological conformation was less studied and ultrastructural data are almost scarce in coronates. But there are some interesting data on the ultrastructure of the female gonad and oogenesis of *Linuche unguiculata* (Eckelbarger & Larson, 1992), and the male and female germ cells and gonad ultrastructure of *Periphylla periphylla* (Tiemann & Jarms, 2010). In the last century, the microscopic anatomy was studied by Claus (1883), Maas (1897), Vanhöffen (1902), Komai (1935), Russell (1956) and Eckelbarger & Larson (1992) mostly focusing in the general description of the gonad structure under light microscopy. Recently Morandini & Silveira (2001), Lucas & Reed (2010), and Tiemann & Jarms (2010) described the histological gonadal features, gametogenesis and exclusive types of cells involved in the process of germ cells maturation. Most of the histological studies on coronates are restricted to traditional descriptions but lacking the histochemical composition of tissues to take their conclusions, even when trichomic or specific stains were used. Detailed descriptions such as tissue and cell compositions (presence of collagen, mucous or musculature) improve our understanding of the main processes that are happening in the structure at the time of preservation and could lead us to infer about the role or specific functions of cells and tissues.

The general histological gonadal organization (Fig. 3), in cross section, is similar to other scyphozoans: an outer and an inner genital epithelium with the oocyte or sperm follicles immersed in the mesoglea (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988; Eckelbarger & Larson 1992; Maas, 1897; Morandini & Silveira, 2001; Widersten, 1965). The gonad has its origin from interstitials cells which migrate from the gastrodermis to mesoglea (Miller, 1983). This region of cell migration was described by Haeckel (1880) as “germinative epithelia” in *Nausithoe challengeri*. Maas (1897) used the term “germinative zone” (“keimzone”) in *Periphylla* and *Atolla* and these cells were

also seen by Komai (1935) as a cluster of "primordial gonad cells". Claus (1883) stated that the structure of the male gonad (called "testis") is similar to that of the female gonad ("ovary") but with some differences in the mesoglea because of the presence of sperm follicles. According to Chapman (1966) the mesoglea is an extracellular matrix composed mainly of collagen and sometimes cells of irregular shape can be seen immersed on it. In some species of *Nausithoe* and *Linuche* no cells can be seen in the gonadal mesoglea while in *Atolla* and *Periphylla* there is few irregular cells with no clear function (Eckelbarger & Larson, 1992; Lucas & Reed, 2010; Morandini & Silveira, 2001).

Lucas & Reed (2010) described the gametogenesis of *Atolla wyvillei* from two different locations: Gulf of Mexico and Cape Hateras. The smaller individual with gonad were 17-22 mm in bell diameter and as the gonads matured, they increase in size and together, the mature male gonads formed an almost continuous ring around the stomach (seen in aboral view). The gonad is organic rich as demonstrated by the high percentage of ash-free dry weights ranging from 67.6 – 96.5% of dry weight. As in other coronates, the gametogenesis of Atollidae is asynchronous with germ cells in all stages of development and presenting a centripetal organization (Maas, 1897; Vanhöffen, 1902). In *A. wyvillei* the spermatogonia and early oocytes (24-53µm) are found in the periphery, with late oocytes (226-263µm) in the center of the mesoglea region. Although mature specimens of *A. gigantea, A. verrili* and *A. valdiviae* were observed, no measurements are provided (Maas, 1897; Vanhöffen, 1902). Concerning *A. wyvillei* oogenesis, the pre-vitellogenic oocytes appeared to be held by a peduncle – as already pointed by Vanhöffen (1902) in *Atolla verrili*.

We only had access to males of *Atolla vanhoeffeni*, so only the spermatogenesis data is added here. As observed in other coronates the follicles are surrounded by mesoglea and consequently by the outer and inner genital epithelium (Fig. 4A) Near the

connection with the gastrodermis it is possible to observe the interstitials cells which gave rise to the gonad. The spermatogonia have an elongated nucleus (10.42-16.52 µm) and are bigger than the spermatocytes (7.65-9.4 µm) which have round nucleus (Fig. 4B). The spermatid has very compact (4.69-6.95 µm) and basophil nucleus (Fig. 4B). The sperm has round basophil head (7.65-13.47 µm) with acidophil flagellum (Fig. 4A). Both sperm head and flagellum are positive to proteins (Fig. 4C-D), negative to PAS (Fig. 4E) and positive to trichomic of Gomori (Fig. 4F).

Bigelow (1909) in the original description of *Atorella vanhoeffeni* provides few details of the oval male gonad which, by the drawing presented is very similar in histological organization of other scyphozoans (outer and inner genital epithelium). The only information about the female gonad is that it is filled with a comparatively small number of large eggs. What made us infer about the gonadal maturation of the female specimen as under development.

Claus (1883) and Morandini & Silveira (2001) are the only studies describing in details the sexual reproduction of nausithoids. Both the female and male gonads are surrounded by an outer and an inner genital epithelium. The oocytes develop from the inner genital epithelium and lie in the mesoglea. Different stages of oocytes are found: the pre-vitellogenic, basophil and acidophil oocytes. The pre-vitellogenic oocytes are round cells (16,5-36,0µm) which stay in the inner genital epithelium. Morandini & Silveira (2001) differentiate two types of oocytes based on the affinity with the hematoxylin and eosin (HE) stain: the basophil oocytes were smaller (31.5-82.5 µm) and the acidophil oocytes were bigger (73.5-133.5 µm). The male germ cells develop centripetally with the spermatogonia near the periphery of follicles and sperm in the center. The spermatogonia are larger (4.8-6.0 µm) cells with round shape and reduced cytoplasm; the spermatocytes are also spherical cells (4.8-6.6 µm) which presents

granular chromatin; the spermatids are round and smaller than spermatocytes (2.4-3.6 µm) and the sperm have conical head (4.2-6.0 µm), midpiece and long flagellum pointing to the center of the follicle.

As we only have access to male polyps of *N. werneri*, only male medusae were cultivated and only the spermatogenesis is detailed here. As already described to *N. maculata* males (Morandini & Silveira, 2001) the gonad of *N. werneri* is inside the gastrovascular cavity near the gastric filaments (Fig. 5A-F). When observed in the oral-aboral section, the germs cells are arranged centripetally inside the follicles surrounded by the outer and inner genital epithelia (Fig. 5B). The spermatogonia are cells with large nucleus (5.42-8.57 µm) and are situated near the follicle periphery (Fig. 5C). The spermatocytes are smaller (4.11-5.82 µm) than spermatogonia and have basophil round nucleus (Fig. 5C). In some cases, it is possible to observe the nucleus in meiotic phases (Fig. 5E). When compared to spermatocytes, the nucleus of spermatids is smaller (2.78-4.64 µm) and more basophil due to the compression of genetic material. The sperm are found in the central part of follicles (Fig. 5B-E) and have a compact conical head (3.94-5.08 µm). The sperm is composed of a basophil head with acidophil midpiece and flagellum (Fig. 5C). The nucleus and flagellum are positive to proteins being the nucleus more reactive than the flagellum (Fig. 5D-E). When compared to *N. maculata*, we can notice a similar pattern of the general morphology and similar measurements.

As *Nausithoe eumedusoides* is a hermaphroditic species, the gametogenesis of this species differs slightly from dioecious nausithoids like *N. maculata* and *N. werneri*. In cross section, the medusoids have a traditional female gonad usually round in shape and a reduced male gonad in which only sperm is observed (Fig. 6A). As in other *Nausithoe*, the female gonad has an outer and an inner genital epithelium with the oocytes in different stages of development surrounded by mesoglea (Fig. 6B). Inside the inner genital

epithelium are the pre-vitellogenic oocytes (6.14-11.29 µm) with round basophil nucleus (Fig. 6B). Differently of the observations of Morandini & Silveira (2001), three stages of oocytes can be recognized (Fig. 6C): an early (Oi), intermediate (109.71-116.52 µm) (Oii) and late (Oiii). The early oocyte (74.83µm) is basophil with few yolk granules, the intermediate is in a transitional acidophil (Fig. 6C) and the late oocyte (154.82-197.11 µm) are highly acidophil and larger (Fig. 6D). No accessory cell is seen surrounding the oocytes and the yolk transport seems to occur directly from the genital epithelia (Fig. 6D). The yolk is composed of glycoproteins being positive to proteins (Fig. 6E) and to neutral polysaccharides (Fig. 6F). Adjacent to the female gonad there is the male gonad (Fig. 6A) in which we can notice the sperm (3.1-5.1 µm) which are free near the gastric cavity (Fig. 5B). The hermaphroditism of *N. eumedusoides* was described by Werner (1973, 1974) and this is the first record of the histological organization.

The histology of the parthenogenetic structure (egg sac) and gonad of *Thecoscyphus zibrowii* was described in detail by Sötje & Jarms (2009). In the female polyps, the upper part above the constriction is transformed into the egg sac: the structure is organized in separate double egg sacs united by a connective tissue. The egg sac detaches and migrate from the bottom to top of the polyp. When mature the eight elongate gonads are apparent located in the adradial axis. Inside the gonad, can be seen eggs in different stages of development. The oocytes developed into planulae and moved to the outside. The egg sac in most cases is pushed out the tube aperture and in rare cases it was incorporated and digested by the polyp.

Eckelbarger & Larson (1992) stated that the ultrastructure of the female gonad (called "ovary") of *Nausithoe atlantica* and *Linuche unguiculata* are very similar. For this reason, the authors provided only the description of *L. unguiculata* female gonad. *Linuche unguiculata* is the only Linuchidae which have the oogenesis studied, it possesses a gonad

that arise from evaginations from the gastrodermal epithelium in the floor of interradial pouches. During the vitellogenesis, pre-vitellogenic oocytes arise from the inner genital epithelium (called "endodermal gastrodermis") and migrate into the mesoglea, where the oocytes develop freely. Numerous intraooplasmic channels are seen in the ooplasm directly associated with rough endoplasmic reticulum, playing some role in the yolk production. Both authosynthetic and heterosynthetic vitellogenesis occurs, being the last one with the support of receptor-mediated endocytosis.

Russell (1956) presented the macroscopical conformation of male follicles inside the gonad of *Paraphyllina ransoni* and pointed that in one of the male specimens the gonad appears fully developed and started to degenerate. The sperm are developed in elongated, oval or slightly branched seminiferous follicles arising from the gonad wall. Concerning the female gonad, as in other coronates, the author only pointed the presence of different stages of development of the oocytes. No schematic drawing of female gonad was provided but an image of a histological section in plate II, made us to infer that the gonad has the same pattern as in other scyphozoans: an outer and inner genital epithelium.

Maas (1897) was the first to study the gametogenesis in *Periphylla periphylla* and recently Lucas & Reed (2010) and Tiemann & Jarms (2010) expanded the knowledge considering three different populations: Gulf of Mexico, Cape Hateras and Lurefjord (Norway). We include here some histochemical observations. The smallest and biggest individuals with gonad are 15 mm and 80 mm in bell diameter, respectively (Lucas & Reed, 2010). Microscopically, oocytes of different sizes were observed embedded singly in the mesoglea (or "gonad tissue" by Tiemann & Jarms, 2010) and both – oocytes and mesoglea – are surrounded by an outer and inner genital epithelium (Fig. 7A-B). Inside the inner genital epithelium ("germ-cell layer of the endoderm" by Tiemann & Jarms, 2010) are the round oogonia and the pre-vitellogenic oocytes. When the oocytes reach

75µm they start the yolk production and already have contact with accessory cells near the inner genital epithelium (with dense plasma at transmission electron microscopy) – called trophocytes by Tiemann & Jarms (2010). Analyzing the female gonad of *P. periphylla*, differently to that described by Tiemann & Jarms (2010), no trophocyte like cell were seen in any of the early oocytes, but ultrastructural studies are needed to confirm this (Fig. 7A-B). Eckelbarger & Hodgson (2021) discussed all terms related to accessory cells in invertebrates and, according to them, these "*P. periphylla* trophocytes cells" are not the same as a typical trophocyte cell, which are an exclusive accessory cell of Discomedusae. Following Eckelbarger & Hodgson (2021) definition, trophocytes are specialized gastrodermal cells that contain abundant apical microvilli, numerous Golgi complexes and extensive vesicles in the apical cytoplasm. Following this definition and observing the ultrastructural schematic drawings from Tiemann & Jarms (2010), we agree with Eckelbarger & Hodgson (2021) that these may not be similar to the Discomedusae trophocyte, but more ultrastructural studies are needed to confirm this hypothesis.

Tiemann & Jarms also described the presence of a second type of accessory cell ("follicles cells") surrounding the oocytes larger than 0.94mm. They identified two types of oocytes: i. Oocytes with large nucleus but no accessory cells and ii. Oocytes with small nucleus and surrounded by accessory cells. The first group of oocytes varied from 0.45mm to 0.9mm and the second group varied from 0.9 to almost 1.2mm. We also observed oocytes without (Fig. 7C-G) or with accessory cells (Fig. 7H), but differently to that described by Tiemann & Jarms (2010) we find small oocytes (0.33-0.55mm) surrounded by accessory cells (Fig. 8). For both types (with or without accessory cells), in the histological sections, we can notice two different types of yolk granules in the ooplasm: small acidophil granules and large acidophil granules (Fig. 7H). The small granules are positive to acid structures (Fig. 7I) and neutral polysaccharides (Fig. 7J),

strongly positive to proteins (Fig. 7K) and stained in blue with Gomori's trichomic (Fig. 7L). While large granules are strongly positive to acid structures (Fig. 7I), positive to neutral polysaccharides (Fig. 7J) and proteins (Fig. 7K) and stained in red with Gomori's trichomic (Fig. 7L). Observing the follicle cells of *P. periphylla*, and adding the definition of the follicle cells type II presented by Eckelbarger & Hodgson (2021): "*squamous or cuboidal epithelial cells that encircle oocytes, that are hypertrophic throughout most of vitellogenesis and contain extensive arrays of rough endoplasmic reticulum (RER) and Golgi complexes that are synthetically active*"; we agree with Tiemann & Jarms (2010) that these may be true follicle cells. However, following the wider definition that follicle cells are somatic cells, we found ourselves in a dilemma. Once all scyphozoan gonads has germ line cells with gastrodermal origin, these follicle cells included, with might propose a different terminology for the "follicle cells". We propose to use the term accessory cell. The mature oocyte of *P. periphylla* are released by an aperture called "pore", surrounded by about 30 circles of tall, cylindrical "mucus cells". Under transmission electron microscopy the mucus cells are more electrondense than the inner genital epithelium cells, and they help the oocyte to be expelled. After spawning some mucus cells, basal lamina and other cells from the genital epithelium form the "follicle remnant" that eventually, is resorbed (Tiemann & Jarms, 2010). This is the first record of both types of cells in coronate jellyfishes. Mucus production are also described to the semaeostome *Pelagia noctiluca* (Rottini-Sandrini & Avian, 1991) but no specific cells are responsible for its production, the trophocytes seems to have large vacuoles full of mucopolysaccharides.

Periphylla periphylla male follicles contain all spermatic cell types – spermatogonia, spermatocytes, spermatids and sperm (Lucas & Reed, 2010) – and maintain contact with nourishing tissue of the male gonad wall (genital epithelium). The

sperm head was 5.2 µm long, with a diameter of 3.2 µm and a flagellum 100 µm long. Near the inner genital epithelium, the sperm are released through a pore into the genital sinus. Surrounding the pore cells are mucus cells that have large vacuoles in the cytoplasm and bean-shaped nucleus (Tiemann & Jarms, 2010). The authors did not make clear if these mucus cells are the same observed in the female gonad and also did not compare both types of cells.

(3) Egg size, life cycle & development and reproductive strategies

Berrill (1949) in his study of the developmental cycle of scyphomedusae stated that the variability of the gastrulation process (either by invagination, ingression or both) is directly correlated to the variability in egg size. Following this hypothesis Lesh-Laurie & Suchy (1991) presented a reasoning in which i. species with small eggs usually gastrulate by ingression; ii. species with intermediate eggs sizes gastrulation occurs by both processes: ingression and invagination; and iii. species with large eggs the gastrulation is by invagination. Following this reasoning, Jarms et al. (1999) based on their findings in *Periphylla periphylla*, added a new topic: iv. species with comparatively huge eggs the gastrulation occurs by delamination. Previously, Larson (1986) in his study of pelagic scyphozoans of the Southern Ocean proposed that large eggs would indicate direct development and this hypothesis was confirmed in *P. periphylla*, but still lack knowledge about the life cycles of atollids and paraphyllinids, which have eggs larger than 1 mm and (Jarms et al., 1999, 2002; Jarms & Morandini, 2019). Other coronates species as *Linuche unguiculata*, *Nausithoe maculata*, *Nausithoe eumedusoides*, *Nausithoe marginata* and *Nausithoe racemosa* have small oocytes with maximum size of 0.240 mm (Conklin, 1908; Jarms, 1990; Metschnikoff, 1886; Morandini & Silveira, 2001) and indirect development with planula larva (Silveira & Morandini, 1997; Jarms, 1990; Komai, 1935). Table 1 summarizes See table 1 for details.

The life cycle of most coronates is metagenetic, following the pattern of many other scyphozoans: the fertilized egg develops into a planula larva that settles and give rise to a polyp. The polyp reproduces asexually (strobilation) giving rise to one or more dioecious free-swimming jellyfish, which grow, mature and reproduce sexually (Bigelow, 1900; Jarms, 2001; Morandini et al., 2004; Schiariti et al., 2008). The strobilation in coronate species is usually polydisc with the formation of several young medusae – ephyrae (Silveira & Morandini, 1997; Jarms, 1990; Komai, 1935). In general, Atorellidae, Linuchidae and Nausithoidae are families which species have a metagenetic life cycle. Among the Nausithoidae, exceptions are the reduced life cycle of *Nausithoe planulophora* (Werner, 1971) in which the strobilation is unique developing 30-50 ephyra-like discs that metamorphose directly into a ciliate planula-like (planuloids); *Nausithoe eumedusoides* (Werner, 1974) strobilates giving rise to hermaphroditic medusoids; *Nausithoe recemosa* (Komai, 1935) strobilates producing male and female medusoids; *Nausithoe marginata* (Jarms, 1990) is a hermaphroditic species with protandric development in the medusa; and *Thecoscyphus zibrowii* (Sötje & Jarms, 1999) which is parthenogenetic. The families Atollidae, Paraphyllidae and Periphyllidae seems to exhibit a holopelagic life cycle in which the planulae and polyp stages are reduced with the egg giving rise directly to a young medusae (Jarms et al., 1999, 2002) – note that this finding is only confirmed in *Periphylla*.

Once only a few species have the gametogenesis of both sexes documented, most of the information about the reproductive strategies came from the taxonomic descriptions or life cycle studies. The majority of coronates seems to be gonochoric (Broch, 1913; Jarms, 1990, 2001; Jarms et al., 2002; Morandini & Silveira, 2001; Tiemann & Jarms, 2010) but some nausithoid species are hermaphroditic – as *N. eumedusoides* and *N. marginata* – or parthenogenetic – as *T. zibrowii* (Jarms, 1990; Sötje

& Jarms, 2009; Werner, 1974). Following the evolutionary trend of the reduced male sex proposed by Sötje & Jarms (1999) and comparing the different reproductive strategies of the species we do support the reduction of the male sex. Although a trend can be seen from the gonochoric *N. maculata*, the progressive hermaphrodite *N. marginata*, reduction of the sexual stage in *N planulophora*, and the parthenogenetic *T. zibrowii*. It is important to consider that *N. maculata* and *N. marginata* are species from shallow waters while *N planulophora* and *T. zibrowii* are found in marine caves, which are completely different environments.

The most recent phylogenetic relationship topology of coronates including at least one species from each family is the tree proposed by Molinari (2019). In her dissertation it is highlighted the monophyly of all families with Nausithoidae as the sister group of a clade defined by the other 5 families: Linuchidae, Atorellidae, Atollidae, Paraphyllinidae and Periphyllidae. We used this topology to discuss all data reviewed here. The main conclusions are highlighted as follow: 1. With the exception of some Nausithoidae species, the majority of coronates are gonochoric and have a typical metagenetic life cycle with indirect development (egg-planula-polyp-medusa); 2. The family Nausithoidae show a larger number of macroscopical gonadal distinct morphologies (nine) with only two types exclusive to the group (the heart-shaped and triangular-faint gonads) and is the only family in which hermaphroditism and parthenogenesis appeared; 3. Atorellidae have two exclusive shapes of gonads (irregular-shaped and fusiform); 4. The holopelagic life cycle and the direct development are seen in the clade (Atollidae (Paraphyllinidae, Periphyllidae)); 5. The W-shaped gonads are exclusive of family Paraphyllinidae; 6. The J and U-shaped gonads are exclusive of Periphyllidae; 7. Periphyllidae is the only family with the presence accessory cells surrounding the oocyte, it is also the only family with mucus and pore cells present in male and female gonads.

Here we presented a review of new and literature data related to general gonad shape and histological observations in Coronatae species. Further morphological investigations, especially using transmission electron microscopy, are needed to enlighten all the gaps in the knowledge of sexual reproduction in coronates. Additional characterization of descriptive and functional morphological data of the gonads in other species of this order combined with knowledge about the evolution and phylogenetic relationships of the group, will provide a robust understanding of the origin and evolutionary trends in the reproductive strategies of the Coronamedusae.

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Tables

Table 1: Sexual reproductive features in coronates. Where - = no data available and (rel) = released.

Family	*Species*	Original Description	Gonad morphology	Gonad color	Number	Histology	Reference	Egg size	Reproductive strategies	Development/Life cycle	Reference
Atollidae	*Atolla bairdii*	Fewkes, 1886	Bean-shaped	Dark red; chestnut brown	8	-	-	-	-	Believed to develop directly/Holopelagic	Jarms et al.,2002
	Atolla chuni	Vanhöffen, 1902	Oval; elongated	Cream-orange	8	Female	Vanhöffen, 1902	-	Gonochoric		
	Atolla gigantea	Maas, 1897	Oval	Cream	8	Female	Maas, 1897	-	Gonochoric		
	Atolla parva	Russel, 1958	Oval	Dark red	-	-	-	-	-		
	Atolla russeli	Repelin, 1962	Bean-shaped with two lobes	-	8	-	-	-	-		
	Atolla tenella	Hartlaub, 1909	-	-	-	-	-	-	-		
	Atolla valdiviae	Vanhöffen, 1902	Oval	Orange	8	Female	Vanhöffen, 1902	-	-		
	Atolla vanhoeffeni	Russel, 1957	Oval	Yellowish brown	4 pairs (8)	Male	Present study	-	Gonochoric		
	Atolla verrilli	Fewkes, 1886	Oblong; bean-shaped	-	4 pairs (8)	Male and Female	Vanhöffen, 1902	-	Gonochoric		
	Atolla wyvillei	Haeckel, 1880	Oval; bean-shaped	Cream-orange	8 sometimes paired	Male and Female	Vanhöffen, 1902; Lucas & Reed, 2010	1 mm; 0.543 mm	Gonochoric		
Atorellidae	*Atorella arcturi*	Bigelow, 1928	Irregular	-	6	-	-	-	-	-	-
	Atorella japonica	Kawaguti & Matsuno, 1981	Fusiform	-	-	-	-	-	-	-	-
	Atorella octogonos	Mills, Larson & Youngbluth, 1987		Cream-tan	8	-	-	-	-	-	-
	Atorella sibogae	Leloup, 1937		-	-	-	-	-	Believed to be metagenetic		Jarms & Morandini, 2019
	Atorella subglobosa	Vanhöffen, 1902	Bean-shaped	Yellowish	4	-	-	-	-	-	-
	Atorella vanhoeffeni	Bigelow, 1909	Oval	Rose to opaque	4	Male	Bigelow, 1909	-	Gonochoric	Indirect	-
Linuchidae	*Linantha lunulata*	Haeckel, 1880	Crescent-shaped	-	4	-	-	-	-	-	-
	Linuche aquila	Haeckel, 1880	Horseshoe-shaped	-	4 pairs (8)	-	-	-	-	-	-
	Linuche draco	Haeckel, 1880	Horseshoe-shaped	-	8	-	-	-	-	Indirect	-
	Linuche unguiculata	Swartz, 1788	Crescent-shaped	Whitish	8	Female	Eckelbarger & Larson, 1992	0.240 mm (rel)	Gonochoric	Indirect	Conklin 1908
Nausithoidae	*Nausithoe albatrossi*	Maas, 1897	Elongated; oval; oblong; bladder-like	-	8	-	-	-	-	-	-
	Nausithoe albida	Gegenbaur, 1856		-	-	-	-	-	-	-	-

	Nausithoe atlantica	Broch, 1913	Oblong	Cream-orange	8	-	-	-	-	-	-
	Nausithoe maculata (=aurea)	Silveira & Morandini, 1997	Round	Pale brown	8	Male and Female	Morandini & Silveira, 2001	0.132-0.208 mm (rel)	Gonochoric	Indirect/ Metagenetic	Morandini & Silveira, 2001
	Nausithoe challengeri	Haeckel, 1880	Bean-shaped	Yellowish-orange	8	-	-	-	-	-	-
	Nausithoe clausi	Vanhöffen, 1892	Round	-	8	-	-	-	-	-	-
	Nausithoe eumedusoides	Werner, 1974	Bean-shaped; oblong	Yellowish to brownish	3 to 6	Male and Female	Present study	0,1875 mm	Hermaphroditic	-	-
	Nausithoe globifera	Broch, 1913	Oblong; quadrangular	Light brownish; white to reddish	8	-	-	-	Gonochoric	Indirect/ Metagenetic	Broch, 1913
	Nausithoe hagenbecki	Jarms, 2001	Round	Pale brown	8	-	-	-	Gonochoric	Indirect/ Metagenetic	Jarms, 2001
	Nausithoe limpida	Hartlaub, 1909	Heart-shaped	-	8	-	-	-	Gonochoric	-	-
	Nausithoe maculata	Jarms, 1990	Round	Dark brown to yellowish-white	8	-	-	-	Gonochoric	Indirect/ Metagenetic	Jarms, 1990
	Nausithoe marginata	Kölliker, 1853	Oval	Male yellowish, female no color	8	-	-	0,230 mm	Hermaphroditic	Indirect/ Metagenetic	Jarms, 1990; Metschnikoff 1886
	Nausithoe picta	Agassiz & Mayer, 1902	Oval	Chocolate brown or carmine	8	-	-	-	-	-	-
	Nausithoe planulophora	Werner, 1971	-	-	-	-	-	-	-	Indirect	-
	Nausithoe punctata	Kölliker, 1853	Horseshoe-shaped	White yellow (more immature); dark brown or blue	8	-	-	-	Gonochoric	Indirect	-
	Nausithoe racemosa	Komai, 1936	Round	-	4	-	-	0,2 mm	Gonochoric	Indirect	Komai, 1936
	Nausithoe rubra	Vanhöffen, 1902	Triangular faint	Orange or dark purple	-	-	-	-	-	-	-
	Nausithoe simplex	Kirkpatrick, 1890	-	-	-	-	-	-	-	-	-
	Nausithoe sorbei	Jarms, Tiemann, & Altuna Prados, 2003	-	-	8	-	-	-	-	Indirect	-
	Nausithoe striata	Vanhöffen, 1910	-	-	-	-	-	-	-	-	-
	Nausithoe thieli	Jarms, 1990	-	-	8	-	-	-	-	Indirect/ Metagenetic	Jarms, 1990
	Nausithoe werneri	Jarms, 1990	Round	Male opaque; female transparent	8	Male	Present study	-	Gonochoric	Indirect/ Metagenetic	Jarms, 1990
	Thecoscyphus zibrowii	Werner, 1984	Oblong?	-	8	Female	Sötje & Jarms, 2009	-	Parthenogenetic	Indirect	Sötje & Jarms, 1999
	Palephyra antiqua	Haeckel, 1880	Crescent-shaped	-	4	-	-	-	-	-	-
	Palephyra indica	Vanhöffen, 1902	Bean-shaped	-	4	-	-	-	-	-	-
	Palephyra pelagica	Haeckel, 1880	Crescent-shaped	-	4	-	-	-	-	-	-
	Paraphyllina intermedia	Maas, 1903	Bean-shaped	Pale cream	4 pairs (8)	-	-	-	-	Believed to develop directly/Holopelagic	Jarms et al., 2002
	Paraphyllina ransoni	Russel, 1956	W-shaped	Colorless	8	Male and Female	Russel, 1956	-	Gonochoric		
Paraphyllinidae	*Paraphyllina rubra*	Neppi, 1915	W-shaped	Dark brown	8	-	-	-	-	Believed to develop directly/Holopelagic	Jarms et al., 2002
	Nauphanthopsis diomedeae	Fewkes, 1886	-	-	-	-	-	-	-	-	-

Periphyllidae	*Pericopla campana*	Haeckel, 1880	Oval	-	8	-	-	-	-	-	-
	Pericolpa quadrigata	Haeckel, 1880	Oval; elongated	-	4 pairs (8)	-	-	-	-	-	-
	Pericopla tetralina	Haeckel, 1880	Oval; elongated	-	4 pairs (8)	-	-	-	-	-	-
	Periphylla periphylla	Péron & Lesueur, 1810	U-shaped; J-shaped	Whitish	4 pairs (8)	Male and Female	Lucas & Reed, 2010; Tiemman & Jarms, 2010; Presen study	2,5mm; 0,777mm	Gonochoric	Holopelagic	Jarms et al., 1999
	Periphyllopsis braueri	Vanhöffen, 1902	C-shaped	Whitish	4 pairs (8)	-	-	-	-	-	-
	Periphyllopsis galatheae	Kramp, 1959	Horseshoe-shaped	-	4	-	-	-	-	-	-

List of Figures

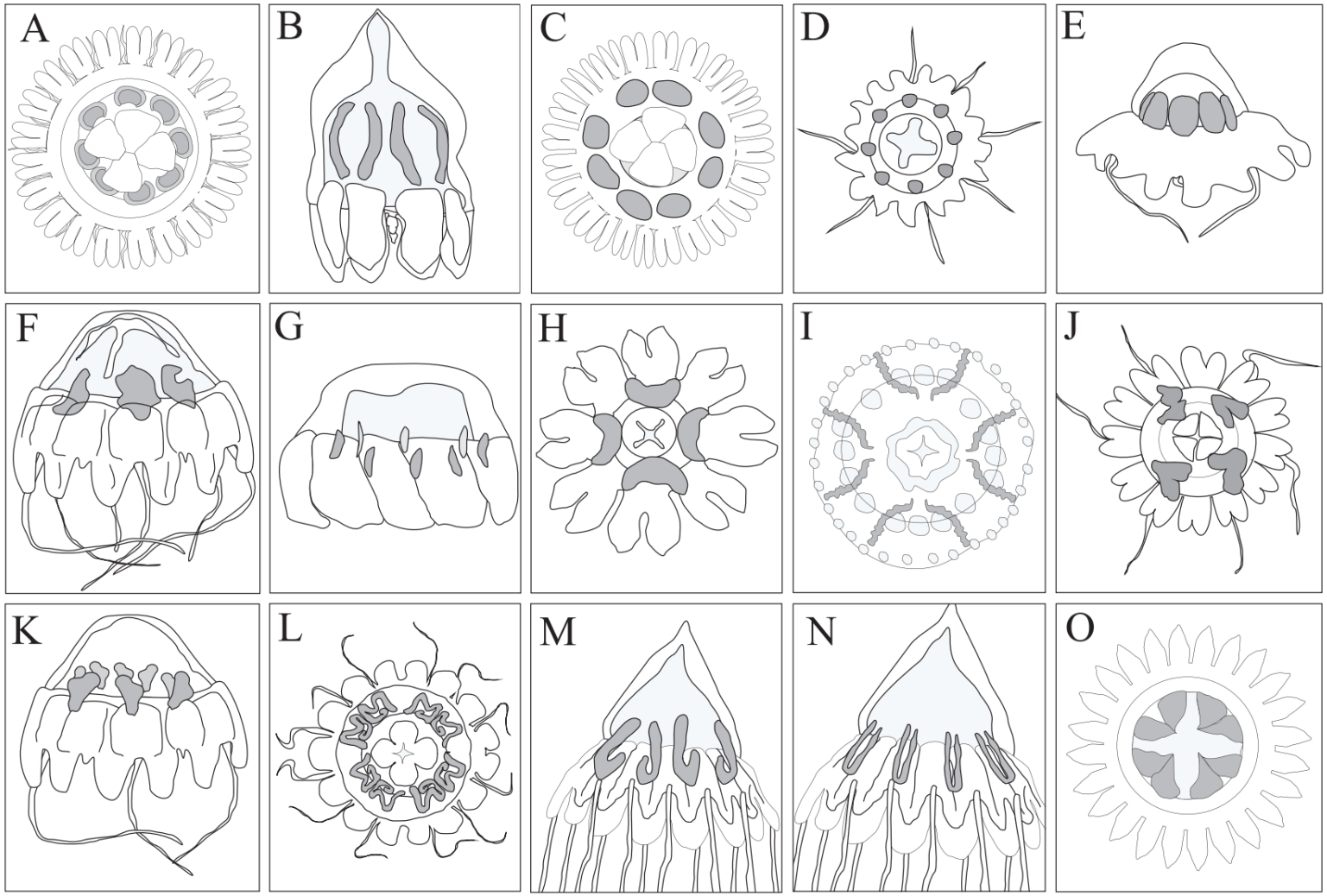

Figure 1: Types of macroscopical gonadal morphology in Coronatae. (A) Bean-shaped. (B) Elongated. (C) Oval. (D) Round. (E) Oblong. (F) Irregular. (G) Fusiform. (H) Crescent-shaped. (I)Horseshoe-shaped. (J) Heart-shaped. (K) Triangular faint. (L) W-shaped. (M) J-shaped. (N) U-shaped. (O) C-shaped.

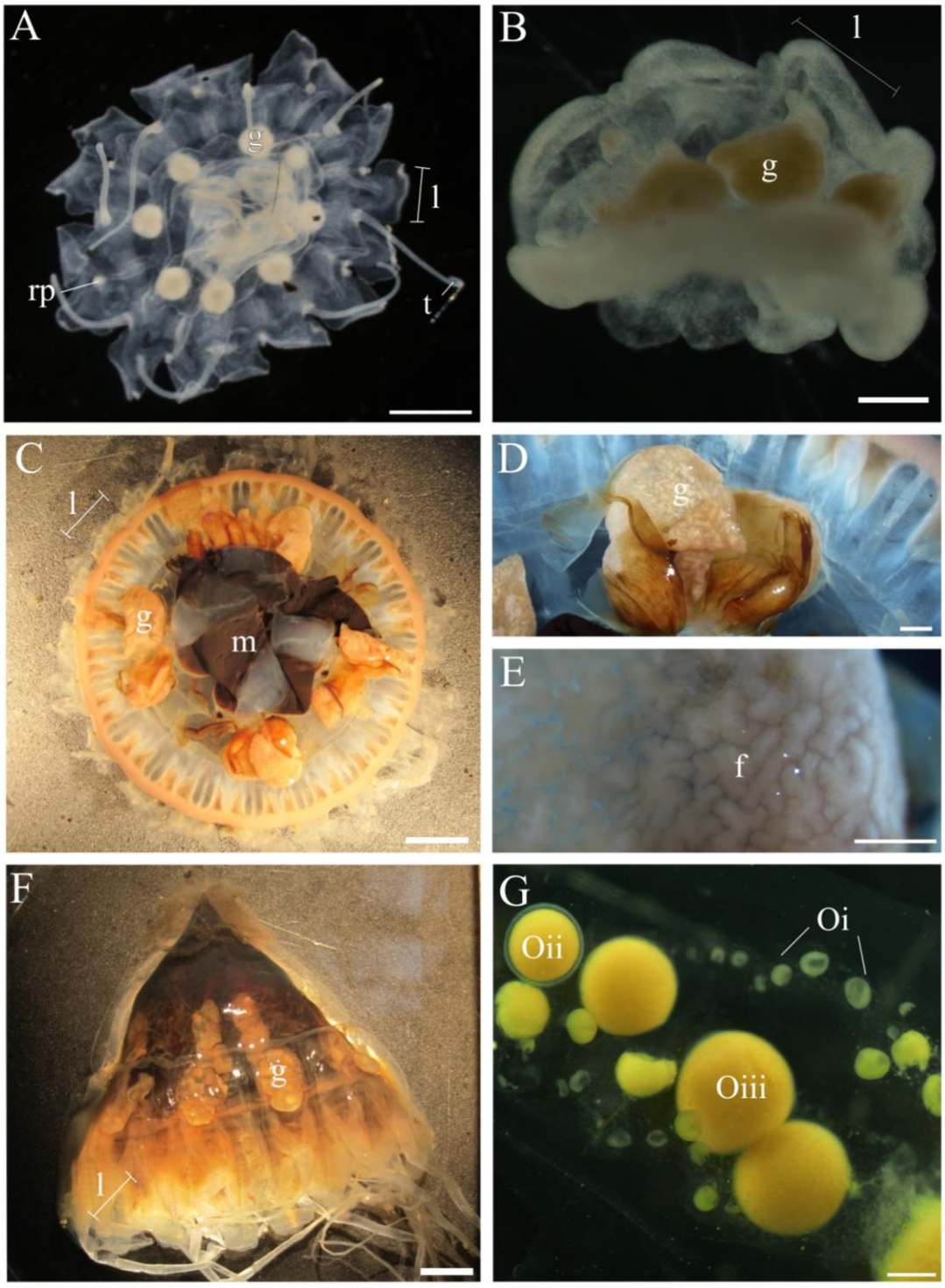

Figure 2: Macroscopical gonadal morphology of Nausithoidae, Atollidae and Periphyllidae representatives. (A) Nausithoe werneri, seen in aboral view, with 8 adradial round gonads, scale: 1mm. (B) The gonads of Nausithoe eumedusoides are usually round but differ from other species to have from 4 to 6 adradial gonads, scale: 0.3mm. (C) Atolla vanhoeffeni, seen in an oral view, with 8 oval yellowish-brown gonads, scale: 5mm. (D) Detail of Atolla vanhoeffeni male gonad, scale: 1mm. (E) The follicles can be seen through a stereomicroscope and a pattern similar to a fingerprint can be noted, scale: 0.5mm. (F) General view of Periphylla periphylla with a J-shaped gonad, scale: 5mm. (G) Female gonad of Periphylla periphylla in detail with early (Oi), intermediate (Oii) and late (Oiii) oocytes, scale: 0,5mm. Follicle = f; gonad = g; lappets = l; manubrium = m; early oocyte = Oi; intermediate oocyte = Oii; late oocyte = Oiii; rhopalium = rp; tentacle = t.

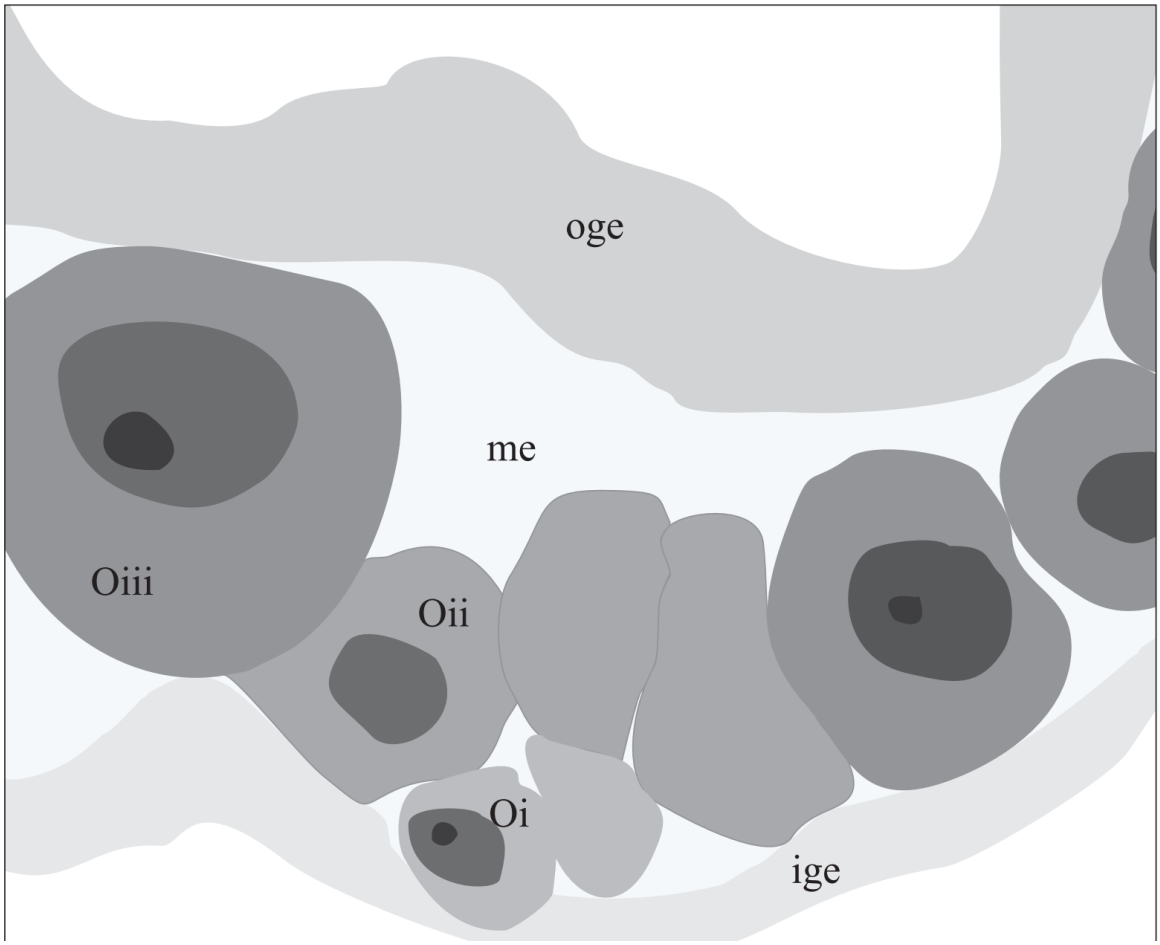

Figure 3: Schematic view of a cross section of Linuche unguiculata female gonad (based on the micrography from Eckelbarger & Larson, 1992). Early oocyte = Oi; inner genital epithelium (ige); mesoglea = me; intermediate oocyte = Oii; late oocyte = Oiii; outer genital epithelium (oge).

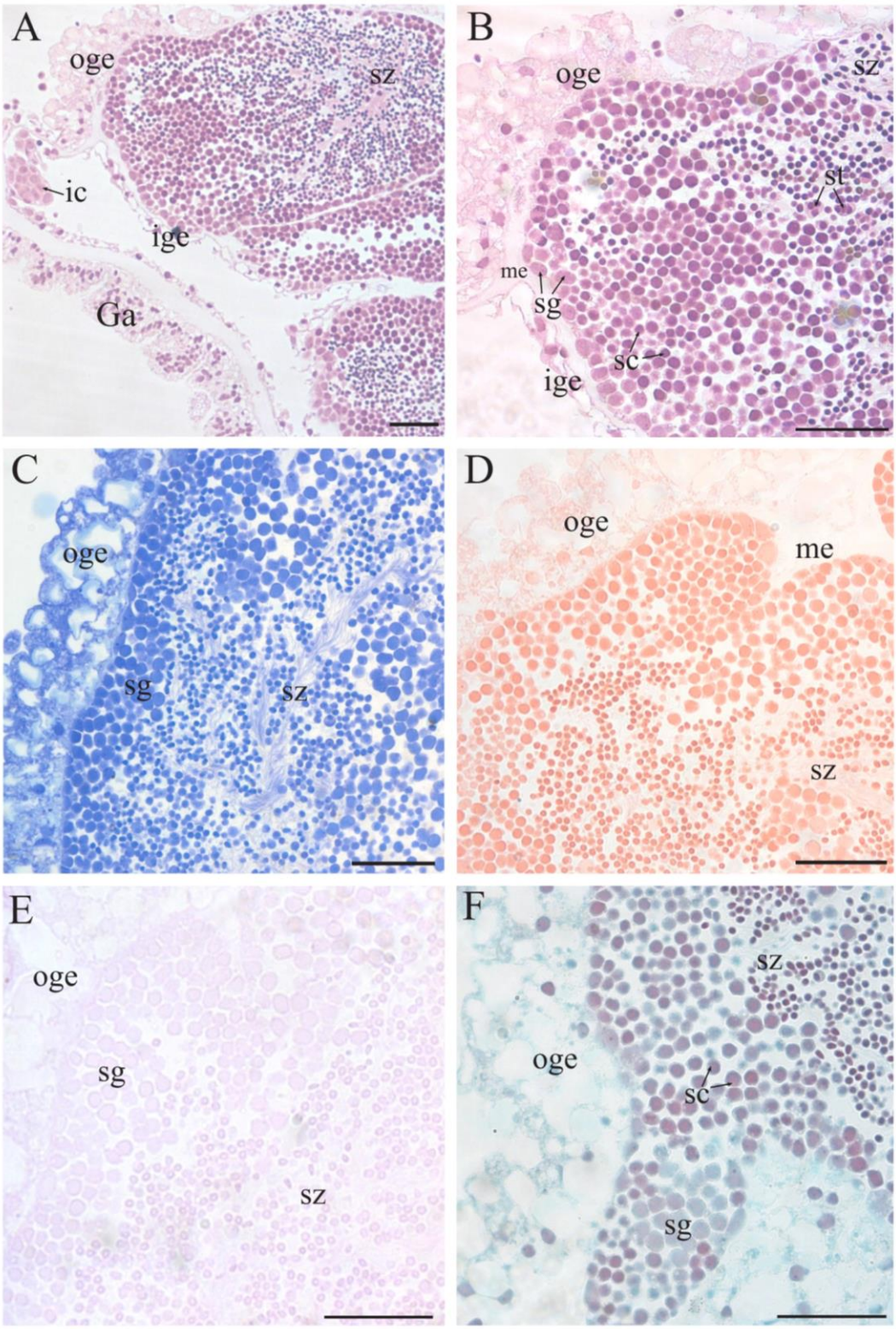

Figure 4: Spermatogenesis in Atolla vanhoeffeni. (A) Hematoxylin & Eosin. Connection of male gonad with gastrodermis. Note the interstitial cells, the outer and genital epithelium, scale: 50 µm. (B) Hematoxylin & Eosin. Detail of the follicle fill with spermatogonia, spermatocyte, spermatid and sperm, scale: 50 µm. (C) Toluidine Blue. Detail of spermatogonia and sperm with nucleus positive to acid structures, scale: 50 µm. (D) Ponceau xylidine. Detail of sperm with flagellum positive to proteins, scale: 50 µm. (E) PAS. Detail of spermatogonia and sperm positive to neutral polysaccharides, scale: 50 µm. (F) Detail of spermatogonia and sperm with nucleus stained in pink and sperm flagellum in blue, scale: 50 µm. Gastrodermis = Ga; inner genital epithelium (ige); interstitial cells = ic; mesoglea = me; outer genital epithelium (oge); spermatid = st; spermatocyte = sc; spermatogonia = sg; sperm =sz.

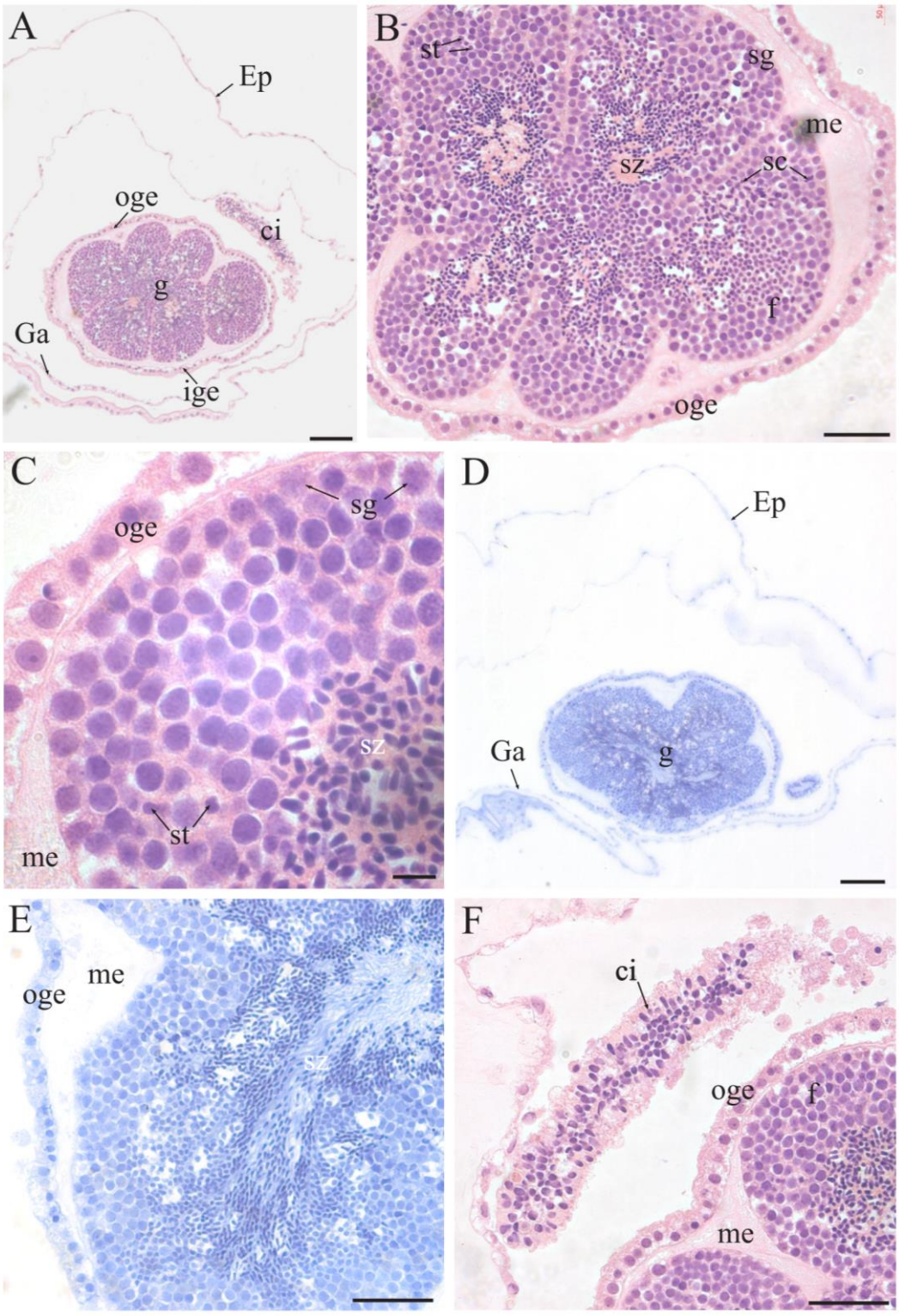

Figure 5: Spermatogenesis in Nausithoe werneri. (A) Hematoxylin & Eosin. Insertion of male gonad inside the gastrovascular cavity seen in aboral-oral section, scale: 100 µm. (B) Hematoxylin & Eosin. Detail of follicles with centripetal organization of germ cells, scale: 50 µm. (C) Hematoxylin & Eosin. Detail of the periphery of follicle with spermatogonia, spermatid and sperm in the center, scale: 10 µm. (D) Mercuric bromophenol blue. Transversal section highlighting the centripetal organization, scale: 100 µm. (E) Mercuric bromophenol blue. Detail of the centripetal organization with sperm flagellum directed to the follicle center, scale: 50 µm. (F) Hematoxylin & Eosin. Detail of gastric filament and the gonad inside the gastrovascular cavity, scale: 50 µm. Epidermis = Ep; follicle = f; gastric filament = ci; gastrodermis = Ga; gonad = g; inner genital epithelium (ige); mesoglea = me; outer genital epithelium (oge); spermatid = st; spermatocyte = sc; spermatogonia = sg; sperm =sz.

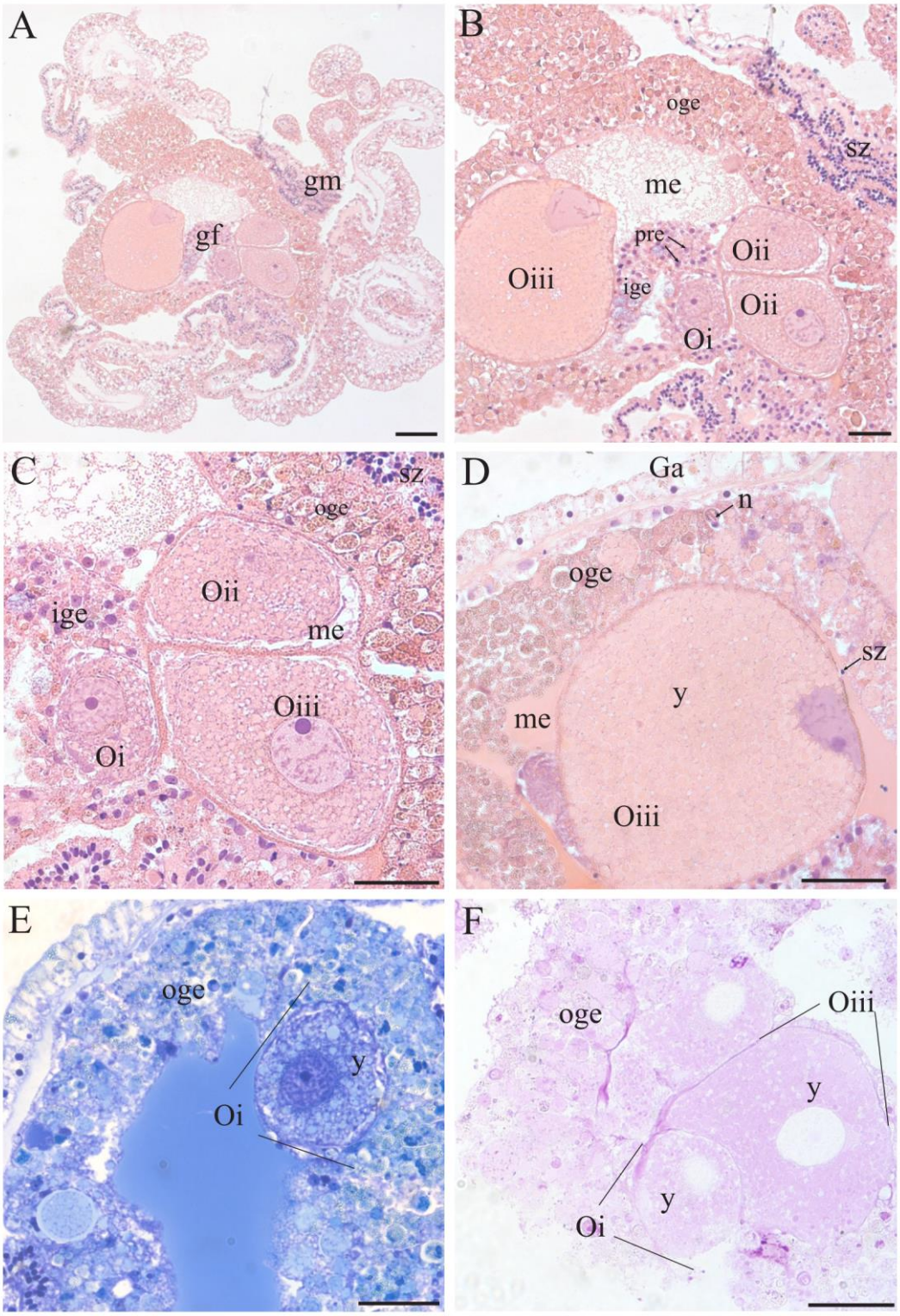

Figure 6: Gametogenesis in Nausithoe eumedusoides. (A) Hematoxylin & Eosin. Cross section of a hermaphrodite medusoid highlighting the female gonad and reduced male gonad, Scale: 100 µm. (B) Hematoxylin & Eosin. Detail of the female gonad with early, intermediate and late oocyte. Note sperm near the outer genital epithelium, scale: 50 µm. (C) Hematoxylin & Eosin. Detail of the central region of female gonad with the three distinct stages of development, scale: 50 µm. (D) Hematoxylin & Eosin. Late oocyte with acidophil cytoplasm, scale: 50 µm. (E) Mercuric bromophenol blue. Early oocyte positive to proteins immersed in the mesoglea, scale: 50 µm. (F) PAS. Intermediate and late oocyte positive to neutral polysaccharides immersed in the mesoglea, scale: 50 µm. Early oocyte = Oi; female gonad =gf; inner genital epithelium (ige); intermediate oocyte = Oii; late oocyte = Oiii; male gonad =gm; mesoglea = me; nematocyst = n; pre-vitellogenic oocyte = pre; outer genital epithelium (oge); sperm = sz; yolk =y.

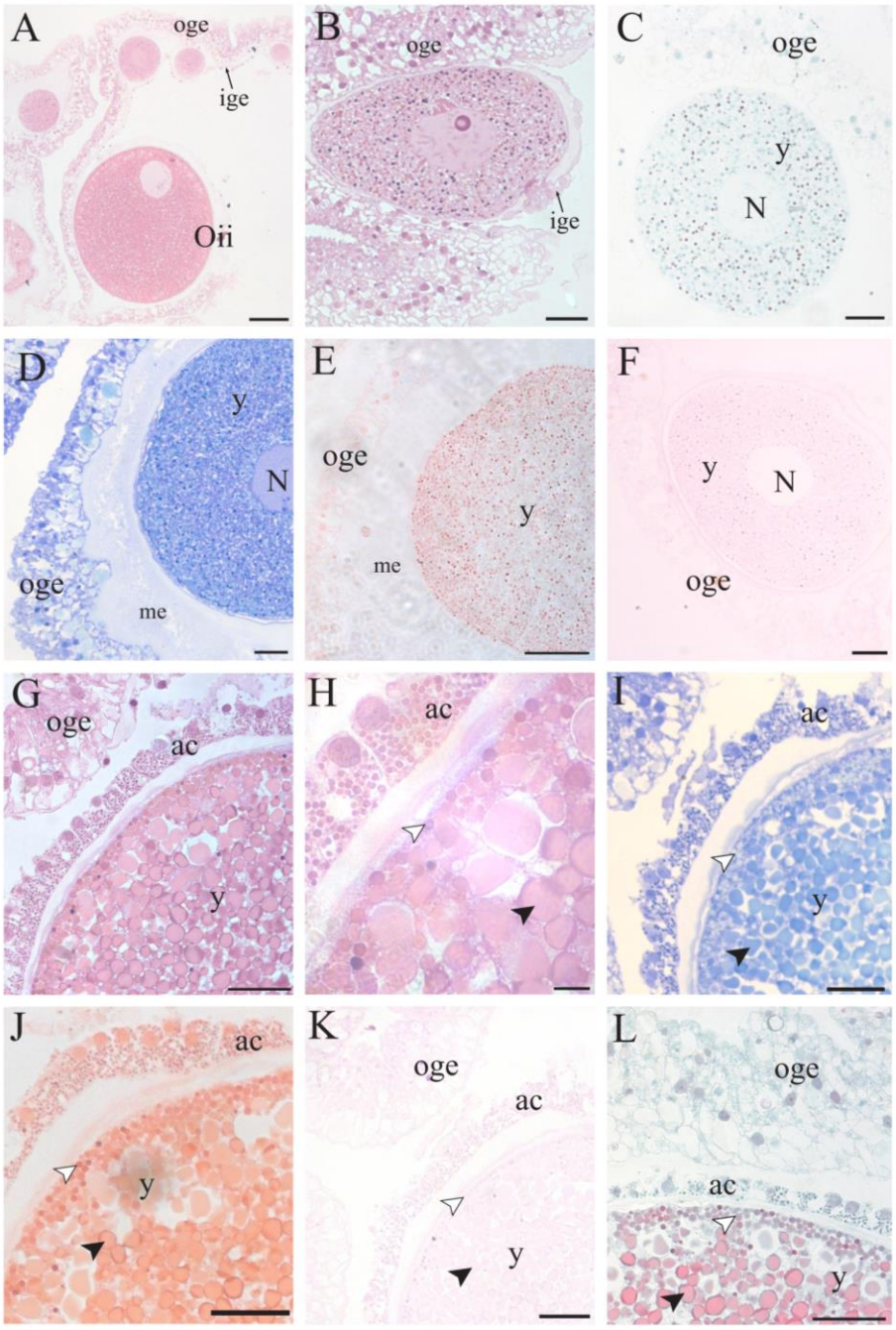

Figure 7: Oogenesis in Periphylla periphylla. (A) Hematoxylin & Eosin. General view of female gonad with oocytes in different stages of development, scale: 200µm. (B) Hematoxylin & Eosin. Early basophil oocyte between the genital epithelium, immersed in the mesoglea, scale: 50 µm. (C) Gomori's trichomic. Intermediate oocyte with two types of granules in the cytoplasm: one stained in blue and another in pink, scale: 50 µm. (D) Toluidine Blue. Intermediate oocyte filled with yolk immersed in the mesoglea, scale: 50 µm. (E) Ponceau xylidine. Intermediate oocyte with yolk positive to proteins, scale: 50 µm. (F) PAS. Intermediate oocyte with yolk positive to neutral polysaccharides, scale: 50 µm. (G) Hematoxylin & Eosin. Acidophil oocyte surrounded by accessory cells, scale: 50 µm. (H) Hematoxylin & Eosin. Detail of previous oocyte with small (white arrowhead) and large (black arrowhead) acidophil granules, scale: 10 µm. (I) Toluidine Blue. The small granules (white arrowhead) positive to acid structures and large granules (black arrowhead) positive to acid structures, scale: 50 µm. (J) Ponceau xylidine. The small granules (white arrowhead) positive to proteins and large granules (black arrowhead) positive to proteins, scale: 50 µm. (K) PAS. The small granules (white arrowhead) positive to neutral polysaccharides and large granules (black arrowhead) positive to neutral polysaccharides, scale: 50 µm. (L) Gomori's trichomic. The small granules (white arrowhead) stained in blue and large granules (black arrowhead) stained in pink, scale: 50 µm. Accessory cell = ac; inner genital epithelium = ige; late oocyte = Oii; mesoglea = me; nucleus = N; outer genital epithelium = oge; yolk = y.

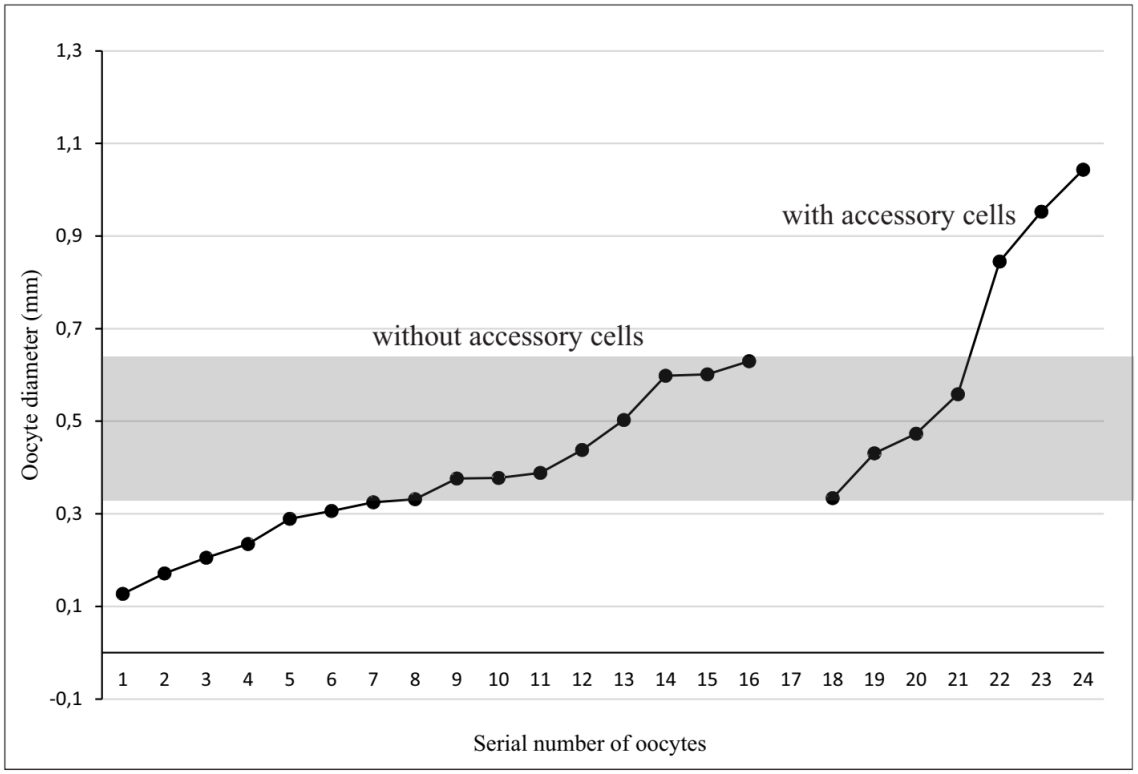

Figure 8: Relation of oocyte diameter and follicle cells in Periphylla periphylla. Oocytes of intermediate and large sizes are surrounded by follicles cells.

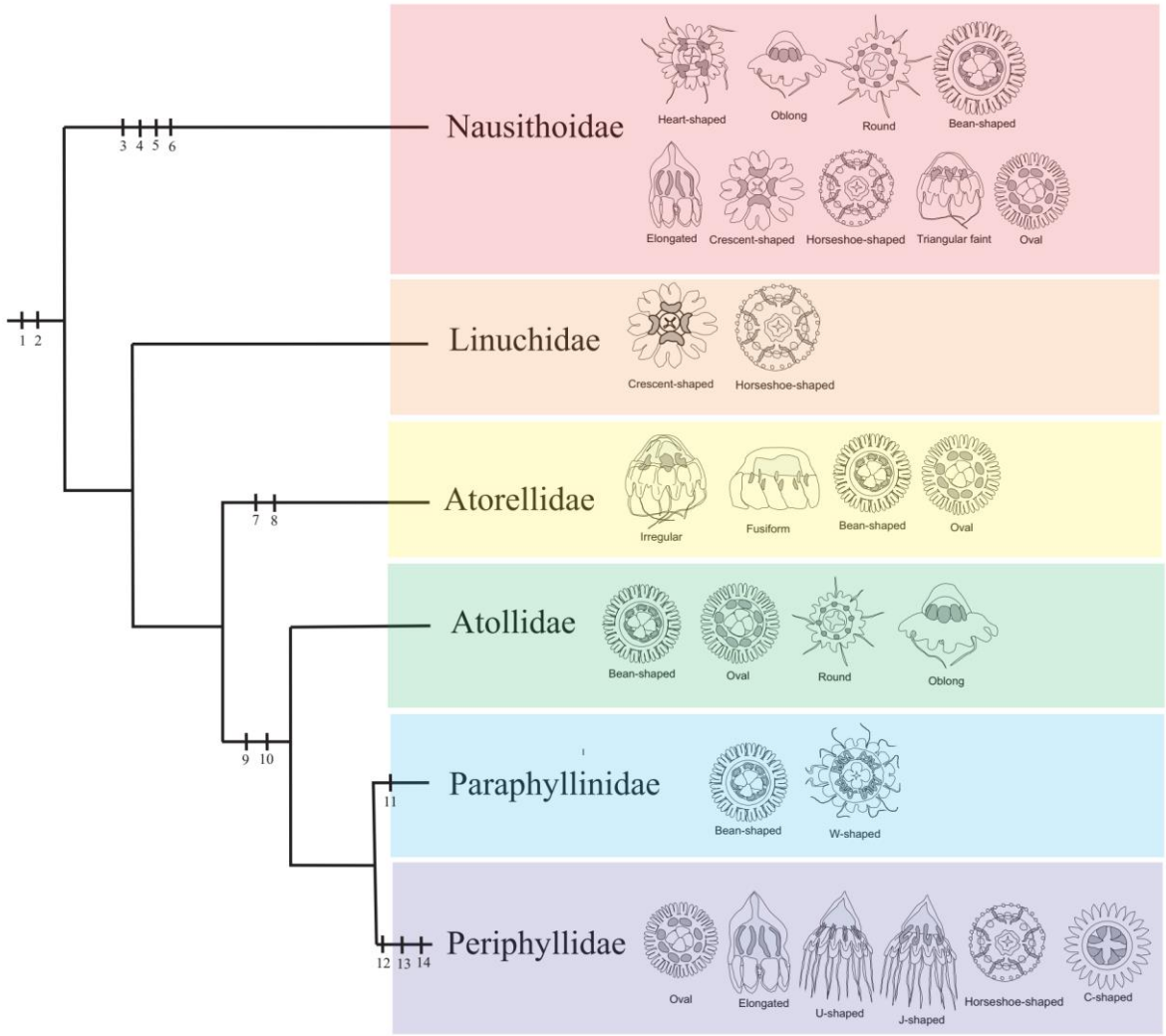

Figure 9: Coronatae gonadal information seen in a phylogenetic framework. (1) Gonochoric. (2) Metagenetic life cycle. (3) Heart-shaped gonad. (4) Triangular-faint gonad. (5) Hermaphroditic. (6) Parthenogenetic. (7) Irregular- shaped gonad. (8) Fusiform gonad. (9) Holopelagic life cycle. (10) direct development; (11) W-shaped gonad. (12) J and U-shaped gonad. (13) Presence of accessory cells around the oocytes. (14) Presence of mucus and pore cells in male and female gonad.

Seminiferous tubule in lower metazoans, insights from scyphozoan Discomedusae (Cnidaria)

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Abstract

Despite several studies focusing on the reproductive biology of Scyphozoa, only a few describe the spermatogenesis and sperm morphology in detail. We described the spermatogenesis of the scyphozoans *Chrysaora lactea*, *Lychnorhiza lucerna* and *Cassiopea maremetens* under light microscopy, histochemistry and ultrastructure, highlighting patterns and differences between sperm production and ultrastructural morphology. The male gonad is composed of a folded epithelium, with three layers: the external genital (outer) epithelium, the mesoglea and the internal genital (inner) epithelium, such organization also observed in other scyphozoans. The spermatogenesis is asynchronous in the three species. The type of sperm transfer is free and by rupture of the follicle wall in *C. lactea* and *L. lucerna* and *spermatozeugmata* in *C. maremetens*. The sperm morphology is also similar to other scyphozoans with small electron-dense vesicles above the nucleus, proximal and distal centrioles, pericentriolar apparatus and four mitochondria but differs mainly in the morphology of the sperm head: ovoid in *L. lucerna*, triangular in *C. lactea* and elongated in *Cassiopea maremetens*. The presence of the constant number of four mitochondria in the sperm midpiece can be considered a synapomorphy of scyphozoans. The sperm ultrastructure allows us to support the clade Rhopaliophora, in which there are always four mitochondria in the midpiece of scyphozoans and six in cubozoans.

Key-words: Reproduction, gonad, gametogenesis, histology, TEM.

1. Introduction

In the Metazoa spermatogenesis is a well-studied process of male germ cell multiplication and differentiation (Staub & Johnson, 2018). It can be divided into two

parts: spermatogenesis and spermiogenesis. During spermatogenesis, germ cells suffer a cycle of several mitotic divisions, leading to meiosis which reduces the chromosome number, then each cell ending in four haploid round spermatids. The spermiogenesis is a complex morphogenetic process that begins with the differentiation of spermatids, progressively, into fully mature spermatozoa to be released into the seminiferous tubules (Cheng & Mruk, 2010; Metz & Monroy, 1985; Staub & Johnson, 2018).

The reproduction in cnidarians is known to be an interesting opportunity for understanding the evolution of sexual and asexual propagation, but as being diverse for the different representatives of the phylum, there is a tendency to generalize some patterns, even with concepts and areas still in need of further investigation (Campbell, 1974; Fautin, 1992). Despite several studies related to reproductive biology of Scyphozoa (Afzelius & Franzén, 1971; Hofmann & Hadfield, 2002; Lucas & Lawes, 1998; Morandini & Silveira, 2001; Rouse & Pitt, 2000), if considering the 222 scyphozoan species diversity (Jarms & Morandini, 2019), there are only a few works describing in detail the spermatogenesis. Sperm production was described for one Coronatae species, three Semaeostomeae and five Rhizostomeae (Ikeda, Ohtsu, & Uye, 2011a; Kikinger, 1992; Lucas & Reed, 2010; Morandini & Silveira, 2001; Schiariti et al., 2012; Tiemann & Jarms, 2010) corresponding to only 4% of scyphozoan species (Table 1).

Most scyphozoans are gonochoric but there are some cases of hermaphroditism – as *C. hysoscella* and *Cassiopea* sp. (Berrill, 1949; Fautin, 1992; Hofmann & Hadfield, 2002; Widersten, 1965). The male gonad originates from the interstitial cells of the gastrodermis, that are transported to the mesoglea and start to develop the follicles (Harrison & Jamieson, 1988; Miller, 1983). In semaeostomes the gonads can be accessed through the subgenital ostia (Morandini & Marques, 2010). They are organized as a semicircle and are highly folded with the gastric filaments at the inner margin (Morandini

& Marques, 2010). In contrast, the gonads in most rhizostomes are described as a cross-shaped structure in which each arm of the cross is a band-like evagination of the gastrodermis forming several folds. The gonads are two-layered in cross-section with a typical genital epithelium (Kikinger, 1992; Schiariti et al., 2012).

In several groups of invertebrates (as annelids, shrimps, crabs and insects) the sperm morphology has been used as a useful character in taxonomic and phylogenetic studies (Bento et al., 2018; Harrison & Jamieson, 1999; Healy et al., 2000; Sampieri et al., 2016; Tudge, 2009). For cnidarians, the ultrastructure of the sperm was studied in the Anthozoa and Hydrozoa (Harrison & Jamieson, 1988) and only in a few scyphozoans. The sperm of Cnidaria consists of a head, midpiece and long flagellum with the absence of the acrosome but the presence of several small electron-dense vesicles above the nucleus (Afzelius & Franzén, 1971; Chapman, 1974; Harrison & Jamieson, 1988; Hinsch & Clark Jr., 1973). The shape of the head is largely determined by the form of the nucleus, which may vary from ovoid to conical or elongated. The midpiece has four or more mitochondria, could have lipid drops, Golgi derivatives, proximal and distal centrioles and the pericentriolar apparatus anchoring the flagellum (Afzelius, 1971; Afzelius & Franzén, 1971; Corbelli et al., 2003; Harrison & Jamieson, 1988; Hinsch & Clark Jr., 1973; Miller, 1983; Rouse & Pitt, 2000).

Cnidarians are divided into two main clades: the subphyla Anthozoa and Medusozoa (Collins, 2002; Collins et al., 2006; Daly et al., 2007) – myxozoans not considered. Even after several years and many studies there are no consensus about the relations between the medusozoans – Scyphozoa, Cubozoa, Staurozoa and Hydrozoa (Collins, 2002; Collins et al., 2006; Marques & Collins, 2004; Miranda et al., 2016). There are also divergences concerning the subgroups of Scyphozoa: Coronatae, Semaeostomeae

and Rhizostomeae (Dawson, 2004; Kayal et al., 2018; Kayal et al., 2013; Marques & Collins, 2004; Thiel, 1966).

In order to provide insights into the relationship between the scyphozoan subgroups, the goals of this study were to describe the histological gonadal structure, spermatogenesis and sperm ultrastructure of some scyphozoan species and compare our results with data from the literature looking for homologous features.

2. Material and Methods

2.1. Sampling

Medusa specimens of *Chrysaora lactea* Eschscholtz, 1829 (Order Semaeostomeae) and *Lychnorhiza lucerna* Haeckel, 1880 (Order Rhizostomeae, Suborder Dactyliophorae) were collected in four localities along São Paulo coast (March 2007 to June 2015) (Table 2). Specimens were collected by hand at the water surface or using artisanal shrimp's trawlers. The animals were transported alive to the laboratory and identified according to Morandini et al. (2005). Polyps of *Cassiopea maremetens* Gershwin, Zeidler & Davie, 2010 (Order Rhizostomeae, Suborder Kolpophorae) were collected at Weipa County, Queensland, Australia and transported alive to the Laboratory at James Cook University in Cairns. They were kept inside in circular aquariums until ephyrae were released. The mature jellyfishes were maintained in tanks with local substrate of the collection site and were fed at least twice a day with *Artemia*.

2.2. Histology and Histochemistry

The specimens were anesthetized by thermal shock (-20°C) for 5 minutes. The male gonad of five individuals of *C. lactea, L. lucerna* and *C. maremetens* was fixed in

4% formaldehyde buffered with a saline solution of 0.2 M sodium phosphate (pH 7.2) for 24 hours. After fixation, the samples were buffered in sodium phosphate 0.2M (pH 7.2) for 24 hours. Then, the samples were dehydrated in ascending ethanol series (30-95%) and embedded in Leica® methacrylate historesin. Serial sections (3-4 μm thick) were made on a microtome. For traditional histological description the tissue samples were stained with hematoxylin and eosin (HE) (modified from Junqueira & Junqueira 1983), avoiding baths in ethanol and xylene (Sant'Anna et al., 2010; Zara et al., 2012). To analyze the chemical composition of the tissues, the slides were stained with mercuric bromophenol blue and ponceau xylidine for proteins (Mello & Vidal, 1980; Pearse, 1960), PAS-Hematoxylin technique to visualize neutral polysaccharides with the groups 1-2-glycol (Junqueira & Junqueira, 1983) and toluidine blue to visualize acid components (modified from Audino et al. 2015).

2.3. Transmission electron microscopy

For transmission electron microscopy (TEM), fragments of $1mm^3$ of the male gonad of *C. lactea* and *L. lucerna* were preserved in Karnovsky solution (modified from Karnovsky, 1965) consisting of 2.5% glutaraldehyde with 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) with 2.5 mM of $CaCl_2$ and sucrose for 24 hours; and samples of the male gonad of *C. maremetens* were fixed in Glutaraldehyde fixative solution consisting of 2.5% glutaraldehyde 0.1 M sodium cacodylate buffered Millipore filtered seawater (pH 7.2-7.4) for 30 minutes at 4ºC. The post-fixation was in 1% osmium tetroxide with the same buffer for one hour at the same temperature. Then, the samples were dehydrated in ascending ethanol series (30 to 100%) and embedded in Spurr's® resin. Semi-thin and ultrathin sections were obtained with a Leica® UC-7 ultramicrotome from the Electron Microscopy Laboratory of the Department of Genetics and

Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil. Grids with sections were contrasted with uranyl acetate and lead citrate, and later examined at a Zeiss Electron Transmission Microscope.

3. Results

3.1. General histological morphology

The general morphology of a scyphozoan male gonad in cross section is shown in Fig. 1. The male gonad composed only of the gastrodermis, but it is divided in three layers: an outer – the external genital cylindrical ciliated layer –, an inner – the internal genital epithelium – and between them lies the mesoglea where the sperm follicles are immersed and develop (Fig. 1). For all three species studied we found cells in different phases of development inside the follicles, highlighting an asynchronous spermatogenesis. We also observed a maturation gradient of cells: from the external to the internal genital epithelium (Figs 2A-C; 4A-B; 6A).

3.2. Spermatogenesis in *Chrysaora lactea*

The external genital epithelium is composed by cells of irregular shape with nucleus in different positions in the cytoplasm (Fig. 2A). The mesoglea is basophil being slightly positive to hematoxylin (Fig. 2B-C). The spermatogonia are in the periphery of the follicles giving rise to the follicular epithelium (Fig. 2C-D). The spermatocytes, with reduced nuclear diameter, have a strongly basophil nucleus (Fig. 2C) in different meiotic phases (Fig. 2D). The spermatids with reduced cytoplasmic volume; have an acidophil flagellum, a basophil nucleus (Fig. 2C), and positive to acid structures (Fig. 2D). The sperm have a rounded basophil head and acidophil flagellum located in the central portion

of the follicle (Fig. 2E-G). The sperm head is strongly positive to acid structures (Fig. 2H), positive to proteins (Fig. 2I), and negative to PAS (Fig. 2J). Follicles in later stages have ovoid shape being filled almost completely by sperm (Fig. 2E). When mature the sperm is released through rupture in the follicle wall (Fig. 2F).

Under TEM the follicular epithelium is composed of spermatogonia and the sperm are found in the center of the follicle (Fig. 3A-B). The spermatogonia have an elongated nucleus with euchromatin and prominent nucleolus (Fig. 3B). Near the spermatogonia, are the spermatocytes in the pachytene meiotic prophases with the synaptonemal complex already formed (Fig. 3C). The spermatids, with reduced cytoplasmic volume, are connected to each other by intercellular bridges (Fig. 3D). The sperm have a triangular head with granular chromatin inside the nucleus (Fig. 3E). Above the nucleus and in the midpiece are observed small electron-dense vesicles (Fig. 3F-G). In the midpiece it is observed 4 mitochondria with several crests (Fig. 3H), between them is the proximal centriole and below the distal centriole (Fig. 3I). The pericentriolar apparatus is a complex structure with primary, secondary, and tertiary processes (Fig. 3J). The flagellum is anchored in the pericentriolar apparatus (Fig. 3K) and presents a simple axoneme with the typical microtubules pattern of 9+2 and dynein arms (Fig. 3L-M). The main observations of the *C. lactea* sperm are summarized in Fig. 4.

3.3. Spermatogenesis in *Lychnorhiza lucerna*

The spermatogenesis starts in the periphery of the follicles (Fig. 5A-C). The spermatogonia are large cells with well-developed rounded nucleus (Fig. 5C). The spermatocytes have a smaller and basophil nucleus (Fig. 5B-C) and it is usual the presence of cells in different meiosis phases (Fig. 5D). The spermiogenesis process begins with the spermatids, characterized by rounded, homogeneous and basophil nucleus (Fig. 5C).

When stained with toluidine blue, the nucleus presents β methacromasy (blue–violet) due to the DNA compaction (Fig. 5D). The sperm have a rounded basophil head with acidophil midpiece and flagellum (Fig. 5E). The rounded nucleus is positive to proteins (Fig. 5F-G), and negative to PAS-Hematoxylin (Fig. 5H). When mature, the sperm are released by rupture of the follicle wall (Fig. 5I).

Under TEM, the external genital epithelium is characterized by cells of irregular shape, with elongated nucleus and evident nucleolus (Fig. 6A). The cytoplasm has many vesicles and in the apical portion can be observed cilia (Fig. 6A-B). Adjacent to this is the follicular epithelium, characterized by the presence of cells of bulky and electron-dense nucleus: the spermatogonia (Fig. 6C). The sperm are clustered in the center of the follicle (Fig.6C-D) and have ovoid electron-dense nucleus (Fig. 6E). Above the nucleus and in the midpiece can be seen little electron-dense vesicles (Fig. 6F-G). In the midpiece can be seen the proximal and distal centrioles (Fig. 6H). From the distal centriole is the pericentriolar apparatus (Fig. 6I-K). Above the pericentriolar apparatus are the Golgi complex (Fig. 6L) and four mitochondria (Fig. 6M). The flagellum presents a simple axoneme with the typical microtubules pattern 9+2 andthe dynein arms (Fig. 6O). The main observations of the *L lucerna* sperm are summarized in Fig. 7.

3.4. Spermatogenesis in *Cassiopea maremetens*

The gonad is a differentiation of the gastrodermis epithelium (Fig. 8A). Below the inner genital epithelium is the genital sinus in which the sperm – clustered by a secretion – are released (Fig 8A-B). Nearby to this chamber is the gastrodermis and bordering it is the cellular mesoglea with several zooxanthellae (Fig. 8A-B). The external genital (outer) layer is a simple columnar epithelium composed of cells juxtaposed to each other, with rounded basophil nucleus and acidophil cytoplasm with large vesicles. These vesicles

show no reaction to hematoxylin and eosin (Fig. 8C). The follicular epithelium is composed of spermatogonia that sometimes are organized in centers (Fig. 8C-E). The spermatocytes have a smaller and basophil nucleus (Fig 8A-C). The spermatids are in the center of the follicle and are surrounded by an acidophil secretion (Fig. 8C). The sperm is positioned near the internal genital epithelium and clustered by a secretion not stained by the hematoxylin and eosin (Fig. 8C). This secretion shows no reaction to acid compounds (Fig. 8D), proteins (Fig. 8E) and neutral polysaccharides (Fig. 8F). However, the sperm present strong reactivity to acid compounds (Fig. 8D), positive reaction to proteins (Fig. 8E) and negative reaction to PAS (Fig. 8F). When mature, all sperm are clustered by this secretion, with the sperm heads organized centripetally and the flagellae radiating outward (Fig 8F). The whole structure, called *spermatozeugmata*, is released in the genital sinus located between the genital and gastrodermal epithelium (Fig. 8F). The secretion of the *spermatozeugmata* is characterized by being non reactive to any of the tested histochemical compounds (Fig. 8G-J).

The follicular epithelium is composed by spermatogonia and the spermatids are immersed in a granular secretion (Fig. 9A-B). The sperm of *C. maremetens* are clustered near the genital epithelium (Fig. 9C) and have an elongated head with electron-dense nucleus (Fig. 9D). In the midpiece it is observed four mitochondria with several crests, the distal (Fig. 9E-H) and proximal centrioles (Fig. 9F), little vesicles adjacent to the mitochondria (Fig. 9F-G) and lamellas (Fig. 9G). The flagellum is anchored in the pericentriolar apparatus (Fig. 9G) and presents a simple axoneme with the typical microtubules pattern of 9+2 (Fig. 9I-J). The main observations of the *Cassiopea maremetens* sperm are summarized in Fig.10.

4. Discussion

The general histological morphology of the male gonad in cross section is like that already described for other scyphozoans like the semaeostomes *Cyanea capilatta*, *Aurelia aurita*, *Chrysaora hysoscella* and for the rhizostome *Rhizostoma pulmo* (Widersten, 1965). Here, we chose not to use the original histologic nomenclature proposed by Widersten (1965) – endodermal (external) layer, mesoglea, and genital (internal) epithelium – because the term "endoderm" is usually used to refer to the embryological internal layer (*sensu* Hyman 1940). Other terms were also used to assign these same structures, as "gastric epithelium" (Kikinger, 1992) and "gastrodermis" (Lucas & Reed, 2010; Morandini & Silveira, 2001; Schiariti et al., 2012). But we decided to use an adaptation of the terms applied by Widersten (1965) as: "outer genital epithelium" – to refer to the upper layer of the gonad – and "inner genital epithelium" – to refer to the lower layer of the gonad (as seen on Fig. 1) – to distinguish both epithelial types like Widersten (1965) suggested. The differential terminology is justified by the presence of cells with different shapes and sizes that probably have different functions, as different groups of cells perform specialized and specific functions (Alberts et al., 2014). When referring to the scyphozoan reproductive tissue, we suggest that the term "gastrodermis" or something related should be avoided.

Spermatogenesis of *C. lactea, L. lucerna* and *C. maremetens* are like other descriptions available for other Discomedusae as *Cyanea capillatta*, *Chrysaora hysoscella*, *Aurelia aurita*, *Catostylus mosaicus* and *Nemopilema nomurai* (Ikeda et al., 2011a; Ohtsu et al., 2007; Pitt & Kingsford, 2000; Widersten, 1965) and Coronamedusae as *N. maculata* (Morandini & Silveira, 2001) and *Peryphylla peryphylla* (Tiemann & Jarms, 2010). For scyphozoan species, the motile sperm could be released by pit, pore or by rupture of follicle epithelium (See Table 3). Even with several studies describing the spermatogenesis process in Scyphozoa, this is the first approach of a complete descriptive

histological, histochemical and ultrastructural analysis of each cell type, and discussed in an evolutionary perspective.

Spermatogenesis in metazoans is a conservative process (Cheng & Mruk, 2010; Giménez, 2013; Grier, Linton, Leatherland, & De Vlaming, 1980; Griswold, 2016; Ilacqua, Francomano, & Aversa, 2018; Leblond & Clermont, 1952; Roosen-Runge & Szollosi, 1965; Sathananthan, 2013; Schulz et al., 2010; Staub & Johnson, 2018; White-Cooper & Bausek, 2010). Thus, it is expected that jellyfishes produce male gametes in an effective way within the gonads (*sensu* Campbell, 1974), even in the absence of systems and organs. In metazoans the testis has two main important functions: to produce (1) germ cells and (2) sexual hormones (androgens). To complete both functions, the testis has two morphologically compartments: i. the exocrine tubular germinative compartment: the seminiferous tubules and ii. the endocrine interstitial compartment: with the Leydig cells, connective tissue and other cellular types found between the seminiferous tubule (Ilacqua et al., 2018; Staub & Johnson, 2018). In mammals, fishes and arthropods there are many studies describing and classifying the testis, its morphology and cell arrangement (Grier et al., 1980; Schulz et al., 2010; Simeó et al., 2009; Staub & Johnson, 2018; Zara et al., 2012). The germinative compartment have germ cells that are in close and continuous interaction with the Sertoli cells (França et al., 2015): a specific type of somatic cell responsible for supporting the germinal epithelium and helping the germ cells to develop and migrate towards the lumen of the tubule (Ilacqua et al., 2018). The Sertoli cell, under light microscopy, can be identified by the presence of a round nucleus in the basal portion of the cell; and under electronic microscopy by (1) the deep indentations or clefts in the nucleus membrane, and (2) the distinctly large and tripartite nucleoli (França et al., 2016). Regarding the three species studied herein and data from the literature only the germinative tubular compartment is observed. The sperm formation has a progressive

organization inside the follicle: spermatogonia in the periphery near the external genital epithelium followed by the spermatocytes, spermatids, and sperm clustered near the internal genital epithelial to be released in the genital sinus (Gohar & Eisawy, 1960; Ikeda et al., 2011a; Kikinger, 1992; Morandini & Silveira, 2001; Pitt & Kingsford, 2000; Smith, 1936; Tiemann & Jarms, 2010; Widersten, 1965). Such organization resembles a seminiferous tubule. This organization was described in *Cassiopea andromeda* and *C. frondosa* (Gohar & Eisawy, 1960; Smith, 1936), and also in *Cotylorhiza tuberculata* (Kikinger, 1992) and is described here to *Cassiopea maremetens*. We hypothesize that the progressive spermatogenesis inside the follicles of jellyfish is similar to the germ cells arrangement in the tubular testis described for Grapsoidea crabs as *Pachygrapsus transversus*, *Pachygrapsus gracilis* (Tiseo, Zara, & Mantelatto, 2014) and *Maja brachydactyla* (Simeó et al., 2009). In these crabs the germ cells are organized in three zones with histological differentiation: (1) germinal zone with spermatogonia located in the pole of the seminiferous tubule; (2) transformation zone with spermatocytes and spermatids in the center of the tube and (3) evacuation zone with sperm released in the tubule lumen. There are still some unanswered questions about the male gonad organization in scyphozoans: (1) are there Sertoli-like cells, perhaps with a different morphology? (2) As the genital epithelium and mesoglea are performing the function of support of germ cells, can they be responsible to execute the function of a Sertoli cell? And (3) once that there are no connective tissue supporting the male gonad, can the genital epithelium be considered a “basal” seminiferous tubule?

In general, Discomedusae seems to have an asynchronous gametogenesis because gametes in different stages are seen at the same time in male gonad (Avian & Rottini-Sandrini, 1991; Iguchi et al., 2010; Lucas & Lawes, 1998; Lucas & Reed, 2010; Pitt & Kingsford, 2000; Schiariti et al., 2008). This same asynchrony is described for *Chrysaora*

lactea, *L. lucerna*, and *Cassiopea maremetens*. For *Cassiopea* spp. and *Cotylorhiza tuberculata*, this asynchrony could be an adaptative feature that improve the production of *spermatozeugmata* as Kikinger (1992) suggested: that the release of the clustered sperm may increase the fertilization in *Co. tuberculata*, which have hundreds of spermatozoa entering the female gastrovascular system with each sperm clump, resulting in simultaneous fertilization of many eggs.

If we consider the type of sperm release, brooding and type of fertilization, we can state that species with external fertilization release the sperm freely and do not have any parental care (*i.e.*, brooding). In contrast, species with sperm released in packages (*i.e., spermatozeugamata*), usually have internal fertilization and brood care (Table 3). But there are always exceptions: *Aurelia aurita* and *Cyanea capilatta* both described by Widersten (1965), which have brooding but sperm freely released. In *L. lucerna* and *C. lactea*, observed here, the sperm release occurs through rupture of the follicular wall with absence of brooding – like in *Na. maculata* and *Ne. nomurai* (Morandini & Silveira 2001; Ikeda et al. 2011). The transfer of sperm through *spermartozeugmata* associated with brooding was restricted only to some rhizostome species (Cassiopeidae, Cepheidae, Mastigiidae) of the suborder Kolpophorae (Table 3). *Spermatozeugmata* was already described for *Cassiopea frondosa* and *Cassiopea andromeda*, but not properly called *spermatozeugmata* (Gohar and Eisawy, 1960; Smith, 1936). This type of sperm transfer is also observed in the reproduction of *Cotylorhiza tuberculata* (Kikinger, 1992). A probable relation between the type of sperm release and brood care was described by Foighil (1989) who reported that the *spermatozeugmata* of the oyster *Ostrea edulis* only work as an efficient sperm transfer mechanism if eggs are retained within the benthic female and if egg masses are at intermediate distances (from 10 cm to 1-2 meters) from spawning males. Among the jellyfish groups there are no suggestions about the

advantages of brooding and the presence of *spermatozeugmata*. But new studies with *Cassiopea* species can try to refute or confirm the hypothesis proposed by Foighil.

In the TEM preparations, the spermatogonia are large cells, with well-developed nucleus and nucleolus. The spermatocytes are round in shape and the spermatids can be united by intercellular bridges. The shape of the spermatogenic cells found in this study is typical and similar to those described for other scyphozoan *Ne. nomurai* (Ikeda et al. 2011) and the hydrozoan *Distichopora* sp. (Gaino et al., 2013). The cnidarian sperm do not possess an acrosome but instead present several electron-dense small vesicles (Harrison & Jamieson, 1988; Miller, 1983) as observed herein to the threes species estudied. Hinsch & Clarck Jr. (1973) found such vesicles in *A. aurita* and suggested that they can be the precursor of the acrosome. Small vesicles were described in the midpiece of *Cassiopea maremetens* sperm as well as in the cubozoans *Carybdea marsupialis* (Corbelli et al., 2003) and *Copula sivickisi* (Garm et al., 2015). The sperm of the three species studied have an electron-dense nucleus, with granular chromatin, but only *Chrysaora lactea* show the nuclear vesicle already described for *Hydra cauliculata* (Moore & Dixon, 1972). The number of mitochondria in the midpiece of the sperm is typically four – which is a feature of scyphozoans. And the typical flagellum pattern (with 9+2 microtubules) with dynein arms observed in the species studies agrees in arrangement with all motile metazoans like mammals (Sathananthan, 2013), fishes (Schulz et al., 2010), bivalves (Introíni et al., 2013) and in the euspermatozoa of gastropods (Giménez, 2013). The anchoring apparatus when seen in cross section had the primary, secondary and tertiary processes clearly distinct and this seems to be the pattern observed in other Rhopaliophora (Corbelli et al., 2003; Hedwig & Schafer, 1986; Helmark & Garm, 2019; Hinsch & Clark Jr., 1973; Hinsch, 1974; Ikeda et al., 2011a). The main morphology of the sperm is similar to that described for *A. aurita* (Hinsch & Clark Jr., 1973) and

Nausithoe sp. (Afzelius & Franzén, 1971). Among scyphozoans, the morphology of the sperm head shows some variation in shape: ovoid (*Nausithoe* sp., *C. hysoscella* and *L. lucerna*); triangular (*C. lactea*); and elongated (*A. aurita*, *R. pulmo* and *Ca. maremetens*). Although interesting, this feature shows no pattern and has no value to distinguish the different scyphozoan orders. Since less than 4% of the scyphomedusae (*sensu* Jarms & Morandini, 2019) was studied concerning the sperm and spermiogenesis, any generalizations seem to be premature and more detailed studies are needed in order to make more accurate evolutionary inferences.

Conclusions

Only a single pattern of gonadal conformation is found at the histological level in scyphozoans, in which two tissue sheets can be recognized: an outer and inner genital epithelium with the mesoglea between them.

The organization of the germ cells inside the follicle (with spermatogonia near the outer genital epithelium and sperm near inner genital epithelium) resembles the seminiferous tubule of Grapsoidea crab's tubular testis.

Sperm release through packages (*spermatozeugmata*) is only found in species with internal fertilization (inside female gonad or in the genital sinus) and brooding (restricted to the group Kolpophorae); while sperm freely released in the water column is found in species with external fertilization.

The sperm ultrastructure shows no pattern that help us distinguish different subgroups among scyphozoans. However, the number of mitochondria is different between scyphozoans (4 mitochondria) and cubozoans (6 mitochondria) and is stated as synapomorphies of these groups.

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Tables

Table 1: Scyphozoan species with gametogenesis and sperm ultrastructure morphology already described.

Order	Species	Spermatogenesis	Oogenesis	References	Sperm morphology	References
Coronatae	*Atolla wyvillei*	✓	✓	Lucas & Reed 2010	-	-
	Linuche unguiculata	-	✓	Eckelbarger & Larson 1992	✓	-
	Nausithoe sp.	-	-	-	✓	Afzelius & Franzen 1974
	Nausithoe maculata	✓	✓	Morandini & Silveira 2001	-	-
	Periphylla periphylla	✓	✓	Tiemann & Jarms 2010; Lucas & Reed 2010	-	-
Semaeostomeae	*Cyanea capillata*	✓	✓	Widersten 1965	-	-
	Chrysaora hysoscella	✓	✓	Widersten 1965	✓	Hedwig & Schafer 1986
	Pelagia noctiluca	-	✓	Avian & Rotttini-Sandrini 1991	✓	-
	Aurelia aurita	✓	✓	Widersten 1965; Eckelbarger & Larson 1988	✓	Hedwig & Schafer 1986; Hinsch 1974
Rhizostomeae	*Cassiopea* sp.	✓	✓	Hofmann & Hadfield 2002	-	-
	Cassiopea andromeda	✓	✓	Gohar & Eisawy 1960	-	-

Cassiopea frondosa	✓	✓	Smith 1936	-	-
Lychnorhiza lucerna	✓	✓	Schiariti et al. 2012	-	-
Catostylus mosaicus	✓	✓	Pitt & Kingsford 2000	-	-
Cotylorhiza tuberculata	✓	✓	Kikinger 1992	-	-
Rhizostoma pulmo	-	✓	Widersten 1965	✓	Hedwig & Schafer 1986
Nemopileama nomurai	✓	✓	Ohtsu et al. 2007; Ikeda et al. 2011	-	-
Stomolophus meleagris	-	✓	Eckelbarger & Larson 1992	-	-

Table 2: Species per sites of collection and their respective coordinates.

Country	Collection site	Coordinates	Species/ Collection site
Brazil	Cananéia county	25°023’955”S/ 47°91’8539”E	*Lychnorhiza lucerna*
Brazil	Santos and São Vicente Bay	23°59’33.9”S/46°22’12.64”E	*L. lucerna; Chrysaora lactea*
Brazil	São Sebastião county	23°27’5”S/45°1’47”E	*L. lucerna; C. lactea*
Brazil	Ubatuba county	23°31’147”S/ 045°05’193”E	*L. lucerna*
Australia	Weipa county	12°38’57.38”S 141°50’48.66”E	*Cassiopea maremetens*

Table 3: Reproductive strategies among members of the class Scyphozoa.

Class	Order	Family	Specie	Sperm release	Fertilization	Brooding	Reference
Scyphozoa	Coronatae	Nausithoidae	*Nausithoe maculata*	Free sperm	External	Absent	Morandini & Silveira, 2001
		Periphylidae	*Periphylla periphylla*	Free sperm	-	-	Lucas & Reed, 2010
		Atollidae	*Atolla wyvillei*	Free sperm	-	-	Lucas & Reed, 2010
	Semaeostomeae	Ulmaridae	*Aurelia aurita*	Free sperm	Internal	Present	Widersten, 1965
		Cyaneidae	*Cyanea capillata*	Free sperm	Internal	Present	Widersten, 1965
		Pelagiidae	*Chrysaora hysoscella*	-	-	Present	Widersten, 1965
			Chrysaora lactea	Free sperm	-	Absent	This study
	Rhizostomeae	Lychnorhizidae	*Lychnorhiza lucerna*	Free sperm	External	Absent	Schiariti et al., 2012; This study
		Rhizostomatidae	*Rhizostoma pulmo*	Free sperm	-	Absent	Paspaleff, 1938; Widersten, 1965
		Stomolophidae	*Nemopilema nomurai*	Free sperm	External	Absent	Ikeda et al., 2011
		Mastigiidae	*Mastigias papua*	Spermatozeugmata	-	Present	Uchida, 1926
		Cassiopeidae	*Cassiopea andromeda*	Spermatozeugmata	-	Present	Hoffmann & Hadfield, 2002
			Cassiopea maremetens.	Spermatozeugmata	-	-	This study

		Cassiopea frondosa	Spermatozeugmata	-	Present	Smith, 1936
	Cepheidae	*Cotylorhiza tuberculata*	Spermatozeugmata	Internal	Present	Kikinger, 1992
	Mastigiidae	*Phyllorhiza punctata*	Spermatozeugmata	-	Present	Rouse & Pitt, 2000
	Catostylidae	*Catostylus mosaicus*	Free sperm	-	Present	Rouse & Pitt, 2000

Classification according to Kramp (1961). - = no data.

Table 4: Comparison between the sperm morphology of the studied species (*Lychnorhiza lucerna*, *Chrysaora lactea* and *Cassiopea maremetens*) with other Scyphozoa and Cubozoa.

	Scyphozoa							**Cubozoa**		
	Coronatae	**Semaeostomeae**			**Rhizostomeae**			**Carybdeida**		
Characteristics	***Nausithoe* sp. [1]**	***Chrysaora lactea* [2]**	***Chrysaora hysoscella* [3]**	***Aurelia aurita* [3;4]**	***Lychnorhiza lucerna* [2]**	***Rhizostoma pulmo* [3]**	***Cassiopea maremetens* [2]**	***Carybdea marsupialis* [5]**	***Copula sivickisi* [6]**	***Tripedalia cystophora* [7]**
Head length	-	2.17 ± 0.61	-	-	2.01 ± 0.41	-	1.9 ± 0.15	3.84 ± 0,34	-	-
Head diameter	-	1.89 ± 0.57	-	-	2.05 ± 0.61	-	1.59 ± 0.29	-	-	-
Midpiece length	-	0.95 ± 0.25	-	-	0.92 ± 0.26	-	0.75 ± 0.11	1.1	-	-
Midpiece diameter	-	2.32 ± 0.75	-	-	2.40 ± 0.54	-	1.35 ± 0.19	2.41 ± 0.36	-	-
Nucleus length	-	1.88 ± 0.59	-	-	1.9 ± 0.35	-	1.76 ± 0.17	2.83 ± 0,27	-	-
Nucleus diameter	-	1.61 ± 0.43	-	-	1.6 ± 0.49	-	1.31 ± 0.21	1.84 ± 0.27	-	-
Head morphology	Ovoid	Ovoid	Ovoid	Elongated	Ovoid	Elongated	Elongated	Ovoid	Elongated*	Elongated*
Nucleus ratio	-	1.22 ± 0.45	-	-	1.29 ± 0.52	-	1.4 ± 0.29	1,54	-	-
Nuclear vesicle	Present	Present	Not observed	Absent	Absent	Not observed	Absent	Absent	-	-
Chromatin	Granular	Granular	Granular	Granular	Granular	Granular	Granular	Granular	Granular*	Granular*
Outer nuclear membrane	Absent	Absent	Absent	Absent	Absent	Not observed	Present	Absent	Absent*	Absent*
Number of Mitochondria	4	4	4	4	4	Not observed	4	6	6	-
Vesicles anteriorly to the nucleus	Present	Present	Not observed	Present	Present	Present	Not observed	Present	Present	-
Vesicle's diameter	-	0.12 ± 0.04	-	-	0.16 ± 0.02	-	-	-	100 nm	-
Electrondense vesicle lateral to the nucleus	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent	Absent
Proximal centriole	Present	Present	Present	Not observed	Present	Present	Present	Present	-	Present*
Distal centriole	Present	Present	Present	Present	Present	Present	Present	Present	-	Present*
Pericentriolar apparatus	Present	Present	Present	Present	Present	Present	Present	Present	-	Present*

Primary process	Present	Present	Not observed	Present	Present	Not observed	Not observed	Present	-	Present*
Interprimary process	Present	Present	Not observed	Present	Present	Not observed	Not observed	Present	-	-
Major striated bands	Present	Present	Not observed	Present	Present	Not observed	Not observed	Present	-	-
Secondary process	Present	Present	Not observed	Not observed	Present	Not observed	Not observed	Present	-	Present*
Tertiary Process	Present	Present	Not observed	Not observed	Present	Not observed	Not observed	Present	-	-
Spurr	Absent	Not observed	Absent	Not observed	Not observed	Not observed	Not observed	Present	-	-
Lamellas	Absent	Not observed	Not observed	Absent	Not observed	Not observed	Not observed	Absent	-	-
Poli-ribosomes	Absent	Present	Not observed	Absent	Present	Not observed	Present	Absent	-	-
Golgi complex	Absent	Absent	Not observed	Absent	Present	Not observed	Present	Absent	Present	Present*
Vesicles in the midpiece	Present	Present	Not observed	Absent	Present	Not observed	Present	Present	Present*	-
Structure of the flagellum	9+2	9 + 2	9+2	9+2	9 + 2	9 + 2	9 + 2	9 + 2	9 + 2*	9 + 2*

[1] Afzelius & Franzen (1974); [2] This study; [3] Hedwig & Schafer (1986); [4] Hinsch (1974); [5] Corbelli et al. (2003); [6] Garm et al. (2015), [7] Helmark & Garm (2019). * = Not described, but observed in the published figure.

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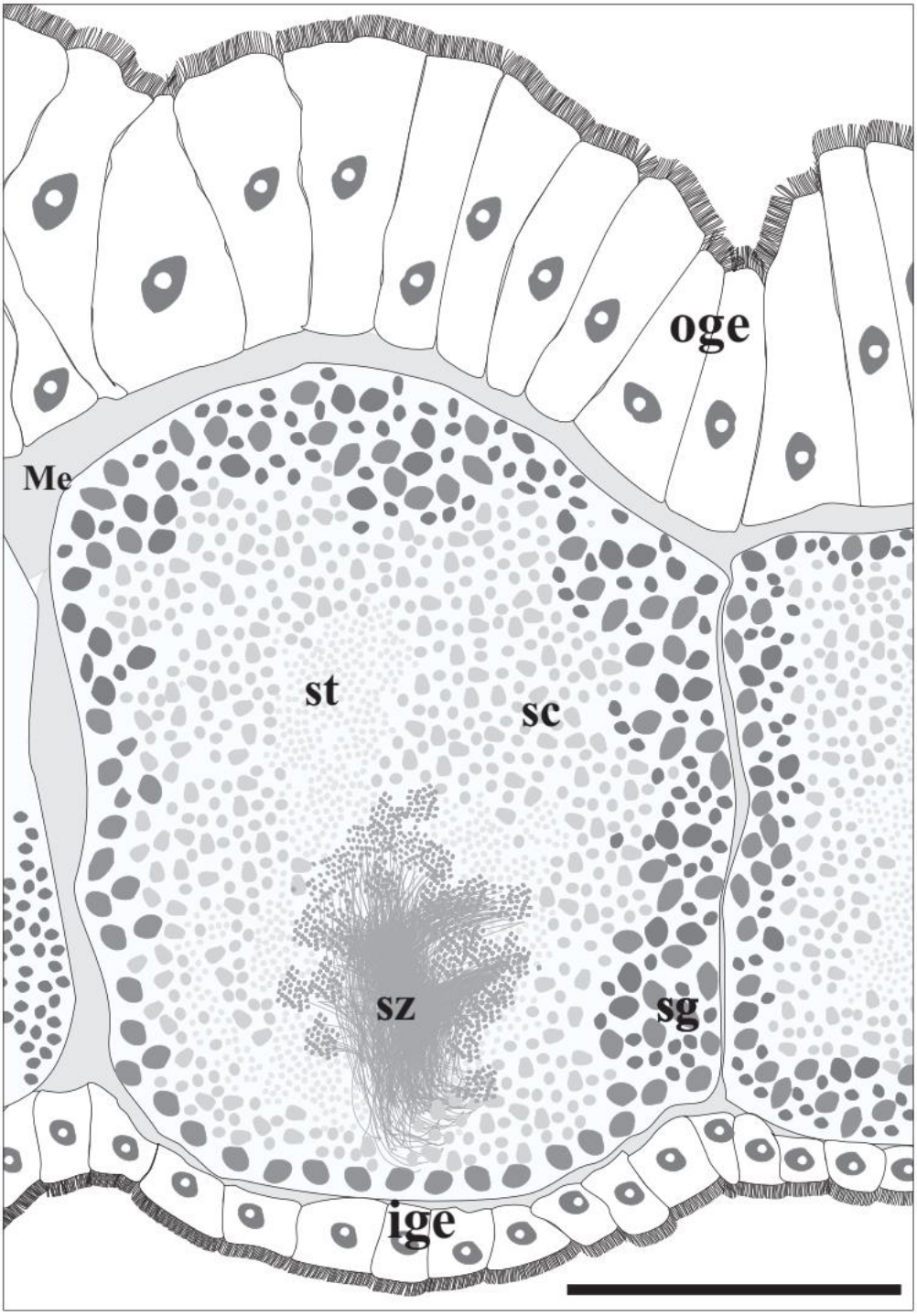

Figure 1: Schematic representation (cross section) of scyphozoan histological view of male gonad, scale: 50µm; Inner genital epithelium= ige; outer genital epithelium= oge mesoglea= m; spermatocyte= sc, spermatogonia= sg; spermatid= st; sperm= sz.

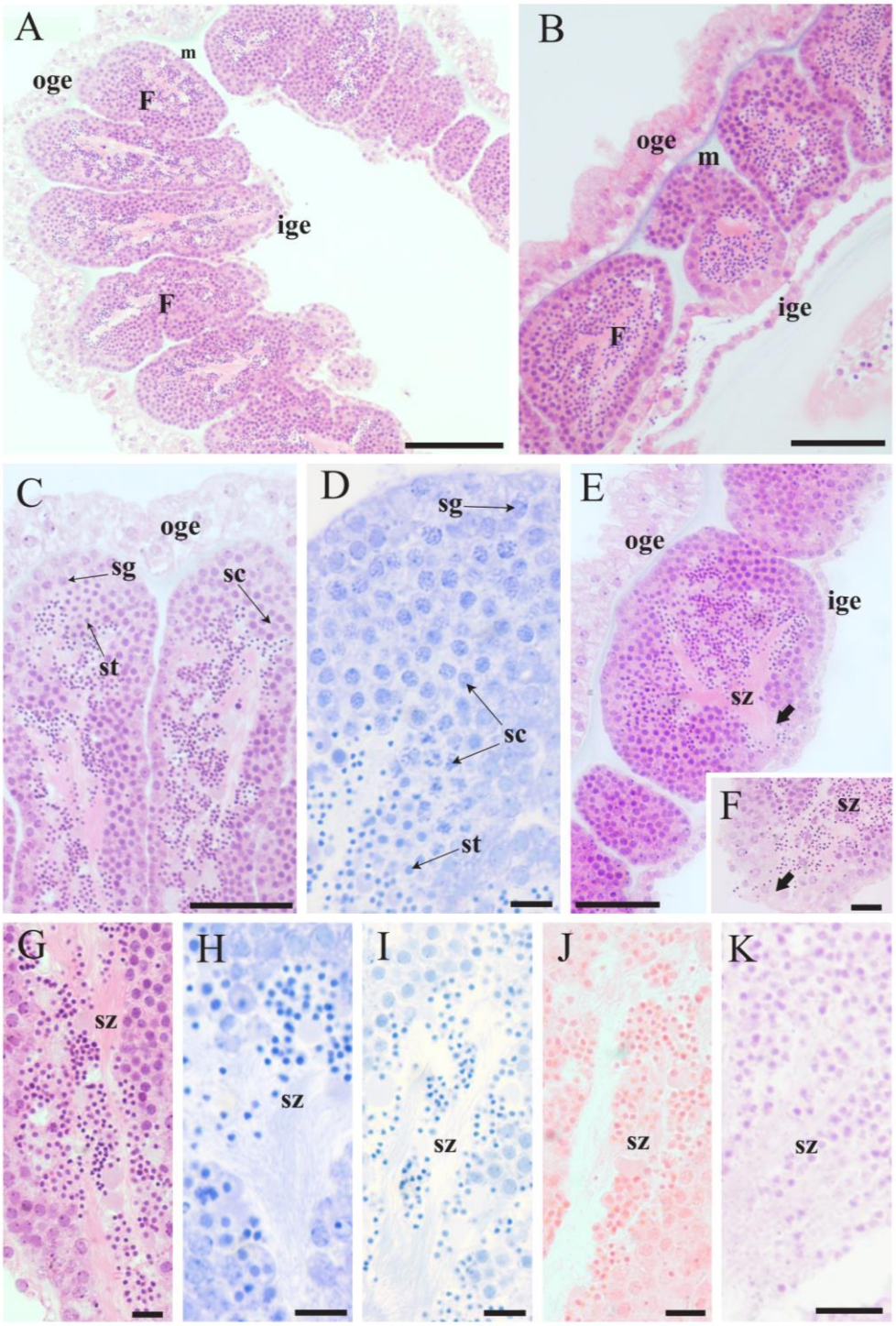

Figure 2: Spermatogenesis in *Chrysaora lactea*, in cross section. (A) Hematoxylin and Eosin. General view of the male gonad, scale:100 µm. (B) Hematoxylin and Eosin. The gonad is divided into outer and inner genital epithelium. The follicles are immersed in the mesoglea, scale: 50 µm. (C) Hematoxylin and Eosin. Detail of the spermatogonia, spermatocytes and spermatids, scale: 50 µm. (D) Toluidine blue. Follicle with cells in different stages of spermatogenesis. Note the spermatocytes in meiosis, scale: 10 µm. (E) Hematoxylin and Eosin. More advanced follicle with the sperm organized in the center (arrow) near the internal genital epithelium, scale: 50 µm. (F) Hematoxylin and Eosin. Sperm released by rupture of follicular wall (arrow), scale: 10 µm. (G) Hematoxylin and Eosin. Detail of sperm with basophil head and acidophil flagellum, scale: 50 µm. (H) Toluidine blue. Sperm heads are positive to acid structures, scale: 10 µm. (I) Mercuric Bromophenol blue. Sperm heads strongly positive to neutral proteins, scale: 10 µm. (J) Ponceau Xylidine. Sperm head is positive to total proteins, scale: 10 µm. (K) PAS. Sperm head negative to neutral polysaccharides, scale: 10 µm. Follicle= F; inner genital epithelium= ige; outer genital epithelium= oge; mesoglea= m; spermatocyte= sc, spermatogonia= sg, spermatid= st, sperm= sz.

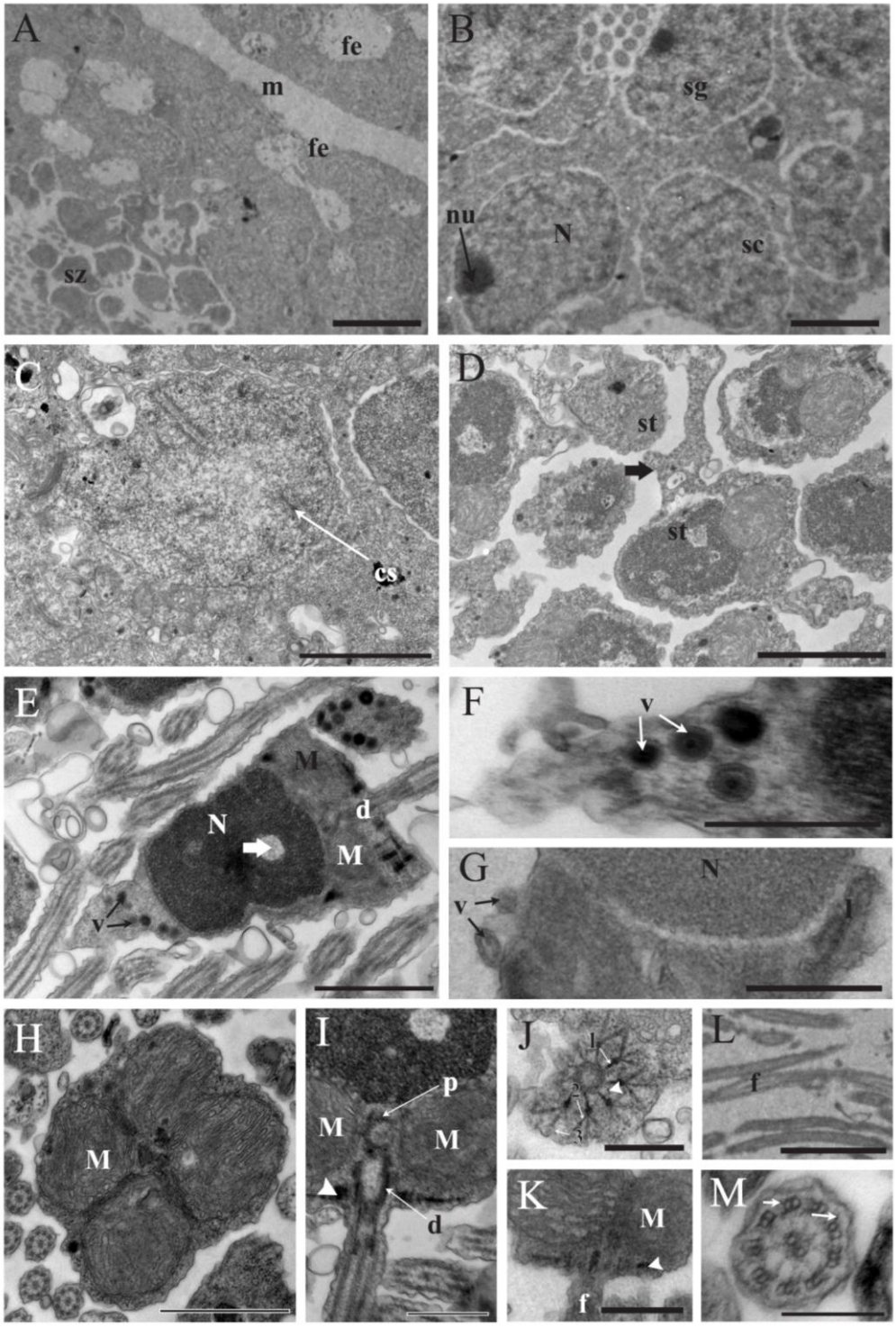

Figure 3: Ultrastructure of spermatogenesis in *Chrysaora lactea*. (A) Cross section of male gonad. Detail of the follicular epithelium with spermatogonia, the sperm clustered in the center, scale: 5 µm. (B) Note the two spermatogonia with evident nucleolus and the spermatocyte with synaptonemal complex, scale: 2 µm. (C) Detail of the spermatocyte in pachytene meiotic prophases with the synaptonemal complex formed, scale: 2 µm. (D) Spermatids connected by intercellular bridges (arrow). (E) Longitudinal section of the sperm with triangular head (arrow), scale: 2 µm. Note the nuclear vesicle, scale: 1 µm. (F) Detail of the sperm head emphasizing the small vesicles precursor of the acrosome, scale: 200 nm. (G) Detail of the sperm midpiece with the small electrodense vesicles, scale: 0,5 µm. (H) Cross section of the midpiece with 4 mitochondria, scale: 1 µm. (I) Detail of the midpiece, highlighting the proximal and distal centriole. From the distal centriole is the pericentriolar apparatus (arrowhead), scale: 500nm. (J) Cross section of the pericentriolar apparatus. Note the primary, secondary and tertiary process. In the arrowhead note the interprimary process, scale: 0,5 µm. (K) Longitudinal section of the pericentriolar apparatus (arrowhead), scale: 0,5 µm. (L) Longitudinal section of the flagellum, scale: 0,5 µm. (M) Cross section of the flagellum. Note the dynein arms (arrow), scale: 200 nm. 1= primary process; 2= secondary process; 3= tertiary process; distal centriole= d; flagellum= f; follicular epithelium= fe; golgi complex= gc; mesoglea= m; mitochondria= M; nucleus= N; nucleolus= Nu; proximal centriole= p; spermatocyte= sc; spermatogonia= sg; spermatid= st; vesicles (v).

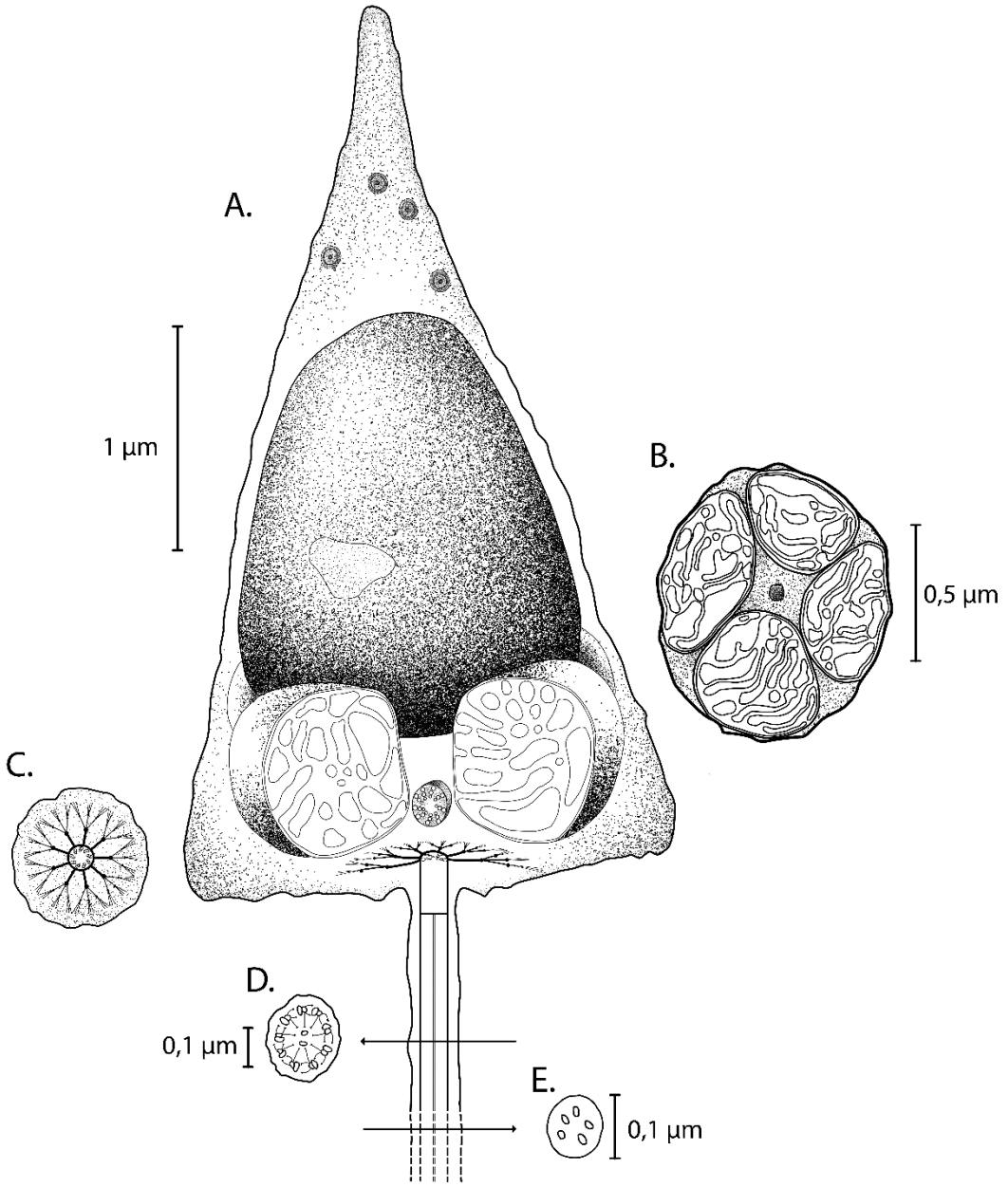

Figure 4: Schematic representation of *Chrysaora lactea* sperm. (A) Longitudinal section of the sperm. (B) Cross section in the midpiece through the mitochondria. (C) Cross section through the pericentriolar apparatus. (D) Cross section of the proximal part of flagellum. (E) Cross section of the median part of flagellum.

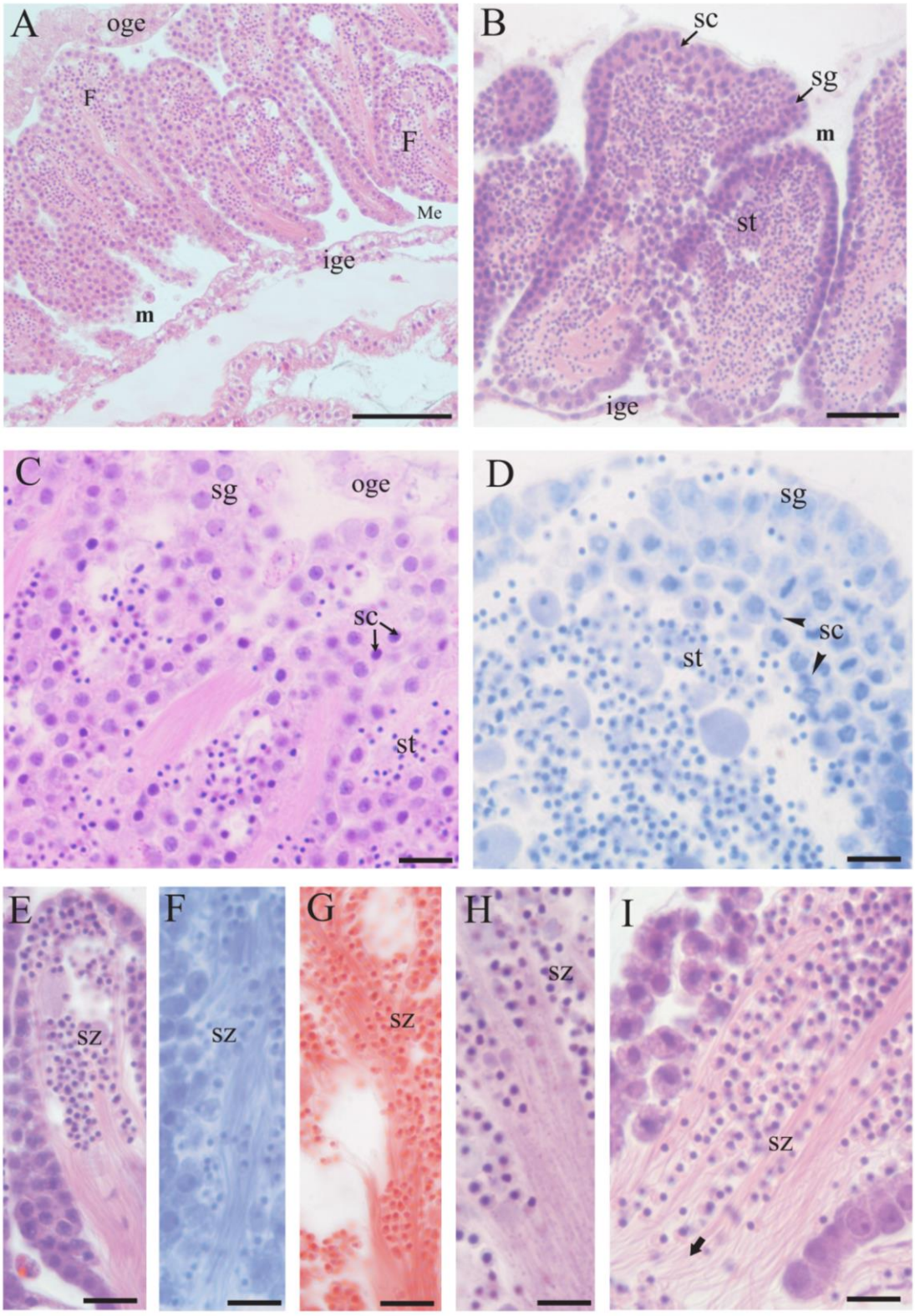

Figure 5: Spermatogenesis in *Lychnorhiza lucerna* in cross section. (A). Hematoxylin and Eosin. General view of the male gonad. divided in outer and inner genital epithelium, scale: 50µm. (B) Hematoxylin and Eosin. Detail of the follicle with spermatogonia and spermatocytes in the periphery and spermatids in the center, scale: 20µm. (C) Hematoxylin and Eosin. Detail of the spermatogonia, spermatocytes and spermatids, scale: 10µm. (D) Toluidine blue. Spermatocytes with elongated nucleus in different stages of meiosis (arrow head) followed by spermatids and sperm in the center of the follicle, scale: 10µm. (E) Hematoxylin and Eosin. Sperm with rounded basophil head and midpiece and flagellum acidophil, scale: 10µm. (F) Mercuric Bromophenol Blue. The sperm presents a positive reaction to basic proteins, scale: 10µm. (G) Ponceau Xylidine. Sperm with positive reaction to total proteins, scale: 10µm. (H) PAS-Hematoxylin. Sperm with negative reaction in the anterior region of the sperm, scale: 10µm. (I) Hematoxylin and eosin. The sperm being released, scale: 10µm. Follicle= F; inner genital epithelium= ige; mesoglea= m; outer genital epithelium= oge; spermatocyte= sc; spermatogonia= sg; spermatid= st; sperm (sz).

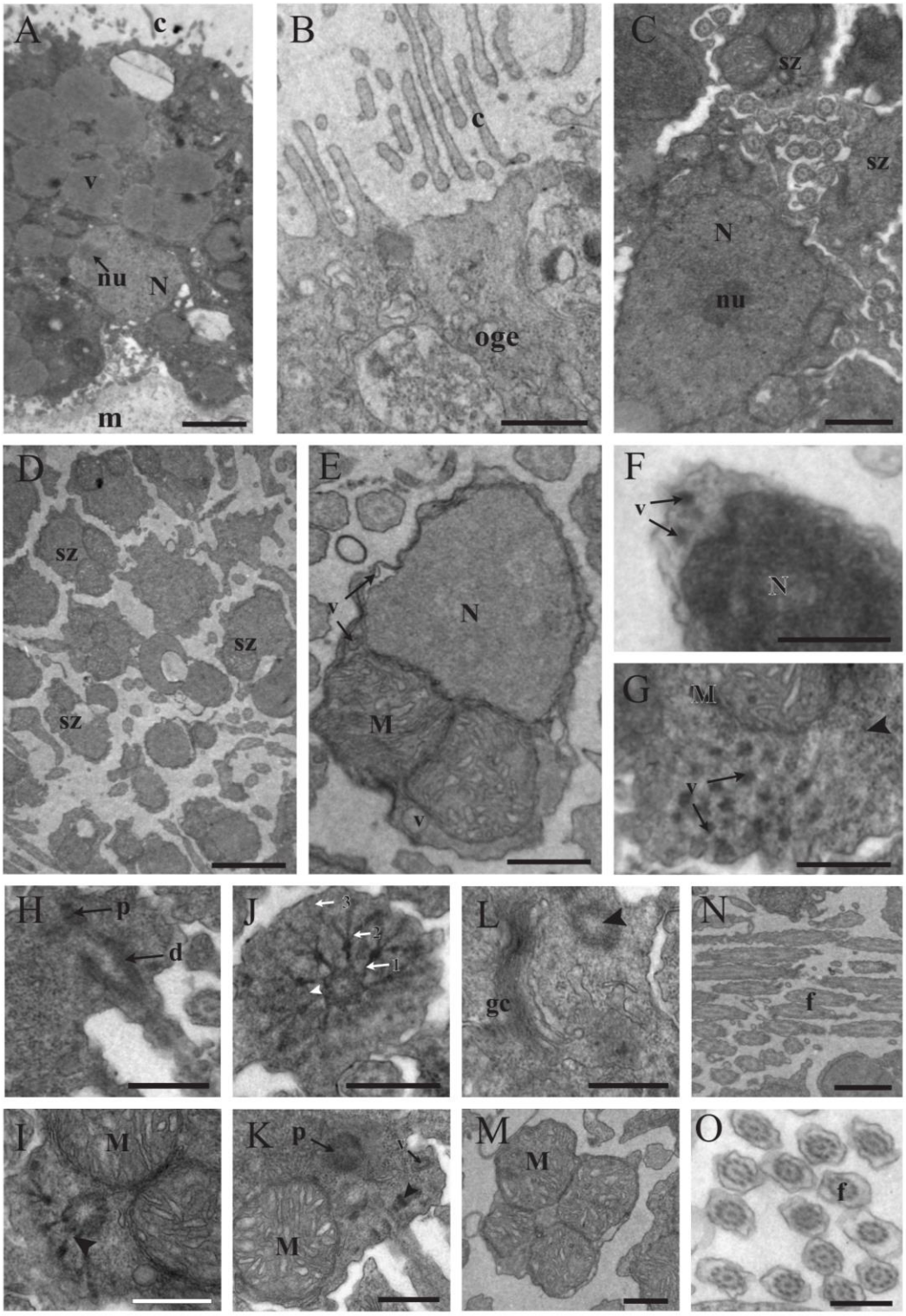

Figure 6: Ultrastructure of spermatogenesis in *Lychnorhiza lucerna*. (A) General view of the external genital epithelium with cells of irregular format and elongated nucleus in different positions of the cytoplasm, scale:1µm. (B) In the apical portion of the cell note the microvilli, scale: 0,5µm. (C) Detail of the follicular epithelium with spermatogonia, scale: 1µm. (D) The sperm in the center of the follicle, scale: 2µm. (E) Longitudinal section of the sperm, scale: 0,5µm. (F) Detail of the sperm head emphasizing the small vesicles precursor of the acrosome, scale: 0,5µm. (G) Detail of the sperm midpiece with the little electrodense vesicles, scale: 0,5µm. (H) Detail of the midpiece, highlighting the proximal and distal centrioles, scale: 0,5µm. (I) Oblique section of the pericentriolar apparatus, scale: 0,5µm. (J) Cross section of the pericentriolar apparatus. Note the primary, secondary and tertiary process. In the arrowhead note the interprimary process, scale: 0,5µm. (K) Oblique section of the pericentriolar apparatus and detail of the proximal centriole, scale: 0,5µm. (L) Golgi complex in the midpiece, scale: 0,5µm. (M) Cross section of the midpiece with 4 mitochondria, scale: 0,5µm. (N) Longitudinal view of the flagellum, scale: 1µm. (O) Cross section of the flagellum, scale: 0,5µm. 1= primary process; 2= secondary process; 3= tertiary process; Cillia= c; distal centriole= d; flagellum= f; golgi complex= gc; mesoglea=m; mitochondria= M; nucleus= N; nucleolus= Nu; outer genital epithelium= oge; proximal centriole= p; spermatogonia= sg; spermatid= st; sperm=sz; vesicles= v.

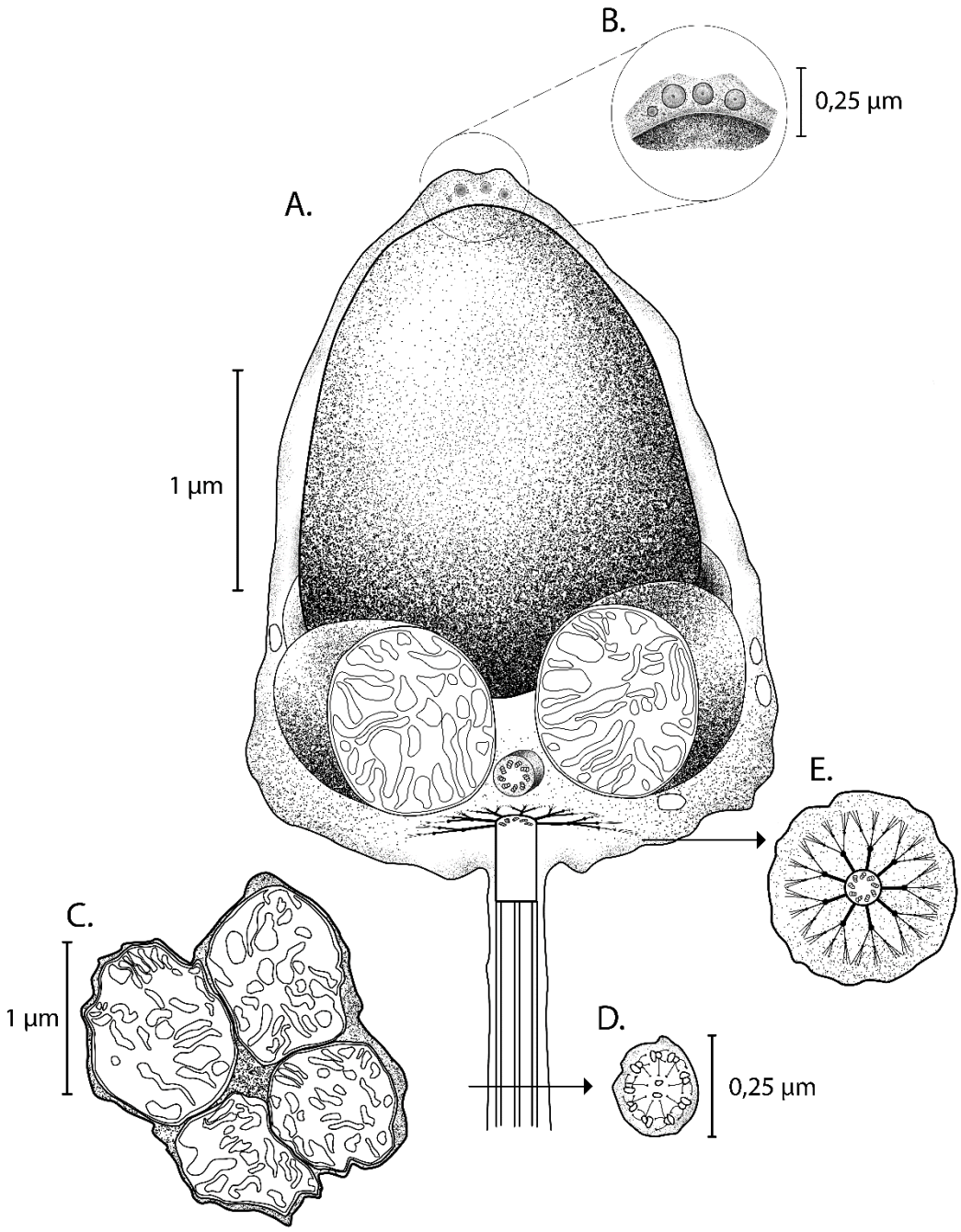

Figure 7: Schematic representation of *Lychnorhiza lucerna* sperm. (A) Longitudinal section of the sperm. (B) Detail of pro-acrosomal vesicles in the sperm head. (C) Cross section in the midpiece near the mitochondria. (D) Cross section of the proximal part of flagellum. (E) Cross section in the pericentriolar apparatus.

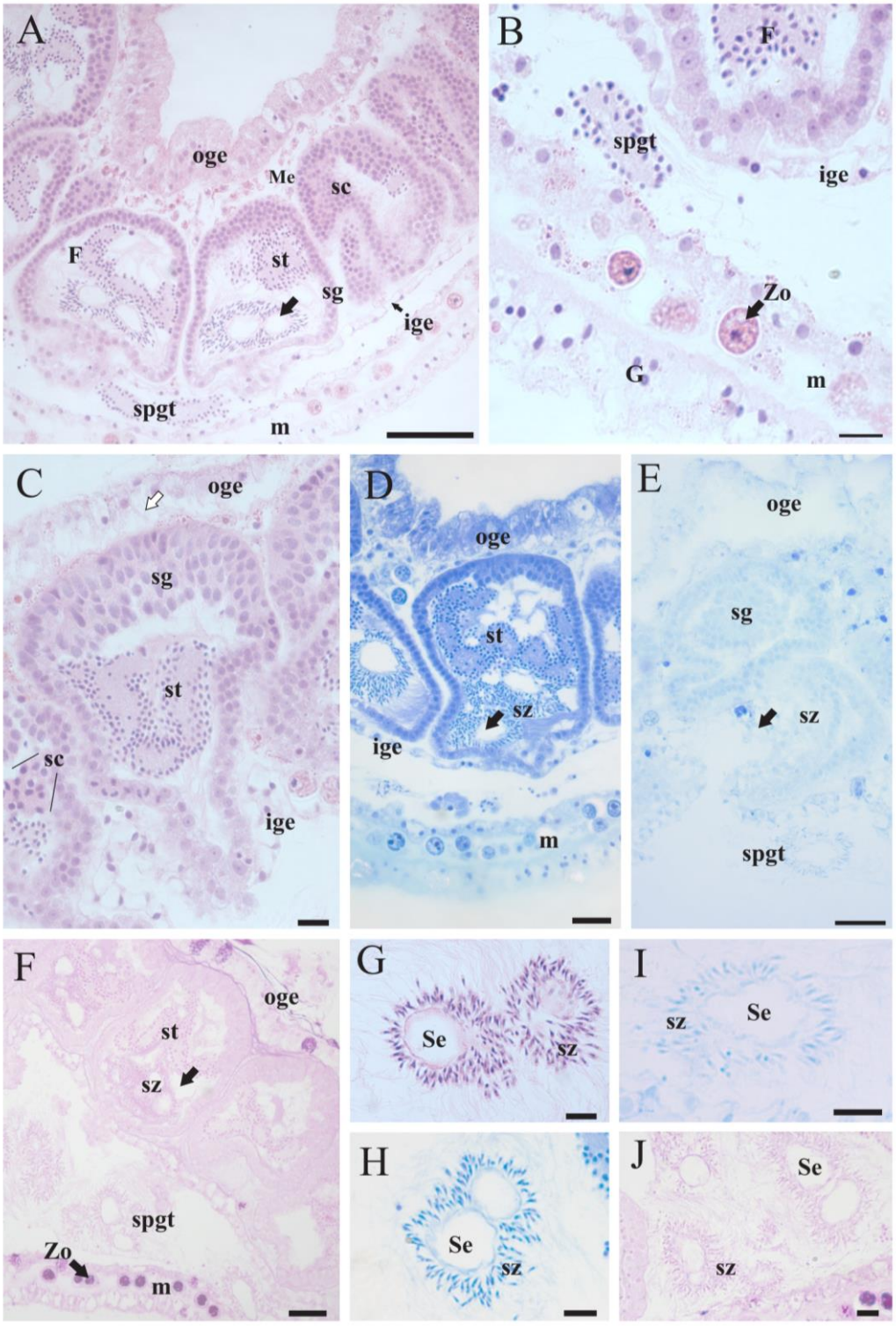

Figure 8: Spermatogenesis in *Cassiopea maremetens* in cross section. (A) Hematoxylin and Eosin. General view of the male gonad. The black arrow indicates the *spermatozeugmata* secretion with absence of reaction to the staining, scale: 50µm. (B) Hematoxylin and Eosin. Detail of the gastrodermis and mesoglea with zooxanthellae, scale: 10µm. (C) Hematoxylin and Eosin. Follicle inside the male gonad with spermatogonia, spermatocytes and spermatids. The white arrow indicates vesicles inside the outer genital epithelial cells, with absence of reaction to the staining, scale: 10µm. (D) Toluidine blue. Mature follicle with sperm immersed in a secretion with no reaction (black arrow) forming the *spermatozeugmata*, scale: 200µm. (E) Mercuric Bromophenol Blue. *Spermatozeugmata* inside the follicle. Note the secretion inside the mature follicle with negative reaction to proteins (black arrow), scale: 200µm. (F) PAS. Mature follicle with *spermatozeugmata*, in which is the secretion with absence of reaction to neutral polysaccharides, scale: 200µm. (G) to (J) Detail of the released *spermatozeugmata*. (G) Hematoxylin and Eosin, scale: 200µm. (H) Toluidine Blue, scale: 200µm. (I) Mercuric Bromophenol Blue, scale: 200µm. (J) PAS, scale: 200µm. Follicle= F; gastrodermis= G; inner genital epithelium= ige; mesoglea= m; outer genital epithelium= oge; spermatocyte= sc; secretion= Se; spermatogonia= sg; spermatid= st; sperm= Sz; spermatozeugmata= spgt; zooxanthellae= Zo.

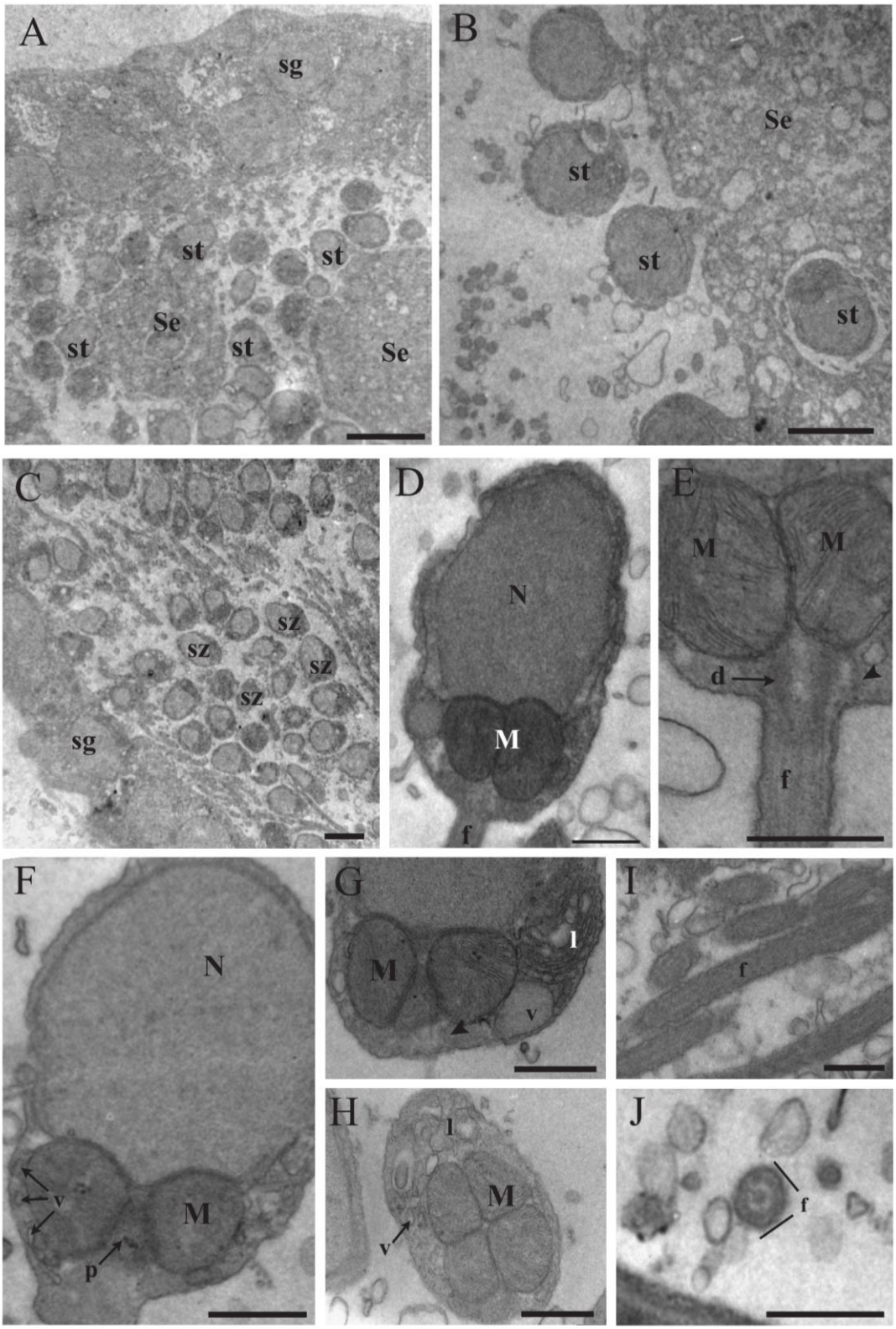

Figure 9: Sperm ultrastructure of *Cassiopea maremetens*. (A) General view of the follicular epithelium with spermatogonia, scale: 5 µm. (B) Mature follicle containing several spermatids immersed in the protein secretion, scale: 2 µm. (C) Detail of the sperm inside the follicle, scale: 2 µm. (D) Longitudinal section of the sperm, scale: 0,5 µm. (E) Detail of the midpiece with mitochondria, the distal centriole and the pericentriolar apparatus (arrowhead), scale: 0,5 µm. (F) Detail of the midpiece with mitochondria, the proximal centriole and the vesicles, scale: 0,5 µm. (G) Detail of the midpiece highlighting the lamella, vesicle and pericentriolar apparatus (arrowhead), scale: 0,5 µm. (H) Cross section of the midpiece with 4 mitochondria, scale: 0,5 µm. (I) Longitudinal section of the flagellum, scale: 0,5 µm. (J) Cross section of the flagellum, scale: 0,5 µm. Distal centriole (d); Flagellum (f); Lamella (l); Mitochondria (M); Nucleus (N); Proximal centriole (p); Secretion (Se); Spermatogonia (sg); Spermatid (st); Sperm (sz); Vesicles (v).

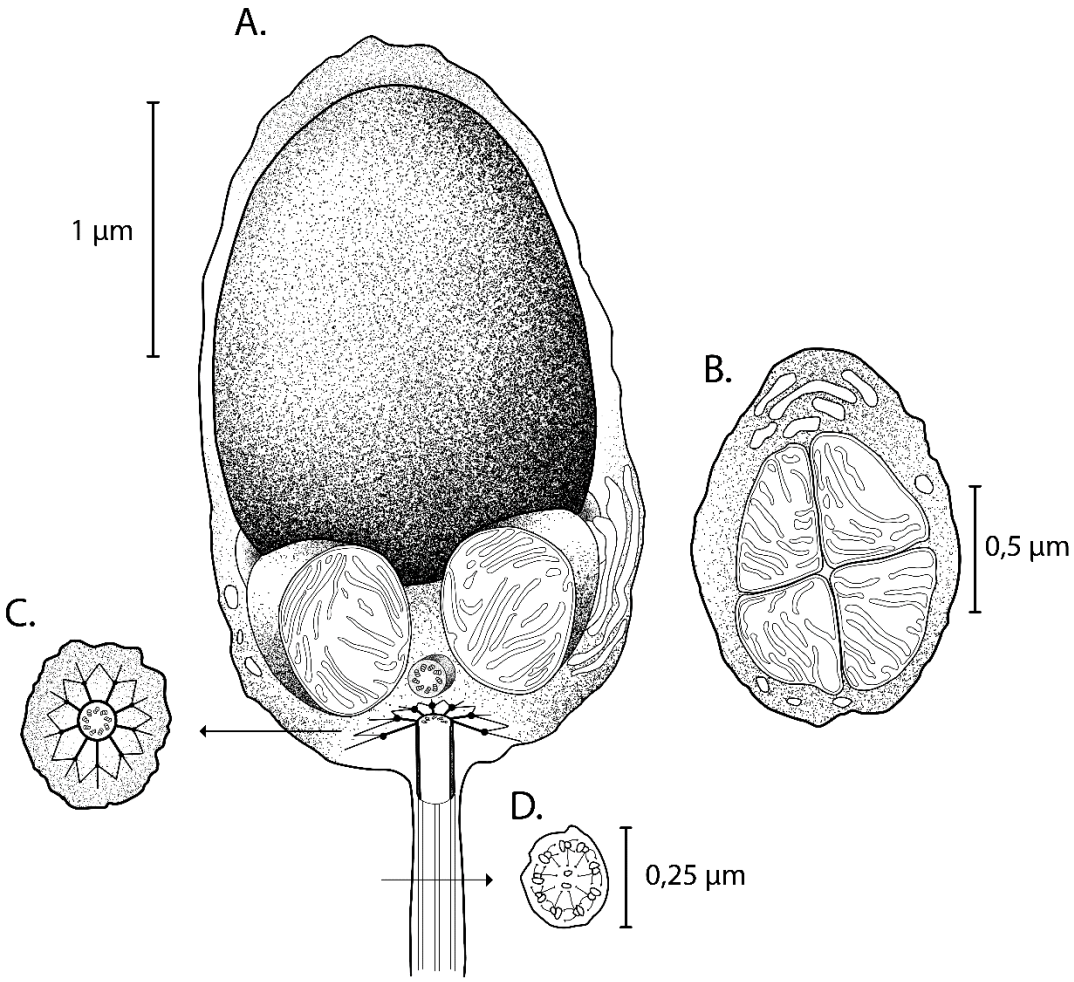

Figure 10: Schematic representation of *Cassiopea maremetens* sperm. (A) Longitudinal section of the sperm. (B) Cross section of the midpiece through the mitochondria. Note the lamella. (C) Cross section through the pericentriolar apparatus. (D) Cross section of the proximal part of flagellum.

Capítulo 3

The role of trophocytes in the vitellogenesis of true jellyfishes (Scyphozoa, Cnidaria)

Running Title: Role of trophocytes in jellyfish vitellogenesis

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Abstract

Jellyfishes are a suitable model to study the process of vitellogenesis specially the communication between the female gamete – oocyte – and the genital epithelium. Here we describe scyphozoan oogenesis highlighting the functions of trophocytes during the process. Samples of female gonads were observed under histological and transmission electron microscopy techniques. Jellyfishes have specific cells (called trophocytes) that have similar functions to the follicular cell of other invertebrates and vertebrates. The trophocytes improve the oocyte maturation (helping in the exogenous transport of yolk) and ovulation (by the disruption of collagen fibers and mucopolysaccharides storage in the trophocytes vacuoles). We can distinguish three different phases of trophocytes and oocytes maturation: early, intermediate and late. In the early stage the oocyte maintains the endogenous yolk production and starts to produce mesoglein (a protein close to ZP of vertebrates). In the intermediate stage, the oocytes increase in size because of the heterosynthetic yolk production and mesoglein starts to accumulate in the oocyte animal pole. The trophocytes modify their morphology with the nucleus moving laterally in the cytoplasm and the vacuoles full of mucus start to appear in the apical central part of the cell. In the late stage the oocyte reaches the larger size and the animal pole starts to protrude through the trophocytes central vacuolar region.

Keywords: Reproduction, gonad, gametogenesis, oogenesis, histology, TEM.

1. Introduction

In metazoans the oogenesis is an effort of intraovarian and/or extraovarian cells and tissues (Eckelbarger and Hodgson, 2021) that involve two main processes: the vitellogenesis and choriogenesis (Eckelbarger, 1994; Hara et al., 2016; Sathananthan,

2013; Zara et al., 2013). The vitellogenesis is the accumulation of yolk in the ooplasm and choriogenesis is the formation of the egg envelope. Both could involve endogenous (autosynthetic) and exogenous (heterosynthetic) protein production (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1992; Hara et al., 2016; Ikeda et al., 2011; Telfer, 2009; Valle, 1993). The endogenous yolk production are proteins produced by the oocyte itself usually recognized – in TEM images – by the presence of electrondense vesicles near the Golgi complex called "nuage" (Abdalla & Cruz-landim, 2003; Eckelbarger & Larson, 1988; Eckelbarger & Hodgson, 2021). The exogenous protein production was thought to be absent in basal metazoans (Eckelbarger, 1994) and is well-documented for many vertebrates – as mammals, reptiles, fishes (Guraya, 1989; Hara et al., 2016; Rahman et al., 2008; Sathananthan, 2013) – and invertebrates – as crustaceans, insects, annelids, platyhelminthes, mollusks and echinoderms (Eckelbarger & Hodgson, 2021; Raikhel & Dhadialla, 1992; Telfer, 2009; Valle, 1993; Zara et al., 2013). The egg envelope (fertilization envelope, vitelline membrane, chorion or zona pellucida) is a macromolecular aggregate or extracellular matrix supplemented by glycoproteins extensions, being essential in the oocyte-sperm recognition (Barresi & Gilbert, 2019; Hara et al., 2016; Mozingo & Chandler, 1991). Both, vitellogenesis and choriongenesis, are facilitated by accessory cells known in most metazoans as follicle or nurse cells (Eckelbarger & Hodgson, 2021; Hara et al., 2016; Sathananthan, 2013). Follicle cells are usually squamous-type somatic cells surrounding the oocyte with the following functions: i. mechanical support, ii. oocyte protection, iii. helping to compound the cellular envelopes around the oocytes, iv. synthesis and transfer of metabolites, yolk precursors, and 5. helping to determine the oocyte animal-vegetal polarity. Nurse cells are germline cells intimately associated with oocytes by cytoplasmic continuity and assisting in the

nutritional transport of macromolecules and organelles (Deng & Bownes, 1998; Eckelbarger & Hodgson, 2021; Lei & Spradling, 2016; Matova & Cooley, 2001).

Considering the diversity of reproductive modes and strategies, basal invertebrates such as jellyfishes, are a suitable model to study the process of vitellogenesis (Eckelbarger & Larson, 1992; Giangrande et al., 1994; Ramirez Llodra, 2002) more specifically, the communication between the female gamete – oocyte – and the genital epithelium. The so-called "true jellyfishes" comprised a taxon with 222 species grouped in two subclasses: Coronamedusae and Discomedusae (Collins et al., 2006; Dawson, 2004; Jarms & Morandini, 2019). In both subclasses the gonadal histological organization is composed of two epithelial layers: an outer – external – and an inner – internal genital epithelium (*sensu* Tiseo et al., submitted). Between these layers the gametes are immersed in the mesoglea (Campbell, 1974; Eckelbarger & Larson, 1992; Ikeda et al., 2011; Kikinger, 1992; Miller, 1983; Ohtsu et al., 2007; Schiariti et al., 2012). Such gametes could be released into the gastrovascular cavity or the environment – characterizing external fertilization (Ikeda et al., 2011; Morandini & Silveira, 2001; Ohtsu et al., 2007; Schiariti et al., 2012; Smith, 1936; Tiseo et al., subimitted) – or be fertilized inside the genital sinus or female gonad – characterizing the internal fertilization (Eckelbarger & Larson, 1988; Kikinger, 1992; Widersten, 1965). For some species a vitelline membrane is described (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1992; Ikeda et al., 2011; Ohtsu et al., 2007; Widersten, 1965). The species *Aurelia aurita* was described to have an exclusive area in the oocyte animal pole called "contact plate" which have a protein agglomerate – mesoglein – homologous to the mammal's zona pellucida (ZP) protein domain (Adonin et al., 2009). In most scyphozoans, the oocytes ripe in a gradient from less to more developed and are connected to the internal genital epithelium by a specific accessory cell type called trophocyte. Among scyphozoans, the trophocytes are exclusive

accessory cell of discomedusae jellyfishes being absent in coronamedusae (Eckelbarger & Larson, 1992; Eckelbarger & Larson, 1988; Ikeda et al., 2011; Morandini & Silveira, 2001; Tiemann & Jarms, 2010; Widersten, 1965). Such cells are electrodense, slightly flattened cells that form a thin plate just over the internal genital epithelium, covering the oocyte animal pole. The plate of trophocytes show cellular junctions in the central portion of the cytoplasm, with the nucleus located laterally near the apical portion of the cell that have several microvilli (Eckelbarger & Larson, 1988).

The target species used to validate the main questions of this work is a gonochoric widespread semaeostomeae jellyfish of Brazilian coast (Migotto et al., 2002; Morandini et al., 2005) which has a greatly folded semi-circle gonad arranged in the gastrovascular cavity that can be reached through the sub-gential ostia (Morandini & Marques, 2010; Morandini et al., 2004). *Chrysaora lactea* has the general scyphozoan histological gonad pattern (Morandini & Marques, 2010; Morandini et al., 2004; Tiseo et al., submitted) and as other Pelagiidae has an asynchrony in gamete production (Rottini-Sandrini & Avian, 1991; Tiseo et al., submitted; Widersten, 1965). Even with a few studies describing reproductive traits of *C. lactea* (Morandini & Marques, 2010; André C Morandini et al., 2005; Morandini et al., 2004; Tiseo et al., submitted) there are still many unanswered questions involving the gametogenesis and especially the oogenesis process. Our study focuses on the widespread semaeostomeae jellyfish from the Brazilian coast: *Chrysaora lactea*. Here, we describe the oogenesis of the species based on light and transmission electron microscopy, highlighting the communication between trophocyte and oocyte. Additionally, we hypothesize about the correlation between the type of trophocyte, the ovulation process and the type of fertilization; as well as discuss the role of trophocytes in the oogenesis process among Discomedusae.

2. **Material and Methods**

2.1. *Sampling*

Specimens of *Chrysaora lactea* (n=8) were collected manually with a small bucket or hand net at the water surface or with shrimp trawls near the Enseada (S23˚41'56.0" W045˚23'26.4") and Jabaquara (S23˚42'49.49" W045˚16'41,8") beaches from January of 2018 to November of 2019. The animals were transported alive to Centro de Biologia Marinha (CEBIMar, USP) in São Sebastião county and were identified and measured according to Morandini et al. (2005).

2.2. *Tissue processing (histology and histochemistry)*

Specimens were anesthetized by thermal shock (-20°C) - placing the medusae in seawater into a freezer - for 5 minutes prior to dissection. Then, samples of gonadal tissue were preserved in 4% formaldehyde solution buffered with a saline solution of 0.2 M sodium phosphate (pH 7.2) for 24 hours. After fixation and buffer washes, the samples were dehydrated in a graded ethanol series (30-95%) and embedded in Leica® methacrylate historesin. Serial sections 3-5μm thick were made on a rotating microtome. For histology the sections were stained with hematoxylin and eosin (HE) without baths in ethanol and xylene (Sant'Anna et al., 2010; Zara et al., 2012). For histochemistry the sections were stained with mercuric bromophenol blue for proteins (Pearse 1960); PAS technique for neutral polysaccharides (Junqueira and Junqueira 1983); toluidine blue stain for acid components (modified from Audino et al. 2015) and Mallory's trichrome stain for collagen (blue).

2.3. *Tissue processing (Transmission Electron Microscopy - TEM)*

For TEM, 1mm^3 fragments of the female gonad were preserved in Karnovsky solution (modified from Karnovsky, 1965) consisting of 2.5% glutaraldehyde with 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) with 2.5 mM of CaCl2 and saccharose for 24 hours, followed by post-fixation in 1% osmium tetroxide solution in the 0.1 M sodium cacodylate buffer, for one hour at the same temperature. Then, samples were dehydrated in a graded ethanol series (30 to 100%) and embedded in Spurr's® resin. Semi-thin and ultrathin sections were cut with a Leica® ultramicrotome. Grids with the ultrathin sections were contrasted with uranyl acetate 2% and lead citrate 0.2% in NaOH 0.1N, and later examined and photographed using a Zeiss EM 900 Transmission Electron Microscope (located in the Electron Microscopy Laboratory from the Department of Genetics, Institute of Biosciences of the University of São Paulo).

2.4. *Cell Measurements*

To determine the length-width ratio of cells we measured length and width of 120 gastrodermal and genital epithelium cells chose randomly. At least 35 measurements of the four oocytes maturation stages (pre-vitellogenic, early, intermediate and late oocytes) were taken under 40x magnification micrographs. All measurements were taken using the software ImageJ with appropriate scale calibrations (Tiseo et al., 2014; Zara et al., 2012).

3. Results

3.1. *Female gonadal organization and pre-vitellogenic oocytes*

Chrysaora lactea is a gonochoric jellyfish species. The gonads form a greatly folded semi-circle arranged in the gastrovascular cavity. The general histological pattern of the gonad is similar to other scyphozoans. The female gonad of *Chrysaora lactea*, seen in longitudinal histological section, is composed of an external genital epithelium (outer),

an internal genital epithelium (inner), and between them lies the basophil mesoglea with oocytes in different stages of maturation: early, intermediate and late oocytes (Figure 1A).

The external genital epithelium is a simple columnar type, has cells taller than wide with a length to width ratio of 4,42 ± 2,23 (average ± standard deviation). This epithelium has an elongated basophil nucleus in the basal portion of acidophil cytoplasm (Figure 1B). Under TEM we observe two types of absorptive cells: one with cilia and Golgi apparatus in the apical portion, nucleus with electrodense nucleolus, several rounded mitochondria (Figure 1C) and another with lipids droplets (Figures 1D and E) microvilli in the apical portion (Figure 1E), Golgi apparatus in the perinuclear zone, several electrodense vesicles in the cytoplasm, nucleus with heterochromatin and euchromatin near the elongated mitochondria (Figure 1D). Both absorptive cell types are supported by a basal lamina and a fibrillar mesoglea (Figure 1F).

The internal genital epithelium is of simple cuboidal type (length to width ratio of 0,98 ± 0,19), with cells containing an elongated basophil nucleus and acidophil cytoplasm (Figure 1B). The internal genital epithelium cells have a large nucleus with a small nucleolus, lipids droplets, elongated mitochondria and a Golgi apparatus in the cytoplasm with cilia in the apical portion (Figure 1G). The cilia are anchored in the cytoplasm by a basal corpuscle with a rootlet (Figure 1H). Pre-vitellogenic oocytes are found inside the internal genital epithelium (9,29 ± 2,09 µm, n=48), with acidophil cytoplasm (Figure 1B), which contain rugous (RER) and smooth endoplasmic reticulum (REL), a large Golgi apparatus with electrodense vesicles, rounded mitochondria with tubular cristae and a large nucleus with prominent nucleolus (Figures 1I and J).

3.2. *Early stage of maturation*

In the early stage of maturation, both the oocytes and trophocytes have specific features: as soon as the oocyte increases in size (early oocyte measures: 19,95 ± 3,86 µm, n=165), they start to protrude into the mesoglea staying attached to the internal genital epithelium through the trophocytes (Figures 2A-E). Trophocytes have an elongated basophil nucleus and a flattened acidophil epithelium with cilia in the apical portion (Figure 2A). The cytoplasm shows positive reaction to acid compounds (Figure 2B), protein (Figure 2C) and neutral polysaccharides (Figure 2D), being negative to collagen (Figure 2E). In the ultrastructure, we observe the nucleus with mostly euchromatin, rounded and elongated mitochondria with tubular cristae and a basal corpuscle in the apical portion anchoring the cilia (Figure 2F). The trophocytes plasma membrane is closely connected to the oocyte plasma membrane through cell interdigitations (Figure 2F).

The early oocyte has a nucleus with prominent nucleolus and basophil cytoplasm (Figure 2A). The ooplasm shows strongly positive affinity to acid compounds (Figure 2B), positive affinity to proteins (Figure 2C) and neutral polysaccharides (Figure 2D) and negative to collagen (Figure 2E). At this stage we can note two types of protein granules in the cytoplasm: the type 1 is basophil and large (1,29 ± 0,26 µm, n=5) and the type 2 is acidophil and small (0,52 ± 0,14 µm, n=5) (Figure 2A). The first types of granules are positives to acid compounds (Figure 2B), not differentiated to basic proteins (Figure 2C), neutral polysaccharides (Figure 2D) and collagen (Figure 2E). The second types of granules are negative to acid compounds (Figure 2B), positive to basic proteins (Figure 2C) and neutral polysaccharides (Figure 2D) but negative to collagen (Figure 2E). The oocyte ultrastructure shows a round nucleus with euchromatin and an ooplasm rich in RER with several mitochondria with tubular cristae in the perinuclear region (Figure 2G). Adjacent to the nucleus we also observe an agglomerate of electrodense vesicles called

"nuage" and few electrodense yolk vesicles indicating the endogenous protein production (Figure 2H and I). At the early stage we also observe few yolk electrodense granules in the trophocyte cytoplasm and few invaginations in the oocyte membrane indicating the beginning of exogenous vitellogenesis (Figure 2J).

3.3. *Intermediate stage of maturation*

At the intermediate stage the trophocytes and oocytes undergoes a few modifications in their cell morphology (Figures 3A-L). The trophocytes nucleus moves laterally in the cytoplasm and several vacuoles are observed, accumulated, in the apical-central part of the cell (Figure 3A). The vacuoles content shows negative affinity to hematoxylin and eosin (Figure 3A) and acid compounds (figure 3B) but strongly positive affinity to neutral polysaccharides (Figure 3C), collagen and mucus (Figure 3D). The trophocytes interdigitations enclosing the oocyte shows positive affinity to neutral polysaccharides (Figure 3C) and collagen (Figure 3D). In the ultrastructure, the trophocytes shows an elongated nucleus with euchromatin, several mitochondria and electrodense yolk granules in the cytoplasm and a large vacuole of fibrous content (Figure 3E). The trophocytes interdigitations are observed laterally surrounding the oocyte and also near the oocyte animal pole – creating an evident intermembranous space between oocyte and trophocyte membranes (Figure 3E). In these intermembranous spaces there is a fibrous deposition and several vesicles that seems to fuse with the oocyte's membranes (Figure 3F).

The intermediate oocyte is characterized by the rapid increase of oocyte size (49,63 ± 12,29 µm, n= 150) and also by the presence of several endogenous and exogenous yolk granules in the ooplasm (Figures 3A to L). At this stage, both types of endogenous protein granules have close sizes and, when compared to the early stage, their location may vary in the cytoplasm. Even with these variations in size and location, the

two types of protein granules can be differentiated by the affinity to the traditional histological compounds: hematoxylin and eosin (Figures 3A to D). The type 1 (basophil) is situated in the animal pole of ooplasm near the connection with trophocytes and the type 2 (acidophil) is situated in the central region of ooplasm (Figure 3A). The second type of granule is also positive to acid compounds (Figure 3B), neutral polysaccharides (Figure 3C) and to Mallory's trichrome (Figure 3D). Ultrastructurally, the first type of granules is more electrodense than the second type (Figure 3E-G). The second type can be distinguished by the presence of several tiny vesicles fusing and forming the final yolk granule (Figures 3G-H) and also by the presence of noticeable crystallized yolk protein (Figure 3L). These endocytic coated vesicles are also observed in the oocyte vegetal pole adjacent the yolk granules (Figures 3G-I). As soon as the coated vesicles enter the oocyte (Figure 3J) they approach and fuse with the electrodense yolk granules (Figures 3K-L) highlighting the exogenous vitellogenesis.

3.4. *Late stage and ovulation*

In the late stage of maturation, both the trophocytes and oocytes maintain the general intermediate morphology with slight modifications that lead to the ovulation process (Figures 4A-L). Continuing the oocyte maturation, we observe an increase in the number of vacuoles in the trophocytes (Figures 4A-G), the nucleus is still situated in the lateral cytoplasm (Figures 4A, G) and the interdigitations surrounding the oocyte are noted laterally (Figure 4F) and in the vegetal pole (Figure 4H). As in the intermediate stage, the vacuoles content shows negative affinity to hematoxylin and eosin (Figure 4A) and acid compounds (Figure 4B) but with positive affinity to basic proteins (Figure 4C), strongly positive affinity to neutral polysaccharides (Figure 4D), collagen and mucus (Figure 4E). At this stage, we can also note the fibrous deposition in the intermembranous space and

the endocytic pits that seem to fuse with the oocyte membranes (Figures 4H-I). The content in the trophocytes vacuoles is clearly a fibrous material like an extracellular matrix (Figures 4J-K). Near the vacuoles, at the apical part of the cell, cilia are anchored by a basal corpuscle (Figures 4K-L).

The late oocyte is characterized by its larger size (105,51 ± 18,21 µm, n=35) with the animal pole protruding through the central vacuolar region of trophocytes (Figures 4A, C). The first type of granule is seen as a cortical agglomerate of basophil protein around the oocyte (Figure 4A), being negative to acid compounds (Figure 4B) and positive to proteins (Figure 4C), neutral polysaccharides (Figure 4D) and Mallory's trichrome (Figure 4E). The second type of granule is seen in the central ooplasm and is acidophil, positive to acid compounds (Figure 4B), strongly positive to proteins (Figure 4C), neutral polysaccharides (Figure 4D) and Mallory's trichrome (Figure 4E). In the ultrastructure, the second type of granule is seen in higher concentrations inside the ooplasm (Figure 4H) and clearly the location of the granules, besides the electrondensity, is eye catching.

4. **Discussion**

Our results support the data presented by Tiseo et al. (submitted) that there is only a single mode of histological organization of the gonads in scyphozoans. The gonad in scyphomedusae – including *C. lactea* presented in this paper – is composed of an external and an internal genital epithelium: the first composed of columnar cells and the last by cuboidal cells, and both with their own basal lamina. The same organization is also observed in 15 of the 19 female gonads studied until now (see Table 1), in which both epithelia are mentioned as the "gastrodermis" (Eckelbarger & Larson, 1992; Lucas & Reed, 2010; Rouse & Pitt, 2000; Schiariti et al., 2012). As suggested by Tiseo et al. (submitted) "cells of different shapes perform different functions" it is clear that both

epithelia should be distinct and named differently as outer (external) and inner (internal) genital epithelia.

Our observations on *C. lactea* reinforce those two main processes are occurring at the same time inside the female gonad: the vitellogenesis and choriogenesis – well known in other invertebrates and vertebrates (Abdalla & Cruz-landim, 2003; Hara et al., 2016; Sathananthan, 2013; Zara et al., 2013). The vitellogenesis in scyphozoans has endogenous and exogenous phase of yolk production (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988, 1992; Ikeda et al., 2011). As Eckelbarger & Larson (1992) pointed, the presence of electrodense aggregations (nuage), surrounded by mitochondria near the Golgi complex, is a feature that indicates the autosynthetic (endogenous) protein production. The "nuage" is described here for the species *C. lactea* and was also observed in other scyphozoans (Avian & Rottini-Sandrini, 1991; Eckelbarger, 1994; Eckelbarger & Larson, 1988, 1992; Ikeda et al., 2011; Ohtsu et al., 2007), staurozoans (Eckelbarger & Larson, 1993), and anthozoans (Eckelbarger et al., 2008) but its function is still not clear (Kloc et al., 2014). The heterosynthetic (or exogenous) protein production was thought to be absent in lower metazoans (Eckelbarger, 1994) but this statement proves to be a speculation as endogenous pinocytic vesicles cover by clathrin was described in the ultrastructural studies of the studied species and other cnidarians (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988, 1992, 1993; Ikeda et al., 2011). In other metazoans, these pinocytic vesicles are responsible to bring yolk precursors as vitellogenin (Hara et al., 2016), confirming the exogenous yolk pathway. Until the present date no proteins similar to the vitellogenin were described in scyphozoans. The ultrastructural observations lead us to conclude that, at least in *C. lactea*, two main uptake routes of yolk production are taking place: i) autosynthetic, and ii) heterosynthetic precursors from the trophocyte contact. Differently from other discomedusae (Avian &

Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988, 1992), in *C. lactea* the connection between the trophocytes and oocytes are not only near the genital epithelium, but also surrounding the entire oocyte. This conclusion is based on: histochemical observations of positive reaction to collagen (Mallory's trichrome stain) around the oocyte; and the ultrastructural observation of interdigitations in the oocyte vegetal pole.

The choriogenesis starts in the earlier stages of the oocytes before yolk accumulation. The precursors of vitelline membrane (egg shell or envelope, zona pellucida or chorion) are produced by the oocyte and imported from the extraovarian organs, tissues or cells (Hara et al., 2016; Sathananthan, 2013). The zona pellucida (ZP) of mammals is composed of fine filaments embedded in a glycoprotein matrix more compact than other ZP's and allows sperm recognition, binding and penetration during the fertilization (Sathananthan, 2013). The egg envelope of cnidarians was thought to have a simple branching microvillus covering the egg surface (Eckelbarger et al., 1998; Eckelbarger & Larson, 1988) but recent studies showed that the oocyte of the semaeostome *Aurelia aurita* produces a glycoprotein – call mesoglein – very similar to the mammals ZP's proteins (Adonin & Podgornaya, 2012). The mesoglein is a protein first isolated from the mesoglea and later described in several stages of oocytes (Adonin, Podgornaya, & Shaposhnikova, 2012), because it binds to antibodies of other metazoans ZP-domain (Adonin et al., 2009). Adonin & Podgornaya (2012) described that in *Aurelia aurita* early oocytes, mesoglein is found centrally in the ooplasm near the nucleus. As soon the oocyte matures the mesoglein disperses to the animal pole near the trophocyte-oocyte association, called "contact plate" in late oocyte (Adonin & Podgornaya, 2012). In *C. lactea* we observed two types of glycoproteins (positive to PAS) in the ooplasm: the first type is a basophil (purple) glycoprotein and the second type an acidophil (pink) glycoprotein. As the first type is found in the ooplasm near the nucleus (in early oocytes)

and starts to disperse to the animal pole (in intermediate oocytes), just like the mesoglein of *A. aurita*, we believe that this glycoprotein is also present in *C. lactea* oocytes. Differently to that observed by Adonin et al. (2012), in *C. lactea* late oocytes the first type glycoprotein, does not form a "contact plate" in the animal pole but is seen in the oocyte cortical ooplasm, resembling the vitelline membrane (ZP or chorion) of other metazoans (Abdalla & Cruz-landim, 2003; Mozingo & Chandler, 1991; Sathananthan, 2013; Zara et al., 2013). Here we are assuming that jellyfishes present a glycoprotein that could have the same functions like mammals ZP protein. A fibrous matrix between the oocyte and trophocyte microvilli was described in *C. lactea* and other scyphozoans (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988, 1992; Ikeda et al., 2011). There are still some questions regarding the scyphozoan's vitelline: I. Do jellyfish have a vitelline envelope properly speaking? II. Is the fibrous matrix composing the vitelline envelope of the oocyte in association with the first type glycoprotein? III. Is the mesoglein protein also present in the oocyte of *C. lactea* and with the same functions of other ZP proteins?

In Semaeostomeae and Rhizostomeae species, the pre-vitellogenic oocytes are seen inside the genital epithelium while the early, intermediate and late oocytes are seen migrating towards the mesoglea but still connected to the genital epithelium through the trophocytes. The contact between the trophocyte and oocyte was mentioned by Pitt & Kingsford (2000), Ikeda et al. (2011) and Schiariti et al. (2012); and all studies state that the trophocytes could be related with oocyte nutrition. As observed in some semaeostomes like *Aurelia aurita* (Eckelbarger & Larson, 1988), *Pelagia noctiluca*, *Discomedusa lobata* and *Rhizostoma pulmo* (Avian & Rottini-Sandrini, 1991), as soon the oocytes maturate, the trophocytes morphology change in order to better assist the oocyte oogenesis. These authors also observed that i. the interdigitations that surround

the oocyte helps in the exogenous vitellogenesis and ii. the trophocytes nucleus are positioned laterally in the cell cytoplasm like described here to *C. lactea*. Except *Aurelia aurita*, *Cotylorhiza tuberculata*, *Rhizostoma pulmo* and *Chrysaora hysoscella*, all other discomedusae possess vacuoles centrally arranged in the trophocyte cytoplasm. Avian & Rottini-Sandrini (1991) report that in *Pelagia noctiluca,* these vacuoles had a secretion of mucopolysaccharides in which the oocytes stay immersed when ovulated. In *C. lactea* we also observed large vacuoles positive to neutral polysaccharides (PAS), surrounded by collagen and the vacuoles are filled with a fibrous material (ultrastructure) positive to collagen and mucopolysaccharides (Mallory's trichrome) like in a secretion of extracellular matrix. Collagen is known to be a protein that can be accumulated by the cell in a procollagen form and provide tensile strength in animal connective tissues (Alberts et al., 2014). In *Chrysaora lactea* we observed the late oocyte stage protrudes into the vacuolar area of the trophocytes rich in mucopolysaccharides, to be ovulated with the disruption of large vacuoles indicating that trophocytes helps in the exogenous vitellogenesis but also in the ovulation process. The spawning of oocytes in a secretion of mucopolysaccharides can be related with the external fertilization of this specie that release the gamete in the water with posterior external fertilization (Morandini et al. 2004; Jarms and Morandini 2019).

Some inferences can be made regarding general features of the reproduction of scyphomedusae while considering the presence of trophocytes, formation of a cup at the base of the oocytes, type of fertilization (external or internal), presence or absence of ovulation and of a contact plate. Usually, species with external fertilization and that ovulate – *C. lactea, Lychnorhiza lucerna, Pelagia noctiluca* and *Nemopilema nomurai* – have tall trophocytes, eletronlucid and with large vacuoles (Avian & Rottini-Sandrini, 1991; Ikeda et al., 2011; Schiariti et al., 2012; Tiseo et al., submitted) while species with

internal fertilization – *Cotylorhiza tuberculata*, *Rhizostoma pulmo* and *Aurelia aurita* – the trophocytes are flattened forming a cup and electrondense (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988; Kikinger, 1992; Widersten, 1965). But there is an exception (*Cassiopea frondosa*) in which Smith (1936) reported a supposed external fertilization but the author did not observe the presence of trophocytes surrounding the oocyte (see Table 2).

In Cnidaria, five types of accessory cell were reported: mesogleal cells, follicle cells, nurse cells, trophonemata and trophocytes. In the black coral *Cirrhipathes* cfr, *anguina* – differently to that described for other Anthozoa – the transport of nutrients occurs by mesogleal cells (Gaino & Scoccia, 2008). Follicle cells were described for the coronate *Peryphylla peryphylla* (Tiemann & Jarms, 2010), staurozoans (Eckelbarger & Larson, 1993), pennatulaceans (Eckelbarger et al., 1998) and gorgonians (Gutiérrez-Rodríguez & Lasker, 2005) and also some hydrozoans (Alexandrova et al., 2005). While nurse cells were described in the hydrozoan *Hydra* sp. (Alexandrova et al., 2005). Trophonemata are exclusively observed in sea anemones (Eckelbarger et al., 2008) and trophocytes are an accessory cell exclusive seen in Discomedusae (Eckelbarger & Larson, 1988, 1992; Ikeda et al., 2011; Rottini-Sandrini & Avian, 1991; Schiariti et al., 2012). The trophocytes were first described by Eckelbarger and Larson (1988) transferring external nutrients to the oocytes (heterosynthetic yolk production) during the vitellogenesis process. Different terms were also used to refer to these same cells: “nurse cells” used by Widersten (1965) and Kikinger (1992) and paraovular body (POB) proposed by Avian & Rottini-Sandrini (1991). We agree with Eckelbarger (1994) that these cells cannot be classified as classical nurse cells because: i. we cannot establish a homology between these three types of cells and ii. by definition, trophocytes cannot be considered a “nurse cell” because they are not connected to the oocyte by cytoplasmic bridges. Even the trophocytes performing most

of the follicle cell functions, they cannot be called true follicles cells because: i. they are not a somatic cell (as all gonadal tissue, trophocytes have a gastrodermal origin as germ line cells) and ii. they are not a squamous epithelial cell type (Deng & Bownes, 1998; Eckelbarger & Hodgson, 2021; Lei & Spradling, 2016; Matova & Cooley, 2001; Miller, 1983). Eckelbarger & Hodgson (2021) summarized all types of accessory cells and in which animal group it occurs: out of the 32 phyla, follicle cells were described in 15 groups, nurse cells in 13 groups and trophocytes only in two. The trophocytes type 1 are exclusive observed in Discomedusae and the type 2 in most Hexapoda.

Conclusions

Jellyfishes have specific cells (called trophocytes) that have similar functions to the follicular cell of other invertebrates and vertebrates. These cells improve the oocyte maturation (helping in the exogenous transport of yolk) and ovulation (by the disruption of collagen fibers storage in the trophocyte vacuoles). The jellyfish oocytes have an endogenous and exogenous vitellogenesis like other metazoans, contrary to what was assumed and we suggest that the vitelline envelope – composed of mesoglein (histology) and of fibrous material (MET) – around the oocyte is produced and secreted by the oocyte during the “choriogenesis" process.

We can distinguish three different stages of trophocytes and oocytes maturation: early, intermediate and late. At the early stage the oocyte maintains the endogenous yolk production and starts to produce mesoglein (a protein close to the ZP of vertebrates). At the intermediate stage, the oocytes increase in size because of the heterosynthetic yolk production and mesoglein starts to accumulate in the animal pole. The trophocytes modify their morphology with the nucleus moving laterally in the cytoplasm and the vacuoles full of collagen start to appear in the apical central part of the cell. At the late stage the oocyte reaches the larger sizes and the animal pole starts to protrude through the

trophocytes central vacuolar region. Trophocytes with large vacuoles, rich in collagen and mucopolysaccharides (histochemistry) and filled with fibrous material (TEM) associated with Oii and Oiii oocytes, indicate that these cells also have a role in the ovulation process.

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Tables

Table 1: Summary of oogenesis studies in scyphozoan jellyfish (where X = present; – = absent).

Order	Species	Histologic description	Ultrastructure description	References
Coronatae	*Atolla wyvillei*	X	–	Lucas & Reed 2010
	Linuche unguiculata	X	X	Eckelbarger & Larson 1992
	Nausithoe aurea	X	–	Morandini & Silveira 2001
	Periphylla periphylla	X	X	Tiemann & Jarms 2010; Lucas & Reed 2010
Semaeostomeae	*Cyanea capillata*	X	–	Widersten 1965
	Chrysaora hysoscella	X	–	Widersten 1965
	Chrysaora lactea	X	X	Present study
	Discomedusa lobata	X	–	Avian & Rotttini-Sandrini 1991
	Pelagia noctiluca	X	X	Avian & Rotttini-Sandrini 1991
	Aurelia aurita	X	X	Widersten 1965; Eckelbarger & Larson 1988;
Rhizostomeae	*Cassiopea sp.*	X	–	Hofmann & Hadfield 2002
	Cassiopea andromeda	x	–	Gohar & Eisawy 1960
	Cassiopea frondosa	X	–	Smith 1936
	Lychnorhiza lucerna	X	–	Schiariti et al. 2012
	Catostylus mosaicus	X	–	Pitt & Kingsford 2000
	Cotylorhiza tuberculata	X	–	Kikinger 1992
	Rhizostoma pulmo	X	–	Widersten 1965
	Mastigias papua	X	–	Uchida 1926
	Nemopileama nomurai	X	X	Ohtsu et al. 2007; Ikeda et al. 2011
	Stomolophus meleagris	X	–	Eckelbarger & Larson 1992

Table 2: Oogenesis and oocyte features compared to types of ovulations and fertilization.

Order	Species	Centrifugal gradient	Trophocytes	Vacuoles	Interdigitations	Intermembranous space	Nuage	Pinocytosis	"Vitelline membrane"	Ovulation	Fertilization	References
Coronatae	*Atolla wyvillei*	Yes	Absent	Absent	No data	No data	No data	No data	No data	No data	No data	Lucas & Reed 2010
	Linuche unguiculata	Yes	Absent	Absent	Yes	Yes	Yes	Yes	Yes	No data	No data	Eckelbarger & Larson 1992
	Nausithoe atlantica	Yes	Absent	Absent	Yes	Yes	Yes	Yes	Yes	No data	No data	Eckelbarger & Larson 1992
	Nausithoe aurea	Yes	Absent	Absent	No data	No data	No data	No data	No data	No data	External	Morandini & Silveira 2001
	Periphylla periphylla	Yes	Absent	Absent	No data	No data	No data	No data	No data	Expelled through pore	No data	Tiemann & Jarms 2010; Lucas and Reed 2010
Semaeostomeae	*Cyanea capillata*	No data	Yes	Absent	No data	No data	No data	No data	Yes	Absent	Internal (sperm entry in the ovary)	Widersten 1965
	Chrysaora hysoscella	No data	Yes	Absent	No data	No data	No data	No data	No data	Absent	No data	Widersten 1965
	Chrysaora lactea	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Disruption in the trophocytes	External	Present study
	Diplumaris antartica	No data	Yes	Yes	No data	No data	No data	No data	No data	No data	No data	Eckelbarger & Larson 1992
	Discomedusa lobata	Yes	Yes	Yes	Yes	No data	No data	Yes	Yes	Disruption in the trophocytes	No data	Avian & Rotttini-Sandrini 1991
	Pelagia noctiluca	Yes	Yes	Yes	Yes	No data	No data	Yes	Yes	Disruption in the trophocytes	No data	Avian & Rotttini-Sandrini 1991
	Aurelia aurita	Yes	Yes	Absent	Yes	Yes	Yes	Yes	Yes	Absent	Internal (sperm entry in the ovary)	Widersten 1965; Eckelbargaer & Larson 1988;
Rhizostomeae	*Cassiopea andromeda*	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	Gohar & Eisawy 1960
	Cassiopea frondosa	No data	No data	No data	No data	No data	No data	No data	No data	Disruption in the subgenital pit	Supposed to be external	Smith 1936
	Cassiopea xamachana	No data	Yes	No data	Yes	No data	No data	No data	No data	No data	No data	Eckelbarger & Larson 1992
	Lychnorhiza lucerna	Yes	Yes	Yes	No data	No data	No data	No data	No data	Expelled through pit	External	Schiariti et al. 2012
	Catostylus mosaicus	Yes	Yes	Yes	No data	No data	No data	No data	No data	Bulged into genital sinus	No data	Pitt & Kingsford 2000
	Cotylorhiza tuberculata	Yes	Yes	Absent	No data	No data	No data	No data	No data	Absent	Internal (sperm entry in the ovary in the genital sinus)	Kikinger 1992

Rhizostoma pulmo	Absent	Yes	Absent	Absent	No data	No data	Yes	Yes	Disruption in the trophocytes	Internal (in the genital sinus)	Widersten 1965
Nemopileama nomurai	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No data	External	Ohtsu et al. 2007; Ikeda et al. 2011
Stomolophus meleagris	No data	Yes	No data	Yes	Yes	Yes	Yes	Yes	No data	No data	Eckelbarger & Larson 1992

Figure captions

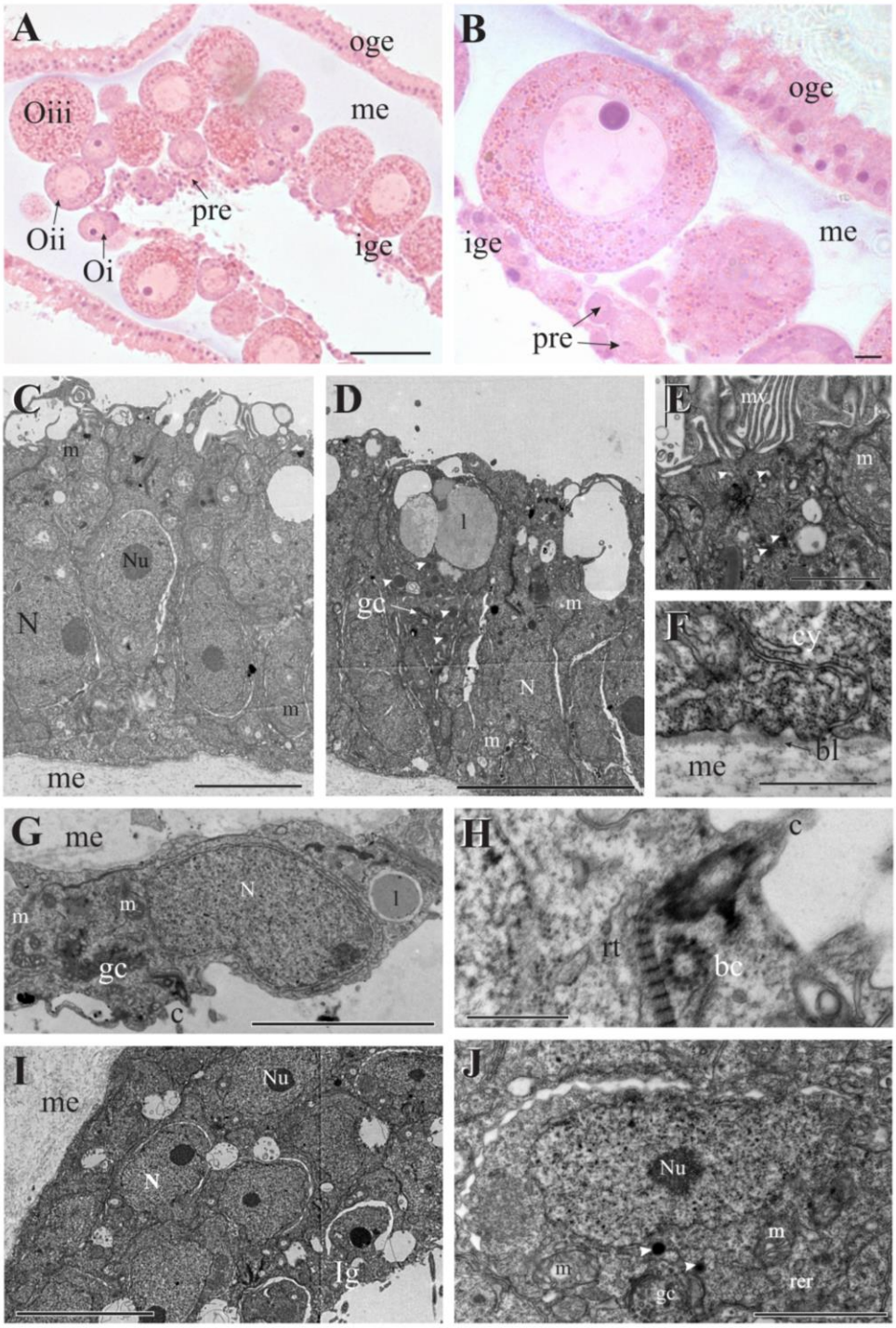

Figure 1: *Chrysaora lactea* female gonad, histologic and histochemistry sections (A and B) and ultrastructure micrographs (C-J). (A-B) Female gonad in longitudinal section stained with Hematoxylin & Eosin. Note the outer External genital epithelium (Eg), the inner Internal genital epithelium (ige) and between then the mesoglea (me) with oocytes in different developmental stages. (C-D) Ultrastructure of external genital epithelium (oge) showing two types of absorptive cells: type one in (C) with cilia anchored by a basal corpuscle (arrowhead) in the apical portion, several mitochondria (m) with tubular cristae, basal nucleus (N) with visible nucleolus (Nu) and type two in (D) with large lipids (l) drops and several electrodense vesicles (arrowhead) near Golgi apparatus (gc). (E) Both absorptive cells have several microvilli (mv) and membranous vesicles (white arrowhead) in the apical portion, occlusive junctions (black arrowhead) and mitochondria with tubular cristae (m). (F) In its basal portion, the cell is supported by the basal lamina (bl) and the fibrillar mesoglea (me). (G) A genital epithelium cell with lipid droplets (l) in the cytoplasm, elongated mitochondria (m) with tubular cristae, Golgi apparatus (gc) and cilia in the apical portion (c). (H) Detail of the rootlet (rt) and basal corpuscle (bc) anchoring the cilia (c). (I) Pre-vitellogenic oocytes inside the internal genital epithelium (Ig). Note the large pre-vitellogenic nucleus (N) and visible nucleolus (Nu). (J) Detail of pre-vitellogenic oocyte with cytoplasm rich in rugous endoplasmic reticulum (rer), Golgi apparatus (gc) with large electrodense vesicles (arrowhead) and mitochondria (m). Scale bars: (A) 50 µm, (B) 10 µm, (C) 5 µm, (D) 10 µm, (E) 2 µm, (F) 1000 nm, (G) 5 µm, (H) 500 nm, (I) 10 µm and (J) 2 µm.

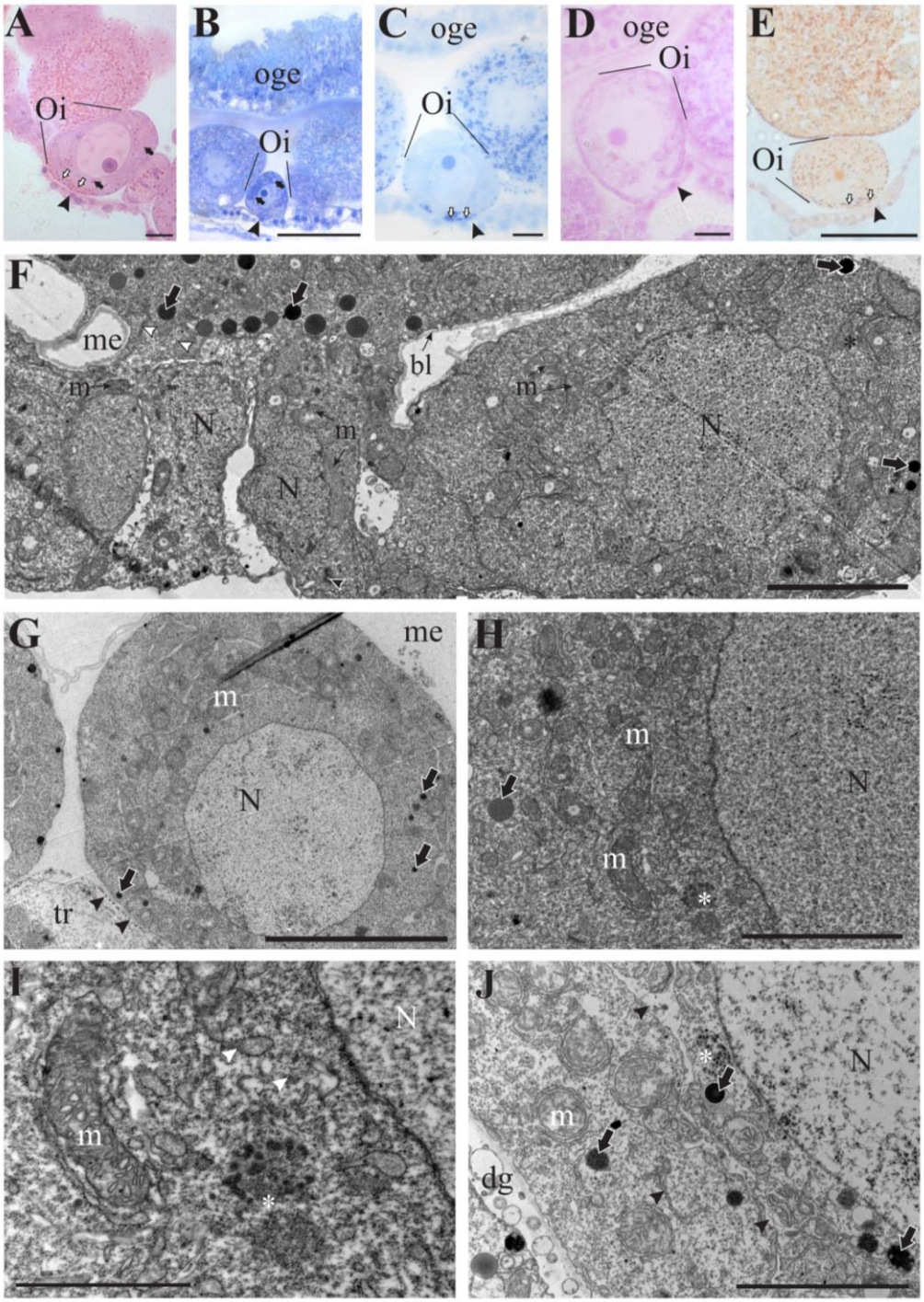

Figure 2: Histochemical sections (A-E) of trophocytes (black arrowhead) associated with early oocytes (Oi) of *Chrysaora lactea*. (A) Early oocyte stained with Hematoxylin & Eosin showing two types of granules in the cytoplasm: one basophil and large (black arrow) and other acidophil and small (white arrow). (B) Only the large basophil granules (black arrow) are positive to acid compounds stained with toluidine blue. (C) Only the small acidophil granules (white arrow) are positive to proteins stained with mercuric bromophenol blue. (D) No granules differentiation is evident when early oocytes are stained with PAS to neutral polysaccharides. (E) Only the small granules are positive to Mallory's trichrome stain. (F) Ultrastructural micrograph of trophocyte (left) associated with early oocyte (above) and pre-vitellogenic oocyte (right). Note the basal lamina (bl) adjacent to mesoglea (me), the nucleus (N), the mitochondria (m), the basal corpuscle (black arrowhead) in the apical cytoplasm of trophocytes and the interdigitations (white arrowhead) between the trophocytes and oocyte membrane. Only the large electrodense granules are present in the ooplasm (black arrow). (G) Detail of early oocyte beneath the mesoglea (Me). Note the large nucleus (N), several mitochondria (m) in the ooplasm and the trophocytes interdigitations (arrowhead) surrounding the oocyte. Only the large granules are present in the cytoplasm (black arrow). (H) Detail of the early ooplasm with several elongated mitochondria (m) and the nuage (*). Only the large granules are present in the cytoplasm (black arrow). (I) Near the nuage (*) electrodense vesicles are in the rugous endoplasmic reticulum (arrowhead) and the elongated mitochondria (m). (J) The large granules (black arrow) can be seen in the ooplasm near the nuage (*). At this stage we also see the parallel smooth membranes (arrowhead) in the perinuclear region. Note the trophocytes interdigitations (dg) around the oocytes. Scale bars: (A) 10 µm, (B) 50 µm, (C) 10 µm, (D) 10 µm, (E) 50 µm, (F) 10 µm, (G) 20 µm, (H) 5 µm, (I) 2 µm and (J) 5 µm.

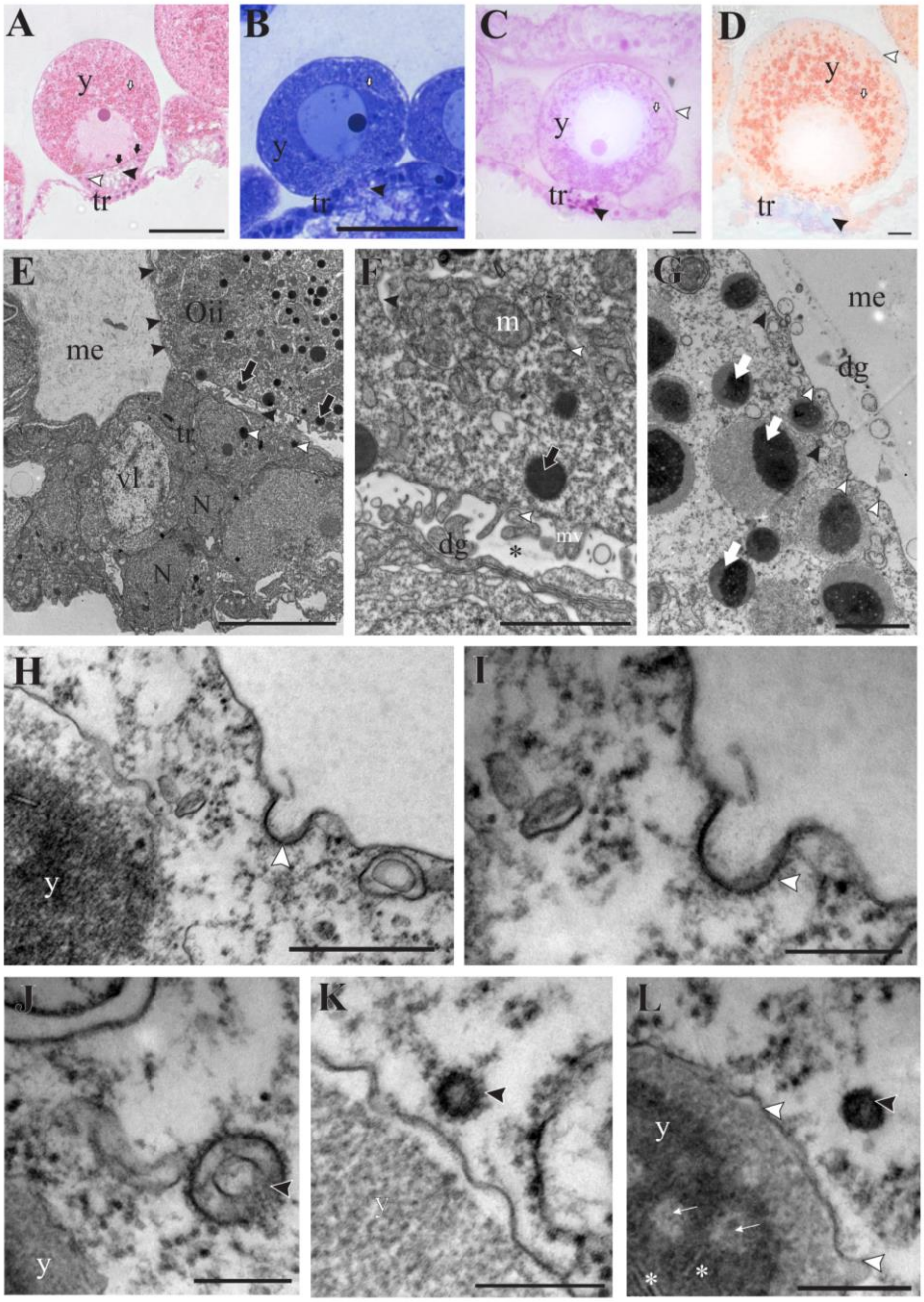

Figure 3: Histochemical sections (A-D) and ultrastructural micrographs (E-L) of trophocytes associated with intermediate oocytes (Oii) of *Chrysaora lactea*. (A) Longitudinal section stained with Hematoxylin & Eosin showing the trophocytes (tr) with lateral nucleus (white arrowhead) and large vacuoles in the cytoplasm (black arrowhead). The black arrow indicates the basophil granules and the white arrow the acidophil granules composing the yolk (y). (B) The trophocytes (tr) are strongly positive to Toluidine Blue stain showing positive reaction to acid compounds in the vacuoles content (arrowhead). Only the type 2 granules can be distinguished (white arrowhead) composing the yolk (y). (C) The content of the vacuoles (black arrowhead) of trophocytes (tr) was strongly positive to neutral polysaccharides (PAS stain). Only the type 2 granules can be distinguished (white arrowhead) composing the yolk (y). Note the positive reaction to the trophocytes interdigitations (white arrowhead) surrounding the oocyte. (D) The content of trophocytes (tr) vacuoles (black arrowhead) and the trophocytes interdigitations (white arrowhead) are positive to collagen in the Mallory's trichrome stain. Note the type 2 granules (white arrowhead) composing the yolk (y) (E) Ultrastructural micrograph of connection between intermediate oocyte (Oii) and trophocytes (tr) with some vacuoles (vl) in the cytoplasm. Note the trophocyte large lateral nucleus (N), the yolk electrodense vesicles (white arrowhead) near the oocyte portion and the interdigitations (black arrowhead) between the trophocyte and the oocyte near the mesoglea (me). In the black arrows note the yolk granules. (F) Detail of connection between the oocyte and trophocytes. Note the trophocyte interdigitations (dg) near the animal pole with microvilli (mv) and between them a fibrous extracellular matrix (*). Inside the ooplasm we can see electrodense granules (black arrow), the rugous endoplasmic reticulum (white arrowhead), ovoid mitochondria (m) and the parallel smooth membranes (black arrowhead) in the perinuclear region. (G) Detail of the contact of the vegetal pole with the trophocyte interdigitations (dg). Note the Mesoglea (Me), the several endocytic pits (white arrowhead), the vesicles (black arrowhead) near the oocyte membrane and the yolk electrodense granules (white arrow) in the cortical ooplasm. (H) Detail of the endocytic pit (white arrowhead) near the yolk (y) granule. (I) Detail of the membrane cell invagination forming the endocytic coated vesicle (arrowhead). (J) Detail of the vesicles already inside of the ooplasm (arrowhead) near the yolk (y). (K) Detail of an endocytic coated vesicle (arrowhead) almost fused with yolk (y) body membrane. (L) Endocytic coated vesicle (black arrowhead) approaching the yolk (y) granule fused with the yolk membrane (white arrowhead). Note the endocytic vesicles (arrow) already fused and inside the yolk body and the electrodense crystallized protein (*) storage inside the yolk body. Scale bars: (A) 50 µm, (B) 50 µm, (C) 10 µm, (D) 10 µm, (E) 10µm, (F) 2 µm, (G) 2 µm, (H) 500 nm, (I) 200 nm, (J) 200 nm, (K) 200 nm and (L) 200nm.

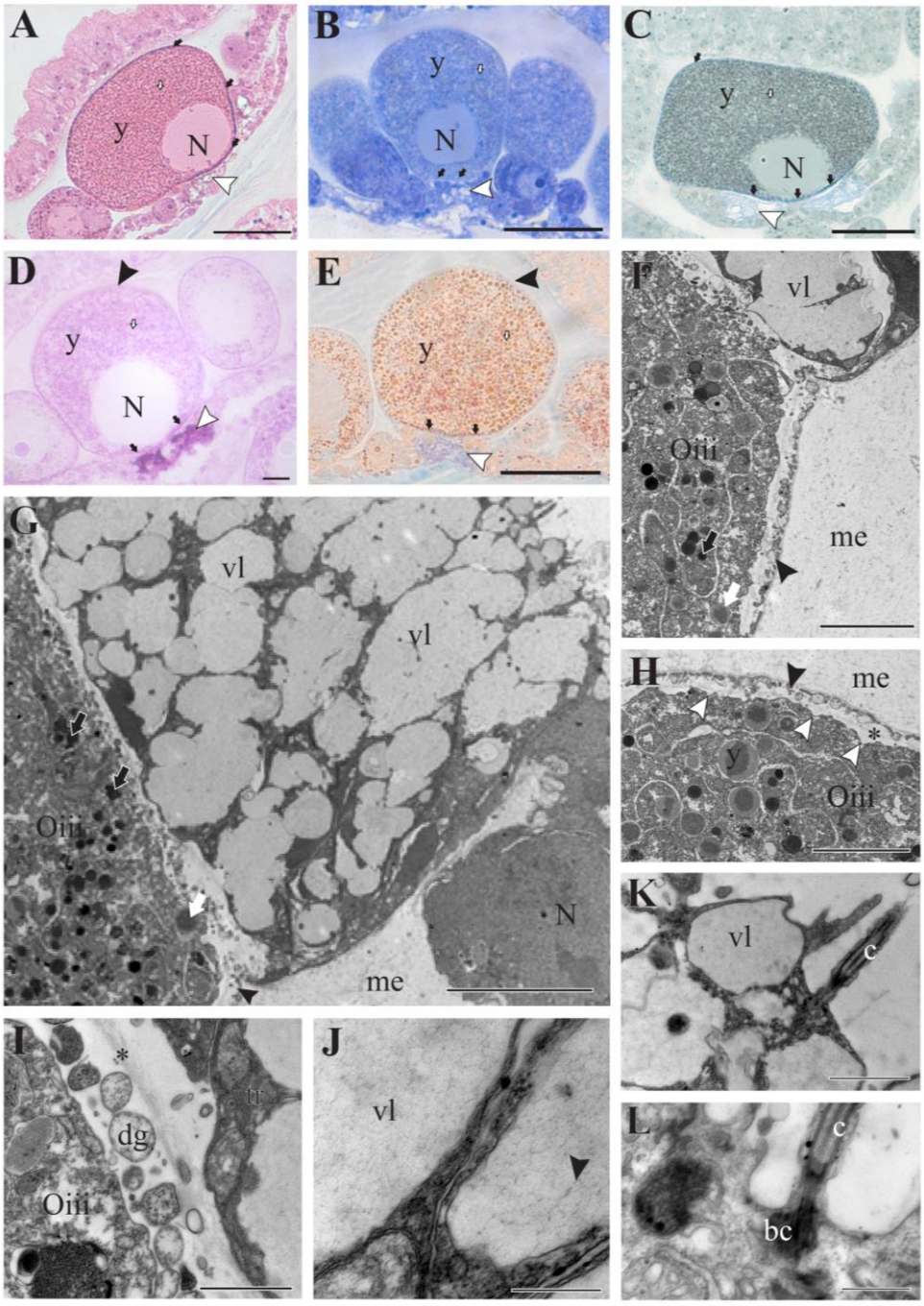

Figure 4: Histochemical sections (A-E) and ultrastructural micrographs (F-L) of trophocytes associated with late oocyte (Oiii) of *Chrysaora lactea*. (A) Late oocyte, stained with Hematoxylin & Eosin, protruding the animal pole in the trophocytes connection region (white arrowhead). Note the basophil granules around the oocyte (black arrow) and acidophil granules in the ooplasm (white arrow) compounding the yolk (y). (B) The content of vacuoles (white arrowhead) shows positive reaction to acid compounds in the slides stained with toluidine blue. Note the positive reaction in the granules around the oocyte (black arrow) and also the yolk acidophil granules (white arrow). (C) The content of the large vacuoles shows positive reaction to proteins (white arrowhead) when stained with the mercuric bromophenol blue. Note the positive reaction in the granules around the oocyte (black arrow) and the strongly positive reaction in the ooplasm granules - yolk (white arrow). (D) The content inside the vacuoles shows a strong reaction to neutral polysaccharides when stained with PAS (white arrowhead). Note the positive reaction around the oocyte (black arrowhead) and the positive reaction to both granules: found in the oocyte animal pole (black arrow) and in the ooplasm (white arrow) composing the yolk (y). (E) The content inside the vacuoles shows a strong reaction to collagen when stained with Mallory's trichrome stain (white arrowhead). Note the positive reaction to the trophocytes interdigitations (black arrowhead) surrounding the oocyte, the positive reaction in the granules in the oocyte animal pole (black arrow) and the strongly positive reaction in the ooplasm granules - yolk (white arrow). (F) Ultrastructural micrograph showing the trophocytes with large vacuoles (vl) near the animal pole (Oiii). Note the trophocytes interdigitations (black arrowhead) surrounding the oocyte lateral surface, isolating the oocyte contact with the mesoglea (Me). Both granules are seen in the late oocyte: the acidophil granule (white arrow) and the basophil (black arrow). (G) Longitudinal view of trophocytes large vacuoles (vl) with lateral nucleus (N). Note the interdigitations (black arrowhead) surrounding the oocyte adjacent to the mesoglea (Me). Both granules are seen in the late oocytes: the acidophil granule (white arrow) and the basophil (black arrow). (H) Trophocytes interdigitations (black arrowhead) surrounding the oocyte vegetal pole. Note the endocytic pits (white arrowhead) in the oocyte membrane that will compound the yolk body (y) and the fibrous extracellular matrix (*) between the oocyte membrane and trophocyte interdigitations. (I) Detail of the contact oocyte (Oiii) and trophocyte (tr) interdigitations (dg) in the animal pole and of the fibrous extracellular matrix (*) between them. (J) Detail of the fibrillar content (arrowhead) inside the trophocytes vacuoles (vl). (K) Apical view of trophocytes cell showing the cilia (c) near vacuoles (vl). (L) Detail of cilia (c) anchored in the apical cytoplasm by the basal corpuscle (bc). Scale bars: (A) 50 µm, (B) 50 µm, (C) 50 µm, (D) 10 µm, (E) 50 µm, (F) 200 nm, (G) 10 µm, (H) 500 nm, (I) 1 µm, (J) 2 µm, (K) 1 µm and (L) 500nm.

Sexual reproduction in three upside-down jellyfish (*Cassiopea* spp.): gonochorism, hermaphroditism and internal fertilization

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Abstract

The upside-down jellyfishes are widely distributed across the world living in estuarine tropical inshore seawaters. Even with the gametogenesis processes known for two of the 12 *Cassiopea* species, there is no comparative and detailed study about the sexual reproduction in this genus. Trying to clarify this subject, we describe the macroscopical and microscopical gonad morphology, gametogenesis and fertilization for the species *Cassiopea xamachana* (from Florida, USA), *Cassiopea andromeda* (from three different Brazilian locations: Cabo Frio, Alagoas and Ceará) and *Cassiopea frondosa* (from Florida, USA). The gametogenesis in *Cassiopea* is similar to that already described for other scyphozoan species with all germ cells occurring at the same time inside the gonad. The oocytes maintain the contact with the inner genital epithelium through trophocytes and have both, vitellogenesis and choriogenesis occurring ate the same time. *Cassiopea* seems to have the mesoglein, a homologue protein from the mammals ZP domains. As other Rhizostomeae species, *Cassiopea* release the sperm in *spermatozeugmata* and have internal fertilization. This is the first histological observation on the presence of a probable sequential hermaphroditism in the specie *Cassiopea frondosa* from Key Largo, Florida (USA).

Key-words: Gametogenesis, histology, Scyphozoa, cnidaria.

1. Introduction

The majority of true jellyfishes (222 scyphozoan species according to Jarms & Morandini, 2019) have a pelagic medusa stage as part of the metagenetic life cycle (Daly et al., 2007). Exceptions are some coronamedusae that have only the polyp stage – as *Nausithoe racemosa* and *Nausithoe eumedusoides* (Werner, 1973, 1974) – and the

rhizostome genus *Cassiopea* which have a metagenetic life cycle but with a semi-sessile medusae stage (Bigelow, 1900; Gohar & Eisawy, 1960; Hofmann et al., 1996). The upside-down jellyfishes (genus *Cassiopea*) are estuarine species which lives in shallow mangroves and tropical inshore seawaters (Holland et al., 2004; Keable & Ahyong, 2016; Kramp, 1961). The genus is widely distributed across the world and have 11 accepted species: *Cassiopea* sp. 2-4, *Cassiopea* sp. 6, *C. andromeda*, *C. culionensis*, *C. frondosa*, *C. maremetens*, *C. mayeri*, *C. ornata* and *C. xamachana* (Gamero-Mora, 2020; Gamero-Mora et al., 2022; Holland et al., 2004; Kramp, 1961; Morandini et al., 2016).

Scyphomedusae have in general separate sexes (Arai, 1997; Fautin, 1992). Exceptions are the protandrous semaeostome *Chrysaora hysoscella* (Berrill, 1949; Widersten, 1965), the coronates *Nausithoe marginata* and *N. eumedusoides* (Jarms, 1990; Werner, 1974, Chapter 3 of this thesis) and one *Cassiopea* unidentified from the Hilton Lagoon in Hawaii (Hofmann & Hadfield, 2002). In their study Hofmann & Hadfield (2002) analyzed the Hilton Lagoon medusae population and found that 29 of 39 specimens were simultaneous hermaphrodites with varying proportions of female and male parts throughout the gonads. In rhizostomes the gonad has a typical cross shape with an outer and inner genital epithelium and the asynchronous gametogenesis takes place inside the genital epithelia with the germ cells immersed in themesoglea (Gohar & Eisawy, 1960; Iguchi et al., 2010; Kikinger, 1992; Pitt & Kingsford, 2000; Schiariti et al., 2012; Smith, 1936, chapter 2 of this thesis). For rhizostomes, it has been registered two strategies of sperm release: free or clustered in spermatozeugmata (Ikeda, Ohtsu, & Uye, 2011; Kikinger, 1992; Ohtsu et al., 2007; Pitt & Kingsford, 2000; Schiariti et al., 2012; Widersten, 1965). Concerning the oogenesis, with the exception of *Cassiopea* species, all other discomedusae have the trophocytes, specific nutritive cells which helps in the vitellogenesis process and ovulation (Avian & Rottini-Sandrini, 1991; Eckelbarger, 1994; Eckelbarger & Larson, 1988, Tiseo & Morandini chapter 3 of this thesis). Adonin et al

(2009) reported a protein agglomerate (called mesoglein) in the animal pole of the oocyte that is homologous to the mammal's zona pellucida proteins: indicating a probable vitellin membrane (or region of sperm recognition) in *Aurelia* oocytes. Based in the histochemistry and ultrastructural findings, Tiseo & Morandini (chapter 3 of this thesis) identified that not only the vitellogenesis is occurring inside the *Chrysaora lactea* oocytes. There is a production of protein (probable mesoglein) involved in the masking of a vitellin membrane in early to late oocytes. Thus, both vitellogenesis and choriogenesis are occurring inside the female gonad.

The gametogenesis was already described for *C. andromeda* from the Red sea (Gohar & Eisawy, 1960) and for *Cassiopea frondosa* from Fort Jefferson (Smith, 1936). Tiseo et al submitted (chapter 2 of this thesis) described the spermatogenesis and the sperm ultrastructure of *Cassiopea maremetens* from Queensland, Australia, being this the only ultrastructural work for the genus. Even with the gametogenesis processes known for two of the 12 *Cassiopea* species, no comparative and detailed studies in the sexual reproduction for this genus were conducted and some questions about the gonochorism and hermaphroditism, the presence of internal fertilization and trophocytes in the oogenesis still remain unanswered. Herein, we describe the macroscopical and microscopical gonad morphology, gametogenesis and fertilization for the species *Cassiopea xamachana* (from Florida, USA), *Cassiopea andromeda* (from three different Brazilian locations: Cabo Frio, Alagoas and Ceará) and *Cassiopea frondosa* (from Florida, USA). Additionally, we made histological observations on the presence of a sequential hermaphroditism in the specie *Cassiopea frondosa* from Key Largo, Florida (USA).

2. Material and Methods

2.1. Sampling

Specimens of *Cassiopea andromeda* from three different localities (11 individuals from Cabo Frio, Rio de Janeiro – Fig. 1A – Coordinates: -22.87555556, -42.01861111; 3 individuals from Foz do Rio Meirim, Maceió, Alagoas – Fig. 1B – Coordinates: -9.54805556, -35.62194444; 4 individuals from Fazenda Aqua Crusta, Acaraú, Ceará – Fig. 1C – Coordinates: -2.88000000, -39.90416667), *Cassiopea xamachana* (7 individuals from Key Largo, Florida, USA, Coordinates: 25.101460, -80.438519) and *Cassiopea frondosa* (6 individuals from Key Largo, Florida, USA – Fig. 1D – Coordinates: 25.101460, -80.438519) were collected and fixed in formaldehyde solution 4% prepared with seawater from the collection site. In the laboratory, all 31 specimens were measured following (Iguchi et al., 2010) and the bell diameter (BD, distance from opposites rhopalia, in cm) were determined. We photographed specimens using a Nikon D7000 digital camera and used a stereomicroscope Zeiss SV11with scales calibrated for the magnification used to register the morphological observations.

2.2. Microscopical techniques

Samples of gonadal tissue already in 4% formaldehyde solution were dehydrated gradually in ethanol series (30-95%), and embedded in Leica® methacrylate historesin. Serial sections 3 μm thick were made on a rotating microtome. Slides with the tissue samples were stained with hematoxylin and eosin (HE) without baths in ethanol and xylene (Sant'Anna et al., 2010; Tiseo et al., 2014, 2019), mercuric bromophenol blue for proteins (Pearse, 1960), PAS technique (Junqueira & Junqueira, 1983) used to visualize neutral polysaccharides, toluidine blue stain (Audino et al., 2015) was used to visualize acid components, Gomori's trichomic and the Mallory's trichromic stain weres used to visualize collagen and mucus (Bancroft & Stevens, 1982; Humason, 1962).

2.3. Cellular Measurements

To organize the types of germ cells, in both females and males, we took, at least, 35 measurements of diameterof the four maturation stages of oocytes (pre-vitellogenic, early, intermediate and late oocytes) and spermatogenic cells (spermatogonia, spermatocyte, spermatid and sperm). Measurements were taken only at the 40x magnification micrographs using the ImageJ software with appropriate scale calibrations (Tiseo et al., 2019). Minimum and maximum diameters are presented inside the parenthesis as follow (minimum – maximum).

3. Results

Structure of *Cassiopea* gonad and gametogenesis

As the main gonadal morphology of all species are practically identical, we use *C. xamachana* as a model, and highlight the specific dissimilarities of each species in separates sections. The gonads of all *Cassiopea* species, are located in the gastrovascular cavity near the gastric cirri in the gastric pouches. From the aboral view, the gonads have the shape of a cross (Fig. 1F) being each arm of the gonad a tissue band-like that originates as an evagination of the gastrodermis (Fig. 1G) that folds in over itself as it grows (Fig. 1H). Seen in a cross section the gonad has two layers of tissue: the outer and inner genital epithelium and between them lies the germinative cells immersed in the mesoglea, where the gametogenesis occurs (Fig. 2A, 5A).

In the female gonad, the inner genital epithelium is located more internally inside the gastric cavity and is of the simple cuboidal type (Fig. 2A). The outer genital epithelium is located more externally in the gastric cavity, has elongated columnar cells with vacuoles not stained with Hematoxylin and Eosin (Fig. 2A), acids structures (Fig. 2B), proteins (Fig. 2C), positive to neutral polysaccharides (Fig. 2D) and mucus (Fig. 2E). Inside the inner genital epithelium of the female gonad are the pre-vitellogenic oocytes (6,4 - 14,75 µm), round cells of basophil nucleus and acidophil cytoplasm (Fig.

2F). Near the pre-vitellogenic oocytes, moving to the mesoglea are the early oocyte (39 - 61,44 µm) usually with visible nucleus and nucleolus round in shape with very basophil cytoplasm (Fig. 2F). The early oocyte cytoplasm (ooplasm) is positive to acid structures (Fig. 2G), proteins (Fig 2H) and neutral polysaccharides (Fig. 2I). The protein granules inside the cytoplasm are mostly blue when stained with Gomori's trichromic (Fig. 2J) and orange in Mallory's trichromic (Fig. 2K), but pink or red granules are seen near the oocyte animal pole (Fig. 2J-K). The intermediate oocyte is bigger than the early oocyte (71,84 – 91,2 µm) and differs from late oocyte by the smaller size and mostly because it is less acidophil (Fig. 3A). Two proteins granules are recognizable in the ooplasm one acidophil and one basophil near the animal pole (Fig. 3A). The acidophil granule is positive to acid structures (Fig. 3B), positive to proteins (Fig. 3C), positive neutral polysaccharides (Fig. 3D), blue in the Gomori's trichromic (Fig. 3E) and orange in Mallory's trichromic (Fig. 3F). The basophil granule is strongly positive to acid structures (Fig. 3B), strongly positive to proteins (Fig. 3C), positive neutral polysaccharides (Fig. 3D), pink in the Gomori's trichromic (Fig. 3E) and red in Mallory's trichromic (Fig. 3F). As soon as the oocyte increase in size, it maintains the contact with the inner genital epithelium via trophocytes, specific cells with basal nucleus (Fig. 3B-C) and with a depression near the animal pole (Fig. 3F). The late oocyte is bigger than early and intermediate oocyte (119,44 – 194,68 µm, Table 1 for details) and have a very acidophil cytoplasm (Fig. 4A). As in the intermediate oocyte both granules are seen in the late oocyte (Fig. 4A-F) but it differs mainly in the locations inside the ooplasm. In late oocyte, the basophil granule (Fig. 4A), strongly positive to acid structures (Fig. 4B), strongly positive to proteins (Fig. 4C), positive neutral polysaccharides (Fig. 4D), pink in the Gomori's trichromic (Fig. 4E) and red in Mallory's trichromic (Fig. 4F) are surrounding the entire oocyte not only located in the animal pole.

The male gonad also has an outer and inner genital epithelium surrounding the follicles with spermatogenic cells immersed in the mesoglea (Fig. 5A). The outer genital epithelium has several vacuoles not stained with hematoxylin and eosin (Fig 5A), acids structures (Fig. 5B), proteins (Fig. 5C), positive to neutral polysaccharides (Fig. 5D) and mucus (Fig. 5E). The follicle is composed by spermatogonia that surround the spermatocytes, spermatids and sperm (Fig. 5A). The spermatogonia are round cells with large nucleus (3,51 – 5,15µm) and visible basophil nucleolus (Fig. 5F-G). The spermatocytes are smaller than spermatogonia (2,69 – 3,21µm), have round basophil and more compact nucleus (Fig. 5F-G). Occasionally spermatocytes can be observed in meiotic stages as metaphasis (Fig.5G). The spermatids are round smaller cells (1,28 – 2,33µm) of basophil nucleus that stay interconnected by cytoplasmic bridges (Fig. 5G). The sperm has a conical elongated head (2,12 – 3,05µm) with basophil nucleus clustered in an extracellular matrix with free flagellum in a structure called *spermatozeugmata* (Fig. 5G). The extracellular matrix is negative to hematoxylin and eosin (Fig. 5H), acid structures (Fig. 5I), positive to proteins (Fig. 5J) and neutral to polysaccharides (Fig. 5K), negative to mucus (Fig. 5L-M).

Cassiopea frondosa (Florida, USA) hermaphrodites' specimens and internal fertilization

The gonad of *C. frondosa* has the cross-shape structure and is located near the gastric cirri similar to that observed in other *Cassiopea* species but with some particularities. For the four individuals analyzed the female gonad was evident (Fig. 6). In the epithelium, near the genital sinus of the smaller specimen (8.5 cm diameter) several clusters of sperm are visibly organized in vesicles (Fig. 6A-C), but only the beginning of the female gonad is recognized. In the second smaller specimen (11.1 cm diameter) the sperm cluster enclosed by the epithelium are seen closer to oocytes in the typical female

gonad (Fig. 6D). While in the two largest specimens (12.4 and 13.4 cm diameters respectively) several free sperm are seen near and inside the typical female gonad (Fig. 7A-E). Inside the genital epithelium of the third specimen (with 12.4 cm diameter), are the pre-vitellogenic oocytes, with several sperm inside the genital sinus (Fig. 7C) closer to the oocytes and trophocytes (Fig.7D). In the largest specimen, free sperm can be seen near the outer genital epithelium (Fig. 7E), inside the genital sinus (Fig. 7F-G), near the genital epithelium of female gonad (Fig. 7G) and crossing the inner genital epithelium through trophocytes (Fig. 7H).

4. Discussion

The gametogenesis in the *Cassiopea* species resembled other descriptions of rhizostomes and semaoestomes jellyfishes (Kikinger, 1992; Ohtsu et al., 2007; Pitt & Kingsford, 2000; Schiariti et al., 2012). The pre-vitellogenic oocytes located inside the inner genital epithelium starts choriogenesis and vitellogenesis bulging inside the mesoglea but maintaining the contact with the genital epithelium through the trophocytes (Avian & Rottini-Sandrini, 1991; Eckelbarger, 1994; Eckelbarger & Larson, 1988; Ikeda et al., 2011). Trophocytes were already described for several discomedusae species as *C. lactea*, *Aurelia aurita*, *Lychnorhiza lucerna*, *Catostylus mosaicus*, *Nemopilema nomurai* and *Stomolophus meleagris* (Eckelbarger & Larson, 1992, 1988; Ikeda et al., 2011; Pitt & Kingsford, 2000; Schiariti et al., 2012, chapter 3 of this thesis), *Cyanea capillatta*, *Chrysaora hysoscella*, *Rhizostoma pulmo, Cotylorhiza tuberculata* (Widersten, 1965 and Kikinger 1992 called as nurse cells by the authors), *Diplumaris antartica*, *Discomedusa lobata* and *Pelagia noctiluca* (which Avian & Rottini-Sandrini 1991 called as paraovular body). Smith (1936) and Gohar & Eisawy (1960) described the oogenesis for *Cassiopea frondosa* and *Cassiopea andromeda*, respectively, but did not mention any kind of accessory cells. In contrast, Eckelbarger & Larson (1992) saw trophocyte like cells in *C.*

xamachana. In our findings trophocytes similar in morphology, to that already described for *Aurelia aurita* (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988), *Cyanea capillatta* and *Rhizostoma pulmo* (Widersten, 1965) were seen in all three species studied: *C. xamachana, C. andromeda* and *C. frondosa*. But with the absence of the large vacuoles with mucus content as described in *P. noctiluca* and *C. lactea* (Avian & Rottini-Sandrini, 1991, chapter 3 of this thesis). Taking into account Eckelbarger and Larson (1992) and Avian & Rottini-Sandrini (1991) findings and our own observations on trophocytes, we suggest that there are two different types of trophocytes in discomedusae: 1. The original trophocyte described by Eckelbarger and Larson (1988) without the large vacuoles and with a crypt as described by Widersten (1965); and 2. The trophocyte described firstly by Avian & Rottini-Sandrini (1991) and observed in *C. lactea* (chapter 3 of this thesis), with large vacuoles positive to neutral polysaccharides and mucus.

In Discomedusae the vitellogenesis is a documented process involving two stages of protein production: one endogenous (autosynthetic) and other exogenous (heterosynthetic) yolk manufacturing (Avian & Rottini-Sandrini, 1991; Eckelbarger, 1994; Eckelbarger & Larson, 1992; Eckelbarger & Larson, 1988; Eckelbarger & Hodgson, 2021; Ikeda et al., 2011). Adonin et al. (2009) pointed that there was some missing information concerning the oogenesis in scyphozoan, as almost no attention has been given to the subject of a vitellin membrane formation. The authors found a specific protein – called mesoglein – homologous to the zona pellucida proteins domains, and trace the existence of it inside *Aurelia aurita* oocyte. Following this statement, Adonin & Podgornaya (2012) described the presence of a contact plate in the animal pole of oocytes of *A. aurita* oocytes stating that this structure is responsible for sperm recognition during the fertilization. Additionally, Tiseo & Morandini (chapter 3 of this thesis) observed that, for *C. lactea*, the vitellogenesis process occurs at the same time of choriogenesis (or vitelline membrane production) and conclude that together with the endogenous yolk

production, the oocyte also produces mesoglein. In *C. lactea* late oocytes, the histochemistry showed that mesoglein surround the entire oocyte. Our observations on histochemistry of the *Cassiopea* female gonad, indicate that all three species studied herein also have two different proteins being produced inside oocytes: one a acidophil and stained by blue at Gomori's trichromic and other basophil stained by pink at Gomori's trichromic. The acidophil proteins are found in the animal pole near the connection with trophocytes just like the contact plate described in *A. aurita* (Adonin & Podgornaya, 2012), suggesting that this structure is also present in the *Cassiopea* oocytes. Even with these positive histochemical evidences, it is necessary to deepen the knowledge looking for ultrastructural and fluorescence details of the mesoglein vitellin membrane in *Cassiopea* oocytes.

Similar to *Mastigias papua* (Uchida, 1926), *C. tuberculata* (Kikinger, 1992), and *Phyllorhiza punctata* (Rouse & Pitt, 2000), *Cassiopea* release the sperm in *spermatozeugmata:* the sperm head stays clustered in an extracellular matrix with free flagella (Gohar & Eisawy, 1960; Smith, 1936, Tiseo et al. chapter 2 of this thesis). Differently what Werner (1973) described for *Tripedalia cystophora* – in which numerous *spermatozeugmata* are enclosed into globular spermatophores and later transferred directly to the female by the tentacles in a mating process – Kikinger (1992) mentioned that the *spermatozeugmata* of *C. tuberculata* make the way out through the follicle wall and genital epithelium reaching the genital sinus, being released in open water still as *spermatozeugmata*. Kikinger (1992) also saw that the *spermatozeugmata* reaches the female genital sinus through the moutharm canals and starts to disintegrate, with the observation of free sperm found near the female gonad. Here we also saw clusters of *spermatozeugmata* to be released in the genital sinus just like Tiseo & Morandini (submitted) described to *Cassiopea maremetens.*

In scyphozoan species the fertilization is believed to occurs inside the gastrovascular cavity and alternatively it may occur in the female gonad itself, on the oral arms or in open sea water (Arai, 1997). Widersten (1965) observed an internal fertilization inside the female gonad for *Cyanea capillata*, *Aurelia aurita, Chrysaora hysoscella* and *Rhizostoma pulmo*. Differently to that observed by Widersten (1965), Russell (1970) saw that *Aurelia aurita* release the oocytes through the trophocytes in the genital sinus where fertilization occurs. Avian & Rottini-Sandrini (1991) described an internal fertilization in *R. pulmo* with the oocytes released through the trophocytes and fertilized in the genital sinus. *Pelagia noctiluca* and *Discomedusa lobata* seems to have an external fertilization as Avian & Rottini-Sandrini (1991) saw these species' oocytes passing through the trophocytes and being spawned into open sea immersed in a mucus strand. Kikinger (1992) described that for *C. tuberculata* the fertilization occurs inside the female gonad as free sperm are seen near the trophocytes. Pitt & Kingsford (2000) saw late oocytes bulging into the genital sinus suggesting that the fertilization in *C. mosaicus* occurs outside the female gonad but inside the medusae as this species broods larvae. Ikeda et al. (2011) and Schiariti et al. (2012) observed that, in the species *N. nomurai* and *L. lucerna*, the oocytes are squeezed out just at the place where the oocytes contact the trophocytes, suggesting that fertilization occurs outside the female gonad. Tiseo & Morandini (submitted) also saw the *C. lactea* oocyte bulge through trophocytes in the genital sinus what corroborates with the external fertilization described by Morandini et al. (2004). Our observations on *C. xamachana* and *C. andromeda* – of the late oocyte bulging into the central region of trophocytes – suggests that the fertilization in these species occurs outside the female gonad and inside the genital sinus just like Smith (1936) described. Gohar & Eisawy (1960) observed the oocytes of *C. frondosa* being "discharged" in the genital sinus. In contrast, no oocytes are seen releasing or bulging but free sperm inside the female gonad near the trophocytes in *C. frondosa* from Key Largo.

Thus, suggesting an internal fertilization inside female gonad like Kikinger (1992) described in *C. tuberculata*. This divergent information led us to conclude that both types of internal fertilization (in the genital sinus and inside the female gonad) occur in *C. frondosa* similar to *Aurelia aurita* (Russel, 1970; Widersten, 1965) and *Rhizostoma pulmo* (Avian & Rottini-Sandrini, 1991; Widersten, 1965).

Among the Discomedusae, the hermaphroditism is observed only in two species: *Chrysaora hysoscella* and *Cassiopea* sp. from Hilton lagoon (Haeckel, 1880; Hofmann & Hadfield, 2002; Widersten, 1965). The hermaphroditism described in *C. hysoscella* is of protandrous type: small specimens have only male gonads; intermediated-sized individuals have a typical female gonad but several follicle-like structures (called "sperm sacs" by Haeckel 1880) seen in different parts of the specimen subumbrellar mesoglea or epithelia; and large specimens have only the female gonad. The hermaphroditism described by Hofmann & Hadfield (2002) in *Cassiopea* sp. is of simultaneous type: all medusa sizes have male and female gonads in different proportions with oocytes and follicles being at the same time in the gonad. In our observations, the smaller specimens of *C. frondosa* have several vesicles with sperm accumulated in the outer genital epithelium while the larger specimens have only the female gonad with several sperm inside of the genital sinus. Kikinger (1992) pointed that, in *C. tuberculata*, the *spermatozeugmata* enters the female gastrovascular cavity through the moutharms and disintegrate on the way to the genital sinus with only the sperm entering the female gonad. Differently to the described to *C. tuberculata*, the sperm in *C. frondosa* seems to be stored by the smaller medusae not entering in the female gonad. As this specific feature was present in large quantities (about 6 vesicles in the gonad) in the smaller specimen (8.5 cm of diameter), in less quantity (about 2 sperm vesicles in the gonad) in the 11.1 cm specimen and being absent in the larger specimens, we believed that the presence of the sperm storage could be an indicative of a sequential hermaphroditism. Our hypothesis is

that smaller specimens of *C. frondosa* (from Key Largo) have only the male gonad producing sperm. As soon as the individuals increase in size, only the remnants of the male gonad are present with the sperm being stored in the vesicles of the outer genital epithelium. Once the medusa reaches larger sizes the vesicles disrupt and release the sperm in the genital sinus where only the female gonad is present.

Conclusions

The gonad in cross shape is in the pattern of Rhizostomeae and the gametogenesis is similar to that already described in other species of Discomedusae. The size of oocytes is also in the average of other Rhizostomeae species. The production of sperm clustered in *spermatozeugmata* is characteristic and could be related with internal fertilization as the presence of sperm inside the genital sinus near the oocytes and also the presence of trophocytes in the female gonad. The sperm clusters described here in *C. frondosa* were never observed in any other scyphozoan, being this the first record of a vesicle filled with sperm enclosed by the outer genital epithelium. Both reproduction strategies, gonochorism and hermaphroditism, seems to be present in species of the genus *Cassiopea*. The hermaphrodite condition in the genus is a trait to be studied, but as it was found in only two different species, we cannot yet discuss anu evolutionary trend within the group.

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Tables

Table 1: Minimum and maximum (min – max) measurements of male and female germ cells of *Cassiopea* species. N= number of measurements; - absence of data.

	Cassiopea xamachana	*Cassiopea andromeda* (Cabo Frio)	*Cassiopea andromeda* (Alagoas)	*Cassiopea andromeda* (Ceará)	*Cassiopea frondosa*
Spermatogonia	3,50 - 5,15 n= 45	4,34 - 7,48 n= 42	-	-	-
Spermatocyte	2,69 - 3,22 n= 36	2,51 - 3,86 n= 39			-
Spermatid	1,28 - 2,33 n= 45	1,26 - 2,73 n= 45			-
Sperm	2,12 - 3,00 n=39	2,17 - 3,02 n= 48			3,19 - 5,59 n= 21
Pre-vitellogenic oocytes	6,4 - 14,75 n= 60	-	8,13 - 15,42 n= 45	9,01 - 17,16 n= 72	8,94 -16,44 n= 25
Early oocytes	39 - 61,44 n= 12		17,08 - 37,73 n= 60	19,23 - 32,94 n= 56	17,32 - 36,36 n= 20
Intermediates oocytes	71,84 - 91,2 n= 28		42,55 - 89 n= 90	38,02 - 87,23 n= 51	46,12 - 90,21 n= 68
Late oocytes	119,44 - 194,68 n= 24		109,49 - 161,08 n= 27	109,26 - 131,37 n= 40	101,76 - 143,79 n= 24

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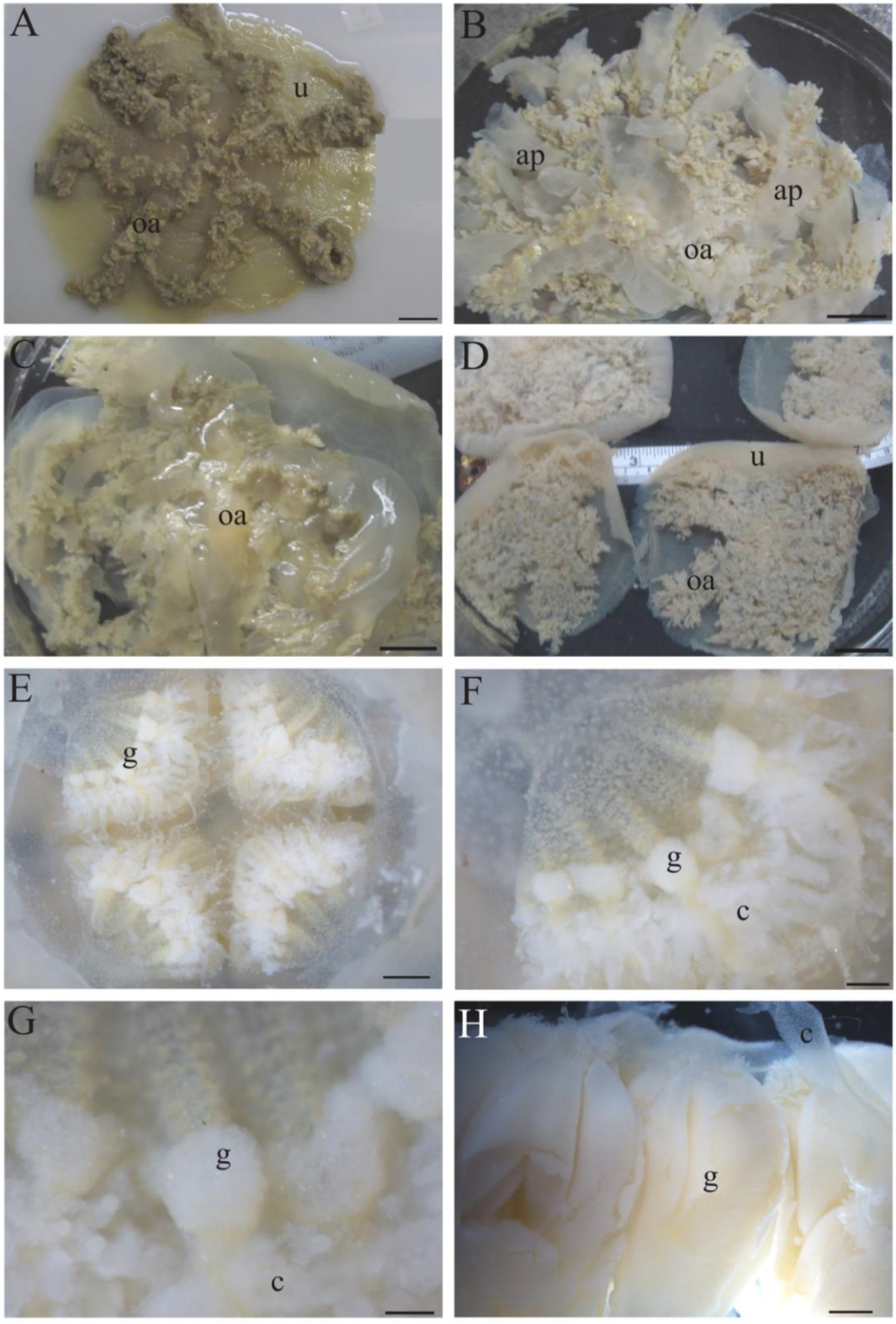

Figure 1: General view of the three target species with gonad details. (A) Oral view of *Cassiopea andromeda* (from Cabo Frio), scale: 2 cm. (B) Oral view of *Cassiopea andromeda* (from Alagoas), scale: 2 cm. (C) Oral view of *Cassiopea andromeda* (from Ceará), scale: 2 cm. (D) Oral view of the four specimens of *Cassiopea frondosa*, scale: 2 cm. (E) Macroscopic view of the four gonads in a cross shape, scale: 500 µm. (F) Detail of one arm of the gonad near the gastric cirri, scale: 200 µm. (G) Gonad with several folds, scale: 100 µm (H) Detail of the larger male gonad with several folds, scale = 1000 µm. Appendages = ap; gastric cirri = c; gonad = g; oral arms = oa; umbrella = u.

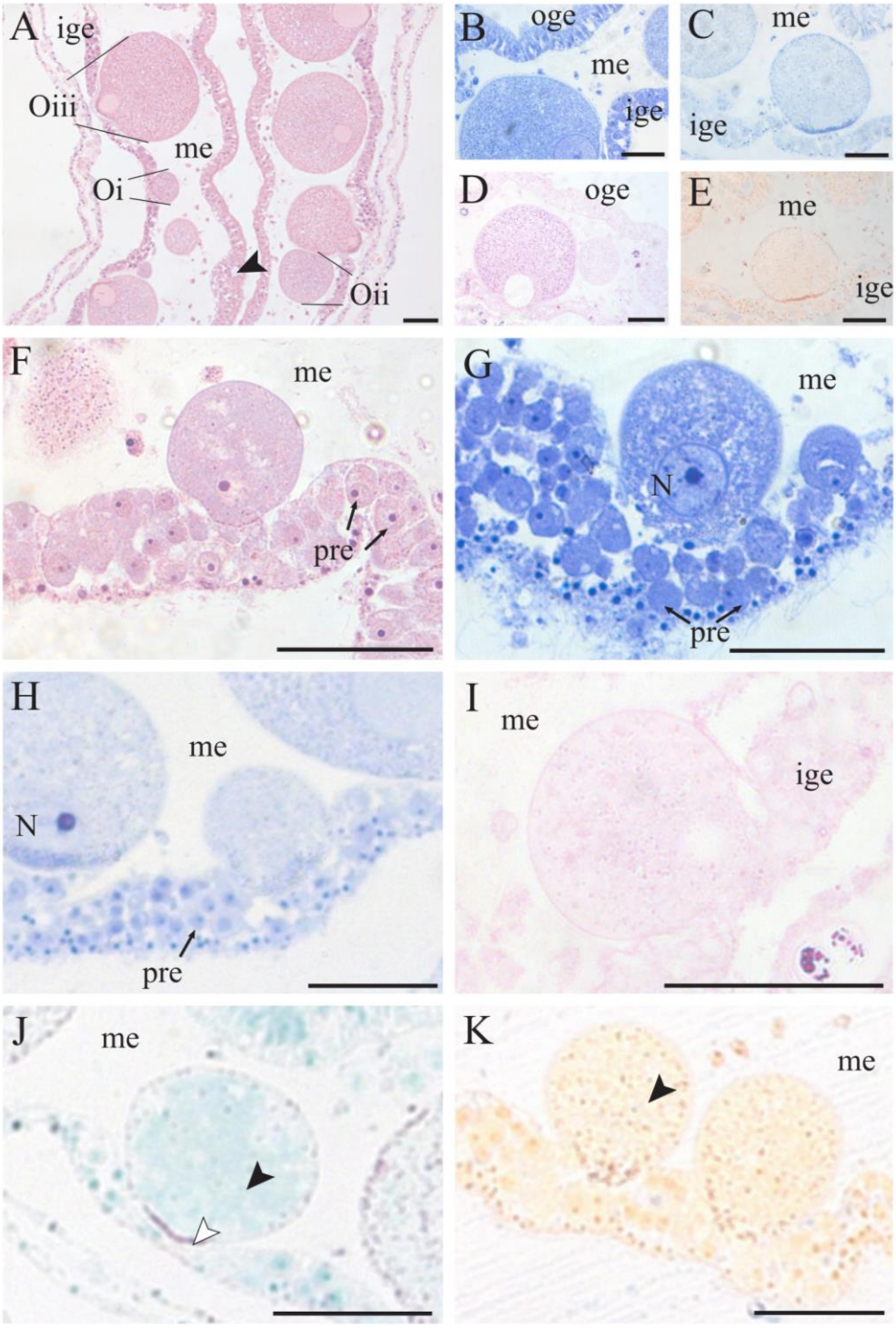

Figure 2: Oogenesis in *Cassiopea xamachana* – pre-vitellogenic and early oocytes. (A) Hematoxylin & Eosin. General view of female gonad. The tree stages of oocytes (early, intermediate and late) are seen immersed in the mesoglea surrounded by an outer and inner genital epithelium. Note the basophil vesicles inside the outer genital epithelium (arrowhead), scale: 100 µm. (B) Toluidine blue. Detail of the outer and inner genital epithelium, scale: 50 µm. (C) Mercuric bromophenol blue. Detail of the outer and inner genital epithelium, scale: 50 µm. (D) PAS. Detail of the outer and inner genital epithelium, scale: 50 µm. (E) Mallory's trichomic. Detail of the outer and inner genital epithelium, scale: 50 µm. (F) Hematoxylin & Eosin. Detail of the inner genital epithelium with the pre-vitellogenic oocytes. Note the basophil early oocyte, scale: 50 µm. (G) Toluidine blue. Inner genital epithelium with pre-vitellogenic oocytes and early oocyte positive to acids structures, scale: 50 µm. (H) Mercuric bromophenol blue. Inner genital epithelium with pre-vitellogenic oocytes and early oocyte positive to proteins, scale: 50 µm. (I) PAS. Inner genital epithelium with pre-vitellogenic oocytes and early oocyte in mesoglea, scale: 50 µm. (J) Gomori's trichomic. Early oocyte with proteins granules stained in blue (black arrowhead) or pink (white arrowhead), scale: 50 µm. (K) Mallory's trichomic. Note the orange granules (arrowhead) in the early ooplasm, scale: 50 µm. Early oocyte = Oi; Inner genital epithelium = ige; intermediate oocyte = Oii; late oocyte = Oiii; mesoglea = m; nucleus = N; outer genital epithelium = oge; pre-vitellogenic oocytes = pre.

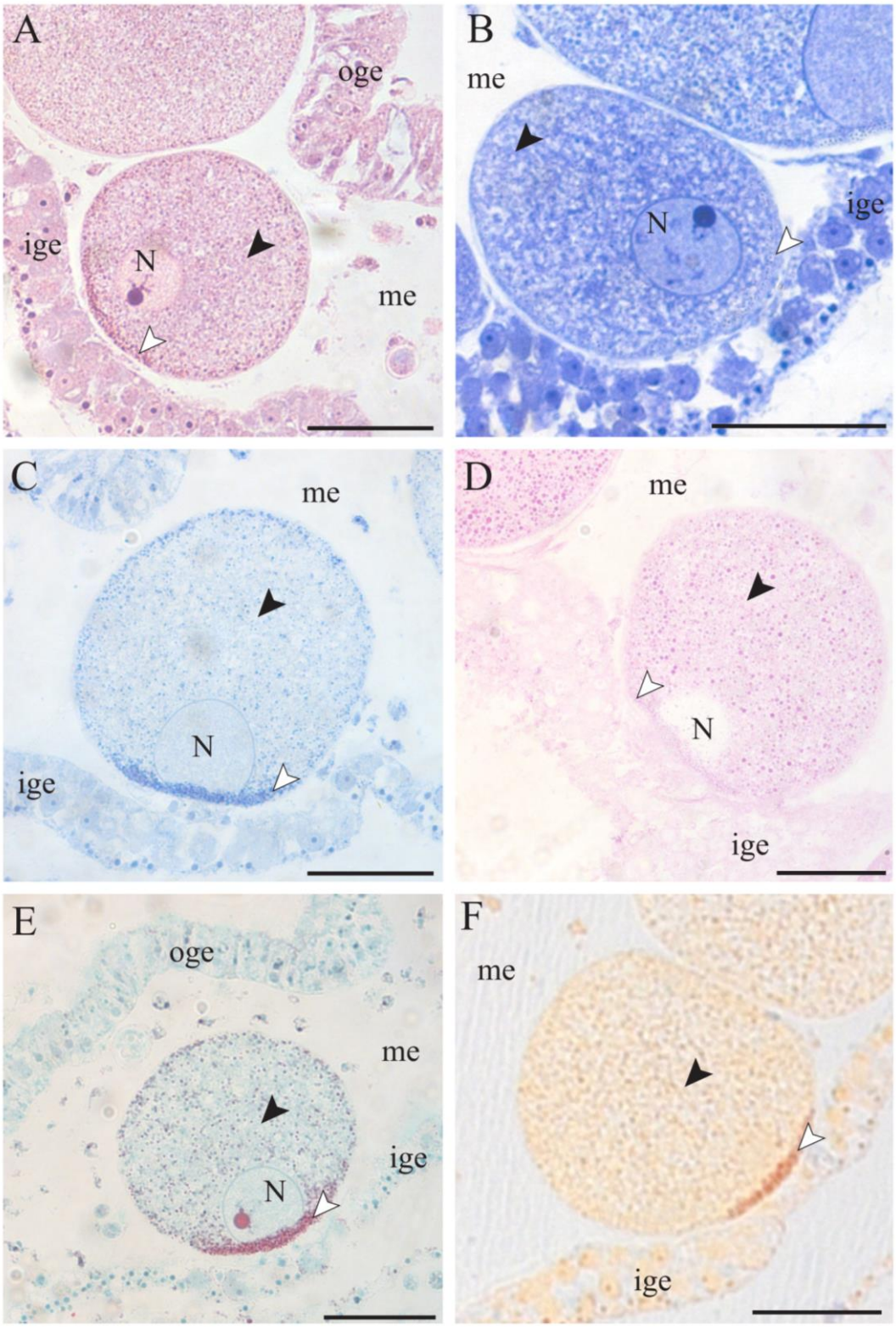

Figure 3: Oogenesis in *Cassiopea xamachana* – Intermediate oocyte. (A) Hematoxylin & Eosin. The intermediate acidophilic oocyte with basophil (white arrowhead) and acidophil (black arrowhead) granules, scale: 50 µm. (B) Toluidine blue. The basophil granules (white arrowhead) are strongly positive to acids structures while the acidophil granules (black arrowhead) are only positive, scale: 50 µm. (C) Mercuric bromophenol blue. The basophil granules (white arrowhead) are strongly positive to proteins while the acidophil granules (black arrowhead) are positive, scale: 50 µm. (D) PAS. The basophil (white arrowhead) and acidophil (black arrowhead) granules are positive to neutral polysaccharides, scale: 50 µm. (E) Gomori's trichomic. The basophil granules (white arrowhead) stain in pink while the acidophil granules (black arrowhead) stain in blue, scale: 50 µm. (F) Mallory's trichomic. The basophil granules (white arrowhead) stain in red while the acidophil granules (black arrowhead) stain in orange, scale: 50 µm. Inner genital epithelium = ige; mesoglea = m; nucleus = N; outer genital epithelium = oge; trophocytes = tr.

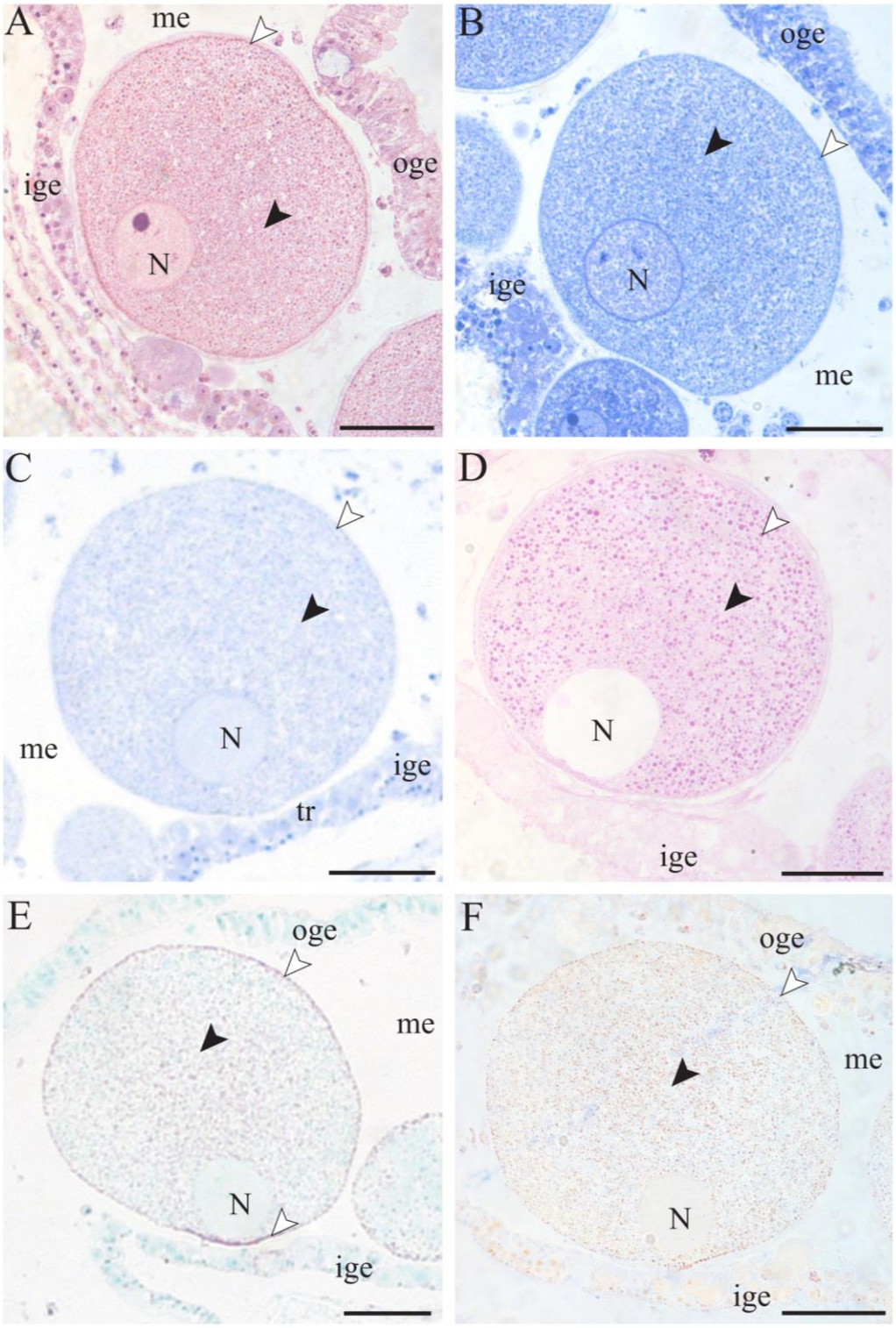

Figure 4: Oogenesis in *Cassiopea xamachana* – Late oocyte. (A) Hematoxylin & Eosin. The late oocyte is very acidophilic and have the basophil granule (white arrowhead) surrounding the oocyte while the acidophil (black arrowhead) proteins granules are centered in the ooplasm, scale: 50 µm. (B) Toluidine blue. The basophil granules (white arrowhead) are strongly positive to acids structures while the acidophil granules (black arrowhead) are only positive, scale: 50 µm. (C) Mercuric bromophenol blue. The basophil granules (white arrowhead) are strongly positive to proteins while the acidophil granules (black arrowhead) are positive, scale: 50 µm. (D) PAS. The basophil (white arrowhead) and acidophil (black arrowhead) granules are positive to neutral polysaccharides, scale: 50 µm. (E) Gomori's trichomic. The basophil granules (white arrowhead) stain in pink while the acidophil granules (black arrowhead) stain in blue, scale: 50 µm. (F) Mallory's trichomic. The basophil granules (white arrowhead) stain in red while the acidophil granules (black arrowhead) stain in orange, scale: 50 µm. Inner genital epithelium = ige; mesoglea = m; nucleus = N; outer genital epithelium = oge; trophocytes = tr.

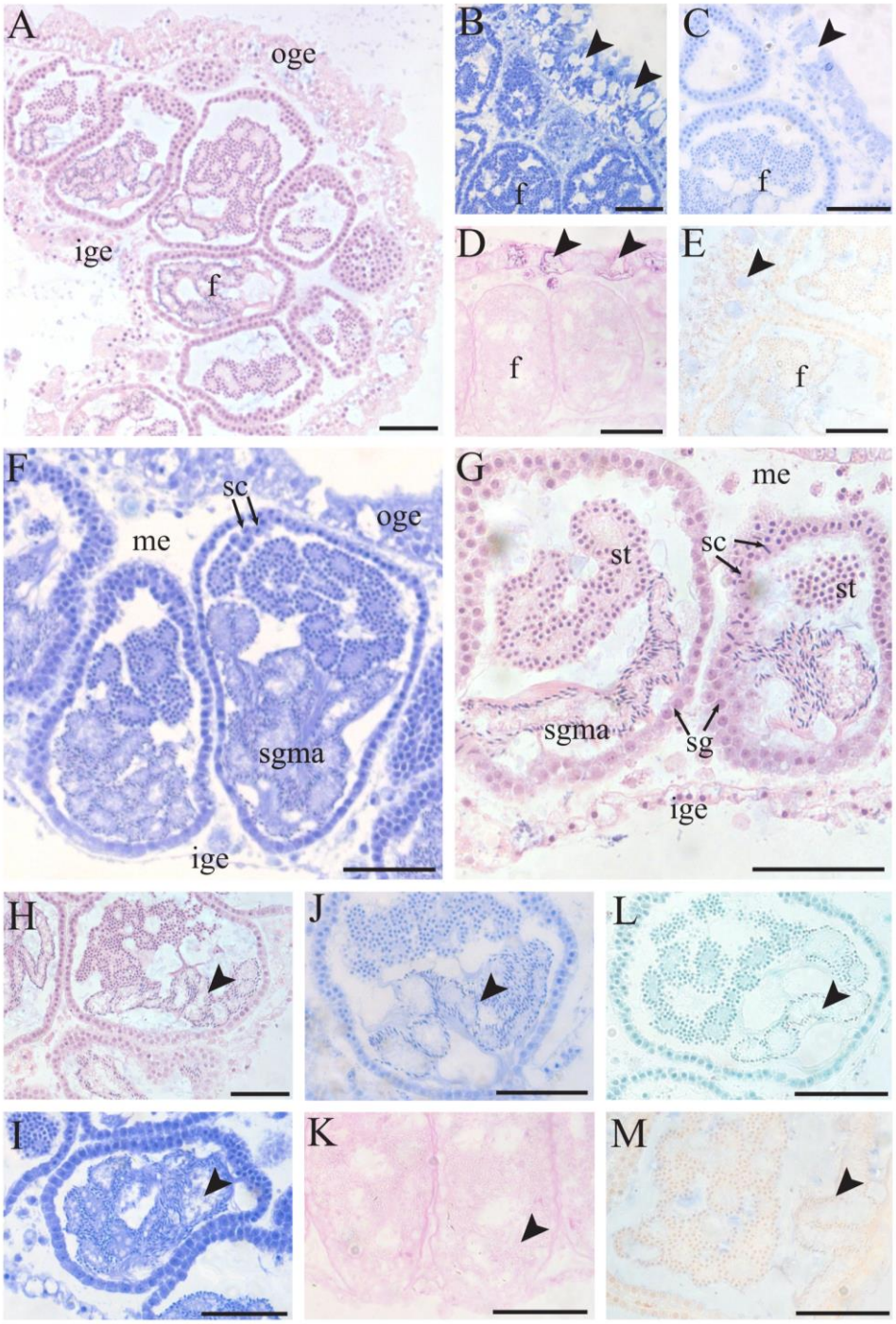

Figure 5: Spermatogenesis in *Cassiopea xamachana*. (A) Hematoxylin & Eosin. General view of male gonad. The follicles are immersed in the mesoglea and surrounded by the inner and outer genital epithelium, scale: 100 µm. (B) Toluidine blue. Detailed of outer genital epithelium with vacuoles (arrowheads) positive to acids structures, scale: 50 µm. (C) Mercuric bromophenol blue. Detailed of outer genital epithelium with vacuoles (arrowhead) positive to proteins, scale: 50 µm. (D) PAS. Detailed of outer genital epithelium with vacuoles (arrowheads) strongly positive to neutral polysaccharides, scale: 50 µm. (E) Mallory's trichomic. Detailed of outer genital epithelium with vacuoles (arrowhead) stained in blue positive to mucus, scale: 50 µm. (F) Toluidine blue. Detailed of spermatic cells inside the male follicles, scale: 50 µm. (G) Hematoxylin & Eosin. Detailed of spermatogonia spermatocytes and spermatids inside the follicles, scale: 50 µm. (H) Hematoxylin & Eosin. The sperm clustered in *spermatozeugmata* (arrowhead) near the genital epithelium, scale: 50 µm. (I) Toluidine blue. Detailed of *spermatozeugmata* (arrowhead) with the extracellular matrix negative to acids structures, scale: 50 µm. (J) Mercuric bromophenol blue. Detailed of *spermatozeugmata* (arrowhead) with the extracellular matrix negative to proteins, scale: 50 µm. (K) PAS. Detailed of *spermatozeugmata* (arrowhead) with the extracellular matrix negative to neutral polysaccharides, scale: 50 µm. (L) Gomori's trichomic. Detailed of *spermatozeugmata* (arrowhead) with the extracellular matrix negative to mucus, scale: 50 µm. (M) Mallory's trichomic. Detailed of *spermatozeugmata* (arrowhead) with the extracellular matrix negative to mucus, scale: 50 µm. Follicles = f; inner genital epithelium = ige; mesoglea = m; outer genital epithelium =oge; spermatocyte = sc; spermatid = st; spermatogonia = sg; spermatozeugamata = sgma.

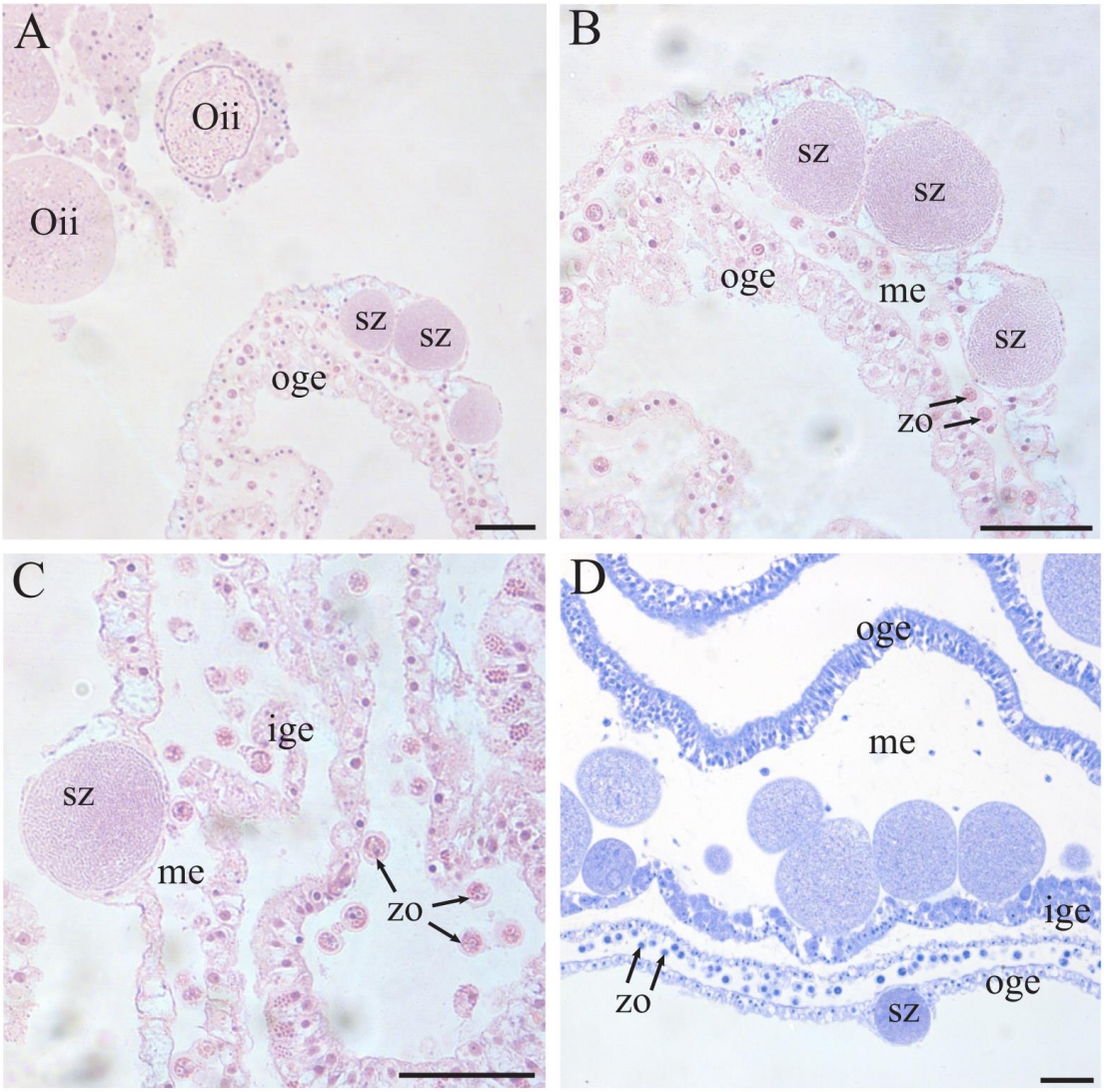

Figure 6: Hermaphroditism in *Cassiopea frondosa*. (A) Hematoxylin & Eosin. Smaller specimen (8,5 cm of bell diameter) with sperm clustered in the vesicles inside the outer genital epithelium of the female gonad, scale: 100 µm. (B) and (C) Hematoxylin & Eosin. Detail of the sperm vesicles enclosed by the outer germinal epithelium, scale: 50 µm. (D) Toluidine blue. Specimen with intermediate size (11,1 cm of bell diameter) with the sperm vesicle near the female gonad, scale: 50 µm. Inner genital epithelium = ige; intermediate oocyte = Oii; mesoglea = m; outer genital epithelium; sperm = sz, zooxantaellae = zo.

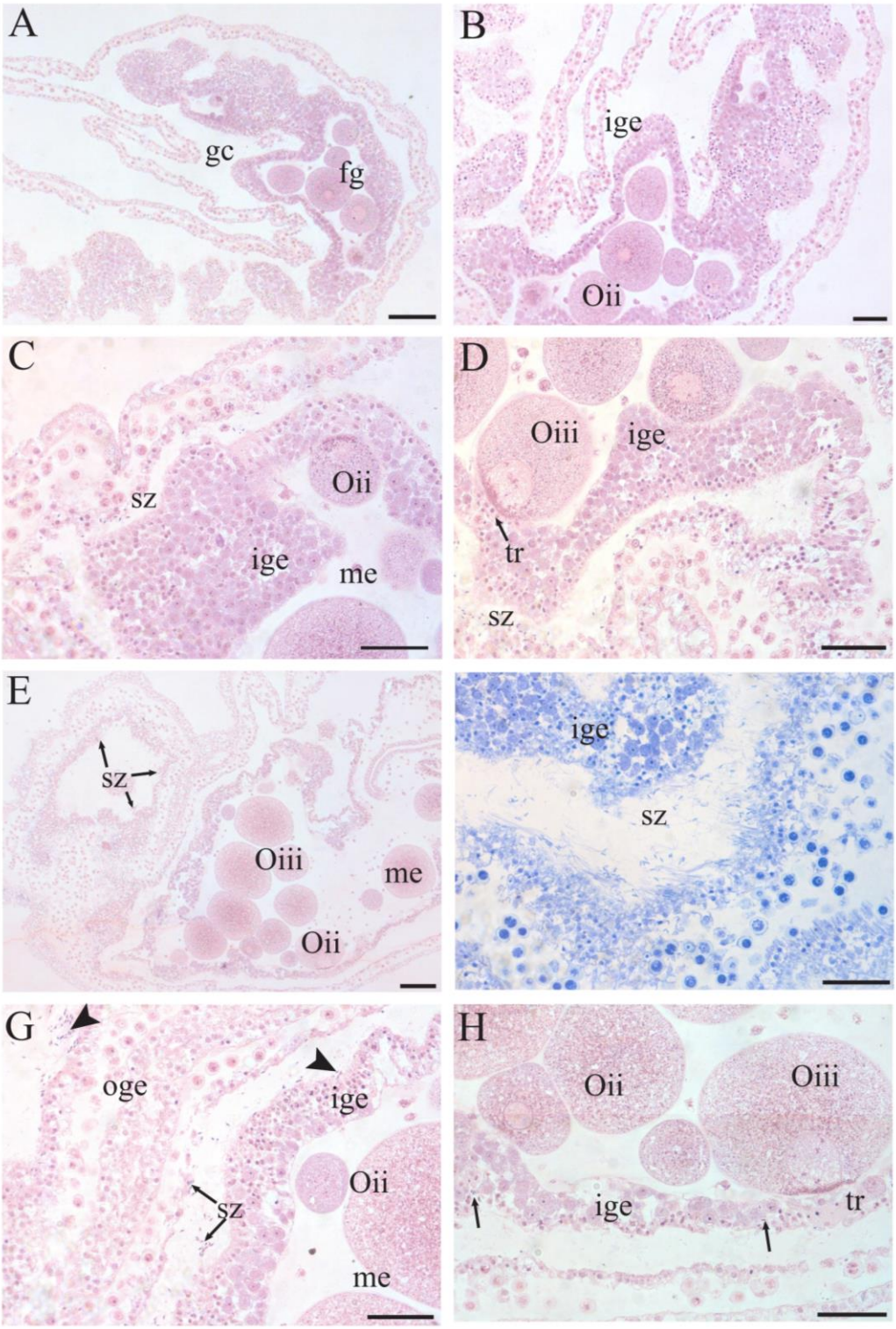

Figure 7: Hermaphroditism in *Cassiopea frondosa*. (A) Hematoxylin & Eosin. General view of female gonad of the specimen with 12,4 cm of bell diameter, scale: 200 µm. (B) Hematoxylin & Eosin. Detail of female gonad. Note the oocytes in different stages of development, scale: 50 µm. (C) Hematoxylin & Eosin. Detail of sperm inside the genital sinus near the inner genital epithelium, scale: 50 µm. (D) Hematoxylin & Eosin. Area with sperm accumulated near the inner genital epithelium, scale: 50 µm. (E) Hematoxylin & Eosin. General view of female gonad of the specimen with 13,4 cm of bell diameter. Note the area with several sperm on the left side of female gonad, scale: 100 µm. (F) Toluidine blue. Detail of the sperm inside the genital sinus near the outer and inner genital epithelium, scale: 50 µm. (G) Hematoxylin & Eosin. Detail of sperm outside (black arrowhead) and inside (white arrowhead) the female gonad, scale: 50 µm. (H) Hematoxylin & Eosin. Sperm near the inner genital epithelium crossing the trophocytes to fertilize the oocyte, scale: 50 µm. Female gonad = fg; genital sinus = gc; inner genital epithelium = ige; intermediate oocyte = Oii; late oocyte = Oiii; mesoglea = m; sperm = sz.

A evolução da estrutura gonadal em Scyphozoa

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Resumo

Apesar da reprodução sexuada em Scyphozoa ter recebido relativamente bastante atenção ao longo dos anos, são poucos os trabalhos relacionando as características das gônadas de águas-vivas em um contexto comparativo e filogenético. Neste trabalho revisamos e comparamos a organização macroscópica, histológica e celular da gônada de cifozoários discutindo os principais tópicos e novidades relacionadas à reprodução sexuada do grupo. Mesmo com mais de 25 morfologias macroscópicas gonadais diferentes a conformação histológica da gônada é a mesma, com as células germinativas imersas na mesogléia e circundadas pelos epitélios genitais. Os padrões de liberação de gametas femininos e masculinos estão relacionados não só com o tipo de fecundação, mas também com o comportamento de incubação dos ovos. Quando consideradas em um contexto filogenético, as características da reprodução sexuada trazem novas percepções e questionamentos que podem contribuir para um melhor entendimento da biologia e evolução do grupo.

Palavras-chave: Reprodução sexuada, águas-vivas, gametogênese, histologia.

1. Introdução

O filo Cnidaria é composto por um grupo diverso de animais relativamente simples agrupados pela presença da cnida (Collins, 2002; Daly et al., 2007; Marques & Collins, 2004; Morandini et al., 2016; Odorico & Miller, 1997; Reft & Daly, 2012; Schuchert, 1993). Seus representantes são divididos em dois grandes clados – Anthozoa e Medusozoa (Collins, 2002; Collins et al., 2006) – que juntos totalizam cerca de 11.000 espécies descritas (Appeltans et al., 2012; Zapata et al., 2015); sem considerarmos a diversidade de Myxozoa. Atualmente, o subfilo Medusozoa é composto por quatro classes (Hydrozoa, Staurozoa, Cubozoa e Scyphozoa) que possuem relações de

parentesco entre si ainda não bem resolvidas (Fig. 1) (Collins et al., 2006; Hyman, 1940; Kayal et al., 2018; Marques & Collins, 2004; Miranda et al., 2016; Thiel, 1966; Werner, 1973a; Zapata et al., 2015). O mesmo acontece com as relações de parentesco das ordens de Scyphozoa, em que a monofilia de Coronatae e Rhizostomeae são resgatadas, porém Semaeostomeae aparece como um agrupamento polifilético (Collins et al., 2006; Kayal et al., 2018; Thiel, 1966). Das quatro filogenias que buscam resolver as relações entre as famílias de cifozoários (Bayha et al., 2010; Dawson, 2004; Gómez Daglio & Dawson, 2017; Straehler-Pohl, 2009), apenas a de Bayha et al. (2010) inclui representantes de quase todas as famílias. Com base em dados moleculares, os autores confirmam a monofilia apenas de Coronatae, com a família Ulmaridae sendo parafilética e grupo irmão de Rhizostomeae e não dos demais semeóstomos.

Historicamente a classe Scyphozoa incluia 5 morfogrupos diferentes: as estauromedusas, cubomedusas, coronadas, rizostomátidas e semeostomas (Hyman, 1940; Kramp, 1961; Mayer, 1910). Porém, atualmente a classe inclui somente três dos grupos citados (Coronatae, Rhizostomeae e Semaeostomeae), uma vez que as ordens Cubomedusae e Stauromedusae foram elevadas ao status de classe (Marques & Collins, 2004; Werner, 1973a). A classe Scyphozoa possui 241 espécies classificadas em 22 famílias (Tabela 1) divididas em dois grandes grupos: as subclasses Coronamedusae (ordem Coronatae) e Discomedusae (ordens Semaeostomeae e Rhizostomeae) (Calder, 2009; Collins et al., 2006; Daly et al., 2007; Mianzan & Cornelius, 1999; Jarms & Morandini, 2019). A ordem Coronatae é composta por 6 famílias de medusas que possuem a presença de um sulco coronal na umbrela. Os representantes de Semaeostomeae são águas-vivas que possuem uma boca central com 4 braços orais enquanto que os Rhizostomeae possuem 8 braços orais com microbocas (Jarms & Morandini, 2019).

O ciclo de vida metagenético é considerado característico de todas as classes de Medusozoa (Bridge et al., 1995; Marques & Collins, 2004; Morandini et al., 2016; Odorico & Miller, 1997). Em Scyphozoa, o pólipo origina uma ou mais medusas jovens (éfiras) através de um mecanismo de reprodução assexuado (chamado estrobilização). A estrobilização pode ser polidisco – formação de várias medusas –, oligodisco – formação de poucas éfiras (2-10) (Fuentes et al., 2011) – ou monodisco – uma éfira é liberada por vez (Bigelow, 1900; Holst et al., 2007; Schiariti et al., 2008; Straehler-Pohl, 2017; Straehler-Pohl & Jarms, 2022). Em Scyphozoa, uma vez que a éfira é liberada na coluna d'água, ela cresce até chegar à fase adulta. É na fase adulta que ocorre o crescimento e desenvolvimento da gônada. Os gametas podem ser liberados na coluna d'água – fecundação externa – ou mantidos na cavidade gastrovascular da fêmea – fecundação interna (Ikeda et al., 2011; Morandini et al., 2004; Schiariti et al., 2012; Tiemann & Jarms, 2010; Tiseo et al submetido capítulo 2; Widersten, 1965). Os ovos fertilizados se desenvolvem em uma larva plânula que assenta em algum substrato e diferencia-se em pólipo (Calder, 2009; Jarms, 2010; Kikinger, 1992; Morandini et al., 2004; Schiariti et al., 2012). Exceções são os ciclos de vida holopelágicos de *Periphylla periphylla* e *Pelagia noctiluca* em que há a ausência dos estágios de larva e pólipo (Jarms et al., 1999; Delap, 1907).

A reprodução em Cnidaria é altamente diversa (Fautin, 2002) e fornece dados significativos para entender a evolução e funcionamento da propagação sexuada e assexuada dentro do grupo (Campbell, 1974). Em sua maioria, os Scyphozoa apresentam gonocorismo (Berrill, 1949; Miranda et al., 2016; Tiseo et al., 2019). No entanto, apesar de raras, existem espécies de cifomedusas como *Chrysaora hysoscella* e exemplares do gênero *Cassiopea* que apresentam hermafroditismo (Berrill, 1949; D. K. Hofmann & Hadfield, 2002). De acordo com a literatura, a gônada dos representantes de Scyphozoa

tem origem endodérmica (Harrison & Jamieson, 1988; Miller, 1983; Miranda et al., 2016; Tiseo et al., 2019; Widersten, 1965) sendo descrita como um espaço preenchido com células germinativas em desenvolvimento que migraram da endoderme para a mesogléia (Harrison & Jamieson, 1988; Miller, 1983).

Tiseo & Morandini (submetido, capítulo 1) revisam os principais tópicos relacionados à reprodução sexuada em cifozoários coronados. Porém em um contexto mais amplo, para Scyphozoa, são poucos os trabalhos descrevendo o processo de formação (Widersten, 1965), a morfologia macroscópica da gônada, sua conformação e organização dentro da cavidade gastrovascular (Eckelbarger & Larson, 1988; Hyman, 1940; Kikinger, 1992; Ohtsu et al., 2007; Schiariti et al., 2012) relacionando as características da reprodução sexuada do grupo em um contexto comparativo e filogenético. Assim, revisamos e comparamos a organização macroscópica, histológica e celular da gônada de cifozoários revisitando os principais tópicos relacionados à reprodução sexuada do grupo, como a liberação dos gametas, tipos de fecundação e incubação de larvas plânulas.

2. Material e Métodos

2.1. *Coleta de dados*

Para a análise da morfologia macroscópica e histológica da gônada foi feita uma revisão bibliográfica de trabalhos originais em base de dados como Web of Science, NCBI, Dedalus e bibliotecas como Biodiversity Heritage Library. A busca se deu através de temas relacionados com a reprodução sexuada, gametogênese e estratégias reprodutivas em águas-vivas Scyphozoa. Também foram inseridos os dados macroscópicos da gônada encontrados nas descrições de todas espécies que compõem a classe Scyphozoa. Adicionalmente, foram feitas observações em espécimes do Museu de

Zoologia (*Cyanea capillata* MZUSP 775, *Cyanea lamarkii* MZUSP 769 e *Rhopilema nomadica* MZUSP 756, *Cassiopea culionensis* MZUSP 8633), 4 espécimes da coleção do Laboratório de Cultivo e Estudos de Cnidaria (*Chrysaora hysoscella* – MZUSP 760, *Sanderia malayensis*, *Aurelia cebimarensis* e *Phyllorhiza punctata*) e em espécies cultivadas em laboratório (*Lychnorhiza lucerna*) ou coletadas na natureza (*Lychnorhiza lucerna, Chrysaora lactea* e *Versuriga anadyomene*).

Éfiras da espécie *Lychnorhiza lucerna* foram cultivadas em laboratório até atingiram a maturidade gonadal. Os indivíduos de *Lychnorhiza lucerna* foram mantidos em potes de plásticos ou aquários cilíndricos "planktonkreisel" (Greve, 1968) com água do mar (Fig. 1E-F) e foram alimentadas com náuplios de *Artemia* sp. enriquecida com ácidos graxos e ovas de peixe três vezes ao dia. Espécimes de *Cassiopea andromeda* (Cabo Frio – Rio de Janeiro -22.87555556, -42.01861111, Foz do Rio Meirim, Maceió - Alagoas -9.54805556, -35.62194444 e Fazenda Água Crusta – Ceará -2.88000000, -39.90416667), *Cassiopea xamachana*, *Cassiopea frondosa* (Key Largo, Flórida 25.101460, -80.438519), *Versuriga anadyomene* (Estuário do rio Richmond em Ballina, Austrália – -28.523632 153.335306) (Fig. 2A), *Lychnorhiza lucerna* e *Chrysaora lactea* (Praia do Jabaquara em Ilha Bela - São Paulo -23.4958 045.2531) (Fig. 2B) foram coletados e fixados em solução de formol 4% com água do mar do local de coleta. Para a análise da morfologia macroscópica da gônada todos os indivíduos foram posicionados de forma que um dos eixos perradiais ficasse perpendicular ao observador (Fig. 2C). O registro fotográfico foi feito com uma câmera Nikon D7000 e o diâmetro umbrelar foi tomado com fita métrica (Fig.1D).

2.2. *Técnicas de Microscopia*

Amostras das gônadas femininas e masculinas dos exemplares de todas as espécies foram removidas e fixadas em paraformaldeído 4% preparado com água do mar por 24 horas. Após a fixação, as amostras foram desidratadas em séries de álcool (70% a 100%), embebidas e incluídas em historesina Leica (Tiseo, 2016). Os cortes seriados de 3-4 µm foram obtidos em micrótomo rotativo e as lâminas coradas com Hematoxilina e Eosina (Junqueira & Junqueira 1983, modificado), evitando banhos com etanol xileno (Sant'Anna et al., 2010; Tiseo et al., 2014; Zara et al., 2012). Para a histoquímica as lâminas foram coradas com azul de toluidina pH 2,5 (modificado de Audino et al. 2015), azul de bromofenol (Pearse, 1960) e xylidine ponceau (Mello & Vidal, 1980) para a demonstração de proteínas; a técnica do ácido periódico de Schiff (PAS) para polissacarídeos com os grupos 1-2-glycol (Junqueira & Junqueira, 1983), a técnica de Azul de Alcian 2,5 para polissacarídeos ácidos e as técnicas Tricômico de Mallory e Gomori para colágeno e mucopolissacarídeos (Bancroft & Stevens, 1982; Humason, 1962).

Uma pequena porção da gônada dos exemplares de *Versuriga anadyomene* foi fixada para microscopia eletrônica de transmissão em solução glutaraldeído 2.5% em tampão cacodilato de sódio (pH 7,4) 0,1M com água do mar do local de coleta ultrafiltrada por 24h a 4ºC. Após este tempo, os materiais passaram por três banhos no mesmo tampão, com duração de 10 minutos cada e foram pós-fixados com tetróxido de ósmio a 2%, durante 1 hora na mesma temperatura. Posteriormente, foram realizadas três lavagens de 10 minutos em água do mar filtrada e a contrastação "*En bloc*" com acetato de uranila 1% (*overnight* a 4ºC). As amostras foram desidratadas em sequência progressiva de etanol (70 a 100%) e foram incluídas em resina Spurr® segundo protocolo de MET. Os cortes semi-finos e ultra-finos foram obtidos no ultra-micrótomo Leica UC7. As grades com os cortes ultra-finos foram contrastadas em citrato de chumbo 0,2% em NaOH 0,1N durante

10 minutos, sendo posteriormente observadas e fotografadas no Microscópio Eletrônico de Transmissão Zeiss EM 900 do Laboratório de Microscopia Eletrônica do Instituto de Biociências.

3. Resultados e Discussão

Organização macroscópica da gônada

A conformação gonadal dos representantes de Scyphozoa é bastante diversa e apresenta mais de 25 morfologias macroscópicas diferentes (Figs 3 e 4). A gônada em Scyphozoa está localizada na cavidade gastrovascular e é formada como uma evaginação do epitélio da gastroderme, em que células intersticiais indiferenciadas migram para a mesogléia e se diferenciam em células germinativas e epitélio genital (Arai, 1997; Miller, 1983; Morandini & Silveira, 2001). Em Coronatae, normalmente, as 8 gônadas adradiais ficam próximas aos filamentos gástricos e ao sulco coronal (Bigelow, 1909; Silveira & Morandini, 1997; Fewkes, 1886; Gegenbaur, 1856; Jarms, 1990, 2001; Komai, 1935; Kramp, 1961; Leloup, 1937; Maas, 1903, 1897; Mills, Larson, & Youngbluth, 1987; Molinari, 2019; Neppi, 1915; Nogueira Júnior et al., 2010; Repelin, 1962; Swartz, 1788; Vanhöffen, 1902; Werner, 1974). Algumas espécies dos gêneros *Atorella*, *Linantha*, *Palephyra*, *Periphyllopsis* e *Nausithoe* fogem do padrão numérico gonadal de Coronatae apresentando quatro ou seis gônadas (Bigelow, 1909; Komai, 1935; Maas, 1897; Russell, 1956; Vanhöffen, 1902; Werner, 1973b) e essa diferença se dá pela possível fusão de gônadas adjacentes (*Palephyra* e *Periphyllopsis*) e ou pela gônada se desenvolver em indivíduos com apenas o estágio de pólipo (*Nausithoe*). De acordo com Tiseo & Morandini (submetido, capítulo 1 desta tese) 4 morfologias gonadais foram descritas para as famílias Atollidae e Atorellidae, 2 para Linuchidae, 9 para Nausithoidae, 2 para

Paraphyllinidae e 6 para Periphyllidae (Fig 3). As espécies *Atolla wyvillei* (Atollidae) e *Atolla vanhoeffeni* possuem 8 gônadas ovais ou em formato de feijão (Haeckel, 1880; Lucas & Reed, 2010; Tiseo & Morandini, capítulo 1 desta tese). As morfologias fusiforme e irregular são exclusivas de Atorellidae, assim como os formatos de coração e triangular são encontrados apenas em Nausithoidae. Em *Linuche unguiculata* (Linuchidae) as 8 gônadas pareadas (Eckelbarger & Larson, 1992; Swartz, 1788) possuem forma crescente e coloração esbranquiçada. A gônada em "W" é característica de Paraphyllinidae e as morfologias em "J" e "U" exclusivas de Periphyllidae. *Periphylla periphylla* (Periphyllidae) possui oito gônadas localizadas na cavidade gastrovascular que aparentam estruturas em formato de "J" (nas medusas pequenas) ou em formato de "U" (nas medusas maiores). Para detalhes veja Lucas & Reed (2010); Russell (1970); Tiemann & Jarms (2010); Vanhöffen (1902). Molinari (2019) e Tiseo & Morandini (capítulo 1 desta tese) discutem que a morfologia das gônadas em Coronatae podem se alterar conforme a maturação da medusa e que, então, as morfologias oval e em formato de feijão; oblonga e irregular podem refletir diferentes estágios de maturação e desenvolvimento gonadal dos espécimes observados. Para mais detalhes veja a Tabela 2 onde um compilado das informações sobre aspectos reprodutivos de diversos cifozoários é apresentado.

Em Discomedusae as quatro gônadas interradiais encontram-se adjacentes aos cirros gástricos e enovelam-se sobre si mesmas formando várias dobras. Com base nas descrições das espécies, a ordem Semaeostomeae possui 8 morfologias diferentes: em formato de "sacos plásticos", em semicírculo, em formato de "W", crescente, ferradura, leque, evertida e crescente fusionada (Fig. 4A-H). Nos semeóstomos das famílias Cyaneidae, Drymonematidae e em espécies do gênero *Phacellophora* a gônada é enovelada e se protrai através do assoalho da umbrela em formato de "sacos plásticos" (Fig. 4A) (Bayha & Dawson, 2010; L.-A. Gershwin & Zeidler, 2008; Haeckel, 1880;

Larson, 1986; Linnaeus, 1758; Péron & Lesueur, 1810; Reynaud, 1830). Para os gêneros *Sanderia* e *Chrysaora* da família Pelagiidae a morfologia é em semicírculo (Fig. 4B) (Eschscholtz, 1829; L.-A. Gershwin & Zeidler, 2008; Goette, 1886; Lesson, 1830; Péron & Lesueur, 1810; Ras et al., 2020; Reynaud, 1830; Vanhöffen, 1902). Outras morfologias também são descritas como em forma de "W" (Fig. 4C) para *Chrysaora pseudoocellata* (Mutlu et al., 2020) ou com o formato de "M" em *C. pentastoma* (Péron & Lesueur, 1810; Gershwin & Zeidler, 2008). Porém, os autores Morandini & Marques (2010) afirmam que este último formato é o mesmo que em "W" apenas visto de um ângulo diferente. Nos Pelagiidae do gênero *Pelagia* e *Mawia* e no Ulmaridae *Stellamedusa* a gônada tem o formato de crescente não fusionada (Fig. 4D) (Eschscholtz, 1829; Péron & Lesueur, 1810; Piraino et al., 2014). Em Ulmaridae, as subfamílias Aureliinae, Poraliinae, Sthenoniinae e Stygiomedusinae possuem a gônada em formato de ferradura direcionada para dentro (Fig. 4E) (Brown et al., 2021; Eschscholtz, 1829; Haeckel, 1880; Lawley et al., 2021; Lesson, 1830; Linnaeus, 1758; Raskoff & Matsumoto, 2004; Vanhöffen, 1902). As gônadas dos representantes das subfamílias Deepstariinae e Tiburoniinae fogem ao padrão de Ulmaridae e possuem formatos de "leque" (Fig. 4F) e "evertida" (Fig.4G), respectivamente (Larson et al., 1988; Russell, 1967). Os representantes da subfamília Ulmarinae também possuem gônada em formato crescente, porém neste caso, fusionadas (Fig. 4H) (Haeckel, 1880; Kishinouye, 1910; Mayer, 1910). De um modo geral, os representantes de Rhizostomeae possuem gônadas em formato de "L" aparecendo como uma diferenciação da gastroderme (Fewkes, 1886; Galil et al., 2010; Galil et al., 2017; Haeckel, 1880; Russell, 1970; Schiariti et al., 2012; Souza & Dawson, 2018; Stiasny, 1920; Uchida, 1926; Vanhöffen, 1902). Conforme a gônada aumenta de tamanho e juntamente com as demais gônadas, o conjunto todo acaba adquirindo um aspecto cruciforme. Além disso, podem estar localizadas em diferentes regiões dentro da cavidade

gástrica mais perifericamente ou mais restrita à região central (Fig. 4I-L). A diferença para os representantes das diferentes famílias está na localização da gônada dentro da cavidade gástrica mais espaçosa nos representantes das famílias Catostylidae, Lobonemidae, Lychnorhizidae, Leptobrachidae, Mastigiidae e Versurigidae (Fig. 4I) e mais restrita nos espécimes de Cepheidae (Fig. 4J) e Cassiopeidae (Fig. 4K). Na gônada cruciforme de Rhizostomatidae e Stomolophidae o "L" tem a curvatura mais aberta que nas demais famílias (Fig. 4L) e em *Nemopilema nomurai* as gônadas, quando maduras, "extravasam" pelo óstio genital, sendo descritas pelos autores Ohtsu et al. (2007) e Ikeda et al. (2011) como "sacos plásticos".

Para *C. capillata* e *C. lamarkii* a gônada é descrita como uma eversão da mesogléia (Fig. 5A, B-D) próxima aos cirros gástricos (Fig. 5C, E-F) (Linnaeus, 1758; Péron & Lesueur, 1810; Widersten, 1965). Em Pelagiidae, nas espécies do gênero *Sanderia* (Fig. 6A) e *Chrysaora* a gônada pode ser observada pelos óstios subgenitais localizada próxima aos filamentos gástricos (Fig. 6B), possui o formato semicircular e apresenta várias dobras sobre si (Morandini & Marques, 2010). Indivíduos de *C. hysoscella* apresentam hermafroditismo protândrico (Haeckel, 1880; Linnaeus, 1758; Russel, 1970; Thiel, 1936) em que espécimes pequenos (Fig. 6C) possuem uma gônada masculina típica (Fig. 6D) e pequenas bolsas com folículos em desenvolvimento em diferentes regiões do corpo, como na mesogléia da cavidade gástrica (Fig. 6E) e braços orais (Fig. 6F-G). Os espécimes maiores de *C. hysoscella* possuem uma gônada feminina típica (Fig. 7A-B) e as pequenas bolsas com folículos em desenvolvimento nas diferentes regiões da subumbrela da cavidade gástrica (Fig. 7C-E). A espécie *Aurelia aurita* (Ulmaridae) possui gônada no formato de ferradura com a abertura direcionada para a boca e óstio genital (Fig. 8A-B). Quando madura a gônada apresenta várias dobras adjacentes aos cirros gástricos (Fig. 8C-E) e pequenos folículos podem ser identificados

(Fig. 8F). Em *Cassiopea* as gônadas podem ser vistas pelo pequeno óstio genital (Fig. 9A) e são enoveladas dentro do pequeno espaço da cavidade gástrica (Fig. 9B-C) próximas aos cirros gástricos (Fig. 9D-F). Em indivíduos pequenos de *Phyllorhiza punctata* (Fig. 10A) a gônada vestigial cruciforme (Fig.10B) já pode ser vista próxima aos cirros gástricos (Fig. 10C-D). Em espécimes maduros de *Versuriga anadyomene* a gônada cruciforme é bastante enovelada e pode ser vista a olho nu (Fig. 10E) próxima aos cirros gástricos (Fig. 10F). Em *L. lucerna*, as gônadas enoveladas são conectadas por uma fina membrana que recobre o canalículo do espaço subgenital que termina no óstio subgenital (Fig. 11A). Em indivíduos imaturos a gônada vestigial pode ser vista como uma fina camada de tecido próximo aos cirros gástricos (Fig. 11B) e os folículos podem ser facilmente reconhecidos (Fig. 11C-D). Em *Rhopilema nomadica* a gônada cruciforme tem o braço em "L" mais aberto (Fig. 11E-F) dando a impressão de ser uma morfologia diferente do padrão cruciforme do grupo.

Em Scyphozoa os diferentes formatos macroscópicos observados, se dão pelo crescimento do tecido no espaço disponível dentro da cavidade gástrica (Fig. 12A). Das oito morfologias descritas para Semaeostomeae, seis seguem o mesmo padrão circular côncavo com a abertura voltada para a boca (Fig. 12B) Já nas rizostomátidas, que não possuem uma boca central, a gônada encontra-se organizada na direção oposta e, eventualmente, chegam a "se fundir" formando uma cruz (Fig. 12C). Uma provável explicação para essa diferença na organização gonadal é a distribuição do alimento na cavidade gástrica que ocorre de maneira diferente em Semaeostomeae e Rhizostomeae. Em Semaeostomeae o alimento sai da boca e vai diretamente para a cavidade gástrica que o distribui (Fig. 12D) (Arai, 1997). Já em Rhizostomeae, o alimento sai dos canais dos braços orais, cai na cavidade gástrica pela região central e se distribui pelos canalículos do centro para a borda da subumbrela (Fig. 12E) (Kikinger, 1992).

Organização histológica da gônada

Em Scyphozoa, a gônada é dividida em três camadas de tecido: uma externa (epitélio genital externo), uma interna (epitélio genital interno) e entre elas encontram-se as células germinativas imersas na mesogléia (Miller, 1983; Tiseo, 2016; Widersten, 1965; Tiseo et al. submetido capítulo 2; Tiseo & Morandini, submetido capítulo 1; Tiseo & morandini capítulo 3 desta tese). O epitélio genital externo é do tipo simples colunar e o epitélio genital interno é do tipo simples, mas cuboidal, ambos com cílios na porção apical (Tiseo & Morandini, capítulo 3 desta tese). Se levarmos em consideração a alta diversidade de Scyphozoa (241 espécies, sensu Jarms & Morandini, 2019), a gametogênese foi relativamente pouco estudada para os representantes da classe. Em apenas 39 espécies a gametogênese é conhecida, sendo que 8 destas descrições se encontram neste trabalho (Tabela 2).

Em Scyphozoa a espermatogênese é assincrônica e acontece dentro dos folículos imersos na mesogléia e envoltos pelo epitélio genital. As espermatogônias e espermatócitos situam-se na periferia do folículo enquanto que as espermátides e espermatozoides ficam na região centro-basal do folículo para serem liberados via poro, fenda ou ruptura do epitélio genital (Ikeda et al., 2011; Kikinger, 1992; Lucas & Reed, 2010; Morandini & Silveira, 2001; Ohtsu et al., 2007; Russell, 1970; Schiariti et al., 2012; Thiel, 1936; Tiemann & Jarms, 2010). Em *Aurelia cebimarensis* (Fig. 13), o epitélio genital externo é do tipo colunar e possui vacúolos com material fortemente basófilo (Fig. 13A), positivo para proteínas (Fig. 13B), muco (Fig. 13C), polissacarídeos ácidos (Fig. 13D) e polissacarídeos neutros (Fig. 13E). Os espermatozóides encontram-se na região central do folículo imersos em uma secreção basófila (Fig. 13F), positiva para muco (Fig. 13C) e não reativa ao tricômico de Gomori (Fig. 13G). Os espermatozoides imersos em

muco são liberados no sino genital por ruptura do epitélio genital interno (Fig. 13H). A gônada de *Rhopilema nomadica* está apoiada na mesogléia adjacente aos cirros gástricos (Fig. 14A). Em corte transversal, observamos a gônada se formando próxima à gastroderme com poucos folículos repletos de espermatogônias em diferenciação (Fig. 14B). Do mesmo modo que em *A. cebimarensis* o epitélio gonadal externo da gônada masculina de *R. nomadica* possui vários vacúolos com conteúdo fortemente basófilo (Fig 14C), negativo para proteínas (Fig. 14D), positivo para polissacarídeos neutros (Fig. 14E), muco (Fig. 14F) e polissacarídeos ácidos (Fig. 14G). Os espermatozoides se encontram na região central dos folículos (Fig. 14H) imersos em uma secreção levemente basófila (Fig. 14I). Com o rompimento do folículo e epitélio genital (Fig. 14J) os espermatozoides são liberados no sino genital (Fig. 14K).

A vitelogênese, tem uma via endógena (o vitelo é produzido pelo próprio ovócito) e exógena (o vitelo é importado do meio extracelular). Com a exceção de *P. periphylla*, em Coronatae a via exógena acontece por meio do epitélio genital, sem o auxílio de uma célula acessória (Lucas & Reed, 2010; Maas, 1903; Morandini & Silveira, 2001; Russell, 1970; Vanhöffen, 1902). Através da ultraestrutura, notou-se a presença de células endodérmicas (denominadas de trofócitos) envolvendo os ovócitos na espécie *P. periphylla* (Tiemann & Jarms, 2010). Eckelbarger & Hodgson (2021) contrapõem o descrito por Tiemann & Jarms (2010) afirmando que os trofócitos do tipo 1 são células presentes apenas em Discomedusae. Tiseo & Morandini (submetido capítulo 1 desta tese), descrevem a presença de células colunares ao redor dos ovócitos tardios de *P. periphylla* e concluem, com base na morfologia diferencial, que estas células realmente não são os trofócitos descritos para discomedusas como afirma Eckelbarger & Hodgson (2021). Porém, uma vez confirmada a presença de células que auxiliam no processo de vitelogênese exógena, Tiseo & Morandini (submetido capítulo 1 desta tese) sugerem que

estas células sejam chamadas, de forma geral, como células acessórias. Para os discomedusae, os ovócitos intermediários e tardios estão envoltos pelos trofócitos, que auxiliam não somente no transporte de vitelo exógeno como também contribuem no processo de ovulação (Avian & Rottini-Sandrini, 1991; Eckelbarger, 1994; Eckelbarger & Larson, 1992; Ikeda et al., 2011; Tiseo & Morandini, capítulo 3 desta tese).

Muito similar ao descrito por Widersten (1965) para *C. capillata*, a gônada feminina de *Cyanea lamarkii* (Fig. 15), *Versuriga anadyomene* (Fig. 16) e *Lychnorhiza lucerna* (Fig. 17) segue o padrão histológico de Scyphozoa com as células germinativas imersas na mesogléia e circundadas pelo epitélio genital interno e externo (Figs 15A; 16A; 17A). Na gônada feminina, ovócitos em três diferentes graus de desenvolvimento são encontrados (Fig. 15A-B). Os ovócitos pré-vitelogênicos encontram-se dentro do epitélio genital interno (Figs 15B; 16B; 17C). Os ovócitos iniciais são basófilos e os intermediários e tardios acidófilos (Figs 15B; 16B; 17B-D). Como descrito para *Aurelia aurita*, *Cotylorhiza tuberculata*, *Cassiopea andromeda*, *Cassiopea frondosa*, *Cassiopea xamacahana* e *Rhizostoma pulmo (*Kikinger, 1992; Widersten, 1965, Tiseo & Morandini, capítulo 4), os ovócitos intermediários e tardios já se encontram quase que totalmente imersos na mesogléia mas mantém a conexão com o epitélio genital pelos trofócitos (Figs 15C; 16C-D). Os trofócitos em *L. lucerna* possuem grandes vacúolos similares aos descritos para as espécies *C. lactea*, *Pelagia noctiluca*, *Diplulmaris antarctica*, *Discomedusa lobata*, *Catostylus mosaicus*, *Nemopilema nomurai* e *Stomolophus meleagris* (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1992; Ikeda et al., 2011; Pitt & Kingsford, 2000; Schiariti et al., 2012; Tiseo & Morandini, capítulo 3). Os ovócitos tardios de *C. lamarkii*, *V. anadyomene* e *L. lucerna* possuem dois tipos de grânulos no ooplasma: um basófilo e outro acidófilo (Figs 15B; 16D-E; 17E). Os grânulos basófilos são positivos para estruturas ácidas (Figs 15C; 16C; 17F), polissacarídeos

neutros (Figs 15D; 16F), corados em rosa pelo Tricômico de Gomori (Fig. 15E-F) e vermelho pelo tricômico de Mallory (Fig. 15G). Adonin et al (2009) ao isolar domínios proteicos da mesogléia de *A. aurita*, descobrem uma proteína (mesogleina) muito similar ao do grupo das proteínas que compõem a zona pelúcida dos ovócitos de mamíferos. Com base no achado, (Adonin & Podgornaya, 2012) investigam a presença desta proteína dentro da gônada feminina de *A. aurita* e notam que a proteína se acumula no ooplasma próximo à região do polo animal do ovócito. Os autores denominam esse aglomerado de mesogleina no polo animal do ovócito de "placa de contato". Através da histoquímica, Tiseo & Morandini (capítulo 3) identificam um aglomerado proteico similar nos ovócitos de *C. lactea* e sugerem que dois processos ocorrem simultaneamente dentro da gônada feminina de Discomedusae: a vitelogênese e a coriogênese. A presença de dois grânulos com características histoquímicas diferentes em *C. lactea*, *Cassiopea andromeda*, *C. xamachana*, *C. frondosa*, *Cyanea lamarkii*, *V. anadyomene* e *L. lucerna* sugerem que a presença de mesogleina nos ovócitos de vários discomedusae é uma característica conservada dentro do grupo e que, consequentemente, os ovócitos de discomedusas produzem uma membrana vitelínica como os demais metazoários.

São poucos os trabalhos descrevendo a ultraestrutura dos gametas de Scyphozoa sendo a morfologia dos ovócitos mais conhecida que a dos espermatozoides (Avian & Rottini-Sandrini, 1991; Eckelbarger & Larson, 1988, 1992; Hedwig & Schafer, 1986; Hinsch, 1974; Ikeda et al., 2011; Rouse & Pitt, 2000; Tiseo et al. submetido capítulo 2). O espermatozoide de Cnidaria não possui acrossomo, porém acredita-se que pequenas vesículas situadas na região anterior ao núcleo ou peça intermediária cumpram sua função (Harrison & Jamieson, 1999; Hinsch, 1974; Miller, 1983). Afzelius & Franzén (1971) descrevem a morfologia do espermatozoide de *Nausithoe* sp. sendo os primeiros a apresentar a ultraestrutura dos espermatozoides de scyphozoa. De um modo geral, a

morfologia do espermatozoide é moldada principalmente pelo formato do núcleo que pode ser alongado, triangular ou ovoide. Logo abaixo do núcleo está a peça intermediária com sempre 4 mitocôndrias, centríolos proximal e distal e aparato de ancoragem do flagelo que se divide em processos primário, secundário e terciário (Afzelius & Franzén, 1971; Hedwig & Schafer, 1986; Hinsch, 1974; Rouse & Pitt, 2000; Tiseo et al., submetido capítulo 2). Quando comparados, a morfologia dos espermatozoides de Scyphozoa diferem principalmente na morfologia da cabeça: ovoide em representantes de *Nausithoe* sp., *C. hysoscella, P. punctata* e *L. lucerna*, triangular em *C. lactea* e alongado em *C. mosaicus, A. aurita, R. pulmo* e *C. maremetens* (Afzelius & Franzén, 1971; Hedwig & Schafer, 1986; Hinsch, 1974; Rouse & Pitt, 2000; Tiseo et al., submetido capítulo 2).

Comparando a ultraestrutura da gônada feminina de cifozoários conseguimos identificar 2 tipos diferentes de trofócitos, confirmar a presença da via exógena de vitelo com a presença de vesículas endocíticas recobertas por clatrina trazendo material extracelular para dentro do ovócito e também de uma matriz extracelular (membrana vitelínica) recobrindo o ovócito. Eckelbarger & Larson (1988) são os primeiros a descrever a ultraestrutura da gônada feminina e detalham não somente a vitelogênese para *A. aurita* mas também são os pioneiros em descrever os trofócitos. Os trofócitos de *A. aurita* são células achatadas eletrondensas com várias microvilosidades em sua porção apical. Do mesmo modo que em *A. aurita* e *R. pulmo* (Avian & Rottini-Sandrini, 1991), os ovócitos pré-vitelogênicos de *Versuriga anadyomene* encontram-se dentro do epitélio genital e possuem um núcleo com nucléolo evidente (Fig. 18A). Os trofócitos, que englobam os ovócitos intermediários e tardios, são eletrondensos e com núcleos centralizados (Fig. 18B-D). Na região de contato entre o ovócito e trofócito são encontradas as vesículas endocíticas cobertas por clatrina que levam os compostos extracelulares para o próprio ovócito produzir o vitelo (Fig. 18E-H). Do mesmo modo

que Adonin et al. (2012) descrevem para *A. aurita*, no ooplasma dos ovócitos de *V. anadyomene*, além dos grânulos de vitelo também são encontrados grânulos proteicos eletrondensos, descritos por Adonin et al. como sendo mesogleina (Fig. 18H). Um segundo tipo de trofócito foi descrito para *P. noctiluca*, *D. lobata* (Avian & Rottini-Sandrini, 1991), *S. meleagris* e *Diplulmaris antarctica* (Eckelbarger & Larson, 1992) e *C. lactea* (Tiseo et al. capítulo 3). Diferentemente do descrito por Eckelbarger & Larson (1988), o trofócito do tipo 2 possui citoplasma de eletrondensidade intermediária e grandes vacúolos com material extracelular fibroso que auxilia no processo de ovulação e manutenção do ovócito na água (Avian & Rottini-Sandrini, 1991; Tiseo et al. capítulo 3). Para Coronatae apenas o trabalho de Tiemann & Jarms (2010) descrevem a ultraestrutura da gônada feminina de *P. periphylla* e identificam dois tipos celulares ainda não descritos para Scyphozoa: as células que compõem o poro e células que liberam muco durante o processo de liberação do ovócito.

Estratégias de liberação de gametas, tipos de fecundação, incubação e semelparidade

Para os cifozoários, existem duas estratégias de liberação de espermatozoides: livres ou agrupados em "*spermatozeugmata"*, em ambos os casos sempre ocorrendo na coluna d'água. Em Scyphozoa os ovócitos sofrem maturação quando imersos na mesogléia e podem ser liberados para o meio externo ou mantidos na cavidade gastrovascular (Avian & Rottini-Sandrini, 1991; Kikinger, 1992; Lucas & Reed, 2010; André C Morandini & Silveira, 2001; Agustín Schiariti et al., 2012; Widersten, 1965). Para a maioria das espécies de Scyphozoa os espermatozoides são liberados livres na cavidade gastrovascular por um poro ou ruptura da parede do folículo e epitélio genital e atingem o meio externo via manúbrio e boca (Ikeda et al., 2011; Lucas & Reed, 2010;

Morandini & Silveira, 2001; Ohtsu et al., 2007; Paspaleff, 1938; Schiariti et al., 2012; Tiemann & Jarms, 2010; Tiseo et al. submetido capítulo 2; Widersten, 1965). Exemplos dessa estratégia são a maioria dos coronados. Em outras espécies a liberação dos espermatozoides se dá em grupos – "spermatozeugmata" – vários espermatozoides imersos em uma matriz extracelular e com flagelos soltos como observado para as espécies do gênero *Cassiopea* (Gohar & Eisawy, 1960; Hofmann & Hadfield, 2002; Smith, 1936; Tiseo et al, capítulo 4), *Mastigias papua* (Uchida, 1926) e *Cotylorhiza tuberculata* (Kikinger, 1992). Rouse & Pitt (2000) descrevem que as espécies *Phyllorhiza punctata* e *Catostylus mosaicus* provavelmente liberam os espermatozoides em "spermatozeugmata". No entanto, esta afirmação dos autores (Rouse & Pitt, 2000) carece de observação e comprovação detalhada; uma vez que nenhum "spermatozeugmata" foi observado nas figuras apresentadas no estudo.

O tipo de fecundação exibido pelas espécies está associado às estratégias de liberação de espermatozoides (livre ou em "*spermatozeugmata"*) e ao tipo de trofócitos na gônada feminina (com ou sem vacúolos). A fecundação externa está atrelada à liberação de espermatozoides livres na água e trofócitos do tipo 2 com grandes vacúolos, que liberam os ovócitos também direto na água imersos em um muco glicoproteico (Tabela 2). Já a fecundação interna (na cavidade gastrovascular ou dentro da gônada feminina) parece estar atrelada à liberação de espermatozoides por "*spermatozegmata*" e a presença do trofócito do tipo 1 (sem os vacúolos). A literatura indica que os representantes do gênero *Cyanea, Aurelia* e *Cassiopea*, as espécies *Cotylorhiza tuberculata*, *Rhizostoma pulmo* e *C. hysoscella*, possuem liberação de espermatozoides via "*spermatozeugmata"*, trofócitos tipo 1, fertilização interna e incubação das plânulas. As demais espécies de Scyphozoa, que liberam espermatozoides livres e possuem trofócito tipo 2 realizam fertilização externa (Tabela 2). De modo geral (Arai, 1997), a

fertilização interna acontece quando a massa de espermatozoides é transferida para a fêmea, mas também quando espermatozoides na coluna d'água são absorvidos pela fêmea através do manúbrio ou microbocas dos braços orais (Arai, 1997). Após a fertilização, os ovos podem ser liberados na coluna d'água ou ficam alojados tanto na gônada feminina, na cavidade gastrovascular, ou em bolsas na superfície mais interna dos braços orais. São nessas bolsas que o desenvolvimento acontece. Algumas espécies de Scyphozoa incubam os ovos, gástrulas ou plânulas dentro do próprio tubo peridérmico – *Nausithoe racemosa* e *Nausithoe planulophora* (Kawaguti & Yoshimoto, 1973; Werner, 1973) –, em bolsas encontradas nos braços orais ou cavidade gástrica – *Cyanea capillata*, *C. lamarkii*, *Chrysaora hysocella* (Widersten, 1965), *Aurelia* sp. (Hamner & Jessen, 1974), *C. tuberculata* (Kikinger, 1992) e como descrito aqui para *Cassiopea culionensis* (Fig. 19).

São poucos os trabalhos de biologia reprodutiva em Cnidaria que abordam o modo de reprodução, se semélparo ou iteróparo. Acredita-se que as águas-vivas sejam semélparas, ou seja, que investem nas gônadas, se reproduzem e morrem (Arai, 1997). No entanto este modo de reprodução foi registrado apenas para espécies de *Cyanea* (Brewer, 1989), que após o pico reprodutivo, as populações da medusa diminuem o diâmetro umbrelar e a gônada sofre redução do peso em massa até desaparecerem.

Gonocorismo e Hermafroditismo

A maioria das espécies de cifozoários são dióicos (Fautin, 1992). Porém, alguns casos de hermafroditismo são descritos para algumas espécies de cifomedusas. Em Coronatae temos as espécies *N. eumedusoides* e *N. marginata* (Jarms, 1990; Werner, 1974) e em Discomedusae as espécies *Pseudorhiza haeckeli, C. hysoscella* (Haeckel, 1880; Widersten, 1965) que é hermafrodita protândrica e algumas *Cassiopea* que são hermafroditas simultâneas ou sequenciais (Hofmann & Hadfield, 2002; Tiseo et al. capítulo 4). Em

Coronatae também são registradas outras estratégias reprodutivas como é o caso de *Thecoscyphus zibrowii* que é partenogenética (Sötje & Jarms, 1999, 2009); *Nausithoe planulophora*, em que o pólipo origina diretamente e incuba planulóides; e *Nausithoe racemosa*, que mesmo sendo gonocórica, mantém os medusóides retidos dentro do tubo peridérmico (Kawaguti & Yoshimoto, 1973). Para os coronados, parece existir uma relação entre as características hermafrodita ou partenogenética com o ambiente cavernícola no qual os animais vivem. Já para as espécies de *Cassiopea* não se sabe ao certo como a condição hermafrodita se manifesta, uma vez que são poucos os registros de hermafroditismo para o gênero e não necessariamente relacionados ao ambiente em que vivem (Tiseo et al, capítulo 4). Mesmo sendo poucos os casos de hermafroditismo em Scyphozoa, ainda não se sabe como essas estratégias reprodutivas se manifestam dentro do cenário evolutivo do grupo e se há um sinal evolutivo suficiente para suportar a tendência evolutiva proposta por Sötje & Jarms (1999) de que há uma redução do sexo masculino na família Nausithoidae (Coronatae).

Bayha et al. (2010) apresentam a mais recente topologia das relações de parentesco de Scyphozoa onde tentam resolver as relações das 22 famílias do grupo. Utilizando esta topologia como um diagrama para discutir diferentes aspectos da reprodução sexuada de cifozoários, pode-se indicar os seguintes pontos (Fig. 20): i. todos os cifozoários possuem gônada de origem gastrodérmica e localização na gastroderme, como os representantes das classes Staurozoa e Cubozoa, diferindo de Hydrozoa que possuem gônadas de origem gastrodérmica e/ou epidérmica e com localização na epiderme (Miller, 1983); ii. em Scyphozoa a morfologia macróscopica da gônada difere bastante entre os grandes grupos (Coronatae vs. Semaeostomeae+Rhizostomeae) e dentro de Coronatae. Estas diferenças estão relacionadas ao espaço relativo para a gônada crescer dentro da cavidade gástrica; iii. a localização e quantidade das gônadas de Coronatae (8

gônadas adradiais) difere dos Discomedusae (4 gônadas interadiais); iv. as 4 gônadas de Semaeostomeae tem as aberturas direcionadas para a boca enquanto as gônadas de Rhizostomeae tem as aberturas direcionadas para fora; v. existe um padrão histológico único para o grupo: a gônada é formada pelo epitélio genital e as células germinativas encontram-se imersas na mesogléia. vi. exceto em *P. periphylla*, os ovócitos dos representantes de Coronatae possuem a via exógena de acúmulo de vitelo mesmo não estando circundados pelos trofócitos; vii. trofócitos são células exclusivas de Discomedusae que auxiliam tanto na nutrição quanto na ovulação dos ovócitos; viii. existem dois tipos de trofócitos encontrados nos diferentes Discomedusae e eles não parecem ser informativos para traçar relações de parentesco entre o grupo; e ix. há uma relação direta das estratégias de liberação de gametas (livres ou *spermatozeugmata*), com a presença dos trofócitos tipo 1 ou 2 e também com o tipo de fecundação e mecanismo de incubação.

Mesmo com o avanço no conhecimento da reprodução sexuada do grupo ainda são muitas as lacunas a serem preenchidas para entender a real diversidade reprodutiva de Scyphozoa. Estudos morfológicos completares, especialmente dentro das áreas de microscopia óptica, de transmissão e fluorescência são urgentemente necessários para ampliar o entendimento da anatomia comparada e dos processos celulares e teciduais do grupo. Análises gerais englobando as características reprodutivas exclusivas de Scyphozoa podem ser informativas e podem trazer novos indicativos para o entendimento da evolução do grupo.

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Tabelas

Tabela 1: Características relacionadas com a reprodução sexuada de Scyphzoa. Onde: n/a = sem dados disponíveis. Lista das espécies válida a partir de Jarms & Morandini (2019).

Família	Subfamília	Espécie	Descrição original	Morfologia gonadal	Cor gonadal	Quantidade	Histologia	Referência
Atollidae		*Atolla bairdii*	Fewkes, 1886	Em forma de feijão	Vermelho escuro; marrom	8	n/a	
		Atolla chuni	Vanhöffen, 1902	Oval; alongada	Creme-laranja	8	Fêmea	Vanhöffen, 1902
		Atolla gigantea	Maas, 1897	Oval	Creme-laranja	8	Fêmea	Mass, 1897
		Atolla parva	Russel, 1958	Oval	Vermelho-escuro	8	n/a	
		Atolla russelli	Repelin, 1962	Em forma de feijão	n/a	8	n/a	
		Atolla tenella	Hartlaub, 1909	n/a	n/a		n/a	
		Atolla valdiviae	Vanhöffen, 1902	Oval	Laranja	8	Fêmea	Vanhöffen, 1902; Russel ,1970
		Atolla vanhoeffeni	Russel, 1957	Oval	Amarelada-marrom	4 pares (8)	Macho	Tiseo et al, 2022 (submetido)
		Atolla verrilli	Fewkes, 1886	Oblonga; em forma de feijão	n/a	4 pares (8)	Macho e Fêmea	Vanhöffen, 1902;
		Atolla wyvillei	Haeckel, 1880	Oval; em forma de feijão	Creme-laranja	8	Macho e Fêmea	Vanhöffen, 1902; Lucas & Reed, 2010
Atorellidae		*Atorella arcturi*	Bigelow, 1928	Irregular	n/a	6	n/a	
		Atorella japonica	Kawaguti & Matsuno, 1981	n/a	n/a		n/a	
		Atorella octogonos	Mills, et al.,1987	Fusiforme	Creme-marrom	8	n/a	
		Atorella sibogae	Leloup, 1937	n/a	n/a	?	n/a	Morandini & Jarms, 2005
		Atorella subglobosa	Vanhöffen, 1902	Em forma de feijão	Amarelada	4	n/a	
		Atorella vanhoeffeni	Bigelow, 1909	Oval	De rosa até opaca	4	Macho	Bigelow, 1909; Grassé, 1993
Linuchidae		*Linantha lunulata*	Haeckel, 1880	Em forma de crescente	n/a	4	n/a	
		Linuche aquila	Haeckel, 1880	Ferradura direcionada para fora	n/a	4 pares (8)	n/a	
		Linuche draco	Haeckel, 1880	Ferradura direcionada para fora	n/a	8	n/a	
		Liuche unguiculata	Swartz, 1788	Em forma de crescente	Esbranquiçada	8	Fêmea	Eckelbarger & Larson, 1992
Nausithoidae		*Nausithoe albatrossi*	Maas, 1897	Alongada; oval; oblonga	n/a	8	n/a	
		Nausithie albida	Gegenbaur, 1856	n/a	n/a	8	n/a	
		Nausithoe atlantica	Broch, 1913	Oblonga	Creme-laranja	8	Fêmea	Eckelbarger & Larson, 1992

	Nausithoe maculata (=aurea)	Silveira & Morandini, 1997	Circular	Marrom-claro	8	Macho e Fêmea	Silveira & Morandini, 2001
	Nausithoe challengeri	Haeckel, 1880	Em forma de feijão	Amarelada-laranja	8	n/a	
	Nausithoe clausi	Vanhöffen, 1892	Circular		8		
	Nausithoe eumedusoides	Werner, 1974	Em forma de feijão; oblonga	Amarelada marrom	3 a 6	Macho e Fêmea	Werner, 1971; Tiseo et al, 2022 (submetido)
	Nausithoe globifera	Broch, 1913	Oblonga; quadrangular	Marrom-claro; vermelho-claro	8	n/a	
	Nausithoe hagenbecki	Jarms, 2001	Circular	Marrom-claro	8	n/a	
	Nausithoe limpida	Hartlaub, 1909	forma de coração	n/a	8	n/a	
	Nausithoe maculata	Jarms, 1990	Circular	Marrom-claro para amarelada-branca	8	n/a	
	Nausithoe marginata	Kölliker, 1853	Oval	Machoa marelada, Fêmea sem cor	8	n/a	
	Nausithoe picta	Agassiz & Mayer, 1902	Oval	Marrom chocolate ou carmim	8	n/a	
	Nausithoe planulophora	Werner, 1971	Ausente	n/a	8	n/a	
	Nausithoe punctata	Kölliker, 1853	Ferradura direcionada para fora	Branca-amarelada (imaturos); marrom-escuro-escuro ou azul	8	n/a	
	Nausithoe racemosa	Komai, 1936	Circular		4	n/a	
	Nausithoe rubra	Vanhöffen, 1902	Triangular	Laranja ou roxo-escuro	8	n/a	
	Nausithoe simplex	Kirkpatrick, 1890	n/a	n/a	8	n/a	
	Nausithoe sorbei	Jarms et al, 2003	n/a	n/a	8	n/a	
	Nausithoe striata	Vanhöffen, 1910	n/a	n/a	8	n/a	
	Nausithoe silveira	Molinari, 2019	n/a	n/a	8	n/a	
	Nausithoe thieli	Jarms, 1990	n/a	n/a	8	n/a	
	Nausithoe werneri	Jarms, 1990	Circular	Macho opaco; fêmea transparente	8	Macho	Jarms, 1990; Tiseo et al, 2022 (submetido)
	Thecoscyphus zibrowii	Werner, 1984	Oblonga	n/a		Fêmea	Sötje & Jarms, 1999
	Palephyra antiqua	Haeckel, 1880	Em forma de crescente	n/a	4	n/a	
	Palephyra indica	Vanhöffen, 1902	Em forma de feijão	n/a	4	n/a	
	Palephyra pelagica	Haeckel, 1880	Em forma de crescente	n/a	4	n/a	
Paraphyllinidae	*Paraphyllina intermedia*	Maas, 1903	Em forma de feijão	Opaco- creme	4 pares (8)	n/a	
	Paraphyllina ransoni	Russel, 1956	Em forma de W	Sem cor	8	Macho e Fêmea	Russel, 1956
	Paraphyllina rubra	Neppi, 1915	Em forma de W	Marrom-escuro	8	n/a	

Periphyllidae	*Nauphanthopsis diomedeae*	Fewkes, 1886	n/a	n/a	n/a	n/a	
	Pericopla campana	Haeckel, 1880	Oval	n/a	8	n/a	
	Pericolpa quadrigata	Haeckel, 1880	Oval; alongada	n/a	4 pares (8)	n/a	
	Pericopla tetralina	Haeckel, 1880	Oval; alongada	n/a	4 pares (8)	n/a	
	Periphylla periphylla	Péron & Lesueur, 1810	Em forma de J ou U	Esbranquiçado	4 pares (8)	Macho e Fêmea	Lucas & Reed, 2010; Tiemman & Jarms, 2010; Tiseo et al, 2022 (submetido)
	Periphyllopsis braueri	Vanhöffen, 1902	Em forma de C	Esbranquiçado	4 pares (8)	n/a	
	Periphyllopsis galatheae	Kramp, 1959	Ferradura direcionada para fora	n/a	4	n/a	
Cyaneidae	*Cyanea annasethe*	Haeckel, 1880	n/a	n/a	4	n/a	
	Cyanea annaskala	von Lendenfeld, 1882	n/a	n/a	4	n/a	
	Cyanea barkeri	Gershwin et al., 2010	n/a	n/a	4	n/a	
	Cyanea buitendijki	Stiasny, 1919	n/a	n/a	4	Fêmea	Widersten, 1965
	Cyanea capillata	Linnaeus, 1758	Em forma de sacos plásticos	Amarelada-laranja	4	Macho e Fêmea	Widersten, 1965; Este estudo
	Cyanea lamarkii	Péron & Lesueur, 1810	Em forma de sacos plásticos	n/a	4	Fêmea	Este estudo
	Cyanea mjöbergi	Stiasny, 1921	n/a	n/a	4	n/a	
	Cyanea mullerianthe	Haacke, 1887	n/a	n/a	4	n/a	
	Cyanea nozakii	Kishinouye, 1891	n/a	n/a	4	n/a	
	Cyanea rosea	Quoy & Gaimar, 1824	n/a	n/a	4	n/a	
	Cyanea tzetlinii	Kolbasova & Neretina, 2015	Em forma de sacos plásticos	n/a	4	n/a	
	Cyanea fulva	Agassiz, 1862	n/a	n/a	4	n/a	
	Cyanea versicolor	Agassiz, 1862	n/a	n/a	4	n/a	
	Cyanea citrea	Kishinouye, 1910	n/a	Amarelada-claro	4	n/a	
	Cyanea ferruginea	Eschscholtz, 1829	n/a	n/a	4	n/a	
	Cyanea postelsi	Brandt, 1835	n/a	n/a	4	n/a	
	Cyanea purpurea	Kishinouye, 1910	n/a	n/a	4	n/a	
	Desmonema comatum	Larson, 1986	Em forma de sacos plásticos	n/a	4	n/a	
	Desmonema gaudichaudi	Lesson, 1830	Em forma de sacos plásticos	n/a	4	n/a	

	Desmonema glaciale	Larson, 1986	Em forma de sacos plásticos	n/a	4	n/a	
	Desmonema scoresbyanna	Gershwin & Zeidler, 2008	Em forma de sacos plásticos	n/a	4	n/a	
Drymonematidae	*Drymonema dalmatinum*	Haeckel, 1880	n/a	Marrom-claro ou rosa-claro	4	n/a	
	Drymonema gorgo	Müller, 1883	n/a	Marrom-claro ou rosa-claro	4	n/a	
	Drymonema larsoni	Bayha & Dawson, 2010	Em forma de sacos plásticos	Marrom-claro ou rosa-claro	4	n/a	
Pelagiidae	*Chrysaora achlyos*	Martin et al., 1997	Semi-círculo	n/a	4	n/a	
	Chrysaora africana	Vanhöffen, 1902	Semi-círculo	n/a	4	n/a	
	Chrysaora agulhensis	Ras et al., 2020	Semi-círculo	n/a	4	n/a	
	Chrysaora chesapeakei	(Papenfuss, 1936)	n/a	n/a	4	n/a	
	Chrysaora chinensis	Vanhöffen, 1888	Semi-círculo	n/a	4	n/a	
	Chrysaora colorata	Russel, 1964	Semi-círculo	n/a	4	n/a	
	Chrysaora fulgida	Reynaud, 1830	Semi-círculo	n/a	4	n/a	
	Chrysaora fuscescens	Brandt, 1835	Semi-círculo	n/a	4	n/a	
	Chrysaora hysoscella	Linnaeus, 1767	Semi-círculo	n/a	4	Macho e Fêmea	Claus, 1883; Widersten, 1965; Este estudo
	Chrysaora lactea	Eschscholtz, 1829	Semi-círculo	Esbranquiçada, amarelada-marrom até rosa claro	4	Macho e Fêmea	Morandini & Jarms, 2004; Tiseo et al 2022 (submetido); Tiseo & Morandini (capítulo3)
	Chrysaora melanaster	Brandt, 1835	Semi-círculo	n/a	4	n/a	
	Chrysaora pacifica	Goette, 1886	Semi-círculo	n/a	4	n/a	
	Chrysaora pentastoma	Péron & Lesueur, 1810	Em forma de crescente e em forma de M	n/a	4	n/a	
	Chrysaora plocamia	Lesson, 1830	Semi-círculo	Esbranquiçada até rosa-claro	4	n/a	
	Chrysaora pseudoocellata	Mutlu, et al. 2020	Em forma de W	Creme-amarelada		n/a	
	Chrysaora quinquecirrha	Desor, 1848	Semi-círculo	Esbranquiçada- rosa claro	4	n/a	
	Mawia benovici	Piraino, et al. 2014	Ferradura direcionada para dentro	Branco	4	n/a	
	Pelagia noctiluca	Forskål, 1775	Alongada	n/a	4	Fêmea	Avian & Rottini-Sandrini, 1991
	Pelagia cyanella	Péron & Lesueur, 1810	n/a	n/a	4	n/a	
	Pelagia discoidea	Eschscholtz, 1829	Crescente	n/a	4	n/a	

		Pelagia flaveola	Eschscholtz, 1829	n/a	n/a	4	n/a	
		Pelagia panopyra	Péron & Lesueur, 1810	n/a	n/a	4	n/a	
		Sanderia malayensis	Goette, 1886	n/a	n/a	4	n/a	
		Sanderia pampinosus	Gershwin & Zeidler, 2008	n/a	n/a	4	n/a	
		Phacellophora camtschatica	Brandt, 1835	Em forma de sacos plásticos	Esbranquiçada; amarelada vivo	4	n/a	
Ulmaridae	Aureliinae	*Aurelia aurita*	Linnaeus, 1758	Ferradura direcionada para dentro	Malva, violeta, avermelhada rosa ou amarelada	4	Macho e Fêmea	Widersten 1965; Eckelbarger & Larson 1988
		Aurelia ayla	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia cebimarensis	Lawley et al, 2021	Ferradura direcionada para dentro	n/a	4	Macho	This study
		Aurelia clausa	Lesson, 1830	n/a	n/a	4	n/a	
		Aurelia coerulea	von Lendenfeld, 1884	Ferradura direcionada para dentro	Branca ou rosa-claro	4	n/a	
		Aurelia colpota	Brandt, 1835	Ferradura direcionada para dentro	Rosa até vermelho	4	n/a	
		Aurelia columbia	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia dubia	Vanhöffen, 1888	n/a	n/a	4	n/a	
		Aurelia hyalina	Brandt, 1835	n/a	n/a	4	n/a	
		Aurelia insularia	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia labiata	Chamisso & Eysenhardt, 1821	Ferradura direcionada para dentro	n/a	4	n/a	
		Aurelia limbata	Brandt, 1835	Ferradura direcionada para dentro	n/a	4	n/a	
		Aurelia malayensis	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia maldivensis	Bigelow, 1904	Ferradura direcionada para dentro	n/a	4	n/a	
		Aurelia marginalis	L. Agassiz, 1862	Ferradura direcionada para dentro	Rosa -claro	4	n/a	
		Aurelia mianzani	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia miyakei	Lawley et al, 2021	n/a	n/a	4	n/a	

		Aurelia montyi	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia mozambica	Brown & Gibbons, 2021	n/a	n/a	4	n/a	
		Aurelia persea	(Forsskål, 1775)	n/a	n/a	4	n/a	
		Aurelia pseudosolida	Garić & Batistić, 2022	n/a	n/a	4	n/a	
		Aurelia rara	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia relicta	Scorrano et al., 2016	n/a	n/a	4	n/a	
		Aurelia smithsoniana	Lawley et al, 2021	n/a	n/a	4	n/a	
		Aurelia solida	Browne, 1905	Ferradura direcionada para dentro (Bastante circular)	n/a	4	n/a	
		Aurelia vitiana	Agassiz & Mayer, 1899	Ferradura direcionada para dentro	Lilás	4	n/a	
		Aurosa furcata	Haeckel, 1880	Ferradura direcionada para dentro	n/a	4	n/a	
	Deepstariinae	*Deepstaria enigmatica*	Russell, 1967	Em forma de leque	n/a	4	n/a	
		Deepstaria reticulum	Larson et al., 1988	n/a	n/a	4	n/a	
	Poraliinae	*Poralia rufescens*	Vanhöffen, 1902	Ferradura direcionada para dentro	n/a	4	n/a	
	Stellamedusinae	*Stellamedusa ventana*	Raskoff & Matsumoto, 2004	Ferradura direcionada para dentro	n/a	4	n/a	
	Sthenoniinae	*Stenonia albida*	Eschscholtz, 1829	Ferradura direcionada para dentro	Esbranquiçada	4	n/a	
	Stygiomedusinae	*Stygiomedusa gigantea*	Browne, 1910	n/a	n/a	4	n/a	
	Tiburoniinae	*Tiburonia granrojo*	Matsumoto et al, 2003	n/a	Esbranquiçada	4	n/a	
		Diplulmaris antarctica	Maas, 1908	n/a	n/a	4	Fêmea	Eckelbarger & Larson, 1992
		Diplulmaris malayensis	Stiasny, 1935	Em forma de crescente	Amarela-rosa	4	n/a	
		Discomedusa lobata	Claus, 1877	Em forma de crescente	Branco	4	Fêmea	Avian & Rottini-Sandrini, 1991
	Ulmarinae	*Discomedusa philippina*	Mayer, 1910	Em forma de crescente	n/a	4	n/a	
		Floresca parthenia	Haeckel, 1880	Em forma de crescente	n/a	4	n/a	
		Parumbrosa polyloobata	Kishinouye, 1910	Em forma de crescente (longa e estreita)	n/a	4	n/a	
		Ulmaris prorotypus	Haeckel, 1880	Em forma de crescente	n/a	4	n/a	
		Ulmaris senlliusi	Stiasny, 1935	Em forma de crescente	n/a	4	n/a	
Cepheidae		*Cephea cephea*	Forskål, 1775	n/a	n/a	4	n/a	

	Cephea coerulea	Vanhöffen, 1902	n/a	n/a	4	n/a	
	Cephea conifera	Haeckel, 1880	n/a	n/a	4	n/a	
	Cephea octostyla	Forskål, 1775	Cruciforme	n/a	4	n/a	
	Cotylorhiza ambulacrata	Haeckel, 1880	Cruciforme	n/a	4	n/a	
	Cotylorhiza erythraea	Stiasny, 1920	n/a	n/a	4	n/a	
	Cotylorhiza tuberculata	Macri, 1778	Cruciforme	n/a	4	Macho e Fêmea	Kikinger, 1992
	Marivagia stellata	Galil & Gershwin, 2010	Cruciforme (crescente)	Rosa-claro	4	n/a	
	Netrostoma coerulescens	Mass, 1903	n/a	n/a	4	n/a	
	Netrostoma dumokuroa	Agassiz & Mayer, 1899	n/a	n/a	4	n/a	
	Netrostoma nuda	Gershwin & Zeidler, 2008	Cruciforme (em formato de U)	Amarelada	4	n/a	
	Netrostoma setouchianum	Kishinouye, 1902	n/a	Rosa	4	n/a	
	Polyrhiza vesiculosa	Ehrenberg, 1837	n/a	n/a	4	n/a	
Cassiopeidae	*Cassiopea andromeda*	Forskål, 1775	Cruciforme	n/a	4	Macho e Fêmea	Gohar & Eisawy, 1960; Tiseo et al (capítulo 4)
	Cassiopea culionensis	Light, 1914	n/a	n/a		n/a	
	Cassiopea depressa	Haeckel, 1880	n/a	n/a	4	n/a	
	Cassiopea frondosa	Pallas, 1774	Cruciforme	n/a	4	Macho e Fêmea	Smith, 1936; Tiseo et al (capítulo 4)
	Cassiopea maremetens	Gershwin et al., 2010	Cruciforme	n/a	4	Macho	Tiseo et al (capítulo 4)
	Casiopea mayeri	Gamero-Mora et al., 2022	n/a	n/a		n/a	
	Cassiopea medusa	Light, 1914	n/a	n/a	4	n/a	
	Cassiopea mertensi	Brandt, 1838	n/a	n/a	4	n/a	
	Cassiopea ndrosia	Agassiz & Mayer, 1899	Cruciforme	n/a	4	n/a	
	Cassiopea ornata	Haeckel, 1880	Cruciforme	n/a	4	n/a	
	Cassiopea vanderhorsti	Stiasny, 1922		n/a	4	n/a	
	Cassiopea xamacahana	Bigelow, 1892	Cruciforme (sacos plásticos)	n/a	4	Macho e Fêmea	Eckelbarger & Larson, 1992; Tiseo et al (capítulo 4)
Mastigiidae	*Mastigias albipunctata*	Stiasny, 1920	n/a	n/a	4	n/a	
	Mastigias andersoni	Stiasny, 1926	n/a	n/a	4	n/a	
	Mastigias gracilis	Vanhöffen, 1888	Cruciforme	n/a	4	n/a	
	Mastigias ocellatus	Modeer, 1791	n/a	n/a	4	n/a	
	Mastigias pantherina	Haeckel, 1880	n/a	n/a	4	n/a	

	Mastigias papua	Lesson, 1830	Cruciforme	n/a	4	Macho	Uchida, 1926
	Mastigias roseus	Reynaud, 1830	Cruciforme	Rosa-escuro	4	n/a	
	Mastigias sidereus	Chun, 1896	n/a	n/a	4	n/a	
	Mastigietta palmipes	Haeckel 1880	Cruciforme	n/a	4	n/a	
	Phyllorhiza luzoni	Mayer, 1915	Cruciforme	n/a	4	n/a	
	Phyllorhiza pacifica	Light, 1921	Cruciforme	n/a	4	n/a	
	Phyllorhiza punctata	von Lendenfeld, 1884	Cruciforme	n/a	4	Macho	Rippingale & Kelly, 1995; Rouse & Pitt, 2000
Leptobrachidae	*Leptobrachia leptopus*	Chamisso & Eysenhardt, 1821	Cruciforme	Amarelada	4	n/a	
	Thysanostoma flagellatum	Haeckel, 1880	Cruciforme (ferradura)	n/a	4	n/a	
	Thysanostoma loriferum	Ehrenberg, 1837	Cruciforme (ferradura)	Avermelhada-amarela	4	n/a	
	Thysanostoma thysanura	Haeckel, 1880	Cruciforme (ferradura)	Amarelada-laranja	4	n/a	
Versurigidae	*Versuriga anadyomene*	Maas, 1908	Cruciforme	Amarronzada-laranja	4	Fêmea	Este estudo
Catostylidae	*Acromitoides purpurus*	Mayer, 1910	n/a	n/a	4	n/a	
	Acromitoides stiphropterus	Schultze, 1898	n/a	n/a	4	n/a	
	Acromitus flagellatus	(Maas, 1903)	Cruciforme	n/a	4	n/a	
	Acromitus hardenbergi	Stiasny, 1934	n/a	n/a	4	n/a	
	Acromitus maculosus	Light, 1914	n/a	n/a	4	n/a	
	Acromitus rabachatu	Annandale, 1915	n/a	n/a	4	n/a	
	Acromitus tankahkeei	Light, 1924			4	n/a	
	Catostylus cruciatus	(Lesson, 1830)	Cruciforme	n/a	4	n/a	
	Catostylus mosaicus	(Quoy & Gaimard, 1824)	Cruciforme	n/a	4	Macho e Fêmea	Pitt & Kingsford, 2000
	Catostylus ornatellus	(Vanhöffen, 1888)	Em forma de U	n/a	4	n/a	
	Catostylus ouwensi	Moestafa & McConnaughey, 1966	Cruciforme	n/a	4	n/a	
	Catostylus perezi	Ranson, 1945	n/a	n/a	4	n/a	
	Catostylus tagi	(Haeckel, 1869)	n/a	n/a	4	n/a	
	Catostylus townsendi	Mayer, 1915		n/a	4	n/a	
	Catostylus tripterus	(Haeckel, 1880)	Cruciforme (ferradura)	n/a	4	n/a	
	Catostylus turgescens	(Schultze, 1898)	n/a	n/a	4	n/a	
	Catostylus viridescens	(Chun, 1896)	n/a	n/a	4	n/a	
	Crambione bartschi	(Mayer, 1910)	Cruciforme	n/a	4	n/a	

	Crambione cooki	Mayer, 1910	n/a	n/a	4	n/a	
	Crambione mastigophora	Maas, 1903	Cruciforme	Rosa ou Vermelho	4	n/a	
	Crambionella annandalei	Rao, 1931	n/a	n/a	4	n/a	
	Crambionella helmbiru	Nishikawa et al., 2015	Cruciforme	n/a	4	n/a	
	Crambionella orsini	(Vanhöffen, 1888)	Cruciforme	n/a	4	n/a	
	Crambionella stuhlmanni	(Chun, 1896)	n/a	n/a	4	n/a	
Lobonematidae	*Lobonema smithii*	Mayer, 1910	n/a	n/a	4	n/a	
	Lobonemoides gracilis	Light, 1914	Cruciforme	n/a	4	n/a	
	Lobonemoides robustus	Stiasny, 1920	n/a	n/a	4	n/a	
	Lobonemoides sewlli	Rao, 1931	n/a	n/a	4	n/a	
Lychnorhizidae	*Anomalorhiza shawi*	Light, 1921	n/a	n/a	4	n/a	
	Lychnorhiza arubae	Stiasny, 1920	n/a	n/a	4	n/a	
	Lychnorhiza lucerna	Haeckel, 1880	Cruciforme	Machos esbranquiçadas ou esverdeadas/ Fêmeas amarronzadas	4	Macho e Fêmea	Schiariti et al., 2008; Schiariti et al, 2012; Tiseo et al, 2022 (submetido); Este estudo
	Lychnorhiza malayensis	Stiasny, 1920	Cruciforme	n/a	4	n/a	
	Pseudorhiza aurosa	von Lendenfeld, 1882	n/a	n/a	4	n/a	
	Pseudrhiza haeckeli	Haacke, 1884	n/a	n/a	4	n/a	
Rhizostomatidae	*Eupilema inexpectata*	Pagès et al, 1992	n/a	n/a	4	n/a	
	Eupilema scapulare	Haeckel, 1880	n/a	n/a	4	n/a	
	Nemopilema nomurai	Kishinouye, 1922	Em forma de sacos plásticos	creme; rosa-claro; rosa; laranja; laranja-escuro; marrom; marrom-escuro	4	Macho e Fêmea	Ohtsu et al, 2007; Ikeda et al, 2010
	Rhizostoma luteum	(Quoy & Gaimard, 1827)	n/a	n/a	4	n/a	
	Rhizostoma octopus	(Gmelin, 1791)	Cruciforme aberta	Macho azul-claro; violeta; Fêmea avermelhada-marromreddih brwon	4	n/a	
	Rhizostoma pulmo	(Macri, 1778)	Cruciforme (em forma de feijão)	Amarelada	4	Macho e Fêmea	Paspalev, 1938; Widersten. 1965
	Rhopilema asamushi	Uchida, 1827		n/a	4	n/a	
	Rhopilema esculentum	Kishinouye, 1891	n/a	Amarela	4	n/a	
	Rhopilema hispidum	(Vanhöffen, 1888)	Cruciforme aberta	n/a	4	n/a	
	Rhopilema nomadica	Galil et al, 1990	Cruciforme aberta	n/a	4	Macho	Este estudo

	Rhopilema rhopalophorum	Haeckel, 1880		n/a	4	n/a	
	Rhopilema verrilli	(Fewkes, 1887)	Cruciforme aberta	n/a	4	n/a	
Stomolophidae	*Stomolophus fritillarius*	Haeckel, 1880		n/a	4	n/a	
	Stomolophus meleagris	L. Agassiz, 1860	Cruciforme aberta	n/a	4	Fêmea	Eckelbarger & Larson, 1992

Tabela 2: Estratégias de Liberação de gametas, fertilização e incubação em cifozoários.

Família	*Espécie*	Célula acessória	Ovulação	Liberação dos espermatozoides	Fertilização	Incubação	Referência
Atollidae	*Atolla chuni*	Ausente	n/a	n/a	n/a	n/a	Vanhöffen, 1902; Russell ,1970;
	Atolla gigantea	Ausente	n/a	n/a	n/a	n/a	Mass, 1897; Russell ,1970
	Atolla valdiviae	Ausente	n/a	n/a	n/a	n/a	Vanhöffen, 1902; Russell ,1970
	Atolla verrilli	Ausente	n/a	n/a	n/a	n/a	Vanhöffen, 1902; Russell ,1970
	Atolla wyvillei	Ausente	n/a	Espermatozoides no sino genital	n/a	n/a	Vanhöffen, 1902; Lucas & Reed, 2010
Linuchidae	*Linuche unguiculata*	Ausente	Liberado na água	Espermatozoides na água	Externa	Ausente	Eckelbarger & Larson, 1992; Silveira & Morandini, 1998
Nausithoidae	*Nausithoe aurea*	Ausente	Liberado na água	Espermatozoides no sino genital e água	Externa	Ausente	Silveira & Morandini, 2001
	Nausithoe eumedusoides	Ausente	Liberados no espaço gástrico	Espermatozoides liberados no espaço gástrico	Interna no espaço gástrico	Ausente	Werner, 1971; Tiseo et al, 2022 (submetido)
	Thecoscyphus zibrowii	Ausente	Ausente	Ausente	Ausente	n/a	Sötje & Jarms, 1999
Paraphyllinidae	*Paraphyllina ransoni*	Ausente	n/a	n/a	n/a	n/a	Russel, 1956
Periphyllidae	*Periphylla periphylla*	Cel. acessória	Liberados no espaço genital com muco	Espermatozoides imersos em muco/ Liberados na água	Externa	Ausente	Lucas & Reed, 2010; Tiemman & Jarms, 2010; Tiseo et al, 2022 (submetido)
Cyaneidae	*Cyanea buitendijki*	Trofócito 1	n/a	n/a	n/a	n/a	Widersten, 1965
	Cyanea capillata	Trofócito 1	Liberados no sino genital/ Mantém dentro da gônada feminina	Espermatozoides na água	Interna na gônada feminina	Retém plânula nos braços orais	Widersten, 1965; Este estudo
	Cyanea lamarkii	Trofócito 1	Liberados no sino genital/ Mantém dentro da gônada feminina	Espermatozoides na água	Interna na gônada feminina	Ausente	Este estudo
	Cyanea fulva					Retém plânula nos braços orais	
	Cyanea versicolor					Retém plânula nos braços orais	
Pelagiidae	*Chrysaora achlyos*		Liberado na água	Liberado na água	Externa		Jarms & Morandini 2019
	Chrysaora africana		Provavelmente liberado na água	Provavelmente liberado na água	Externa		Jarms & Morandini 2019
	Chrysaora colorata		Liberado na água	Liberado na água	Externa		Jarms & Morandini 2019
	Chrysaora fuscescens		Liberado na água	Liberado na água	Externa		Straehler-Pohl et al 2011
	Chrysaora hysoscella	Trofócito 1	Liberado na água	Liberado na água	Interna na gônada feminina	Embriões se desenvolvem até a gástrula na gônada feminina	Claus, 1883; Widersten, 1965; Este estudo
	Chrysaora lactea	Trofócito 2	Liberados no espaço genital com muco; liberado na água	Liberado na água	Externa		Morandini & Jarms, 2004; Tiseo et al (submetido); Tiseo & Morandini (capítulo3)

	Chrysaora pacifica		Liberado na água	Liberado na água	Externa		Jarms & Morandini 2019
	Chrysaora plocamia		Liberado na água	Liberado na água	Externa		Jarms & Morandini 2019
	Chrysaora quinquecirrha		Liberado na cavidade gástrica e na água	Liberado na água	Na cavidade gástrica ou Externa		Littleford, 1939
	Pelagia noctiluca	Trofócito 2	Liberados no espaço genital com muco; liberado na água	Liberado na água	Externa		Avian & Rottini-Sandrini, 1991
	Sanderia malayensis		Liberado na água	Liberado na água			Straehler-Pohl et al 2011
Ulmaridae	*Aurelia aurita*	Trofócito 1	Liberados no sino genital/ Mantém dentro da gônada feminina	Espermatozoides imersos em muco/ Liberados na água	Interna na gônada feminina ou sino genital	Plânula se desenvolve em bolsas nos braços orais	Widersten 1965; Russell, 1970; Eckelbarger & Larson 1988;
	Aurelia cebimarensis			Espermatozoides imersos em muco			Este estudo
	Stygiomedusa gigantea					Viviparous	Russel & Rees 1960
	Diplulmaris antarctica	Trofócito 2					Eckelbarger & Larson, 1992
	Discomedusa lobata	Trofócito 2					Avian & Rottini-Sandrini, 1991
Cepheidae	*Cephea cephea*					Retém plânula nos braços orais	Thiel, 1936
	Cotylorhiza tuberculata	Trofócito 1	Liberado no sino genital	*Spermatozeugmata*	Interna no sino genital	Retém plânula nos braços orais	Berril, 1949; Kikinger, 1992
Cassiopeidae	*Cassiopea andromeda*	Trofócito 1	Liberado no sino genital	*Spermatozeugmata*		Retém plânula na cavidade gástrica ou os braços orais	Gohar & Eisawy, 1960; Hofmann et al., 1996 Tiseo et al (capítulo 4)
	Cassiopea culionensis					Retém plânula nos braços orais	Este estudo
	Cassiopea frondosa	Trofócito 1	Liberados no sino genital/ Mantém dentro da gônada feminina				Smith, 1936; Tiseo et al (capítulo 4)
	Cassiopea maremetens			*Spermatozeugmata*		Retém plânula nos braços orais	Tiseo et al, 2022 (submetido)
	Cassiopea xamacahana	Trofócito 1		*Spermatozeugmata*		Retém plânula nos braços orais	Eckelbarger & Larson, 1992; Tiseo et al (capitulo 4)
Mastigiidae	*Mastigias papua*			*Spermatozeugmata*		Retém plânula nos braços orais	Uchida, 1926; Jarms & Morandini, 2019
	Phyllorhiza punctata				Parece ser interna	Parece reter plânula nos braços orais	Rippingale & Kelly, 1995; Rouse & Pitt, 2000
Versurigidae	*Versuriga anadyomene*	Trofócito 1				Ausente	Este estudo
Catostylidae	*Catostylus mosaicus*	Trofócito 2		Espermatozoides livres na água		Parece reter plânula nos braços orais	Pitt & Kingsford, 2000
Lychnorhizidae	*Lychnorhiza lucerna*	Trofócito 2	Liberado na água	Espermatozoides livres	Externa	Ausente	Schiariti et al., 2012; Tiseo et al, 2022 (submetido); Este estudo
Rhizostomatidae	*Nemopilema nomurai*	Trofócito 2	Liberado na água	Espermatozoides livres	Externa	Ausente	Kawahara et al, 2006; Ohtsu et al., 2007; Ikeda et al., 2011
	Rhizostoma pulmo	Trofócito 1	Liberados no sino genital/ Mantém dentro da gônada feminina	Espermatozoides livres	Interna na gônada feminina ou sino genital	Ausente	Paspaleff, 1938; Widersten, 1965
	Rhopilema esculentum					Ausente	Ding & Chen 1981; Dong et al. 2006

	Rhopilema nomadica		Espermatozoides imersos em muco/ Liberados na água	Ausente	Lotan et al., 1992; Lotan et al., 1994; Este estudo
	Rhopilema verrilli			Retém plânula nos braços orais	Cargo, 1971; Calder, 1973
Stomolophidae	*Stomolophus meleagris*	Trofócito 2		Ausente	Calder, 1982; Eckelbarger & Larson, 1992

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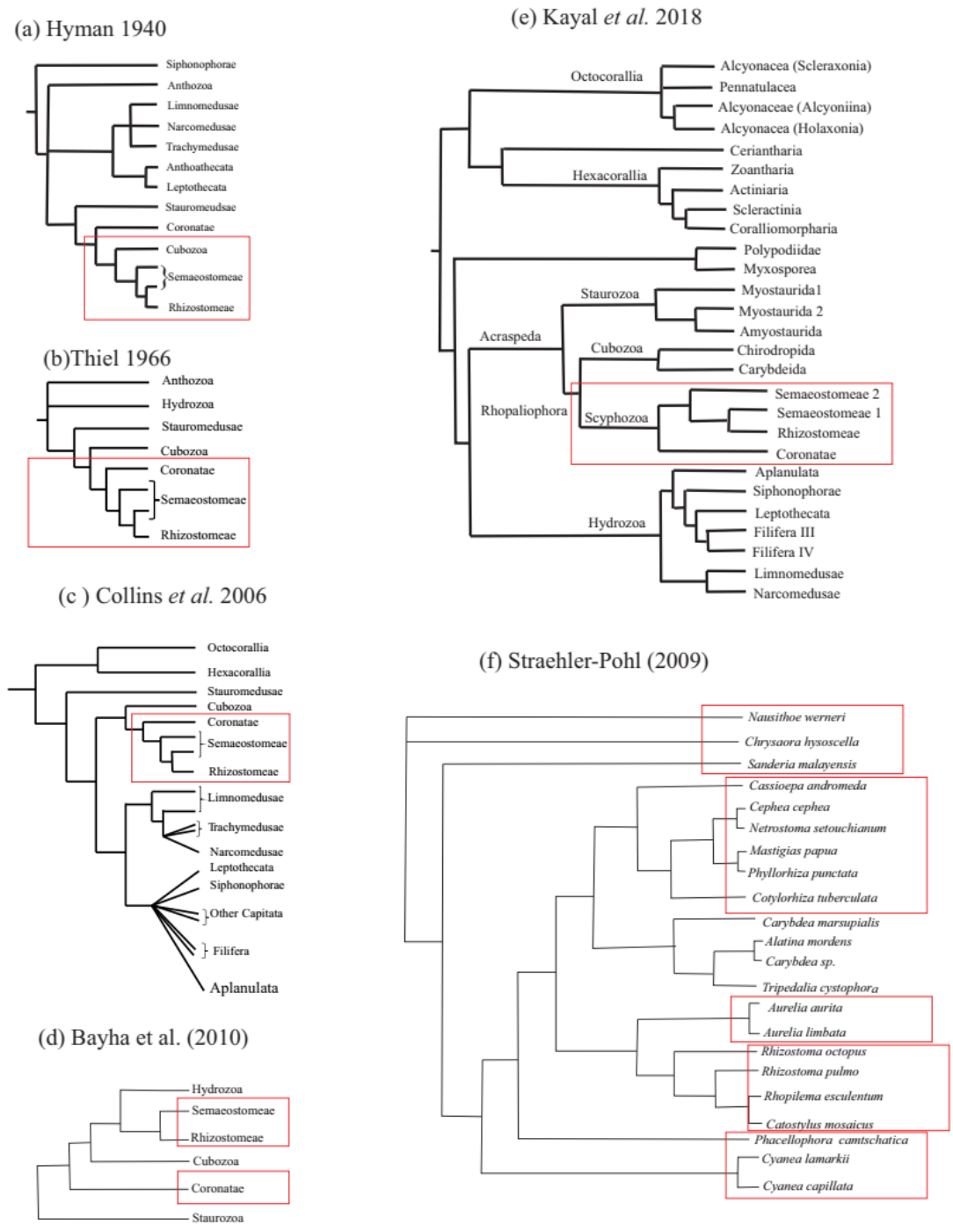

Figura 1: Comparação de algumas hipóteses filogenéticas propostas para o subfilo Medusozoa e que incluem a classe Scyphozoa. No retângulo vermelho filogenias que recuperam ou não a parafilia de Semaeostomeae ou Rhizostomeae. Ressaltando apenas as que apresentam diferenças que serão relevantes dentro do escopo deste trabalho. (A) Hyman, 1940; (B) Thiel, 1966; (C) Collins et al., 2006; (D) Bayha et al., 2010; (E) Kayal et al., 2018; (F) Straehler-Pohl, 2009.

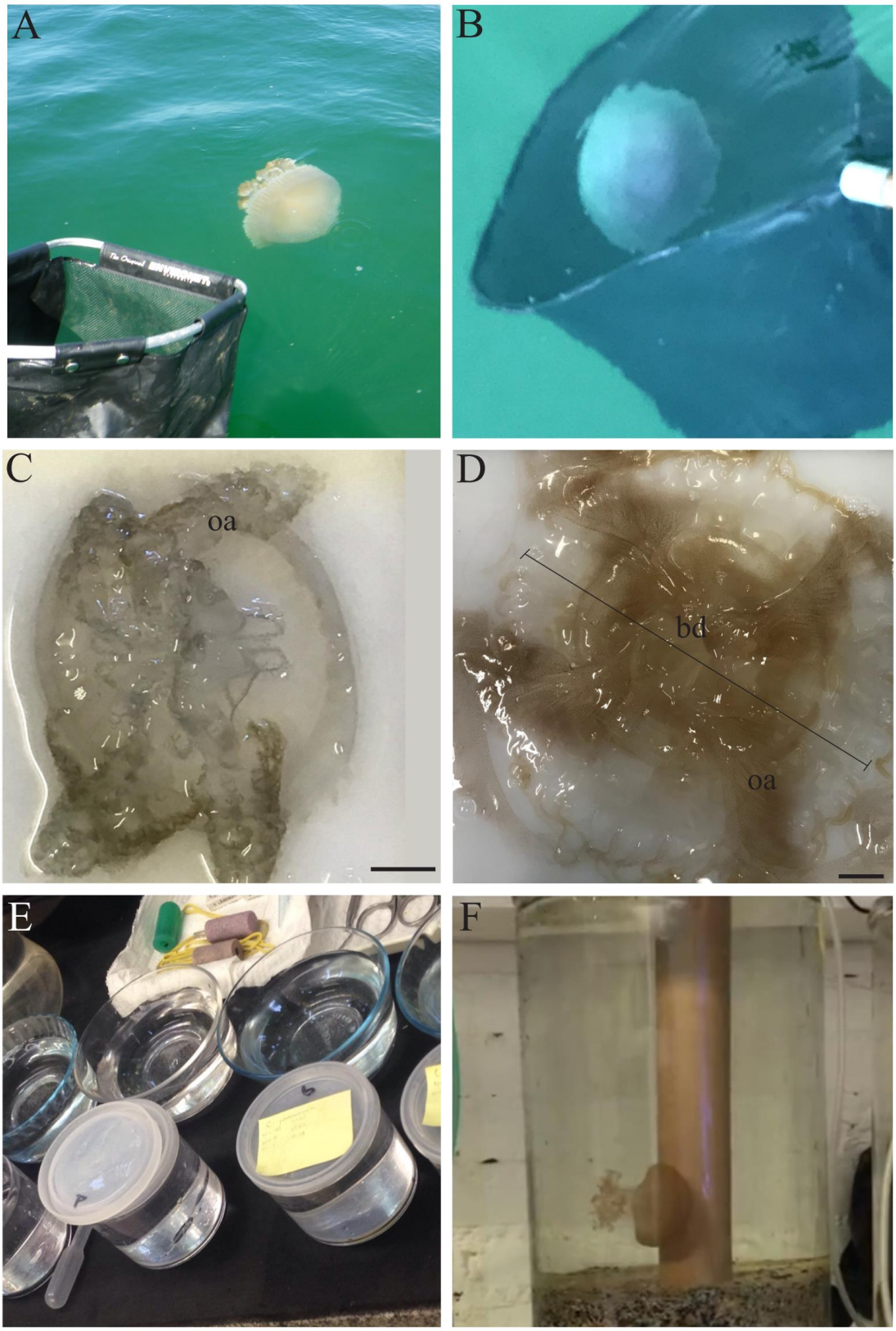

Figura 2: Coleta de espécimes, medidas corporais e instalações de cultivo. (A) Coleta de espécime de *Versuriga anadyomene* no estuário de Ballina, Austrália. (B) Coleta de espécime de *Chrysaora lactea* na superfície da água, Praia do Jabaquara, São Sebastião. (C) Posicionamento dos indivíduos em eixo perradial perpendicular ao observador de forma a padronizar a análise da morfologia das gônadas, escala: 4 cm. (D) Medida do diâmetro umbrelar (BD) das medusas, escala: 2 cm. (E) Cultivo de éfiras de *Lychnorhiza lucerna* em potes circulares de acrílico e vidro. (F) Cultivo de medusas de *Lychnorhiza lucerna* em planktonkreisel cilíndrico.

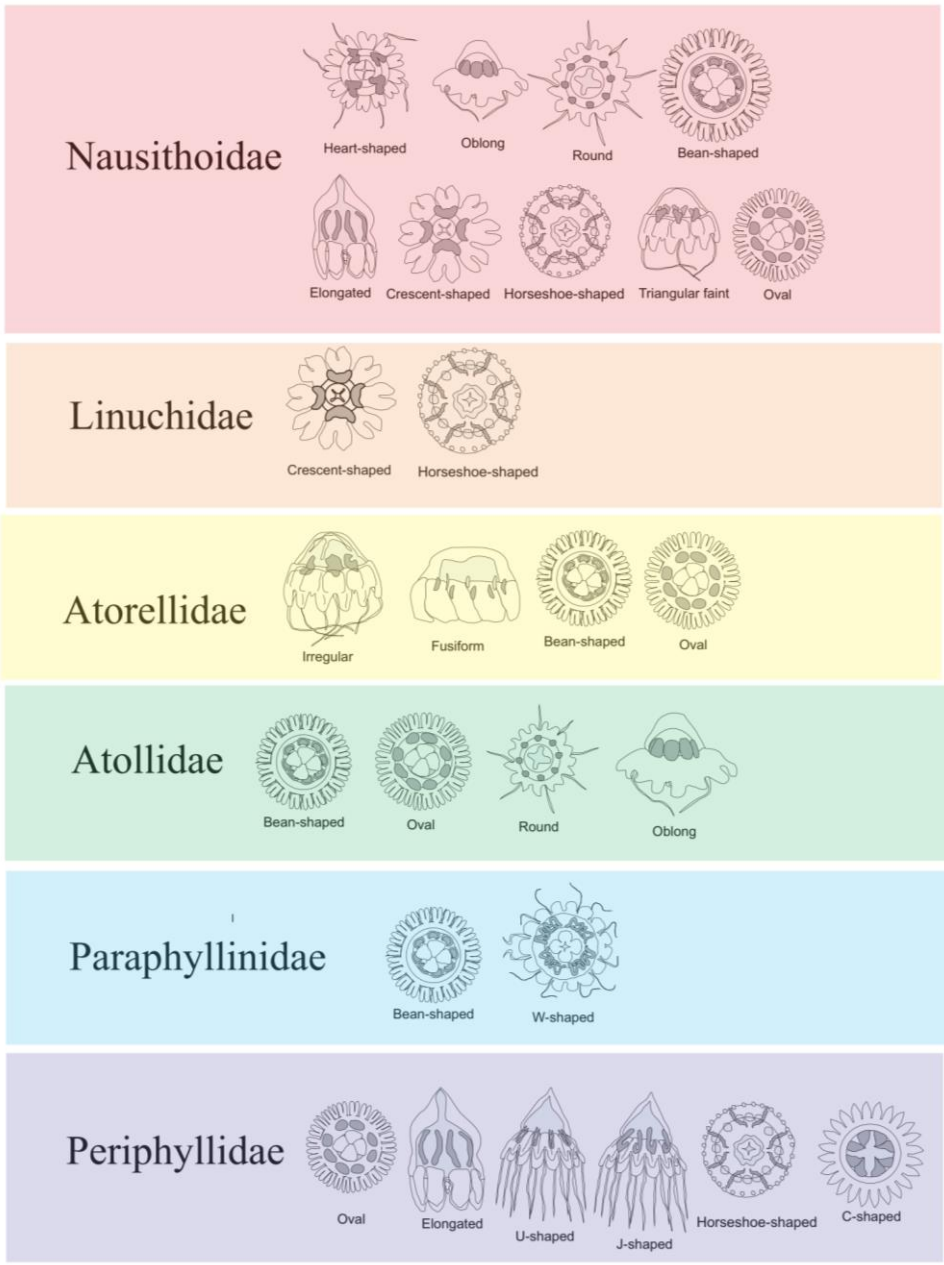

Figura 3: Morfologias gonadais macroscópicas de medusas de Coronatae organizadas e distribuídas por famílias (Diagrama modificado de Tiseo et al., submetido).

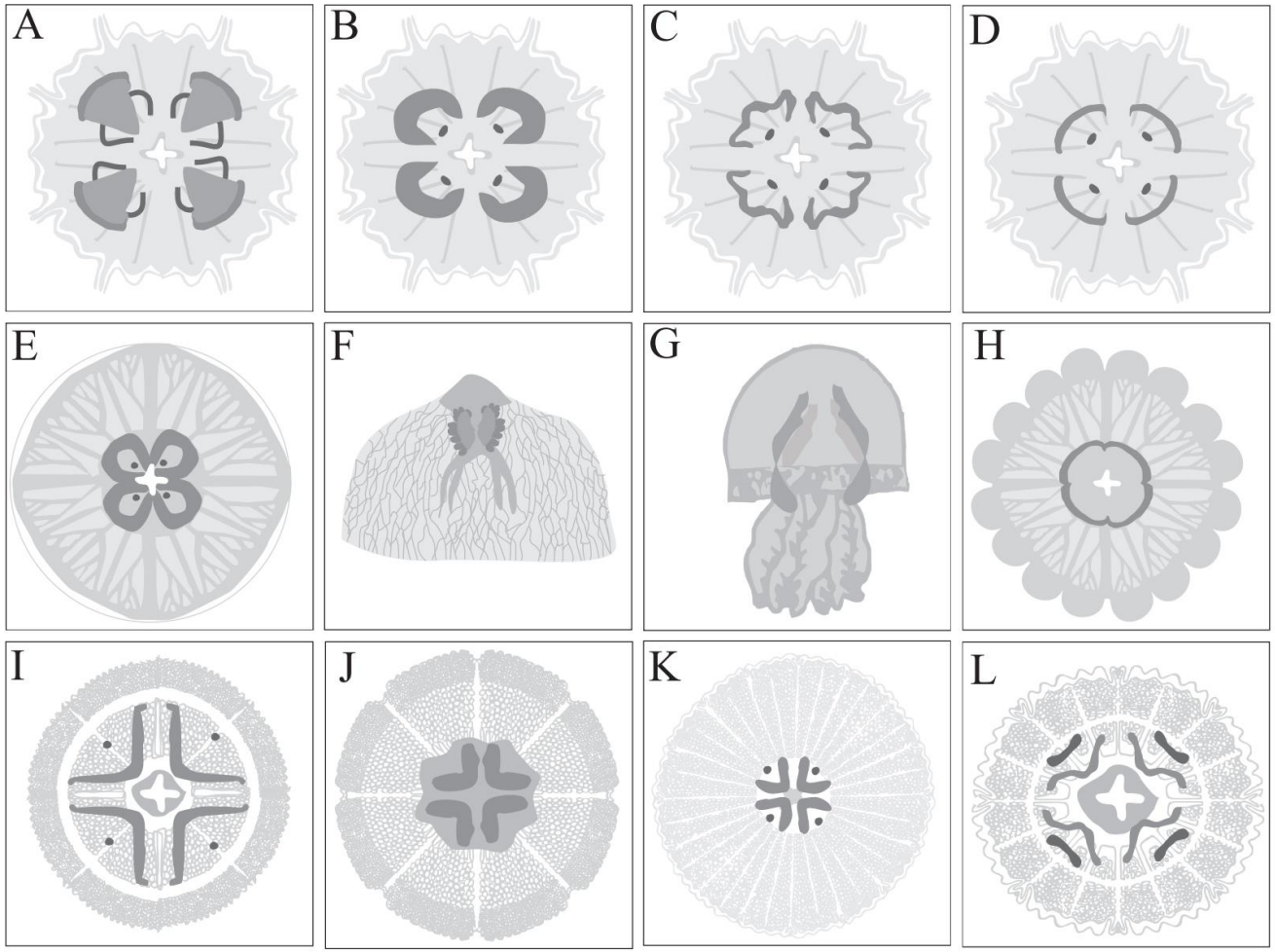

Figura 4: Morfologias gonadais macroscópicas de Discomedusae. (A) Gônada em forma de sacos plásticos presente nos representantes de Cyaneidae, Drymonematidae e *Phacellophora*. (B) Gônada semicircular presente nos representantes dos gêneros *Chrysaora* e *Sanderia* (Pelagiidae). (C) Gônada em forma de "W" presente na espécie *Chrysaora pseudocellata*. (D) Gônada em forma de crescente presente nos representantes dos gêneros *Pelagia*, *Mawia* (Pelagiidae) e *Stellamedusa* (Ulmaridae). (E) Gônada em forma de ferradura direcionada para dentro típica dos representantes de Aureliinae, Poraliinae, Sthenoniinae e Stygiomedusinae (Ulmaridae). (F) Gônada em leque das espécies do gênero Deepstarinae (Ulmaridae). (G) Gônada evertida das espécies do gênero *Tiburonia* (Ulmaridae). (H) Gônada em forma de crescente "fusionada" dos espécimes de Ulmariinae *Discomedusa*, *Diplulmaris*, *Floresca*, *Parumbrosa* e *Ulmaris* (Ulmaridae). (I) Gônada cruciforme das espécies das famílias Catostylidae, Lobonemidae, Lychnorhizidae, Leptobrachidae, Mastigiidae e Versurigidae. (J) Gônada cruciforme das espécies de Cepheidae. (K) Gônada cruciforme das espécies de Cassiopeidae. (L) Gônada cruciforme "aberta" das espécies das famílias Rhizostomatidae e Stomolophidae.

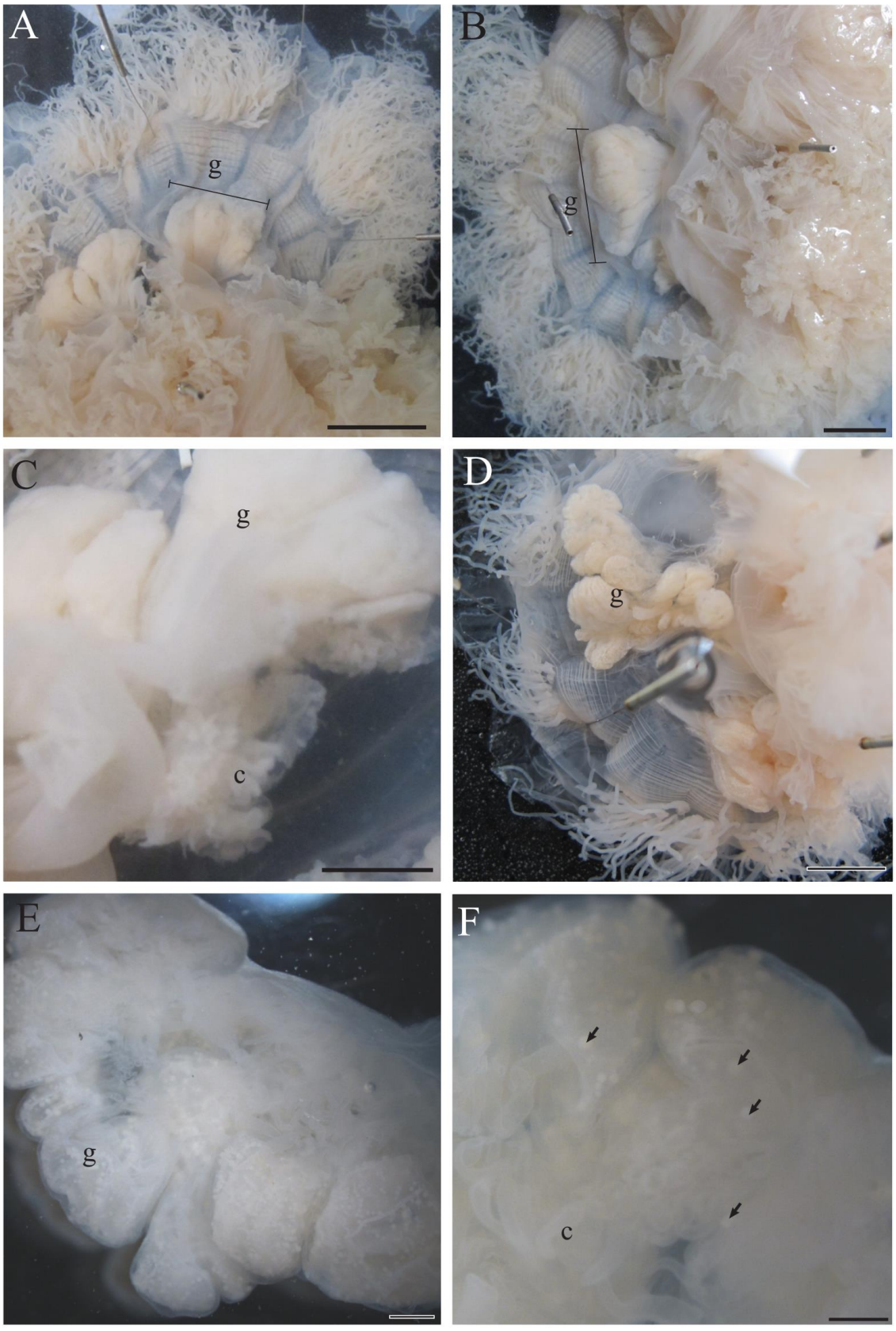

Figura 5: Morfologia gonadal de Semaeostomeae. (A) Gônada em forma de "sacos plásticos" de *Cyanea capillata*, escala: 2 cm. (B) Detalhe da gônada de *C. capillata* evertida pela subumbrela, escala: 2 cm. (C) Aumento da gônada de *C. capillata* próxima aos cirros gástricos, escala: 0,5 cm. (D) Detalhe da gônada de *Cyanea lamarkii* evertida pela subumbrela, escala: 1 cm. (E) Aumento da gônada de *C. lamarkii* próxima aos cirros gástricos, escala: 1 mm. (F) Aumento da gônada de *C. lamarkii* evidenciando os ovócitos (setas), escala: escala: 500 µm. Cirros gástricos = c; Gônada= g.

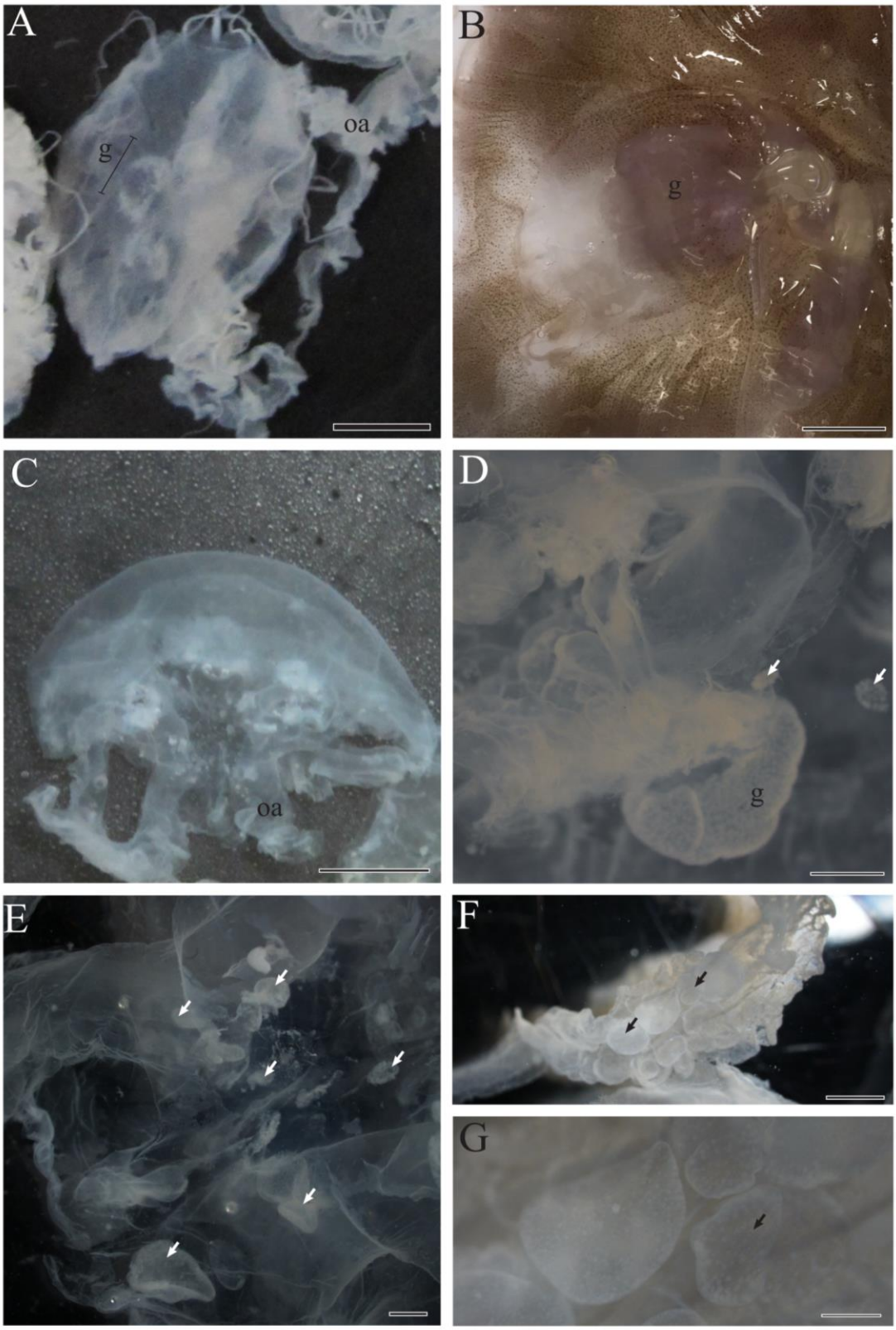

Figura 6: Morfologia gonadal de Semaeostomeae. (A) Gônada em semicírculo de *Sanderia malayensis*, escala: 1 cm. (B) Gônada em semicírculo de *Chrysaora lactea*, escala: 2 cm. (C) Indivíduo macho pequeno de *Chrysaora hysoscella*, escala: 1 cm. (D) Detalhe da gônada masculina típica de *Chrysaora hysoscella* dentro da cavidade gástrica. Nas setas as bolsas com folículos espermáticos na mesogléia, escala: 1 mm. (E) Detalhe das bolsas com folículos espermáticos de *Chrysaora hysoscella* dentro da cavidade gástrica (setas), escala: 1 mm. (F) Bolsas com folículos espermáticos de *Chrysaora hysoscella* nos braços orais (setas), escala: 500 µ. (G) Detalhe das bolsas com folículos espermáticos de *Chrysaora hysoscella* nos braços orais (setas), escala: 500 µm. Braços orais = oa; Gônada= g.

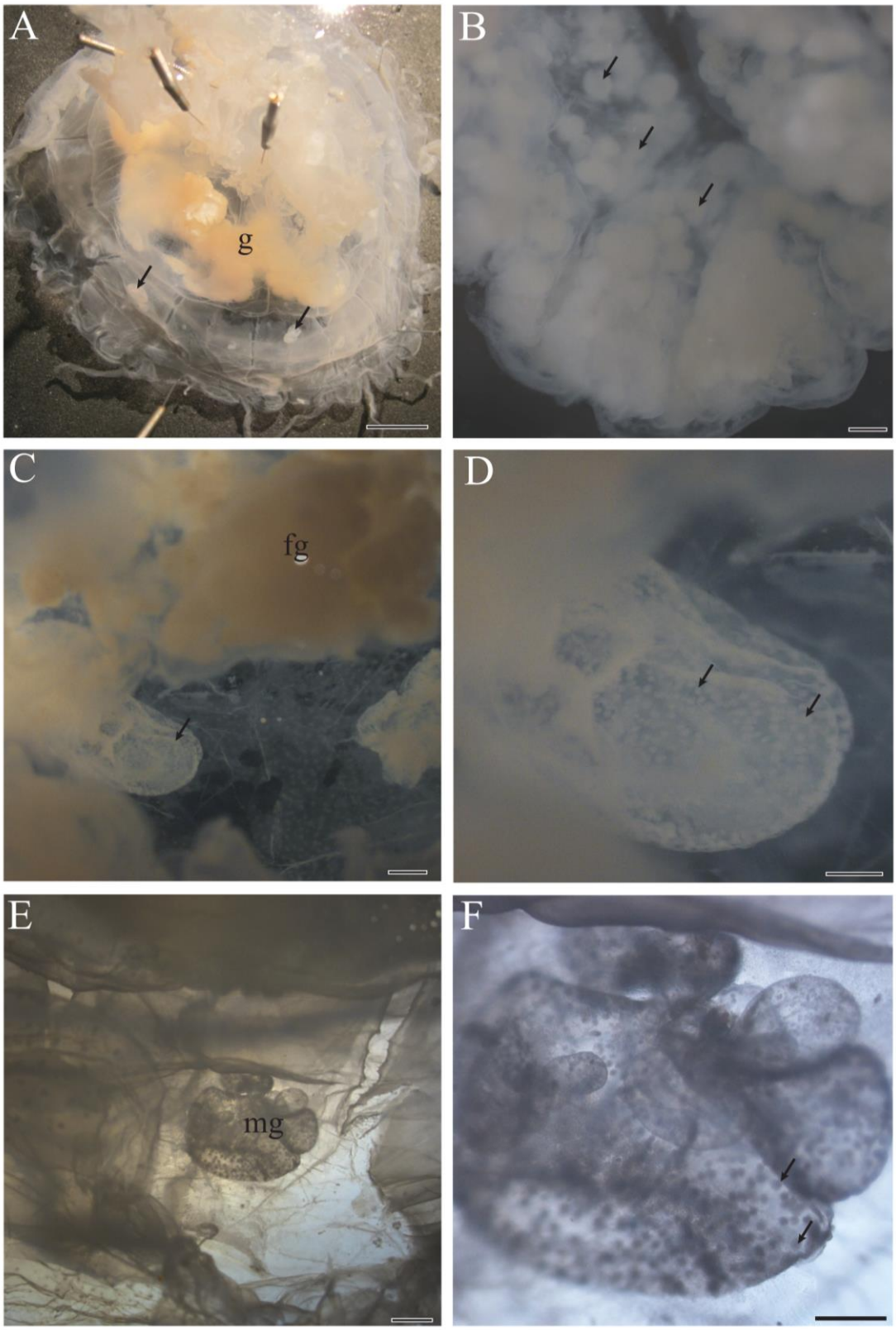

Figura 7: Morfologia gonadal de Semaeostomeae. (A) Gônada em semicírculo do indivíduo feminino maior de *Chrysaora hysoscella*, escala: 1 cm. Nas setas as bolsas com folículos espermáticos na mesogléia. (B) Detalhe da gônada feminina de *Chrysaora hysoscella*. Nas setas ovócitos, escala: 250 µm. (C) Detalhe da gônada feminina e das bolsas com folículos espermáticos de *Chrysaora hysoscella* dentro da cavidade gástrica (setas), escala: 1 mm. (D) Detalhe das bolsas com folículos espermáticos de *Chrysaora hysoscella* dentro da cavidade gástrica. Nas setas note os folículos espermáticos, escala: 500 µm. (E) Bolsa com folículos espermáticos de *Chrysaora hysoscella* próxima ao manúbrio, escala: 1 mm. (F) Detalhe da bolsa com folículos espermáticos (setas) de *Chrysaora hysoscella*, escala: 500 µm. Gônada= g; gônada feminina = fg; gônada masculina = mg.

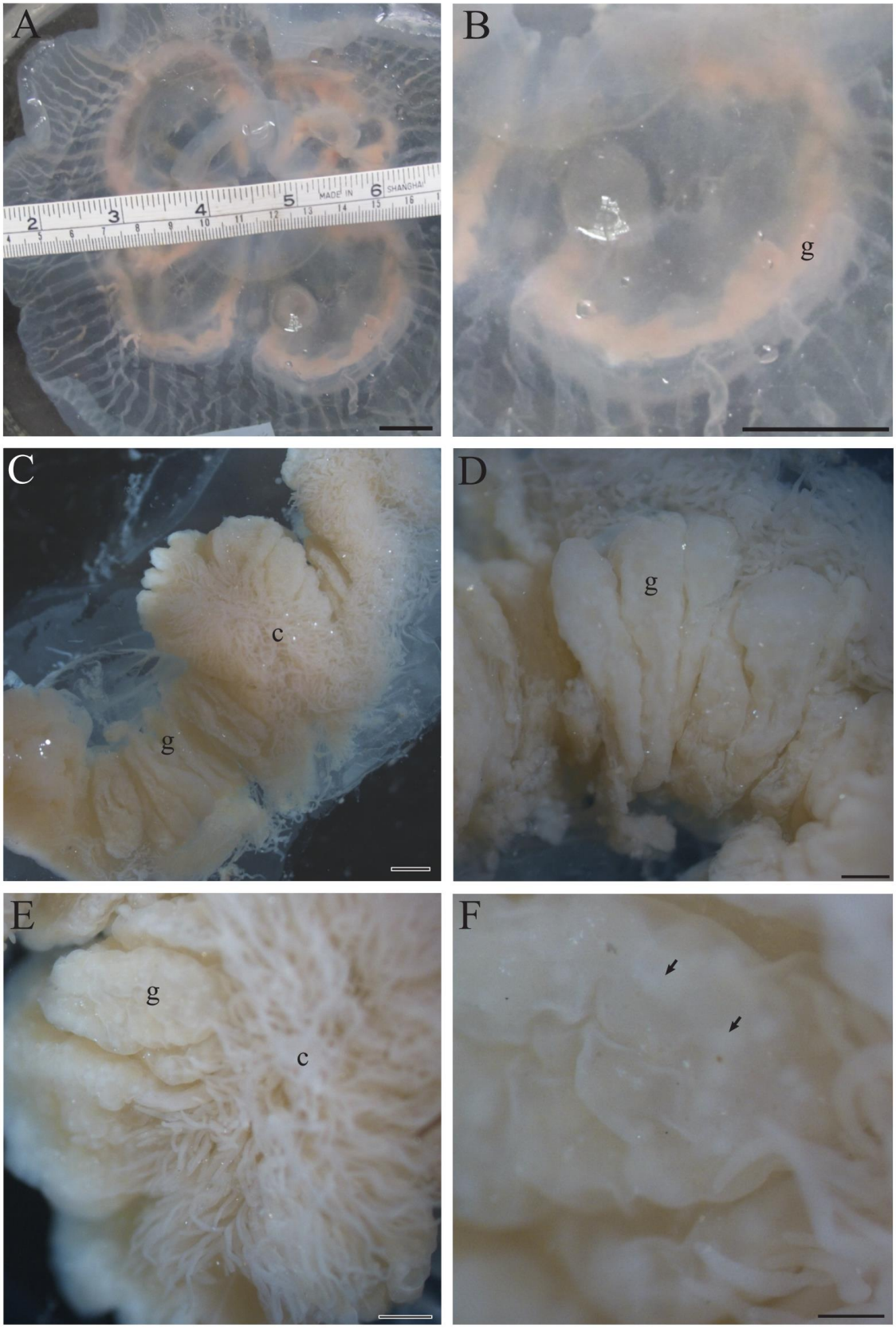

Figura 8: Morfologia gonadal de Semaeostomeae. (A) Gônada em ferradura voltada para dentro de *Aurelia cebimarensis*, escala: 1 cm. (B) Detalhe da gônada em ferradura de *Aurelia cebimarensis*, escala: 2 cm. (C) Detalhe da gônada de *Aurelia cebimarensis*, escala: 1 mm. (D) Note o enovelamento da gônada formando grandes dobras sobre si mesma, escala: 500 µm. (E) Detalhe da gônada de *Aurelia cebimarensis* próxima dos cirros gástricos, escala: 500 µm. (F) Detalhe da gônada evidenciando os folículos espermáticos (setas), escala: 500 µm. Cirros gástricos = c; Gônada= g.

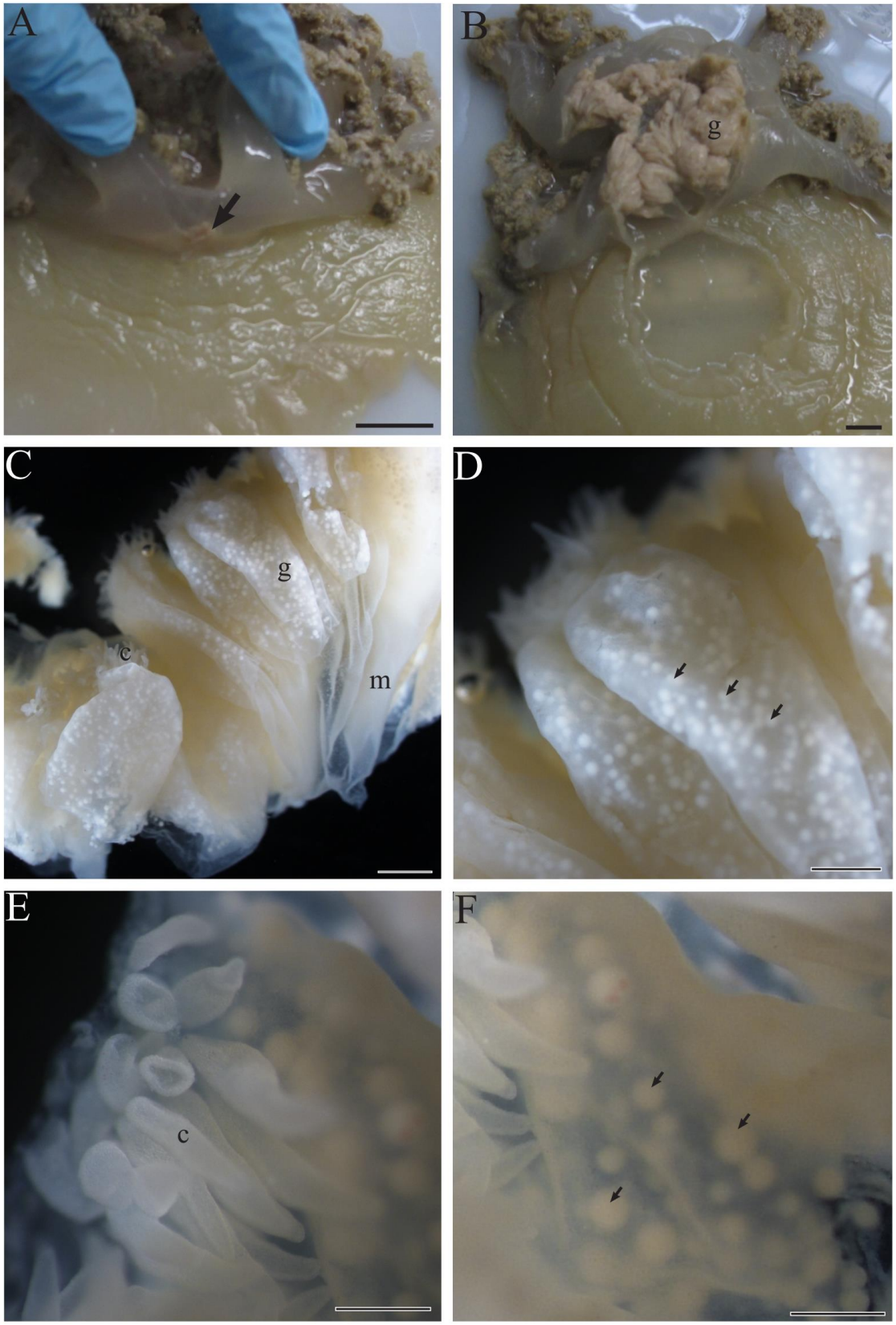

Figura 9: Morfologia gonadal de Rhizostomeae. (A) Gônada de *Cassiopea andromeda* saindo pelo óstio genital, escala: 2 cm. (B) Gônada cruciforme de *Cassiopea andromeda*, escala: 1 cm. (C) Gônada enovelada vista em maior aumento adjacente aos cirros gástricos, escala: 1 mm. (D) Detalhe da gônada de *Cassiopea culionensis*. Na seta ovócitos, escala: 500 µm. (E) Detalhe dos cirros gástricos próximos à gônada feminina de *C. culionensis*, escala: 200 µm. (F) Detalhe da gônada feminina evidenciando os ovócitos (setas), escala: 200 µm. Cirros gástricos = c; gônada = g; mesogléia =m.

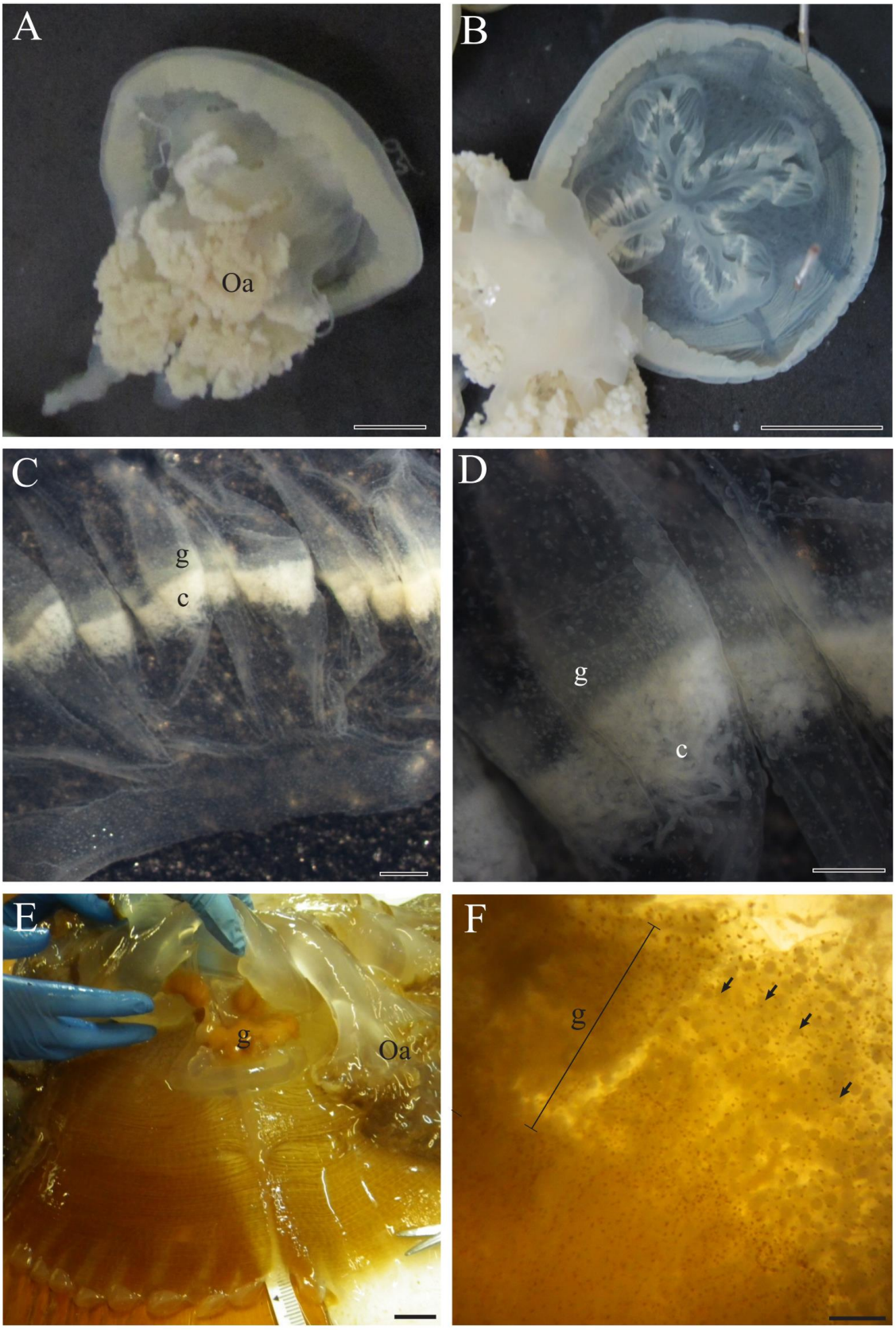

Figura 10: Morfologia gonadal de Rhizostomeae. (A) Indivíduo pequeno de *Phyllorhiza punctata*, escala: 1 cm. (B) Gônada cruciforme de *P. punctata*, escala: 2 cm. (C) Gônada vestigial de *P. punctata* na mesogléia, escala: 1 mm. (D) Detalhe da gônada vestigial de *P. punctata* próxima aos cirros gástricos, escala: 500 µm. (E) Gônada cruciforme de *Versuriga anadyomene* vista pelo óstio genital, escala: 2 cm. (F) Detalhe dos ovócitos (setas) dentro da gônada feminina de *V. anadyomene*, escala: 100 µm. Braços orais =ao; Cirros gástricos = c Gônada= g.

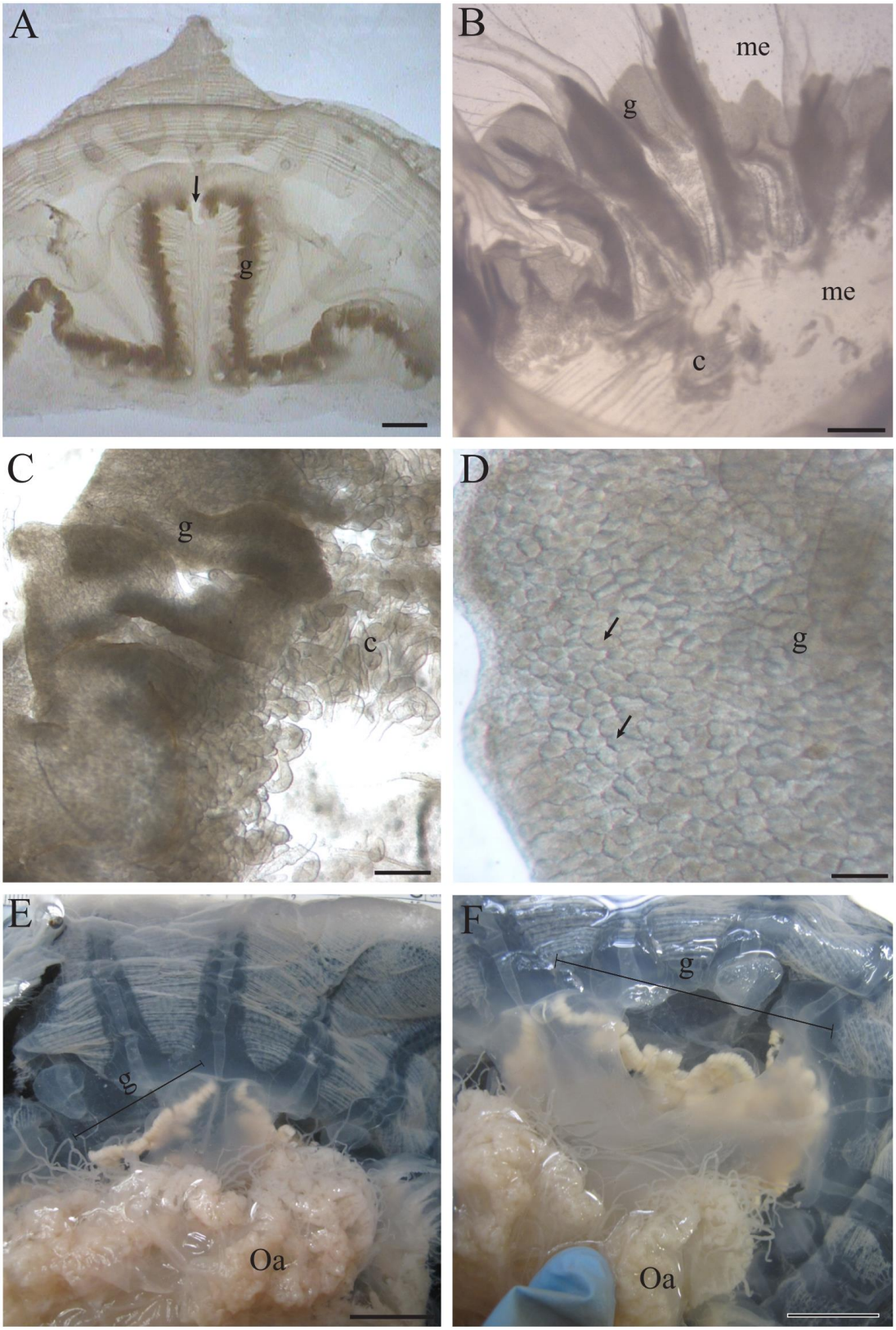

Figura 11: Morfologia gonadal de Rhizostomeae. (A) Dois dos braços em "L" da gônada cruciforme de *Lychnorhiza lucerna* conectados. Note o canalículo (seta) por entre os braços em "L" da gônada, escala: 2 cm. (B) Observação *in vivo* da gônada vestigial de *L. lucerna* vista pelo óstio, escala: 100 µm. (C) Gônada masculina de *L. lucerna* próxima aos cirros gástricos, escala: 250 µm. (D) Detalhe da gônada masculina de *L. lucerna* evidenciando os folículos (setas), escala: 100 µm. (E) Visão geral da gônada cruciforme aberta de *Rhopilema nomadica*, escala: 2 cm. (F) Detalhe da gônada cruciforme de *R. nomadica* dentro da cavidade gástrica, escala: 1 cm. Braços orais = ao; cirros gástricos = c; gônada =g; mesogléia =m.

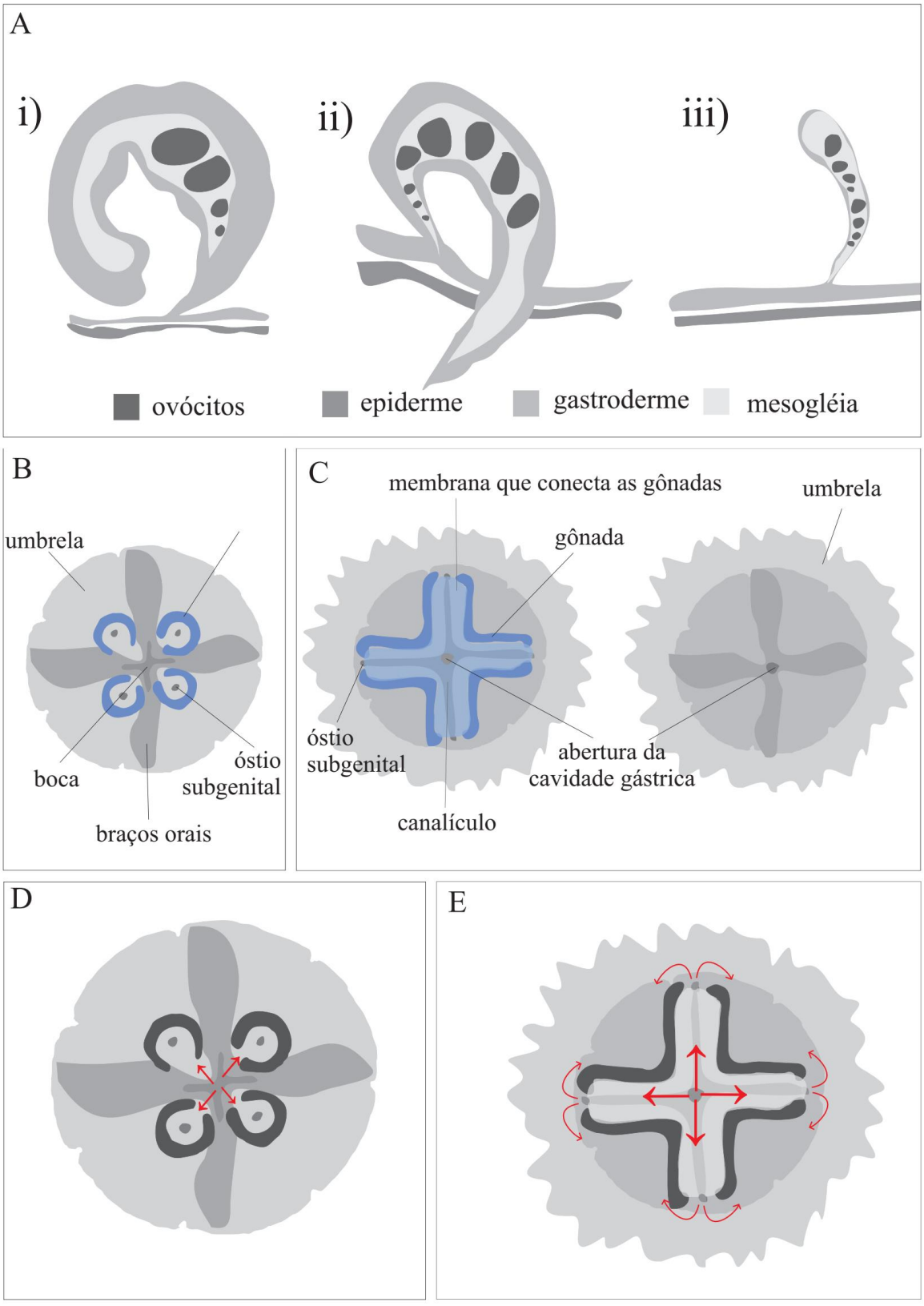

Figura 12: Formação da gônada de Coronatae e comparação da gônada de Discomedusae. (A) Desenho esquemático da origem da gônada de Coronatae em (i) Nausithoidae em (ii) Periphyllidae e em (iii) Linuchidae. (B) Desenho esquemático da localização e organização da gônada de Semaeostomeae voltada para a boca (centro). (C) Desenho esquemático da localização e organização da gônada de Rhizostomeae voltada para a direção oposta a boca (margem), formando uma cruz. (D) Desenho esquemático simplificado do caminho do alimento (setas vermelhas) em Semaeostomeae. (E) Desenho esquemático simplificado do caminho do alimento (setas vermelhas) em Rhizostomeae.

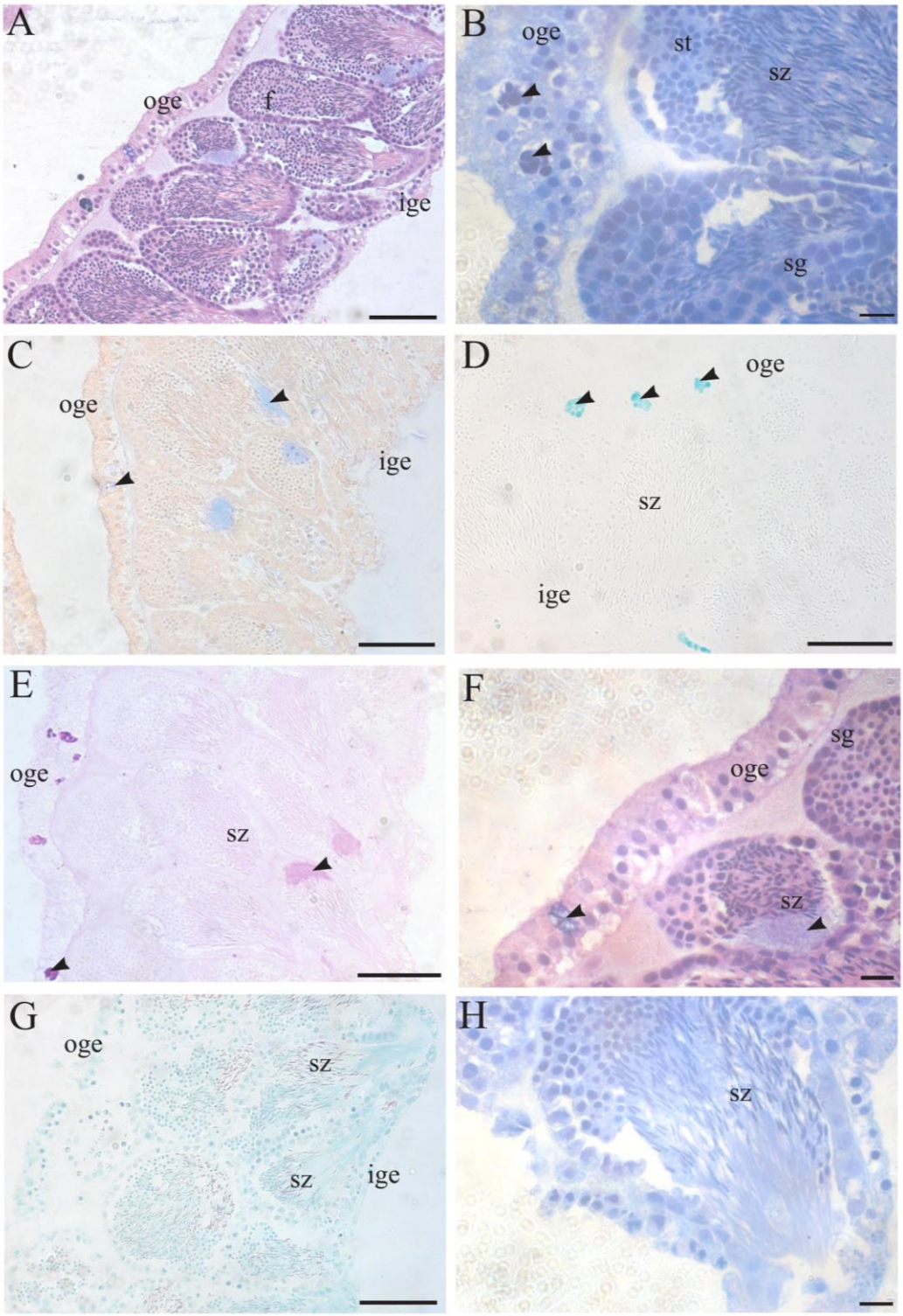

Figura 13: Espermatogênese em *Aurelia cebimarensis*. (A) Hematoxilina e Eosina. Corte transversal da gônada evidenciando os epitélios genitais externo e interno e as células espermáticas imersas na mesogléia, escala: 50 µm. (B) Azul de toluidina. Detalhe dos vacúolos no epitélio genital externo com material fortemente positivo para estruturas ácidas (cabeça de seta). Note os espermatozoides nos centros dos folículos, escala: 10 µm. (C) Tricrômico de Mallory. Material nos vacúolos do epitélio genital e a secreção por entre os espermatozoides positivas a muco (cabeças de seta), escala: 50 µm. (D) Azul de alcian pH 2,5. Material nos vacúolos do epitélio genital positivos para polissacarídeos ácidos (cabeças de seta), escala: 10 µm. (E) PAS. Material nos vacúolos do epitélio genital positivos para polissacarídeos neutros (cabeças de seta), escala: 50 µm. (F) Hematoxilina e Eosina, Material nos vacúolos do epitélio genital externo e a secreção por entre os espermatozoides basófila (cabeças de seta), escala: 10 µm. (G) Tricrômico de Gomori. Espermatozoides acumulados próximos ao epitélio genital para serem liberados, escala: 50 µm. (H) Azul de Toluidina. Espermatozoides sendo liberados por ruptura do folículo e epitélio genital, escala: 10 µm. Epitélio genital externo =oge; Epitélio genital interno =ige; espermatócito= sc; espermátide =st; espermatogônia= sg; espermatozoide=sz; folículo=f.

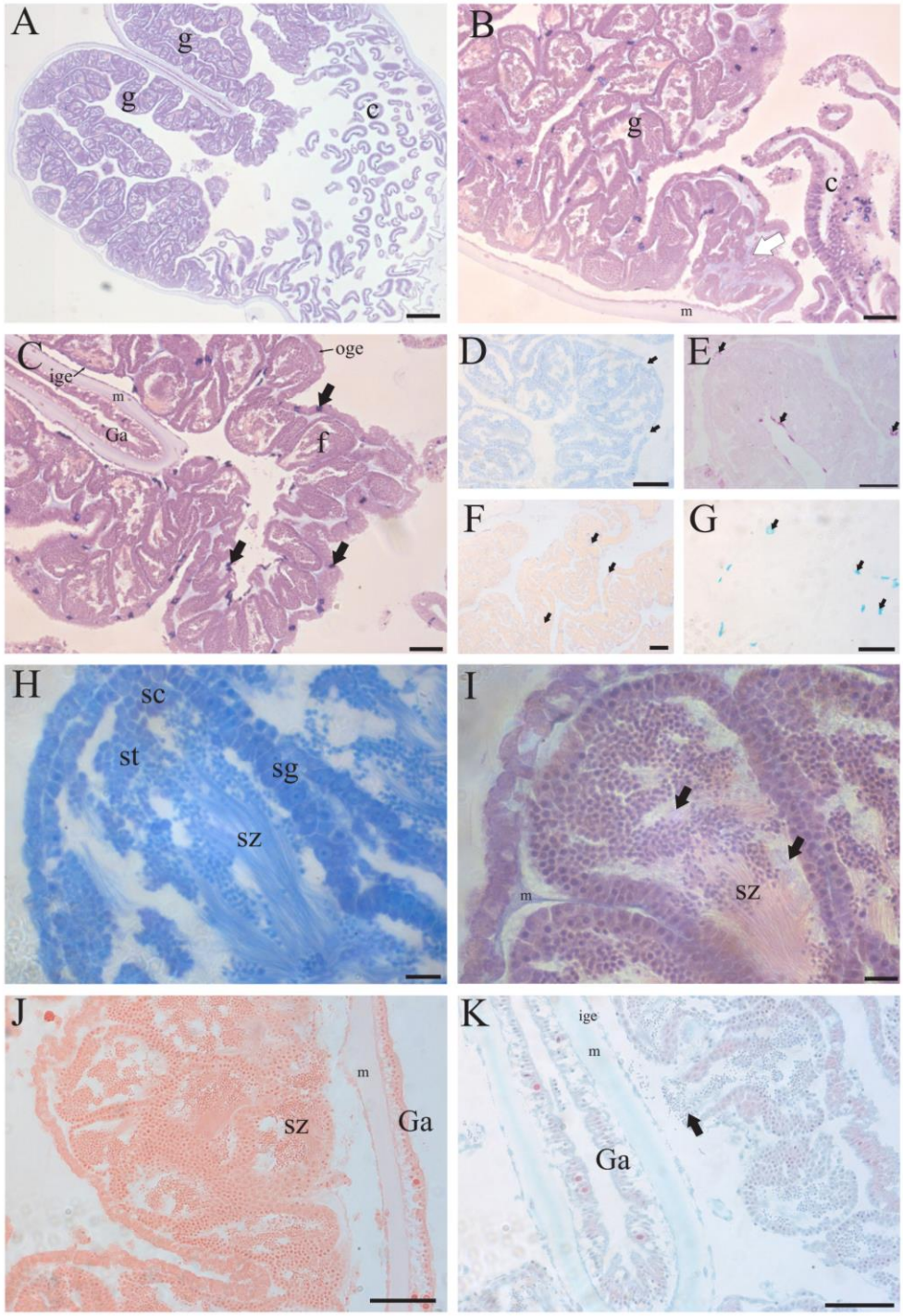

Figura 14: Espermatogênese em *Rhopilema nomadica*. (A) Hematoxilina e Eosina. Corte transversal da gônada masculina de *Rhopilema nomadica* próxima aos cirros gástricos, escala: 200 µm. (B) Hematoxilina e Eosina. Detalhe da região de evaginação da gônada (seta), escala: 50 µm. Note os folículos apenas com espermatogônias e a mesogléia basófila. (C) Hematoxilina e Eosina. Epitélio genital externo e interno da gônada de *Rhopilema nomadica* circundados pela mesogléia e gastroderme. Nas setas grandes vacúolos com secreção fortemente basófila nas células do epitélio genital externo, escala: 50 µm. (D) Azul de Bromofeneol. A secreção dos vacúolos teve reação negativa para proteínas (setas), escala: 50 µm. (E) PAS. A secreção dos vacúolos teve reação fortemente positiva para polissacarídeos neutros (setas), escala: 50 µm. (F) Tricrômico de Mallory. A secreção dos vacúolos teve reação positiva para muco (setas), escala: 50 µm. (G) Azul de Alcian. A secreção dos vacúolos teve reação fortemente positiva para polissacarídeos ácidos (setas), escala: 50 µm. (H) Azul de Toluidina. Detalhe dos folículos com as células germinativas imersas na mesogléia, escala: 10 µm. (I) Hematoxilina e Eosina. Detalhe dos espermatozoides imersos em secreção basófila (setas), escala: 10 µm. (J) Xylidine Ponceau. Os espermatozoides acumulados na região centro basal dos folículos prontos para serem liberados, escala: 50 µm. (K) Tricrômico de Gomori. Espermatozoides sendo liberados por ruptura do folículo e epitélio genital no sino genital, escala: 50 µm. Cirros gástricos=c; epitélio genital externo =oge; epitélio genital interno =ige; espermatócito= sc; espermátide =st; espermatogônia= sg; espermatozoide=sz; folículo=f; gônada=g; gastroderme= Ga; mesogléia=m.

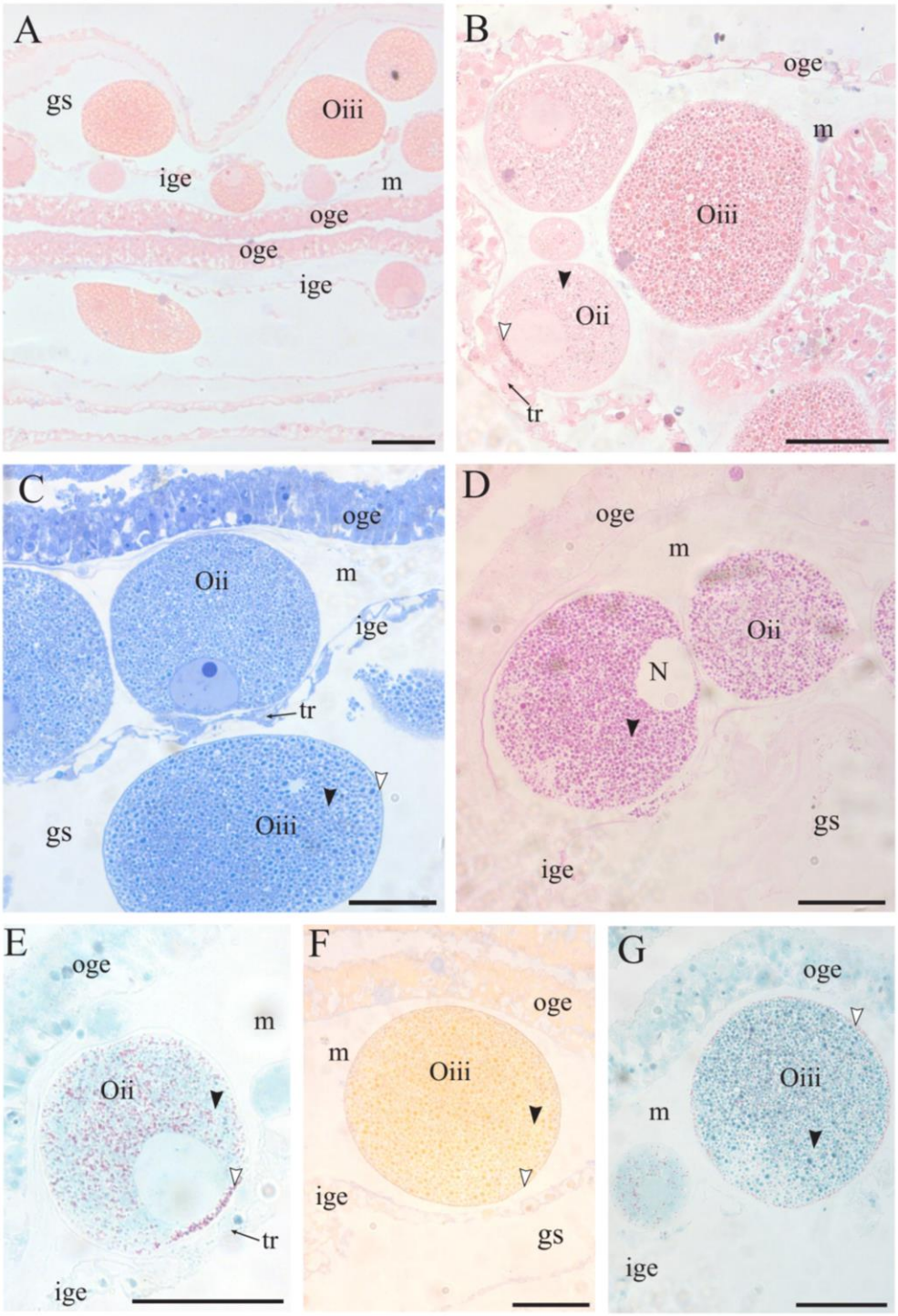

Figura 15: Ovogênese em *Cyanea lamarkii*. (A) Hematoxilina e Eosina. Corte transversal da gônada evidenciando os epitélios genitais externo e interno. Note os ovócitos em diferentes graus de desenvolvimento e um ovócito já maduro liberado no sino genital, escala: 100 µm. (B) Hematoxilina e Eosina. Ovócitos imersos na mesogléia e mantendo o contato com o epitélio genital via trofócitos do tipo 1. Note os diferentes grânulos no ooplasma do ovócito: basófilo (cabeça de seta branca) e acidófilo (cabeça de seta preta), escala: 50 µm. (C) Azul de Toluidina. Detalhe dos trofócitos em forma de cripta e de ambos os grânulos positivos para estruturas ácidas (cabeça de seta branca e preta), escala: 50 µm. (D) PAS. Detalhe de ambos os grânulos positivos para PAS (cabeça de seta preta), escala: 50 µm. (E) Tricômico de Gomori. Detalhe do ovócito intermediário com grânulos basófilos corados em rosa (cabeça de seta branca) e os grânulos acidófilos corados em azul (cabeça de seta preta), escala: 50 µm. (F) Tricrômico de Mallory. Detalhe dos ovócitos tardios com grânulos fortemente positivos para Mallory (cabeça de seta branca) e positivos para Mallory (cabeça de seta preta), escala: 50 µm. (G) Tricômico de Gomori. Ovócito tardio com os grânulos basófilos circundando o ovócito, corados em rosa (cabeça de seta branca) e grânulos acidófilos na região central do ooplasma corados em azul (cabeça de seta preta), escala: 50 µm. Epitélio genital externo =oge; epitélio genital interno =ige; mesogléia=m; núcleo = N; ovócito inicial = Oi; ovócito intermediário = Oii; ovócito tardio=Oiii; sino genital=gs; trofócitos = tr.

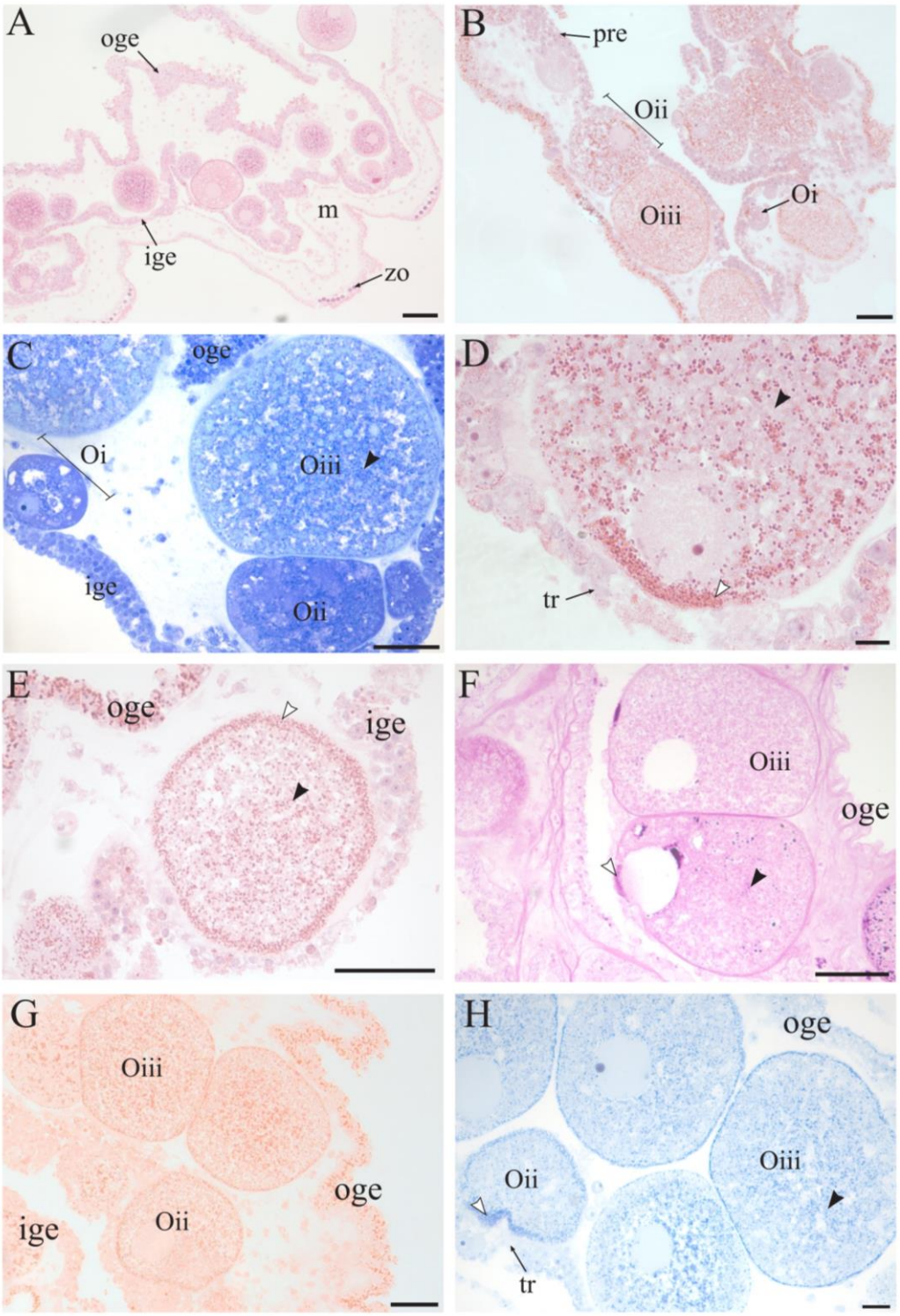

Figura 16: Ovogênese em *Versuriga anadyomene*. (A) Hematoxilina e Eosina. Corte transversal da gônada evidenciando os epitélios genitais externo e interno. Note as zooxantelas na mesogléia, escala: 100 µm. (B) Hematoxilina e Eosina. ovócitos em diferentes graus de desenvolvimento, escala: 50 µm. (C) Azul de Toluidina. Ovócitos inicial, intermediário e tardio. Na cabeça de seta os grânulos acidófilos positivos para estruturas ácidas, escala: 50 µm. (D) Hematoxilina e Eosina. Região de contato do ovócito com os trofócitos tipo 1. Na cabeça de seta branca os grânulos acidófilos e na cabeça de seta preta os grânulos basófilos, escala: 10 µm. (E) Hematoxilina e Eosina. Ovócito tardio com ambos os tipos de grânulos: acidófilos (cabeça de seta branca) e basófilo (cabeça de seta preta), escala: 50 µm. (F) PAS. Detalhe dos ovócitos tardios com ambos os grânulos positivos para polissacarídeos neutros, escala: 50 µm. (G) Xylidine Ponceau. Ovócitos em diferentes fases de desenvolvimento positivos para proteínas totais, escala: 50 µm. (H) Azul de Bromofenol. Ovócitos em diferentes fases de desenvolvimento positivos para proteínas neutras, escala: 20 µm. Epitélio genital externo =oge; epitélio genital interno =ige; mesogléia=m; núcleo = N; ovócitos pré-vitelogênicos =pre; ovócito inicial = Oi; ovócito intermediário = Oii; ovócito tardio=Oiii; sino genital=gs; trofócitos = tr.

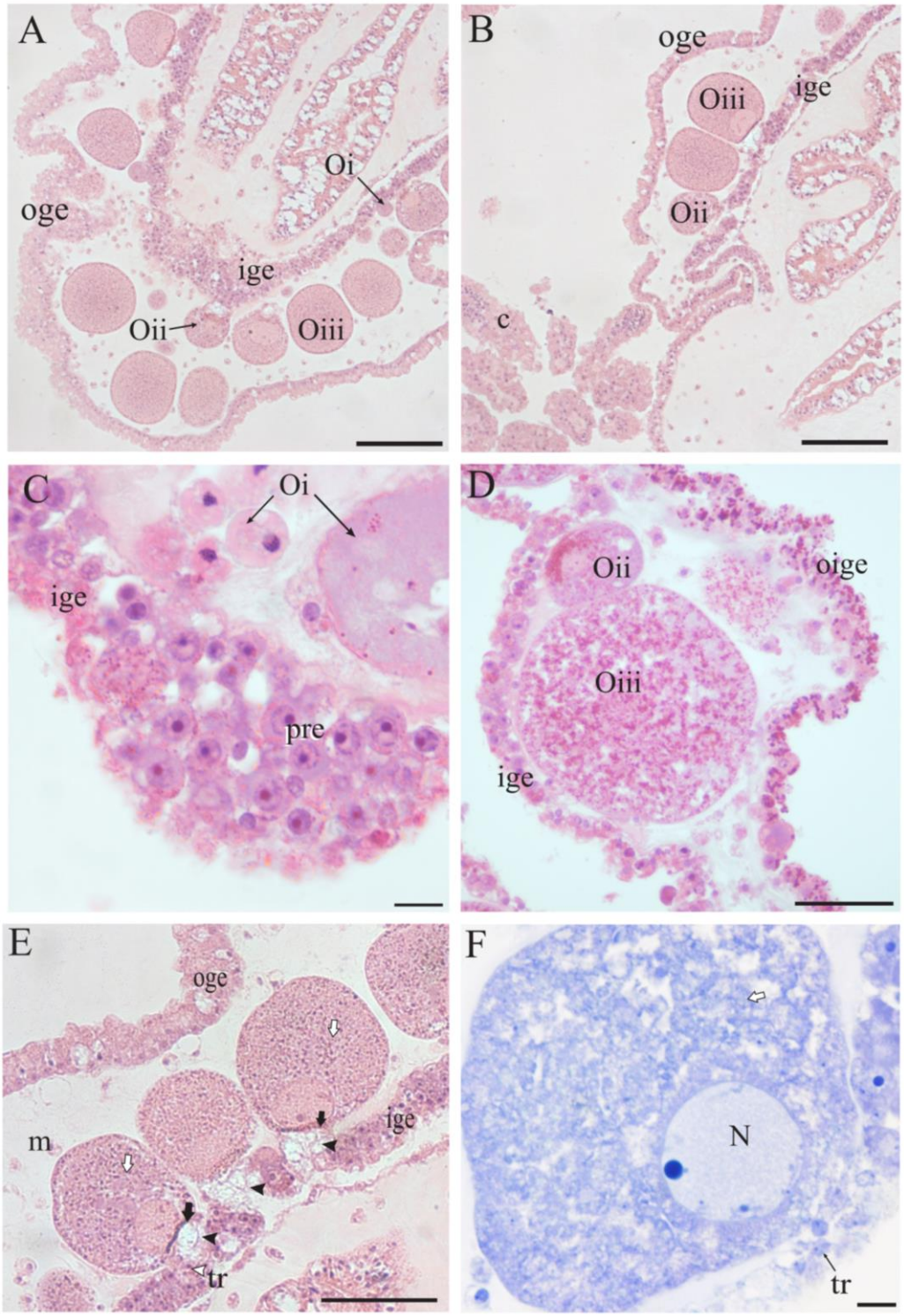

Figura 17: Ovogênese em *Lychnorhiza lucerna*. (A) Hematoxilina e Eosina. Corte transversal da gônada com ovócitos em diferentes fases de desenvolvimento imersos na mesogléia, escala: 50 µm. (B) Hematoxilina e Eosina. Região de evaginação da gônada feminina. Note os cirros gástricos próximo à gônada, escala: 50 µm. (C) Hematoxilina e Eosina. Detalhe dos ovócitos pré-vitelogênicos dentro do epitélio genital interno, escala: 10 µm. (D) Hematoxilina e Eosina. Detalhe do ovócito tardio, escala: 50 µm. (E) Hematoxilina e Eosina. Corte transversal da gônada com ovócitos intermediários e tardios em contato com os trofócitos tipo 2. Na cabeça de seta note os grandes vacúolos. Nas setas pretas os grânulos basófilos e nas setas brancas os grânulos acidófilos, escala: 50 µm. (F) Azul de Toluidina. Ambos os grânulos positivos a estruturas ácidas, escala: 10 µm. Epitélio genital externo =oge; epitélio genital interno =ige; ovócitos pré-vitelogênicos =pre; ovócito inicial = Oi; ovócito intermediário = Oii; ovócito tardio=Oiii; trofócitos = tr.

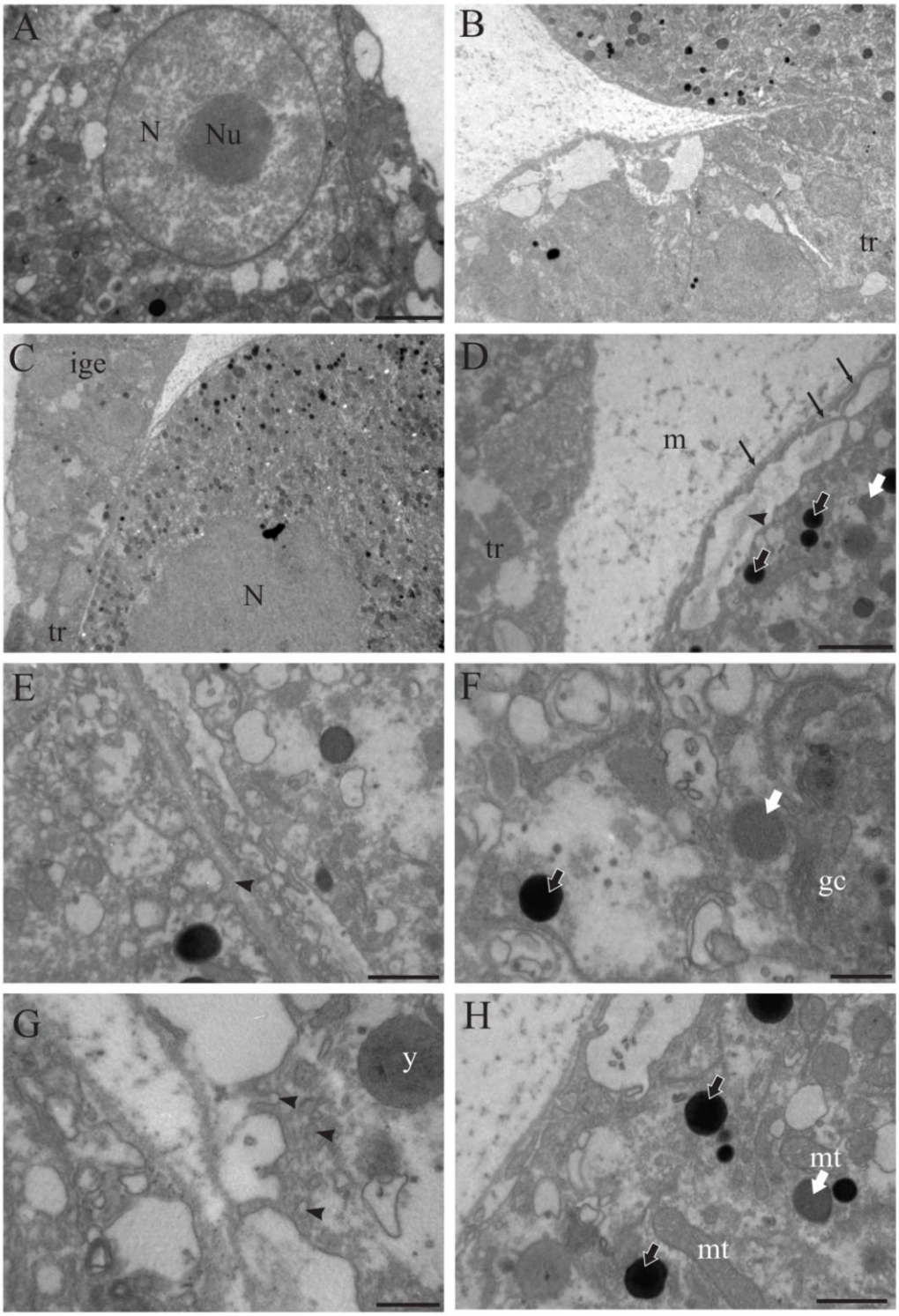

Figura 18: Ultraestrutura da gônada de *Versuriga anadyomene*. (A) Ovócito pré-vitelogênico dentro do epitélio genital interno, escala: 2 µm. (B) Contato do ovócito intermediário com os trofócitos. (C) Detalhe do ovócito tardio em contato com os trofócitos. (D) Os trofócitos englobam os ovócitos através de prolongamentos da membrana plasmática (setas finas). Note os grânulos de vitelo nas setas brancas e os de mesogleína nas setas pretas. Na cabeça de seta a membrana vitelínica sendo formada, escala: 2 µm. (E) Detalhe da região entre ovócito e trofócito. Na cabeça de seta preta a membrana vitelínica, escala: 1 µm. (F) Detalhe dos grânulos de vitelo (seta branca) e dos grânulos de mesogleina (seta preta) no ooplasma, escala: 0,5 µm. (G) Detalhes das vesículas endocíticas sendo formadas no pólo animal do ovócito, escala: 0,5 µm. (H) Detalhe do pólo vegetal do ovócito com os grânulos de vitelo (seta branca) e grânulos de mesogleina (seta preta), escala: 1 µm. Complexo de Golgi =gc; epitélio genital interno=; ige; mesogléia=m; mitocôndria= mt; núcleo=N; nucléolo=Nu; trofócitos= tr; vitelo=y.

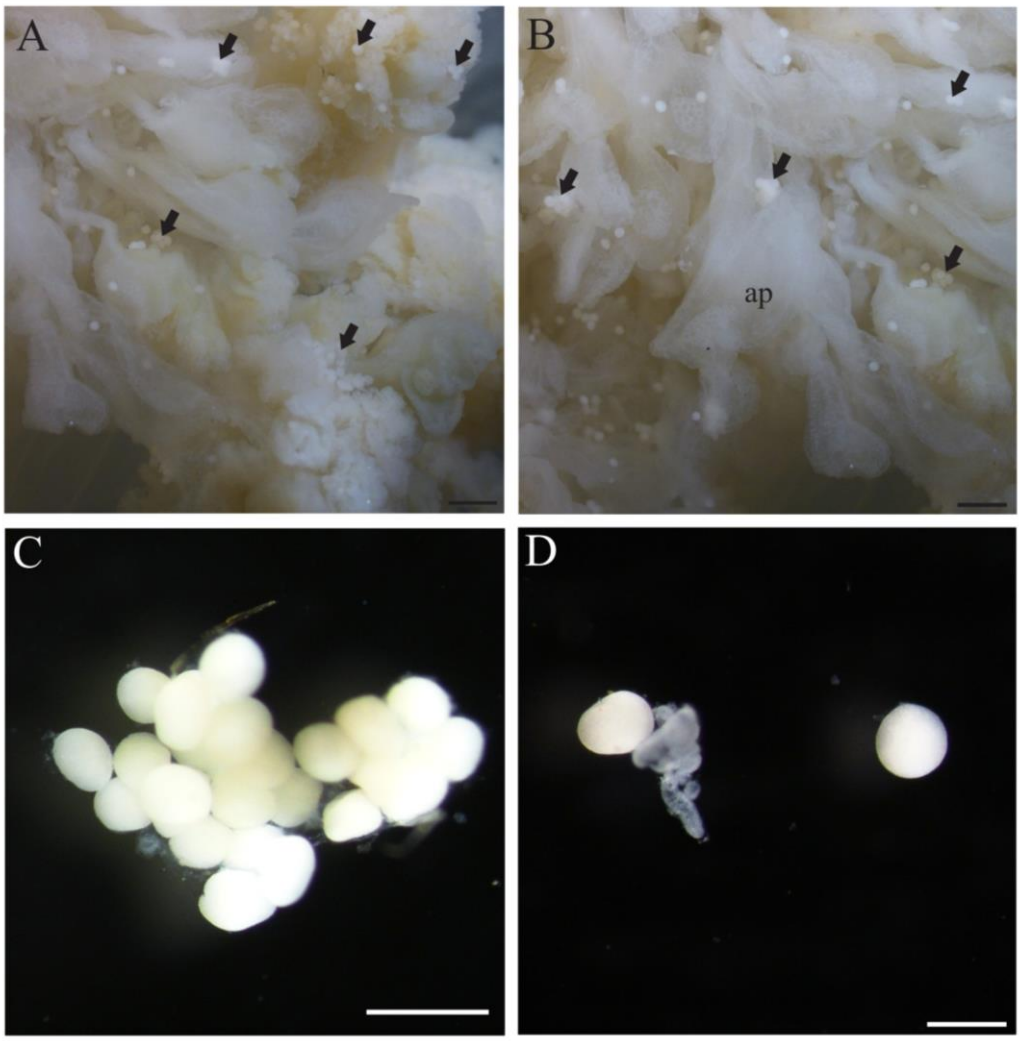

Figura 19: Incubação de plânulas. (A) Plânulas (setas) nos braços orais de *Cassiopea culionensis*, escala: 1mm. (B) Plânulas próximas aos apêndices nos braços orais de *Cassiopea culionensis*, escala: 1mm. (C) Plânulas ovaladas a circulares removidas dos braços orais, escala: 200µm. (D) Detalhes das plânulas de *C. culionensis*, escala: 200µm.

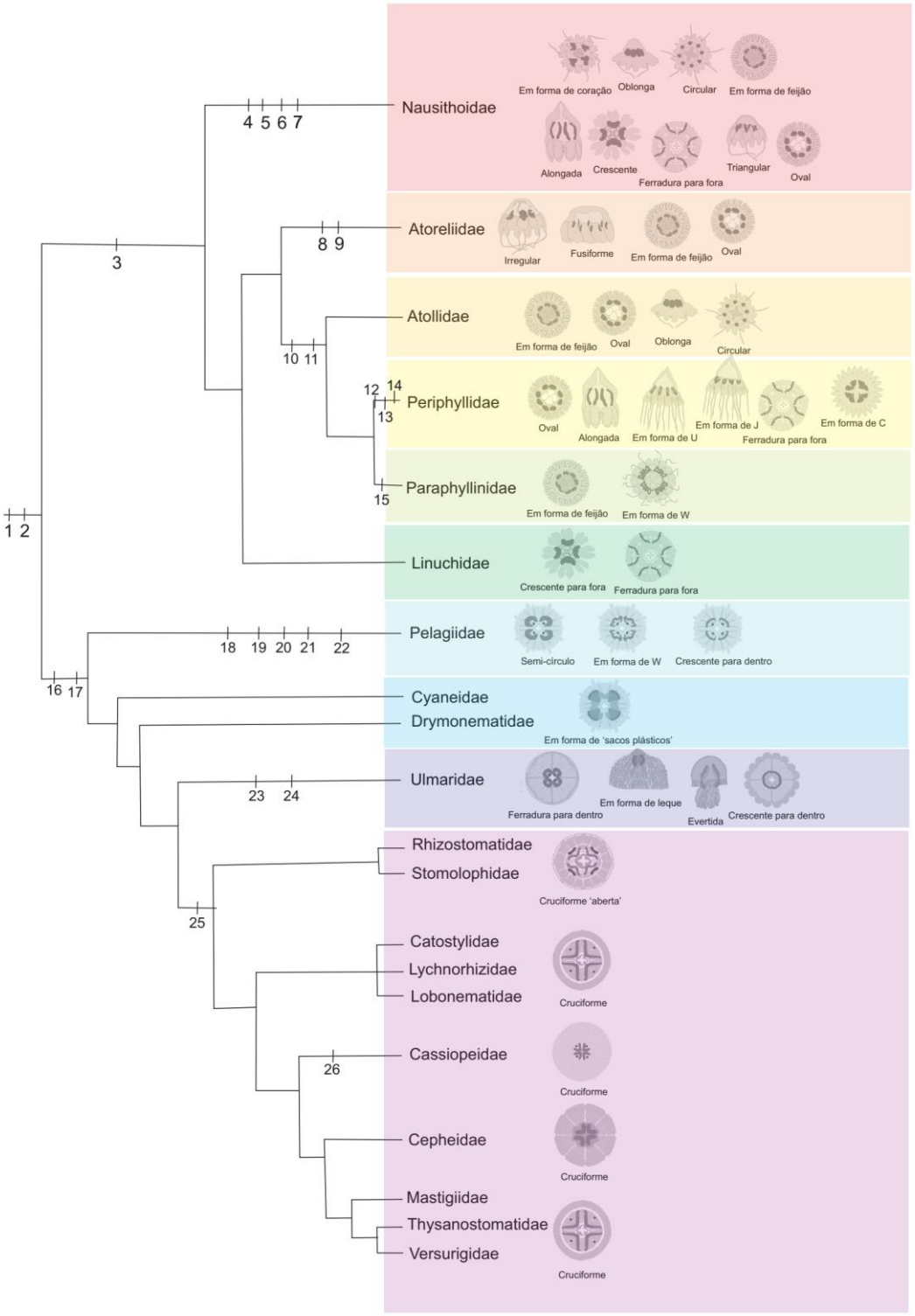

Figura 20: Diagrama de relações de parentesco (baseado na topologia de Bayha et al., 2010) resumindo os conhecimentos da reprodução sexuada de cifozoários. (1) Gonocórico. (2) Ciclo de vida metagenético. (3) Gônada adradiais. (4) Gônada em forma de coração. (5) Gônada triangular (6) Hermafrodita. (7) Partenogenético. (8) gônada em forma irregular. (9) gônada fusiforme. (10) Ciclo de vida holopelágico. (11) Desenvolvimento direto. (12) Presença de muco nas gônadas femininas e masculinas. (13) Gônada em forma de U ou J. (14) Presença de células acessórias. (15) Gônada em forma de W. (16) Gônadas interradiais. (17) Trofócitos. (18) Gônada em semicírculo. (19) gônada em forma de W. (20) Presença de muco na gônada feminina. (21) Ciclo de vida holopelágico (22) Desenvolvimento direto. (23) Gônada na forma de Ferradura voltada pra dentro. (24) Gônada na forma de leque. (25) Gônada cruciforme. (26) Hermafrodita.

Considerações Finais

Mesmo com a reprodução sexuada sendo descrita para vários representantes das três ordens de cifozoários, frente a grande diversidade do grupo (241 espécies sensu Jarms and Morandini, 2019) podemos dizer que ainda são poucos os trabalhos que descrevem, em detalhes, a conformação gonadal, histologia da gônada e gametogênese do grupo (Maas 1897; Vanhöffen 1902; Bigelow 1909; Eckelbarger and Larson 1988, 1992; Avian and Rottini-Sandrini 1991; Morandini and Silveira 2001; Ohtsu et al. 2007; Sötje and Jarms 2009; Lucas and Reed 2010; Tiemann and Jarms 2010; Ikeda et al. 2011; Schiariti et al. 2012). A primeira descrição de um cifozoário foi há 264 anos atrás, e desde então nenhuma revisão sobre a reprodução sexuada do grupo, dentro de um contexto comparativo e filogenético, foi feita. Desta forma, esta tese detalha não somente os processos microscópicos e tipos celulares dentro do grande tema da reprodução sexuada de scyphozoa – como nos capítulos dois, três e quatro – mas também estuda e discute a morfologia gonadal macroscópica, histológica e várias das estratégias reprodutivas de seus representantes – capítulos um e cinco – dentro do cenário filogenético e evolutivo mais recente do grupo (Collins et al. 2006; Straehler-Pohl 2009; Bayha et al. 2010; Kayal et al. 2018).

Nossos resultados evidenciaram uma alta diversidade de morfologias macroscópicas de gônadas, com Coronatae apresentado a maior variedade (15 no total), seguida por Semaeostomeae (6 morfologias gonadais) e Rhizostomeae com apenas uma. Para a gônada de todos os representantes, um padrão histológico foi encontrado com a gônada sendo uma evaginação da região da gastroderme, tendo um epitélio colunar externo, um cuboidal interno e por entre eles a mesogléia com as células germinativas. A

presença de trofócitos exclusiva de Discomedusae e aqui descrevemos dois tipos diferentes relacionados com o tipo de liberação de gametas e fecundação, se interna ou externa. Adicionalmente ao proposto por Adonin & Podgornaya (2012), aqui descrevemos a presença da mesogleína nos ovócitos de várias espécies de scyphozoa e acreditamos que esta proteína – produzida pelo próprio ovócito – esteja presente nos ovócito dos demais representantes de cifozoários, comprovando a existência de uma membrana vitelínica para os Scyphozoa.

Utilizando uma das filogenias mais recentes de Scyphozoa – Bayah et al. (2010) – para discutir sobre a localização das gônadas nos representantes das diferentes ordens de cifozoários, coronados possuem gônadas adradiais enquanto que discomedusas possuem gônadas interadiais (Fig. 1). A presença de células acessórias dentro da classe separa os discomedusae dos coronados (Fig. 2) porém a diferenciação dos trofócitos em tipo um ou dois parece estar mais relacionada com a biologia reprodutiva do grupo não mostrando ser um caráter acurado para resgatar aspectos evolutivos do grupo. Em um contexto mais abrangente, ao reconstruir a ancestralidade da presença de células acessórias em Acraspeda (grupo formado pelas classes Scyphzoa, Cubozoa e Staurozoa, recentemente resgatado por Kayal et al. 2018), tal característica parece ser informativa em um contexto evolutivo a nível de classes (Fig. 3).

Mesmo esta tese aumentando significativamente a quantidade de descrições e estudos sobre a reprodução sexuada em cifozoários (a nível de microscopia de luz e de transmissão), são muitas ainda, as lacunas a serem preenchidas para que possamos ter um entendimento mais conclusivo e acurado do cenário reprodutivo evolutivo do grupo. Desta forma, encorajamos fortemente o cultivo de espécies de medusas, coletas de espécimes e de materiais para a microscopia de luz e transmissão para que possamos dar continuidade às análises aqui iniciadas.

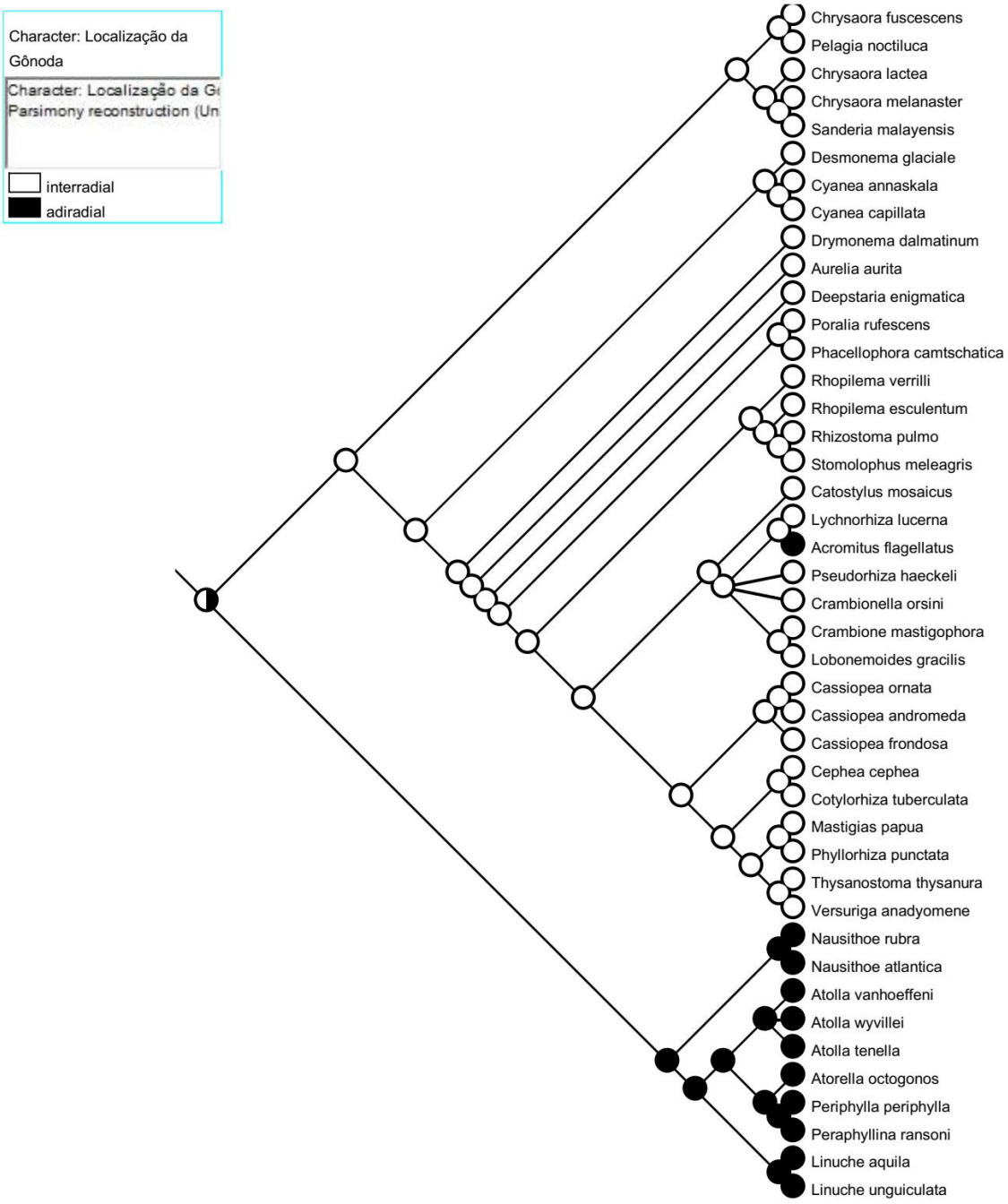

Figura 1: Reconstrução ancestral da localização da gônada dos diferentes representantes de Scyphozoa. Diagrama construído com base na filogenia proposta por Bayha et al. (2010).

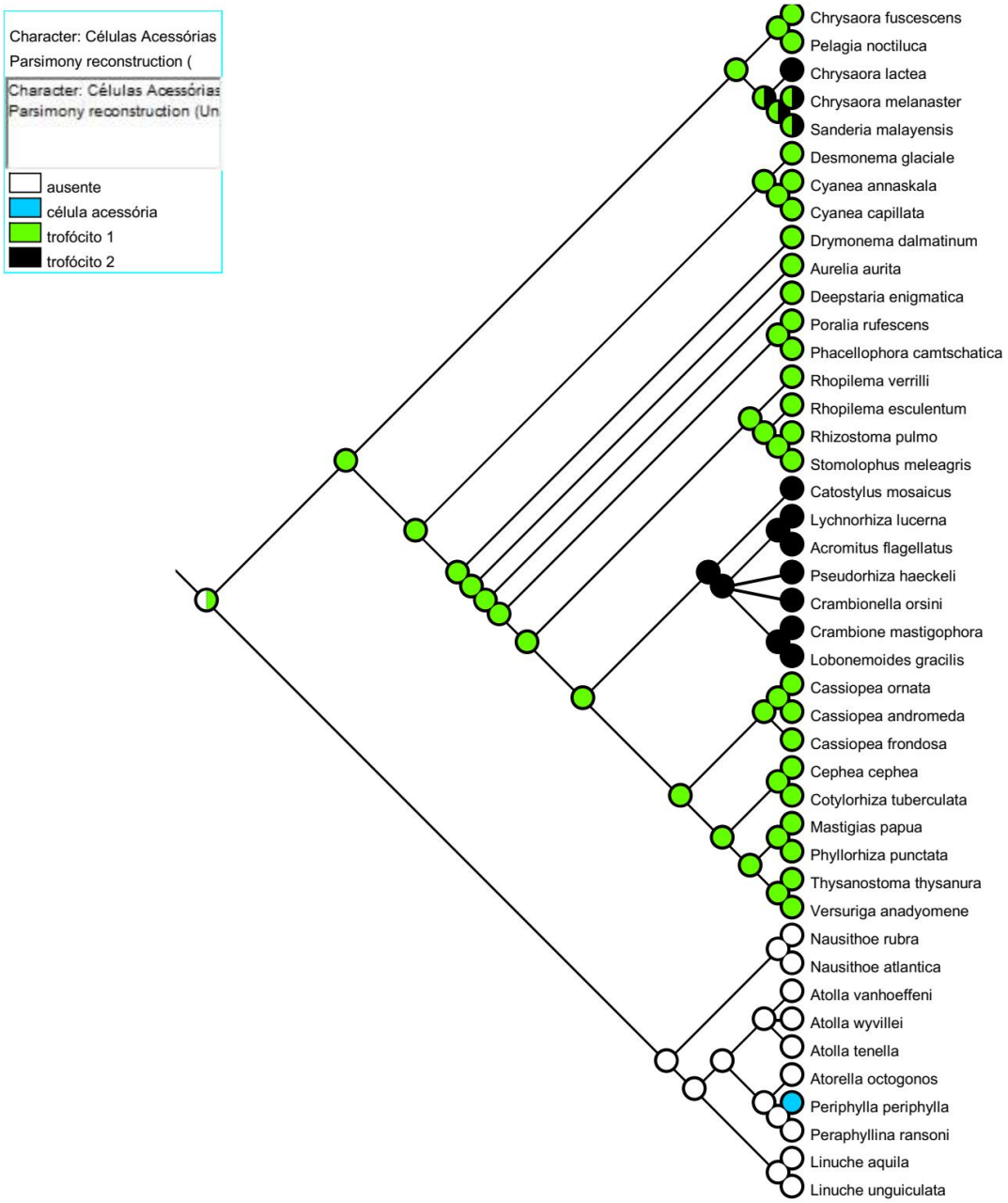

Figura 2: Reconstrução ancestral da presença de células acessórias dos diferentes representantes de Scyphozoa. Diagrama construído com base na filogenia proposta por Bayha et al. (2010).

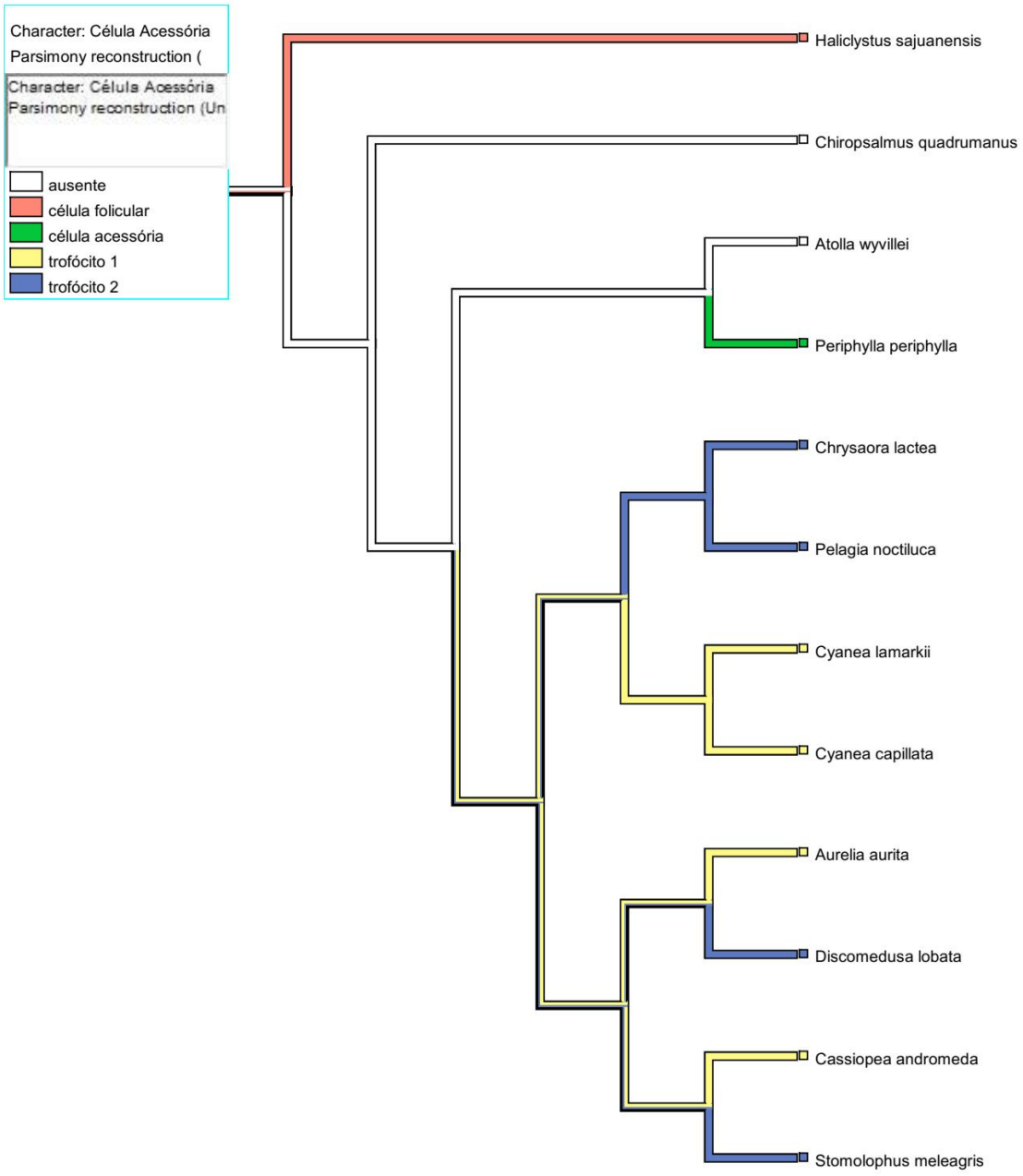

Figura 3: Reconstrução ancestral da presença de células acessórias em representantes de Acraspeda. Diagrama construído com base na filogenia proposta por Kayal et al. (2018).

Resumo Geral

Dentro de Medusozoa, a classe Scyphozoa é uma das quatro que compõem o subfilo possuindo 241 morfoespécies descritas. Seus representantes são comumente chamados de “águas-vivas verdadeiras” e, atualmente, encontram-se divididos em duas subclasses: Coronamedusae e Discomedusae. Coronamedusae é conhecida por possuir espécies de profundidade, porém a diversidade do grupo (57 espécies descritas) também engloba medusas coronadas de águas rasas. A subclasse Discomedusae possui 184 espécies dividas em duas ordens: Semaeostomeae e Rhizostomeae, sendo seus representantes conhecidos por serem planctônicos com biomassa de até 96% de água. Apesar da reprodução sexuada em Scyphozoa ter recebido relativamente bastante atenção ao longo dos anos, são poucos os trabalhos relacionando as características das gônadas de águas-vivas em um contexto comparativo e filogenético. Esta tese traz dados morfológicos sobre a reprodução sexuada em cifozoários e, comparativamente, identifica padrões evolutivos relevantes para futuro suporte na classificação dos táxons que compõem tal classe. No capítulo 1 nós revisamos o estado da arte da reprodução sexuada em medusas coronadas trazendo novos dados microscópicos da gônada e discutindo as principais considerações evolutivas para o grupo. No capítulo 2 descrevemos a estrutura microscópica da gônada de Scyphozoa, a espermatogênese e ultraestrutura do espermatozoide para três espécies de discomedusas discutindo a similaridade da organização histológica da gônada masculina com o túbulo seminífero presentes em outros metazoários. No capítulo 3 apresentamos dados da ovogênese do Semaeostomeae *Chysaora lactea* discutindo sobre os importantes papéis realizados pelos trofócitos durante a vitelogênese, coriogênese e ovulação do gameta. No capítulo 4 revisamos a gametogênese para as medusas semi-sésseis do gênero *Cassiopea*, registrando um caso de hermafroditismo para *C. frondosa*. Por fim, no capítulo 5 revisamos e comparamos a organização macroscópica, histológica e celular da gônada de cifozoários discutindo os principais tópicos e novidades evolutivas relacionadas à reprodução sexuada da classe Scyphozoa.

Palavras-chave: Evolução gonadal, Gametogênese, histologia, MET.

Abstract

Among Medusozoa, the class Scyphozoa is one of the four that composes the subphylum with 241 described morphospecies. Its representatives are commonly called “true jellyfishes” and are currently divided into two subclasses: Coronamedusae and Discomedusae. Coronamedusae is known to have deep-sea species, but the diversity of the group (57 described species) also includes shallow-water species. The subclass Discomedusae has 184 species divided into two orders: Semaeostomeae and Rhizostomeae, and their representatives are known to be planktonic with a biomass of up to 96% of water. Although sexual reproduction in Scyphozoa has received relatively considerable attention over the years, there are few studies relating the characteristics related to jellyfish gonads in a comparative and phylogenetic context. This thesis brings new morphological data on sexual reproduction of scyphozoans and, comparatively, identifies relevant evolutionary patterns for future support in the classification of the taxa. In chapter 1 we review the state of the knowledge of sexual reproduction in coronate medusae, bringing new microscopic data from the gonads and discussing the main evolutionary trends for the group. In chapter 2 we describe the microscopic structure of the gonad in Scyphozoa, spermatogenesis and sperm ultrastructure in three species of discomedusae and discuss the similarity of the histological organization of the male gonad with the seminiferous tubule found in other metazoans. In chapter 3 we present data on the oogenesis of the semaeostomeae *Chysaora lactea* discussing the important roles played by trophocytes during vitellogenesis, choriogenesis and spawning. In chapter 4 we review gametogenesis for semi-sessile jellyfish of the genus *Cassiopea*, reporting a case of hermaphroditism in *C. frondosa*. Finally, in chapter 5 we review and compare the macroscopic, histological and cellular organization of the scyphozoan gonads, discussing the main topics and evolutionary novelties related to the sexual reproduction of the class Scyphozoa.

Key-words: Gonadal evolution, gametogenesis, histology, TEM.

A**pêndice I - Protocolo de Fixador para Histologia**

Paraformaldeído 4% em Tampão Fosfato de Sódio 0,2 M pH 7,2

1. Preparação de solução estoque de paraformaldeído 10%;

2. Preparação de solução estoque de Tampão Fosfato de Sódio 0,4M

3. Preparação do Fixador.

1. Preparação de solução estoque de paraformaldeído 10%

Água destilada ..... 250 ml;

Paraformaldeído .....30 g.

a. Preparação:

- Aquecer a água destilada a 55ºC e separar em uma proveta 10 ml;

- Adicionar 240 ml em um bécker;

- Pesar 30 g de paraformaldeído;

- Adicionar o paraformaldeído no bécker com água destilada;

- Adicionar os 10 ml restantes e misturar em agitador magnético;

- Adicionar 4 pastilhas de Hidróxido de Sódio;

- Esperar até que solução fique transparente.

2. Preparação de Solução estoque de Tampão Fosfato de Sódio 0,4 M

Solução A:

Fosfato de Sódio Monobásico..... 7,78g

Água destilada.....140ml

Solução B:

Fosfato de Sódio Bibásico.....20,42g

Água destilada.....360ml

a. Preparação:

Para 500 ml de solução final:

- Misturar em um bécker Solução A e B;

- Corrigir o pH se necessário (pH 7,2 – 7,4).

3. Preparação do Fixador

Água destilada.....40 ml;

Tampão Fosfato de Sódio Na 0,4M.....200ml;

Paraformaldeído 10%.....160ml.

a. Preparação:

Para 400ml de solução final:

- Em um bécker adicionar 40ml de água destilada;

- Adicionar 200 ml de Tampão Fosfato de Sódio 0,4M;

- Adicionar 160 ml de Paraformaldeído 10%;

- Misturar em agitador magnético;

- Confirmar o pH (pH 7,2 – 7,4).

Apêndice II - Protocolo de Histologia em Historesina

1. Fixação

Colocar as pequenas amostras de tecido em solução de paraformaldeído a 4% em tampão fosfato de sódio 0,2M por 24 horas. Sempre na proporção de 10:1 (10 partes de fixador para 1 de amostra).

2. Lavagem

Retirar o fixador e fazer 1 lavagem com o tampão fosfato de sódio por 24 horas em geladeira.

3. Desidratação

- Retirar todo o tampão fosfato de sódio e colocar o material em séries de álcoois de 30% a 100%:
- Álcool 30%..... 30 min;
- Álcool 50%..... 30 min;
- Álcool 70%..... 30 min;
- Álcool 80%..... 30 min;
- Álcool 90%..... 30 min;
- Álcool 95%..... 30 min;

- Álcool 100%..... 1 hora.

4. Infiltração

- Retirar o álcool e colocar solução 1:1 de historesina + álcool. Deixar por 2 dias na geladeira.
- Retirar a solução anterior e colocar em resina pura por 2 horas no vácuo.
- Trocar a resina pura por uma nova solução e deixar 48 horas na geladeira.

5. Inclusão

- Antes de começar a inclusão do material, organizar as amostras e anotar em um caderno
todas as informações necessárias para posterior identificação dos bloquinhos, como nome
da espécie, material, tipo de fixador, orientação da amostra (longitudinal ou transversal), entre outras.
- Em um bécker adicionar resina líquida e o endurecedor (15ml de resina para 1ml de endurecedor) e misturar em agitador magnético.
- Como a resina endurece em aproximadamente 30 minutos, colocar o bécker com a resina (devidamente coberto com parafilm) em um pequeno vidro com gelo para que ela endureça mais devagar.
- Após, com uma pipeta pasteur, adicionar um pouco de resina no molde desejado, orientar a amostra o mais rápido possivel dentro do molde, e completar o restante do

molde com resina.

- Deixar polimerizar em estufa a 37ºC.

6. Fixação do bloco de resina em bloco de madeira

- Retirar os blocos de resina do molde pressionando o lado externo .

- Colar o bloco de resina no bloco de madeira utilizando cola especial* ou com cola superbonder.

- Identificar a madeira com o nome do material.

* Araldite Profissional – Misturar partes iguais dos dois tubos até ficar uma mistura homogênea de cor esbranquiçada, a cola seca em aproximadamente uma hora e definitivamente em 24 horas

- Passar cola na base do bloco de resina e uma quantidade mais generosa no bloco de madeira

- Pressionar o bloco de resina sobre o de madeira

- Cuidadosamente rearranjar a cola (com auxilio de um palito) pra que forme uma base larga, que os lados estejam distribuídos uniformemente e que a altura da cola não alcance a amostra.

7. Microtomia

- Limpar as lâminas com álcool e identificá-las.

- Cortar o material de modo contínuo a 10µm até chegar na amostra.

- Diminuir espessura dos cortes para 3 a 5µm.

- Depositar o corte na superfície da água destilada e esperar o corte esticar.

- Recolher com auxílio de um pincel posicionando na lâmina da forma desejada.

- Colocar a lâmina para secar na chapa aquecida;

- Após o término, deixar lâminas na estufa por pelo menos 15 min para secarem bem.

Apêndice III - Técnicas Histológicas e Histoquímicas de Colorações

Hematoxilina e Eosina (HE) (Behmer *et al.*, 1976; Llewellyn, 2013)

1) Hematoxilina de Mayer (20min a 37^{0}C);

2) Água de torneira corrente (3min) – Lavar algumas vezes (2 ou 3) a cuba retirando o excesso de corante e depois deixar apenas um fio de água não muito forte que elimine o resto do excesso de corante;

3) Lavagem rápida em água destilada – Secar e analisar as lâminas sob microscópio para verificar a intensidade da coloração;

4) Eosina (5min a 37^{0}C);

5) Lavar em água destilada (2min x 3) - Mergulhar para tirar o excesso;

6) Água destilada - analisar sob microscópio;

7) Secagem em chapa aquecedora (37^{0}C);

8) Secar em estufa (15-20 min);

9) Montagem final das lâminas com meio de montagem (*Entellan* ou *Permount*) e lamínula.

Preparação dos corantes:

Hematoxilina de Mayer (1891):

- Dissolver 1g de hematoxilina em 50ml de álcool a 95%;
- Dissolver 50g de sulfato de amônio e alumínio em 1000ml de água destilada;

- Misturar em agitador magnético;

- Deixar o corante maturar por meses (o frasco deve ser tampado com um chumaço de algodão);

- Pode-se adicionar posteriormente 20ml de ácido acético glacial.

Eosina Y (solução aquosa com ácido acético) (Behmer *et al.,* 1976):

- Dissolver 10g de eosina Y em 1000ml de água destilada;

- Adicionar 2ml de ácido acético glacial;

Azul de Toluidina com borax (Junqueira, 1995)

Para estruturas ácidas

1) Azul de Toluidina (1-2min) – Ajustar o tempo de acordo com a amostra aumentando gradativamente o tempo até obter a coloração desejada;

2) Mergulhar a lâmina em outra cuba com água destilada (2 ou 3 vezes até o excesso de corante ser removido);

3) Secar em chapa quente e observar a intensidade da coloração;

4) Secar em estufa (15-20 min);

5) Montagem final das lâminas com meio de montagem (*Entellan* ou *Permount*) e lamínula.

Preparação do corante:

- Azul de toluidina 0,1 g.

- Borato de sódio 1 g;

- Água destilada 100 ml.

PAS – Ácido Periódico e Reativo de Schiff (McManus, 1946)

(Bancroft & Stevens, 1982; Kiernan, 1990)

Para polissacarídeos neutros

1) Ácido periódico (10-15min);

2) Lavar em água destilada (deixar por 2min x 3);

3) Reativo de Schiff (30min);

4) Água de torneira corrente (5min);

5) Água destilada (lavagem rápida);

6) Secar e observar sob microscópio se houve reação positiva;

Opcional:

7) Hematoxilina de Mayer (10-20min a 37^{0}C);

8) Água de torneira corrente (3min);

9) Água destilada (lavagem rápida);

10) Secagem em chapa aquecedora (37^{0}C);

11) Montagem final das lâminas com meio de montagem (*Entellan* ou *Permount*) e lamínula.

Preparação do corante e reagente:

Ácido Periódico:

- Ácido periódico 1g;

- Água destilada 200ml.

Reativo de Schiff:

- Dissolver 1g de fucsina básica em 200 ml de água fervente (um pouco antes da fervura);

- Reduzir a temperatura da solução para 50°C;

- Adicionar 2g de metabissulfito de potássio e misturar em agitador magnético;

- Deixar a solução chegar à temperatura ambiente;

- Adicionar 2ml de ácido clorídrico concentrado;

- Adicionar 2g de carvão ativado, com agitação.

- Armazenar o frasco à temperatura ambiente e ao abrigo da luz.

- No dia seguinte, filtrar e manter o reagente em frasco âmbar e a 4°C.

Azul de Alcian (pH 2,5) – Alcian Blue (Bancrofft & Stevens, 1982)

Para polisacarídeos ácidos

1) Azul de Alcian a 1% em ácido acético a 3% (10-30 min) (melhor 60 min para resina);

2) Água de torneira corrente (3 min);

3) Água destilada (lavagem rápida);

4) Secar e observar sob microscópio se houve reação positiva;

5) Hematoxilina de Mayer (10-20 min a 37°C);

6) Água de torneira corrente (3 min);

7) Água destilada (lavagem rápida);

8) Secagem em chapa aquecedora (37ºC);

9) Montagem final das lâminas com meio de montagem (*Entellan* ou *Permount*) e lamínula.

Preparação do corante:

Azul de Alcian (pH 2,5);

- Azul de Alcian - 2g;
- Ácido acético glacial a 3% (pH 2,5) - 200ml.

Azul de Bromofenol (Pearse, 1985)

Proteínas Básicas

1) Corar por 1 hora em temperatura ambiente;

2) Lavar em ácido acético 0,5% por 5 minutos;

3) Passar em Água corrente por 15 minutos;

4) Secagem em chapa aquecedora (37ºC);

5) Montagem final das lâminas com meio de montagem (*Entellan* ou *Permount*) e lamínula.

Preparação do corante e reagente:

Corante:

- Bicloreto de mercúrio 30 g;

- Azul de bromofenol 300 mg;

- Etanol (95%) 300 ml.

Ácido acético:

- 1 ml de Ácido acético glacial;

- 200 ml de água destilada.

Xylidine Ponceau (Pearse, 1985)

Proteínas Totais

1) Corar por 30 minutos;

2) Passar no Tampão Acetato de Sódio por 1 minuto;

Observação: Medir o pH antes de usar o tampão (o pH 2,5 é o ideal);

3) Lavar em água destilada;

4) Secagem em chapa aquecedora (37^{0}C);

5) Montagem final das lâminas com meio de montagem (*Entellan* ou *Permount*) e lamínula.

Preparação do corante e reagente:

Xylidine

- 0,25 g de Xylidine;

- 250 ml de ácido acético a 2% (pH 2,5)

Ácido Acético 2%

- 5 ml de ácido acético glacial;

-250 ml de água destilada.

Tampão Acetato de Sódio

Solução A:

- 6,8 g de Acetato de Sódio

- 250 ml de água destilada.

Solução B:

- 15 ml de ácido acético;

- 250 ml de água destilada

- Adicionar solução B em A até atingir o pH desejado (2,5 a 3,5);

Observação: Fazer sempre a Solução B em maior quantidade.

Apêndice IV - Protocolos dos Fixadores para Microscopia Eletrônica de Transmissão

Fixador Karnovsky

(OBS.: realizar todos os procedimentos em CAPELA e utilizando LUVAS e AVENTAL.)

Para 200 ml de solução fixadora:
- Dissolver 4 g de paraformaldeído (pó) em 50 ml de água destilada aquecida a 60-70˚C (em agitador magnético). Adicione algumas gotas de NaOH 1N até a solução ficar transparente.

CUIDADO, TÓXICO!

- Resfriar à temperatura ambiente e adicionar 20 ml de glutaraldeído a 25%.

CUIDADO, TÓXICO!

- Adicionar 100 ml de tampão cacodilato de sódio 0.2M (4.28 g de cacodilato de sódio em 95 ml de
água destilada). CUIDADO, EXTREMAMENTE TÓXICO!
- Ajustar o pH para aprox. 7.4 com HCl 0.2N. CUIDADO, CORROSIVO!
- Adicionar 5 ml de $CaCl_2$ 0.1M (0.027g de $CaCl_2$ em 5ml de água destilada)
- Adicionar 40 g de sacarose.
- Se necessário, adicionar água destilada para completar o volume de 200 ml (volume medido no
béquer graduado).

Obs.1: manter refrigerado. Retire somente o necessário para fixar (cobrir) suas amostras. A fixação deve ser realizada em geladeira. Após o término do tempo de fixação, trocar a solução fixadora pela solução tampão, descrita abaixo. Não descarte o fixador na pia (siga as normas para descarte).
Obs.2: concentrações finais do fixador: paraformaldeído a 2%, glutaraldeído a 2.5%, tampão cacodilato de sódio (pH 7.4) a 0.1M e $CaCl_2$ a 2.5mM.

Solução tampão cacodilato de sódio a 0.1M (para 200ml):

-Adicionar 100 ml de tampão cacodilato de sódio 0.2M (4.28 g de cacodilato de sódio em 95 ml de
água destilada). Utilize um agitador magnético. CUIDADO, EXTREMAMENTE

TÓXICO!
- Ajustar o pH para aprox. 7.4 com HCl 0.2N.
- Adicionar 5 ml de $CaCl_2$ 0.1M (0.027 g de $CaCl_2$ em 5 ml de água destilada)
- Adicionar 40 g de sacarose.
- Completar o volume de 200 ml com água destilada (volume medido no béquer graduado).

Obs.: manter refrigerado. Retire somente o necessário para cobrir as amostras fixadas. Após trocar a solução fixadora pela solução tampão, mantenha as amostras em geladeira. Não descarte o tampão na pia (siga as normas para descarte).

Apêndice V- Protocolo de Microscopia Eletrônica de Transmissão

1. Fixação I

Fixador Karnovsky: glutaraldeído a 2.5% + paraformaldeido 2% em tampão cacodilato de sódio (pH 7.4) a 0.1M e $CaCl_2$ a 2.5mM por 24 horas a 4ºC (espécies do Brasil).
Glutaraldeído 2,5% em Tampão Cacodilato 0,1M em água do mar filtrada a vácuo Millipore (pH 7.2-7.4) – por 30 minutos a 4ºC (espécies da Austrália).

2. Lavagem I

Mesmo Tampão Cacodilato utilizado para fazer o fixador 0,1M – 3 x 10 min a 4ºC.

3. Fixação II (Pós-Fixação)

Ósmio 1% em Tampão Cacodilato 0,1M - 1 hora a 4ºC
Receita para 10 ml:
_ Tetróxido de Ósmio a 2% aquoso ..... 5 ml
_ Tampão Cacodilato 0,2M ..... 5 ml

4. Lavagem II

Opção 1 (com "En Bloc Staining" – amostras Brasil)
_ Água do mar filtrada ..... 20 ml

Opção 2 (sem "En Bloc Staining" - amostras Austrália)
Tampão Cacodilato de Sódio 0,1M em água do mar – 3 x 10 min a 4ºC
Receita:
_ Tampão Cacodilato 0,1M
Obs: Nessa etapa, caso seja necessário, pode-se deixar o material na geladeira por alguns dias.

5. "En Bloc Staining"

Acetato de Uranila 1% aquosa – 15 a 18 horas a 4ºC

6. Desidratação (temperatura ambiente)
Álcool 50% ..... 10 min

Álcool 70% ..... 10 min
Álcool 95% ..... 15 min
Álcool 100% ..... 2 X 10 min

7. Embebição (temperatura ambiente)

Álcool 100% 2:1 Spurr ..... 1 hora
Álcool 100% 1:1 Spurr ..... 1 hora
Álcool 100% 1:2 Spurr ..... 1 hora
Spurr puro ..... 2 x 2 horas

8. Emblocagem

Em molde de borracha ou cápsula de gelatina – 72 horas a 58ºC (Amostras do Brasil);
Em molde de borracha ou cápsula de gelatina – 8 horas a 70ºC (Amostras da Austrália).

Receita:

1. Spurr tipo Standard
p / + 30 ml p / + 15 ml
_ ERL 4206 ..... 10 g ..... 5 g
_ DER 736 ..... 6 g ..... 3 g
_ NSA ..... 26 g ..... 13 g
_ S-1 (DMAE) ..... 0,4 g ..... 0,2 g

2. Uranila 1% aquosa

_ Acetato de uranila ..... 1 g
_ água bi-destilada ..... 100 ml (q.s.p.)