

The background of the cover is a microscopic image showing a dense field of circular, reddish-brown cells. These cells appear to be arranged in a somewhat regular pattern, possibly representing a cross-section of a biological tissue or a specific stage of cell division. The lighting is soft, highlighting the texture and color of the cells.

Lucília Souza Miranda

Taxonomia e biologia de  
*Haliclystus antarcticus*:  
inferências para a evolução de  
Staurozoa (Cnidaria)

São Paulo  
2010

Lucília Souza Miranda

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Taxonomy and biology of *Haliclystus antarcticus*:  
inferences for the evolution of Staurozoa (Cnidaria)

Dissertação apresentada ao Instituto de  
Biotecnologia da Universidade de São Paulo, para  
a obtenção de Título de Mestre em Ciências,  
na Área de Zoologia.

Orientador: Prof. Dr. Antonio Carlos Marques

São Paulo  
2010

Miranda, Lucília S.

Taxonomia e biologia de *Haliclystus antarcticus*: inferências para a evolução de Staurozoa (Cnidaria)

130 páginas

Dissertação (Mestrado) - Instituto de Biociências da Universidade de São Paulo. Departamento de Zoologia.

1. Stauromedusae 2. taxonomia 3. ciclo de vida I. Universidade de São Paulo. Instituto de Biociências. Departamento de Zoologia.

## Comissão Julgadora

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Prof. Dr. Antonio Carlos Marques

*Com carinho,  
a minha mãe e à Camilinha.*

*“We must not mistake a similarity for an identity, any more than that the cry of a child would identify it with a cat, because their voices sound alike, and cannot always be distinguished the one from the other by any single faculty of our senses.”*

Henry J. Clark, 1862

*“Comme l’observation directe me paraît peu possible, nous sommes réduits pour le moment à cette hypothèse qui n’a d’ailleurs rien d’invraisemblable.”*

Wietrzykowski, 1912

(Sobre seus estudos com estauromedusas)

# Agradecimentos

---

Ao Antonio Carlos Marques (Tim) pela orientação, apoio, confiança e brincadeiras. Por sua ajuda e incentivo nos momentos mais importantes.

Ao Allen G. Collins pelas conversas, por toda sua ajuda, por acreditar no meu trabalho e por compartilhar o amor pelos mesmos animais.

Ao André C. Morandini, que sempre me ajudou em tudo que precisei, pelas discussões, pelos animais (o grande coletor de estauromedusas!), obrigada!

Ao CNPq, pela bolsa de Mestrado e pelo Edital Universal (474672/2007-7) concedidos.

Ao Instituto de Biociências, pela estrutura oferecida durante a execução deste estudo. Aos técnicos Eduardo Matos (*in memoriam*) e Enio Matos, pela grande ajuda durante a histologia e as sessões de microscopia eletrônica, pelas conversas, histórias, e pelo livro de magia.

Ao pessoal do LEM Amanda, Elaine, Juliana, Mariana, Max, Nathalia e Thaís, e do LSM Fernando P.L. Marques, Maíra, Sabrina e Teté, por toda a ajuda, pelos conselhos, pelas risadas. Muito obrigada mesmo!

Aos amigos Dri, Gabi, Nando, Gustavo, Gabriel, Sheila, Daniel, Rafael, Aline, Pri, Fausto, Dorta e Thiago, por mesmo sem saber o que eu fazia de verdade (“Então é um pólipó que teve vontade de virar medusa, mas não conseguiu? Meio frustrado?”), sempre estiveram do meu lado, às vezes me tirando do trabalho. A Marina e Isabel, que estão distantes, mas presentes! A Cynthia, sempre uma mestra, e Thaís Santos, pelas bagunças. A Ju e Nathy, pelas viagens, conversas, palhaçadas. Ao Té, um parceiro, pela enorme ajuda, pela força, pela tranquilidade, e por me fazer tão bem!

A Camilinha, minha irmã, pelo amor e pelas loucuras! Sempre crianças.

A minha família, pelo apoio que me permite trabalhar com o que eu amo.

Obrigada!

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# Capítulo 1

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## Introdução

### INTRODUÇÃO GERAL AOS ESTUDOS DE STAUROZOA

#### Contexto filogenético

As relações entre as linhagens de Cnidaria vêm sendo melhor definidas nos últimos anos, consequência da integração de dados moleculares, morfológicos e do ciclo de vida. Entretanto, uma parte das hipóteses evolutivas para o grupo continua inconclusiva e, por isso, ainda se busca uma maior estabilidade topológica para a compreensão da evolução das espécies e sua diversificação em riqueza, morfologia e desenvolvimento (Bridge et al., 1992, 1995; Odorico & Miller, 1997; Kim et al., 1999; Collins 2000, 2002; Medina et al., 2001; Marques & Collins, 2004; van Iten et al., 2006; Collins et al., 2006).

Sucessivos estudos filogenéticos do filo Cnidaria sugerem um consenso em que Anthozoa é grupo-irmão dos outros cnidários, os quais são coletivamente denominados Medusozoa (Petersen, 1979; Salvini-Plawen, 1987; Collins, 2002; Marques & Collins, 2004). O monofiletismo de Medusozoa é corroborado pelo compartilhamento de DNA mitocondrial linear, um caráter único em relação aos Anthozoa e aos outros Metazoa (Bridge et al., 1992). Nos últimos anos, as relações entre as classes de Medusozoa foram melhor definidas, e as topologias apresentadas em diferentes trabalhos vêm demonstrando resultados parcialmente congruentes (Collins, 2002; Dawson, 2004; Marques & Collins, 2004; Collins et al., 2006; van Iten et al., 2006).



Marques & Collins (2004), baseados em dados morfológicos e do ciclo de vida, concluíram que os Scyphozoa (Coronatae, Rhizostomeae, Semaestomeae e Stauromedusae) não constituem um grupo monofilético, e propuseram uma nova classe, denominada Staurozoa, clado este formado por Stauromedusae e Conulatae (grupo fóssil), e relacionado aos Cubozoa. Os resultados encontrados naquele trabalho são congruentes com hipóteses baseadas em marcadores moleculares nucleares (Collins, 2002 com 18S; Dawson, 2004 com 5.8S e 28S parcial).

Van Iken et al. (2006) re-investigaram a posição sistemática do grupo-fóssil Conulatae, corrigindo a codificação de alguns caracteres morfológicos e do ciclo de vida que fora proposta por Marques & Collins (2004), e concluíram que o grupo fóssil Conulatae era grupo-irmão de Coronatae. Assim, Staurozoa foi redefinida como uma nova classe restrita aos Stauromedusae, ocupando uma posição basal em relação aos outros Medusozoa. Esse posicionamento filogenético de Staurozoa como grupo-irmão de todos os outros Medusozoa, proposto por van Iken et al. (2006), foi independentemente obtido com base no gene mitocondrial 16S (Collins & Daly, 2005) e em genes nucleares ribossomais (Collins et al., 2006).

As discussões evolutivas sobre os Staurozoa têm sido focadas nas suas relações com outros grupos de Cnidaria, deixando as relações entre seus componentes em segundo plano (Collins & Daly, 2005). Os raros esforços em determinar as relações filogenéticas entre os Staurozoa são recentes (Collins & Daly, 2005; Lutz et al., 2006). Em geral, famílias e gêneros são reconhecidos por um mosaico de características, muitas das quais não são exclusivas, ou que sugerem agrupamentos contraditórios (Collins & Daly, 2005). De fato, inferências filogenéticas realizadas para subgrupos de Staurozoa por meio de dados moleculares (mitocondrial 16S e nuclear 18S),

demonstraram a necessidade de uma reavaliação de clados supragenéricos, uma vez que a maioria deles não é monofilético (Collins & Daly, 2005).

### **Características gerais da classe Staurozoa**

Staurozoa (do grego “*stauros*”, cruz) é um grupo de cnidários bentônicos, em formato de cálice, com um pedúnculo aboral que se fixa a diferentes substratos por um disco adesivo basal (Mayer, 1910). O grupo possui algumas características que o distingue dos demais Medusozoa, como a ultra-estrutura dos ovários e ocelos (Eckelbarger, 1994; Blumer et al., 1995); uma larva não ciliada rastejante com 16 células endodérmicas (Otto, 1976, 1978); pedúnculo com quatro câmaras e oito conjuntos de tentáculos capitados adradiais (Mayer, 1910; Collins & Daly, 2005).

A classe Staurozoa possui apenas uma ordem, Stauromedusae. Dentro dessa ordem estão compreendidas seis famílias, 15 gêneros e cerca de 50 espécies (Tabela 1). A família Lucernariidae conta com o maior número de espécies (23), o que corresponde quase à metade da diversidade total conhecida para a classe. Essa família inclui os gêneros *Haliclystus*, *Lucernaria*, *Stenoscyphus* e *Stylocoronella*. O gênero *Haliclystus* é o que possui o maior número de espécies (12, sendo duas espécies *nomida nuda*) dentre os Staurozoa (Bisby et al., 2009). No entanto o embasamento para esta classificação não é filogenético, uma vez que já foi demonstrado que há grupos classicamente adotados que não são monofiléticos (Collins & Daly, 2005).

As estauromedusas, como um todo, possuem uma distribuição cosmopolita (Lutz et al., 1998). Todavia são mais comumente encontradas em regiões entre-marés, em águas temperadas ou polares, com poucos registros de espécies para águas tropicais ou subtropicais (Mayer, 1910; Grohmann et al., 1999; Mills & Hirano, 2007;

Bisby et al., 2009). Cerca de 40 espécies ocorrem no hemisfério Norte e apenas 10 são registradas para o hemisfério Sul (Grohmann et al., 1999; Bisby et al., 2009; Miranda et al., 2009). Os indivíduos encontram-se fixos a rochas ou a algas, e geralmente sua coloração combina com a da alga, em uma forma de camuflagem (Larson, 1988). Portanto, a taxonomia de Staurozoa é ainda mais dificultada pelo fato de muitas espécies do grupo serem dificilmente encontradas, dados seus hábitos crípticos e sua ocorrência restrita a poucas regiões do planeta (cf. Mayer, 1910; Larson, 1990; Zagal, 2004a; Collins & Daly, 2005). Como consequência, muitas espécies de Staurozoa estão mal descritas, relegadas a poucos exemplares (como *Kishinouyea hawaiiensis*, cuja descrição é baseada em apenas dois indivíduos – ver Edmondson, 1930) ou a informações de trabalhos antigos, muitas vezes incompletas, havendo necessidade de uma melhor taxonomia para o grupo.

### **Desenvolvimento e ciclo de vida**

A questão da natureza ancestral dos Cnidaria, se um pólipó ou uma medusa, foi motivo de debate por muito tempo, desde Brooks (1886; cf. Marques & Collins, 2004; Collins et al., 2006). Por um lado, há estudos que defendem o ancestral medusóide pelágico, com perda deste estágio na linhagem que originou os Anthozoa (Hyman, 1940; Hand, 1959; Schuchert, 1993). Por outro lado, diversos trabalhos defendem que o ancestral de Cnidaria era um animal sésil com a forma de um pólipó (Hadzi, 1953; Salvini-Plawen, 1987, entre outros; ver Marques & Collins, 2004). Um exemplo de como esta questão ainda não chegou a um consenso é que, embora apresentem a mesma topologia em seus trabalhos, [(Anthozoa(Hydrozoa(Scyphozoa,Cubozoa)))],

Salvini-Plawen (1987) e Schuchert (1993) defendem teorias opostas, o que significa interpretações divergentes até para um mesmo padrão filogenético.

A classe Staurozoa parece ter algumas características “chaves” para uma boa compreensão sobre a evolução do ciclo de vida nos Cnidaria. Entretanto o ciclo de vida de Staurozoa é pouco estudado. Apenas o estágio de plânula de *Haliclystus “sanjuanensis”* (previamente identificada como *H. stejnegeri*) e *H. salpinx* (Otto, 1976, 1978); os estágios pós-metamorfose de algumas estauromedusas (Hirano, 1986); e o desenvolvimento completo de *H. octoradiatus* e *Stylocoronella riedli* (Wietrzykowski, 1910, 1912; Kikinger & Salvini-Plawen, 1995) foram documentados. Em Staurozoa ocorre uma transformação apical do indivíduo que não é seguida por uma fissão transversal, resultando em um adulto sésil semelhante a um pólipó (Wietrzykowski, 1912; Kikinger & Salvini-Plawen, 1995). A plânula dos Staurozoa é uma larva rastejante não ciliada (Otto, 1976, 1978) que, após o assentamento, desenvolve-se em um pólipó jovem (Wietrzykowski, 1912). Mais tarde, a extremidade oral do pólipó sofre uma metamorfose (Wietrzykowski, 1912; Uchida, 1929; Kikinger & Salvini-Plawen, 1995), desenvolvendo características que lembram aquelas encontradas em medusas adultas de Scyphozoa e Cubozoa, como músculos circulares coronais, gônadas, filamentos gástricos e órgãos adesivos ou ropalióides (Uchida, 1929; Hirano, 1986; Kikinger & Salvini-Plawen, 1995). Ao mesmo tempo, a parte aboral do adulto retém algumas características de pólipó, apresentando septos gástricos e musculatura longitudinal associada aos infundíbulos, o que é observado em alguns pólipos de Scyphozoa (Collins, 2002; Collins et al., 2006; Daly et al., 2007).

Inferências sobre o ciclo de vida peculiar dos Staurozoa na evolução do grupo são sugeridas na literatura. Como os dados morfológicos e moleculares atuais sugerem

que Staurozoa é a linhagem basal que diverge no clado dos Medusozoa, Collins et al. (2006) e van Iten et al. (2006) sugeriram que a medusa pelágica teria evoluído como um estágio de vida secundário, uma característica derivada dentro de Medusozoa, sinapomorfia do clado Scyphozoa+Cubozoa+Hydrozoa, enfraquecendo o argumento do ancestral medusóide pelágico dos Cnidaria. Dawson (2004) também sugere um ancestral polipóide para Medusozoa – entretanto, para Dawson, Medusozoa está restrito a Cubozoa+Scyphozoa+Hydrozoa, ou seja, é um clado que contém apenas medusas pelágicas (um estágio de vida secundário), excluindo os Staurozoa, que para o autor é uma forma de pólipóide modificada.

#### **A espécie *Haliclystus antarcticus* Pfeffer, 1889**

Como o gênero mais rico em número de espécies dentre os Staurozoa, o gênero *Haliclystus* já foi objeto de estudo em trabalhos morfológicos e evolutivos. Clark (1878) realizou um estudo detalhado acerca da biologia de *Haliclystus auricula*. Mayer (1910), Uchida (1929), Kramp (1961), Hirano (1986) e Zagal (2004a, 2004b, 2008) são outros exemplos de trabalhos que contribuíram para uma melhor compreensão morfológica e ecológica do grupo. O trabalho de Collins & Daly (2005) foi o primeiro a apresentar um posicionamento filogenético para algumas espécies do gênero, o que também foi realizado posteriormente por Lutz et al. (2006), sendo que nos dois trabalhos o gênero aparece como monofilético. Das 11 espécies do gênero, apenas três foram registradas para o hemisfério sul, dentre elas *Haliclystus antarcticus* Pfeffer, 1889, que ocorre na região sub-antártica da Ilha Geórgia do Sul e Ilha Paulet (Pfeffer, 1889; Carlgren, 1930; Davenport, 1998).

Poucos estudos foram realizados com *Haliclystus antarcticus*. A espécie foi descrita inicialmente por Pfeffer (1889), subsequentemente estudada por Carlgren (1930, taxonomia e histologia) e, posteriormente, listada em trabalhos compilatórios (Mayer, 1910; Thiel, 1936; Kramp, 1961). O trabalho mais recente encontrado sobre *H. antarcticus* é de Davenport (1998), o qual consiste em uma nota sobre as relações tróficas da espécie. Desta forma, há carência de informações sobre a biologia, diversidade intraespecífica, ciclo de vida, posicionamento filogenético e distribuição geográfica da espécie.

#### **OBJETIVOS GERAIS DO ESTUDO**

A partir do estudo de *Haliclystus antarcticus* como um modelo biológico de Staurozoa, este estudo visa:

- (1) revisar caracteres tradicionalmente utilizados na taxonomia do gênero *Haliclystus*, alguns destes adotados amplamente para Staurozoa;
- (2) abordar questões relacionadas à ontogenia e à biologia desses animais, em um contexto comparado e evolutivo;
- (3) discutir e revisar relações de homologia entre Staurozoa e as outras classes de Medusozoa, subsidiando inferências sobre a evolução do grupo e de seu ciclo de vida.

Para a consecução destes objetivos principais, estudos histo-morfológicos bem como a re-descrição de *H. antarcticus* foram relacionados ao conhecimento sobre a biologia e taxonomia do grupo. Dados moleculares, morfológicos e ecológicos foram utilizados para inferências acerca da ontogenia e ciclo de vida de *H. antarcticus*. Os estudos histo-morfológicos também proporcionaram uma discussão sobre as relações de homologia e ciclo de vida entre as classes de Medusozoa.

## ORGANIZAÇÃO DA DISSERTAÇÃO

Além do capítulo atual, a dissertação está dividida em mais quatro capítulos com objetivos independentes, porém complementares.

O **capítulo 2**, “Taxonomic review of *Haliclystus antarcticus* Pfeffer, 1889 (Stauromedusae, Staurozoa, Cnidaria), with remarks on the genus *Haliclystus* Clark, 1863” (Miranda et al. 2009), é uma re-descrição da espécie *H. antarcticus*, uma das poucas do gênero encontradas no hemisfério Sul. Com a oportunidade de trabalhar com centenas de espécimes, realizamos um estudo de variação morfológica intraespecífica, raramente possível para Staurozoa. Além disso, revimos alguns caracteres tradicionalmente usados na taxonomia do grupo, bem como a distribuição do gênero.

O **capítulo 3**, “Molecules clarify a cnidarian life cycle – The “hydrozoan” *Microhydrula limopsicola* is an early life stage of the staurozoan *Haliclystus antarcticus*” (Miranda et al., submetido e em revisão) é um estudo realizado a partir de dados moleculares (ITS1, ITS2 e 16S), com posteriores considerações morfológicas e ecológicas, por meio dos quais demonstramos que a espécie *Microhydrula limopsicola* Jarms & Tiemann, 1996, até então um Hydrozoa, é na verdade uma fase do ciclo de vida de *H. antarcticus*, provavelmente posterior à fixação da plânula, elucidando uma etapa do ciclo de vida do grupo, que pode ter influência direta em sua estrutura populacional.

O **capítulo 4**, “Histological anatomy of *Haliclystus antarcticus* (Cnidaria, Staurozoa) with a comparative discussion of staurozoan morphology” (Miranda et al., a ser submetido) possui, como propósito, a caracterização histológica da espécie visando contribuir para uma melhor taxonomia do grupo e para entender questões morfo-

funcionais dos caracteres. Além disso, realizamos uma discussão sobre as relações de homologia com as outras classes de Medusozoa, bem como a revisão de padrões encontrados durante o ciclo de vida desse grupo.

O **capítulo 5** traz as considerações finais do nosso estudo, destacando questões taxonômicas, do ciclo de vida e evolutivas, levantadas durante os capítulos anteriores.

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**Tabela 1** Classificação tradicional da classe Staurozoa (baseada em Mills, 2009; Bisby et al., 2009). \* Segundo a proposta de Marques & Collins (2004). \*\*Antiga ordem de Scyphozoa, agora ordem de Staurozoa.

CLASSE STAUROZOA*	ORDEM STAURUMEDUSAE**	Subordem Cleistocarpida	Família Depastridae	Gênero <i>Craterolophus</i>	<i>C. convolvulus</i> (Johnston, 1835) <i>C. macrocystis</i> von Lendenfeld, 1884
				Gênero <i>Depastromorpha</i>	<i>D. africana</i> Carlgren, 1935
				Gênero <i>Depastrum</i>	<i>D. cyathiforme</i> (M. Sars, 1846)
				Gênero <i>Halimocyathus</i>	<i>H. platypus</i> Clark, 1863
				Gênero <i>Manania</i>	<i>M. atlantica</i> (Berril, 1962)
					<i>M. auricula</i> (Fabricius, 1780)
					<i>M. distincta</i> (Kishinouye, 1910)
					<i>M. gwilliami</i> Larson & Fautin, 1989
					<i>M. handi</i> Larson & Fautin, 1989
					<i>M. hexaradiata</i> (Broch, 1907)
		Família Kishinouyeidae	Gênero <i>Kishinouyea</i>	<i>K. corbini</i> Larson, 1980	
				<i>K. hawaiiensis</i> Edmondson, 1930	
				<i>K. nagatensis</i> (Oka, 1897)	
			Gênero <i>Lucernariopsis</i>	<i>L. campanulata</i> (Lamouroux, 1815)	
				<i>L. capensis</i> Carlgren, 1938	
				<i>L. cruxmelitensis</i> Corbin, 1978	
				<i>L. vanhoeffeni</i> (Browne, 1910)	
			Gênero <i>Sasakiella</i>	<i>S. cruciformis</i> Okubo, 1917	
				<i>S. tsingtaoensis</i> Ling, 1937	
			Família Kyopodiidae	Gênero <i>Kyopoda</i>	<i>K. lamberti</i> Larson, 1988
		Família Lipkeidae	Gênero <i>Lipkea</i>	<i>L. ruspoliana</i> Vogt, 1886 <i>L. stephensoni</i> Carlgren, 1933 <i>L. sturdzii</i> (Antipa, 1893)	
		Família Lucernariidae	Gênero <i>Haliclystus</i>	<i>H. antarcticus</i> Pfeffer, 1889	
				<i>H. auricula</i> (Rathke, 1806)	
				<i>H. borealis</i> Uchida, 1933	
				<i>H. "californiensis" nomen nudum</i>	
				<i>H. kerguelensis</i> Vanhöffen, 1908	
				<i>H. monstrosus</i> (Naumov, 1961)	
				<i>H. octoradiatus</i> (Lamarck, 1816)	
				<i>H. salpinx</i> Clark, 1863	
				<i>H. "sanjuanensis" nomen nudum</i>	
<i>H. sinensis</i> Ling, 1937					
<i>H. stejnegeri</i> Kishinouye, 1899					
<i>H. tenuis</i> Kishinouye, 1910					
Gênero <i>Lucernaria</i>	<i>L. australis</i> Vanhöffen, 1908				
	<i>L. bathyphila</i> Haeckel, 1880				
	<i>L. haeckeli</i> (Antipa, 1891)				
	<i>L. infundibulum</i> Haeckel, 1880				
	<i>L. janetae</i> Collins & Daly, 2005				
	<i>L. quadricornis</i> Müller, 1776				
	<i>L. sainthilarei</i> (Radicorzew)				
<i>L. walteri</i> (Antipa, 1891)					
Gênero <i>Stenoscyphus</i>	<i>S. inabai</i> (Kishinouye, 1893)				
Gênero <i>Stylocoronella</i>	<i>S. riedli</i> Salvini-Plawen, 1966				
	<i>S. variabilis</i> Salvini-Plawen, 1987				
Família Tesseranthidae	Gênero <i>Tesserantha</i>	<i>T. connectens</i> Haeckel, 1880			

## Capítulo 2

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### **Taxonomic review of *Haliclystus antarcticus* Pfeffer, 1889 (Stauromedusae, Staurozoa, Cnidaria), with remarks on the genus *Haliclystus* Clark, 1863**

#### **ABSTRACT**

Difficulties concerning the taxonomy of stauromedusae are long known, and there is a clear need for taxonomic revision of the genus *Haliclystus*, as well as the reevaluation of some species. *Haliclystus antarcticus* Pfeffer, 1889 is recorded from Admiralty Bay, King George Island, Antarctic Peninsula. Due to the lack of detailed information on this species, we provide a redescription, presenting new data on the cnidome, morphometry, geographical distribution and intraspecific variation. Based on these characters, we propose that our specimens and *Haliclystus auricula* from Chile and Argentina are synonymous and should be classified as *H. antarcticus*. We also review the worldwide distribution of the genus *Haliclystus* Clark, 1863 and discuss taxonomic issues, concluding that some characters traditionally used in the taxonomy of the group should be used cautiously.

#### **RESUMO**

Dificuldades relacionadas à taxonomia de estauromedusas são bem conhecidas, e há nítida necessidade de uma revisão do gênero *Haliclystus*, como também da reavaliação de algumas espécies. *Haliclystus antarcticus* Pfeffer, 1889 é registrada para a Baía do Almirantado, Ilha do Rei George, Península Antártica. Devido à falta de

informações detalhadas sobre a espécie, nós realizamos uma re-descrição, apresentando novos dados de cnidoma, morfometria, distribuição geográfica e variação intraespecífica. Baseados nessas características, nós propusemos que nossos espécimes e *Haliclystus auricula* do Chile e Argentina são sinônimos e devem ser classificados como *H. antarcticus*. Além disso, revimos a distribuição global do gênero *Haliclystus* Clark, 1863 e discutimos questões taxonômicas, concluindo que alguns caracteres tradicionalmente utilizados na taxonomia do grupo devem ser cuidadosamente empregados.

## INTRODUCTION

Staurozoa was recently proposed as a distinct class of the phylum Cnidaria (Marques & Collins, 2004). Phylogenetic analyses based on morphological and molecular data indicate its basal position or early divergence within Medusozoa (Marques & Collins, 2004; Collins et al., 2006; van Iken et al., 2006). The class Staurozoa has only one order, Stauromedusae, comprising six families, 15 genera and approximately 50 species (Daly et al., 2007; Bisby et al., 2008; Mills, 2009). This traditional classification does not yet have a phylogenetic framework, and it includes some non-monophyletic groups (Collins & Daly, 2005 for the Lucernariidae and Depastridae).

The stauromedusae, or stalked jellyfishes, have a cosmopolitan distribution (Lutz et al., 1998), but the latitudinal distribution of the species is unequal. Approximately 80% of the species are found in the northern hemisphere, viz. North Pacific, North Atlantic, Arctic Ocean and Mediterranean (maybe related to the fact that the northern hemisphere has about 80% of the land mass of the earth). Only 11

species have been recorded from the southern hemisphere (Grohmann et al., 1999; Bisby et al., 2008), and most of them are found in higher latitudes, both temperate and polar waters (Mayer, 1910; Bisby et al., 2008).

Notwithstanding its broad distribution, the group has been scarcely studied and knowledge concerning its biology and ecology is limited, especially regarding the southern hemisphere (Davenport, 1998; Zagal, 2004a). Indeed, despite broader research programs focusing on the biodiversity of the Southern Ocean (Arntz, 1997), the research effort applied to different taxonomic groups has not been uniform (López-González & Gili, 2000).

Eight formally described species and two commonly adopted names (*nomina nuda*, *Haliclystus "californiensis"* and *Haliclystus "sanjuanensis"*) of the genus *Haliclystus* are recorded for the northern hemisphere, and only three species for the southern hemisphere (Figs. 1, 2; see also Bisby et al., 2008). Among the austral species, *Haliclystus antarcticus* was originally described by Pfeffer (1889), and subsequently studied by Carlgren (1930, taxonomy and histology) and Davenport (1998, trophic relationships). Other literature dealing with *H. antarcticus* consists only of compilations (Mayer, 1910; Thiel, 1936; Kramp, 1961).

Hirano (1997:251) discussed some taxonomic issues of *Haliclystus*, noting the importance of more detailed studies that include southern species. Indeed, we consider further studies on the biology, morphological intraspecific diversity and geographical distribution of *H. antarcticus* to be of utmost importance in understanding the genus. Therefore, the goal of this study is to fully redescribe the species, presenting new data on its cnidome, morphometry and distribution.



## MATERIALS AND METHODS

Specimens of *H. antarcticus* were collected manually during low tide (tide prediction between 0.2 and 0.4 m) on two beaches in Admiralty Bay, King George Island, Antarctic Peninsula (Fig. 3): (A) Pieter Lenie, in Copacabana (North American Refuge, 62°10'S, 58°26'W) and (B) Shag Point, in Arctowski (Polish Station, 62°10'S, 58°31'W). Material was collected on 16 and 17 February, and 1 March 2007, during the XXV Brazilian Antarctic Program. Individuals of *H. antarcticus* were attached to algae *Iridaea cordata* (Rhodophyta) or rocks (Fig. 4) around 0.1–1.0 m deep. Specimens of *Haliclystus* from Chile were also collected manually, during low tide, on Los Molinos beach, Valdivia, Chile. Material was collected on 11 March 2009. Individuals were attached to algae *Gracilaria chilensis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta). They were all preserved directly in 4% formaldehyde solution in seawater or in 80% ethanol. Qualitative observations were made based on 184 individuals (134 from Antarctica and 50 from Chile) and measurements of material were made with 60 individuals (50 from Antarctica and 10 from Chile) preserved in formaldehyde solution. Nematocyst types and distribution were determined using a light microscope. Only capsules of undischarged nematocysts were measured ( $n= 10$ ). Selected parts of individuals for scanning electron microscopy were dehydrated in a graded series of ethanol, dried in a critical point dryer, and sputter-coated with gold (modified from Migotto & Marques, 1999). Studied materials were deposited in the Cnidarian Collection of the Museu de Zoologia of the Universidade de São Paulo (MZUSP), São Paulo, Brazil; in the Cnidarian Collection of Zoology Department of the Universidade Federal do Rio de Janeiro (DZUFRJ), and in the Cnidarian Collection of the Museu Nacional da Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil.

## TAXONOMY

*Haliclystus antarcticus* Pfeffer, 1889 (Figs. 4–8)

*Haliclystus antarcticus*, Pfeffer, 1889:52–53; Mayer, 1910:536; Thiel, 1936:167; Uchida, 1929:154; Carlgren, 1930:1–6, Figures 1–9; Kramp 1961:292; Davenport, 1998:663–664; Grohmann et al., 1999:386.

*Haliclystus auricula*, Amor, 1962:89–96, Figures 1–2; Zagal, 2004a:331–336; 2004b:337–340; 2008:259–262, Figure 1 [non *Lucernaria auricula* Rathke, 1806, = *Haliclystus auricula* (Rathke, 1806)]

### Type series

The material used by Pfeffer (1889) to describe the species is lost. We also did not find other materials from South Georgia Island studied by Carlgren (1930, no reference in the paper) and Davenport (1998, no reference in the paper and no reply to our contacts). We, therefore, designate as Neotype of the species the specimen MZUSP 1551, from Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4 m), fertile specimen on alga *Iridaea cordata*, formaldehyde solution, col. and det. A.C. Morandini.

### Type locality

South Georgia Island, Antarctica (Pfeffer, 1889).

## Material examined

Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4 m), fertile specimen on algae *Iridaea cordata*, formaldehyde solution, col. and det. A.C. Morandini, NEOTYPE MZUSP 1551; Shag Point, Arctowski Polish Station, Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°31'W, 16 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4 m), fertile specimens on algae *Iridaea cordata*, formaldehyde solution, col. and det. A.C. Morandini, 3 individuals MZUSP 1552; Shag Point, Arctowski Polish Station, Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°31'W, 16 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4 m), fertile specimens on algae *Iridaea cordata*, ethanol, col. and det. A.C. Morandini, 2 individuals MZUSP 1553; Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4m), fertile specimens on algae *Iridaea cordata* or rocks, formaldehyde solution, col. and det. A.C. Morandini, 5 individuals MZUSP 1554; Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4 m), fertile specimens on algae *Iridaea cordata* or rocks, formaldehyde solution, col. and det. A.C. Morandini, 4 individuals MZUSP 1555; Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4 m), fertile specimens on algae *Iridaea cordata* or rocks, formaldehyde solution, col. and det. A.C. Morandini, 50 individuals MZUSP 1556; Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.5 m (tide

prediction 0.2–0.4 m), fertile specimens on algae *Iridaea cordata* or rocks, formaldehyde solution, col. and det. A.C. Morandini, 35 individuals MZUSP 1557; Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.5 m (tide prediction 0.2–0.4 m), fertile specimens on algae *Iridaea cordata* or rocks, ethanol, col. and det. A.C. Morandini, 30 individuals MZUSP 1558; Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 17 Feb 2007, depth 0.2 m (tide prediction 0.2–0.4 m), fertile specimens on rocks, formaldehyde solution, col. and det. A.C. Morandini, 2 individuals DZUFRJ 1-047; Pieter Lenie Beach, Copacabana Refuge (USA), Admiralty Bay, King George Island, Antarctic Peninsula, 62°10'S 58°26'W, 01 Mar 2007, depth 0.2 m (tide prediction 0.2–0.4 m), fertile specimens on rocks, formaldehyde solution, col. and det. A.C. Morandini, 2 individuals MNRJ 7671; Los Molinos, Valdivia, Chile, 11 Mar 2009, specimens on algae *Gracilaria chilensis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta), formaldehyde solution, col. J.P. Didier, 20 individuals MZUSP 1559; Los Molinos, Valdivia, Chile, 11 Mar 2009, specimens on algae *Gracilaria chilensis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta), ethanol solution, col. J.P. Didier, 30 individuals MZUSP 1560.

#### Description

Calyx conical (Fig. 4a), maximum diameter 1.25 times wider than high, height 3.5–16.2 mm (mean  $9.9 \pm 3.2$  mm,  $n = 50$ ) excluding tentacular clusters, width 4.1–23.4 mm ( $12.4 \pm 4.4$  mm), semi-translucent, separated from stalk. Stalk morphologically distinct from calyx (umbrella) (Fig. 4a), sometimes marked by thin stria, sometimes with no constriction at all. Outer surface of calyx (exumbrella) smooth (Fig. 4).

Eight adradial arms (Figs. 4c, 5) (abnormal specimens with 7–12), length 0.3–6.0 mm ( $2.3 \pm 1.1$  mm) excluding tentacular clusters. Distal internal region of arms thicker in mature specimens due to gonadal content, forming a groove about half of an arm midline (Fig. 6b). Arms generally equidistant (Fig. 5), distance from the distal end of arms (base of tentacular cluster) to anchors 0.3–6.6 mm ( $2.7 \pm 1.3$  mm). Distal region of each arm with 6–224 ( $97 \pm 40$ ) capitate tentacles (Figs. 4c, 6a, g); centripetal (towards mouth/subumbrella) tentacles smaller, larger tentacles at the central area of the cluster, centrifugal tentacles (towards margin/exumbrella) of intermediate size (Figs. 6h, 8a, b). Each tentacle with hollow stem and distal globular end (Fig. 6i) covered with nematocysts. Internal base of tentacular cluster with intertentacular lobules (Fig. 6j, k).

“U”-shaped coronal muscle in eight segments, each segment extending between distal regions of successive arms (Figs. 5, 7b, 8i). Radial muscle arranged in eight bands (four perradial and four interradian), centripetal to the coronal muscle, from the subumbrellar rim to the manubrium (Figs. 5, 8a).

One anchor centered between successive arms (abnormal specimens with 0–3) (Fig. 4c), located at the umbrella rim, projecting towards the exumbrella, height 0.3–3.1 mm ( $1.6 \pm 0.6$  mm), width 0.4–2.7 mm ( $1.2 \pm 0.4$  mm), totaling eight anchors, four perradial and four interradian (Fig. 6g). Anchors stalked, height of cylindrical stalk 0.05–0.51 mm ( $0.2 \pm 0.1$  mm) (Figs. 7a, 8g). The anchor morphology varies during ontogeny: at early ontogenetic stage (after metamorphosis to medusae) the anchors are circular, sometimes with a tentacular knob; juvenile anchors triangular, with a shallow groove at the basal region; median to late ontogenetic stages apparently cross constricted at

the central region, approximately “8”-shaped, forming a longitudinal groove at the central region (Figs. 7d–g, 8d–f). Adult anchors generally with wrinkled aspect.

Aboral stalk (Fig. 4a) four-chambered internally over the entire length; length 2.3–10.7 mm ( $6.5 \pm 2.0$  mm), externally divided into four regions (four furrows, not necessarily clear in juveniles) by four interradial longitudinal muscles (Fig. 4a), which extend all along the stalk upwards, reaching the manubrium, and subdivided towards the subumbrellar rim of the arms (Figs. 4, 5, 6m). Base of stalk with adhesive disc of wrinkled aspect, diameter 1.5–8.0 mm ( $4.2 \pm 1.5$  mm) (Figs. 6n, 8j).

Manubrium (Figs. 4b, 5) four-sided in cross section, each interradial face cross-striated from base of manubrium up to three-fourths of its height; each face separated by four bands of perradial muscles extending from mouth corners to perradial anchors (Fig. 5). Mouth four-sided, delicately pleated and frilled lips; interradial face projected outwards at the buccal rim, which is cut out in the area corresponding to the cross striations of the manubrium (Fig. 6a, c, e, f).

Four perradial pouches at the gastrovascular cavity (not visible in juveniles), extending from the manubrium to the perradial anchors, separated from each other by four interradial septa (Figs. 4b, 5, 6c). Pouches with gonads on the inner wall. Numerous gastric cirri at the perradial corner (=pouch centrifugal end) of the gastrovascular cavity (Fig. 6d).

Eight adradial gonads extending from the manubrium to the distal end of arms (Fig. 5), visible through translucent calyx (Fig. 4c), organized into four pairs (Figs. 4b, 5). Gonadal length 1.6–12.9 mm ( $6.9 \pm 2.2$  mm). Each pair of gonads arising centripetally at perradii, gonads somewhat parallel up to half of their length, then separating adradially (Figs. 4b, 5, 6a). Successive pairs of gonads completely separated at the

interradii by interradial septa (Figs. 4b, 5). Gonads comprised of numerous small vesicles, organized in 6–12 rows, each vesicle circular to hexagonal in shape (probably due to tightly packed arrangement), maximum width 0.5–0.65 mm ( $0.6 \pm 0.04$  mm) (Figs. 6d, 8c). Gonads mature in specimens with calyx height of about 11.72 mm and above.

Living specimens red-orange in colour with light-red gonads (Fig. 4) in King George Island, Antarctica; and specimens red or/and green in colour (some individuals presented calyx and stalk green and tentacles red, for example, others are completely green or red) in Valdivia, Chile. Oral surface largely unpigmented. Preserved individuals rosy-cream in colour.

Capitulum of tentacles with two types of nematocysts (Fig. 7k–m): isorhiza (atrichous or holotrichous—light microscopy was insufficient to distinguish the spines), abundant, length 14.0–17.0  $\mu\text{m}$  ( $15.3 \pm 0.08$   $\mu\text{m}$ ), diameter 4.0–6.0  $\mu\text{m}$  ( $5.0 \pm 0.05$   $\mu\text{m}$ ); microbasic heterotrichous, scarce, length 15.0–20.0  $\mu\text{m}$  ( $17.8 \pm 0.15$ ), diameter 8.0–10.0  $\mu\text{m}$  ( $9.0 \pm 0.05$   $\mu\text{m}$ ). Gastric cirri with microbasic heterotrichous, abundant, length 10.0–12.0  $\mu\text{m}$  ( $10.6 \pm 0.06$   $\mu\text{m}$ ), diameter 5.0–9.0  $\mu\text{m}$  ( $6.3 \pm 0.11$   $\mu\text{m}$ ), and isorhiza (atrichous or holotrichous), scarce, length 11.0–14.0  $\mu\text{m}$  ( $12.3 \pm 0.12$   $\mu\text{m}$ ), diameter 3.0–4.0  $\mu\text{m}$  ( $3.5 \pm 0.05$   $\mu\text{m}$ ). Anchors with scarce microbasic heterotrichous, length 8.0–10.0  $\mu\text{m}$  ( $9.0 \pm 0.14$   $\mu\text{m}$ ), diameter 5.0–6.0  $\mu\text{m}$  ( $5.5 \pm 0.07$   $\mu\text{m}$ ). Exumbrella with scattered microbasic heterotrichous, abundant, length 7.0–10.0  $\mu\text{m}$  ( $9.2 \pm 0.12$   $\mu\text{m}$ ), diameter 4.0–6.0  $\mu\text{m}$  ( $4.9 \pm 0.05$   $\mu\text{m}$ ). Subumbrella with two types of nematocysts: isorhiza (atrichous or holotrichous), abundant, length 10.0–14.0  $\mu\text{m}$  ( $12.4 \pm 0.13$   $\mu\text{m}$ ), diameter 3.0–4.0  $\mu\text{m}$  ( $3.1 \pm 0.03$   $\mu\text{m}$ ); microbasic heterotrichous, scarce, length 10.0–20.0  $\mu\text{m}$  ( $15.8 \pm 0.44$   $\mu\text{m}$ ), diameter 6.0–10.0  $\mu\text{m}$  ( $9.2 \pm 0.17$   $\mu\text{m}$ ).

## Distribution

Antarctica: South Georgia Island (Pfeffer, 1889; Carlgren, 1930; Davenport, 1998); King George Island, Admiralty Bay, Shag Point (this study); King George Island, Admiralty Bay, Pieter Lenie (this study); Graham Land, Paulet Island (Carlgren, 1930). Argentina: Chubut and Santa Cruz (Amor, 1962; Mianzan, 1989; as *H. auricula*). Chile: Valdivia (this study and Zagal, 2004a, 2004b, 2008; as *H. auricula*).

## DISCUSSION

Difficulties concerning the taxonomy of stauromedusae are long known (Mayer, 1910:522; Gwilliam, 1956:61; Kramp, 1961:293), and the need for taxonomic revision of the genus *Haliclystus*, as well as the reevaluation of some species, has been urged by Hirano (1997). We have documented intraspecific and ontogenetic variation in the following characteristics that have been used in the taxonomy of Staurozoa: presence/absence of anchors and their morphology, arrangement of arms, number and shape of tentacles, relations between “size of stalk” and “size of calyx”, and morphological separation between calyx and stalk (Mayer, 1910; Kramp, 1961; Kikinger & Salvini-Plawen, 1995), besides differences in number of gonadal sacs per gonad (Gwilliam, 1956). These characters, then, must be carefully assessed when employed in the taxonomy of the group.

Hirano (1997:247) reviewed several species of *Haliclystus* having a circumboreal distribution and recognized two important characters defining groups (Table 1): the morphology of the base of the tentacle clusters and the location of white spots of nematocysts on the subumbrella. These characters led her to distinguish two complexes of species, those with intertentacular lobules located internally at the base



of the tentacular cluster [*H. auricula*; *Haliclystus octoradiatus* (Lamarck, 1816); *H. "sanjuanensis"*, *nomen nudum*; *Haliclystus stejneri* Kishinouye, 1899] and those with a "U"-shaped interspace and without intertentacular lobules at that region (*Haliclystus tenuis* Kishinouye, 1910; *Haliclystus borealis* Uchida, 1933; *H. "californiensis"* of Gwilliam, 1956). These two complexes are subdivided according to the location of white spots of nematocysts. *Haliclystus antarcticus* possesses the same pattern as *H. auricula*, with intertentacular lobules at the internal base of the tentacular cluster and without white spots of nematocysts.

Another character that seemed to divide the genus *Haliclystus* into two groups is the arrangement of the gonads. Apparently, according to the literature, there are two different patterns: the "Y-pattern" in which the two halves of each pair of gonads are close together at their perradial point of origin at the base of the bell (present in *H. antarcticus* and *H. octoradiatus*; see Browne, 1895), and the pattern in which the proximal half of the gonads is connected in pairs at the interradii, but are completely separated in the perradii (present in *H. auricula*, *Haliclystus monstrosus* Naumov, 1961 and *H. borealis*; see Clark, 1878; Hirano, 1986; Kramp, 1961; Naumov, 1961). The pattern for *H. stejneri* is controversial: Hirano (1986) considers the pattern similar to that of *H. antarcticus*, and Uchida (1929) and Kramp (1961) consider it similar to that of *H. auricula*. However, in our observations of several specimens of the genus *Haliclystus* of the collection of the National Museum of Natural History, Smithsonian Institution, all species analysed [identified as *H. auricula* (USNM 54518), *H. stejneri* (USNM 1106655, USNM 1106935, USNM 1106936, USNM 1106937, USNM 1106938, USNM 1106939), *H. "sanjuanensis"* (USNM 1106653, 1106663, 1106664, 1106665), *H. borealis* (USNM 1106650) and *H. tenuis* (USNM 1106651, 1106652)] have the gonads

united at the periradii. So that characteristic needs to be reevaluated for the different species of *Haliclystus*.

The problem of finding clues to relationships among the stauromedusae is aggravated by the relatively few macromorphological characters that may be used for taxonomy (Hirano 1997). The species *H. octoradiatus*, *H. "sanjuanensis"*, *H. tenuis*, for example, have all been considered by some authors to be synonyms with *H. auricula* (Kramp, 1961; Gwilliam, 1956). However, from our point of view, there are enough characteristics to distinguish these species (Table 1), and they should be recognized as valid. Indeed, similarities between *H. antarcticus* and other species of the genus, such as *H. octoradiatus* (Carlgren, 1930), *H. stejnegeri* (Hirano, 1986) and *Haliclystus kerguelensis* Vanhöffen, 1908 (Mayer, 1910), have been noted in literature, with differences mainly based on morphometrical patterns (e.g. differences in proportions of the body parts). Among them, *H. kerguelensis* is the only other Antarctic species of the class (besides *H. antarcticus*, Fig. 1), and it was recorded from Kerguelen Island (Vanhöffen, 1908; Kramp, 1957). Mayer (1910:536) emphasized the similarities between *H. kerguelensis* and *H. antarcticus*, but they pointed out that *H. kerguelensis* has "fewer tentacles, smaller anchors and apparently a longer peduncle, although the stalk of *H. antarcticus* was probably contracted in Pfeffer's preserved specimens", and had different colours. Indeed, *H. kerguelensis* has up to 50 tentacles (Kramp, 1961) and *H. antarcticus* has about 97 tentacles per cluster (Table 1), but as we pointed out, the number of tentacles can vary according to the life cycle stage (*H. antarcticus* presents a variation of 6-224 tentacles per cluster). In fact, anchors of *H. kerguelensis* seem to be smaller than anchors of *H. antarcticus*: the first one is one-third as wide as the diameter of the stalk (Mayer, 1910) and the second one is about one-half to two-thirds

as wide as the diameter of the stalk (Table 1). Concerning the stalk, *H. kerguelensis* has the “peduncle about twice as long as height of calyx” (Kramp, 1961), and our analysis with relaxed individuals show that it is one-half to two-thirds as long as the height of the calyx for *H. antarcticus*. So, this is another difference that can be valid. The colours of *H. kerguelensis* can be rather different from those described by Vanhöffen (Kramp, 1957), likewise colours in *H. antarcticus* can vary (same population of *H. antarcticus* in Chile presented individuals completely red or green), and this characteristic seems not to be useful in the taxonomy of the group. One “similarity” between the species is the arms that are 45° apart (Vanhöffen, 1908; Mayer, 1910; Kramp, 1961), but as we observed for *H. antarcticus*, this characteristic can present intraspecific variation (see discussion below). According to these characteristics (relative size of the anchors and the stalk), we maintain the traditional view in distinguishing these species, but more detailed comparisons should be accomplished.

In this respect, specimens of *H. antarcticus* are similar to the specimens of *H. auricula* described by Amor (1962) and Zagal (2004a, 2004b, 2008), from Argentina and Chile, respectively, which raised questions about their identification. Our observations of animals from Los Molinos, Valdivia, Chile (same locality as in Zagal, 2004a, 2004b, 2008) strongly suggest that these materials are misidentified and that their true identity is *H. antarcticus*. Support for the synonymy between *H. antarcticus* and “*H. auricula*” (from Chile and Argentina) is suggested by the relative size of the anchors, the average number of tentacles per cluster, the proportion of calyx/stalk and the arrangement of the gonads (see Tables 1, 2). However, usually the anchors of *H. antarcticus* from Chile have a tentacular knob, rarely observed in *H. antarcticus* from King George Island at about the same life cycle stage. This tentacular knob in the

anchors was related to the earlier stages of *H. antarcticus* by Pfeffer (1889) and Carlgren (1930), and this difference might be an intraspecific and/or ontogenetic variation. Based on these characteristics, we propose that the species from Antarctica, Chile and possibly Argentina (although for this locality we do not have material and our conclusions are restricted to information from Amor, 1962) are synonymous and should be classified as *H. antarcticus*. There is an apparent similarity between *H. auricula* and *H. antarcticus* (Table 1); however, the proportion between stalk and calyx, and the height of anchors x diameter of stalk are different (Table 1). Several authors attributed different characteristics for *H. auricula* (Rathke, 1806; Clark, 1863; Clark, 1878; Uchida, 1927; Gwilliam, 1956; Hirano, 1997) and it is possible that there are some misidentifications, raising problems about the taxonomy of the genus *Haliclystus*, and about its distribution.

Previous studies have proposed distinctive characters for *H. antarcticus* that have proven to be ineffective because of ontogenetic variation or high plasticity in the species (see below). Mayer (1910) distinguished *H. antarcticus* from *H. auricula* based on the arms being united in pairs in the former. However, in our large series of animals ( $n = 184$ ), we have observed that the majority of *H. antarcticus* specimens have equidistant ( $45^\circ$ ) arms, though they are internally united “in pairs” by common bundles of interradial muscles. But we note that, in the early stages of development, the arms can be slightly united in pairs. According to Gwilliam for the genus *Haliclystus* (1956:52), “the arms pairing is not a consistent characteristic of the species and it is most probably an attitude caused by contractions of the marginal muscle.” Another putatively distinctive character, the number of tentacles, adopted by Mayer (1910) and Kramp (1961), is highly variable (6-224, for *H. antarcticus*), and its use should be

employed cautiously in the taxonomy of the group (Table 1, see also Gwilliam, 1956:53).

The morphological plasticity in the group also includes an array of abnormal features that have been described for other species of *Haliclystus* (Browne, 1895:3, Plate 1), and these features are also present in *H. antarcticus* (as in Zagal, 2008:260, tentatively identified as *H. auricula*), such as: variation in the number of arms; the morphology, number and location of anchors (Fig. 7c, h–j); the presence of two stalks (Fig. 6o). Earlier authors have hypothesized different environmental and biological causes of these abnormalities. Absence of arms, for instance, was proposed to be caused by physical disturbance such as wave action or predation (Zagal, 2008). Browne (1895) reported cases of congenital variation and imperfect regeneration of organs damaged or completely destroyed by injury. Amor (1962) merged physical and biological reasons and pointed out that losses in body parts of stauromedusae due to their intertidal habit are compensated by their strong regeneration ability, occasionally leading to “supernumerary” members (see also Mayer, 1910:522 and 532). However, it is unclear whether variation in symmetry is caused by genetic, environmental or developmental factors (variability in symmetric expression), or a combination of these factors (Gershwin, 1999).

The cnidome found in our studies is similar to that described by Carlgren (1930), who reported oval and rod-shaped nematocysts in the tentacles and gastric cirri, with similar proportions to those we had observed. The “oval” nematocysts are probably the microbasic heterotrichous, and the “rod-shaped” ones are the isorhiza (atrichous or holotrichous). The type of nematocysts seems to be not helpful in distinguishing species because isorhizas and heterotrichous (euryteles) have already

been described for other species of Staurozoa (Uchida, 1929; Weill, 1934; Larson, 1980, 1988; Calder, 1983; Hirano, 1986; Larson & Fautin, 1989; Collins & Daly, 2005). However, Gwilliam (1956) used the morphometry of nematocysts to distinguish some species of *Haliclystus*, so their possible use in taxonomy needs further investigation.

Collins & Daly (2005:221) emphasized that evolutionary discussions of stauromedusae have largely focused on their relationship to other groups of Cnidaria rather than on those among its component subgroups. As a result, families and genera are recognized by a mosaic of features, many of which are not exclusive, or suggest contradictory groupings. In fact, the suprageneric affinities among stauromedusae are also not well founded (Daly et al., 2007), partly because of imprecise or incorrect information found in literature. Mayer (1910) described *H. antarcticus* with a single-chambered stalk, suggesting a close relationship between the genera *Haliclystus* (for which other species frequently have four-chambered stalks) and *Lucernaria* (species with single-chambered stalks). However, this apparent error by Mayer contrasts with the original description by Pfeffer (1889) and the subsequent study by Carlgren (1930), who also described a four-chambered stalk for the species. Our sections of the specimens corroborated the four-chambered stalk of the original description. This hypothesis of a close relationship between *Haliclystus* and *Lucernaria*, pointed out by Mayer (1910), was also refused based on molecular markers, since these genera do not form a clade corresponding to the family Lucernariidae (Collins & Daly, 2005).

The lack of basic knowledge about most species of stauromedusae makes it difficult to address biogeographical questions, even though they are interesting animals for that approach, because of their life cycles and natural history. A possible justification for the lack of attention to this class is related to their cryptic habit and

occurrence in specific habitats (Larson, 1990; Salvini-Plawen, 2006). Indeed, our specimens were found attached to rocks or to red or green algae, to which their coloration matches closely, effectively providing camouflage (see also information for other species in Larson, 1980; Larson & Fautin, 1989). It was most likely because of this lack of attention and taxonomic expertise that large populations of *H. antarcticus* have been overlooked in the Southern Ocean. Our study is the second record of the species outside its type locality, the previous record, by Carlgren (1930:2, for Paulet Island), was not noticed by several authors (Kramp, 1961:292; Davenport, 1998:663; Grohmann et al., 1999:386). To these, we also propose to encompass the materials from Argentina (Amor, 1962) and Chile (Zagal, 2004a, 2004b, 2008).

Despite these difficulties discussed above, we have shown that what was thought to be a cosmopolitan species, *H. auricula*, appears not to be distributed in the southern hemisphere. Studies dealing with the distribution of these animals, mainly in the southern hemisphere, should be encouraged, since they contribute significantly to a better understanding of the evolution of the group.

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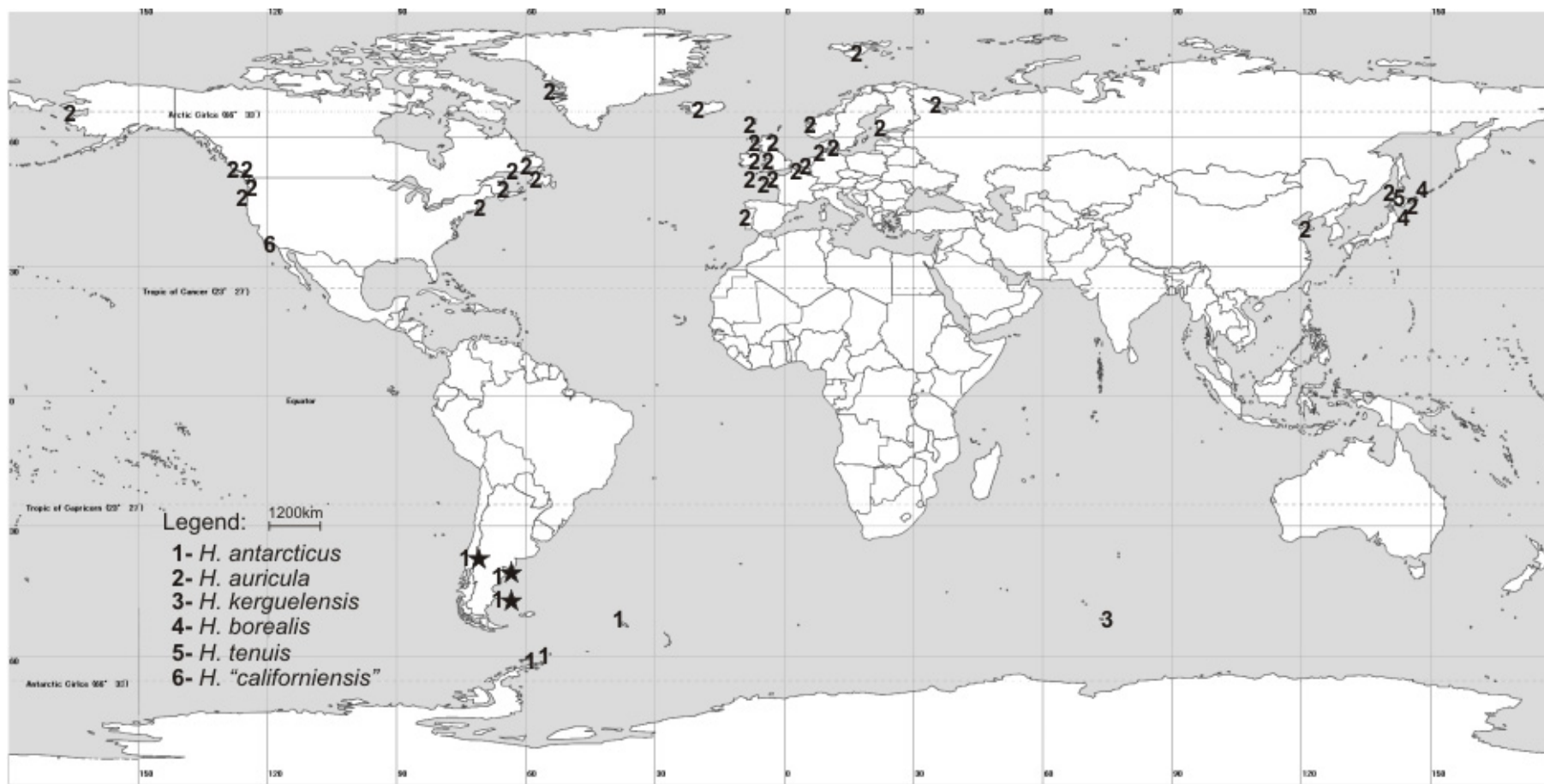


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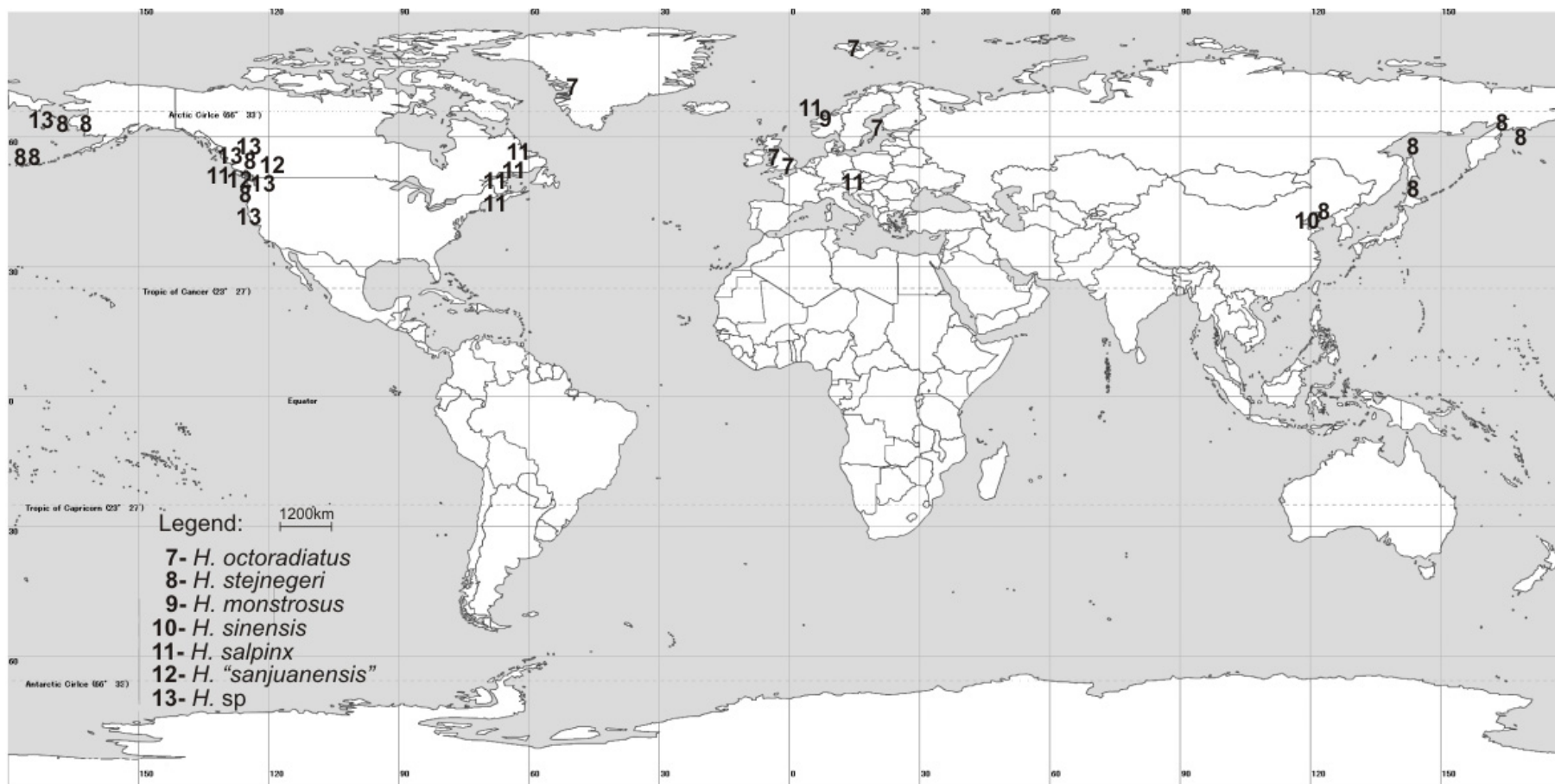
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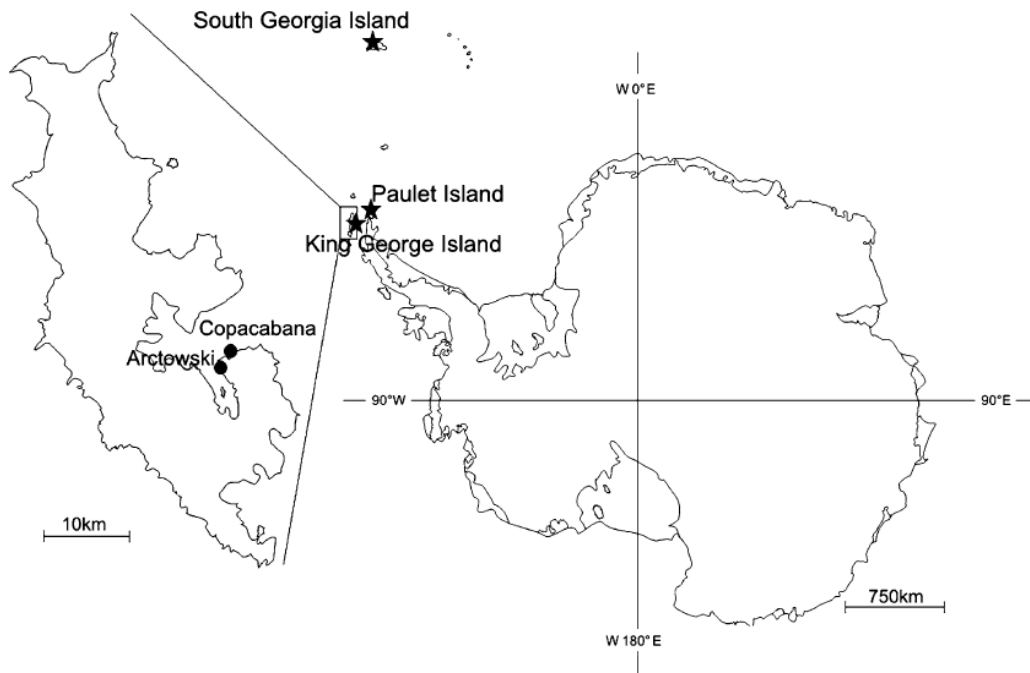
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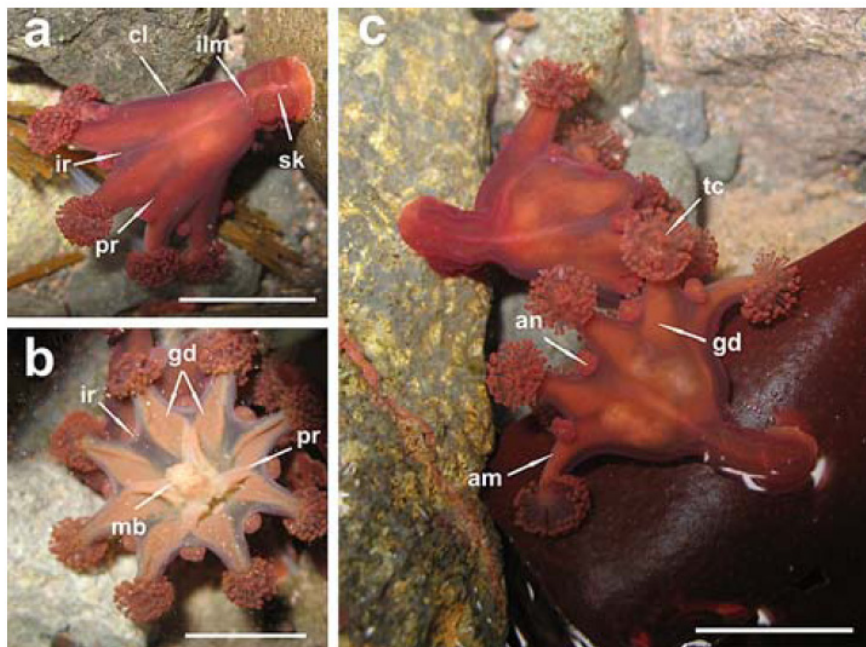
**Fig. 1** Global distribution of selected species of the genus *Haliclystus* (part 1). *H. antarcticus*, *H. auricula* (unspecific locality for Greenland coast), *H. kerguelensis*, *H. borealis*, *H. tenuis* and *H. "californiensis"* (Pfeffer, 1889; Kishinouye, 1910; Mayer, 1910; Uchida, 1929; Carlgren, 1930; Kramp, 1961; Amor, 1962; Corbin, 1979; Hirano, 1986, 1997; Mianzan, 1989; Davenport, 1998; Hansson, 1998; Grohmann et al., 1999; Zagal, 2004a, 2004b, 2008; Mills & Larson, 2007; and Smithsonian online database (<http://nhbacsmith2.si.edu/emuwebizweb/pages/nmnh/iz/Query.php>. Accessed 03 Jun 2008) *H. antarcticus* highlighted with a star were originally misidentified as *H. auricula*, see text.



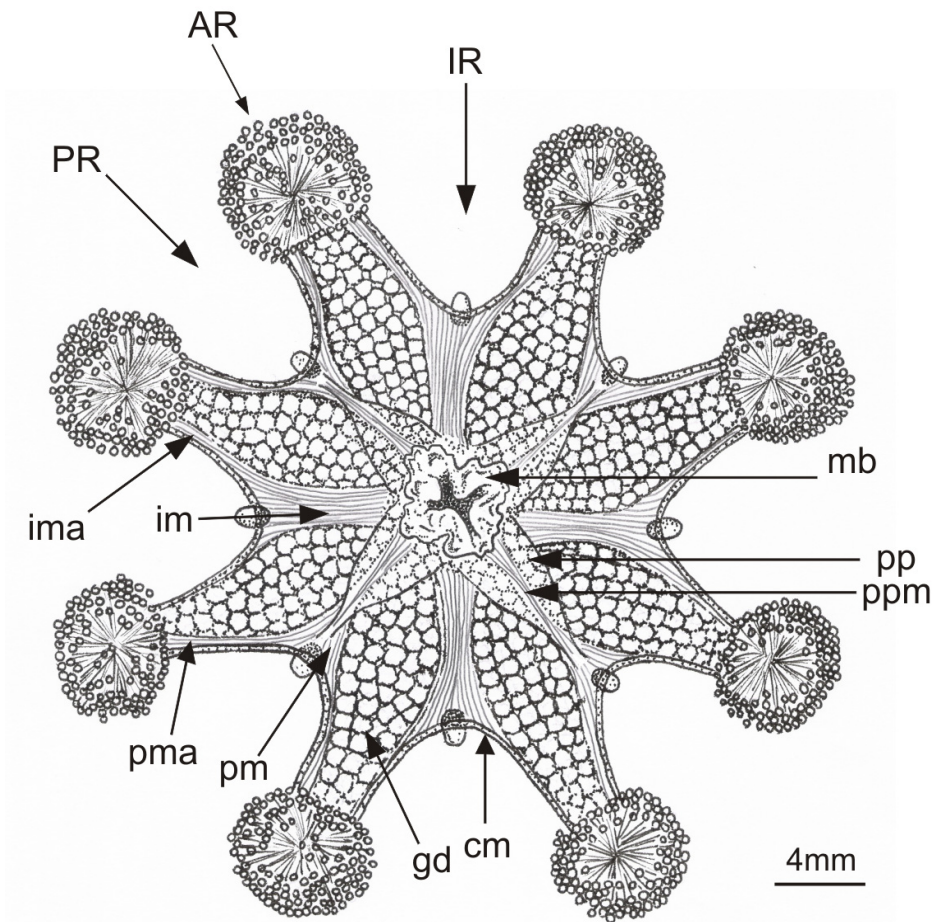
**Fig. 2** Global distribution of selected species of the genus *Haliclystus* (part 2). *H. octoradiatus* (unspecific locality for Greenland coast), *H. stejnegeri*, *H. monstrosus*, *H. sinensis*, *H. salpinx*, *H. "sanjuanensis"* and *Haliclystus* sp. (Browne, 1895; Mayer, 1910; Uchida, 1929; Ling, 1937; Hirano, 1986; Kramp, 1961; Otto, 1976; Hansson, 1998; and Smithsonian online database (<http://nhbacsmit2.si.edu/emuwebizweb/pages/nmnh/iz/Query.php>. Accessed 03 Jun 2008).



**Fig. 3** Map of Antarctica, highlighting the South Georgia Island, type locality of *Haliclystus antarcticus*, Paulet Island, and King George Island, new record for the species. Detail of King George Island, evidencing the collection points of *H. antarcticus*, Polish Arctowski Station and the US Copacabana Refuge.



**Fig. 4** Living specimens of *Haliclystus antarcticus* in the field **a** Specimen in side view with contracted stalk, attached to rock: *cl* calyx, *ilm* interradian longitudinal muscle, *ir* interradius, *pr* perradius, *sk* stalk; **b** specimen in oral view: *gd* gonads, *ir* interradius, *mb* manubrium, *pr* perradius; **c** specimens in side view attached to rock and algae (*Rhodophyta Iridaea cordata*): *am* arms, *an* anchors, *gd* gonads, *tc* tentacle cluster. Scale = 1.2 cm.

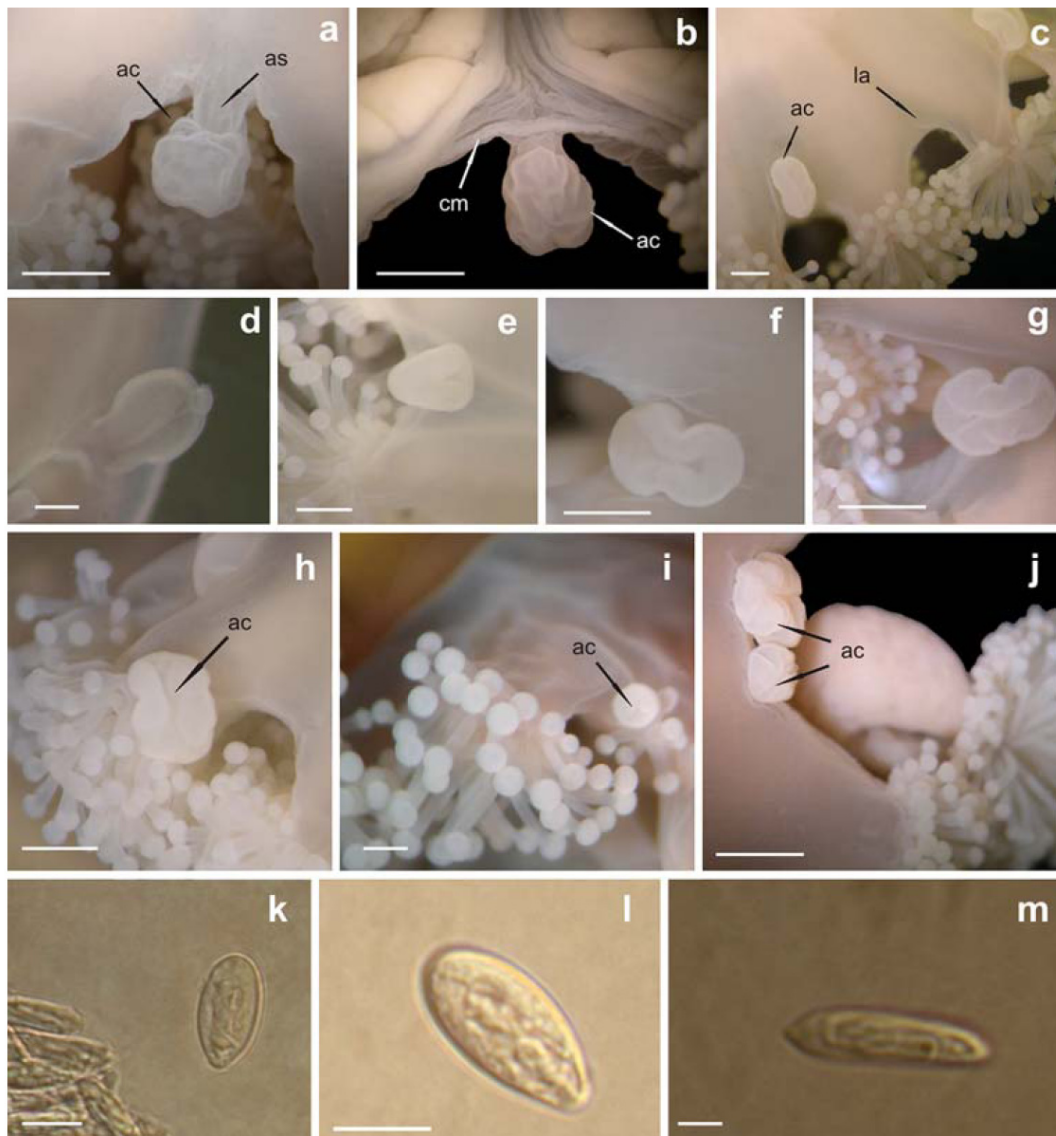


**Fig. 5** Representation of the oral view of *Haliclystus antarcticus*: *AR* adradius, *IR* interradius, *PR* perradius, *im* interradial muscle, *ima* interradial muscles extending to the tip of the arm, *pm* perradial muscle, *pma* perradial muscle extending to the tip of the arm, *gd* gonads, *cm* coronal muscle, *mb* manubrium, *pp* perradial pouches, *ppm* perradial pouches muscle.

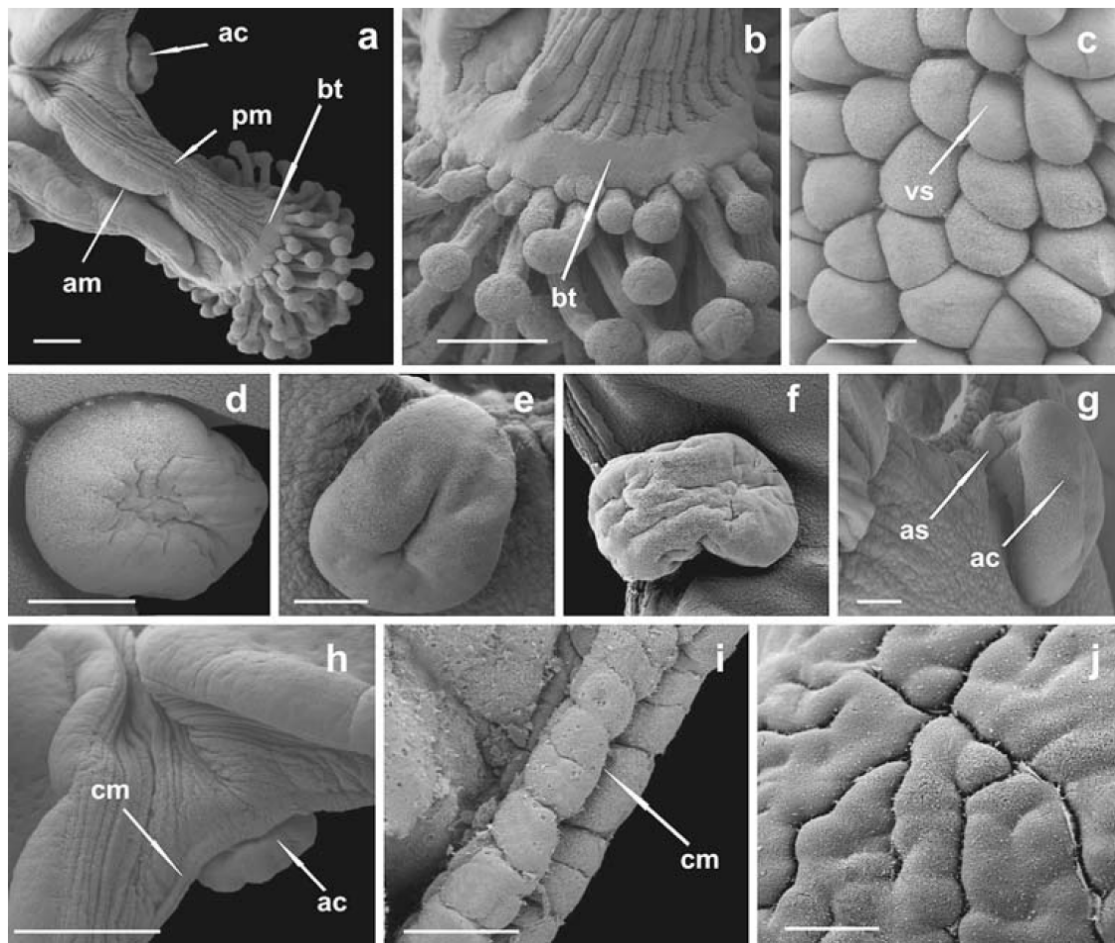




**Fig. 6** Morphological characters of preserved *Haliclystus antarcticus*. **a** *gd* gonads, *ir* interradius, *pr* perradius, *tc* tentacular cluster; **b** *am* arm, *gv* groove over about half of arm midline; **c** *ir* interradii, *mb* manubrium, *pp* perradial pouches; **d** *gc* gastric cirri, *gd* gonads, *pp* perradial pouches with gonads at inner wall; **e** *lp* frilled lips of the mouth, *mb* manubrium; **f** *st* manubrium striations; **g** *ac* anchor, *am* arm, *tc* tentacular cluster; **h** *lt* larger tentacles, *st* smaller tentacles; **i** *ge* distal globular end of tentacle, *hs* hollow stem of tentacle; **j** longitudinal section of arm, showing internal space with intertentacular lobules (*il*); **k** detail of intertentacular lobules (*il*); **l** gonads (*gd*) extending until the end of the arms; **m** *sk* stalk; **n** *ad* adhesive disc at the base of stalk; **o** individual with two stalks (*sk*). Scales: **a–j** 1.2 mm, **k** 1.0 mm, **l** 0.60 mm, and **m–o** 1.05 mm.



**Fig. 7** Details of anchor, tentacle cluster and nematocysts of preserved *Haliclystus antarcticus*. **a** *ac* anchor, *as* anchor stalk; **b** *ac* anchor, *cm* coronal muscle; **c** *ac* anchor, *la* lacking anchor; **d–g** anchor development: **d** anchor with a tentacular knob at young specimen (without gonads); **e** triangular anchor, with a shallow groove at basal region; **f** median ontogenetic stage cross constricted at central region, approximately “8”-shaped, forming a longitudinal groove at central region; **g** mature specimen, with wrinkled aspect; **h** anchor (*ac*) together with tentacular cluster; **i** abnormal anchor (*ac*) together with tentacular cluster; **j** specimen with two anchors (*ac*); **k** nematocysts; **l** microbasic heterotranchous; **m** isorhiza (atrachous or holotranchous). Scales **a–c**, **f–h**, **j** 2.3 mm, **d**, **i** 0.4 mm, **e** 1.0 mm, **k**, **l** 9.0  $\mu\text{m}$  and **m** 5.0  $\mu\text{m}$ .



**Fig. 8** Scanning electron micrographs of *Haliclystus antarcticus*. **a** *ac* anchor, *am* arm, *bt* rectangular internal space at the base of tentacular cluster, *pm* perradial muscles; **b** *bt* detail of rectangular internal space at the base of tentacular cluster; **c** *vs* gonadal vesicles; **d** circular anchor at young specimen (without gonads); **e** triangular anchor, with a shallow groove at basal region; **f** mature specimen, with wrinkled aspect; **g** *ac* anchor, *as* anchor stalk; **h** *ac* anchor, *cm* coronal muscle; **i** *cm* coronal muscle in detail; **j** base of stalk with adhesive disc of wrinkled aspect. *Scales* **a**, **b**, **f**, **h** 1.2 mm, **c** and **e** 0.6 mm, **d** 0.4 mm; **g** 0.2 mm; **i** 0.13 mm; and **j** 1.0 mm.

**Table 1** Comparisons of diagnostic characteristics of the species of *Haliclystus*.

Species	White spots of nematocysts		Location of white spot of nematocysts	Number of tentacles per cluster	Stalk	Shape of anchors	High of anchors x diameter of stalk	Rows of sacs per gonad	Internal base of tentacular cluster		References
	Present	Absent							"U-shaped", without intertentacular lobules	Shallow internal space, with intertentacular lobules	
<i>Haliclystus antarcticus</i>	-	X	-	6-224	1/2 to 2/3 as long as height of calyx	Oval	1/2 to 2/3 as wide as diameter of the stalk	6-12	-	X	current data
<i>Haliclystus auricula</i>	-	X	-	30-120	About as long as height of calyx	Oval	1/3 to 1/4 as wide as diameter of stalk	6-8	-	X	Rathke, 1806; Clark, 1863; Mayer, 1910; Gwilliam, 1956; Kramp, 1961; Hirano, 1997
<i>Haliclystus borealis</i>	X	-	Subumbrellar bell margin, in the perradii and interradii	20-30	Less than 1/3 as long as height of the calyx	Circular	2/3 as wide as diameter of the stalk	3-4	X	-	Uchida, 1933; Uchida & Hanaoka, 1934; Kramp, 1961; Hirano, 1986, 1997
<i>Haliclystus "californiensis"</i>	X	-	Subumbrellar bell margin in the perradii and interradii	60-70	4 to 5 times shorter than the calyx	Large cushion imperfectly divided in two	1/2 as wide as diameter of the stalk	3-4 abreast in the widest portion of the gonad	X	-	Gwilliam, 1956; Hirano, 1997
<i>Haliclystus kerguelensis</i>	?	?	?	Up to 50	Twice as long as height of calyx	Oval	1/3 as wide as diameter of the stalk	?	?	?	Vanhöffen, 1908; Mayer, 1910; Kramp, 1957, 1961
<i>Haliclystus monstrosus</i>	?	?	?	100	1/2 as long as height of the calyx	Trumpet shaped	2/3 to 1/2 as wide as diameter of the stalk	4-6	?	?	Naumov, 1961
<i>Haliclystus octoradiatus</i>	X	-	Subumbrellar bell margin in the interradii, and at the top of the gonads in the perradii	30-60	About as long as height of calyx	Oval	1/2 as wide as diameter of stalk	2	-	X	Lamarck, 1816; Clark, 1863; Browne, 1895; Mayer, 1910; Hirano, 1997
<i>Haliclystus salpinx</i>	X	-	Subumbrellar bell margin and along the perradial margin of the gonads	60-250	Longer than height of calyx	Trumpet shaped	About as long as wide as diameter of the stalk	4	X	-	Clark, 1863; Mayer, 1910; Gwilliam, 1956; Kramp, 1961, current observations
<i>Haliclystus "sanjuanensis"</i>	X	-	Perradially at the top of the gonads	Up to 130	2/3 as long as height of calyx	Oval	1/2 as wide as diameter of the stalk	10-22 irregularly arranged (not in rows), abreast in the widest portion of the gonad	-	X	Hyman, 1940; Hirano, 1997; Mills & Larson, 2007
<i>Haliclystus sinensis</i>	X	-	Subumbrellar bell margin	22	Half as long as height of calyx	Circular, wider than high	1/2 to 1/3 as wide as diameter of the stalk	1	?	?	Ling, 1937
<i>Haliclystus stejneri</i>	X	-	Subumbrellar bell margin, in perradii, and along the edge of the gonads	70-100	1/2 as long as height of the calyx	Oval	1/2 as wide as diameter of the stalk	6-8, irregularly arranged (not in rows), abreast in the widest portion of the gonad	-	X	Kishinouye, 1899; Mayer, 1910; Gwilliam, 1956; Kramp, 1961; Hirano, 1986, 1997
<i>Haliclystus tenuis</i>	X	-	Subumbrellar bell margin in the interradii and perradii, and along subumbrella in the perradii	35-45	1/2 as long as height of the calyx	Circular, slightly heart shaped	Diameter as long as that of the stalk	2	X	-	Kishinouye, 1910; Hirano, 1997

**Table 2** Measures (maximum, minimum and mean; in mm) of morphological characters of *Haliclystus antarcticus* from Antarctica (n=50), *Haliclystus* sp. from Chile (n=10) and *H. auricula* from Argentina (\*only mean, cf. Amor, 1962).

	<b>Calyx height</b>	<b>Calyx width</b>	<b>Anchors height</b>	<b>Anchors width</b>	<b>Arms length</b>	<b>Distance from distal end of arms to anchors</b>	<b>Stalk length</b>	<b>Base of stalk</b>
<i>Haliclystus antarcticus</i>	<b>3.5-16.2 (9.9)</b>	<b>4.1-23.4 (12.4)</b>	<b>0.3-3.1 (1.6)</b>	<b>0.4-2.7 (1.2)</b>	<b>0.3-6.0 (2.3)</b>	<b>0.3-6.6 (2.7)</b>	<b>2.3-10.7 (6.5)</b>	<b>1.5-8.0 (4.2)</b>
<i>Haliclystus</i> sp. (Chile)	4.1-7 (5.74)	6.9-13.8 (10.3)	0.7-1.5 (1.09)	0.6 -1.3 (0.83)	1.0-2.5 (1.69)	1.2-3.0 (1.95)	2.9-5.0 (3.63)	1.7-3.1 (2.57)
<i>Haliclystus auricula</i> (Argentina)*	(9.0)	(17.0)	(2.0)	(1.5)	?	?	(6.0)	(4.0)

## Capítulo 3

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### **Molecules clarify a cnidarian life cycle – The “hydrozoan” *Microhydrula limopsicola* is an early life stage of the staurozoan *Haliclystus antarcticus***

#### **ABSTRACT**

Life cycles of medusozoan cnidarians vary widely, and have been difficult to document, especially in the most recently proposed class Staurozoa. However, molecular data can be a useful tool to elucidate medusozoan life cycles by tying together different life history stages. Genetic data from fast-evolving molecular markers (mitochondrial 16S, nuclear ITS1, and nuclear ITS2) show that animals that were presumed to be a hydrozoan, *Microhydrula limopsicola* (Limnomedusae, Microhydrulidae), are actually an early stage of the life cycle of the staurozoan *Haliclystus antarcticus* (Stauromedusae, Lucernariidae), expanding our understanding of the staurozoan life cycle, which was thought to be more straightforward and simple. A synthetic discussion of prior observations makes sense of the morphological, histological and behavioral similarities/congruence between Microhydrulidae and Stauromedusae. The consequences are likely to be replicated in other medusozoan groups.

#### **RESUMO**

Os ciclos de vida dos cnidários medusozoários variam amplamente, e são difíceis de documentar, especialmente na classe Staurozoa, recentemente proposta. Entretanto,

dados moleculares podem ser uma ferramenta útil para elucidar ciclos de vida por unir seus diferentes estágios. Marcadores moleculares de evolução rápida (mitocondrial 16S, e nucleares ITS1 e ITS2) mostram que animais previamente classificados como um hidrozóário, *Microhydrula limopsicola* (Limnomedusae, Microhydrulidae), são na verdade um estágio do ciclo de vida da estauromedusa *Haliclystus antarcticus* (Stauromedusae, Lucernariidae), expandindo nosso conhecimento sobre o ciclo de vida de Staurozoa, o qual se pensava ser mais direto e simples. Observações acerca de similaridades/congruências morfológicas, histológicas e comportamentais entre Microhydrulidae e Stauromedusae corroboram os resultados moleculares. As consequências deste estudo provavelmente podem ser replicadas em outros grupos de Medusozoa.

## INTRODUCTION

Medusozoan (i.e., non-Anthozoan cnidarians) life cycles are highly complex and diverse, with combinations of planulae, benthic polyps (occasionally planktonic), creeping frustules, and/or pelagic medusae (occasionally benthic). As with other organisms displaying complex life cycles, documenting all the life history stages in medusozoan species is an enormous challenge. The usual approach has been to attempt to rear species through their various life stages in the laboratory. However, each life stage is adapted for different and often unknown conditions, making the task difficult, time consuming, and in many cases so far, impossible. Because the genome is the same in different life history stages of any given species, molecular data provide another tool that can help elucidate medusozoan life cycles by tying together different life stages.

While there is great variation in medusozoan life cycles, there exist some broad-scale patterns of congruence between life cycle differences and the origins of major medusozoan taxa (Collins, 2002; Marques & Collins, 2004), suggesting that evolutionary changes in life cycle have sometimes corresponded to the establishment of distinct lineages. One of the most intriguing findings from these phylogenetic studies has been the hypothesis that the Stauromedusae (so-called stalked jellyfishes) form an early-diverging medusozoan clade that is separate from Scyphozoa (Coronatae and Discomedusae), within which Stauromedusae was traditionally classified (Marques & Collins, 2004; Collins & Daly, 2005; Collins et al., 2006; van Iten et al., 2006). Because of its distinct origin and some putatively unique life history characteristics, Marques & Collins (2004) established the class Staurozoa and noted that the finding raises important issues about the evolution of cnidarian development and life cycles.

The present view holds that the life cycle of staurozoans is relatively simple, consisting of a planula larva that attaches to the substrate and grows into a primary polyp, which subsequently undergoes an apical transformation into the adult form. Because the transformation to adult takes place without fission or budding, this development results in a mosaic individual, in which the structures of the oral part are similar to those of an adult medusa (particularly scyphozoans and cubozoans), whereas the basal part retains characteristics of the sessile polyp (Wietrzykowski, 1912; Kikinger & Salvini-Plawen, 1995). However, knowledge of staurozoan development is based on a handful of observations on a small number of species. Studies about juvenile stauropolyp development include only *Haliclystus octoradiatus* (Lamarck, 1816) (Wietrzykowski, 1910, 1912) and two species of



*Stylocoronella*, *S. riedli* Salvini-Plawen, 1966 and *S. variabilis* Salvini-Plawen, 1987 (Salvini-Plawen, 1987; Kikinger & Salvini-Plawen, 1995). Polyps of these latter species are interstitial and it is unknown whether or not this psammic condition is common in the group.

Based on analyses of nuclear genes coding for the small and large subunits of the ribosome (SSU or 18S and LSU or 28S, respectively), Collins et al. (2008) suggested that the diminutive polyp form of the Antarctic species *Microhydrula limopsicola*, originally described by Jarms & Tiemann (1996) in the class Hydrozoa (Trachylina, Limnomedusae), could be an unknown life stage of a species of Stauromedusae. The hypothesis was immersed in a broader analysis of the phylogeny and evolution of Trachylina, and many issues remain unattended: (1) To which staurozoan species should *M. limopsicola* be synonymized? (2) Which stage of the stauromedusan life cycle does it represent? (3) How can its morphology be interpreted in relation to what is known about staurozoans? and (4) What are the consequences of this unknown stage for our understanding of the biology and biogeography of staurozoans? The goal of this study is to address these questions, bringing new molecular and morphological evidence to this conundrum.

## **MATERIALS AND METHODS**

We were provided with a few live polyps of *Microhydrula limopsicola* from a culture maintained by Gerhard Jarms at the Universität Hamburg. The culture, which has been maintained since December, 1991, derived from the original (and unique) sampling of this species on the shells of five specimens, 3-4 mm bivalves *Limopsis hirtella* at 31 m

deep in firm mud near the Argentine Antarctic Station “Jubany” (King George Island, South Shetland Islands 62°13.979’S 58°41.812’W; Fig. 1, Table 1) (Jarms & Tiemann, 1996; Collins et al., 2008). Specimens of *Haliclystus antarcticus* Pfeffer, 1889 from Antarctica (Fig. 2) were collected manually during low tide (tide prediction between 0.2 and 0.4 m) on two beaches in the Admiralty Bay, King George Island, Antarctic Peninsula: (A) Pieter Lenie, Copacabana, North American Refuge, 62°10’S, 58°26’W; and (B) Shag Point, Arctowski, Polish Station, 62°10’S, 58°31’W (Fig. 1, Table 1) and initially preserved in 80% ethanol (Miranda et al., 2009). Specimens of the Chilean *Haliclystus antarcticus*, originally (mis)identified as *Haliclystus auricula* (Rathke, 1806) (Miranda et al., 2009), came from Los Molinos beach, Valdivia, southern Chile (39°47’S 73°20’W; Fig. 1, Table 1), and were collected during low tide by C.J. Zagal (Zagal, 2004a, 2004b, 2008) and J.P. Didier (Miranda et al., 2009). Tissue samples from the tentacle clusters were then dissected and preserved in pure ethanol stored at -20°C.

Sequences included in our analysis were derived for this study or have come from GenBank (Table 1). Fast evolving molecular markers (mitochondrial 16S, nuclear ITS1, and nuclear ITS2) were targeted for analysis. The markers were already adopted and proved to be efficient for the species level identification in Medusozoans (16S- Miglietta et al., 2007; Galea & LeClère, 2007; Miglietta & Lessios, 2009; ITS- Dawson, 2004). DNA extraction was carried out with InstaGene (Bio-Rad). Genes were amplified using PCR, then purified with AMPure® (Agencourt®). PCR primers were CB1 (forward) and CB2 (reverse) (Cunningham & Buss, 1993) for mitochondrial DNA 16S; JFITS1-5f (forward) (Dawson & Jacobs, 2001) and CAS28SB1d (reverse) (Ji et al., 2003) for nuclear ribosomal DNA (ITS1 and ITS2). DNA

sequencing was made using the BigDye® Terminator v3.1 kit (Applied Biosystems) and the same primers for PCR, except by the use of ITS1-R (reverse) (Schroth et al., 2002) for ITS1 and ITS2. The procedure was carried out on an ABI PRISM®3100 genetic analyzer (Hitachi). Samples of *M. limopsicola* and *H. antarcticus* were extracted and amplified at different times and at different laboratories (NMNH, USA and USP, Brazil, respectively), without risk of contamination. To confirm molecular data, the sequences of *M. limopsicola* (based on an independent DNA extraction from a second sampling of the original culture) and *H. antarcticus* (based on 10 individuals for each locality – King George Island, Antarctica and Valdivia, Chile – Table 1) were repeated at the same laboratories. Sequences of *M. limopsicola* were included in an analysis with mitochondrial 16S and nuclear ITS1 and ITS2 sequences of *H. antarcticus* (from Antarctica and Chile), *Haliclystus “sanjuanensis”* (*nomen nudum*) (Mills & Larson, 2007), *Haliclystus stejnegeri* Kishinouye, 1899, *Haliclystus tenuis* Kishinouye, 1910, *Depastromorpha africana* Carlgren, 1935, and *Lucernaria janetae* Collins & Daly, 2005 as an outgroup for rooting the topology (Table 1).

Contig sequences were edited in SEQUENCHER™ 4.6 (Gene Codes Corporation), aligned using BioEdit© “ClustalW Multiple Alignment” (Hall, 1999), resulting in three alignments: (1) mitochondrial 16S sequences, (2) ITS1+ITS2 sequences, and (3) combined 16S+ITS1+ITS2 sequences. Uncorrected pairwise distances were calculated in Bioedit. Gaps were treated as missing data. Maximum Parsimony (MP) analyses were performed using branch and bound algorithm in PAUP 4.1 (Swofford, 2002). Maximum Likelihood (ML) analyses were performed using PALM (Phylogenetic Inference with Automatic Likelihood Model Selectors) (Chen et al., 2009). The most appropriate model for each of

the datasets was chosen by employing the Akaike information criterion (AIC). The model 'GTR+I+G' was applied to 16S, 'TIMeF' to ITS1 and ITS2, and 'GTR+G' to combined data (16S+ITS1+ITS2). Branch support was estimated by bootstrapping (Felsenstein, 1985) with 1000 replicates for the MP (PAUP 4.1) and ML (PALM) analyses.

## RESULTS

The MP and ML topologies are identical for 16S and combined data. The ML topology for ITS1+ITS2 is congruent with MP topology, however less resolved (Fig. 3). The cladograms show that *M. limopsicola* from Antarctica falls within *H. antarcticus*, and in fact, has no differences from *H. antarcticus* of Antarctica. *Haliclystus antarcticus* from Chile forms a clade with these specimens and is only slightly diverged from them.

All ten sequenced specimens of *H. antarcticus* from Antarctica possess a unique haplotype for the three markers, and this is identical (for 16S, ITS1 and ITS2 - Table 2) to the haplotype found for *M. limopsicola* and slightly different (distance of 0.40-0.77%, depending on the marker - Table 2) from the haplotype of the *H. antarcticus* from Chile. Similar to *H. antarcticus* from Antarctica, the population from Chile (n=10) has no genetic variation for the studied molecular markers, presenting a unique haplotype (Tables 1 and 2). Nucleotide differences among 16S, ITS1 and ITS2 sequences from other species of Stauromedusae (*H. "sanjuanensis"*, *H. stejneri*, *H. tenuis*, *D. africana* and *L. janetae*) are higher (3.75-33.78% for 16S; 1.22-50.28% for ITS1; and 0.94-61.15% for ITS2; Table 2 - phylograms in Fig. 3). Species of stauromedusae, for which we have more than one haplotype (*H. antarcticus*, *H. "sanjuanensis"* and *H. stejneri*) show that the intraspecific

difference is between 0.19%-0.77% for 16S, 0.00%-0.40% for ITS1 and 0.00%-0.47% for ITS2 (Table 3).

## DISCUSSION

### *Microhydrula limopsicola* is *Haliclystus antarcticus*

Collins et al. (2008) did not formally establish a synonym for *Microhydrula limopsicola*. However, a very close relationship of *M. limopsicola* with *H. octoradiatus* and also with “the species of *Haliclystus* from southern Chile reported on by Zagal (2004)” was noted, although the latter was not formally included in their analysis (Collins et al., 2008:9]. Our analyses are obviously constrained by the non-availability of other cultures or samples of *M. limopsicola*, which as far as we know has only been observed by Jarms & Tiemann (1996), but our results are based on the most complete data available at this time. With increased taxon sampling and data from fast-evolving markers, we conclude that *M. limopsicola* is actually *H. antarcticus*. Several points support this conclusion.

First, we document 100% identity of three fast-evolving markers of *Microhydrula limopsicola* with those of *H. antarcticus* from Antarctica, all of which differ slightly from conspecific samples of *H. antarcticus* from Chile. While intraspecific variation of these genetic markers is not very well known for species of stauromedusae, available data indicate that some intraspecific genetic variation exists, and that it is smaller than observed interspecific variation (compare Table 3 with Table 2). This would suggest that identity in these genetic markers can only happen if the samples are taken from the same species. Finally, one might question whether there are other Antarctic species of

*Haliclystus* that could confound our identification of *Microhydrula limopsicola* as *H. antarcticus*. There is one additional species known from the southern hemisphere, *Haliclystus kerguelensis* Vanhöffen, 1908 from Kerguelen Island, southern Indian Ocean, but this species is readily differentiated from *H. antarcticus* by its morphology (Miranda et al., 2009) and thus should have a different genetic signature; no specimens of *H. kerguelensis* suitable for genetic study were available to us.

With the remainder of the discussion, we synthesize the relevant historical literature to address the implications of our identification of *M. limopsicola* as *H. antarcticus* on taxonomy, morphology, and life history of Stauromedusae.

#### Taxonomy

The implications of this synonymy for the family Microhydrulidae were only briefly touched upon by Collins et al. (2008). The family Microhydrulidae Bouillon & Deroux, 1967 (Hydrozoa, Limnomedusae) encompassed three species in two genera: *Microhydrula pontica* Valkanov, 1965, *M. limopsicola* and *Rhaptapagis cantacuzenei* Bouillon & Deroux, 1967. One is now clearly established as a stauromedusa. It remains to be explicitly tested whether *M. pontica* and *R. cantacuzenei* are also early stages of the life cycle of local species of Stauromedusae, but we think this is likely to be the case, since similarities between these species and the preserved larvae of stauromedusae have been previously recorded (Bouillon & Deroux, 1967). We note that *M. pontica* and *R. cantacuzenei* have been found in abundance living in marine surface biofilms at the Station Biologique in Roscoff, France, on the English Channel, in the same vicinity where several species of

Staurozoa including *Haliclystus auricula*, *H. octoradiatus*, *Depastrum cyanthiforme* (M. Sars, 1846), *Lucernariopsis campanulata* (Lamouroux, 1815) and *Craterolophus convolvulus* (Johnston, 1835) (Wietrzykowski, 1912; Kramp, 1961; Bouillon & Deroux, 1967; Hartog, 1976) have also been found. Of course, additional data, particularly genetic data, from other species of Microhydrulidae are necessary to test our hypothesis that these species represent stages in the development of species of Stauromedusae.

### Morphology

Medusozoan plesiomorphies (e.g., primitive widespread cnidarian characters such as the presence of microbasic euryteles nematocysts), homoplasies (e.g., presence of convergent morphological characters such as periderm and life history characters such as asexual frustules), and the very simple morphology (small solitary hydroids without tentacles and sexual stage (Bouillon & Deroux, 1967; Jarms & Tiemann, 1996)) of *M. limopsicola* evidently worked as obstacles in correctly identifying *M. limopsicola* when it was discovered. As a result, *M. limopsicola* bears a closely resemblance to the very simple hydropolyps such as those of *Craspedacusta* and *Monobrachium*. No doubt the scarce literature on early stages of staurozoans made it difficult to establish reliable comparisons among taxa. Even though dissimilarities are evident (Collins et al., 2008), there are several morphological similarities between *M. limopsicola* and Staurozoa.

First, the hemispherical shape of the settled planulae of *Haliclystus octoradiatus* (Wietrzykowski, 1912:43, Figure XXIII) is similar to the general shape of Microhydrulidae “polyps” (Jarms & Tiemann, 1996:11, Figure 1b) (Fig. 4). Moreover, both lack mouth and a

permanent gastrovascular cavity necessitating intracellular digestion (Wietrzykowski, 1912; Bouillon & Deroux, 1967; Jarms & Tiemann, 1996). A further similarity is the production of frustules (Wietrzykowski, 1912; Jarms & Tiemann, 1996). More specifically, the settled, rounded up planula of *H. octoradiatus* produces lateral protuberances, which become buds for the process of frustulation (Wietrzykowski, 1912), similar to the “young polyps of *M. limopsicola*”, which can also produce frustules (Jarms & Tiemann, 1996) (Fig. 4). Likewise, the frustules of Microhydrulidae are also formed from a lateral budding of the body (Bouillon & Deroux, 1967:254). The planulae of *H. octoradiatus* can also have small pronounced expansions, more or less regular, not related to the process of frustulation. These protuberances, which are provided with numerous nematocysts, result from a local thickening of the ectoderm and seem to play an important role in prey capture prior to the development of tentacles (Wietrzykowski, 1912:29). Homology between the protuberances, found in *H. octoradiatus* planulae larvae (Wietrzykowski, 1912) and the similar morphology in *M. limopsicola* is not clear. One possible interpretation is that these expansions correspond to the cauliflower structure bearing numerous nematocysts found at one end of “elder” individuals of *M. limopsicola* (Jarms & Tiemann, 1996). However, the cauliflower structure can also be a simple result of a strong aggregation of larvae (see below) (Wietrzykowski, 1912) since at a more advanced stage, the larva of *H. octoradiatus* increases considerably in size, and its contour, previously more or less rounded, develop four lobules (Wietrzykowski, 1910) in a similar arrangement to the cauliflower structure (Fig. 4). Another interpretation is that the development of *H. antarcticus* differs from that of *H. octoradiatus*, with the cauliflower



structure corresponding to the early development of the primary tentacles. Recently metamorphosed individuals of *Haliclystus borealis* Uchida, 1933, *H. stejnegeri*, and *H. auricula*, as well as their juvenile medusae stages, have eight primary tentacles (Hirano, 1986:184, 187, 189, Figures 2, 4, 6), which might be correlated to these cauliflower structures.

Histological similarities between *M. limopsicola* and *H. antarcticus* are also of note. The “polyps” of *M. limopsicola* are attached to the substratum with a slightly widened base, whose ectoderm produces a flat thin fibrous periderm plate (Jarms & Tiemann, 1996, Figure 2). Stalks of adult individuals of the genus *Haliclystus* also present fibrillar components at the attachment sites (Lesh-Laurie & Suchy, 1991). In addition, the endoderm of *M. limopsicola* and of the settled planula of *H. octoradiatus* is composed by vacuolar cylindrical cells, which touch at the terminal end, without leaving any space (Wietrzykowski, 1912:43; Jarms & Tiemann, 1996:113).

One feature remarked by Jarms & Tiemann (1996) and Collins et al. (2008) is the cnidome, with microbasic euryteles being present in *M. limopsicola*. This nematocyst type is a common feature of *H. antarcticus* (Carlgren, 1930; Miranda et al., 2009), but microbasic euryteles are plesiomorphic for Staurozoa (Uchida, 1929; Weill, 1934; Larson, 1980, 1988; Calder, 1983; Hirano, 1986; Larson & Fautin, 1989; Collins & Daly, 2005) and not particularly useful for staurozoan taxonomy, besides its ubiquitous presence in other medusozoan groups (see data matrix in Marques & Collins, 2004). Adults of *H. antarcticus* also possess isorhizas (Carlgren, 1930; Miranda et al., 2009), which were not recorded for *M. limopsicola* (Jarms & Tiemann, 1996). Accepting the identity of *M. limopsicola* with *H.*

*antarcticus* suggests that the cnidome of this species varies ontogenetically (Calder, 1971, 1973, 1983). Indeed, nematocysts of the creeping planula larvae of *Haliclystus salpinx* Clark, 1863 are different from the adults of the same species (Otto, 1978). The planula larval stage of *H. salpinx* also has only microbasic euryteles, whereas the adults have both microbasic euryteles and isorhizas (Otto, 1978).

#### Life cycle inference

Based on our finding that *M. limopsicola* is synonymous with *H. antarcticus* we propose that *M. limopsicola* is an early life cycle stage of *H. antarcticus* (Fig. 5). Thus far, early stages of the life cycle of *H. antarcticus* have never been recorded. In fact, few staurozoan pre-adult stages are known. Only the creeping benthic planula stage of *H. "sanjuanensis"* (misidentified as *H. stejnegeri*) and *H. salpinx* (Otto, 1976, 1978), the post-metamorphosis stages of some stauromedusae (Hirano, 1986) and the complete development of *H. octoradiatus* and *S. riedli* (Wietrzykowski, 1910, 1912; Kikinger & Salvini-Plawen, 1995) have been documented. Therefore, it is presently impossible to assert on the universality of this "microhydrula" stage in all species of staurozoans. However, as well as unique frustules stages found in other stauromedusae (Wietrzykowski, 1912), a hidden diversity of life cycle stages and strategies in Staurozoa may be in the study of sediments or in associations with overlooked substrata (e.g., bivalves), instead the present focus on the well known settlement of stauromedusae on rocks and macrophytes.

The non-sexually-reproductive “microhydrula” stage most likely occurs before the development of the stauropolyp and its apical metamorphosis into an adult medusa in the life cycle. We hypothesize that this stage occurs right after the settlement of the planula larvae (or sometime after the settlement of the frustule), adding a stage (Fig. 5) to the hypothetical life cycle proposed for staurozoans, since the best known life cycle of a staurozoan, the odd psammic *Stylocoronella* spp., apparently does not contain a “microhydrula” stage (Kikinger & Salvini-Plawen, 1995, Figure 13). Settlement of planulae occurs in groups of 3-20 individuals in *H. octoradiatus* (Wietrzykowski, 1912). Experimental procedures restricting larval aggregation of *H. octoradiatus* to groups of 1-3 larvae demonstrated that none of these larvae were successful in undertaking further development, probably due to not being able to capture sufficient food when growing in small aggregations (Wietrzykowski, 1912). The same gregarious behavior has been described for “adults” of Microhydrulidae, also hypothesized to enhance efficiency in prey capture (Bouillon & Deroux, 1967; Jarms & Tiemann, 1996) (Fig. 4). Furthermore, similar to planula settlement in *H. octoradiatus*, the frustules of Microhydrulidae attach to the substrate by adhesion of a portion of the surface lacking nematocysts (Wietrzykowski, 1912; Bouillon & Deroux, 1967; Jarms & Tiemann, 1996).

It is important to note, however, that the juvenile stauropolyps of *S. riedli* and *S. variabilis* can produce frustules by budding of the long filiform tentacles (Kikinger & Salvini-Plawen, 1995). Such a polyp has not been described for any other staurozoan. Asexual reproduction via frustulation found in the “microhydrula” stage (and possibly also in the polyp, since cnidarians demonstrate different kinds of budding (Boero et al., 2002;

Morandini et al., 2009)) would increase the potential of large populations in isolated areas of a larger fragmented seascape, which is consistent with the patchy distribution of *H. antarcticus*, for example. In fact, 130 specimens of *H. antarcticus* were found in an area of ca. 150m<sup>2</sup> in Copacabana, King George Island, Antarctica (Miranda et al., 2009), and ca. 385 individuals/m<sup>2</sup> in Valdivia, Chile (Zagal, 2004b). Further, intense asexual reproduction would lead to low genetic diversity. This is consistent with our finding of just single 16S, ITS1 and ITS2 haplotypes (Table 2) in each sampled *H. antarcticus* population (Antarctica and Chile).

Ecological constraints of the “microhydrula” stage may restrict the distribution of *H. antarcticus*. *Microhydrula limopsicola* was described living attached to the upper valve of the very small, subtidal lamellibranch bivalve *Limopsis hirtella* (Rochebrune & Mabile, 1889) on King George Island (Jarms & Tiemann, 1996). Jarms & Tiemann (1996:115) suggested that the association between the clam *Limopsis hirtella* and the hydroid *Microhydrula limopsicola* is to be regarded as “highly specific”. The authors have kept *M. limopsicola* alive on glass for nearly 20 years and, to our knowledge, no development other than asexual frustulation has been observed. Since *M. limopsicola* can be kept alive in the lab, the association between *M. limopsicola* and *L. hirtella* may be regarded as a coincidence of co-distribution. However, the absence of further development in the lab leads us to believe that *L. hirtella* provides vital cues enabling further development of *M. limopsicola*. Such relationships among epibiont and host are not uncommon in marine ecosystems (Puce et al., 2005). *Limopsis hirtella* is spread across the Magellanic Province, the Falkland Islands and the western part of the Antarctic (Mühlenhardt-Siegel, 1989).

Coincidentally, this is the area where *H. antarcticus* is recorded: Antarctica Peninsula (Pfeffer, 1889; Carlgren, 1930; Davenport, 1998; Miranda et al., 2009) and southern South America (Mianzan, 1989; Zagal, 2004a, 2004b, 2008; Miranda et al., 2009) (Fig. 1).

## CONCLUSIONS

Documenting medusozoan life cycles is an enormous challenge. In this work we show that molecular data can be a useful tool to identify an unknown life cycle stage by tying together different life history stages with the same haplotype and from it derive a hypothesis about the life history of the species. Similarity between the haplotypes of three markers of *Microhydrula limopsicola* and *Haliclystus antarcticus* settles the identity of these taxa, expanding our understanding of the staurozoan life cycle, which was thought to be more straightforward and simple. Frustration was recorded for *Haliclystus* for the second time, in a different life stage from that was recorded for *Stylocoronella*. This knowledge sheds light on morphological, biogeographical, and evolutionary issues, mainly because *Haliclystus* is the most diverse genus in Staurozoa. However molecular analysis will not replace additional investigations. Continued exploration of the meiofauna and integrated analysis encompassing morphology, ecology, molecules, life cycles and biology will be needed to solve outstanding evolutionary and biogeographical questions like those addressed here.

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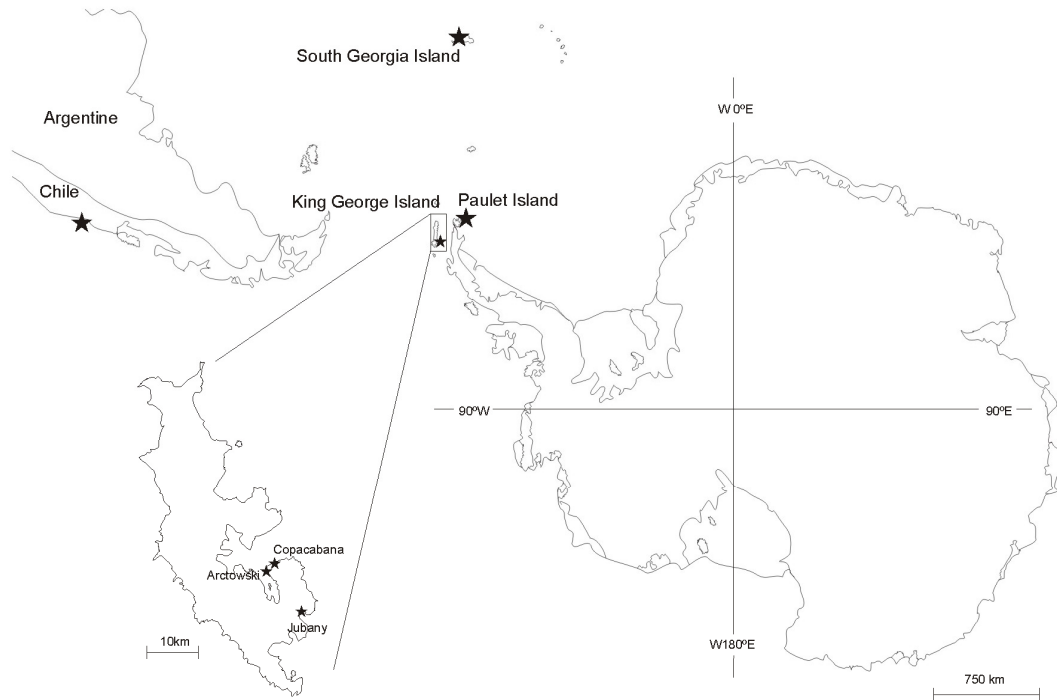
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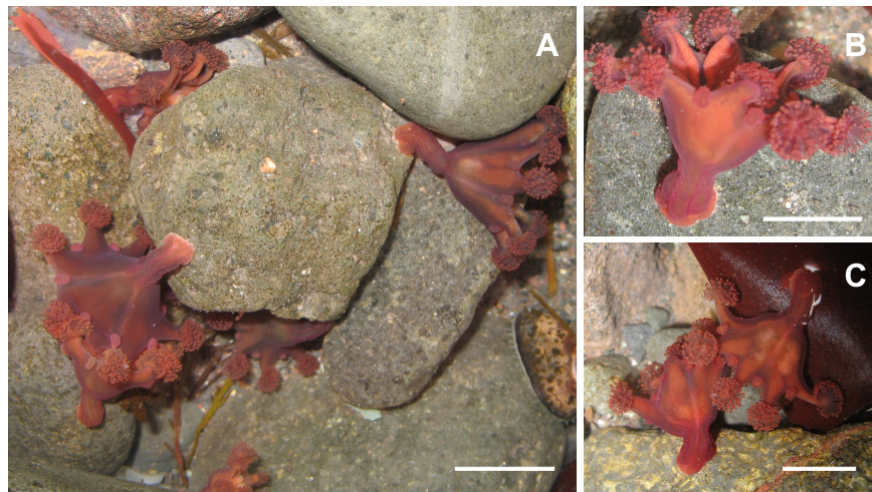
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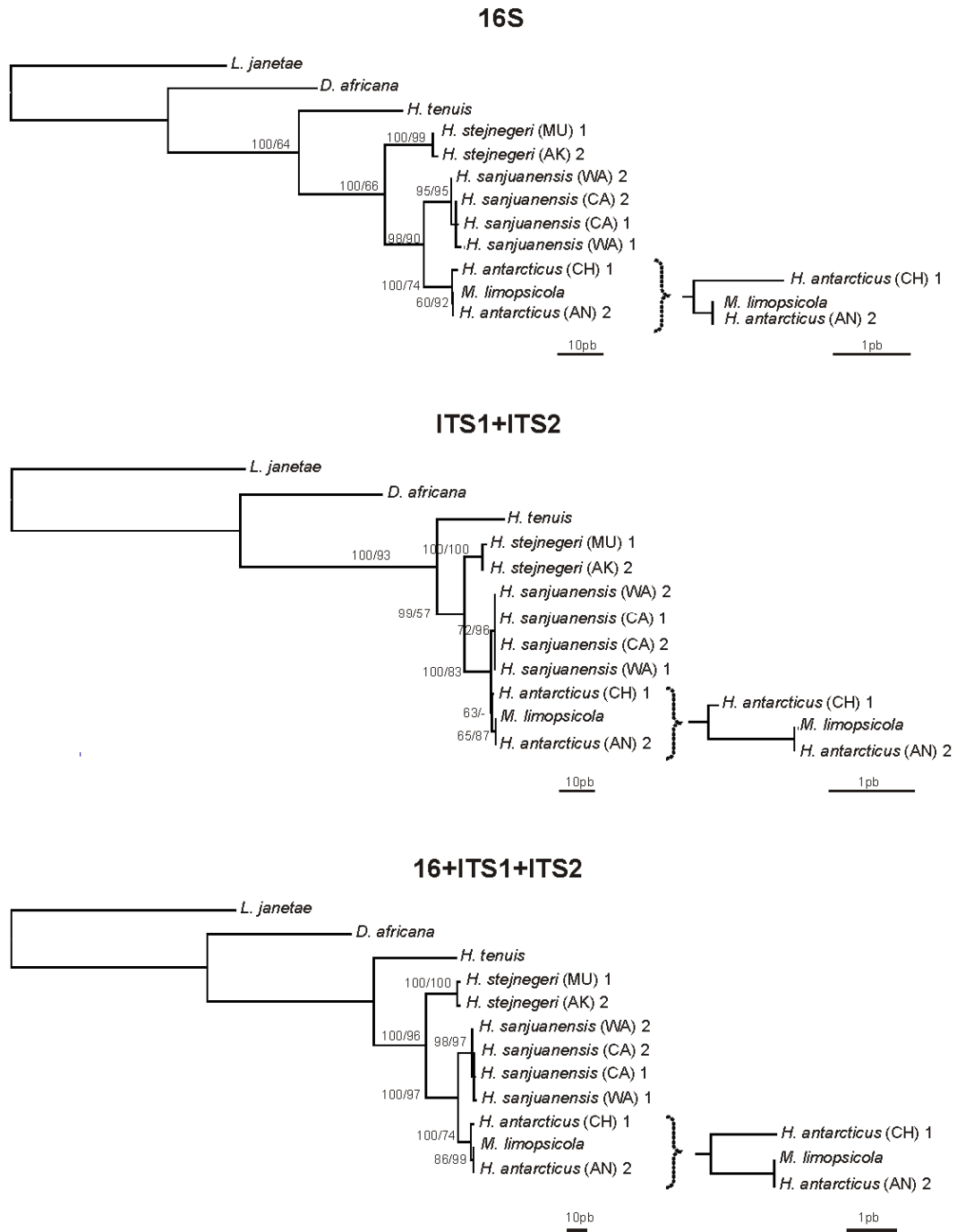
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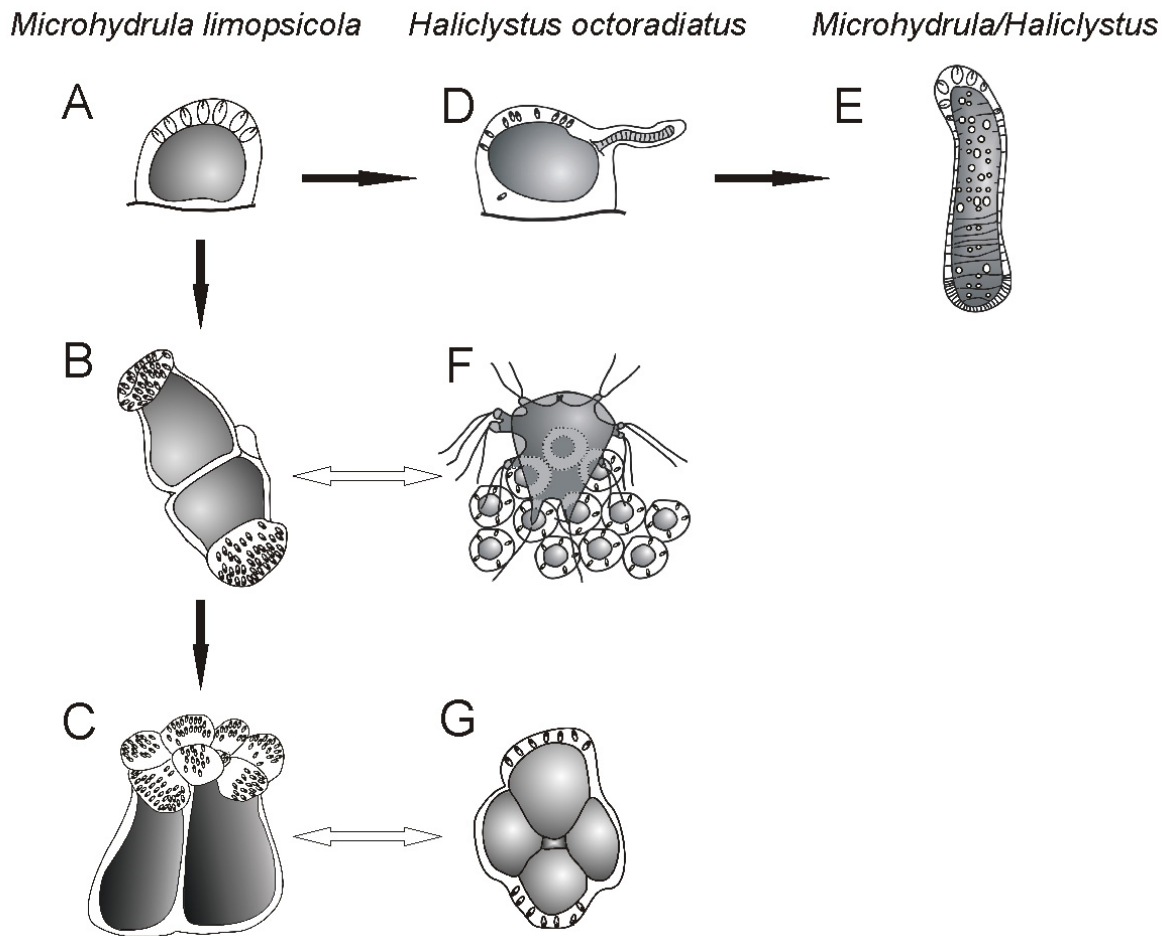
**Fig. 1** Map of Antarctica and southernmost part of Chile. Stars are records of *Haliclystus antarcticus*: South Georgia Island, Paulet Island, King George Island (Polish “Arctowski” Station, US “Copacabana” Refuge and Argentinean Antarctic Station “Jubany”) and Chile (Valdivia).



**Fig. 2** Living specimens of *Haliclystus antarcticus* in the field. **A and B** Side view, attached to rock; **C** Side view attached to rock and algae (*Rhodophyta Iridaea cordata*). Pictures from Morandini, AC. Scale= 1.2 cm.



**Fig. 3** Phylogenetic hypothesis (MP) based on mitochondrial 16S, nuclear ITS1+ITS2 and combined data. AN (King George Island, Antarctica), AK (Akkeshi, Hokkaido, Japan), CA (Franklin Point, California, USA), CH (Valdivia, Chile), MU (Muroran, Hokkaido, Japan), WA (San Juan Island, Washington, USA). "1" and "2" refers to the different haplotypes found for each species. Bootstrap indices under both MP and ML (respectively) at each node. Topologies are congruent under MP and ML analysis.



**Fig. 4** Comparisons between the *M. limopsicola* polyp and the *H. octoradiatus* settled planula. **A-C** different stages of *M. limopsicola* (Jarms & Tiemann, 1996): **A** newly settled “polyp”; **B** closely attached “polyps”, with expansions provided with nematocysts; **C** later stage, with a cauliflower-shaped head. **D-E** process of frustulation observed in both species: **D** planula of *H. octoradiatus* producing lateral protuberances, which become frustules (Wietrzykowski, 1912); **E** frustules (Jarms & Tiemann, 1996). **F-G** possible correspondences of stages of both species: **F** a group of *H. octoradiatus* larvae, capturing a nauplius (Wietrzykowski, 1912); **G** superior view of a settled planula of *H. octoradiatus* at an advanced stage, showing four lobes (Wietrzykowski, 1910). The hemispherical shape and the production of frustules (**A**, **D**, **E**) are similar in settled planulae of *H. octoradiatus* and “polyps” of *M. limopsicola*. The same gregarious behavior to feeding was observed in both species (**B**, **F**). At a more advanced stage, the larva of *H. octoradiatus* presents four lobes (**G**), that might be associated with the cauliflower structure seen in later stages of *M. limopsicola* (**C**), which possibly is an aggregation of more than one individual. Figures modified from Wietrzykowski (1910, 1912) and Jarms & Tiemann (1996).



**Table 1** Localities, GenBank codes (\*sequences produced in this study) and number of specimens used in molecular analysis for each species and for each molecular marker.

Species	Locality	GenBank code		Number of specimens	Voucher
		16S	ITS1+ITS2		
<i>H. antarcticus</i>	Copacabana and Arctowski Sta (King George Island, Antarctica)	FJ874775*	FJ858787*	10	MZUSP 1558
<i>H. antarcticus</i>	Los Molinos (Valdivia, Chile)	submitted*	FJ874777*	10	MZUSP 1560
" <i>M. limopsicola</i> "	Jubany Sta (King George Island, Antarctica)	EU294003	FJ874779*	1	G. Jarms culture
<i>H. "sanjuanensis"</i>	San Juan Island (Washington, USA)	submitted*	submitted*	1	USNM 1106935
		AY845339*	submitted*	1	USNM 1073340
<i>H. "sanjuanensis"</i>	Franklin Point (California, USA)	submitted*	FJ874776*	3	USNM 1106653
		submitted*	submitted*	1	USNM 1073341
<i>H. stejnegeri</i>	Muroran (Hokkaido, Japan)	submitted*	submitted*	1	USNM 1106655
<i>H. stejnegeri</i>	Akkeshi (Hokkaido, Japan)	submitted*	submitted*	1	KUNHM 002673-B
<i>H. tenuis</i>	Muroran (Hokkaido, Japan)	submitted*	submitted*	1	USNM 1106651
<i>D. africana</i>	False Bay (South Africa)	AY845341	submitted*	1	none
<i>L. janetae</i>	East Pacific Rise (8°36.745N, 104°12.740W)	AY845342	FJ874778*	1	FMNH 10329



**Table 2** DNA distance matrix between *Haliclystus antarcticus* from Antarctica and: “*Microhydrula limopsicola*”, *Haliclystus antarcticus* from Chile, *Haliclystus “sanjuanensis”*, *Haliclystus stejnegeri*, *Haliclystus tenuis*, *Depastromorpha africana* (family Depastridae) and *Lucernaria janetae* (family Lucernariidae).

	<i>H. antarcticus</i> (King George Island, Antarctica)		
	16S	ITS1	ITS2
<b>“<i>M. limopsicola</i>”</b> <b>(King George Island,</b> <b>Antarctica)</b>	0.00%	0.00%	0.00%
<b><i>H. antarcticus</i></b> <b>(Valdivia, Chile)</b>	0.77%	0.40%	0.47%
<b><i>H. “sanjuanensis”</i></b> <b>(San Juan Island,</b> <b>Washington)</b> <b>haplotype 1</b>	3.75%	1.22%	0.94%
<b><i>H. “sanjuanensis”</i></b> <b>(San Juan Island,</b> <b>Washington)</b> <b>haplotype 2</b>	3.75%	1.22%	0.94%
<b><i>H. “sanjuanensis”</i></b> <b>(Franklin Point,</b> <b>California)</b> <b>haplotype 1</b>	3.75%	1.22%	0.94%
<b><i>H. “sanjuanensis”</i></b> <b>(Franklin Point,</b> <b>California)</b> <b>haplotype 2</b>	3.96%	1.22%	1.41%
<b><i>H. stejnegeri</i></b> <b>(Muroran, Japan)</b> <b>haplotype 1</b>	7.16%	2.05%	6.00%
<b><i>H. stejnegeri</i></b> <b>(Akkeshi, Japan)</b> <b>haplotype 2</b>	7.37%	2.47%	6.00%
<b><i>H. tenuis</i></b>	16.41%	7.95%	13.35%
<b><i>D. africana</i></b>	22.06%	20.74%	26.24%
<b><i>L. janetae</i></b>	33.78%	50.38%	61.15%

**Table 3** Intraspecific variation for three species of Staurozoa in 16S, ITS1 and ITS2, highlighting the number of specimens, the number of haplotypes found and the range of divergence of each molecular marker; the linear distance refers to the distance between populations.

Species	<i>H. antarcticus</i>	<i>H. "sanjuanensis"</i>	<i>H. stejneri</i>
Locality	King George Island (Antarctica) and Valdivia (Chile)	Washington and California (Pacific Coast, USA)	Akkeshi and Muroran (Hokkaido, Japan)
Linear distance	2,700 km	1,270 km	330 km
Population	2	2	2
Specimens	20	6	2
<b>16S</b> Haplotypes	2	4	2
Divergence	0.77%	0.19-0.39%	0.19%
Specimens	20	4	2
<b>ITS1</b> Haplotypes	2	1	2
Divergence	0.40%	-	0.40%
Specimens	20	4	2
<b>ITS2</b> Haplotypes	2	2	1
Divergence	0.47%	0.46%	-

# Capítulo 4

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## **Histological anatomy of *Haliclystus antarcticus* (Cnidaria, Staurozoa) with a comparative discussion of staurozoan morphology**

### **ABSTRACT**

Stauromedusae present relatively few macromorphological characters useful for their taxonomy, making their identification a difficult task. For this reason, internal anatomy and histological characters are additionally employed to characterize species. The aim of this study is to describe the histomorphology of *Haliclystus antarcticus* Pfeffer, 1889, presenting new data for the species and addressing some evolutionary, ecological, and taxonomical issues. The organization of the body of *H. antarcticus* was used in a comparative analysis of medusozoan morphology to better understand the early evolution in the history of Medusozoa. This histological investigation provides a framework for similar studies of other species, and allows for an investigation of relationships and morphological evolution within Staurozoa. We conclude that in spite of stauromedusae having a combination of polyp and medusa characteristics, the last common ancestor of Staurozoa cannot be assumed to be either a degenerated medusa or a polyp with interrupted development at a certain stage of the life cycle.

### **RESUMO**

Estauromedusas possuem relativamente poucos caracteres macromorfológicos úteis para sua taxonomia, tornando sua identificação uma tarefa difícil. Por essa razão, a anatomia interna e alguns caracteres histológicos são adicionalmente

empregados para caracterizar as espécies. O objetivo desse estudo é descrever a histomorfologia de *Haliclystus antarcticus* Pfeffer, 1889, apresentando novos dados para a espécie e abordar algumas questões evolutivas, ecológicas e taxonômicas do grupo. A organização do corpo de *H. antarcticus* foi utilizada em uma análise comparativa com a morfologia de Medusozoa para melhor entender a sua evolução. Essa investigação histológica provê uma base para estudos similares com outras espécies, e permite uma investigação das relações e da evolução morfológica dentro de Staurozoa. Nós concluímos que apesar das estauromedusas terem uma combinação de características de pólipos e medusa, o último ancestral comum de Staurozoa não pode ser considerado nem uma medusa degenerada, nem um pólipos com o desenvolvimento interrompido em certo estágio do ciclo de vida.

## INTRODUCTION

Staurozoa is the most recently proposed class for the phylum Cnidaria (Marques & Collins, 2004). Species of this clade do not produce pelagic medusae, a synapomorphy of the clade Scyphozoa+Cubozoa+Hydrozoa (Collins et al., 2006; van Iken et al., 2006). Instead, the so-called stauropolyp apically metamorphoses into an adult stauromedusa, which lives attached to the substrate by a peduncle (Mayer, 1910). The apical half of the metamorphosed stauromedusa bears similar characters to those present in adult medusae of scyphozoans and cubozoans, e.g. hollow structures of tentacular origin (rhopalioids/rhopalia), circular coronal muscles and gonads. The aboral region, on the other hand, retains polypoid characters such as gastric septa and four longitudinal muscles associated with the four peristomal pits surrounding the mouth (Collins, 2002; Stangl et al., 2002). Therefore, the particular life cycle of the

stauromedusae is tightly connected to their unique anatomy, in which polypoid and medusoid characters are expressed in a single individual (Collins et al., 2006), making detailed knowledge of this group essential for the correct understanding of the evolution of the life cycles in Cnidaria in general.

Stauromedusae present relatively few macromorphological characters useful for taxonomy (Hirano, 1997). Besides, these characters exhibit significant intraspecific variation (Miranda et al., 2009), making identifications of staurozoan species a difficult task. For this reason, internal/histological characters are additionally employed to characterize species. For instance, the presence of four intramesogleal muscles of the peduncle (e.g., present in *Haliclystus*, but absent in *Kishinouyea*; Uchida, 1929), the number (one or four) of chambers in the peduncle (e.g. four chambers in *Haliclystus* and one in *Lucernaria*; Kramp, 1961), and the presence and anatomy of the claustrum (a tissue that transversely divides the four gastric pockets; Collins & Daly, 2005) are used to distinguish different species, or different higher taxa within Staurozoa.

Although the internal anatomy is essential for the taxonomy of the Staurozoa and helpful for understanding of its interrelationships with other Medusozoa, histological accounts for species of staurozoans are still rare. A few studies employ general histological characters (e.g., sections of the peduncle) in the taxonomy of the class (e.g., Larson, 1980, 1988; Hirano, 1986; Larson & Fautin, 1989), but there are examples in which even the general characters adopted were misunderstood or misinterpreted (e.g., the number of chambers in the peduncle of *Kishinouyea hawaiiensis*, actually one chamber, subdivided into four, and not four chambers as described by Edmondson, 1930). On the other hand, there are some detailed histological descriptions (Clark, 1878; Wietrzykowski, 1912; Uchida, 1929; Uchida &

Hanaoka, 1933, 1934; Ling, 1939). Nevertheless, efforts to understand body plan evolution of stauromedusae through comparative analysis with different stages of the life cycle of the other medusozoan classes are practically nonexistent (Berrill, 1963; Thiel, 1966).

Miranda et al. (2009) redescribed *Haliclystus antarcticus* Pfeffer, 1889, providing data on the intraspecific variation of some characters used in taxonomy of the genus *Haliclystus*. The aim of the present study is to augment this description with new data on the histomorphology of *H. antarcticus*. These data are also used in a comparative analysis to better understand morphological evolution early in the history of Medusozoa, as well as within Staurozoa. To do this, the organization of the body of *H. antarcticus* is compared with general body plans of polyps and medusae of the other medusozoan classes, as well as to other representatives within Staurozoa, in light of a working hypothesis of phylogenetic relationships.

## **MATERIALS AND METHODS**

Specimens of *Haliclystus antarcticus* were collected manually during low tide on two beaches in Admiralty Bay, King George Island, Antarctic Peninsula: (1) Pieter Lenie, in Copacabana (North American Refuge, 62°10'S, 58°26'W) and (2) Shag Point, in Arctowski (Polish Station, 62°10'S, 58°31'W), during the XXV Brazilian Antarctic Program (February-March 2007). Materials were fixed directly in 4% formaldehyde solution with seawater. Routine histological procedures for cnidarians were carried out (Appendix I, II and III; modified from Mahoney, 1966; Marques, 2001) for nine individuals of different sizes, both for transverse and longitudinal sections (10 µm thick), stained with acid fuchsin, aniline blue - chromotrope 2R (Masson trichrome

stain) and acetic aniline blue (Mallory). The body plan of *H. antarcticus* was studied and photographed under microscope. The main terminology of morphological features followed Uchida (1929) and Uchida & Hanaoka (1934). The slides will be deposited in the collection of the Lab of Medusozoa of the University of São Paulo.

## RESULTS

### General body anatomy (Fig. 1)

Aboral region formed by pedal disc with increased surface area by having many invaginations (Fig. 2A). Peduncle with four perradial chambers (= gastric canals), alternating with four strong interradial longitudinal muscle bands embedded in mesoglea (Figs. 1A, B; 3A), circular in cross section; chambers and muscles developed throughout peduncle except at pedal disc. Perradial chambers with some invaginations basally, mainly at central region, becoming more oval and smooth towards oral region; perradial chambers fusing at junction of peduncle and calyx (Fig. 1C), connecting with gastrovascular cavity by a median aperture. Gastrodermis envelops muscle bands at basal region of calyx, forming four interradial septa (= gastric septa) (Figs. 1D; 4A). Each muscle band compressed medially, due to one small interradial aperture surrounded by epidermis (infundibulum or peristomal pit) (Figs. 1E,F; 5A; B). All four infundibula funnel-shaped with blind end (Fig. 5A), deeply developed up to base of calyx, widening orally, apertures located at subumbrella. Septal gastrodermis fuse forming four perradial regions dividing gastrovascular cavity, representing basalmost part of manubrium and perradial pockets (i.e., central part of gastrodermis of each septum join originating four-sided manubrial gastrodermis; lateral part of adjacent septa join forming perradial pockets) (Fig. 1H). Similarly, infundibular epidermis also

progressively fuse: central part of each infundibular epidermis originates manubrial epidermis; epidermis of adjacent infundibula form epidermis of perradial pockets (Fig. 1I). Four perradial gastric pockets laterally separated from each other by septa; each perradial pocket connecting with gastrovascular cavity by median aperture; manubrium extending above this aperture (Fig. 1J).

Gastrovascular cavity without claustrum. Each perradial pocket formed by fusion of gastrodermis and epidermis of adjacent septa, manubrium formed by fusion of all four septa. Each perradial pocket extending throughout calyx margin, subsequently continuing into two adradial arms and respective tentacular clusters. Subumbrellar epidermis marginally merges with exumbrellar epidermis, dividing perradial pockets at origin of arms (Fig. 1K-N). Gonads formed by evagination of gastrodermal layer of septa, perradial pockets and arms; gonads of same perradial pocket formed by gastrodermis of different interradial septa (two neighboring septa). Gastric filaments formed by evagination of gastrodermal layer of septa (Fig. 1G-N).

Anchors (or rhopalioids), such as tentacles, hollow, formed by evagination of body surface (Figs. 6, 7). Eight large anchors organized as four perradial and four interradial, each anchor located between adjacent arms at calyx margin.

Large interradial muscles bundles located at septa between adjacent perradial pockets branch into smaller muscles bundles, going towards arms and respective tentacles. Eight groups of coronal muscles present at calyx margin, each between adjacent arms (Fig. 8).



**Cell and tissue description (Figs. 2-13)**

PEDAL DISC AND PEDUNCLE - Aborally, epidermis of pedal disc comprising high and thick epithelial cells, characterized by glandular cells highly granulated, stained with fuchsin; vacuolated glandular cells and supporting cells less common (Fig. 2A-D). Still at peduncle, although more orally, epidermis thinner and with sinuous surface because of differentiated size of its cells; supporting cells become more conspicuous, vacuolated and granulated glandular cells rarer, few nematocysts present (Fig. 3).

Gastrodermis of peduncle chambers mainly comprising vacuolated cells (external, juxtaposed to mesoglea) and granulated glandular cells (juxtaposed to chamber). Granulated glandular cells organized in two layers defining two different natures: one intermediate layer densely stained with fuchsin, one internal (closer to chamber) stained with aniline blue (Fig. 3C, G). Gastrodermis almost uniform throughout peduncle, but thinner at aboral end of peduncle at level in which four chambers are not fully developed (Fig. 2E, F), where they contain only a few granulated and vacuolated cells. Interradial longitudinal musculature composed by myoepithelial cells stained with fuchsin, interspersed by thin layers of mesoglea stained blue. Pack of myoepithelial muscle fibers with reticulated appearance because of presence of empty spaces among them (Fig. 3C, F).

Mesoglea strongly colored at central portion of peduncle (Fig. 3A), peripheral region divided into two main layers: one strongly stained region, dense, located internally close to perradial chambers, one loose weakly stained with aniline blue region, more external. Another mesogleal layer strongly stained juxtaposed to epidermis only visible in higher magnification because it becomes indistinct with epidermis itself (Fig. 3B).

CALYX AND INFUNDIBULA- epidermis of aboral region of calyx similar to that of upper region of peduncle, although the number of nematocysts progressively increases (Fig. 4C). Septal mesoglea strongly stained in blue, respective gastrodermis quite vacuolated, muscles embedded in septa surrounded by layer of dense mesoglea (Fig. 4A, B). Septal mesoglea becomes thinner orally. Epidermis of infundibula (similar to subumbrellar epidermis) divided into two main regions: lateral side, facing perradii, composed by thicker cells including many granulated glandular cells and nematocysts; interradiial side facing muscles and central region comprised by thinner cells. Gastrodermis of infundibula of similar thickness, therefore gastrodermis comparatively thinner or thicker than epidermis at infundibula. Thin layer of mesoglea between the gastrodermis and epidermis of infundibula (Fig. 5).

GASTRIC FILAMENTS - Interradiial gastric filaments apparently elliptical in cross sections, formed by gastrodermal evagination, with internal mesoglea (Fig. 9A-C). Nematocysts present, as well as glandular cells with well stained granules (Fig. 9D, E).

GONADS - formed as gastrodermal evagination at lateral regions of interradiial septa, therefore younger ones located near interradii and older ones in perradii. Thin layer of mesoglea separates outer gastrodermis from inner gonadal content (Fig. 10).

MANUBRIUM AND PERRADIAL POCKETS - manubrium internally composed by gastrodermis, externally by epidermis (Fig. 11A). Manubrial gastrodermis thick, continuous and similar to that of central region of septa, but joint region at perradii with taller granulated glandular cells (Fig. 11B-D). Manubrial epidermis thin, continuous and similar to that of infundibula. Perradiial pockets formed by lateral regions of septa (Fig. 10D), whose gastrodermis forms gonads, and by thick and granular epidermis, including sparsely distributed nematocysts.

ANCHORS - epidermis formed by tall supporting cells, additionally with vesiculated glandular cells. Nematocysts not present. Mesogleal layer between epidermis and gastrodermis thinner at apex of anchor, relatively thicker on lateral and basal regions. Gastrodermis thick with many dense stained granules (Fig. 7).

ARMS - thick and granulated subumbrellar epidermis, similar and continuous to lateral epidermis of infundibula, thinner exumbrellar epidermis with numerous supporting cells. Gonads present inside arms, formed by gastrodermis continuous to that of perradial pockets. Distal end of arm with intertentacular lobes containing thin layer of mesoglea and gastrodermis intensely granulated (Fig. 12K, L).

SECONDARY TENTACLES - secondary hollow tentacles composed by two parts, knob and stem (Figs. 12, 13). At stem base, tentacles tightly joined, separated only by thin layer of mesoglea, with a beehive appearance in cross section (Fig. 12G, H, I). Each tentacle with loose inner layer of gastrodermis with highly vacuolated cells (Fig. 12I, J). Epidermis of stem interspersed by longitudinal muscles (Figs. 12J, 13H), stained with fuchsin, extending throughout tentacular stem in longitudinal sections (Fig. 13A, C-D), visible as close together dots in cross section at basal region of stem (Fig. 13F, G, H, I). Tentacular knob region with very tall epidermal cells (Fig. 12A-F), especially supporting and glandular cells, with numerous nematocysts (mainly isorhizae) on its apex (Fig. 12C, F); nematocysts strongly stained with fuchsin and aniline blue. Basal region of epidermis with the presence of vacuoles (Fig. 12F). Knob gastrodermis more densely stained than that of stem, containing many granules (Fig. 12E). Probable nematogenic region at tentacular base, with numerous nematocysts, not organized (Fig. 13B, I, J).

## DISCUSSION

### Working hypothesis of evolutionary relationships

Marques & Collins (2004), based on morphological and life history data, concluded that the traditional scyphozoan groups (Coronatae, Rhizostomeae, Semaestomeae, and Stauromedusae) do not constitute a monophyletic group, and suggested a new class, Staurozoa, encompassing Stauromedusae and an enigmatic fossil group Conulatae. Van Iten et al. (2006) reinvestigated the systematic position of fossil group Conulatae, concluding that they were sister group of Coronatae. Consequently Staurozoa would be a new class restricted only to Stauromedusae, which was found to be the earliest diverging lineage of Medusozoa. This phylogenetic position of Staurozoa as the sister group to all other Medusozoa was independently derived on the basis of mitochondrial 16S data (Collins & Daly, 2005) and nuclear ribosomal gene data (Collins et al., 2006) (Fig. 14). The synapomorphies pointed out for Medusozoa (Staurozoa+Scyphozoa+Cubozoa+Hydrozoa) were four intramesogleal muscles associated with four peristomal pits, transformation of primary polyp tentacles into hollow structures, gastric filaments and coronal muscles (Collins et al., 2006). According to the same analysis, the synapomorphies for Staurozoa are a non-ciliated creeping planula and complex ovaries with follicle cells. A pelagic medusa phase as adult is a characteristic present in the last common ancestor of the clade Scyphozoa+Cubozoa+Hydrozoa (Collins et al., 2006) (Fig. 14).

Discussions of the evolution of stauromedusae have largely focused on their relationship to other groups of Cnidaria, rather than on relationships among them (Collins & Daly, 2005). A preliminary analysis with relatively poor taxon sampling, however, began to clarify relationships within the group (Collins & Daly, 2005). The

main characters used as probable synapomorphies were the claustrum, the number of chambers in peduncle and the anchors (or rhopalioids). While limited by the lack of taxon sampling, this study demonstrates the need for a reassessment of suprageneric clades, since some of them are not monophyletic (Collins & Daly, 2005). This lack of correspondence between traditional taxa and initial molecular-based phylogenies is not too surprising, given that families and genera are not recognized by exclusive features (Daly et al., 2007). In the absence of a comprehensive phylogenetic analysis of Stauromedusae, we incorporate the results of Collins & Daly (2005), Collins et al. (2006) and van Iten et al. (2006) in our working hypothesis of Staurozoa and Medusozoa (Fig. 14), which we use to guide the comparative analysis provided below.

#### **Fine anatomy and the taxonomy of Staurozoa**

Some internal characters of Staurozoa like the presence of four intramesogleal muscles of the peduncle, the number (one or four) of chambers in the peduncle, and the presence and anatomy of the claustrum have been considered useful in the taxonomy of the group (Uchida, 1929; Kramp, 1961; Collins & Daly, 2005). The histo-anatomical study seems particularly valuable in this regard, especially because of the few macromorphological characters available for the identification of species (Hirano, 1997). However, the use of histo-morphological characters in the literature may be also misguided. For instance, Mayer (1910) pointed out that *Haliclystus* is closely related to *Lucernaria* because *H. antarcticus*, like *Lucernaria*, has one chamber in the peduncle. However, we (as well as Pfeffer, 1889 and Carlgren, 1930) observed that this remark is incorrect and, therefore, any relationship based on this character is spurious.

Besides of incorrect interpretations, general morphology proved to be inefficient in defining more inclusive groups. Recent molecular analyses (Collins & Daly, 2005) showed that, although genera definitions seems to be reasonable, traditional families and suborders are generally non-monophyletic (Daly et al., 2007). As far as we noticed, characters that might be helpful such as the peduncular chambers and muscles have not been applied in suprageneric classifications of Staurozoa (cf. Uchida, 1929; Mills, 2009). For instance, present classification (Mills, 2009) includes genera with one (*Lucernaria*) or four (*Haliclystus*) chambers in the peduncle in the same family (Lucernariidae; see Kramp, 1961), as well as peduncular muscles present (*Manania*, with four interradial longitudinal muscles) or not (*Craterolophus*) (Depastridae; see Uchida, 1929; Larson & Fautin, 1989).

Another character with taxonomical significance is the claustrum, that has played a fundamental role in the systematics of Stauromedusae. The group has long been divided into two primary groups, Cleistocarpida and Eleutherocarpida (Clark, 1863), on the basis of presence or absence of claustrum, respectively (Daly et al., 2007). Molecular data suggested that these structure evolved homoplastically in different groups, besides of have been lost in more than one occasion (Collins & Daly, 2005; Daly et al., 2007). Although the present molecular phylogeny is based on a restricted species sample, at this point it seems likely that the claustrum is not useful character for diagnosing subgroups within Staurozoa (Collins & Daly, 2005).

Histological characters may be useful to the taxonomy of Staurozoa, but reinterpretations, universality and literature checking have to be done to avoid more cumbersome taxonomy.

### **Body organization and comparison with other Medusozoa**

Staurozoa has particular features distinguishing them from the other classes of Cnidaria, and presumably placing them as the first diverging class among the Medusozoa (Marques & Collins, 2004; Collins et al., 2006; van Iten et al., 2006). However, some peculiar characters led the class to be previously considered either as an order of Scyphozoa, or with close affinities with Cubozoa (Uchida, 1929; Collins, 2002; Marques & Collins, 2004). We used histological details to shed light on gross morphology and, consequently, try to review and discuss some of these characteristics in a new context, as well as their implications in the evolution of life cycles and relationship of Staurozoa with other medusozoan classes.

Polyp.—The development of Staurozoa is poorly known, and only the planula stage of some species (Otto, 1976, 1978) and the complete development of *Haliclystus octoradiatus* and *Stylocoronella riedli* (Wietrzykowski, 1912; Kikinger & Salvini-Plawen, 1995) have been described. Young polyps of *H. octoradiatus* are radially symmetrical in the peduncle and calyx until the stage of four primary tentacles (Wietrzykowski, 1912), similar to radial hydropolyps. However, later stages have important modifications in their bodies, resulting in an appearance more similar to a scyphopolyp (Wietrzykowski, 1912).

Scyphozoan polyps exhibit tetramerous symmetry due to the presence of four gastric septa diving the gastric cavity, four gastric perradial pockets, and four intramesogleal interradial longitudinal muscle strands, associated to the infundibula, all common features to stauropolyps (and stauromedusae) (Thiel, 1966).

According to recent and more inclusive phylogeny, the ancestral medusozoan would have a similar polyp, with gastric septa and four longitudinal muscle fibers running through the mesoglea and connecting the infundibula to the base of the polyp, a symplesiomorphy independently lost in Cubozoa and Hydrozoa (Collins et al., 2006). In fact, polyps of cubozoan and hydrozoan are essentially radial with no signs of tetramery, which has led some authors to argue for a sister group relationship of these classes (e.g., Werner, 1973). Although cubopolyps does not have four gastric septa and interradial muscle fibers, they kept intramesogleal muscles (like Staurozoa) but never concentrated in four bands (Chapman, 1978).

The polyp features persist in the sedentary adult stauromedusae, though modified by medusoid characteristics (Thiel, 1966), but the region in which these polypoid traits are present is variable among Staurozoa. For instance, four gastric septa, four infundibula, and four gastric perradial pockets are still present in the calyx of *H. antarcticus*, but not in the peduncle, which only has four chambers and four intramesogleal longitudinal interradial muscles bands. However, *Lucernaria* was described with gastric septa in the peduncle (Berrill, 1963, Figure: 4C,G; Collins & Daly, 2005, Figure: 2A, B). Consequently, the general understanding that the peduncle region retains the polypoid characters such as gastric septa (Stangl et al., 2002) is not appropriate for all staurozoans and, therefore, the universality of this trait must be verified.

The peduncle of Staurozoa may contain characteristics not found in any other polyp of Cnidaria, like the four perradial chambers (which by the way are described as absent in *Lucernaria*; Kramp, 1961). These chambers begin to be formed after the 16 tentacle stage, and complete their arrangement later in the development



(Wietrzykowski, 1912), so they shall not be considered typical “polypoid” structures. Therefore, it seems reasonable to consider this characteristic as a synapomorphy of Staurozoa (cf. Collins & Daly, 2005).

**Ephyra.**— In the life cycle of Staurozoa, the polyp metamorphoses into a medusa, not presenting an ephyra stage (Wietrzykowski, 1912; Kikinger & Salvini-Plawen, 1995). Collins (2002) proposed that the stauromedusae are separated from other Scyphozoa because of the absence of the strobilation process (consequently, not producing ephyrae as well). The absence of strobilation and ephyrae, among other features, led some authors to the conclusion that Stauromedusae is closely related to Cubozoa (Uchida, 1929; Collins, 2002; Marques & Collins, 2004).

**Medusa.**— The oral region of the metamorphosed medusa bears many similarities with adult medusae of scyphozoans and cubozoans, e.g., hollow structures of tentacular origin (rhopalioids/rhopalia), circular coronal muscles, gonads, and gastric filaments (Collins et al., 2006).

**Claustrum.** The gastrovascular cavity of stauromedusae is divided by four interradial septa that separate a central gut from four perradial pockets (Thiel, 1966; Collins & Daly, 2005), similar to Cubozoa and Scyphozoa. Some Stauromedusae may also have a claustrum dividing the four perradial pockets (Collins & Daly, 2005), like in adults of Cubozoa (Uchida, 1929). However, the gonads of the Staurozoa with claustrum are in the mesogon, while in Cubozoa they are in the exogon (Berrill, 1963). The presence of a claustrum in Staurozoa and Cubozoa raised the hypothesis that this character was present in the ancestral medusozoan and subsequently independently lost in Hydrozoa

and Scyphozoa (Collins & Daly, 2005), although, as discussed, the claustrum may have different origins even in Staurozoa.

**Gastric filaments and gonads.** Gastric filaments of Staurozoa, Cubozoa and Scyphozoa are thought to be homologous, a structure probably lost in Hydrozoa (Collins & Daly, 2005). Filaments project from the gastrodermis into the gastric cavity (Thiel, 1966), similar to the gonads, also of gastrodermal origin (cf. Hydrozoa with gonads located at the epidermis). Despite this similarity, ultrastructural studies have shown that stauromedusan ovaries are unique. In Scyphozoa, the oocytes develop within the mesoglea, but in the Staurozoa the ovaries are more complex, with the young developing oocytes near periphery and mature eggs at the central lumen of spherical follicles, formed by the evagination of the gastrodermis (Clark, 1862:50; Eckelbarger, 1994). They do not develop within the mesoglea (like Scyphozoa), which forms a thin layer around the outer region of the ovarian follicle (Eckelbarger, 1994). These particularities of the ovaries were considered a distinguishable character of Staurozoa (Collins et al., 2006).

**Ocelli.** In Staurozoa, ocelli are only known for the polyp stage of the species *Stylocoronella riedli*. These ocelli have a different ultrastructure from all known types of photoreceptive cilia within Eumetazoa (Blumer et al., 1995). It is important to notice that the generality of ocelli among Staurozoa is disputable, as they have been studied for this single species.

**Infundibula.** In polyps, as well as in medusae of Staurozoa, Cubozoa and Scyphozoa, infundibula lie aside the manubrium in the interradial of the subumbrella. Specifically in *H. antarcticus*, the infundibula go along most of the calyx, together with the gastric septa, dividing the stomach cavity and finishing in a blind end. They open interradially

in calyx, at the subumbrella. In scyphomedusae, there are called genital funnels (or subgenital pits) appearing to be invaginations in the interradial of the subumbrella, but they do not extend into the gastric cavity. Besides, in the Semaestomeae and Rhizostomeae, the musculature is not connected to the peristomal pits (Thiel, 1966). According to Thiel (1966) whether these pits or subgenital cavities are related to the gonads/germ products or whether they have another function is not known, but they are considered to be homologous. In fact, the infundibula of adult stauromedusae have the same structure of that of Scyphozoa. However, differently from Staurozoa, the infundibula reduce during the development of the ephyra of Scyphozoa (Eggers & Jarms, 2007), no more dividing the gastric cavity of adult medusae, although they are still associated with gastric filaments and gonads (Thiel, 1966).

**Musculature.** In adults of Staurozoa, the musculature is similar to that of the polyp, with longitudinal interradial musculature in the peduncle, distally continuing into the tentacles. However, stauromedusae also present coronal musculature. In the medusae of Scyphozoa, the coronal muscle assumes the important role in the swimming activity, but all orders of Scyphozoa still have radial longitudinal musculature running perpendicularly to the coronal musculature (Thiel, 1966). In *H. antarcticus*, the longitudinal musculature also runs perpendicularly to the coronal musculature in the calyx. The coronal muscle is a synapomorphy of Medusozoa (Collins et al., 2006), probably lost in the ancestry of Hydrozoa.

**Nervous System.** Maybe because their sessile way of life, Staurozoa has a quite simple nervous system. Nerve cells are located on the tentacular tips and at the bases of the rhopalioids, as well as in Scyphozoa and Cubozoa, for which the nervous system is

located on tentacles and/or rhopalia. These arrangements seem to be homologous (Thiel, 1966).

**Marginal structures.** Tentacles, rhopalioids (anchors) and rhopalia are marginal structures. These structures are roughly presumed to be homologous in Staurozoa, Cubozoa, and Scyphozoa, because they are all of tentacular origins (Thiel, 1966), and they are located at the perradii and interradii. Ontogenetically, the polyp of *Haliclystus* first develop two primary perradial tentacles, then other two perradial tentacles arise, followed by the appearance of four interradial tentacles. With the development of more tentacles, there is the formation of a glandular region at the base of the primary interradial and perradial tentacles. These glandular regions develop, the primary tentacles reduce, thus forming the rhopalioids or anchors (Wietrzykowski, 1912). Indeed, in some specimens of *Haliclystus antarcticus* a small tentacle knob can be seen in the upper portion of the rhopalioid, as a remnant of the primary tentacle, which gradually retracts and disappears during development. In this sense, the homology of the primary tentacles between cubozoans, scyphozoans, and stauromedusans seems to be undisputable, a character not present in the Hydrozoa (Collins & Daly, 2005), in which primary polyp tentacles do not develop in more complex hollow structures (Collins et al., 2006). However, although rhopalia and rhopalioid have the same tentacular origin, the relationship between primary tentacles metamorphosing into rhopalioids in Stauromedusae and into the rhopalia in Cubozoa and Scyphozoa is not clear, because the development of this structures in Staurozoa is little known and variable. First, the eight perradial and interradial primary tentacles modified into rhopalioids during ontogeny are reported for species of only three genera, *Haliclystus*, *Stenoscyphus*, and *Manania*, (Kramp, 1961; Collins & Daly, 2005). Additionally, in some

species, such as *Kishinouyea corbini*, the primary tentacles are present in the juvenile stauromedusae, but they completely disappear along the development (Grohmann et al., 1999). Therefore, it is likely that anchors have been derived more than once within Stauromedusae and that their evolutionary origin (or origins) would be independent from that of cubozoan and scyphozoan rhopalia (Collins & Daly, 2005).

### **Functional aspects of cellular and tissue data**

The cellular composition and microscopic anatomy is directly related to the natural history and life cycle of the stauromedusa. General tissue and cellular organization of *Haliclystus antarcticus* is qualitatively similar to those described for its congeners (cf. Clark, 1878; Wietrzykowski, 1912; Uchida, 1929; Uchida & Hanaoka, 1933, 1934).

The numerous invaginations of the pedal disc as well as the presence of numerous non-vacuolated gland cells containing granules at the basal portion of the peduncle enhance attachment to the surface of the substrate, usually algae and rocks for *H. antarcticus* (cf. Miranda et al., 2009) and many other stauromedusae (Mayer, 1910; Uchida, 1929; Edmondson, 1930; Uchida & Hanaoka, 1934; Larson, 1980; Larson & Fautin, 1989). Indeed, adherence is even more difficult for those species living in the intertidal zone, where hydrodynamic stress from current and wave action is very high (Franc, 1994). Similar cellular content have been described for *Haliclystus* (Uchida, 1929), and detailed studies of the pedal disc of the genus characterized it as highly specialized for attachment, with adhesive, supportive and mucous cells, whose secretions may polymerize after being released and become fibrillar components at the region of contact with the substrate (Lesh-Laurie & Suchy, 1991).

The muscular system must be regarded as an important character for sessile animals such as Staurozoa (Uchida, 1929). The developed longitudinal musculature of *H. antarcticus* helps in the attachment of the medusa to loosely substrata such as the leaves of the macrophytes. Additionally, the contraction of the coronal musculature (responsible for the calyx movement) causes a reduction of the calyx opening. The contraction of the coronal muscles of the calyx is simultaneous with the contraction of the longitudinal muscles of the peduncle and arms, making the arms folding over the manubrium and keeping the animal tightly fixed to the substrate when disturbed. The combination of these movements considerably reduces the total volume of the animal, making its adherence in highly hydrodynamic habitats more efficient (pers. obs. of *H. antarcticus* in Valdivia, Chile). The myoepithelial cells longitudinally organized along the arms are also related to the feeding behavior, contracting to bring the food to the manubrium (pers. obs.).

As for most other cnidarians, the tentacles act in protecting the animal and capturing prey, with the urticant and adhesive cnidae, respectively. Touching the tentacles starts the feeding reaction, consisting of quick bending of one arm so that the tentacle cluster touches the manubrium (Hyman, 1940). The nematocysts and the glandular cells of gastrodermis, present in the gastric filaments and at the region of the manubrium, are also important to subdue the prey and help in digestion, releasing enzymes (Heeger & Möller, 1987).

Additionally tentacles also play a role in the locomotion of the medusa, adhering the oralmost region to the substrate, serving as a counterpart of the pedal disc. In this respect, tentacles work together with the anchors, glandular structures that also allow momentary adhesion to the substrate through the abundant adhesive

and supporting cells of their epithelium (Franc, 1994:662; Hyman, 1940). Not coincidentally, all adhesive regions of the body of a stauromedusa, viz. peduncle and pedal disc, tentacular knob and anchors, have the epidermis composed by tall cells, mainly with glandular and supporting functions.

### **Homologies and life cycle of Stauromedusae in a medusozoan context**

As discussed above, stauromedusae do not produce free living ephyrae or medusae by strobilation or lateral budding. The stauropolyps develop into a sessile medusa by metamorphosis, mainly at the apical region (Collins et al., 2006). This form of medusa appearance resembles the metamorphosis of Cubozoa, in which the entire polyp undergoes through an apical metamorphosis and becomes a medusa (Werner, 1973). This would contrast with the monodisc strobilation of Scyphozoa (cf. Stangl et al., 2002), because scyphopolyps remain intact after transversal fission of the medusa, and continue to propagate asexually.

Nevertheless, a second type of metamorphosis was described for the cubozoans, in which a regenerative remnant basal part of the polyp persists and may regenerate into fully developed polyps, in a process suggested to be related to the strobilation of Scyphozoa (Straehler-Pohl & Jarms, 2005). Indeed, scyphozoan transformation begins at the oral end and continues aborally (Calder, 1982; Kroiher et al., 2000; Stangl et al., 2002). Besides, there is a horizontal groove in both, strobilating Scyphozoa and metamorphosing Cubozoa, delimiting the transforming distal end of the polyps (Hofmann et al., 1978; Laska-Mehnert, 1985; Eggers, 2002; Stangl et al., 2002). These similarities (among others) would corroborate the hypothesis that the

metamorphosis of the Cubozoa is derived from the strobilation (mainly monodisc) of the Scyphozoa (Straehler-Pohl & Jarms, 2005).

Staurozoa assumes an important role to understand the origin of the medusa. Strikingly, we have observed a horizontal groove between the calyx and the peduncle in some adults of *Haliclystus antarcticus*. Whether this groove has any relationship with that of Scyphozoa/Cubozoa or whether the life cycle of stauromedusae should be also regarded as a “monodisc strobilation” are still open questions. Ruppert et al. (2005:180) suggested that a stauromedusa is a great monodisc strobilus, sexually mature, with its ephyrae still attached. However, this is not consensual in the literature, and the traditional idea that stauromedusae are persistent polypoid stages with modified medusoid characters are still in vogue (Thiel, 1966).

The understanding of the individual nature of the Stauromedusae faces basic concepts in cnidarian biology, such as the definition of ‘medusa’. Some authors understand the medusa as a detached polyp which has changed to a free-swimming way of life (Werner, 1973). In this teleological concept, assuming the appearance of the medusa as the synapomorphy of Medusozoa (cf. Marques & Collins, 2004; Collins et al., 2006; van Iken et al., 2006), the medusoid ancestor of the clade would be merely a functional phase of a cnidarian life cycle, without further evolutionary consequences. However, the basal lineage of the Medusozoa, the Staurozoa, does not swim – instead, its morphology is undoubtedly adapted to the sessile life habit (Uchida, 1929). Therefore, the corollary of Werner’s understanding is that stauromedusae are derived medusae adapted to sessile habit, even though they represent the basal lineage of the medusozoans. Oral metamorphosis, however, may also indicate that the ancestor of the medusozoans could simply be a non-free-swimming individual with derived



morphology comprising medusoid traits, such as subumbrella, arms, and rophalioids. This ancestor eventually became a detached medusa (most likely by a transverse division, like a strobilation), and all the exaptations became useful (see also consonant a scenario given by Wietrzykowski, 1912).

Another open question concerns the homologies of the stauropolyp and the polyps of other medusozoans. Peduncles of younger developmental stages of stauropolyps may be similar to the peduncle of the polyps of Cubozoa and Scyphozoa. However, the peduncle of later developmental stages has four chambers (at least in some species – e.g., in *H. octoradiatus* the chambers are complete only at the stage of pre-developed gonads, see Wietrzykowski, 1912:64). In this sense, in spite of the stauromedusae have a combination of both polyp and medusa characteristics, the last common ancestor of Staurozoa cannot be assumed to be either a degenerated medusa or a polyp with interrupted development, because ontogenetically the whole body (peduncle and calyx) diverges from the other Medusozoa since primary juvenile stages of the stauropolyps, and continues suffering modifications in an independent way in advanced life stages. Maybe the answers to all these questions are in future developmental biology studies.

## **CONCLUSIONS**

We have characterized a species of Staurozoa and used these data to address some evolutionary, ecological, and taxonomical issues. The main similarities and differences between Staurozoa and its phenotypically similar classes, Scyphozoa and Cubozoa, were reviewed. Although clear similarities have being depicted, especially at specific stages of life cycle, there is no doubt on the distinctiveness of the class, as

proposed by Marques & Collins (2004) and van Iken et al. (2006), corroborated by molecular data in Collins et al. (2006). Besides the ultrastructure of the gonads and ocelli, and the non-ciliated creeping planula, the stauromedusae may have four chambers in the peduncle, a feature not present in extant Medusozoa (though present in the fossil group Conulatae). Another particular feature is the infundibula of stauromedusae dividing the gastrovascular cavity of the calyx, and not reduced to the subumbrellar surface as presented by the Scyphozoa. We conclude that in spite of the stauromedusae have a combination of both polyp and medusa characteristics, the last common ancestor of Staurozoa cannot be assumed to be either a degenerated medusa or a polyp with interrupted development at a certain stage of the life cycle.

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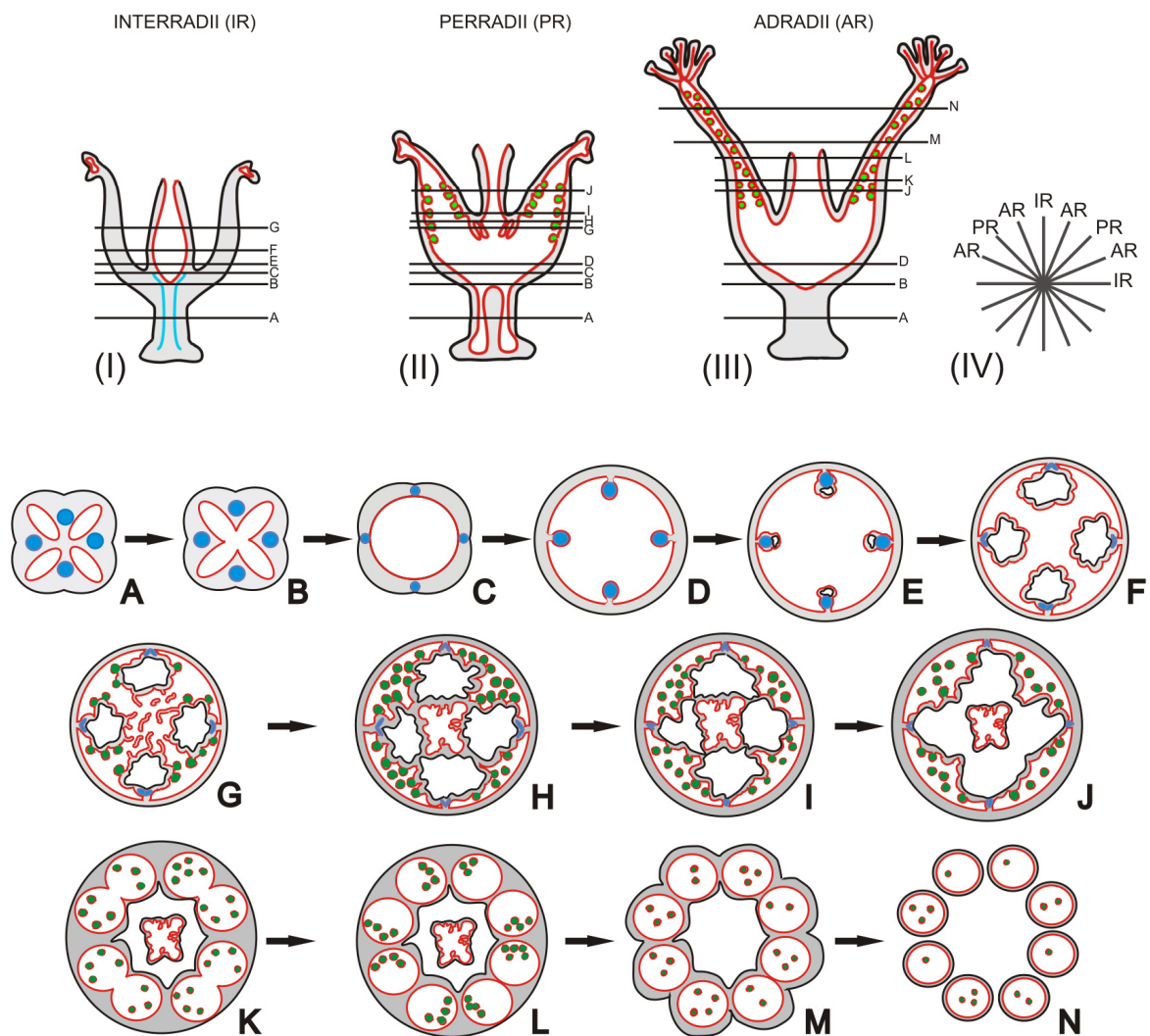
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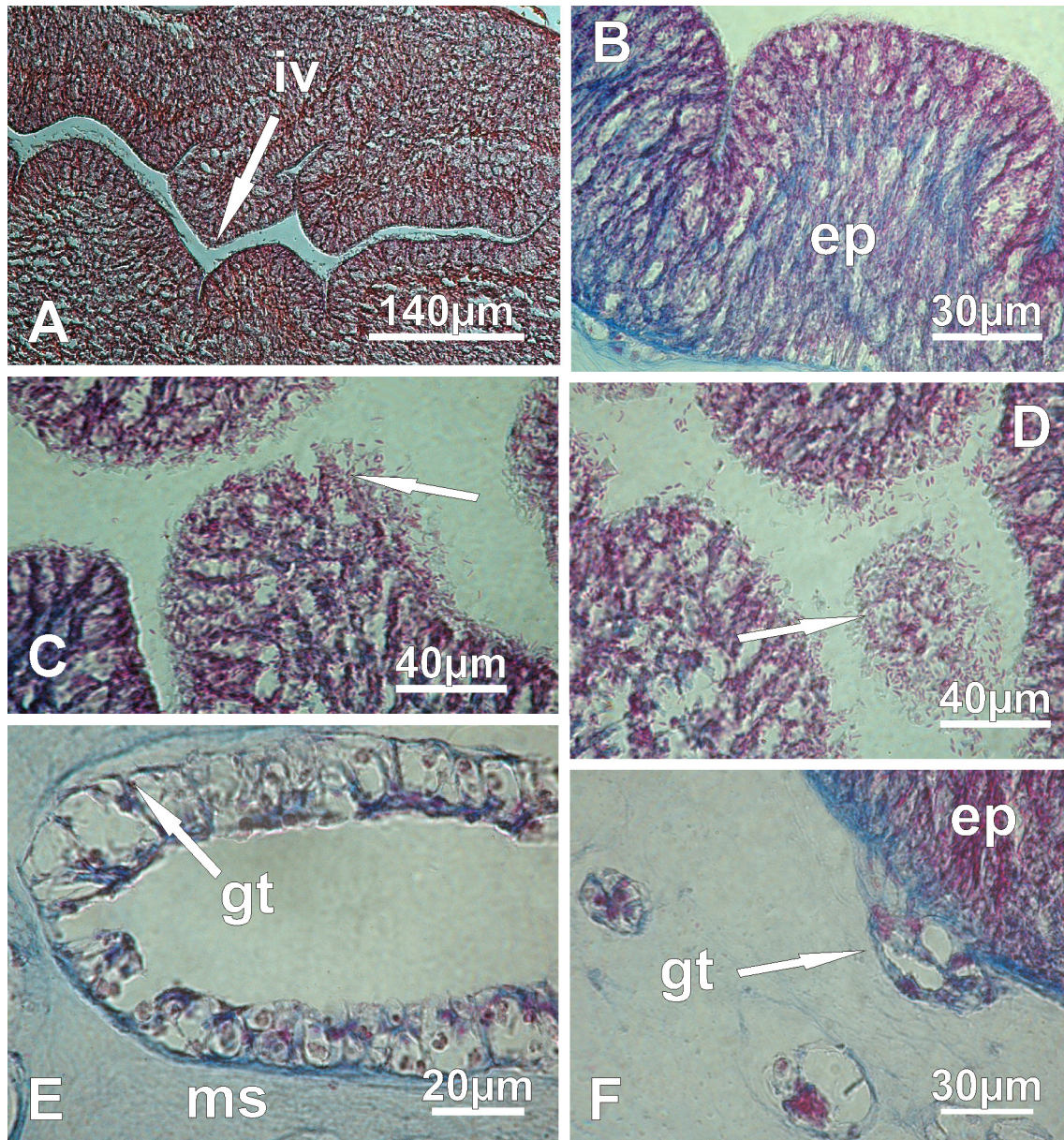
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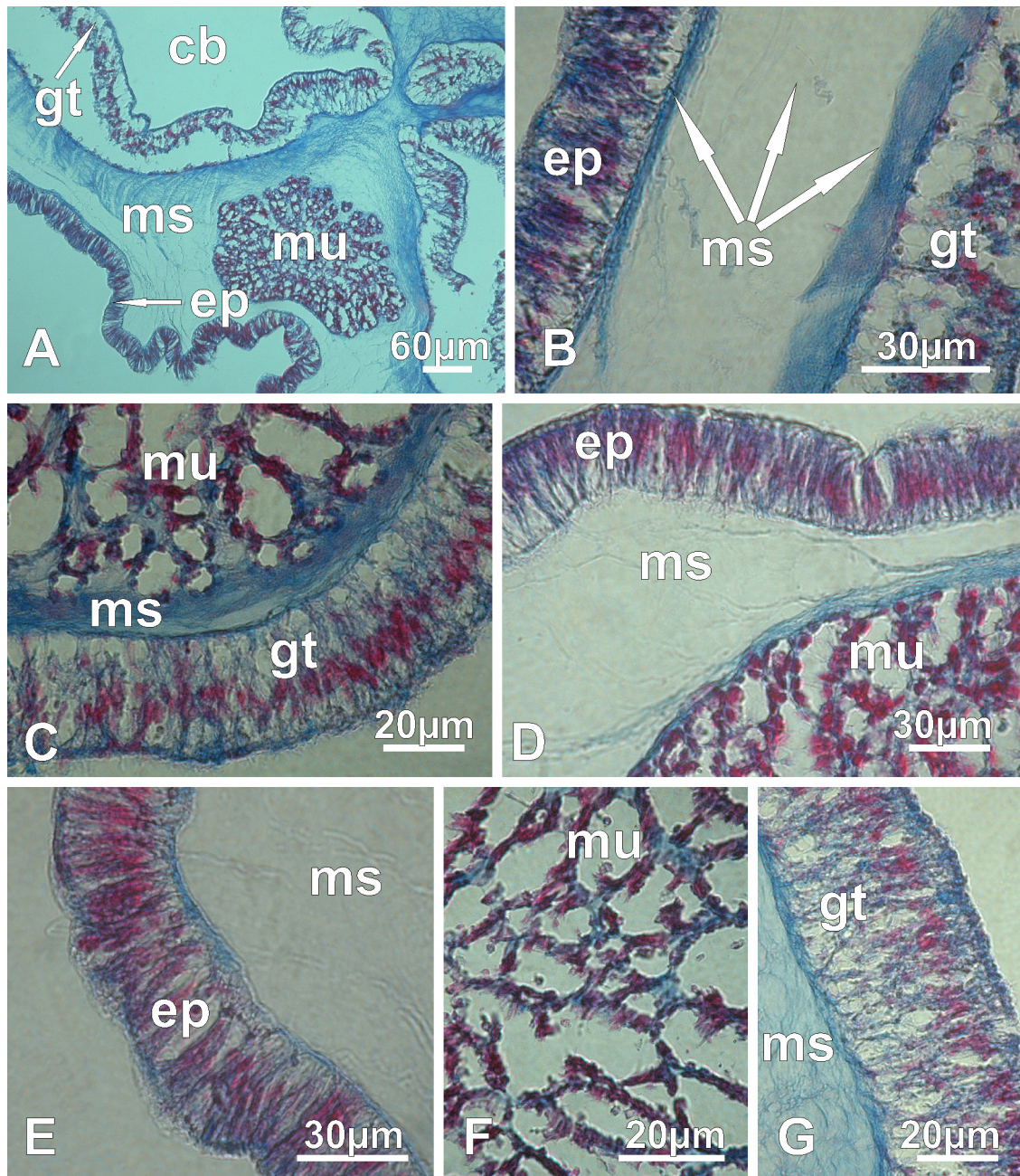


**Fig. 1** Internal anatomy of *Haliclystus antarcticus*. I-III Longitudinal section of the body, showing the sites where the cross sections were made. I interradii; II perradii; III adradii; IV orientation of the cross sections (A-N). Cross section of the body, from the basalmost (A) to the apicalmost (N) region. A peduncle with four perradial chambers and four longitudinal interradial muscles; B apical region of peduncle, with the four chambers fused; C connection region between calyx and peduncle; D basalmost region of the calyx with gastrodermis enveloping the interradial longitudinal muscle, forming the four septa; E basalmost region of the four infundibula, within the septa; F enlargement of infundibula dividing the interradial longitudinal muscles into two regions in each septum; G evagination of gastrodermis forming the gastric filaments and gonads; H gastrodermis of adjacent septa fused forming the gastrodermis of manubrium and perradial gastric pockets; I-J epidermis of adjacent septa fused completing the formation of manubrium and perradial pockets; K-L division of perradial pockets into eight regions, towards the eight arms; M-N fusion of subumbrellar epidermis with exumbrellar epidermis at region of the arms. Legend: epidermis- black; gastrodermis- red; mesoglea- gray; longitudinal interradial muscles- blue; internal content of the gonads- green.

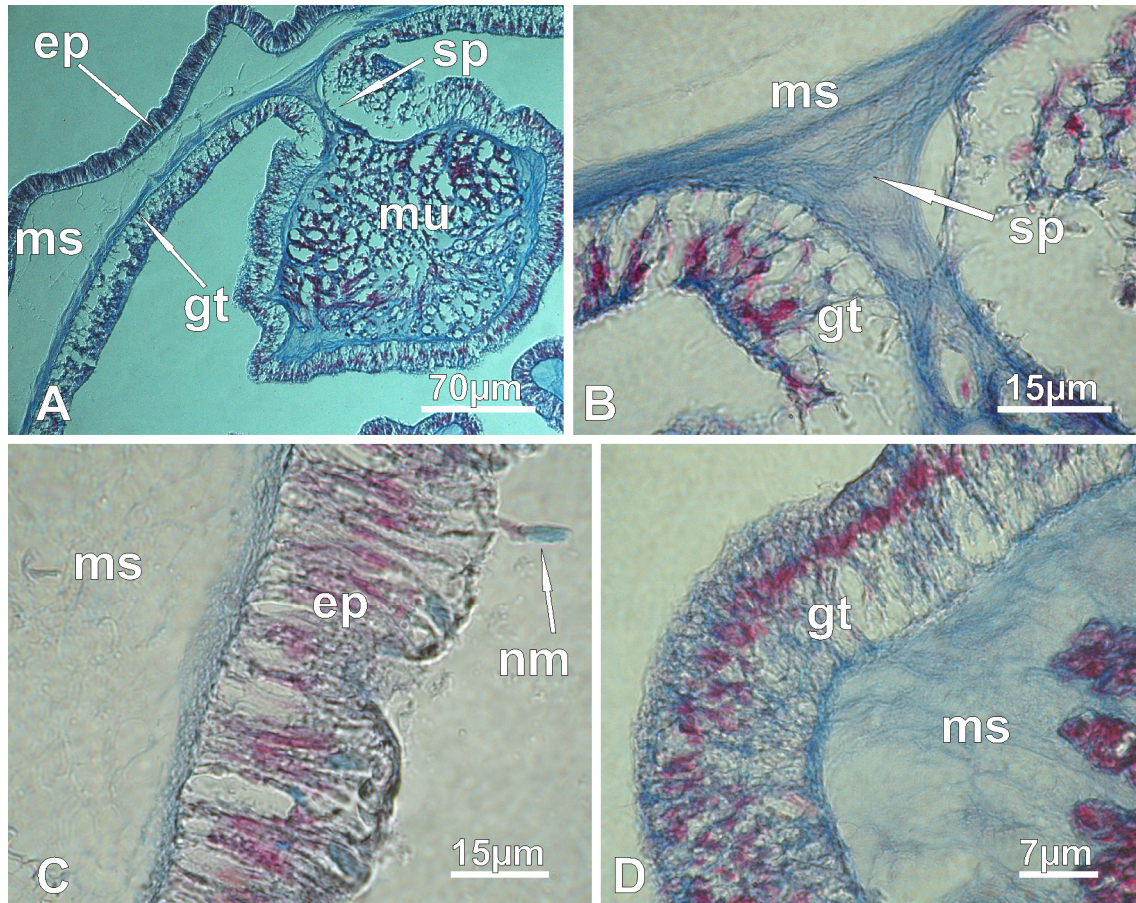


**Fig. 2** Basalmost region of peduncle. **A** invagination of pedal disc; **B** tall epidermis of pedal disc; **C-D** granules produced by glandular cells at pedal disc; **E** gastrodermis lining the chamber; **F** formation of chambers at the region close to mesoglea and epidermis. ep- epidermis; gt- gastrodermis; iv- invagination; ms-mesoglea.

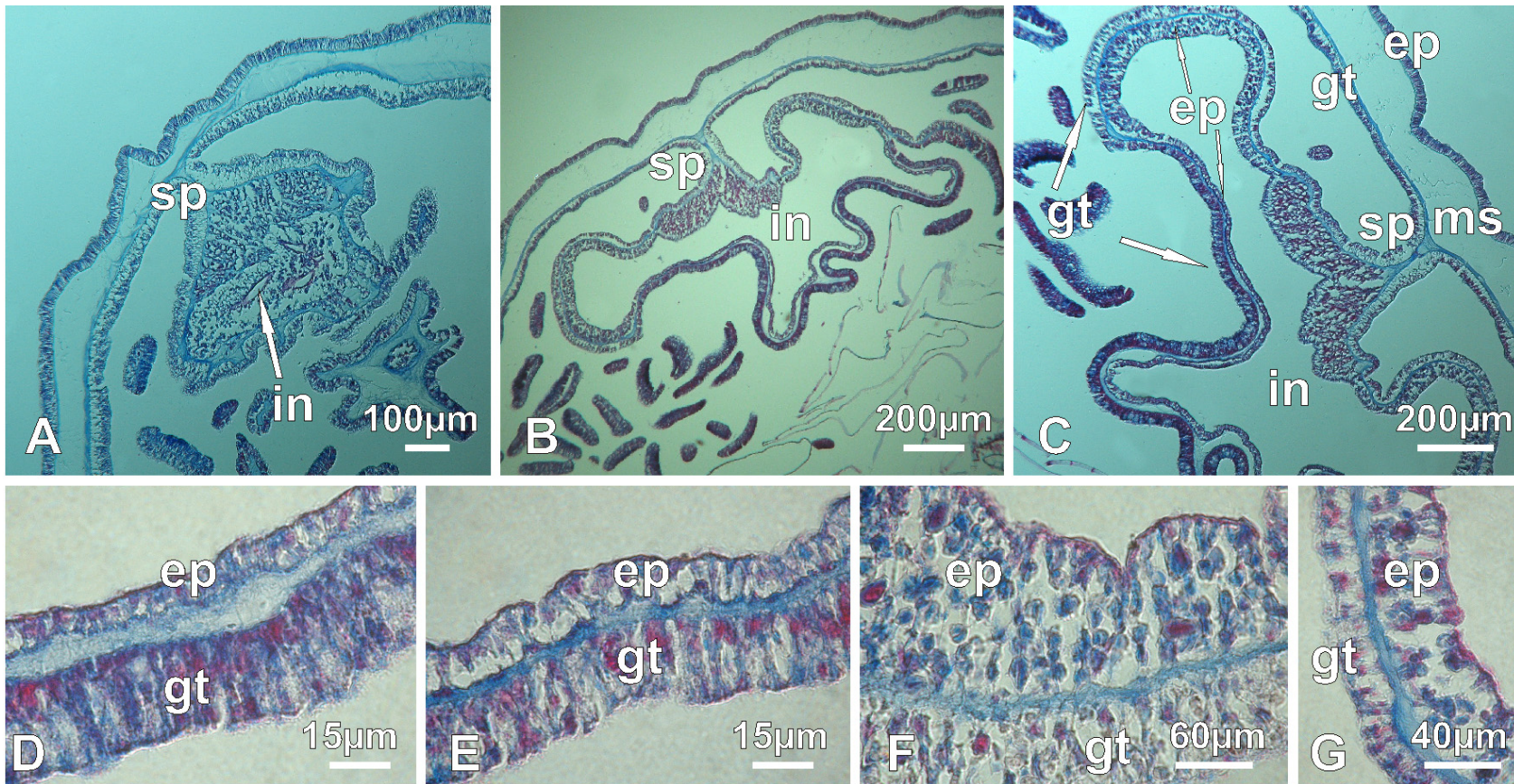




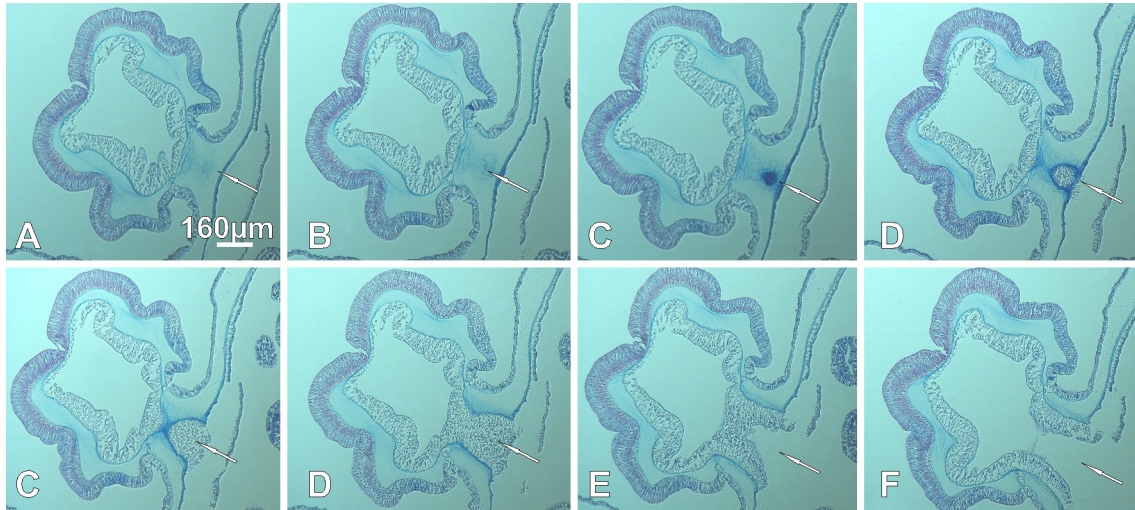
**Fig. 3** Median region of peduncle. **A** epidermis, interrarial longitudinal muscle embedded in mesoglea and chambers lined by gastrodermis; **B** mesoglea organized into three layers; **C** gastrodermis lining the chambers close to muscle; **D** epidermis close to muscle; **E** detail of epidermis; **F** detail of interrarial longitudinal muscle; **G** detail of gastrodermis with three layers (vacuolar cells juxtaposed to mesoglea; glandular cells stained with fuchsin; and glandular cells stained with aniline blue juxtaposed to chamber). cb- periradial chamber; ep- epidermis; gt- gastrodermis; ms- mesoglea; mu- interrarial longitudinal muscle.



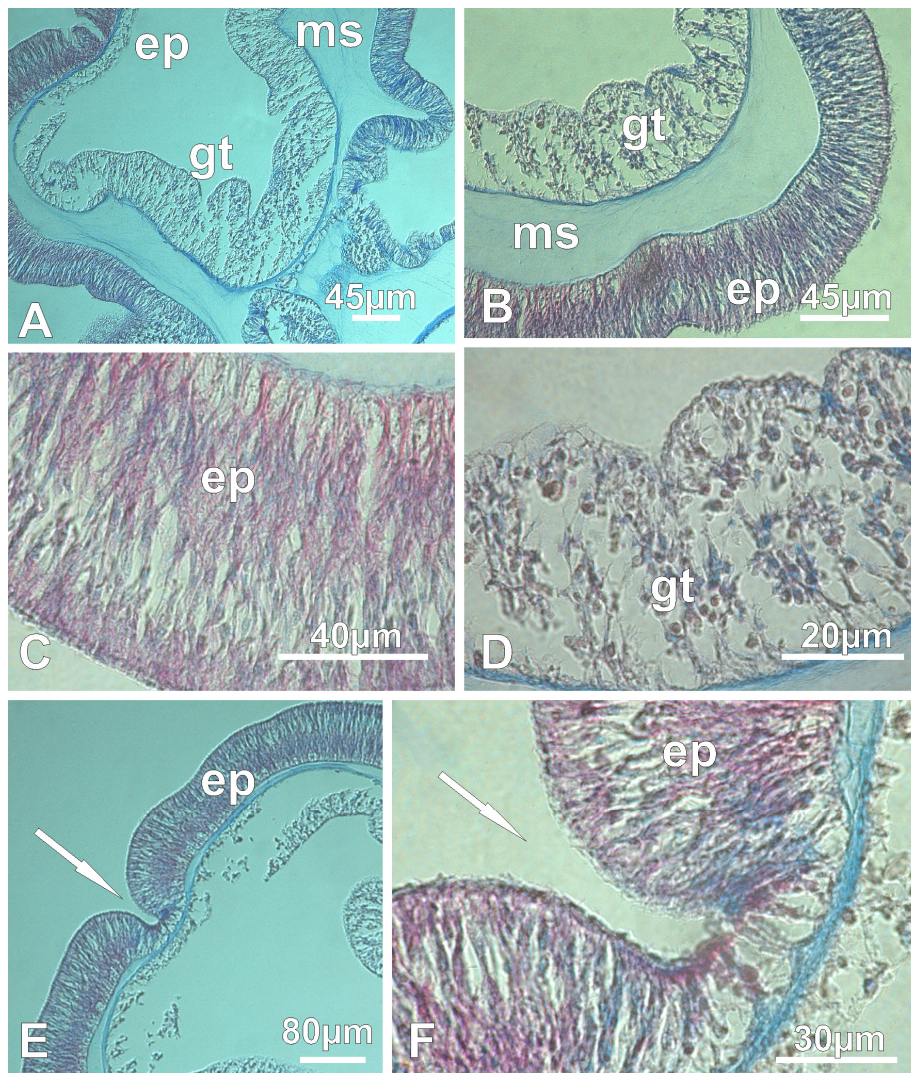
**Fig 4** Basalmost region of calyx. **A** gastrodermis envelops the interradial longitudinal muscle, forming the septum; **B** detail of septal region; **C** detail of epidermis with nematocysts; **D** detail of gastrodermis, with cells organized in three layers. ep- epidermis; gt- gastrodermis; ms- mesoglea; nm- nematocyst; sp- septum; mu- interradial longitudinal muscle.



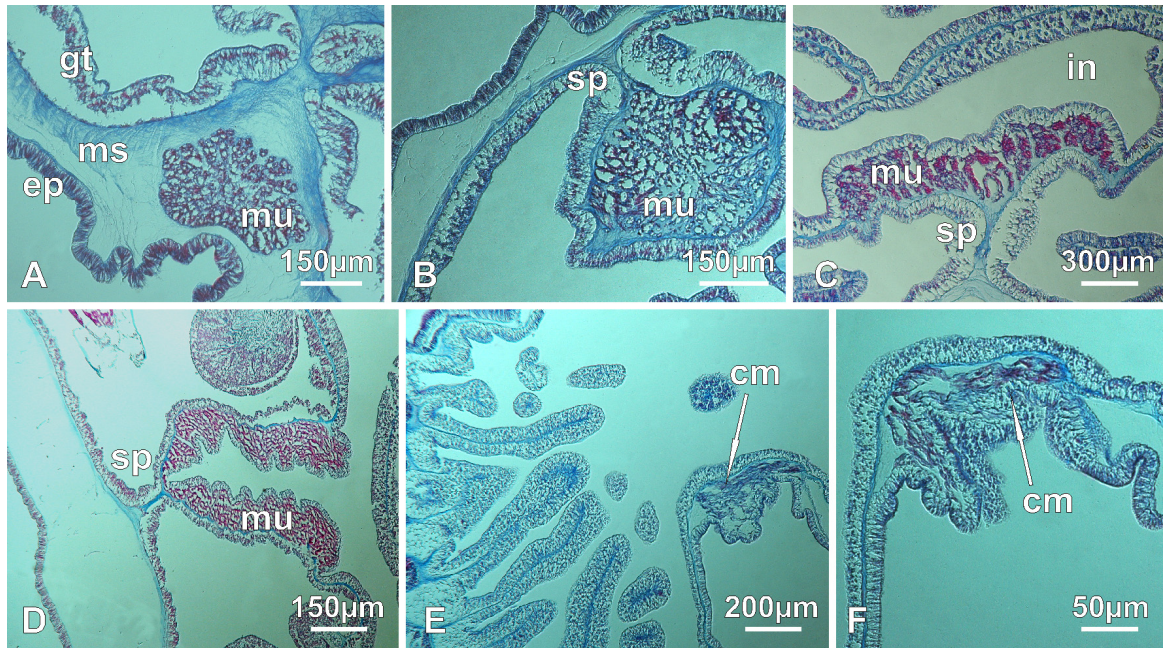
**Fig. 5** Infundibulum. **A** basalmost region of infundibulum; **B** infundibulum at the median region of the calyx; **C** epidermis of infundibulum, thicker at lateral region and thinner in front of muscle and central region; **D-E** detail of gastrodermis, thicker than epidermis at central region and in front of muscle; **F-G** detail of gastrodermis thinner than epidermis (with many granules and nematocysts) at lateral sides. ep- epidermis; gt- gastrodermis; in- infundibula; ms- mesoglea; sp- septum.



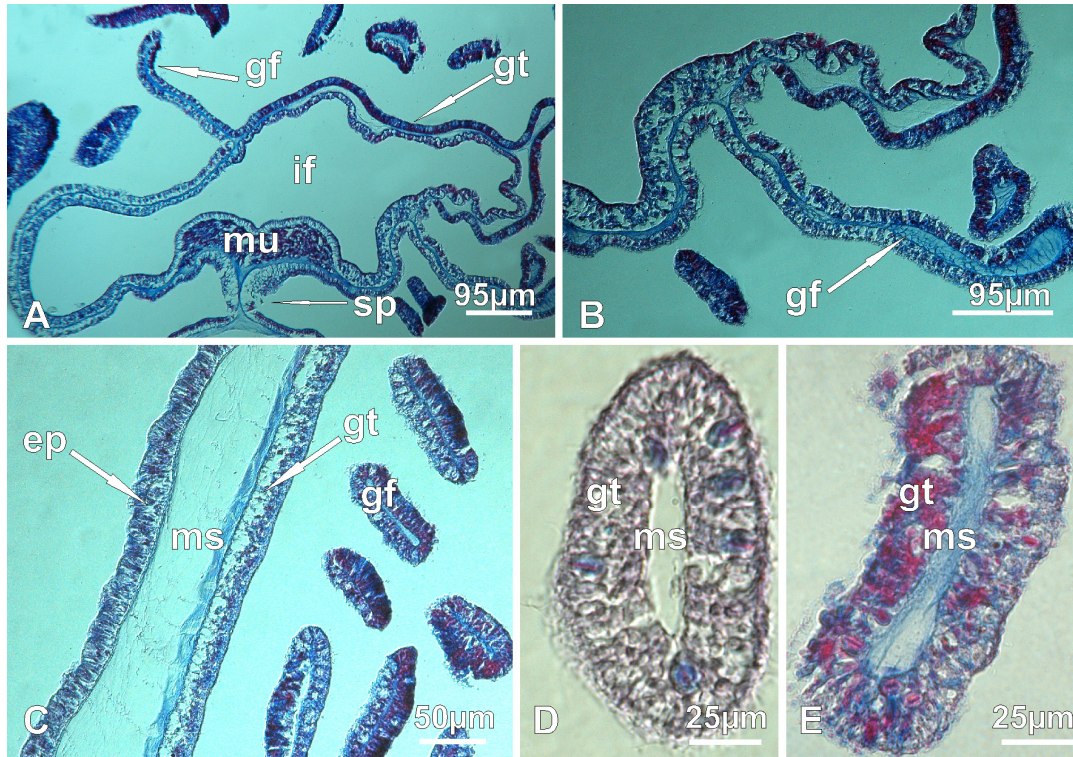
**Fig. 6** Anchor. **A-F** Hollow canal connecting the hollow anchors with the gastrodermis of the calyx. Sequence indicated by the arrow.



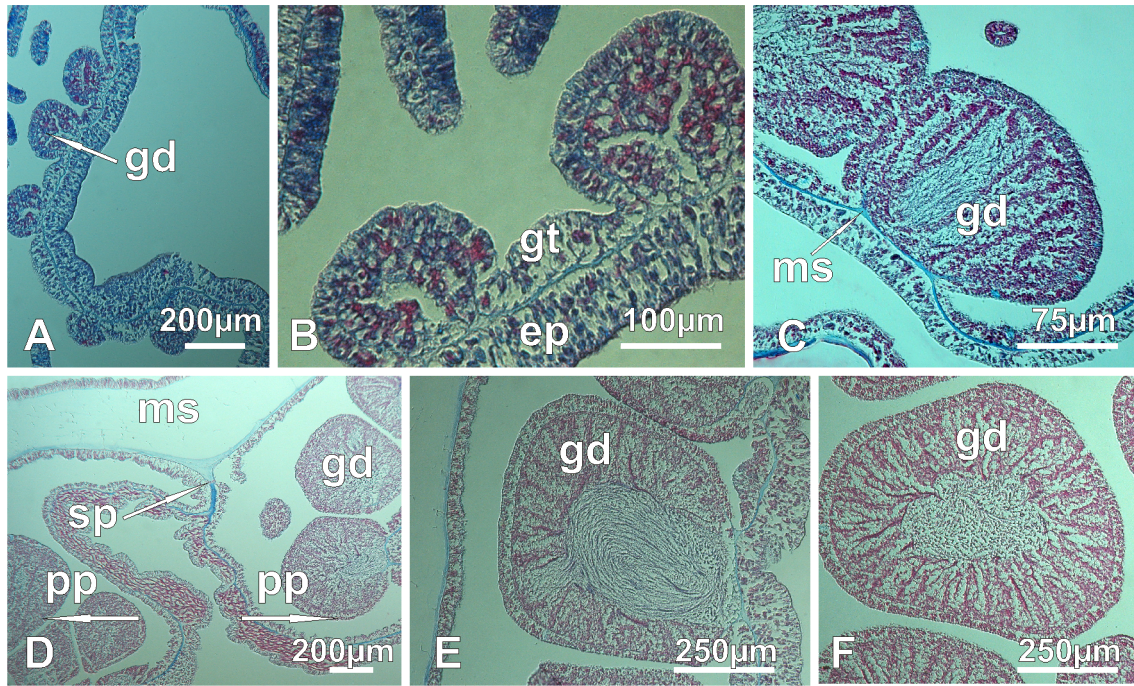
**Fig. 7** Anchor. **A** general structure; **B** detail of epidermis, mesoglea, and gastrodermis; **C** detail of epidermis with tall cells; **D** detail of gastrodermis with granules inside cells; **E-F** region of reduction of the primary tentacle. ep- epidermis, gt- gastrodermis, ms- mesoglea.



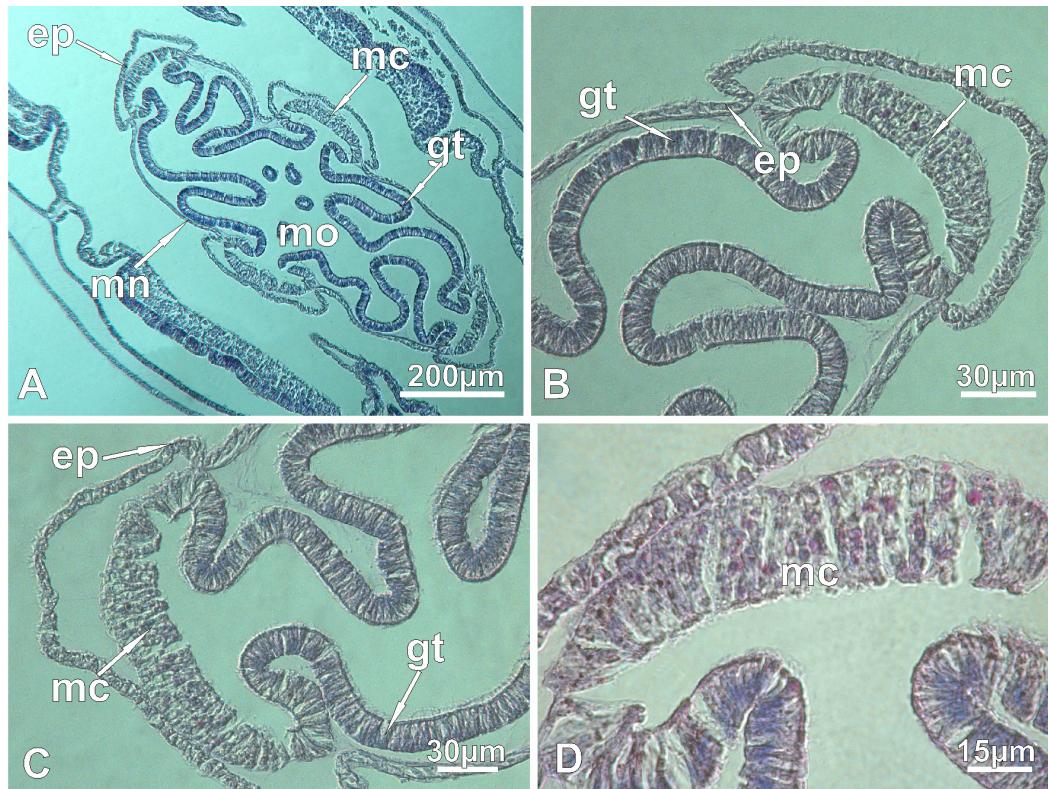
**Fig. 8** Musculature. **A** interradial longitudinal muscle in the peduncle, embedded in mesoglea; **B** gastrodermis involves interradial longitudinal muscle, forming the septum; **C** compression of interradial longitudinal muscle at the calyx by the infundibulum; **D** interradial longitudinal muscle at the calyx divided into two regions separated by the infundibulum; **E-F** coronal musculature close to the arms. cm- coronal musculature; ep- epidermis; gt- gastrodermis; in- infundibula; mu- muscle; ms- mesoglea; sp- septum.



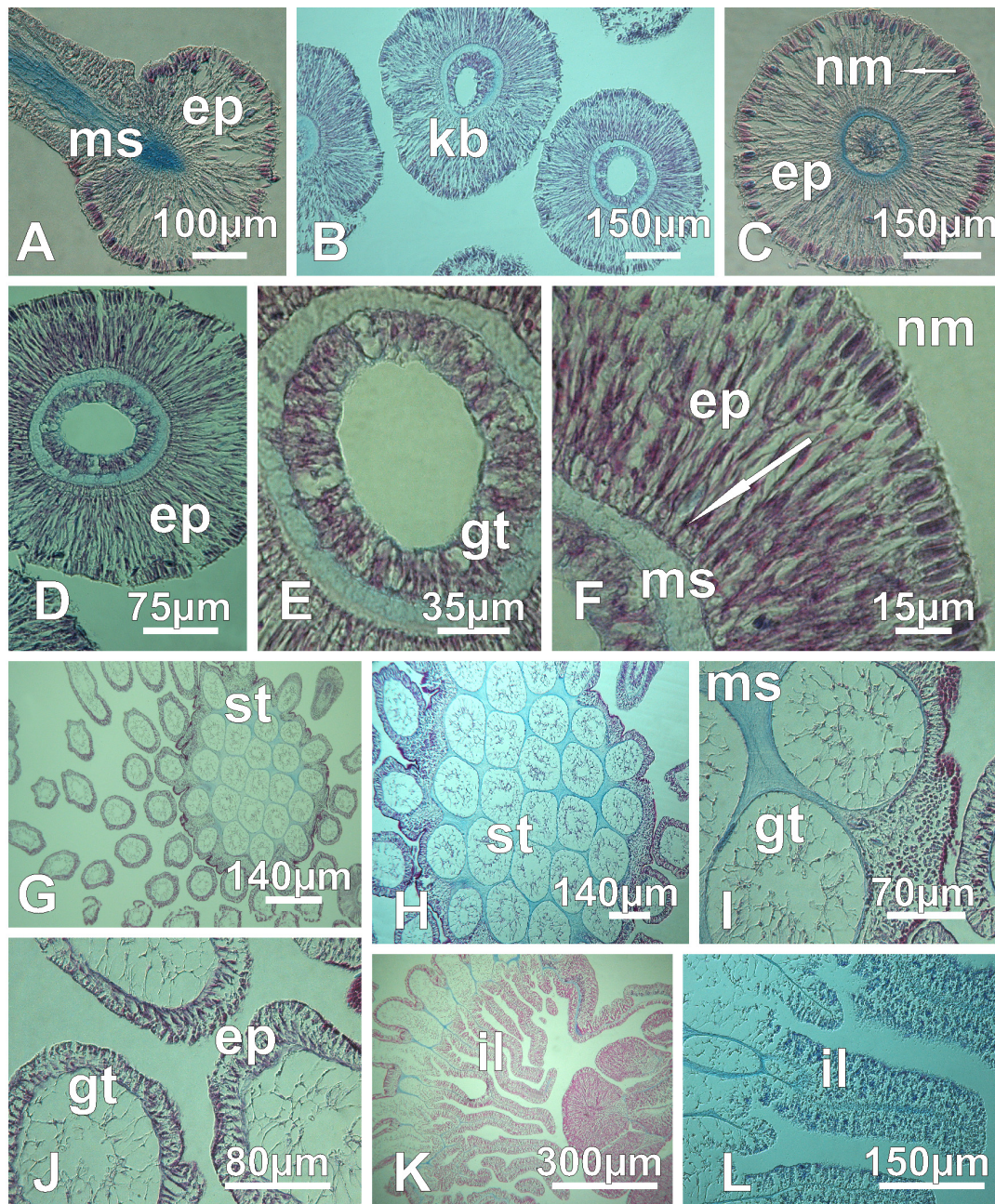
**Fig. 9** Gastric filament. **A** formation of gastric filament by evagination of septal gastrodermis; **B** detail of gastric filament formation; **C** sections of parts of the gastric filaments; **D-E** detail of gastric filament with gastrodermal nematocysts and internal mesoglea. ep- epidermis; gf- gastric filament; gt- gastrodermis; if- infundibula; mu- muscle; ms- mesoglea; sp- septum.



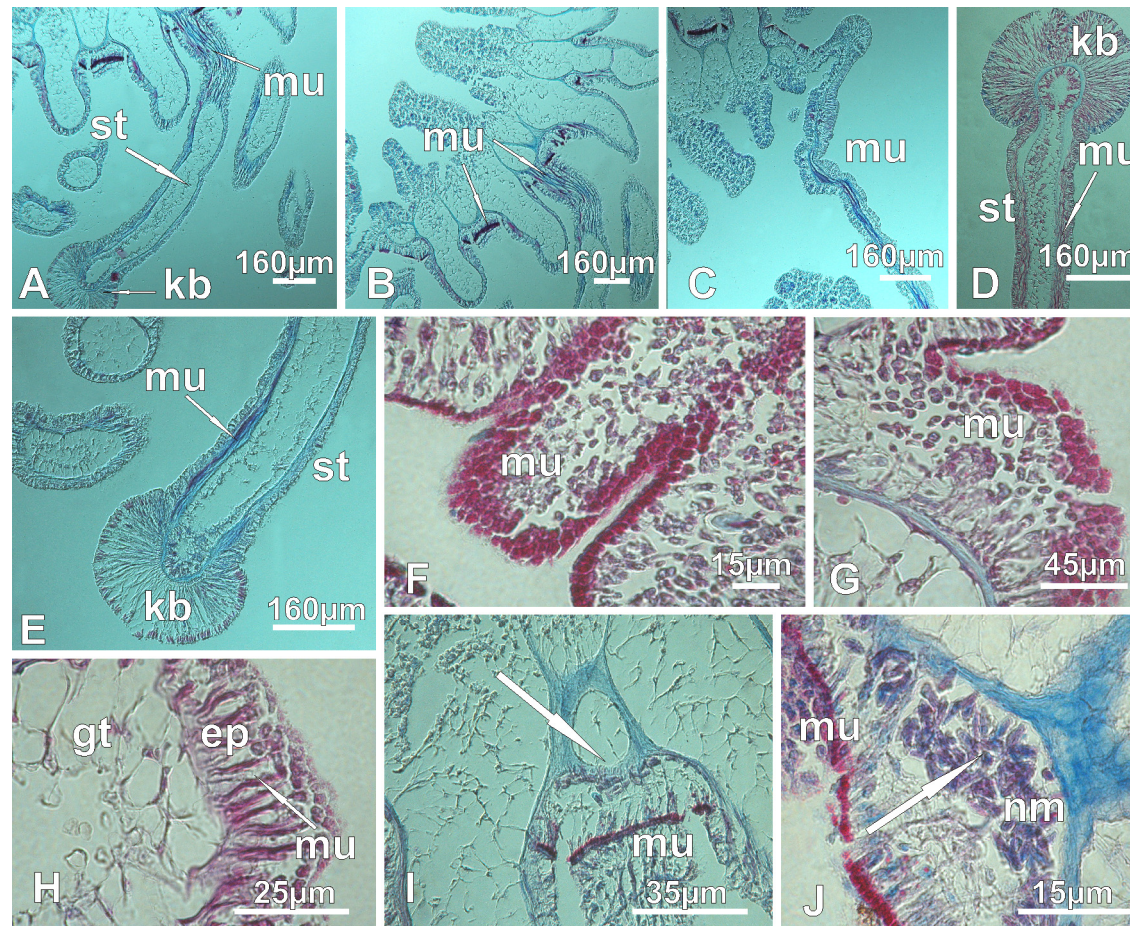
**Fig. 10** Gonads. **A-B** gonads formation by evagination of the gastrodermis; **C** thin layer of mesoglea separating the gastrodermis of the gonadal content; **D** perradial pockets separated by the septum, with the gonads in their interior; **E** longitudinal section of a developed male gonad; **F** cross section of a developed male gonad. ep- infundibular epidermis; gd- gonad, gt- gastrodermis, ms- mesoglea, pp- perradial pockets, sp- septum.



**Fig. 11** Manubrium. **A** cross section with general view of manubrium; **B-D** detail of perradial corner of manubrium. ep- epidermis; gt- gastrodermis; mc- manubrial corner; mo- mouth; mn- manubrium.

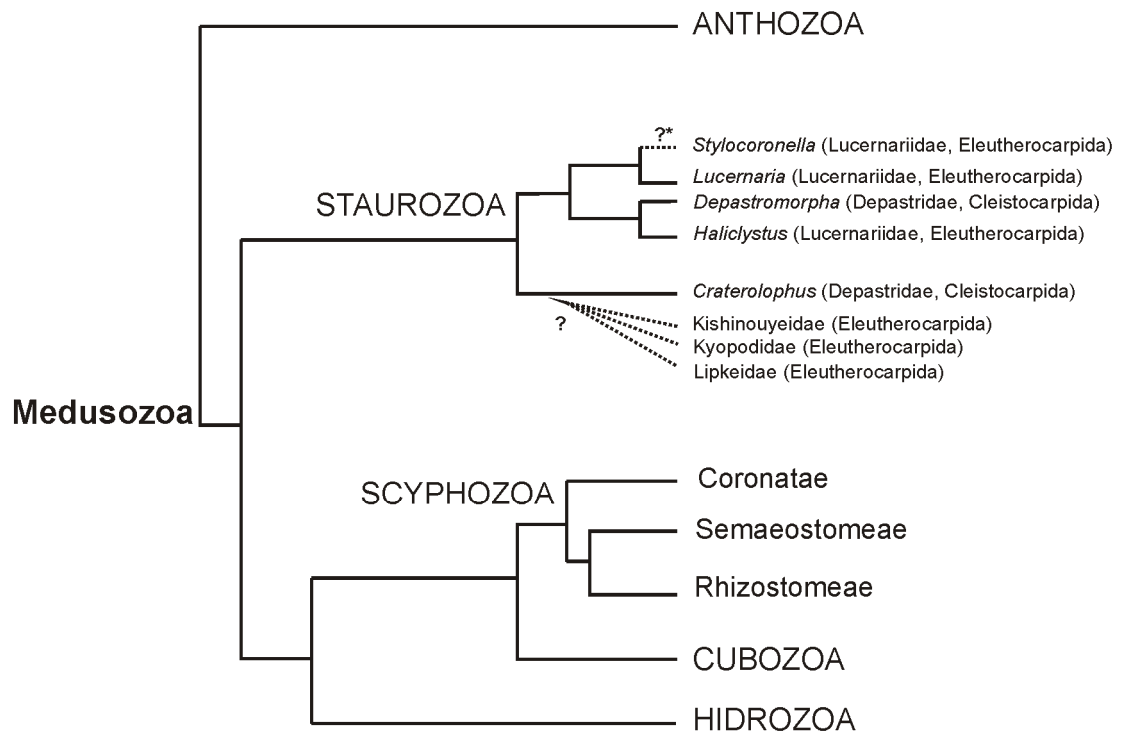


**Fig. 12** Tentacles. **A** knob tentacle, highlighting the tall epidermis and mesoglea inside tentacle; **B** cross sections of tentacular knobs; **C-D** tentacular knob with tall epidermis and nematocysts on its apex; **E** gastrodermis of tentacular knob with vacuolated glandular cells; **F** basal region of epidermis with the presence of vacuoles; **G-H** cross section of stem basis; **I-J** stem base highlighting the longitudinal muscle and gastrodermis with vacuolated cells; **K-L** longitudinal section of tentacular basis showing intertentacular lobes. ep- epidermis; gt- gastrodermis; il- intertentacular lobes; kb- knob; ms- mesoglea; nm- nematocyst; sb- stem base.



**Fig. 13** Tentacles. **A** longitudinal muscle throughout tentacular stem; **B** longitudinal and cross section of longitudinal muscles (dots stained with fuchsin); **C** longitudinal muscle along the arms, towards tentacular tips; **D-E** detail of longitudinal muscle of stem; **F-G** cross section of longitudinal muscle at tentacular base; **H** cross section of the base of the stem showing epidermis interspersed by longitudinal muscles; **I-J** region forming nematocysts at the tentacular base. ep- epidermis; gt- gastrodermis; kb- knob; mu- muscle; nm- nematocyst; st- stem.





**Fig. 14** Working phylogenetic hypothesis of cnidarian relationships, with an emphasis on Staurozoa. Based on Collins & Daly (2005), van Iken et al. (2006) and Collins et al. (2006). \*relationship was inferred based on Salvini-Plawen (2006). See text for a discussion of the studies underlying this composite hypothesis.

# Capítulo 5

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## Considerações finais

### DISCUSSÃO GERAL E CONCLUSÕES

Dificuldades relacionadas à taxonomia de estauromedusas são bem conhecidas (Mayer, 1910; Gwilliam, 1956; Kramp, 1961), e a necessidade de uma revisão do gênero *Haliclystus*, o mais diverso da classe, bem como a reavaliação de algumas espécies do gênero, foi enfatizada por Hirano (2007). Neste estudo, documentamos variações intraespecíficas e ontogenéticas em alguns caracteres que são tradicionalmente utilizados na taxonomia de Staurozoa. Esses caracteres devem ser cuidadosamente avaliados quando empregados. Como um exemplo dessas dificuldades, nós mostramos que a espécie *Haliclystus auricula*, até então considerada cosmopolita e bipolar, aparentemente não ocorre no hemisfério Sul. Os espécimes identificados como *H. auricula* no Chile e Argentina (Amor, 1962; Mianzan, 1989; Zagal, 2004a, 2004b, 2008) são propostos como *Haliclystus antarcticus*, ampliando a distribuição desta espécie, até então restrita a Península Antártica.

Atualmente, famílias e gêneros de Staurozoa são reconhecidos por um mosaico de características, muitas das quais não exclusivas ou que sugerem agrupamentos contraditórios (Collins & Daly, 2005). Estudos que tratem da distribuição e taxonomia desses animais, englobando dados morfológicos e moleculares, devem ser encorajados, uma vez que esse tipo de informação ainda é raro e já demonstrou contribuir significativamente para melhor compreensão da evolução do grupo.

Outro desafio para o estudo da classe é a documentação do seu ciclo de vida. Apesar de Staurozoa ter características “chaves” para uma melhor compreensão da evolução do ciclo de vida nos Cnidaria, pois reúne características de pólipos e medusa em um único indivíduo (Collins et al., 2006), apenas duas espécies tiveram seu ciclo de vida totalmente descrito (*Haliclystus octoradiatus*, por Wietrzykowski, 1912; *Stylocoronella riedli*, por Kikinger & Salvini-Plawen, 1995). As poucas características passíveis de distinção em estágios precoces do ciclo de vida desses animais dificultam ainda mais sua identificação. Ferramentas de biologia molecular foram então utilizadas para esclarecer o ciclo de vida de Staurozoa. Similaridades entre os haplótipos de três marcadores de *Microhydrula limopsicola*, até então classificado com um Hydrozoa (Jarms & Tiemann, 1996), e *H. antarcticus*, provam que os indivíduos estudados pertencem à mesma espécie. Dados morfológicos e ecológicos sugerem que *M. limopsicola* seja um estágio do ciclo de vida de *H. antarcticus* posterior à fixação da plânula. Ademais, a produção de frústulas foi registrada para o gênero *Haliclystus* pela segunda vez (Wietrzykowski, 1912), em um estágio de vida diferente do documentado em *Stylocoronella* (Kikinger & Salvini-Plawen, 1995). Aparentemente, a produção de frústulas é uma estratégia de reprodução assexuada intensa em *H. antarcticus*, pois apenas um haplótipo foi encontrado para cada região estudada (Chile e Antártica). Análises integradas englobando morfologia, ecologia, moléculas e ciclo de vida são necessárias para resolver questões evolutivas e biogeográficas, pouco desenvolvidas para o grupo.

Questões relacionadas ao ciclo de vida e evolução de Cnidaria, mais especificamente em Medusozoa, foram abordadas nesse estudo, com base em caracteres histo-morfológicos. Paralelos entre a metamorfose de Staurozoa, Cubozoa e

Scyphozoa foram estabelecidos e possíveis conexões foram discutidas. A anatomia interna também é importante na taxonomia de Staurozoa, uma vez que o grupo possui poucos caracteres macro-morfológicos que podem ser utilizados na diferenciação de espécies. Entretanto, análises moleculares recentes (Collins & Daly, 2005) mostram que a utilização desses caracteres deve ser reavaliada em classificações supragenéricas. Uma análise mais ampla visando abordar as particularidades dessas características em Staurozoa, bem como um estudo de desenvolvimento comparativo em Medusozoa, contribuirão para responder questões sobre a evolução do ciclo de vida em Cnidaria.

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# Resumo

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A recente proposta de uma nova classe de Cnidaria, Staurozoa, contribuiu para um melhor conhecimento sobre o grupo. Entretanto, os dados e hipóteses sobre a evolução, taxonomia, biologia e ecologia de Staurozoa ainda são limitados, especialmente com relação às espécies do hemisfério Sul. O objetivo deste estudo é: (1) rever caracteres tradicionalmente utilizados na taxonomia do gênero *Haliclystus* (alguns deles amplamente utilizados na taxonomia de Staurozoa em geral), (2) abordar questões relacionadas à ontogenia e ao ciclo de vida desses animais e (3) discutir e revisar homologias de Staurozoa e de outras classes de Medusozoa, subsidiando inferências evolutivas para o grupo. A espécie *Haliclystus antarcticus* foi utilizada como um modelo para Staurozoa em geral a fim de atingir os objetivos propostos. Contribuições para o conhecimento sobre a biologia e taxonomia do grupo provieram de estudos histo-morfológicos e da re-descrição de *H. antarcticus*. Dados moleculares, morfológicos e ecológicos foram utilizados em inferências sobre a ontogenia e o ciclo de vida de *H. antarcticus*. Discussões sobre homologias e ciclo de vida de outros Medusozoa provieram de dados histo-morfológicos. As principais consequências do nosso estudo são: (a) os espécimes provenientes do Chile foram identificados erroneamente como *H. auricula*, e devem ser classificados como *H. antarcticus*; (b) muitos caracteres tradicionalmente utilizados na taxonomia do grupo são variáveis (intraespecificamente e ontogeneticamente) ou são interpretados incorretamente; (c) dados moleculares suportam a identificação da espécie *Microhydrula limopsicola* (Limnomedusae, Hydrozoa) como um estágio da vida de *H. antarcticus*, o que esclarece o ciclo de vida e a morfologia do grupo; (d) o significado evolutivo de alguns caracteres morfológicos e do ciclo de vida da classe Staurozoa foram revisados e discutidos em uma perspectiva mais ampla entre os Medusozoa.

# Abstract

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The recent proposal of a new class of Cnidaria, Staurozoa, has contributed to a better knowledge on the group. However, data and hypotheses on evolution, taxonomy, biology, and ecology of Staurozoa are still limited, especially concerning species of the Southern Hemisphere. The aim of this study is: (1) to review characters traditionally adopted in the taxonomy of the genus *Haliclystus* (some of them widely used for Staurozoa taxonomy in general), (2) to address issues concerning the ontogeny and life history of these animals, and (3) to discuss and review homologies of Staurozoa and other classes of Medusozoa, subsidizing evolutionary inferences for the group. The species *Haliclystus antarcticus* was used as a model for Staurozoa in general in order to achieve the proposed goals. Contributions to the knowledge on the biology and taxonomy of the group came from histo-morphological studies and the re-description of *H. antarcticus*. Molecular, morphological, and ecological data were used in inferences concerning the ontogeny and life cycle of *H. antarcticus*. Discussions on homologies and life cycle of other Medusozoa also came from histo-morphological data. The main consequences of our studies are: (a) specimens from Chile were misidentified as *H. auricula*, and they should be assigned to *H. antarcticus*; (b) many characters traditionally adopted in the taxonomy of the group were demonstrated to be either variable or incorrectly interpreted; (c) molecular data have supported the assignment of the Hydrozoa Limnomedusae species *Microhydrula limopsicola* as a phase of the life cycle of *H. antarcticus*, what shed light on the understanding of the life cycle of the group and its morphology; (d) the evolutionary meaning of some morphological and life cycle characters in the class Staurozoa were reviewed and discussed in a broader perspective among the Medusozoa.

# Apêndice I

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## Preparação do material

- Lavar com água destilada: 30 minutos
- Manter em álcool 70%: 15 minutos
- Manter em álcool 80%: 15 minutos
- Manter em álcool 90%: 15 minutos
- Manter em álcool 95%: 15 minutos
- Manter em álcool 100% I: 15 minutos
- Manter em álcool 100% II: 15 minutos
- Manter em álcool 100% III: 15 minutos
- Manter em xilol I: 15 minutos
- Manter em xilol II: 15 minutos
- Manter em xilol III: 15 minutos
- 1º banho - parafina + xilol (1:1): colocar na estufa com tampa durante 1 hora
- 2º banho - parafina pura: colocar na estufa sem tampa durante 1 hora
- 3º banho - parafina pura: colocar na estufa sem tampa durante 1 hora



## Apêndice II

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### Coloração das lâminas histológicas

- Manter as lâminas na estufa por 10 minutos
- Xilol I: 10 minutos
- Xilol II: 5 mergulhos + 2 minutos
- Álcool 100% I: 2 minutos
- Álcool 100% II: 2 minutos
- Álcool 90%: 1 minuto
- Álcool 80%: 1 minuto
- Álcool 70%: 1 minuto
- Água destilada: 10 mergulhos + 1 minuto
- Fucsina ácida: 5 mergulhos + 15 minutos
- Água destilada: 5 mergulhos
- Azul de anilina Cromotrope 2R: 2 mergulhos + 5 minutos
- Água destilada: 5 mergulhos
- Azul de anilina: 2 mergulhos + 3 minutos
- Água destilada: 5 mergulhos
- Álcool 70%: 5 mergulhos
- Álcool 90%: 1 minuto
- Álcool 100% I: 1 minuto
- Álcool 100% II: 1 minuto
- Xilol I: 5 minutos
- Xilol II: 5 minutos

# Apêndice III

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## Preparação dos corantes

### Fucsina ácida

Fucsina ácida .....	1g
Água destilada.....	100ml

### Azul de Anilina – Cromotrope 2R (Tricômio de Masson)

Azul de anilina .....	0.3g
Cromotrope 2R.....	0.6g
Ácido Acético .....	0.8g
Água destilada.....	100ml

### Azul de anilina acética (Mallory)

Azul de anilina .....	10g
Água destilada.....	400ml
Ácido Acético .....	10ml

Dissolver a quente o azul de anilina e manter fervendo a solução por alguns minutos.

Adicionar ácido acético, esfriar e filtrar.