JULIA SILVA BENETI

Revisão sistemática do gênero *Bunodosoma* Verrill, 1899 (Cnidaria: Actiniaria: Actiniidae) e estudo de populações do Atlântico Sul

Systematic review of the genus *Bunodosoma* Verrill, 1899 (Cnidaria: Actiniaria: Actiniidae) and study of populations from the South Atlantic Ocean

São Paulo

2016

Julia Silva Beneti

Revisão sistemática do gênero *Bunodosoma* Verrill, 1899 (Cnidaria: Actiniaria: Actiniidae) e estudo de populações do Atlântico Sul

Systematic review of the genus *Bunodosoma* Verrill, 1899(Cnidaria: Actiniaria: Actiniidae) and study of populations from the South Atlantic Ocean

Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências Biológicas na Área de Zoologia.

Orientador: Prof. Dr. André Carrara Morandini

Coorientadora: Dra. Luciana C. F. R. de Gusmão

São Paulo

2016

Beneti, Julia Silva

Revisão sistemática do gênero *Bunodosoma* Verrill, 1899 (Cnidaria: Actiniaria: Actiniidae) e estudo de populações do Atlântico Sul

236 p.

Tese (Doutorado) - Instituto de Biociências da Universidade de São Paulo. Departamento de Zoologia.

1. Anêmonas do mar 2. Delimitação de espécies 3. Delimitação de populações
I. Universidade de São Paulo. Instituto de Biociências. Departamento de Zoologia.

Comissão Julgadora:

_____ _____

Prof(a). Dr (a). Prof(a). Dr (a).

_____ _____

Prof(a). Dr (a). Prof(a). Dr (a).

_____

Prof. Dr. André Carrara Morandini

Orientador

Agradecimentos

Ao meu orientador, Prof. Dr. André Carrara Morandini, por sua enorme contribuição na minha formação científica e por aceitar a me orientar mesmo eu não querendo estudar medusas! Além disso, agradeço pela grande ajuda nas coletas, pelas conversas e por corrigir meus textos e responder e-mails super rápido!

Aos colegas e amigos, presentes e passados, do Laboratório de Cultivo e Estudos em Cnidaria, Renato Nagata, Gisele Tiseo, Henrique Alves, Leandro Santos, Clarissa Molinari, Edgar Gamero, Mayara Jordano e Jonathan Lawley, pela divertida convivência (comigo e com a minha bagunça). E em especial ao Sérgio N. Stampar e Max M. Maronna pela paciência para me ensinar e ajudar infinitamente com os estudos moleculares.

À minha co-orientadora Luciana Gusmão, pela ajuda em coletas e no laboratório, por me acolher em Recife e em Nova Iorque, e pela amizade (e a ioga, é claro).

À Dra. Estefania Rodríguez, que me recebeu e disponibilizou toda a estrutura em seu Laboratório no American Museum of Natural History, e me ajudou a compreender várias coisas que eu não entendia ainda sobre as lindas anêmonas.

À todos os funcionários do Instituto de Biociências, em especial ao Ênio, Manuel, Bia, Sabrina e Phillipe por me salvarem inúmeras vezes ao longo desses 4 anos e meio, e me ajudarem prontamente com dúvidas de histologia ou molecular que foram surgindo pelo caminho! E à Lilian e Erika pela enorme ajuda nas questões burocráticas!

A todo mundo que me ajudou em coletas: André Morandini, Sérgio Stampar, Karla Paresque, Luciana Gusmão, Renato Nagata, Nathalia Padovanni, Thomás Banha, Alexandre Nascimento, Felipe Rocha, Nathalia Hristov, María Mendoza Becerril, Amanda Cunha, Fabián Acuña, Agustín Garese, Fabrizio Scarabino, Valentina Leoni, Romina Trinchin e Bárbara Romera, e muitas outras pessoas.

Ao Prof. Dr. Fabián Acuña e Dr. Agustín Garese pela ajuda com o estudo dos cnidocistos e pela ótima recepção em Mar del Plata. E à Irene Deserti por tornar essa semana na Argentina muito agradável com os passeios.

À Profa. Dra. Vera Solferini e seus alunos, principalmente Fernanda, Cecília e Elen, por me ajudarem com as análises de população.

Aos amigos da Zoo e agregados, pelas conversas, boicotes ao Bobó, Churras da Zoo, CVZoos, e tudo mais que aconteceu nesses 4 anos e meio, além da ajuda na tese: Pedro H., Jorge, Loboda, Bárbara, Pedro G., Isa, Rach, Mari, Rafa, Adri, Alípio, Jhon, Anne, Juan, Thalles, Avatar, Gabriel (os dois), Carol (as duas), Victor, Gordo, Bruna, Bala, Tama, Nilvea, Caios peixes e Antunes, etc.

A todas minhas companheiras de casa, que dividiram comigo meus e seus momentos de alegria, fofocas e estresse: Ruli, Paula, Alejandra, Nati, Flávia, Melina, Camila, Natália, Vivi.

Às minhas amigas queridas (entre outras coisas), que mesmo longe estão sempre perto ciberneticamente, falando besteira e contando histórias de mundos longínquos: Rach, Ruli, Cobrinha, Esther e Paula.

Aos melhores amigos da M11, simplesmente por existirem e por ser a melhor turma que alguém pode ter! À Sofia, melhor amiga pra dividir coisas do doutorado e da vida! E à Tati, que de uma forma ou de outra está desde sempre presente em tudo que eu faço na vida, seja com palavras inteligentes ou de carinho pra dar!

À Nara, a gata mais charmosa e cheia de personalidade que já existiu, por me fazer companhia todo santo dia (dormindo, mas tudo bem!), principalmente agora no fim da tese.

À minha família maravilhosa e divertida, Cesar, Claudia, Lucas e Sara, sempre presentes e bem humorados, dando forças nos momentos em que mais preciso. Nada de nada na minha vida seria possível sem vocês. E um agradecimento mais do que especial e merecido pra pequena irmã Sara que editou grande parte das fotos dessa tese (quase) sem reclamar. E à minha família canina Chico, Bela, Malu, Frida, por me fazerem companhia na sala de casa enquanto eu editava minhas tabelas infinitas de cnidocistos (Zeca, Lia, Nina, Meg e Mila estavam lá zoando em espírito, certamente).

Obrigada a todos!!!

Índice

Resumo

Bunodosoma é um gênero cosmopolita de anêmonas que apresentam pólipos grandes e conspícuos, sendo comuns em ambientes costeiros. Atualmente compreende 14 espécies válidas e, apesar de estas serem utilizadas em estudos filogenéticos dentro da ordem Actiniaria, não existem abordagens visando resolver os problemas intragenéricos, e o conhecimento da diversidade do gênero é limitado por problemas taxonômicos e de identificação. Dessa forma, é necessário que se estabeleçam os limites entre a variação inter e intraespecífica dos pólipos, e para isso devem-se integrar informações morfológicas e moleculares. As espécies *B. cangicum*, *B. caissarum* e *B. zamponii*, endêmicas do Atlântico Sul e com diversos registros ao longo da costa, são um interessante modelo para o estudo destas variações. Portanto, os objetivos desse projeto são: 1) realizar a revisão sistemática e propor uma hipótese filogenética para o gênero *Bunodosoma* com base em evidências moleculares; 2) explorar a viabilidade da utilização do marcador molecular ITS nos estudos de populações de anêmonas e testar outros marcadores nucleares que possam representar as diferenças genéticas inter e intraespecíficas; e 3) verificar a variabilidade morfológica (utilizando dados de tamanhos de cnidocistos) e molecular intraespecífica das espécies *B. caissarum* e *B. cangicum*. A revisão sistemática, apresentada no Capítulo 1, evidenciou que 14 espécies podem ser reconhecidas, apesar de o status taxonômico de três delas permanecer aberto a testes. A análise filogenética com os dados moleculares disponíveis não fornece evidência para o monofiletismo de *Bunodosoma*, que foi recuperado parafilético em relação a outras espécies da família Actiniidae. No Capítulo 2, as análises filogenéticas e filogeográficas com o marcador ITS forneceram suporte para o reconhecimento de dois clados: o primeiro composto por indivíduos de *B. caissarum* e o segundo por indivíduos de *B. cangicum* e *B. zamponii*. Apesar de se mostrar útil na delimitação de grandes grupos, o ITS é um marcador eficiente na delimitação de populações. Em relação à variação morfométrica dos cnidocistos, os resultados apresentados no Capítulo 3 demonstram que, caso bem empregados e analisados, alguns tipos de nematocistos podem ter valor taxonômico na delimitação de populações de anêmonas. O conjunto dos resultados do presente estudo contribuem para um melhor conhecimento da sistemática e biologia desses organismos, além de gerar subsídios para a compreensão de aspectos evolutivos da ordem Actiniaria.

Palavras-chave: taxonomia, sistemática molecular, populações, cnidocysts.

Abstract

Bunodosoma is a cosmopolite genus of sea anemones of large and conspicuous polyps, common in costal environments. It currently comprises 14 species and, despite being employed in phylogenetic studies within the order Actiniaria, no study so far has tried to solve intrageneric problems in *Bunodosoma*, so the knowledge about the genus' diversity is limited by problems of taxonomy and identification. Thus, it is necessary to establish the limits of inter and intraspecific variation of the polyps. To accomplish that, integrative studies using morphological and molecular data must be carried out. The species *B. cangicum*, *B. caissarum*, and *B. zamponii* are endemic of the South Atlantic Ocean. They represent an interesting model to study the aforementioned variation. Therefore, the goals of this study are: 1) to review the systematics and to propose a phylogenetic hypothesis for the genus *Bunodosoma* based on molecular evidence; 2) to explore the usage of the molecular marker ITS in population studies of sea anemones, as well as to test other nuclear regions that may show inter and intraspecific variation; and 3) to access the phenotypic (based on cnidocyst size variation) and molecular intraspecific variability of *B. caissarum* and *B. cangicum.* The systematic review, presented in Chapter 1, showed that there are 14 recognized species; three of them need further analyses. The molecular phylogenetic analysis did not provide evidences for the monophyly of *Bunodosoma*, which was recovered paraphyletic regarding other species of the family Actiniidae. In Chapter 2, the phylogenetic and phylogeographic analysis of ITS provided support to recognize two major clades: one composed by *B. caissarum* individuals and the other by individuals of *B. cangicum* plus *B. zamponii*. Despite been useful to delimit major groups, the ITS marker is not efficient to delimit populations. Regarding the morphometric variation of the cnidocysts, the results presented in Chapter 3 demonstrated that, when employed with caution, some types may present taxonomic value in population delimitation. The results of the present study contribute to a better understanding of the systematics and the biology of *Bunodosoma*, and provide support to the comprehension of evolutionary aspects of the order Actiniaria.

Key words: taxonomy, molecular systematics, populations, cnidocysts.

INTRODUÇÃO

As anêmonas-do-mar (Filo Cnidaria, Classe Anthozoa, Ordem Actiniaria) estão entre os invertebrados mais conspícuos dos ambientes marinhos costeiros, principalmente em costões rochosos e praias arenosas. Tais animais apresentam uma grande diversidade morfológica e ecológica, sendo encontrados em diferentes tipos de habitats marinhos, em todas as profundidades e latitudes, o que faz com que estejam entre os membros mais diversos e bem sucedidos entre os antozoários (Daly *et al*., 2008).

Dentre os cnidários, os antozoários compreendem aproximadamente 7.500 espécies (Daly *et al.*, 2007), sendo a classe mais diversa do filo. Os antozoários são diagnosticados por apresentarem exclusivamente formas polipoides no ciclo de vida, actinofaringe, sifonóglife e mesentérios (Daly *et al.*, 2007). Atualmente a classe é definida como apresentando duas linhagens monofiléticas caracterizadas basicamente por meio da simetria corporal octâmera, no caso dos octocorais, e hexâmera para os hexacorais (embora variações possam existir). Stampar *et al* (2014) propõem que a ordem Ceriantharia, tradicionalmente incluída em Hexacorallia, seja considerada como um clado à parte, mas que sua posição dentro de Anthozoa ou de Cnidaria deve ser melhor estudada antes de ser definida.

Ordem Actiniaria

Um pólipo de anêmona do mar consiste basicamente em uma coluna, estrutura tubular que na maioria das espécies tem grande capacidade de contração, em cuja base (porção proximal) geralmente há uma estrutura de adesão ao substrato, o disco pedal. A porção distal

da coluna é o disco oral, na qual há uma boca oval ou em forma de fenda circundada pelo perístoma. Na ordem Actiniaria, a coluna pode apresentar estruturas (verrugas, vesículas, esférulas marginais, etc.), divisões (escapo, escapulo, capítulo, etc.) ou pode ser lisa. Na margem distal da coluna estão os tentáculos, que estão frequentemente dispostos em ciclos em torno do perístoma.

Internamente à boca em sentido distal-proximal segue a actinofaringe, uma estrutura tubular e lateralmente comprimida que faz a comunicação do ambiente externo com a cavidade gastrovascular. Esta apresenta fundo cego e é multifuncional, pois atua na digestão extracelular, circulação, excreção, reprodução e suporte por esqueleto hidrostático (Shick, 1991). As anêmonas apresentam simetria corporal bilateral ou birradial, evidenciada pelo fato de terem a faringe comprimida e pela presença de um ou dois sifonóglifes, sulcos ciliados que têm início em cantos opostos da boca e que se estendem ao longo do comprimento da faringe.

A cavidade gastrovascular é dividida em câmaras radiais por mesentérios, que são dobras longitudinais de gastroderme (endoderme) e mesogléia. Os prolongamentos distais das câmaras radiais formam os tentáculos ocos. Os tentáculos dos ciclos mais internos são denominados endocélicos, pois suas cavidades são contínuas com as câmaras formadas por mesentérios do mesmo par, denominadas endoceles. Já os tentáculos do último ciclo são exocélicos, por estarem ligados às exoceles, espaços entre mesentérios de pares distintos.

Os mesentérios ocorrem aos pares e podem ser de dois tipos: completos ou perfeitos, são os que se ligam à parede da coluna da anêmona e à actinofaringe; ou incompletos ou imperfeitos, aqueles que se ligam apenas à parede da coluna da anêmona. A margem do mesentério é denominada filamento mesentérico, e é formada por três regiões: de ambos os lados os tratos ciliados e no meio o trato cnidoglandular. Atrás do filamento mesentérico está a porção do mesentério na qual o tecido reprodutivo se desenvolve. O número de mesentérios

completos ou incompletos e a presença de tecido reprodutivo em determinados mesentérios constitui um caráter taxonômico relevante.

Os principais músculos utilizados na taxonomia de anêmonas do mar são o esfíncter marginal e os músculos retratores, parietobasilares e basilares dos mesentérios. O esfíncter marginal, quando presente, está localizado logo abaixo ou um pouco abaixo da margem distal da coluna, sendo uma camada de musculatura circular concentrada, que pode ser revestida por endoderme ou mesogléia e cuja função primária é dobrar a margem da coluna por cima dos tentáculos durante a retração do pólipo. Sua presença, localização (endoderme ou mesogléia) e forma são caracteres taxonômicos. Os músculos retratores encontram-se nas faces internas dos pares de mesentérios (exceto nos mesentérios diretivos, no qual estão nas faces externas dos pares), e se ligam ao disco oral e à base da anêmona com a função principal de puxar a parte distal do animal para baixo quando necessário. O músculo parietobasilar está na junção entre a base do mesentério e a coluna do pólipo. Suas fibras são diagonais e, geralmente, formam uma aba em cada lado do mesentério que pode variar em seu comprimento e espessura. Por fim, a musculatura basilar está na extremidade proximal dos mesentérios, paralelamente à inserção deste no disco pedal. A direção das fibras desse músculo é horizontal e auxiliam na fixação do pólipo ao substrato. Espécies que se enterram (como em Edwardsiidae – ver Daly & Ljubenkov, 2008) podem apresentar a musculatura parietal. Esta está na junção do mesentério com a coluna, mas se difere da musculatura parietobasilar por ser simétrica, não formar abas, e por seus feixes serem longitudinais. Outras descrições de morfologia de anêmonas do mar com características relevantes para a taxonomia podem ser encontradas em Stephenson (1928), Carlgren (1949), Corrêa (1964) e Shick (1991).

Dentro dos hexacorais, as anêmonas estão agrupadas na ordem Actiniaria Hertwig, 1882 (com ~ 1.100 espécies válidas, segundo Fautin *et al.*, 2007), para a qual diferentes autores apresentam distintas propostas classificatórias (*e.g.* Stephenson, 1921; Carlgren, 1949;

Schmidt, 1974, Rodríguez *et al.*, 2014). A proposta de classificação sugerida por Carlgren é historicamente a mais aceita (Daly *et al.*, 2007), e divide as anêmonas em três subordens: Protantheae Carlgren, 1891, Endocoelantheae Carlgren, 1925 e Nynantheae Carlgren, 1899. Os caracteres morfológicos utilizados na separação das subordens são: grau de desenvolvimento do disco pedal e musculatura basilar; presença e tipo de esfíncter; presença ou ausência de tratos ciliados nos filamentos mesentéricos; e arranjo dos mesentérios. No entanto, Rodríguez *et al*. (2014) sugerem que esta classificação pode não refletir a história evolutiva do grupo e, baseado em dados moleculares, propuseram a separação das anêmonas em duas subordens, Anenthemonae Rodríguez & Daly, 2014 e Enthemonae Rodríguez & Daly, 2014, que se diferenciam pelo arranjo dos mesentérios.

A monofilia de Actiniaria foi testada recentemente (Rodríguez *et al.*, 2014), apesar de alguns trabalhos já terem indicado que seus agrupamentos são polifiléticos, inclusive grande parte das famílias (*e.g.* Bernston *et al.*, 1999; Won *et al*., 2001; Daly *et al.*, 2003, 2008; Rodríguez *et al.*, 2012). De acordo com a análise de Rodríguez *et al.* (2014), a ordem Actiniaria não é monofilética devido à espécie *Relicanthus daphneae* (Daly, 2006) (que antes pertencia à infraordem Boloceroidaria Carlgren, 1924, dentro de Nynantheae) ter sido recuperada como grupo irmão de um clado que contém os membros da ordem Zoanthidea. Neste cenário, Actiniaria é caracterizada pelas seguintes características, sendo nenhuma delas exclusivas para a ordem: ausência de esqueleto, pólipos solitários, presença de apenas um ciclo marginal de tentáculos (Rodríguez *et al.* 2014). Um possível caráter fenotípico para os membros da ordem, o que também depende do posicionamento de *R. daphneae*, é a presença de abas apicais nos nematocistos, que consistem em três elementos triangulares no ápice das cápsulas que se dobram para fora no momento do disparo (ver Reft & Daly, 2012; Rodríguez *et al.*, 2014).

A subordem Enthemonae contém a maioria das espécies de Actiniaria, incluindo a maioria dos membros de Nynantheae (*sensu* Carlgren, 1949), e seus representantes apresentam o típico arranjo de mesentérios dos actiniários: ciclos de mesentérios dispostos de forma hexâmera, sendo que os pares novos de mesentérios surgem das exoceles (Rodríguez *et al*., 2014). Dentro desta subordem estão contidas três superfamílias: Actinostoloidea Carlgren, 1932, Metridioidea Carlgen, 1893 e Actinioidea Rafinesque, 1815. Esta última compreende espécies que apresentam musculatura basilar e esfíncter marginal endodérmico ou ausente. Além disso, a maioria dos membros habita ambientes rasos, e grande parte apresenta algum tipo de protuberância na coluna ou margem (vesículas, verrugas, esférulas marginais, etc.) (Rodríguez *et al.,* 2014). Actinioidea inclui membros da subtribo Endomyaria Stephenson, 1921 (*sensu* Carlgren, 1949; inclui espécies com musculatura basilar – tribo Thenaria - e que possuem esfíncter marginal endodérmico) e algumas famílias da tribo Athenaria Carlgren, 1899 (*sensu* Carlgren, 1949; maioria das espécies de anêmonas sem musculatura basilar). Carlgren (1949) dividiu Endomyaria em famílias sem a intenção de refletir a filogenia do grupo, de forma que os caracteres morfológicos escolhidos geraram agrupamentos artificiais em estudos posteriores (Daly *et al.*, 2008; Rodríguez *et al.*, 2012, 2014), apesar de muitas das famílias ainda serem válidas (Fautin, 2013).

A família Actiniidae

A família Actiniidae Rafinesque, 1815 compreende 44 gêneros e mais de 200 espécies (Daly *et al.*, 2007), sendo um dos maiores grupos da ordem. Constitui a família melhor representada em levantamentos faunísticos realizados no mundo, inclusive no Brasil (*e.g.* Zamponi *et al.*, 1998; Amaral *et al.*, 2000), e seus representantes são encontrados principalmente em ambientes de águas rasas e costões rochosos (Häussermann, 2004). Apesar

disso, assim como no restante da ordem Actiniaria, a distinção de gêneros e espécies dentro de Actiniidae é bastante complicada e muitos grupos necessitam ser reexaminados (Daly, 2003, 2004; Daly *et al.*, 2008; Gomes *et al.*, 2012).

Os representantes da família são em geral pólipos relativamente grandes (diâmetro maior que 2 cm) e algumas espécies apresentam características diagnósticas facilmente observadas. Projeções da coluna são umas das características mais relevantes para a sistemática do grupo. Tradicionalmente, estruturas na coluna como verrugas, vesículas, acrorrágios e pseudoacrorrágios são usados para distinguir os gêneros de Actiniidae, enquanto caracteres histológicos e distribuição dos tipos de nematocistos separam as espécies (Stephenson, 1921, 1935; Carlgren, 1949; Häussermann, 2004). No entanto, a simplicidade do pólipo faz com que exista um baixo número de características taxonomicamente informativas, e a escassez de descrições detalhadas e chaves de identificação dificulta o reconhecimento da grande maioria das espécies (Daly, 2003; Häussermann, 2004)

Estudos propondo filogenias moleculares para Actiniaria sugerem a parafilia da família Actiniidae (Won *et al.*, 2001; Daly *et al.*, 2003, 2008, Rodríguez *et al.,* 2014), e uma das principais causas apontadas é o fato da família ser definida, assim como de toda a ordem, com base na ausência de características ao invés da utilização de sinapomorfias (Daly *et al.* 2008; Gusmão & Daly, 2010). Outro problema encontrado nesta família é a interpretação das projeções das colunas que, além de apresentarem uma grande plasticidade (Daly, 2003, 2004; Gomes *et al.*, 2012), são geralmente difíceis de diferenciar em espécimes preservados (Häussermann, 2004). Devido a isso, as definições dos diferentes tipos de projeções da coluna são controversas o que torna difícil a tarefa de inferir uma direção para a evolução desses caracteres (Daly, 2003, 2004).

Gênero *Bunodosoma*

Segundo Fautin (2013), o gênero *Bunodosoma* Verrill, 1899 apresenta atualmente 13 espécies válidas. Estas são, em sua maioria, de águas tropicais e subtropicais e ocorrem em ambas as costas do oceano Atlântico, na costa leste do Oceano Pacífico e no Indo-Pacífico (Fautin, 2013; Gomes *et al.*, 2012). Gomes *et al.* (2012) descreveram uma nova espécie para o gênero, *Bunodosoma zamponii* Gomes, Schama & Solé-Cava, 2012, encontrada na costa da Argentina, e que até então era identificada equivocadamente como *Phymactis papillosa* (Lesson, 1830) (Gomes *et al.*, 2012). Portanto, as espécies válidas do gênero *Bunodosoma* são: *Bunodosoma biscayense* (Fisher, 1874), *Bunodosoma caissarum* Corrêa *in* Belém, 1987, *Bunodosoma californicum* (Carlgren, 1951), *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973, *Bunodosoma capense* (Lesson, 1830), *Bunodosoma cavernatum* (Bosc, 1802), *Bunodosoma diadema* (Drayton in Dana, 1846), *Bunodosoma fallax* (Pax, 1922), *Bunodosoma goanense* den Hartog & Vennam, 1993, *Bunodosoma grande* (Verrill, 1869), *Bunodosoma granuliferum* (Le Sueur, 1817), *Bunodosoma kuekenthali* Pax, 1910, *Bunodosoma sphaerulatum* Duerden, 1902 e *B. zamponii.*

Os representantes do gênero *Bunodosoma* são caracterizados pela presença de um esfíncter marginal circunscrito e endodérmico, acrorrágios, vesículas não aderentes e mesentérios férteis com musculatura bem desenvolvida (Haussermann, 2004; Gomes *et al.*, 2012). A presença de acrorrágios e vesículas não aderentes, as principais características de morfologia externa utilizadas na identificação deste gênero, são compartilhadas com os gêneros *Phymactis* Milne-Edwards, 1857 e *Phymanthea* Carlgren, 1959, e têm sido a principal causa de identificações equivocadas (Häussermann, 2004; Gomes *et al.*, 2012). A relação filogenética entre os três gêneros ainda não foi explorada.

As semelhanças entre os gêneros *Anthopleura* Duchassaing de Fobressin & Michelotti, 1860 e *Bunodosoma* foram exploradas em trabalhos direcionados para o estudo do primeiro gênero (*e.g.* Daly, 2004; Heestand, 2009). Segundo Daly (2004), *Anthopleura* é atualmente reconhecido pela presença de acrorrágios e verrugas adesivas. Daly (2003) infere que o acrorrágio é homólogo entre os membros dos gêneros *Actinia* Linnaeus, 1767, *Anemonia* Risso, 1826, *Anthopleura* e *Bunodosoma,* e outros trabalhos sugerem que *Anthopleura* é parafilético com relação a *Bunodosoma* (McCommas, 1991; Daly, 2004). Segundo Daly (2004), as vesículas arredondadas e não adesivas são uma característica derivada compartilhada (sinapomorfia) de *Bunodosoma*, enquanto as verrugas adesivas, encontradas em *Anthopleura*, seriam a condição primitiva compartilhada (simplesiomorfia) deste caráter.

Häussermann (2004) fornece uma lista de questões dentro do gênero *Bunodosoma* que necessitam ser analisadas. Segundo a autora algumas das espécies atualmente válidas necessitam ser reexaminadas, pois podem pertencer a outro gênero, como é o caso de *B. capense* (Lesson, 1830). *B. fallax* (Pax, 1922) tem um *status* desconhecido, pois a autora afirma que o material depositado em coleção não permite identificar o espécime como sendo pertencente ao gênero *Bunodosoma.* Já a espécie *B. biscayense* pode pertencer ao gênero *Anthopleura* (Häussermann, 2004; Daly, 2004), pois apresenta atributos anatômicos característicos desse gênero: os acrorrágios e as verrugas adesivas. O problema da espécie *B. cavernatum* (Bosc, 1802) foi apontado por McCommas (1991), que observou espécimes capazes de aderir pedaços de conchas quebradas às suas "vesículas" que, quando analisadas em cortes finos, mostram a aparência esperada de uma verruga adesiva.

O gênero *Bunodosoma* é comumente representado nas propostas de filogenia que utilizam dados moleculares (McCommas, 1991; Daly, 2004; Daly *et al.*, 2008; Rodríguez *et al.,* 2014), no entanto, a amostragem taxonômica é bastante restrita. McCommas (1991)

incluiu *B. cavernatum*, *B. californicum* e *B. granuliferum* em suas análises, enquanto a análise de Daly (2004) incluiu as duas primeiras espécies e *B. biscayense*. Daly *et al*. (2008) e Rodríguez *et al.* (2014) incluíram apenas *B. grande* (Verrill, 1869) em sua análise da ordem Actiniaria. As espécies brasileiras do gênero, *B. cangicum* e *B. caissarum*, e a argentina *B. zamponii* foram incluídas apenas nas análises de Gomes *et al.* (2012). Ou seja, os problemas do gênero não foram foco de nenhum trabalho e o conhecimento de sua diversidade é limitado por problemas taxonômicos e de identificação.

Cnidocistos como caracteres taxonômicos

A utilização das cnidas (espirocistos, pticocistos e nematocistos) como ferramenta para estudos filogenéticos em Cnidaria sempre entusiasmou os pesquisadores principalmente devido à complexidade e variação encontrada nestas organelas, o que contrasta com a simplicidade estrutural da maioria dos representantes do filo (Fautin, 2009). Os estudos para a diferenciação de nematocistos (onde a variabilidade é maior) em Cnidaria iniciaram com trabalhos em Actiniaria de Möbius (1866), Bedot (1896) e Will (1909 *apud* Shostak *et al.*, 2005) e na ordem Trachymedusae com Iwanzoff (1895 *apud* Shostak *et al*., 2005). Contudo, Weill (1934) foi o primeiro a propor uma classificação formal baseando-se na forma e tamanho das cápsulas dos nematocistos e da ornamentação (espinhos) que pode estar presente ou não nos filamentos. O sistema de classificação proposto por Weill (1934) sofreu diversas modificações desde sua publicação (*e.g.* Werner, 1965; Mariscal, 1974; England, 1991; Östman, 2000). Especificamente para Anthozoa, Schmidt (1972, 1974) criou uma nova proposta ao realizar um estudo detalhado dos nematocistos levando em conta as variações dos arranjos dos espinhos na base do túbulo do nematocisto. Schmidt (1974) propôs uma filogenia para Anthozoa baseando-se em sua proposta de classificação dos nematocistos. Sua proposta

de nomenclatura é até hoje utilizada por alguns autores e para alguns grupos específicos, como para as anêmonas Acontiaria (Acuña *et al.*, 2003). No entanto, a terminologia proposta por Weill (1934), com suas subsequentes modificações, é a mais utilizada em trabalhos recentes de descrição de anêmonas (*e.g.* Rodriguez *et al.*, 2009; Gusmão, 2010). Östman (2000) realizou a mais recente redefinição de categorias de nematocistos baseando-se principalmente na classificação de Weill e nas posteriores modificações realizadas por Carlgren (1940, 1945), Cutress (1955), Mariscal (1974) e Calder (1977 *apud* Östman, 2000). Estudos continuam sendo realizados para tentar melhorar a compreensão da evolução das formas dos cnidocistos (*e.g.* Reft *et al.*, 2009).

Os representantes de Anthozoa apresentam menos tipos de nematocistos do que os medusozoários (Fautin, 2009), mas, apesar disso, as cnidas são parte essencial da sistemática deste primeiro táxon. Segundo a proposta de classificação de Weill (1934) os diferentes tipos de nematocistos podem ser divididos em 16 categorias agrupadas em dois conjuntos mais amplos: haplonemos e heteronemos. Os haplonemos se caracterizam por apresentarem filamento e espinhos (quando presentes) sem distinção de regiões, enquanto os heteronemos sempre possuem espinhos e um espessamento basal no filamento denominado *shaft* (ressalta-se que na literatura em língua portuguesa raramente se utilizam termos específicos para as diferentes partes dos nematocistos, e, quando isso ocorre, preferencialmente se utilizam os termos em inglês). Dentre os haplonemos há dois tipos: os átricos, que não apresentam espinhos, e os holótricos, que apresentam espinhos geralmente ao longo de todo o comprimento do filamento. O holótricos por sua vez podem ser isorriza ou anisorriza, sendo que estes últimos apresentam uma gradual, porém óbvia mudança no diâmetro ou nos espinhos ao longo de seu comprimento.

Entre os heteronemos há duas características importantes que definem a nomenclatura do nematocisto: o comprimento do filamento em comparação com a cápsula e a forma da

região que conecta o *shaft* ao restante do filamento. O nematocisto é denominado macrobásico quando o *shaft* é mais longo que a cápsula (e encontra-se dobrado dentro da cápsula não disparada), e microbásico quando o *shaft* é mais curto que a cápsula. Se o *shaft* for mais largo que o restante do filamento e na junção entre as duas partes formar-se um funil em forma de V, bastante característico na cnida não disparada, ele é denominado p-mastigóforo. Caso essa forma de funil esteja ausente, ele será denominado b-mastigóforo ou basítrico, sendo que este último apresentará espinhos na base do *shaft* e o primeiro não. Caso ocorra um nematocisto *p*-mastigóforo em que não exista filamento após o *shaft* este é denominado amastigóforo. A modificação mais relevante proposta por Östman (2000) para esses nematocistos foi a modificação do termo amastigóforo para *p*-amastigóforo. Dessa forma geram-se os seguintes nomes para os nematocistos: átricos, holótricos isorrizas e anisorrizas, macrobásico *p*-mastigóforo, macrobásico *b*-mastigóforo, macrobásico *p*-amastigóforo, microbásico *p*-mastigóforo, microbásico *b*-mastigóforo, microbásico *p*-amastigóforo e basítrico. Todos esses tipos de cnidas são possíveis de serem encontrados em anêmonas do mar, além dos espirocistos.

Denomina-se cnidoma o conjunto de cnidocistos presentes nas diversas estruturas corporais de um indivíduo ou espécie de cnidário. Na descrição de uma espécie de anêmona do mar informações detalhadas referentes ao cnidoma são essenciais, tanto com relação aos tipos de cnidocistos encontrados quanto seus tamanhos (Fautin, 1988; 2009) e especifica-se o conjunto de cnidocistos para cada região do pólipo (tentáculos, coluna, actinofaringe, filamentos, especializações). Um dos motivos para isso é que a ordem Actiniaria é a que possui o cnidoma mais variado entre os demais Anthozoa (Schmidt, 1974). Em decorrência desse fato as cnidas se tornaram características taxonômicas importantes dentre as anêmonas não somente para definir táxons menos inclusivos como também para identificar espécies em

grupos complexos (ver exemplos em Manuel, 1981). No entanto, a tradição em se dar um grande peso taxonômico ao cnidoma gera bastante discussão.

O valor dos dados de distribuição qualitativa (tipos de nematocistos) nos tecidos para definir espécies ou táxons superiores é constantemente questionado, pois se sabe que existem fatores biológicos que podem afetar as categorias de cnidas que uma espécie ou indivíduo apresenta. Um exemplo disso é o nematocisto do tipo holótrico: algumas espécies são definidas pela presença deste nematocisto em seus tentáculos ou em estruturas especializadas. No entanto, Watson & Mariscal (1983) observaram que em *Diadumene lineata* (= *Haliplanella luciae)* eles aparecem em resposta a condições externas (agressão intraespecífica), quando há formação dos *catch-tentacles*. Além disso, há diversos casos de presença de uma determinada cnida em somente parte de uma população (Fautin, 1988). Já o uso de informações referentes ao tamanho das cnidas é ainda mais problemático. A apresentação destes valores é uma prática bem estabelecida, mas muitos autores discutem a importância destes dados (Fautin, 1988). Uma das principais justificativas para se a utilização destes é que se presume que as cnidas tenham seu crescimento determinado e, portanto, só são reconhecidas depois de estarem prontas, podendo ter um tamanho mais ou menos definido para uma determinada espécie (Fautin, 1988). No entanto, enquanto Ardelean & Fautin (2004) observaram que o tamanho do nematocisto em um mesmo tecido tem tamanhos semelhantes, Acuña *et al.*, (2003) registram a alta variabilidade nos tamanhos dos nematocistos entre indivíduos da mesma espécie. Além disso, já se demonstrou que o tamanho da cnida pode variar com a idade ou tamanho dos pólipos (Francis, 2004; Acuña *et al*. 2003) e com a variação em parâmetros ecológicos ou geográficos, como profundidade ou latitude (Zamponi & Acuña, 1994). Como o tamanho das cnidas pode ser afetado por características intrínsecas ao indivíduo e pelas condições do hábitat, as informações quantitativas (tamanho) das cnidas são as que causam maior desconfiança entre os pesquisadores.

Tantos impedimentos levam à conclusão de que não se deve dar um peso taxonômico muito grande para o cnidoma. No entanto, devido à existência de tão poucas alternativas de caracteres taxonômicos em anêmonas, o potencial dos cnidocistos como indicador de informações filogenéticas ou filogeográficas ainda deve ser estudado e melhor compreendido. Uma abordagem ainda pouco explorada para se delimitar variações interespecíficas, interpopulacionais e interindividuais é a integração de dados de morfologia do pólipo e cnidoma com dados moleculares. Poucas famílias ou gêneros têm sido foco de estudos filogenéticos (*e.g.* Gusmão, 2010) e estudos levando em conta as variações populacionais são extremamente escassos (*e.g.* Gomes, 2002). Neste contexto, o gênero *Bunodosoma* pode ser uma fonte de informações bastante interessantes para elucidar diversas questões, já que as espécies *B. caissarum* e *B. cangicum* apresentam distribuição ampla ao longo da costa brasileira, o que possibilita a observação de variações populacionais ao longo de uma ampla escala geográfica.

Dificuldades no estudo das anêmonas do mar e o uso de dados moleculares

O fato de as anêmonas apresentarem um pólipo bastante simples, aliado à elevada riqueza em espécies, faz com que os taxonomistas do grupo tenham dificuldade para estabelecer caracteres taxonômicos suficientes para serem utilizados na separação dos táxons (Fautin, 1988). Como comentado anteriormente, as descrições atuais de espécies de anêmonas incluem caracteres taxonômicos baseados na morfologia e anatomia do pólipo, além de informações sobre os tipos e medidas (variação e média do tamanho) das cnidas presentes em cada região do pólipo. Há, no entanto, controvérsias a respeito da forma com que os caracteres têm sido utilizados tanto na separação de espécies quanto de grupos em níveis hierárquicos mais elevados (Daly *et al.*, 2007).

Certos caracteres de morfologia do pólipo aos quais tem se dado valor taxonômico, como musculatura do esfíncter marginal e projeções da coluna, estão sujeitos a reavaliações ou são difíceis de serem distinguidos, o que torna sua utilização bastante problemática (Daly *et al.*, 2007). Alguns autores vêm sugerindo novas formas de estudar as cnidas, tanto com relação às características morfológicas dos diferentes tipos de nematocistos, com a utilização da técnica de microscopia eletrônica de varredura e transmissão (*e.g.* Reft *et al*., 2009), quanto com relação à análise das medidas, com a utilização de tratamentos estatísticos mais adequados como os Modelos Lineares Generalizados (em inglês GLM) (Nelder & Wedderburn, 1972) (*e.g.* Acuña *et al.*, 2004). Esses dois tipos de abordagens ainda são pouco utilizadas nos estudos dentro do grupo e, portanto, sua eficácia na resolução dos problemas do estudo do cnidoma ainda não é bem conhecida.

Poucas famílias ou gêneros têm sido foco de estudos filogenéticos (*e.g.* Daly, 2004; Gusmão, 2010), e há uma grande necessidade de revisões de espécies e gêneros, para que se possa definir quais os limites entre a variação inter e intraespecífica (Rodriguez *et al*., 2012). A alta plasticidade morfológica encontrada em espécies de anêmonas aliada à falta de um consenso para a filogenia baseada em caracteres morfológicos faz com que a utilização de dados moleculares seja uma ferramenta importante (Daly *et al.*, 2010). Portanto, no panorama atual dos estudos em Actiniaria é fundamental a realização de estudos que integrem informações morfológicas e dados moleculares para que seja possível a determinação de uma espécie de anêmona.

Estudos moleculares tem se mostrado particularmente interessantes para resolver questões de delimitação de espécies de anêmonas, pois muito do que se considera como sendo variação morfológica entre indivíduos de uma mesma espécie pode representar de fato diferenças entre espécies (Knowlton, 2000; Gomes *et al.*, 2012). Problemas de definição de espécies em grupos que apresentam poucos caracteres taxonômicos e grande plasticidade

morfológica ocorrem em outros grupos de Cnidaria, como, por exemplo, Zoantharia (Sinniger *et al.*, 2008), Ceriantharia (Stampar *et al*., 2012) e Scyphozoa (Dawson, 2005).

A classe Anthozoa apresenta uma das taxas de relógio molecular mais baixas dentre os metazoários para marcadores mitocondriais (Shearer *et al.,* 2002). Entretanto, a utilização de marcadores nucleares vem se mostrando extremamente efetiva para a delimitação de espécies em Anthozoa (Torres-Pratts *et al*., 2011; Stampar *et al*., 2012). A estimativa de divergências acumuladas nas regiões espaçadoras (ITSs) dos genes ribossomais (rDNA nuclear) permite, muitas vezes, a detecção de diferenças entre espécimes coespecíficos e portanto, pode ser uma ferramenta útil para o estudo de populações ou espécies proximamente relacionadas de antozoários (referências em Daly *et al.*, 2010). A variação do marcador ITS foi explorada em alguns grupos de anêmonas até o momento, apresentando uma grande diversidade intraespecífica e, até mesmo, intragenômica nas espécies *Diadumene lineata* (Verrill, 1869) (Ting & Geller, 2000) e *Condylactis gigantea* (Weinland, 1860) (Stoletzki & Schierwater, 2005). Porém, estes marcadores apresentaram pouca variação intraespecífica em espécies dos gêneros *Aulactinia* Verrill, 1864 (Acuña *et al*., 2007) e *Calliactis* Verrill, 1869 (Gusmão, 2010), sendo, portanto, necessário que outros marcadores moleculares sejam explorados para reforçar ou se contrapor aos resultados encontrados para o marcador ITS.

As espécies endêmicas da América do Sul, *B. caissarum* e *B. cangicum,* apresentam uma ampla distribuição ao longo da costa brasileira e um estudo integrando informações morfológicas e moleculares em suas populações pode auxiliar na compreensão dos limites entre a variação interespecífica, entre populações e entre indivíduos de uma mesma espécie. Gomes (2002) estudou características morfológicas e genéticas (isoenzimas e ITS) de populações de *B. cangicum*, *B. caissarum* e *B. zamponii*, e relacionou estes fatores a características biológicas, ecológicas e de distribuição destas espécies. No atual estudo, complementamos seus resultados com amostragem mais ampla das espécies brasileiras,

buscamos novos marcadores moleculares de interesse para estudos em nível populacional e específico em anêmonas do mar, e relacionamos isso com a variação do tamanho dos cnidocistos entre populações, um conjunto de estudos essencial para compreensão das variações entre espécies próximas e a variação intraespecífica em anêmonas do mar.

Estrutura da Tese

No Capítulo 1, apresento a revisão taxonômica do gênero *Bunodosoma*, realizada por meio de observações morfológicas, anatômicas e de cnidoma, e proponho uma hipótese filogenética para parte do gênero *Bunodosoma*, utilizando dados moleculares. No Capítulo 2, testo a viabilidade da utilização do marcador ITS nos estudos de populações de *Bunodosoma*, além de testar outros dois marcadores moleculares nucleares com o intuito de representar as diferenças genéticas existentes entre espécies, populações e indivíduos de uma mesma população de *B. caissarum*, *B. cangicum* e *B. zamponii*. No Capítulo 3, dados de morfometria dos cnidocistos são usados para buscar diferenças entre as populações de *B. caissarum* e *B. cangicum.* Desta forma, analisamos a variação dentro do gênero *Bunodosoma* e propomos que seus resultados sejam utilizados como modelo a ser testado na delimitação de espécies e populações de outras anêmonas do mar.

Referências Bibliográficas

Acuña, F. H.; Excoffon, A. C.; Zamponi, M. O. & Ricci, L. 2003. Importance of nematocysts in taxonomy of acontiarian sea anemones (Cnidaria, Actiniaria): a statistical comparative study. *Zoologischer Anzeiger* 242: 75 – 81.

Acuña, F. H.; Ricci, L; Excoffon, A. C. & Zamponi, M. O. 2004. A novel statistical analysis of cnidocysts in acontiarian sea anemones (Cnidaria, Actiniaria) using generalized linear models with gamma errors. *Zoologischer Anzeiger* 243: 47 - 52.

Acuña, F. H.; Excoffon, A. C.; McKinstry, S. R. & Martínez, D. E. 2007. Characterization of Aulactinia (Actiniaria: Actiniidae) species from Mar del Plata (Argentina) using morphological and molecular data. *Hydrobiologia* 592: 249 - 256.

Amaral, F. D.; Hudson, M.M.; da Silveira, F. L.; Migotto, A.E.; Pinto, S.M. & Longo, L. 2000. Cnidarians of Saint Peter and St. Paul Archipelago, Northeast Brazil. In: *International Coral Reef Symposium, 9. Bali: International Coral Reef Society* 1: 567 –572.

Ardelean, A. & Fautin, D. G. 2004. Variability in nematocysts from a single individual of the sea anemone Actinodendron arboretum (Cnidaria: Anthozoa: Actiniaria). *Hydrobiologia* 530/531: 189 – 197.

Bedot, M. 1896. Note sur les cellules urticantes. *Revue Suisse Zoologie* 3: 533-539.

Berntson, E. A.; France, S. C. & Mullineaux, L. S. 1999. Phylogenetic relationships within the class Anthozoa (phylum Cnidaria) based on nuclear 18S rDNA sequences. *Molecular Phylogenetics and Evolution* 13: 417 – 433.

Carlgren, O. 1940. A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. *Kungliga Fysiografiska Sällskapets Handlingar* 51 (3): 1 – 62.

Carlgren, O. 1945. Further contributions to the knowledge of the cnidom in the Anthozoa especially in the Actiniaria. *Kungliga Fysiografiska Sällskapets Handlingar* 56 (9): 1 – 24.

Carlgren, O. 1949. A Survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskapsakademiens Handlingar* 1(1): 1 – 121.

Corrêa D.D. 1964. *Corallimorpharia e Actiniaria do Atlântico Oeste Tropical*. Universidade de São Paulo, Ph.D Thesis, São Paulo, Brazil, 139 p.

Cutress, C.E. 1955. An interpretation of the structure and distribution of Cnidae in Anthozoa. *Systematic Zoology* 4: 120-137.

Daly, M. 2003. The anatomy, terminology, and homology of acrorhagi and pseudoacrorhagi in sea anemones. *Zoologishe Mededelingen* 345: 89 – 102.

Daly, M. 2004. Phylogeny and biogeography of Anthopleura in the North Atlantic Ocean. *Hydrobiologia* 530/531: 241 – 248.

Daly, M.; Fautin, D. G. & Cappola, V. A. 2003. Systematics of the Hexacorallia (Cnidaria: Anthozoa). *Zoological Journal of the Linnean Society* 139: 419 – 437.

Daly, M.; Brugler, M. R.; Cartwright, P.; Collins, A.G.; Dawson, M. N.; Fautin, D.; France, S. C.; McFadden, C.S.; Opresko, D. M.; Rodriguez, E.; Romano, S. & Stake, J. 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. In: Zhang, Z.-Q. & Shear, W.A. (Eds). Linnaeus Tercentenary: Progress in Invertebrate Taxonomy. *Zootaxa* 1668: 127 - 182.

Daly, M.; Chaudhuri, A.; Gusmão, L. & Rodríguez, E. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular Phylogenetics and Evolution* 48: 292 – 301.

Daly, M.; Gusmão, L. C.; Reft, A. B.; Rodríguez, E. 2010. Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (Cnidaria, Actiniaria). *Integrative and Comparative Biology* 50 (3): 371 – 388.

Daly, M. & Ljubenkov, JC. 2008 Edwardsiid sea anemones of California (Cnidaria: Actiniaria: Edwardsiidae), with descriptions of eight new species. *Zootaxa* 1860: 1–27

Dawson, M. N. 2005. Renaissance taxonomy: integrative evolutionary analyses in the classification of Scyphozoa. *Journal of the Marine Biological Association of the United Kingdom* 85: 733 – 739.

England, K. W. 1991. Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia: Cnidaria): nomenclature. *Hydrobiologia* 216/217: 691 – 697.

Fautin, D. G. 1988. Importance of nematocysts to actinian taxonomy. In: Hessinger, D. A. & Lenhoff, H. M (Ed.). *The Biology of Nematocysts*. San Diego and other cities: Academic Press. p. 487 – 500.

Fautin, D. G., Zelenchuk, T. & Raveendran, D. 2007. Genera of orders Actiniaria and Corallimorpharia (Cnidaria, Anthozoa, Hexacorallia), and their type species. *Zootaxa* 1668: 183 - 244.

Fautin, D.G. 2009. Structural diversity, systematics, and evolution of cnidae. *Toxicon* 54: 1054–1064.

Fautin, D. G. 2013. Hexacorallians of the World. Avaliable at: <http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm> [Access on May, 10th , 2016]

Francis, L. 2004. Microscaling: Why larger anemones have longer cnidae. *Biological Bulletin* 207: 116 - 129.

Gomes, P.B. 2002. Estudio de la vicariancia en algunas especies de anémonas de mar (Cnidaria, Actiniaria) del intermareal de Brasil y Argentina con el uso de datos morfológicos y genéticos. Ph.D Thesis – Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. 101pp.

Gomes, P.B.; Schama, R. & Solé-Cava. 2012. Molecular and morphological evidence that Phymactis papillosa from Argentina is, in fact, a new species of the genus Bunodosoma (Cnidaria: Actiniidae*). Journal of the Marine Biological Association of the United Kingdom* 92 (5): 895–910.

Gusmão, L. C. 2010. Systematics and evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. Ph.D. Thesis - The Ohio State University, USA. 337 pp.

Gusmão, L. C. & Daly, M. 2010. Evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. *Molecular Phylogenetics and Evolution* 56: 868 – 877.

Häussermann, V. 2004. Re-description of Phymactis papillosa (Lesson, 1830) and Phymanthea pluvia (Drayton in Dana, 1846) (Cnidaria:Anthozoa), two common actiniid sea anemones from the south Pacific with a discussion of related genera. *Zoologische Mededelingen* 78: 345-381.

Heestand, E.N. 2009. Phylogeny and Evolution of Anthopleura (Cnidaria:Anthozoa:Actiniaria). MSc dissertation - The Ohio State University. 42 pp.

Knowlton, N. 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* 420: 73-90.

Manuel, R. L. 1981. British Anthozoa - *Synopses of the British Fauna*: new series 18. London: Academic Press. 241 pp.

Mariscal, R. N. 1974. Nematocysts. In: Muscatine, L. & Lenhoff, H. M. (Eds.). *Coelenterate Biology. Reviews and New Perspectives*. New York: Academic Press. pp. 129 – 178.

McCommas, S.A. 1991. Relationships within the family Actiniidae (Cnidaria, Actiniaria) based on molecular characters. *Hydrobiologia* 216/217: 509-512.

Möbius, K.A., 1866. Ueber den Bau, den Mechanismus und die Entwicklung der Nesselkapseln einiger Polypen und Quallen. *Abhandlungen aus dem Gebiete der Naturwissenschaften* Ver. Hamburg V(1): 1-22, pls. 1-2.

Nelder, J. A. & Wedderburn, R. W. M. 1972. Generalized Linear Models. *Journal of the Royal Statistical Society* 135 (3): 370- 384.

Östman, C. 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. *Scientia Marina* 64 (Supl. 1): 31 – 46.

Reft, A. J.; Westfall, J. A. & Fautin, D. G. 2009. Formation of the apical flaps in nematocysts of sea anemones (Cnidaria: Actiniaria). *Biological Bulletin* 217: 25 - 34.

Reft, A. & Daly, M. 2012. Morphology, distribution, and evolution of apical structure of nematocysts in Hexacorallia. *Journal of Morphology* 273: 121–136.

Rodríguez, E.; López-González, P. J. & Daly, M. 2009. New family of sea anemones (Actiniaria, Acontiaria) from deep polar seas. *Polar Biology* 32: 703 - 717.

Rodríguez, E.; Barbeitos, M.; Daly, M.; Gusmão, L.C.; Hausserman, V. 2012. Towards a natural classification: phylogeny of Acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). *Cladistics* 28: 375 – 392.

Rodríguez, E; Barbeitos, MS; Brugler, MR; Crowley, LM; Grajales, A; Gusmão, L; Häussermann, V.; Reft, A. & Daly, M. 2014. Hidden among Sea Anemones: The First Comprehensive Phylogenetic Reconstruction of the Order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) Reveals a Novel Group of Hexacorals. *PLoS ONE* 9(5): e96998. doi:10.1371/journal.pone.0096998

Shick J. M. 1991. *A Functional Biology of Sea Anemones*. London: Chapmann and Hall.

Schmidt, H. 1972. Die nesselkapseln der Anthozoen und ihre bedeutung für die phylogenetische systematik. *Helgoländer wissenschaftliche Meeresuntersuchungen* 23: 422 – 458.

Schmidt, H. 1974. On evolution in Anthozoa. *Proceedings of the 2nd International Coral Reef Symposium* 1: 533 – 560.

Shearer, T.L.; van Oppen, M. J. H.; Romanos, S. L. & Wörheide, G. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology* 11: 2475-2487.

Shostak, S. 1993. A symbiogenetic theory for the origins of cnidocysts in Cnidaria. *Biosystems* 29: 49-58.

Sinniger, F.; Reimer, J. D. & Pawlowski, J. 2008. Potential of DNA sequences to identify Zoanthids (Cnidaria: Zoantharia). *Zoological science* 25: 1253 – 1260.

Stampar, S.N.; Maronna, M.M.; Vermeij, M.J.A.; Silveira, F.L. & Morandini, A.C. 2012. Evolutionary diversification of banded tube-dwelling anemones (Cnidaria; Ceriantharia; Isarachnanthus) in the Atlantic Ocean. *PLoS One* 7: e41091.

Stampar, S.N., Maronna MM, Kitahara MV, Reimer JD & Morandini AC. 2014. Fast-Evolving Mitochondrial DNA in Ceriantharia: A Reflection of Hexacorallia Paraphyly? *PLoS ONE* 9(1): e86612. doi:10.1371/journal.pone.0086612

Stephenson, T. A. 1921. On the classification of Actiniaria. Part II. Consideration of the whole group and its relationships, with special reference to forms not treated in Part I. *Quarterly Journal of Microscopical Sciences* 65: 493 - 576.

Stephenson, T.A. 1928. *The British Sea Anemones - volume I.* London: The Ray Society. 148 pp.

Stephenson, T.A. 1935. *The British Sea Anemones - volume II*. London: The Ray Society. 426 pp.

Stoletzki, N. & Schierwater, B. 2005. Genetic and color morph differentiation in the Caribbean sea anemone Condylactis gigantea. *Marine Biology* 147: 747 -754.

Ting, J. H. & Geller, J. B. 2000. Clonal diversity in introduced populations of an asian sea anemone in North America. *Biological Invasions* 2: 23 - 32.

Torres-Pratts, H.; Lado-Insua,T.; Rhyne, A.; Rodriguez-Matos, L. & Schizas, N. 2011. Two distinct, geographically overlapping lineages of the corallimorpharian Ricordea florida (Cnidaria: Hexacorallia: Rocordeidae). *Coral Reefs* 30: 391 – 396.

Watson, G. M. & Mariscal, R. N. 1983. The development of a sea anemone tentacle specialized for aggression: morphogenesis and regression of the catch-tentacle of Haliplanella luciae (Cnidaria, Anthozoa). *Biological Bulletin* 164 (3): 506 – 517.

Weill, R. 1934. Contribution à l'etude des cnidaires et leurs nématocyctes I e II. *Travaux de la Station Zoologique de Wimeraux* 10 - 11: 1 – 701.

Werner, B. 1965. Die Nesselkapseln der Cnidaria, mit besonderer Beriicksichtigung der Hydroida. I. Klassifikation und Bedeulung für die Systematik und Evolution. *Helgoländer Wissenschaftliche Meeresuntersuchungen* 12: 1 – 39.

Won, J. H.; Rho, B. J. & Song, J-I. 2001. A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. *Coral Reefs* 20: 39 – 50.

Zamponi, M. O. & Acuña. F. H. 1994. La variabilidad de los cnidocistos y su importancia en la determinación de clines. *Physis* 49: 7 – 18.

Zamponi, M. O; Belém, M. J. C.; Schlenz, E & Acuña, F. H. 1998. Distribution and some ecological aspects of Corallimorpharia and Actiniaria from shallow waters of the South American Atlantic coasts. *Physis, Secc. A* 55: 31 - 45.

Capítulo 1

Systematic review and phylogeny of the sea anemone genus *Bunodosoma* Verrill, 1899 (Anthozoa: Actiniaria: Actiniidae)

Abstract

Bunodosoma is a worldwide-distributed genus of sea anemones, currently composed of 14 valid species. Its representatives are relatively large and conspicuous solitary polyps found in rocky shores. Although members of the genus are commonly used in phylogenetic studies of the order Actiniaria, there are no studies solving intrageneric species boundaries. Thus, the knowledge on diversity of the genus is limited by taxonomical and identification problems. A revision of the genus would allow a better comprehension of the evolution of Actiniaria, and also will help to understand the importance of polyp's column projections. Therefore, we performed a systematic review and proposed a phylogenetic hypothesis for *Bunodosoma* based on morphological and molecular data. This study increases the knowledge on systematics and biology of these organisms, besides generating information that will help the comprehension of some important evolutionary aspects among the order Actiniaria.

Key words: taxonomy, morphology, molecular systematics

Introduction

Sea anemones (phylum Cnidaria, class Anthozoa) are among the most conspicuous invertebrates in coastal marine environments, especially rocky shores and sandy beaches. They are grouped in the order Actiniaria Hertwig, 1882, and accounting ~ 1,200 species for which there are some different classification proposals (*e.g.* Stephenson, 1921; Carlgren, 1949; Schmidt, 1974, Rodríguez *et al.*, 2014). The system proposed by Carlgren (1949) has been, historically, the most followed (Daly *et al.*, 2007) and divides sea anemones into three suborders: Protantheae Carlgren, 1891, Endocoelantheae Carlgren, 1925 and Nynantheae Carlgren, 1899. The morphological characters used in this suborder division are: level of development of the pedal disc and basilar muscles; presence and type of marginal sphincter muscle; presence or absence of ciliated tracts on the mesenterial filaments; and arrangement of the mesenteries (Daly *et al.*, 2010). Rodríguez *et al*. (2014) found that this classification does not reflect the evolutionary history of the group and, based on molecular data, proposed the separation of the sea anemones into two suborders, Anenthemonae Rodríguez & Daly, 2014 and Enthemonae Rodríguez & Daly, 2014, based in the arrangement of the mesenteries.

The monophyly of Actiniaria was not specifically tested until recently (Rodríguez *et al.* 2014), although based on molecular data many of its traditional groupings had been shown to be para or polyphyletic, including most families (*e.g.* Bernston *et al.*, 1999; Won *et al*., 2001; Daly *et al.*, 2003, 2008; Rodríguez *et al.*, 2012). According to the analysis of Rodríguez *et al.* (2014), Actiniaria is not monophyletic because *Relicanthus daphneae* (Daly, 2006) (previously belonging to the infraorder Boloceroidaria Carlgren, 1924, within Nynantheae) is recovered as sister-group to the clade containing members of order Zoanthidea. In this scenario, Actiniaria is characterized by the following features, none of them unique for the order: lack of skeleton, solitary polyps, and only one marginal ring of tentacles (Rodríguez *et*

al. 2014). A possible unique phenotypic feature for members of the order, which also depends on resolving the position of *R. daphneae* within the order, are apical flaps on the nematocysts, three triangular elements in the apex of the capsules that flex outward during discharge (Reft & Daly, 2012; Rodríguez *et al.*, 2014).

The suborder Enthemonae comprises the majority of actiniarians, including most members of Nynantheae (*sensu* Carlgren, 1949), and its representatives have the typical arrangement of mesenteries for actiniarians (primarily hexamerous cycles in which pairs of mesenteries arise in the exocoels) (Rodríguez *et al*., 2014). It contains three superfamilies: Actinostoloidea Carlgren, 1932, Metridioidea Carlgen, 1893 and Actinioidea Rafinesque, 1815. The latter is comprised of species that, as a rule, have basilar muscles and endodermal or no marginal sphincter muscle. Also, most members are shallow-water forms and many of them have some type of column or marginal protuberance (vesicles, verrucae, spherules, etc.) (Rodríguez *et al.,* 2014). Actinioidea includes members of former Endomyaria Stephenson, 1921 (species with endodermal marginal sphincter) and some families of the formerly Athenaria Carlgren, 1899 (most species without basilar muscles). Carlgren (1949) divided Endomyaria into families with no intention of reflecting the phylogeny of the groups, so the morphological characters chosen generated artificial groups (Daly *et al.*, 2008; Rodríguez *et al.*, 2012, 2014), although most families are still valid (Fautin, 2013).

The family Actiniidae Rafinesque, 1815 comprises 44 genera and over 200 species (Daly *et al.*, 2007), being one of the largest groups within the order. It constitutes the most well sampled families in faunistic surveys all around the world, including Brazil (*e.g.* Zamponi *et al.* 1998a; Amaral *et al.*, 2000) and its representatives are found mainly in shallow waters and rocky shores (Häussermann, 2004). Nevertheless, as in the rest of the order, the distinction between many genera and species is quite poorly resolved and many groups must be reexamined (*e.g*., Daly, 2003, 2004; Daly *et al.*, 2008; Gomes *et al.*, 2012). Most members

of the family Actiniidae are large polyps (diameter larger than 2cm) and some species have conspicuous diagnostic features (*e.g.* vesicles or verrucae). Structure of the column and marginal projections are one of the most relevant characteristics for the systematics of the group. In general, structures as verrucae, vesicles, acrorhagi and pseudoacrorhagi are used to distinguish genera within Actiniidae whereas histological and cnidae characters separate species (Stephenson, 1921, 1935; Carlgren, 1949; Häussermann, 2004). However, the scarcity of detailed definitions of the structures, descriptions and identification keys makes the recognition of most species even more difficult (Daly, 2003; Häussermann, 2004).

Molecular phylogenies for Actiniaria have demonstrated that Actiniidae is a paraphyletic group (Won *et al.*, 2001; Daly *et al.*, 2003, 2008, Rodríguez *et al.,* 2014), and one of the main causes pointed out is the fact that the family, as the entire order, is defined by the absence of features instead of synapomorphies (Daly *et al.* 2008; Gusmão & Daly, 2010). Another problem found in this family is the interpretation of column projections that, besides presenting great plasticity (Daly, 2003, 2004; Gomes *et al.*, 2012), are usually quite difficult to differentiate in preserved specimens (Häussermann, 2004). Thus, the definitions of the types of column projections are controversial, which makes it difficult to propose any hypothesis for the evolution of such characters (Daly, 2003, 2004). According to Daly (2004), vesicles and verrucae are both hollow columnar outgrowths, but they bear some differential characteristics that can be observed in histological sections. On one hand, the adhesive verrucae (as in *Anthopleura* Duchassaing de Fonbressin & Michelotti, 1860) are cup-shaped, and have a specialized region (upper part of the cup) with a bilayered appearance of ectoderm near the junction with the mesoglea, and thin, densely packed epitheliomuscular cells in the ectoderm. All three layers of column wall are present in the evagination of the verrucae, in which the ectoderm is thick, glandular and lacks nematocysts, the mesoglea is relatively thin and the endoderm is also relatively thin and without musculature. On the other hand, the non-

adhesive (as in *Bunodosoma*) or weakly adhesive vesicles are rounded, may or may not have nematocysts and there may not be any clear difference in the layers at the apex and lateral parts of the structure (Daly, 2004).

The genus *Bunodosoma* currently has 13 valid species (Fautin, 2013). Most of them are from tropical and subtropical waters, and are found in both coasts of the Atlantic, the Pacific and the Indian Oceans. On the East Atlantic coast is *Bunodosoma biscayense* (Fisher, 1874), *Bunodosoma capense* (Lesson, 1830) and *Bunodosoma diadema* (Drayton in Dana, 1846), and on the West Atlantic coast is *Bunodosoma caissarum* Corrêa *in* Belém, 1987, *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973, *Bunodosoma cavernatum* (Bosc, 1802), *Bunodosoma granuliferum* (Le Sueur, 1817), *Bunodosoma kukenthali* Pax, 1910, *Bunodosoma sphaerulatum* Duerden, 1902. On the East Pacific Ocean are *Bunodosoma californicum* (Carlgren, 1951) and *Bunodosoma grande* (Verrill, 1869) (see Fautin, 2013; Gomes *et al.*, 2012). *Bunodosoma goanense* den Hartog & Vennam, 1993 and *Bunodosoma fallax* (Pax, 1922) are the only species recorded for the Indian Ocean. Gomes *et al.* (2012) described a new species for the genus, *Bunodosoma zamponii* Gomes, Schama & Solé-Cava, 2012, which was found on the coast of Argentina, and for a long time misidentified as *Phymactis papillosa* (Lesson, 1830) (Gomes *et al.*, 2012).

Members of *Bunodosoma* are characterized by an endodermal and circumscribed marginal sphincter muscle, acrorhagi, non-adherent vesicles and perfect fertile mesenteries with well-developed muscles (Häussermann, 2004; Gomes *et al.*, 2012). Acrorhagi and non-adherent vesicles, the main features used in the diagnosis of this genus, are shared with the genera *Phymactis* Milne-Edwards, 1857 and *Phymanthea* Carlgren, 1959, and have been the main cause of misidentifications (Häussermann, 2004; Gomes *et al.*, 2012). The phylogenetic relationships among the three genera have not yet been explored, but they can be distinguished based on the shape of the marginal sphincter muscle, diffuse in the latter two

genera (Häussermann, 2004). Similarities between the genera *Anthopleura* and *Bunodosoma* were explored in studies in which the main focus was the former genus (*e.g.* Daly, 2004; Heestand, 2009). According to Daly (2004), *Anthopleura* is currently recognized by the presence of acrorhagi and adhesive verrucae, and the latter would be a primitive condition (simplesiomorphy) of the round and non-adhesive vesicles, a sinapomorphy of *Bunodosoma.* Daly (2004) draws attention to the fact that *B. biscayense* does not have the group sinapomorphy, non-adhesive vesicles, but instead it has adhesive vesicles (den Hartog, 1987).

Many authors have pointed out issues in *Bunodosoma* that must be studied. Häussermann (2004) proposes that some species of *Bunodosoma* currently valid must be reexamined, as they may belong to other genera, like *B. biscayense* and *B. capense*. *Bunodosoma fallax* has an unknown status, as Häussermann (2004) claims that the material deposited in the collection does not allow the specimen to be identified as belonging to *Bunodosoma*. A problem with the species *B. cavernatum* (Bosc, 1802) was pointed out by McCommas (1991), who observed specimens that were able to adhere pieces of broken shells to their "vesicles". According to the author histological observations showed that the vesicles have the appearance expected of a verrucae. As for the Brazilian species, *B. caissarum* and *B. cangicum*, which were originally described in an unpublished thesis by Corrêa (1964), the correct authority must be discussed and established. Finally, the status and description of the species from the Gulf of Mexico and Caribbean Sea *B. cavernatum*, *B. granuliferum*, *B. kukenthali*, and *B. sphaerulatum*, must be revised because distinction is not clear based on the information available (González-Muñoz, *et al.* 2013).

Although *Bunodosoma* has been commonly represented in phylogenetic hypotheses that use molecular data (McCommas, 1991; Daly, 2004; Daly *et al.*, 2008; Rodríguez *et al.,* 2014), taxonomic sampling is always very restricted. McCommas (1991) included three species (*B. cavernatum*, *B. californicum* and *B. granuliferum*) in his analyses, while Daly

(2004) included the first two and also *B. biscayense*. Daly *et al*. (2008) and Rodríguez *et al.* (2014) included only *B. grande* in their analysis of the order Actiniaria. *Bunodosoma cangicum, B. caissarum* and *B. zamponii* were included only in the analyses of Gomes *et al.* (2012). There are no studies addressing intraspecific issues within species of *Bunodosoma* and the knowledge on the diversity of the genus is limited by taxonomical and identification problems. A revision of the genus would allow a better comprehension of the evolution of Actiniaria, within the frame work of the homology of column projections of the polyps. Therefore, here we perform a systematic review and propose a phylogenetic hypothesis for *Bunodosoma*.

Material & Methods

Redescriptions based on morphology. The species of the genus *Bunodosoma* were redescribed based on specimens obtained by museum loans or newly collected material. When possible, the information was based on type specimens. Museum loans were made from the following collections: Naturalis Biodiversity Center, Leiden (RMNH.Coel), Museum für Naturkunde der Humboldt Universität, Berlin (ZMB), United States National Museum of Natural History, Smithsonian Institute, Washington DC (USNM), Museum of Comparative Zoology, Harvard University, Cambridge (MCZ), Peabody Museum of Natural History, Yale University, New Haven (YPM). Newly collected material was deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

All specimens were examined whole and some were dissected, in cross and longitudinal sections. Measurements were made on preserved and live (when possible) animals. All morphological features relevant to the taxonomy of the group (*e.g.* size and form

of pedal disc, arrangement of the projections on the column, number and arrangement of tentacles and mesenteries, etc.) were examined.

For most species fragments were dehydrated, embedded in paraffin and histological sections 7-10 µm thick were stained with Mayer Hematoxylin-Eosin (Humason, 1967), Ramon y Cajal's Triple Stain (Gabe, 1968) and Masson's trichrome (Presnell & Schreibman 1997). The characters observed in the histological sections were: nature of the projections on the column, nature and shape of the marginal sphincter muscle, number and arrangement of mesenteries and reproductive tissue, and tentacle, retractor, parietobasilar and basilar muscles.

Cnidae capsules were identified and measured from squash preparations made of preserved pieces of tissue from acrorhagi, tentacles, column, actinopharynx and filaments. The preparations were examined under an optical microscope at 1000X magnification (Nikon Eclipse 80i) and at least 20 undischarged capsules were measured for each type of cnidae found in each tissue (for very scarce types, less capsules were measured). Cnidae terminology follows Östman (2000). For each type, range, mean and standard deviation are provided to give a qualitative idea of the distribution of sizes, though these values are not statistically significant. Frequencies for each type of cnidocyst were given, but are subjective impressions based on all the capsules seen on the slides.

Molecular analysis and phylogeny. Total DNA was obtained from samples of tentacles and/or pedal disc of *B. cavernatum*, *B. caissarum*, *B. cangicum*, *B. granuliferum* and *B. zamponii*, using the Qiagen DNeasy Blood & Tissue Kit. Standard PCR techniques were applied to perform the amplification of three mitochondrial (12S, 16S rDNA and COIII) and two nuclear markers (18S – in 3 fragments – and 28S rDNA – in 5 fragments). Primers used and PCR protocols followed are in Table 1.1. All PCR products were cleaned using ExoSAP-IT™ (USB Corp.) and cycle sequencing reactions used 0.6-4µl of purified PCR product to

produce forward sequences. Cycle sequencing products were cleaned through gel filtration with Sephadex G-50 (Sigma–Aldrich Corp.) and sequenced via traditional Sanger-based capillary electrophoresis at the sequencing facilities of the American Museum of Natural History (AMNH), New York, USA. Sequences were assembled and manually inspected (checking ambiguous base calls and removing primer sequences) using Geneious™ 9.0.5 (Kearse *et al.*, 2012). All sequences were compared (via BLAST) against the nucleotide database of GenBank to confirm if the target locus and organism were sequenced. Sequence alignment was done in MAFFT v.7 (Katoh & Standley, 2013) in default parameters to make a preliminary alignment so that all sequences ad similar lengths. For 18S and 28S rDNA each fragment was aligned separately, generating a total of 8 data matrices for nuclear markers, which were posteriorly concatenated. Sequences used by Rodríguez *et al.*, 2014 were downloaded from GenBank and included in the matrices.

A phylogenetic analysis was conducted under the parsimony optimality criteria. Parsimony was historically advocated by many researches (Hennig, 1966; Farris, 1983; Kluge, 2001) and its epistemological justification resides on the anti-superfluity principle (Barnes, 2000; Baker, 2003; Kluge & Grant, 2006; Grant & Kluge, 2009), in which optimal hypothesis are those that require the smallest number of causal explanations. Nucleotide homology was accessed simultaneously with the tree search under the direct optimization of POY 5.1.1. (Varón *et al.*, 2010). Such approach provides an objective criterion to choose among the multiple alignments hypothesis (Wheeler, 1996; Kluge and Grant, 2009; Padial *et al.*, 2014; see also Slowinsk, 1998). Searches were performed using the command “Search”, which implements a driven search building Wagner trees using random addition sequences (RAS), Tree Bisection and Reconnection (TBR) branch swapping followed by Ratchet (Nixon, 1999), and Tree Fusion (Goloboff, 1999). This command stores the shortest tree of each independent run and does final tree fusing using the pooled trees as a source of

topological diversity. The resulting topologies were submitted to a final round of TBR using iterative pass optimization (Wheeler, 2003). The resulting tree was rooted in the clade containing members of Edwardsioidea (*sensu* Rodríguez *et al.*, 2014).

Table 1.1. Primers used for amplifying and sequencing the molecular markers used in this study and PCR protocols.

Marker	Primer	Sequence	PCR protocol	Primer reference
12S	12S-F	5'-AGC CAC ACT TTC ACT GAA ACA AGG-3'	94°C/2min; 40x(94°C/20s; 55°C/30s; 72°C/50s); 72°C/5min	Chen *et al.* (2002)
	12S-R	5'-GTT CCC YYW CYC TYA CYA TGT TAC GAC-3'		
16S	16S-Forward	5'-CAC TGA CCG TGA TAA TGT AGC GT-3'	94°C/2min; 35x(94°C/20s; 51.5°C/30s; 72°C/45s); 72°C/4min	Geller & Walton (2001)
	16S-Reverse	5'-CCC CAT GGT AGC TTT TAT TCG-3'		
CO3	CO3-F	5'-CAT TTA GTT GAT CCT AGG CCT TGA CC-3'	94°C/2min; 40x(94°C/20s; 51°C/30s; 72°C/45s); 72°C/5min	Geller & Walton (2001)
	CO3-Reverse	5'-CAA ACC ACA TCT ACA AAA TGC CAA TAT C-3'		
18S	18S-A	5'-AAC CTG GTT GAT CCT GCC AGT-3'	94°C/5min; 40x(94°C/30s; 52.5°C/45s; 72°C/45s); 72°C/6min	Apakupakul *et al.* (1999)
	18S-L	5'-CCA ACT ACG AGC TTT TTA ACT G-3'		
	18S-C	5'-CGG TAA TTC CAG CTC CAA TAG-3'	94°C/5min; 40x(94°C/30s; 53.5°C/45s; 72°C/45s); 72°C/6min	
	18S-Y	5'-CAG ACA AAT CGC TCC ACC AAC-3'		
	18S-O	5'-AAG GGA CCA CCA GGA GTG GAG-3'	94°C/5min; 40x(94°C/30s; 58.5°C/45s; 72°C/45s); 72°C/6min	
	18S-B	5'-TGA TCC TTC CGC AGG TTC ACC T-3'		
28S	5S	5'-GCC GAC CCG CTG AAT TCA AGC ATA T-3'	94°C/5min; 40x(94°C/30s; 56.5°C/45s; 72°C/45s); 72°C/6min	Medina *et al.* (2001)
	R635SQ	5'-GGT CCG TGT TTC AAG ACG G-3'		
	Sequence – F635	5'-CCG TCT TGA AAC ACG GAC C-3'	94°C/5min; 40x(94°C/30s; 56°C/45s; 72°C/45s); 72°C/6min	
	R1630	5'-CCY TTC YCC WCT CRG YCT TC-3'		
	Sequence – F1379	5'-GAC AGC AGG ACG GTG GYC ATG G-3'	94°C/5min; 40x(94°C/30s; 60°C/45s; 72°C/45s); 72°C/6min	
	R2077sq	5'-GAG CCA ATC CTT WTC CCG ARG TT-3'		
	F2076sq	5'-TAA CYT CGG GAW AAG GAT TGG CTC-3'	94°C/5min; 40x(94°C/30s; 58.5°C/45s; 72°C/45s); 72°C/6min	
	Sequence – R2800	5'-GAG CTY RCC TTA GGA CAC CTG C-3'		
	Sequence – F2800	5'-GCA GGT GTC CTA AGG YRA GCT C-3'	94°C/5min; 40x(94°C/30s; 55°C/45s; 72°C/40s); 72°C/6min	
	R3264	5'-TTC YGA CTT AGA GGC GTT CAG-3'		

Results

Systematics

Order Actiniaria Hertwig, 1882

Suborder Enthemonae Rodríguez & Daly, 2014

Superfamily Actinioidea Rafinesque 1815

Family Actiniidae Rafinesque, 1815

Diagnosis (modified from Belém, 1987, changes in bold made by the present authors). **Actinioidea** with flattened basal disc and basilar muscles. Column smooth or verrucose; **acrorhagi**, **pseudoacrorhagi** or vesicles. Tentacles in **4 to 7** cycles. No more than one tentacle in each endo or exocoele. Numerous pairs of perfect mesenteries. **Reproductive tissue** and filaments in younger **mesenteries** distal to those in older **mesenteries**. **Reproductive tissue** found exclusively in the area of the cnidogladular tract. Sphincter from diffuse to **circumscript** or absent. Parietobasilar muscles formed of an obvious fold. Cnidom: **Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores.**

Included genera. See Fautin (2013).

Remarks. The modifications in bold were made to include more accurate nomenclature of structures and the current classification. The diagnosis of Actiniidae does not include any exclusive attribute (not seen in other Actiniaria) (Daly *et al.,* 2007). Studies using molecular data including specimens from Actiniidae have failed to recover the family as a monophyletic group (Won *et al.*, 2001; Daly *et al.*, 2003, 2008; Rodríguez *et al*. 2014).

Genus *Bunodosoma* Verrill, 1899

Type species. *Actinia granulifera* Le Sueur, 1817

Diagnosis (modified from Belém, 1987, changes in bold made by the present authors). Actiniids with well-developed basal disc; all or almost all the column covered with uniform, rounded vesicles **which may have** weak nematocyst batteries; **acrorhagi** in the fossa rarely absent, containing great numbers of holotrichs as well as spirocysts and**, sometimes,** basitrichs; **marginal sphincter muscle endodermal and circumscript**; tentacles and mesenteries arranged hexamerously; generally two pairs of siphonoglyphs and two pairs of directives; rarely several siphonoglyphs not linked to directives; pairs of perfect mesenteries generally numerous; all stronger mesenteries fertile (excepting the directives in some species). Cnidom: **Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores, and microbasic *p*-mastigophores.**

Remarks. Some modifications were made in the diagnosis to include a more accurate nomenclature of structures.

Bunodosoma biscayense (Fischer, 1874)

(Figs. 1.1-1.2, Table 1.2)

Synonymy list

Bunodes biscayensis Fischer, 1874: 229-231 (original description).

Bunodes Biscayensis – Fischer, 1889: 254, 271-271, 304.

Bunodactis Biscayensis – Carlgren, 1949: 65.

Bunodactis verrucosa – Schmidt, 1972: 82-86.

Aulactinia biscayensis – Dunn, Chia and Levine, 1980: 2078.

Bunodosoma biscayensis – den Hartog, 1987: 533-556.

Bunodosoma biscayense – Fautin, 2013 (spelling corrected).

Material examined

RMNH Coel. 17487: 2 dissected specimens; France, SW coast Ilbarritz plage, between Boarritz [sic] and Bidart.

RMNH Coel. 17489: 1 dissected specimen; France « Côte Basque », Bidart, north of Plage du Centre.

RMNH Coel. 17490: 3 dissected specimens; France « Côte Basque », near Bayonne south of Adour.

Diagnosis. *Bunodosoma* (?) with up to 45 transverse rows of vesicles in the column; vesicles from the upper part of the column may be adhesive (=verrucae); usually 96 tentacles arranged in 5 cycles (6+6+12+24+48); mesenteries pairs usually 48, hexamerously distributed in 4 cycles (6+6+12+24) at the actinopharynx level; mesenteries of first and second cycles perfect over entire length of the actinopharynx, third and fourth reach actinopharynx only at its most distal part; all mesenteries fertile, fifth cycle infertile when present.

Description

Pedal disc. Adherent, circular to semi-circular, 25-46 mm in diameter in preserved specimens.

Column. Cylindrical, up to 42mm in diameter and up to 36mm height in preserved specimens. Column densely covered with vesicles in 96 longitudinal rows. Small specimens with up to 30 transverse rows of vesicles, in larger specimens there may be up to 45 rows. The vesicles/verrucae are endocoelic and exocoelic, may be adhesive and cup-shaped.

Vesicles/verrucae become compound distally in the last 2-4 transversal rows. Each longitudinal row of vesicles ends distally in a short marginal projection with a distinct acrorhagus on its oral side. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two irregular alternate rows, inner row endocoelic, second exocoelic; inner row with larger acrorhagi (up to 2.5 mm in diameter) than second one (up to 1 mm in diameter). Smaller specimens may have only one row of acrorhagi. Preserved specimens studied are not very relaxed, so fosse seems to be very pronounced.

Oral disc. Up to 36mm in diameter, in the preserved specimens, it has no special characters.

Tentacles. Long, length up to 12 mm in preserved specimens, tapered and pointed. Tentacles of first and second rows may be longer than the rest. 96 arranged in 5 cycles (6+6+12+24+48). Some specimens with fewer tentacles in 4 cycles and a fifth incomplete one.

Internal anatomy. Mesenteries pairs usually 48, hexamerously arranged in 4 cycles (6+6+12+24) at the actinopharynx level. Some specimens with an incomplete fourth cycle, or a few additional pairs of mesenteries in a fifth cycle. Number of mesenteries equal proximally and distally. Two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first and second cycles perfect over entire length of the actinopharynx, third and fourth reach actinopharynx only at its most distal part. All mesenteries fertile, fifth cycle infertile when present. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal slightly bilobed and circumscript, main mesogleal lamella relatively thick and long. Retractors restricted and moderately strong. Parietobasilar muscles with short and thick mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores, and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.2.

Color. In preserved specimens, pedal and oral discs cream, tentacles cream or grey. Column may be striped in some preserved specimens, with 2-4 columns longitudinal rows of vesicles forming dark grey strips and 2-3 light cream.

Distribution. This species is registered for localities on the south-west coast of France, and its type-locality is Arcachon, Moulleau (Fischer, 1874; den Hartog, 1987). It was found in the intertidal zone, fixed to sand-covered, rocky substrate (for more ecological information, see den Hartog, 1987).

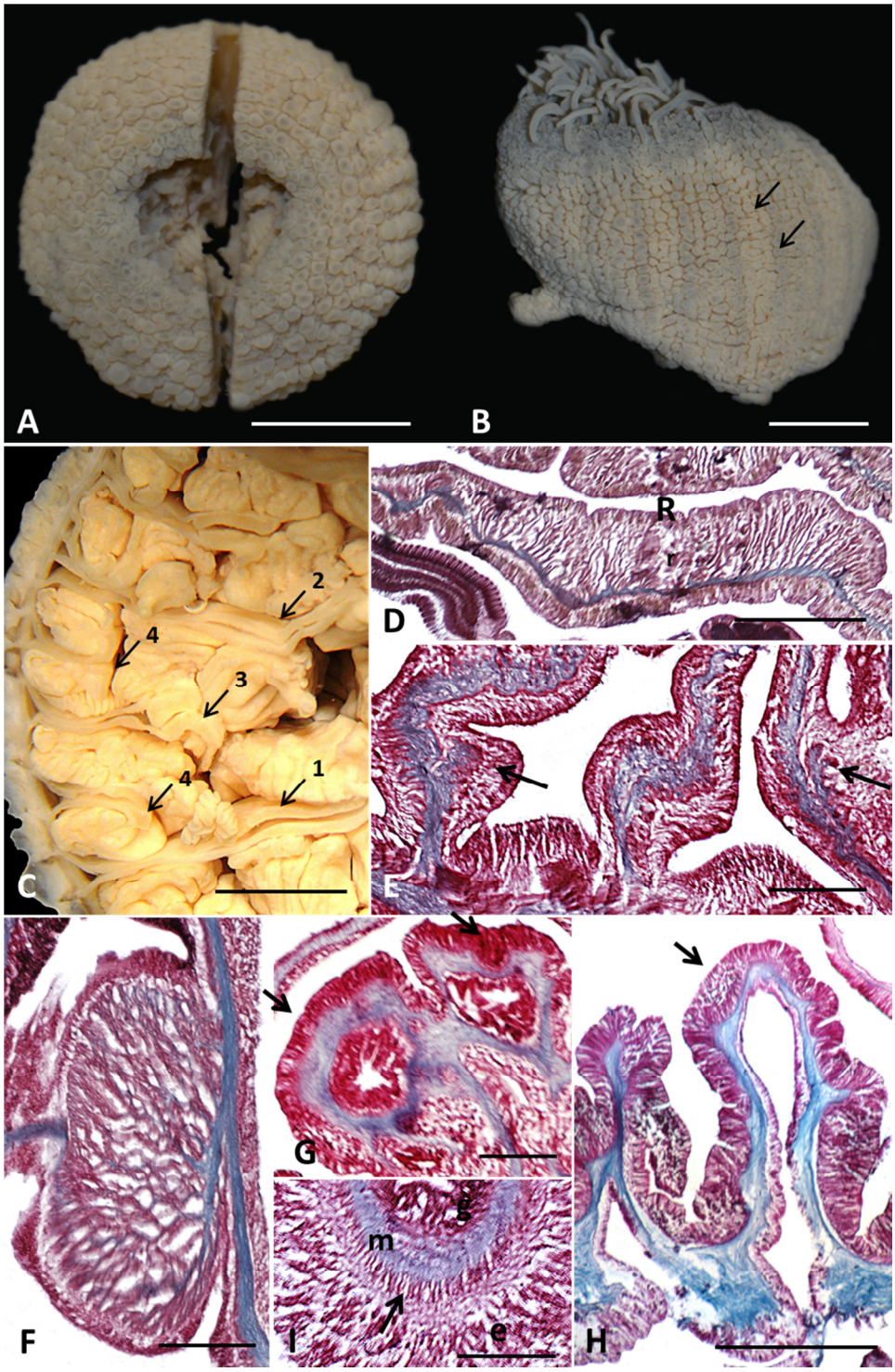

Fig. 1.1. *Bunodosoma biscayense* (Fischer, 1874). A) RMNH. Coel. 17489. Oral view. Scale = 1 cm. B) RMNH. Coel.17490. Lateral view, arrows indicate light and dark stripes. Scale = 1 cm. Internal anatomy: C-J. C) Cross section of the column, just below actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 0.5 mm. D) Cross section of retractor muscles. Scale = 0.02 mm. E) Cross section of parietobasilar muscles, arrows indicate mesogleal pennon. Scale = 0.01 mm. F) Longitudinal section through marginal sphincter muscle. Scale = 0.5 mm. G) Longitudinal section of the margin showing two acrorhagi, arrows indicate ectoderm with cnidocysts. Scale = 0.4 mm. H) Cross section of columnar vesicles (ou verrucae). Scale = 1 mm. I) Cross section of tentacle. Scale = 0.3 mm. Abbreviations: e–epidermis; g–gastrodermis; m-mesoglea; R–retractor muscles; s–siphonoglyph; t–tentacle; v–vesicle.

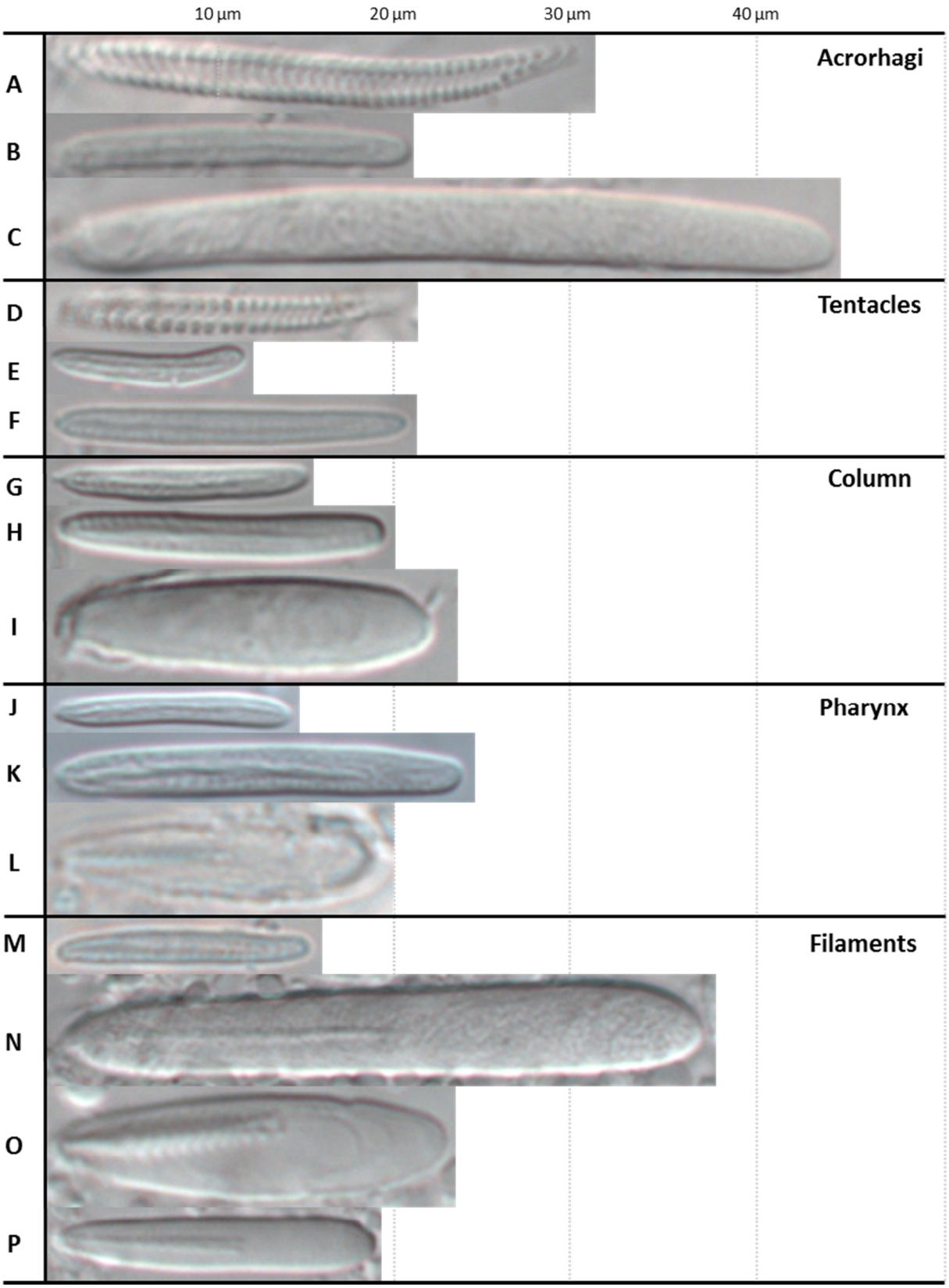

Fig 1.2. Distribution of cnidae in *Bunodosoma biscayense* (Fischer, 1874). A, D: Spirocyst; B, E, F, G, H, J, K, M: Basitrich; C, I: Holotrich; N: Microbasic *b*-mastigophore; L, O, P: Microbasic *p*-mastigophore.

Table 1.2. Size and distribution of cnidae of *Bunodosoma biscayense* (Fischer, 1874). Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 6); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	15.529 - 44.888 x 1.467 - 4.068	31.055 ± 5.451 x 3.010 ± 0.525	42	6/6	++
Basitrich	13.034 - 21.606 x 1.151 - 2.748	15.068 ± 2.035 x 2.064 ± 0.379	22	6/6	+
Holotrich	31.886 - 54.786 x 2.186 - 6.663	43.173 ± 5.039 x 5.236 ± 0.568	153	6/6	+++
Tentacles					
Spirocyst	14.709 - 26.609 x 1.438 - 3.764	21.405 ± 3.176 x 2.398 ± 0.443	76	6/6	++
Basitrich 1	11.177 - 14.042 x 1.514 - 2.368	12.412 ± 0.953 x 1.899 ± 0.274	8	2/6	+
Basitrich 2	17.145 - 25.206 x 1.465 - 3.477	21.322 ± 1.765 x 2.414 ± 0.375	152	6/6	+++
Column					
Basitrich 1	12.991 - 17.455 x 0.996 - 3.014	15.940 ± 0.989 x 1.926 ± 0.386	111	6/6	+++
Basitrich 2	18.931 - 22.381 x 1.439 - 3.058	19.990 ± 0.981 x 2.279 ± 0.387	30	6/6	++
Holotrich	21.291 - 25.603 x 4.325 - 4.936	22.872 ± 2.375 x 4.666 ± 0.312	3	3/6	+
Pharynx					
Basitrich 1	11.276 - 19.251 x 1.249 - 3.091	14.877 ± 1.885 x 2.080 ± 0.400	49	6/6	++
Basitrich 2	20.269 - 27.789 x 2.192 - 4.309	24.417 ± 1.680 x 3.156 ± 0.448	88	6/6	+++
Microbasic *p*-mastigophore	19.165 - 21.055 x 4.672 - 5.747	20.238 ± 0.971 x 5.154 ± 0.546	3	2/6	+
Filaments					
Basitrich	10.307 - 19.118 x 1.487 - 2.718	14.942 ± 2.170 x 2.157 ± 0.374	13	4/6	+
Microbasic *b*-mastigophore	29.158 - 47.364 x 4.472 - 9.015	38.639 ± 3.398 x 5.992 ± 0.759	95	6/6	+++
Microbasic *p*-mastigophore 1	15.912 - 24.519 x 3.384 - 6.731	21.896 ± 1.680 x 5.066 ± 0.645	68	6/6	++
Microbasic *p*-mastigophore 2	17.076 - 21.263 x 2.515 - 4.794	19.400 ± 1.419 x 3.449 ± 0.497	14	6/6	+

Taxonomical remarks. Due to the presence of "distinct, vesicular, adhesive verrucae" shown by den Hartog (1987) in his redescription, this species is probably a representative of the genus *Anthopleura*. Fautin (2013) corrected the spelling of the epithet, from "biscayensis" to the neuter "biscayense", according to the Latin declension of the neuter genus name.

Discussion. No type specimen of *Bunodes biscayensis*, as described in Fischer (1874), were found, so we considered the specimens collected by den Hartog as being the representatives of the species. In his description of *B. biscayense*, den Hartog (1987) states that this species' column has "distinct, vesicular, adhesive verrucae" and that, as he found no cnidae in the columnar outgrowths of *B. biscayense*, the columnar evaginations of this species should not be called "vesicles". His figures of histological sections of the distal columnar projections of this species indicate that there is in fact an area of differentiated adhesive ectoderm, and it fits into the definition of verrucae, presented by Daly (2004): "a verruca is an adhesive, hollow evagination of all three layers of the column wall, with thick, glandular ectoderm that lacks nematocysts, relatively thin mesoglea (especially at the center, where it may form a cinclid), and relatively thin, unmuscular endoderm". This feature, therefore, puts *B. biscayense* into the genus *Anthopleura*. In our histological sections, we interpreted the projections as being vesicles. Possibly due to the state of contraction in the specimens we observed the cup-shaped verrucae were deformed and, therefore, overlooked. But, for now, we choose to maintain this species in *Bunodosoma*.

The species' redescription by den Hartog (1987) was already quite thorough, although we found some discrepancies concerning histological and cnidae aspects. Den Hartog (1987) described a trilobed condition of the marginal sphincter muscle, whereas we found it to be bilobed. In general, the types and sizes of cnidae found in the acrorhagi, tentacles, column,

and actinopharynx are the same as observed by den Hartog (1987); however, we did not find the long basitrichs that he described in the filaments.

The striped pattern of the column in live specimens was described by Fischer (1889, p. 272), who comments that the darker stripes are grey and the lighter ones white. Den Hartog (1987) also observed this pattern in the specimens he collected. This pattern is maintained in the preserved specimens. Also according to den Hartog (1987), the sexes of this species are separate and he assumes that it may be oviparous, as he found no indication of viviparity. The anatomical irregularities may be a result of developmental variations, and should not be considered as a proof of asexual reproduction. Den Hartog (1987) comments that he observed some individuals in aquarium for two years and no event of asexual reproduction was observed, and that he has observed external scars in field organisms, which are probably a result of mechanical damages or intra/interspecific relations rather than a sign of asexual reproduction.

Bunodosoma caissarum Corrêa *in* Belém, 1988

(Figs. 1.3-1.5, Table 1.3)

Synonymy list

Bunodosoma caissarum Corrêa, 1964: 49, 63-67, 72, 73. [*nomen nudum*]

Bunodosoma caissarum Corrêa *in* Belém, 1987: 365-376 (original description).

Bunodosoma caiçarum – Oigman-Pszczol, Figueiredo & Creed, 2004: 177.

Bunodosoma caissarium – Monroy-Estrada *et al.,* 2007: 401.

Material examined

1 specimen; Cabelo Gordo beach, São Sebastião, SP, Brazil (23°49'S 45°25'W); Col. J.S. Beneti; anesthetized with $MgCl_2$, preserved in formalin.

5 specimens; Cabelo Gordo beach, São Sebastião, SP, Brazil (23°49'S 45°25'W); Col. J.S. Beneti; anesthetized with $MgCl_2$, preserved in formalin.

5 specimens; Arraial do Cabo, RJ, Brazil (22°57'S 42°01'W); Col. J.S. Beneti, A.C. Morandini and G. Tiseo; anesthetized with $MgCl_2$,preserved in formalin.

5 specimens; Conchas beach, Cabo Frio, RJ, Brazil. (22°52'S 41°58'W); Col. J.S. Beneti, A.C. Morandini and G. Tiseo; anesthetized with $MgCl_2$, preserved in formalin.

Diagnosis. *Bunodosoma* with up to 70 transverse rows of vesicles densely distributed in the column; usually 192 tentacles in six cycles and 96 mesenteries in five cycles; mesenteries of first, second and third cycles perfect over entire length of the actinopharynx, fourth cycle becomes imperfect before the most proximal part of the actinopharynx, fifth cycle reaches actinopharynx only at its most oral part; all mesenteries fertile, except last cycle and directives.

Description

Pedal disc. Strongly adherent, circular to semi-circular, up to 70 mm in diameter in live expanded specimens, up to 65 mm in diameter in preserved specimens.

Column. Cylindrical, live specimens with column narrower than pedal and oral discs, 20-58 mm in diameter and up to 80 mm height in live specimens, and 15-50 mm in diameter and up to 70 mm height in preserved specimens. Column densely covered with vesicles in 96 longitudinal rows. Larger specimens have up to 70 transverse rows. Vesicles endocoelic, rounded, non-adhesive, non-cup-shaped, mesoglea and ectoderm of similar thickness at sides

and apex, ectoderm with similar cells at sides and apex. The vesicles of the 2-3 most distal rows may be compound, with 2 or 3 lobes each. Each longitudinal row of vesicles ends distally in a short marginal projection with a distinct acrorhagus on its oral side. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic, second exocoelic; inner row with larger acrorhagi (up to 3 mm in diameter) than second one (up to 1.6 mm in diameter). Smaller specimens may have only one row of acrorhagi. Fosse pronounced. Specimens with two oral discs are very uncommon, but may be found (Fig. 1.3D). Contracted specimens often dome-shaped.

Oral disc: Wide, up to 35 mm in diameter in preserved and live specimens. Mouth with slightly raised lips in some specimens. In the preserved specimens, it has no special characters.

Tentacles: Up to 17 mm in length in live specimens and up to 9 mm in preserved ones, tapered and pointed. Usually all of the same size and set very close together. Usually 192 in 6 cycles (6+6+12+24+48+96). Some specimens with up to 202 tentacles, with a seventh incomplete cycle. Tentacles of the last cycle are exocoelic.

Internal anatomy. Mesenteries pairs usually 96, hexamerously arranged in 5 cycles (6+6+12+24+48) at the actinopharynx level. Number of mesenteries equal proximally and distally. Two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first, second and third cycles perfect over entire length of the actinopharynx, fourth cycle becomes imperfect before the proximal end of the actinopharynx, fifth cycle reaches actinopharynx only at its most distal part. All mesenteries fertile, except last cycle and directives. No indication of asexual reproduction. Gonochoric, all specimens observed with oocytes or sterile. Marginal sphincter muscle endodermal and circumscript, main mesogleal lamella thick and short. Retractors restricted and moderately strong. Parietobasilar muscles

with slightly long and thick mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.3.

Color. Pedal disc cream with insertion of mesenteries visible as red lines in live specimens. The column always has a very uniform color, deep red to maroon. Acrorhagi are lighter than the column in live specimens, usually of a cream color. Oral disc with color same as column and insertion of mesenteries may be visible as red lines in live specimens. . Tentacles' color may be same as column or maroon to purple in live specimens. When preserved, the column has a grey to brown color, tentacles are of the same color or may be slightly green, and acrorhagi remain lighter than column and tentacles.

Distribution. South Atlantic Ocean, in Brazil, from the south coast of the Espírito Santo state (Guarapari) to Rio Grande do Sul state. It is also found in the Archipelago of Fernando de Noronha and São Pedro e São Paulo, respectively around 500 and 1,000km off the Brazilian shore. It is usually in the lower intertidal zone, fixed to rock crevices and protected from sun exposure.

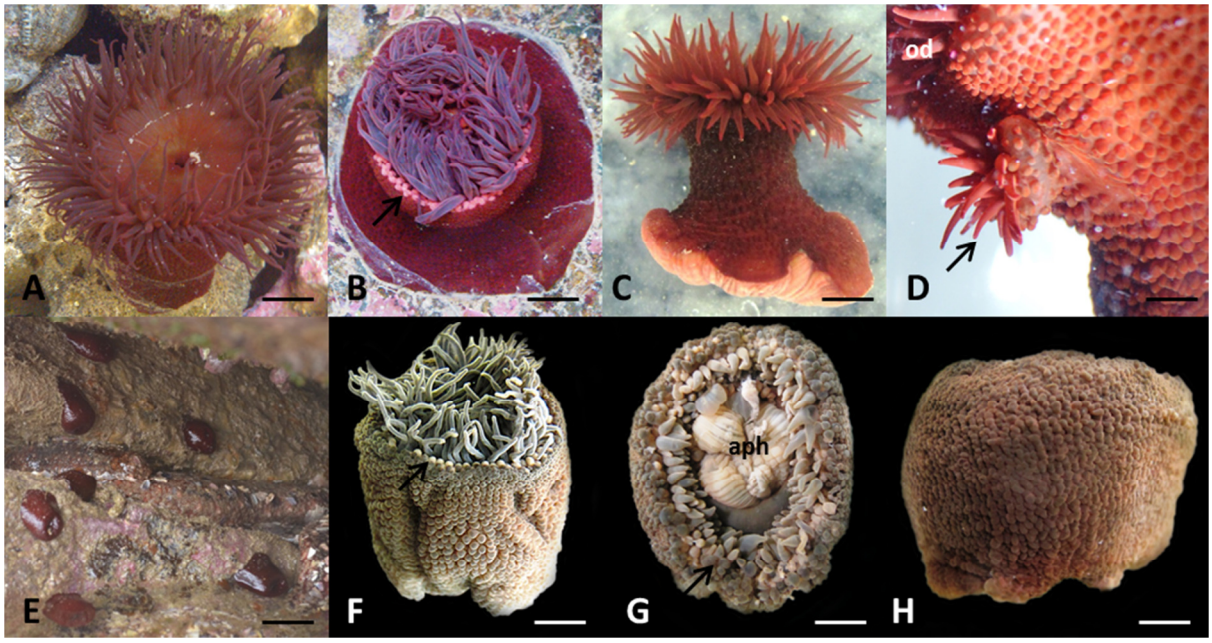

Fig. 1.3. *Bunodosoma caissarum* Corrêa *in* Belém, 1988. A) Live specimen from São Sebastião, SP, Brazil, oral view. Scale = 1 cm. B) Live specimen from São Sebastião, SP, Brazil, tentacle color variation, arrow indicates acrorhagi. Scale = 1.5 cm. C) Typical form of live specimen from Cabo Frio, RJ, Brazil, expanded at the base and margin, lateral view. Scale = 1.5 cm. D) Live specimen from Cabo Frio, RJ, Brazil, with a second oral disc, possibly due to regeneration, arrow indicates second oral disc. Scale = 0.5 cm. E) Specimens in the field in Cabo Frio, RJ, Brazil, before collection. Scale = 5 cm. F) Preserved specimen from Cabo Frio, RJ, Brazil, lateral view, arrow indicates acrorhagi. Scale = 1 cm. G) Preserved specimen from Arraial do Cabo, RJ, Brazil, oral view, arrow indicates compound vesicles. Scale = 1 cm. H) Preserved specimen from Arraial do Cabo, Rio de Janeiro, lateral view. Scale = 1 cm. Abbreviations: aph-actinopharynx; od-oral disc.

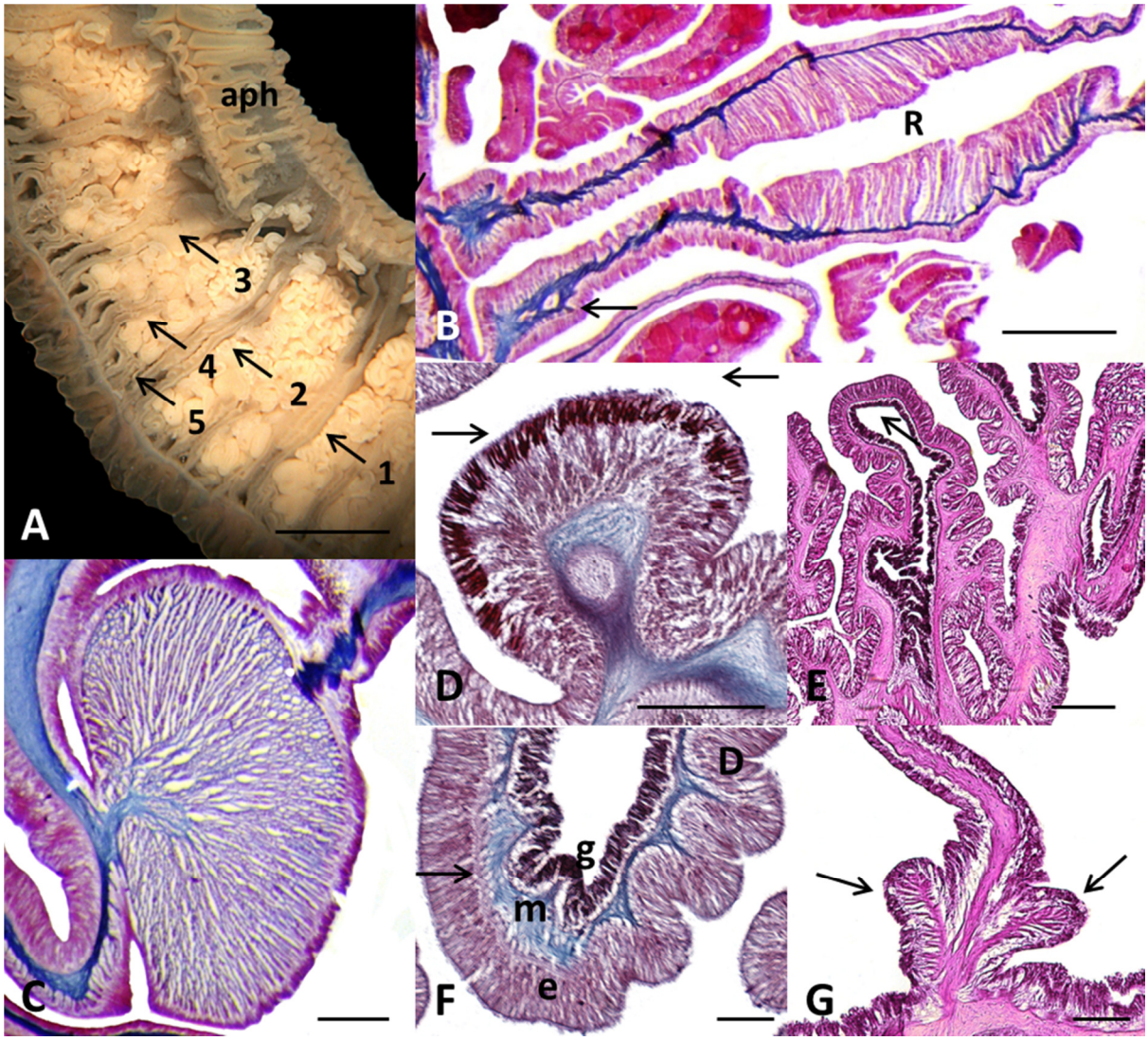

Fig. 1.4. Internal anatomy of *Bunodosoma caissarum* Corrêa *in* Belém, 1988. A) Cross section of mesenteries, proximal to the actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 0.5mm. B) Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 0.05 mm. C) Longitudinal section through marginal sphincter muscle. Scale = 0.2 mm. D) Longitudinal section of the margin showing acrorhagus, arrow indicates ectoderm with cnidocysts. Scale = 0.2 mm. E) Cross section of columnar vesicles. Scale = 0.2 mm. F) Cross section of tentacle. Scale = 0.2 mm. G) Cross section of basilar muscle. Scale = 0.2 Abbreviations: aph–actinopharynx; e–epidermis; g–gastrodermis; m-mesoglea; R–retractor muscles.

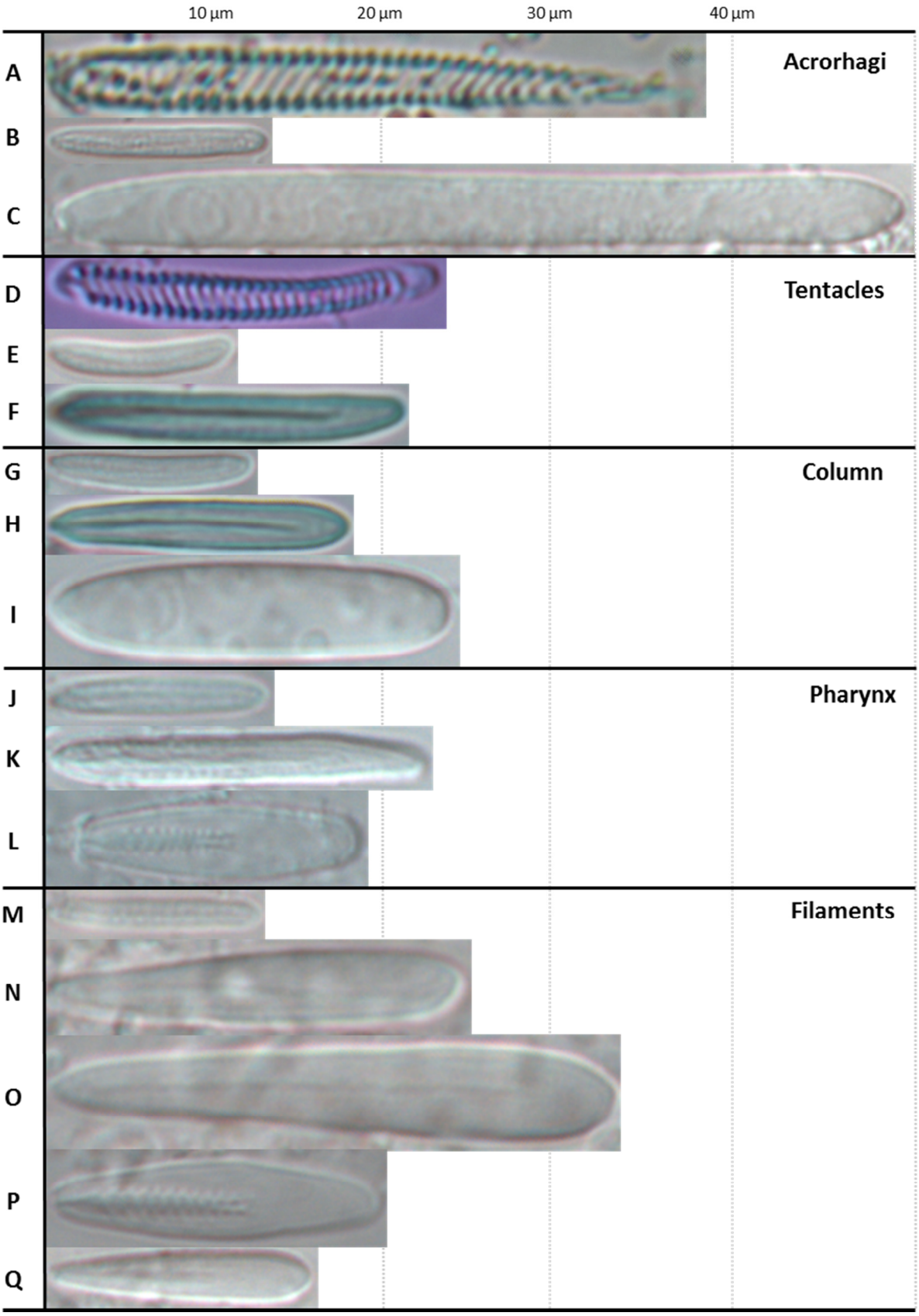

Fig 1.5. Distribution of cnidae in *Bunodosoma caissarum* Corrêa *in* Belém, 1988. A, D: Spirocyst; B, E, F, G, H, J, K, M: Basitrich; C, I: Holotrich; N, O: Microbasic *b*-mastigophore; L, P, Q: Microbasic *p*-mastigophore.

Table 1.3. Size and distribution of cnidae of *Bunodosoma caissarum* Corrêa *in* Belém, 1988. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 6); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	30.390 - 46.187 x 2.620 - 4.553	36.966 ± 3.812 x 3.688 ± 0.481	34	4/4	++
Basitrich	10.192 - 17.115 x 1.924 - 2.653	13.769 ± 2.359 x 2.299 ± 0.191	15	3/4	+
Holotrich	40.383 - 62.609 x 4.126 - 6.425	49.896 ± 4.792 x 5.136 ± 0.463	139	4/4	+++
Tentacles					
Spirocyst	16.170 - 28.750 x 2.020 - 4.156	23.525 ± 3.235 x 3.208 ± 0.442	29	4/4	++
Basitrich 1	11.201 - 12.295 x 2.030 - 2.441	11.767 ± 0.448 x 2.209 ± 0.211	4	2/4	+
Basitrich 2	18.167 - 27.233 x 1.924 - 3.282	21.456 ± 1.595 x 2.509 ± 0.247	143	4/4	+++
Column					
Basitrich 1	9.998 - 13.685 x 1.553 - 2.821	11.834 ± 1.208 x 2.269 ± 0.325	15	3/4	+
Basitrich 2	15.456 - 25.473 x 1.865 - 3.443	18.220 ± 1.864 x 2.629 ± 0.248	161	4/4	+++
Holotrich	22.982 - 25.007 x 4.082 - 5.943	23.874 ± 0.750 x 5.135 ± 0.591	9	3/4	+
Pharynx					
Basitrich 1	11.418 - 14.918 x 1.755 - 2.830	13.054 ± 1.078 x 2.275 ± 0.249	21	4/4	++
Basitrich 2	18.248 - 26.196 x 2.354 - 3.630	22.587 ± 1.811 x 3.086 ± 0.296	80	4/4	+++
Microbasic *p*-mastigophore	18.622 - 21.619 x 4.795 - 5.226	19.757 ± 1.546 x 5.074 ± 0.169	5	3/4	+
Filaments					
Basitrich	10.919 - 16.755 x 1.035 - 2.593	13.008 ± 1.384 x 2.026 ± 0.298	35	4/4	++
Microbasic *b*-mastigophore 1	19.867 - 29.959 x 3.455 - 5.112	25.380 ± 2.944 x 4.547 ± 0.402	21	4/4	+
Microbasic *b*-mastigophore 2	30.840 - 38.533 x 4.425 - 6.521	33.314 ± 2.378 x 5.523 ± 0.524	12	3/4	+
Microbasic *p*-mastigophore 1	16.303 - 23.737 x 4.092 - 5.872	20.175 ± 1.782 x 5.117 ± 0.323	52	4/4	+++
Microbasic *p*-mastigophore 2	14.050 - 18.432 x 2.690 - 4.166	16.450 ± 0.939 x 3.449 ± 0.305	70	4/4	+++

Taxonomical Remarks. This species was first described in a cathedratic thesis presented by Corrêa (1964) at the University of São Paulo, Brazil. However, as pointed out by Fautin (2013) this work cannot be considered a valid publication. Thus, the proper description was only available by Belém (1987), who published a very thorough description of the species morphology but basing her data on the information provided by Corrêa and on her specimens. Since then, this species has appeared in many studies on sea anemones from Brazil, as it is extremely common, especially on the rocky shores of beaches between the states of Rio de Janeiro and north of Rio Grande do Sul. According to Belém (1987), the holotype is a specimen deposited by Corrêa (1964) identified as A1 at the Department of Zoology (Biosciences Institute, University of São Paulo). Apparently this collection was transferred to the Museum of Zoology of the University of São Paulo (MZUSP), but the holotype was not found. We, therefore, propose that one of the specimens from Cabelo Gordo beach, São Sebastião, SP, Brazil, the same locality as the Corrêa's holotype, should now be considered as the neotype.

Discussion. The vesicles found in this species agree with the definition of these columnar projections provided by Daly (2004), so it belongs, in fact, to the genus *Bunodosoma*. Our description agrees mostly with what was presented by Belém (1987) in terms of morphology and internal anatomy, but we have made some different interpretations concerning the cnidae of the tentacles, pharynx and filaments. Belém (1987) described one type of basitrich for the tentacles and pharynx, but, in both cases, we decided to separate them in two distinct size classes. Also, Belém (1987) identified one size class of microbasic *b*-mastigophore in the filaments, while we divided them into two classes.

As pointed out by den Hartog & Vennam (1993), this species is indeed very similar to *B. goanense.* Both species share the same number of tentacles and are basically of the same

color (also according to the color described by Fautin *et al.*, 2015). However, specimens of *B. caissarum* usually have more transverse rows of vesicles (up to 70) than *B. goanense* (less than 40). And *B. caissarum* seems to be capable of extending more its column, whereas *B. goanense* usually has a shorter column. A difference in their internal anatomy is that, whereas the fifth (youngest) mesentery cycle is sterile in *B. caissarum*, it may be fertile in *B. goanense* (according to Den Hartog & Vennam, 1993; it was infertile in most specimens we observed). The cnidom of the two species are similar (as in all species of the genus), but some differences can be pointed out: *B. caissarum* has two types of basitrichs in the column, and *B. goanense* has only one; the microbasic *p*-mastigophores are slightly more common in the pharynx of *B. caissarum* than in *B. goanense*; *B. goanense* has three types of basitrichs and one of microbasic *b*-mastigophore in the filaments, while *B. caissarum* has respectively, one and two size classes of each nematocyst.

Also, as pointed out by Corrêa (1964) and Belém (1987), this species shares some features with *B. capense.* Besides having the same number of tentacles and mesenteries, *B. capense* seems to also share the color of *B. caissarum* (Carlgren, 1928, 1938), although the author comments that the former species has fourteen color varieties. As for cnidocyst types, on one hand, both species have two size classes of basitrichs in the column, which are rare in both species. On the other hand, *B. capense* only has one size class of basitrichs in the tentacles and one of microbasic *b*-mastigophore in the filaments.

B. caissarum occurs in sympatry with *B. cangicum*, but they tend to occupy different parts of the rocks of the intertidal zone: the former species is usually at the lower intertidal zone, while the latter is at the upper intertidal zone. When present, *B. caissarum* is more abundant (more than 3 specimens per/m2, personal obs.). Juveniles (diameter less than 15mm) are usually found only at the lower intertidal zone, in small cavities between rocks, very protected from sunlight and desiccation.

Corrêa (1964) commented on the finding of a "two-headed" individual, and we have also found one at Concha beach, Cabo Frio (Fig 1.3D). As commented by Corrêa (1964), this situation was probably not caused by a longitudinal fission as both "heads" are of very unequal sizes. It is probably a result of regeneration after some kind of ecological interaction.

Bunodosoma californicum Carlgren, 1951

(Figs. 1.6-1.8, Table 1.4)

Synonymy list

Bunodosoma californica Carlgren, 1949: 52. [*nomen nudum*]

Bunodosoma californica Carlgren, 1951: 420-421 (original description).

Bunodosoma californicum – Fautin, 2013 (spelling corrected).

Material examined

USNM 49447: Lectotype, 1 dissected specimen; North Pacific Ocean, Gulf of California, Mexico, Baja California, Puerto Escondido; Collected in March 26th 1940. Col. E. F. Rickleffs. Identified by O. Carlgren. 70% Ethanol. Lot size originally 2.5 specimens. Syntype of *Bunodosoma californica* according to Daphne G. Fautin in May, 2002. Designated Lectotype of *B. californica* by M. Daly, 2003.

USNM 1013363: Paralectotype, 1 whole specimen; North Pacific Ocean, Gulf of California, Mexico, Baja California, Puerto Escondido; Collected in March 26th 1940. Col. E. F. Rickleffs. Identified by O. Carlgren. 70% Ethanol. (Lot size originally 2.5 specimens, as

USNM 49447. Syntype according to Daphne G. Fautin in May, 2002. This specimen was designated paralectotype of *B. californica* by M. Daly, 2003).

USNM 49449: 2 dissected specimens; North Pacific Ocean, United States, California, Angeles Bay; Collected in April 1st 1940. Col. E. F. Rickleffs. Identified by O. Carlgren. 70% Ethanol.

Diagnosis. *Bunodosoma* with up to 25 transverse rows of vesicles in the column; no compound vesicles; 48 tentacles arranged in 4 cycles (6+6+12+24) or up to 100 tentacles in 5 cycles (6+6+12+24+48); Mesenteries pairs up to 96, usually hexamerously distributed in 4 cycles (6+6+12+24) or 4 cycles (6+6+12+24+48). Large specimens with three first cycles perfect, and all perfect mesenteries fertile, directives excluded.

Description

Pedal disc. Adherent, circular to semi-circular, 9-28 mm in diameter in preserved specimens.

Column. Cylindrical, more or less of the same diameter as pedal disc, 10-28 mm in diameter and up to 30 mm height in preserved specimens. Column densely covered with vesicles in around 96 longitudinal rows and up to 25 transverse rows. Vesicles are endocoelic and exocoelic, rounded, non-adhesive, may be slightly cup-shaped; mesoglea and ectoderm of similar thickness at the sides and apex, ectoderm with similar cells at sides and apex. No compound vesicles. In some specimens, each longitudinal row of vesicles ends distally in a short marginal projection, on its inner aspect provided with a distinct acrorhagus. Acrorhagi endocoelic with holotrichs and spirocysts, arranged into one cycle (0.1-0.9 mm in diameter). Individuals from lot USNM 49449 have marginal projections, but with no holotrichs. Fosse pronounced.

Oral disc. 6-16 mm in diameter and without markings in the preserved specimens.

Tentacles. Short, 2-6 mm in preserved specimens, stout, tapered and pointed. Tentacles of the inner cycle the same length as the outer. Small specimen (USNM 1013363) with 48 tentacles in 4 cycles (6+6+12+24); larger specimen (USNM 49447) with up to 100 tentacles in 5 cycles (6+6+12+24+48) or a sixth incomplete cycle. Tentacles of the last cycle are exocoelic.

Internal anatomy. Mesenteries pairs up to 96, usually hexamerously arranged in 4 cycles (6+6+12+24) or 5 cycles (6+6+12+24+48). Same number of mesenteries distally and proximally. Usually two pairs of directives connected with two distinct siphonoglyphs. Large specimens with first three or four cycles perfect over entire length of the actinopharynx, and all perfect mesenteries fertile, excluding directives. May show indication of asexual reproduction (irregular distribution of mesenteries and number of siphonoglyphs). Gonochoric. Marginal sphincter muscle endodermal and circumscript, main mesogleal lamella thin and short. Retractors restricted and moderately strong. Parietobasilar muscles with long and thick mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.4.

Color. Preserved specimens entirely light brown to grey, acrorhagi of a lighter color. Live specimens with reddish brown to pink column; tentacles may be pink, orange, or purple, with an opaque white longitudinal stripe and white crossbars, and tip may be lighter than base; pedal disc same color as distal column; oral disk same color as column, but may be marked with opaque white or yellowish rays (Daly, 2004).

Distribution. North Pacific Ocean: Gulf of California, Mexico, to El Salvador (Daly, 2004).

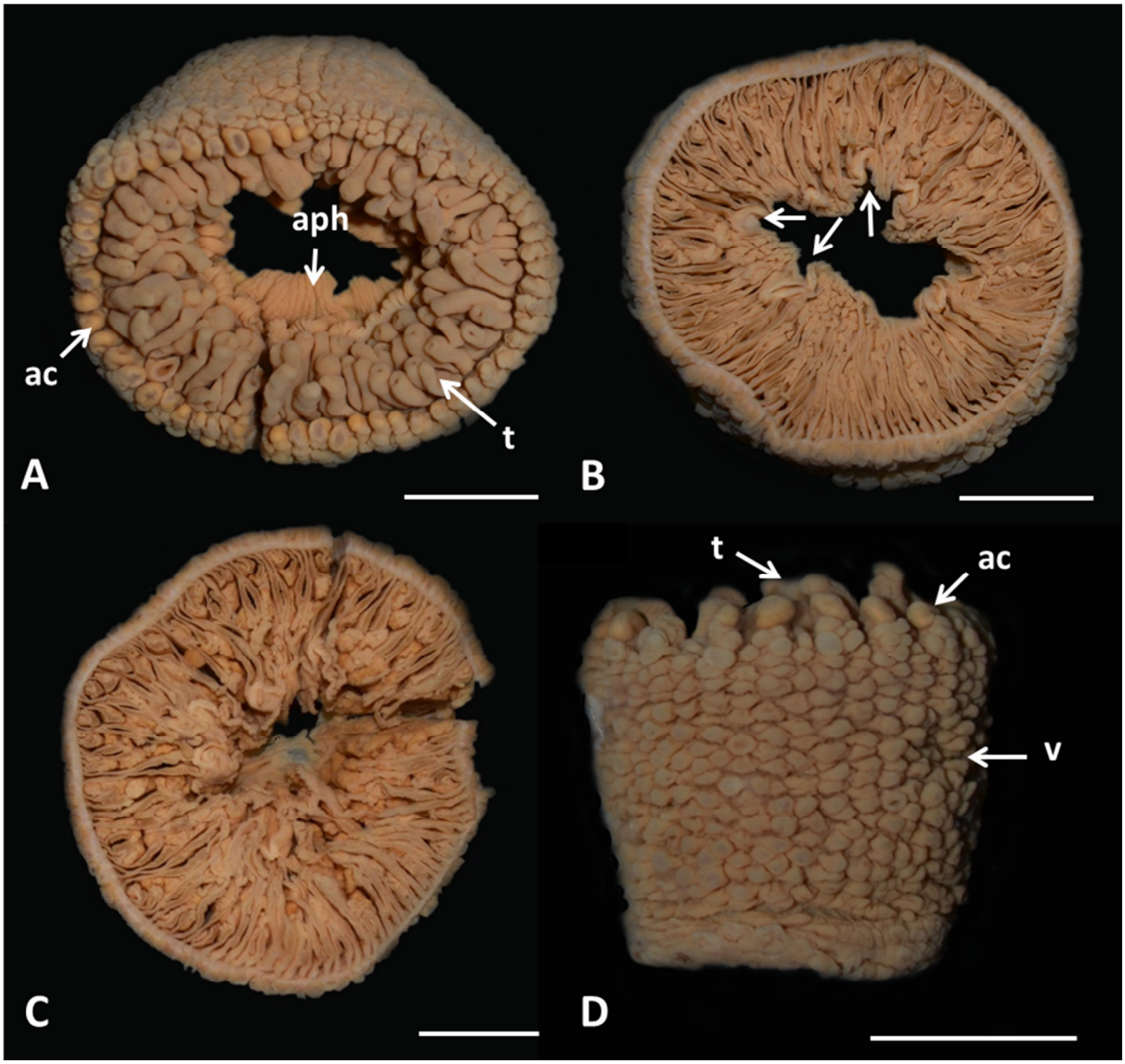

Fig. 1.6. *Bunodosoma californicum* Carlgren, 1951. A) USNM 49447, Lectotype. Oral view. Scale = 7 mm. B) USNM 49447, Lectotype. Cross section at actinopharynx level, arrows indicate siphonoglyphs. Scale = 7 mm. C) USNM 49447, Lectotype. Cross section below actinopharynx level. Scale = 6 mm. D) USNM 1013363. Paralectotype, lateral view. Scale = 10 mm. Abbreviations: ac–acrorhagus; aph–actinopharynx; t–tentacle; v–vesicle.

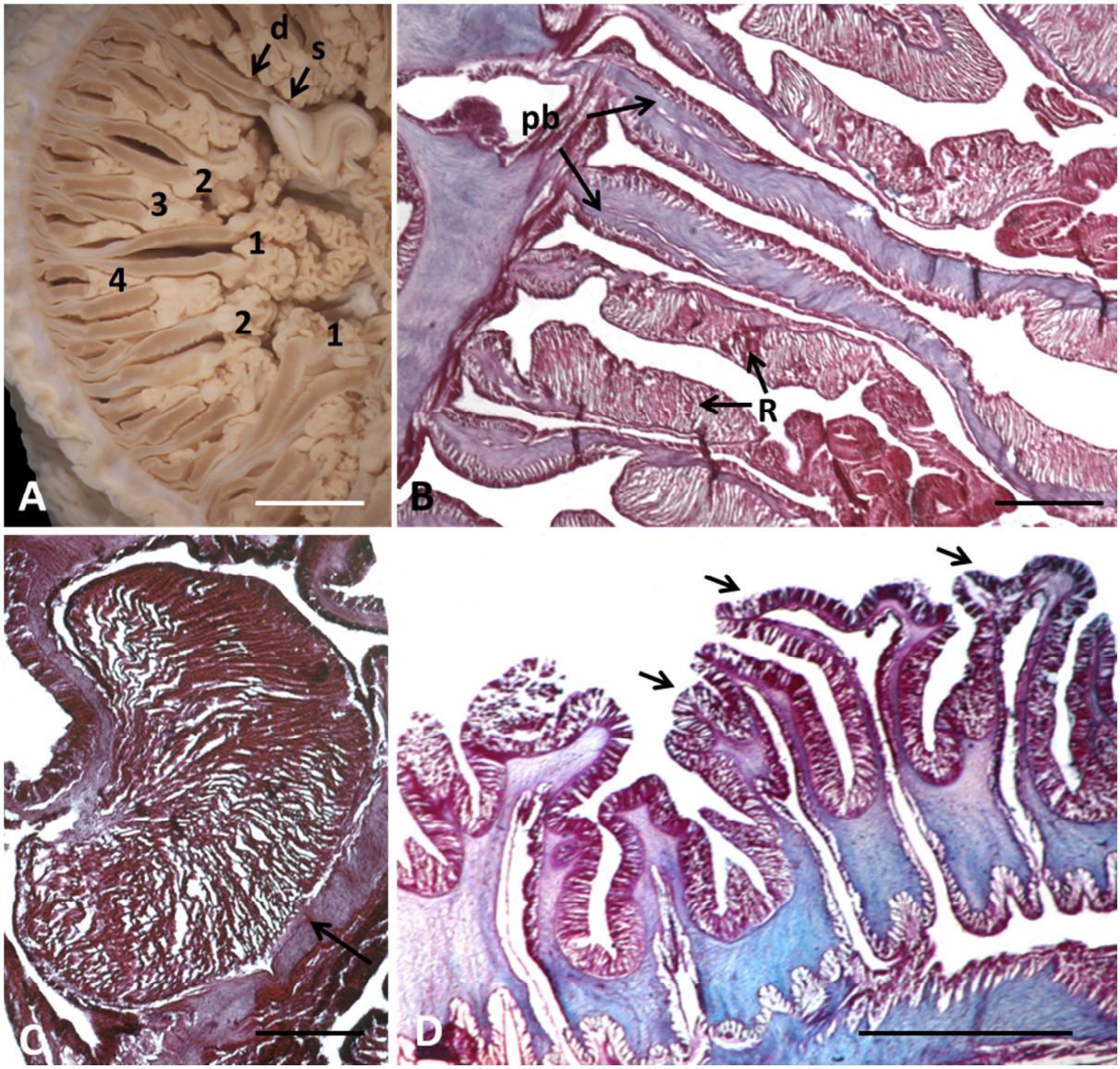

Fig. 1.7. Internal anatomy of *Bunodosoma californicum* Carlgren, 1951. A) Cross section of mesenteries, just below actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries Scale = 0.3 mm. B) Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 0.05 mm. C) Longitudinal section through marginal sphincter muscle. Scale = 0.3 mm. D) Cross section through columnar vesicles. Scale = 1 mm. Abbreviations: d–pair of directive mesenteries; R–retractor muscles; s–siphonoglyph.

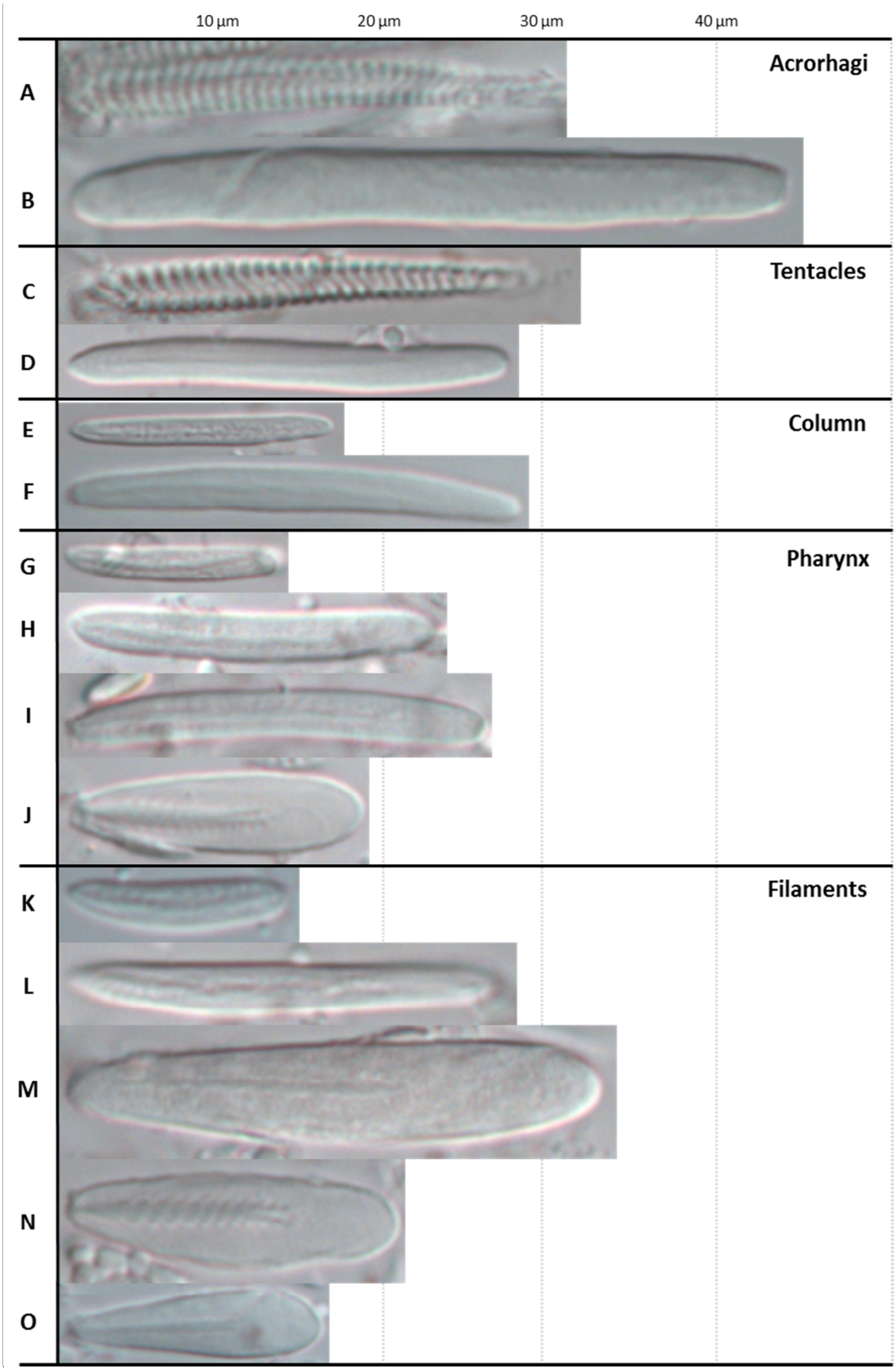

Fig 1.8. Distribution of cnidae *Bunodosoma californicum* Carlgren, 1951. A, C: Spirocyst; D, E, F, G, H, J, I, K, L: Basitrich; B: Holotrich; M: Microbasic *b*-mastigophore; J, N, O: Microbasic *p*-mastigophore.

Table 1.4. Size and distribution of cnidae of *Bunodosoma californicum* Carlgren, 1951. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 3; not all parts were analyzed in all specimens); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	26.615 - 34.925 x 2.409 - 4.069	31.399 ± 2.731 x 3.362 ± 0.515	14	3/3	+
Holotrich	39.642 - 48.985 x 3.758 - 5.852	43.725 ± 2.136 x 4.572 ± 0.407	56	1/3	+++
Tentacles					
Spirocyst	20.699 - 36.963 x 1.698 - 4.839	31.239 ± 4.053 x 3.104 ± 0.588	30	3/3	++
Basitrich	21.616 - 34.089 x 2.222 - 3.724	27.523 ± 2.512 x 2.906 ± 0.341	90	3/3	+++
Column					
Basitrich 1	15.160 - 20.569 x 2.162 - 3.165	18.172 ± 1.370 x 2.664 ± 0.228	46	2/2	++
Basitrich 2	22.377 - 32.353 x 2.909 - 4.259	28.453 ± 1.922 x 3.691 ± 0.307	66	2/2	++
Pharynx					
Basitrich 1	10.818 - 17.756 x 2.133 - 3.717	14.080 ± 2.050 x 2.469 ± 0.377	18	2/2	+
Basitrich 2	21.436 - 24.224 x 3.032 - 4.024	23.087 ± 0.692 x 3.367 ± 0.241	25	2/2	++
Basitrich 3	25.105 - 30.334 x 2.856 - 4.214	27.082 ± 1.318 x 3.445 ± 0.282	39	2/2	++
Microbasic *p*-mastigophore	16.674 - 21.241 x 3.823 - 6.478	19.373 ± 1.243 x 5.018 ± 0.648	15	2/2	+
Filaments					
Basitrich 1	11.360 - 17.498 x 1.917 - 2.728	14.144 ± 1.482 x 2.269 ± 0.224	26	2/2	++
Basitrich 2	26.254 - 29.401 x 3.652 - 4.038	28.357 ± 1.452 x 3.878 ± 0.165	4	1/2	+
Microbasic *b*-mastigophore	30.051 - 37.892 x 4.965 - 7.882	33.805 ± 1.886 x 6.543 ± 0.668	19	2/2	++
Microbasic *p*-mastigophore 1	18.586 - 25.140 x 4.361 - 6.522	21.454 ± 1.408 x 5.310 ± 0.543	53	2/2	+++
Microbasic *p*-mastigophore 2	13.013 - 21.463 x 3.615 - 6.176	17.167 ± 2.247 x 4.744 ± 0.680	12	2/2	+

Taxonomical Remarks. Fautin (2013) corrected the spelling of the epithet, changing it from "californica" to the neuter "californicum", according to the Latin declension of the neuter genus name.

Discussion. This species was first cited as being a member of the genus *Bunodosoma* by Carlgren (1949), but a description was only published in Carlgren (1951). Later, Daly (2004) provided a more complete description of the species, and observed that the original type series of *B. californicum* was heterogenous, as one of the specimens in the lot belonged to the species *Anthopleura dowii* Verrill, 1869. To solve this problem, she designated a specimen as Lectotype (USNM 49447) of *B. californicum* and the other two as paralectotypes (USNM 1013363 and USNM 10133634). Specimens from the lot USNM 49449, identified by O. Carlgren, were included in the analysis as their features were consistent with type specimens.

Daly (2004) had already studied the columnar projections of this species and confirmed that it does in fact belong to the genus *Bunodosoma*. Also, no material was found attached to the vesicles of the specimens, as pointed out by Daly (2004), who states that in this species the vesicles are nonadhesive, both in the field and in museum species. Daly (2004) also provided the following diagnosis for this species: "Actiniidae with reddish brown to pink column covered from margin to limbus with rows of endocoelic nonadhesive vesicles. Margin with endocoelic vesicles atop marginal projections; each projection bears a single holotrichous acrorhagus on its oral surface. Tentacles may be pink, orang, or purple, with an opaque white longitudinal stripe and white crossbars. Tentacles arranged in three or four cycles, approximately 80 in total". We cannot account for the color of live specimens, as we studied only preserved museum lots, however, besides that, concerning the species morphology and anatomy, our observations are in agreement with what she described.

On the other hand, there are some disagreements between the study of cnidae provided by Daly (2004) and the one we carried out. The cnidae we found in the tentacles and column are the same as provided by Daly (2004). Basitrichs were only found in acrorhagus with no holotrichs (USNM 49449), and they were of the same size as the basitrichs from the columns, meaning that, although the projection had the external form of an acrorhagus, it may not have been completely developed. Therefore they were not considered as a characteristic of the real acrorhagus (only one of the specimens examined, USNM 1013363, proved to have real acrorhagus). There is a difference in the cnidae of the actinopharynx as we considered an intermediate class of basitrichs, which was not considered by the former author. As for the filaments, the cnidae we identified as being the type 2 of microbasic *p*-mastigophore was considered by the former author to be a small type of *b*-mastigophore.

This species seems to be the only *Bunodosoma* with an irregular arrangement of mesenteries and siphonoglyphs, indicating asexual reproduction. Daly (2004) indicates that the cause might be regeneration. The ability to undergo fission is unknown, but, although all specimens seen by her in the field were solitary, several specimens beared scars indicative of regeneration (Daly, 2004). This species does not seem to occur in sympatry with *B. grande*, the only other species to occur in the Pacific Ocean. Nevertheless, both species may be easily distinguished, especially by the number of tentacles.

Bunodosoma cangicum Corrêa *in* Belém & Preslercravo, 1973

(Figs. 1.9-1.11, Table 1.5)

Synonymy list

Bunodosoma cangicum Corrêa, 1964: 49-50, 67-72, 73, 86. [*nomen nudum*]

Bunodosoma cangicum Corrêa *in* Belém & Preslercravo, 1973: 4-6, 8, 10, 13 (original description).

Material examined

1 specimen; Flamengo Bay, Ubatuba, SP, Brazil (23°29'S 45°05'W); Col. J.S. Beneti, S.N. Stampar & A.C. Morandini; anesthetized with $MgCl_2$, preserved in formalin.

1 specimen, Flamengo Bay, Ubatuba, SP, Brazil (23°29'S 45°05'W); Col. J.S. Beneti, S.N. Stampar & A.C. Morandini; anesthetized with $MgCl_2$, preserved in formalin.

5 specimens; Cabelo Gordo beach, São Sebastião, SP, Brazil (23°49'S 45°25'W); Col. J.S. Beneti; anesthetized with $MgCl_2$, preserved in formalin.

5 specimens; Arraial do Cabo, RJ, Brazil (22°57'S 42°01'W); Col. J.S. Beneti, A.C. Morandini and G.Tiseo; anesthetized with $MgCl_2$, preserved in formalin.

5 specimens; Coqueiral, Aracruz, ES, Brazil (19°56'S 40°08'W); Col. J.S. Beneti; anesthetized with $MgCl_2$, preserved in formalin.

Diagnosis. *Bunodosoma* with up to 45 transverse rows of vesicles densely distributed in the column; 96 tentacles in five cycles and 48 mesenteries in four cycles; mesenteries of first and second cycles perfect over entire length of the actinopharynx, third and fourth cycles reach actinopharynx only at its most distal part; all mesenteries fertile, except last cycle and directives.

Description

Pedal disc. Strongly adherent, circular to semi-circular, diameter up to 80 mm in live expanded specimens, and up to 72 mm in preserved specimens.

Column. Cylindrical, live specimens with column narrower than pedal and oral discs, 20-58 mm in diameter and up to 85 mm height; preserved specimens with up to 50 mm in diameter and up to 73 mm height. Column densely covered with vesicles in 96 longitudinal rows, large specimens have up to 45 transverse rows. Vesicles endocoelic and exocoelic, rounded, non-adhesive, non-cup-shaped, mesoglea and ectoderm of similar thickness at the sides and apex, ectoderm with similar cells at sides and apex. Vesicles of the 2-3 most distal rows may be compound, usually with 2 or 3 lobes each, but may have 5 lobes. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic, second exocoelic, both with up to 48 acrorhagi; inner row with larger acrorhagi (up to 3 mm in diameter), the second one smaller (up to 1.5 mm in diameter). Smaller specimens may have only one row of acrorhagi. Fosse pronounced. Contracted specimens often dome-shaped.

Oral disc. Up to 40 mm in diameter. Mouth with slightly raised lips in some specimens. In the preserved specimens, it has no special characters.

Tentacles. Long, length up to 28 mm in live specimens and up to 22 mm in fixed specimens, tapered, pointed. Most individuals with 96 tentacles arranged in 5 cycles (6+6+12+24+48). Some specimens with up to 102 tentacles, in a sixth incomplete cycle. Tentacles of the last cycle are exocoelic and may be shorter than the others.

Internal anatomy. Mesenteries pairs usually 48, hexamerously arranged in 4 cycles (6+6+12+24) at the actinopharynx level. Some specimens may have up to 52 mesenteries pairs, in which case there is a fifth incomplete cycle proximally. Number of mesenteries usually equal proximally and distally. Two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first and second cycles perfect over the entire pharynx's

length, third and fourth cycles reach actinopharynx only at its most oral part. All mesenteries fertile, except last cycle and directives. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript, main mesogleal lamella thin and short. Retractors diffuse to restricted and moderately strong. Parietobasilar muscles with long and thin mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.5.

Color. Pedal disc cream with insertion of mesenteries visible in live specimens. Column usually of a green to brown color, but in some specimens may be grey or bluish, acrorhagi are lighter than the column in live specimens, usually of a cream color. Oral disc same as column, or light green or pink, vesicles often darker, insertion of mesenteries may be visible as lines in live specimens. Tentacles may be same as column or light green or pink. When preserved, the column has a grey to brown color; tentacles are of the same color or lighter, and acrorhagi usually of color similar to column or tentacles.

Distribution. South Atlantic Ocean from the coast of Pará State, Brazil, to Rocha department, Uruguay. It is also found in the Archipelago of Fernando de Noronha and São Pedro e São Paulo, respectively around 500 and 1,000km off the Brazilian shore. Found in the lower of middle intertidal zone, fixed to rocks and buried in the sand.

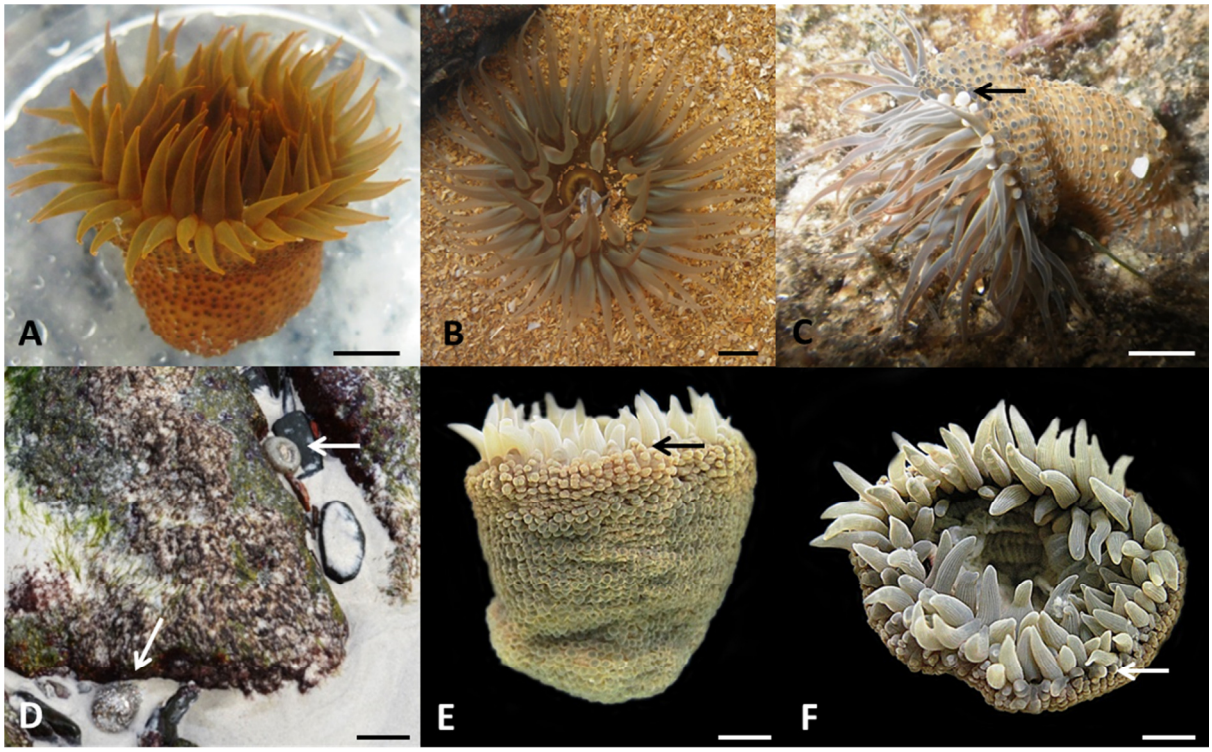

Fig. 1.9. *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973. A) Live specimen from Flamengo Bay, Ubatuba, SP, Brazil, removed from the substrate. Scale = 1 cm. B) Live specimen at natural environment from Aracruz, ES, Brazil; note tentacle color variation. Scale = 1 cm. C) Live specimen at natural environment from Aracruz, ES, Brazil, lateral view, arrow indicates acrorhagus. Scale = 1.7 cm. D) Specimens in the field during low tide at Arraial do Cabo, RJ, Brazil, before collection, arrows indicate specimens. Scale = 6 cm. E) Preserved specimen from Arraial do Cabo, RJ, Brazil, lateral view, arrow indicates compound vesicle. Scale = 1 cm. F) Preserved specimen from Arraial do Cabo, RJ, Brazil, oral view, arrow indicates acrorhagus. Scale = 1 cm.

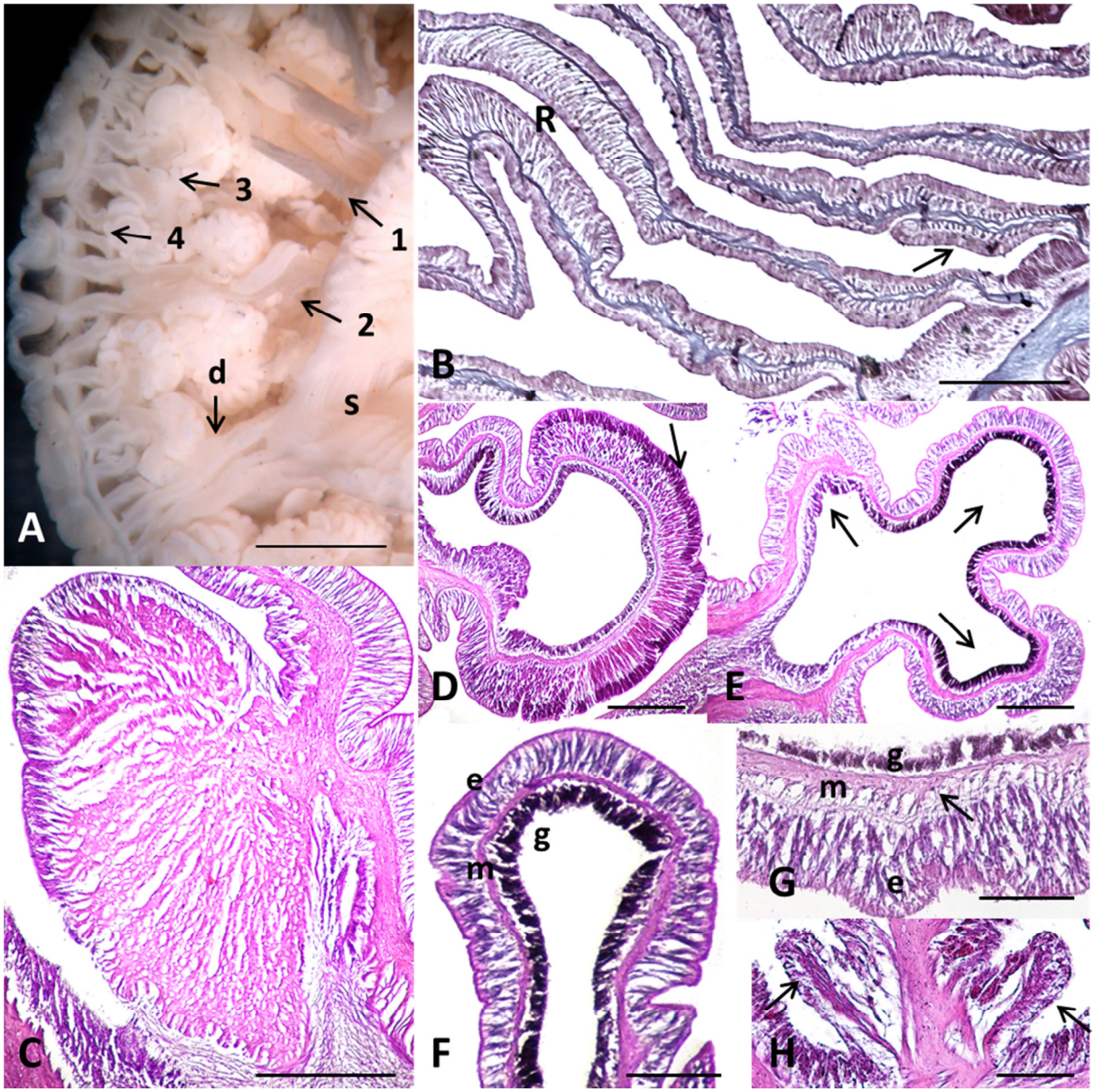

Fig. 1.10. Internal anatomy of *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973. A) Cross section of the column, proximal to the actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 5 mm. B) Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 0.05 mm. C) Cross section through marginal sphincter muscle. Scale = 0.2 mm. D) Longitudinal section of the margin showing acrorhagus, arrow indicates ectoderm with cnidocysts. Scale = 0.4 mm. E) Cross section of trilobed compound vesicle, arrows indicate lobes. Scale = 0.3 mm. E) Cross section of columnar vesicle. Scale = 1 mm. F) Cross section of tentacle. Scale = 0.2 mm .G) Cross section of basilar muscle. Scale = 0.1 mm . Abbreviations: d- directive pair of mesenteries; e–epidermis; g–gastrodermis; m-mesoglea; R–retractor muscles; s-siphonoglyph.

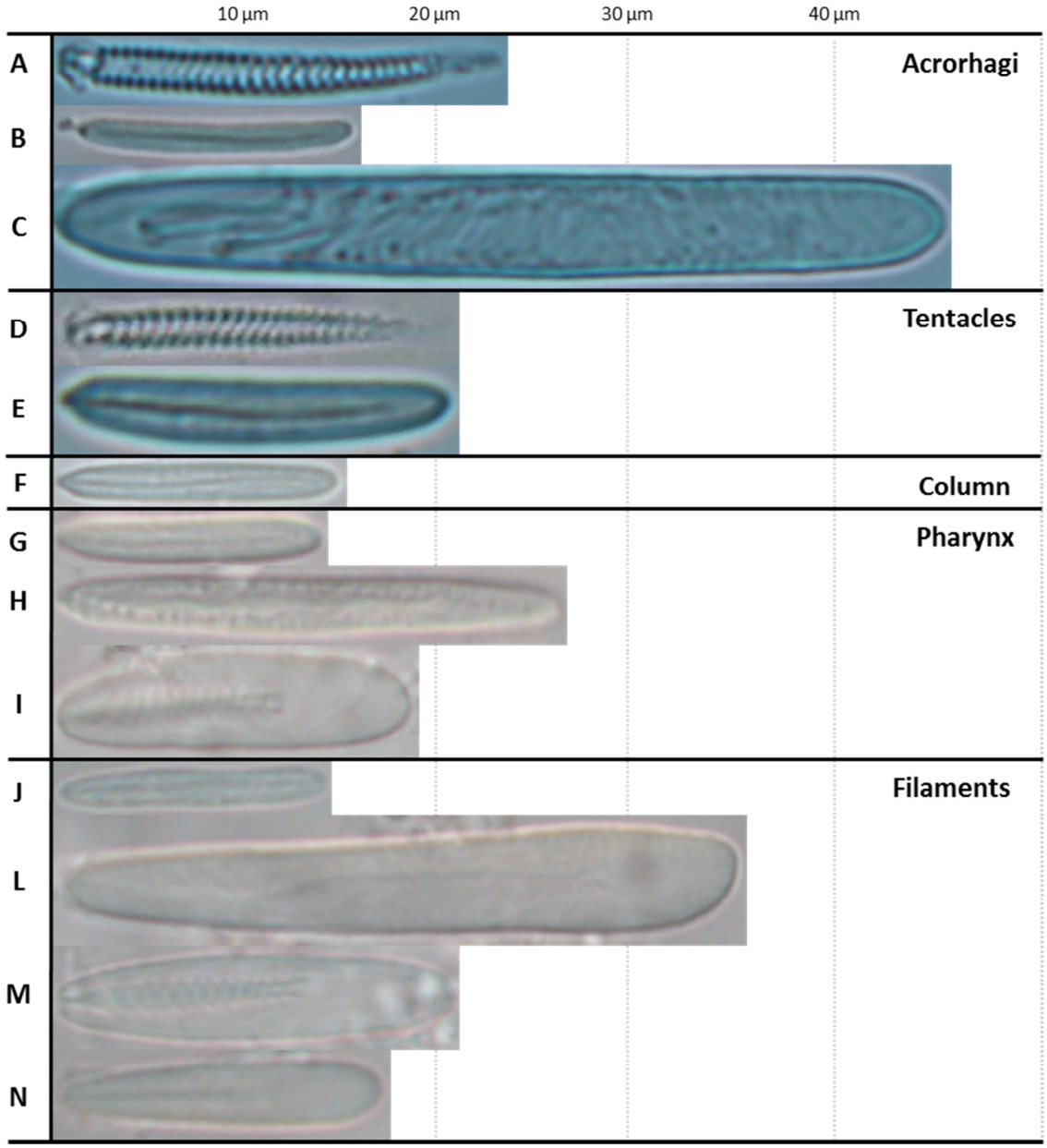

Fig 1.11. Distribution of cnidae in *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973. A, D: Spirocyst; B, E, F, G, H, J: Basitrich; C: Holotrich; L: Microbasic *b*-mastigophore; M, N: Microbasic *p*-mastigophore.

Table 1.5. Size and distribution of cnidae of *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 9); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	12.340 - 44.120 X 1.750 - 3.780	22.495 ± 7.732 x 2.590 ± 0.463	78	8/9	+
Basitrich	12.342 - 27.660 X 1.370 - 3.500	16.909 ± 2.291 x 2.533 ± 0.373	92	8/9	+
Holotrich	34.626 - 57.870 X 3.260 - 7.640	45.146 ± 5.048 x 4.962 ± 0.674	200	9/9	+++
Tentacles					
Spirocyst	13.070 - 35.390 X 1.530 - 4.180	21.287 ± 3.711 x 2.580 ± 0.444	200	9/9	+++
Basitrich	12.680 - 25.840 x 1.590 - 3.890	21.228 ± 1.980 x 2.600 ± 0.353	179	9/9	+++
Column					
Basitrich	12.615 - 21.664 x 1.906 - 2.903	16.612 ± 1.696 x 2.414 ± 0.223	115	4/4	+++
Pharynx					
Basitrich 1	12.003 - 18.651 x 1.116 - 2.795	14.825 ± 1.607 x 2.250 ± 0.409	22	4/4	++
Basitrich 2	21.219 - 32.832 x 2.245 - 4.331	27.812 ± 2.452 x 3.089 ± 0.322	103	4/4	+++
Microbasic *p*-mastigophore	17.658 - 20.956 x 4.951 - 5.731	19.373 ± 1.653 x 5.306 ± 0.395	3	2/4	+
Filaments					
Basitrich	11.150 - 18.894 x 1.241 - 2.376	14.013 ± 1.644 x 1.965 ± 0.261	25	4/4	++
Microbasic *b*-mastigophore	29.112 - 42.484 x 3.998 - 6.158	36.618 ± 2.977 x 5.455 ± 0.536	29	4/4	++
Microbasic *p*-mastigophore 1	18.260 - 25.290 x 4.168 - 5.968	21.245 ± 1.768 x 5.023 ± 0.334	47	4/4	+++
Microbasic *p*-mastigophore 2	13.222 - 21.954 x 2.352 - 3.204	17.579 ± 2.554 x 2.865 ± 0.309	12	4/4	++

Taxonomical Remarks. This species was first described in a cathedratic thesis presented by Corrêa (1964) at the University of São Paulo, Brazil. However, as pointed out by Fautin (2013) this work cannot be considered a valid publication. Thus, the proper description was only available by Belém & Preslercravo (1973), who refer to Corrêa (1964) as being the original description and based their observations in such previous work. As *B. caissarum*, it has also appeared in many studies on sea anemones in Brazil, although it has a much larger distribution than the former species. Belém & Preslercravo (1973) do not mention observation of a holotype for the species, but Corrêa (1964) proposed that the holotype was a specimen identified as A2 at the Department of Zoology (Biosciences Institute, University of São Paulo). This collection is now at the Museum of Zoology of the University of São Paulo (MZUSP), but the holotype was not found. We, therefore, propose that one specimen from Flamengo Bay, Ubatuba, SP, Brazil, from the same location as the holotype, should now be considered the Neotype.

Discussion. The vesicles in this species agree with the definition of these columnar projections provided by Daly (2004), so it belongs, in fact, to the genus *Bunodosoma*. The descriptions of Corrêa (1964) and Belém & Preslercravo (1973) are in agreement with what we observed for external morphology and internal anatomy. The major difference is in the cnidae of the column, in which Corrêa (1964) stated to be basitrichs and holotrichs, whereas we observed only basitrichs. Holotrichs and spirocysts may be found in the column of some individuals from populations of *B. cangicum* from other parts of the Brazilian coast (JSB, personal observations), but none was found in the populations herein studied. This information is more thoroughly studied comparing different Brazilian populations in Chapter 3.

B. cangicum is very similar morphologically and ecologically to *B. zamponii,* from Argentina. According to Gomes *et al.* (2012), the major morphological differences between them are the number of mesentery cycles (four in *B. zamponii* and three in *B. cangicum*) and type of retractor muscle (circumscribed to diffuse strong in *B. zamponii*, strong and circumscript in *B. cangicum*). However, we observed that *B. cangicum* also has four cycles of mesenteries (as was also commented by Corrêa, 1964) and the retractor muscle has the same morphology, diffuse to restricted and moderately strong, in both species. The differences we found between these two species are the presence of perfect mesenteries of first and second cycles in *B. cangicum*, whereas in *B. zamponii* the third cycle is also perfect over also the entire length of the actinopharynx. Also, in *B. cangicum* the last cycle of mesenteries is not fertile, and in *B. zamponii* it may be. We cannot confirm if these features in fact depict a difference between the species or if they represent mainly different levels of the same feature. Nevertheless, we chose to keep these species separate until a larger study, including various populations of both species and using appropriate data is carried out (Chapter 2).

This species occurs in sympatry with *B. caissarum*, but they tend to occupy different parts of the rocks of the intertidal zone: *B. caissarum* is usually at the lower intertidal zone, whereas *B. cangicum* is at the upper intertidal zone. When *B. caissarum* is present, *B. cangicum* individuals are scarce. However, when the first species is not present, the latter is usually very abundant (more than 5 specimens/m^2, personal observations). Although *B. cangicum* has some color variations, it is never of the same color as *B. caissarum* (dark red). This information allied to the fact that the former species has half the number of tentacles and longer tentacles than the latter makes it very difficult for both species to be confused in the field.

Bunodosoma capense (Lesson, 1830)

(Figs. 1.12-1.14, Table 1.6)

Synonymy list

Actinia capensis Lesson, 1830: 76, Plate II Fig. 4 (original description).

Phymactis capensis – Milne Edwards, 1857: 274.

Bunodosoma capensis – Carlgren, 1928: 169-172 [47-50].

Bunodosoma capense – Fautin, 2013 (spelling corrected).

Material examined

3 specimens, Kalk Bay, South Africa; intertidal, 8 May 2013; Col. S.N. Stampar & A.C. Morandini; anesthetized with $MgCl_2$ preserved in 4% formaldehyde solution.

Diagnosis. *Bunodosoma* with up to 32 transverse rows of vesicles densely distributed in the column; usually 192 tentacles in six cycles and 96 mesenteries in five cycles; mesenteries of first and second cycles perfect over entire length of the actinopharynx, third cycle reaches the actinopharynx from the middle to its distal part, fourth and fifth cycles reach actinopharynx only at its most oral part; all mesenteries fertile, except for last cycle and directives.

Description

Pedal disc. Adherent, circular to semi-circular, 20-27 mm in diameter in preserved specimens. *Column*. Cylindrical, may be narrower or the same diameter of the pedal disc, 19-27 mm in diameter and up to 22 mm height in preserved specimens. Column densely covered with vesicles in 96 longitudinal rows in up to 32 transverse rows. Vesicles endocoelic, rounded, non-adhesive, non-cup-shaped, mesoglea and ectoderm of similar thickness at the sides and

apex, ectoderm with similar cells at sides and apex. No compound vesicles. Each longitudinal row of vesicles ends distally in a short marginal projection with a distinct acrorhagus on its oral side. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic, second exocoelic; inner row with with larger acrorhagi (0.9-1 mm in diameter) than the second incomplete one (0.3-0.5 mm in diameter). May have only one row of acrorhagi. Fosse pronounced.

Oral disc. Wide, 12-18 mm in diameter in preserved specimens, may be of a larger diameter than the pedal disk; without markings in preserved specimens; mouth with slightly raised lips in some specimens.

Tentacles. Short, usually 4-7 mm in length in preserved specimens, tapered and pointed. Tentacles from the inner cycle longer. Up to 192 in 6 cycles (6+6+12+24+48+96); some specimens with up to 200 tentacles in an incomplete seventh cycle. Those of the last cycle are exocoelic.

Internal anatomy. Mesenteries pairs usually 96, hexamerously arranged in 5 cycles (6+6+12+24+48) at the actinopharynx level. Number of mesenteries equal proximally and distally. Two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first and second cycles perfect over entire length of the actinopharynx, third cycle reaches the actinopharynx from the middle to its distal part fourth and fifth cycles reach actinopharynx only at its most distal part. All mesenteries fertile, except for last cycle and directives. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript, very strong, main mesogleal lamella thin and long. Retractors restricted and moderately strong. Parietobasilar muscles with long and thin mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.14.

Color. Carlgren (1928) and Carlgren (1938, *in* Corrêa, 1964) refer to 14 color varieties in this species. In Kalk Bay we observed the following color varieties: dark red to maroon column, vesicles, tentacles and acrorhagi (Fig. 1.12A, B); dark red to marron column and tentacles, blue to purple vesicles, acrorhagi light red (Fig. 1.12 C); blue column and tentacles, dark blue vesicles, light blue acrorhagi (Fig. 1.12 D); orange column and tentacles, blue to purple vesicles and light orange acrorhagi (Fig. 1.12 E). Preserved specimens with light cream to grey column, acrorhagi and oral disk, tentacles darker grey (Fig. 1.12 F).

Distribution. Southern coast of the African continent, from Lüderitz Bay (Nigeria), to Durban (South Africa) (Acuña & Griffiths, 2004). At the intertidal zone, attached on rocks or smaller stones.

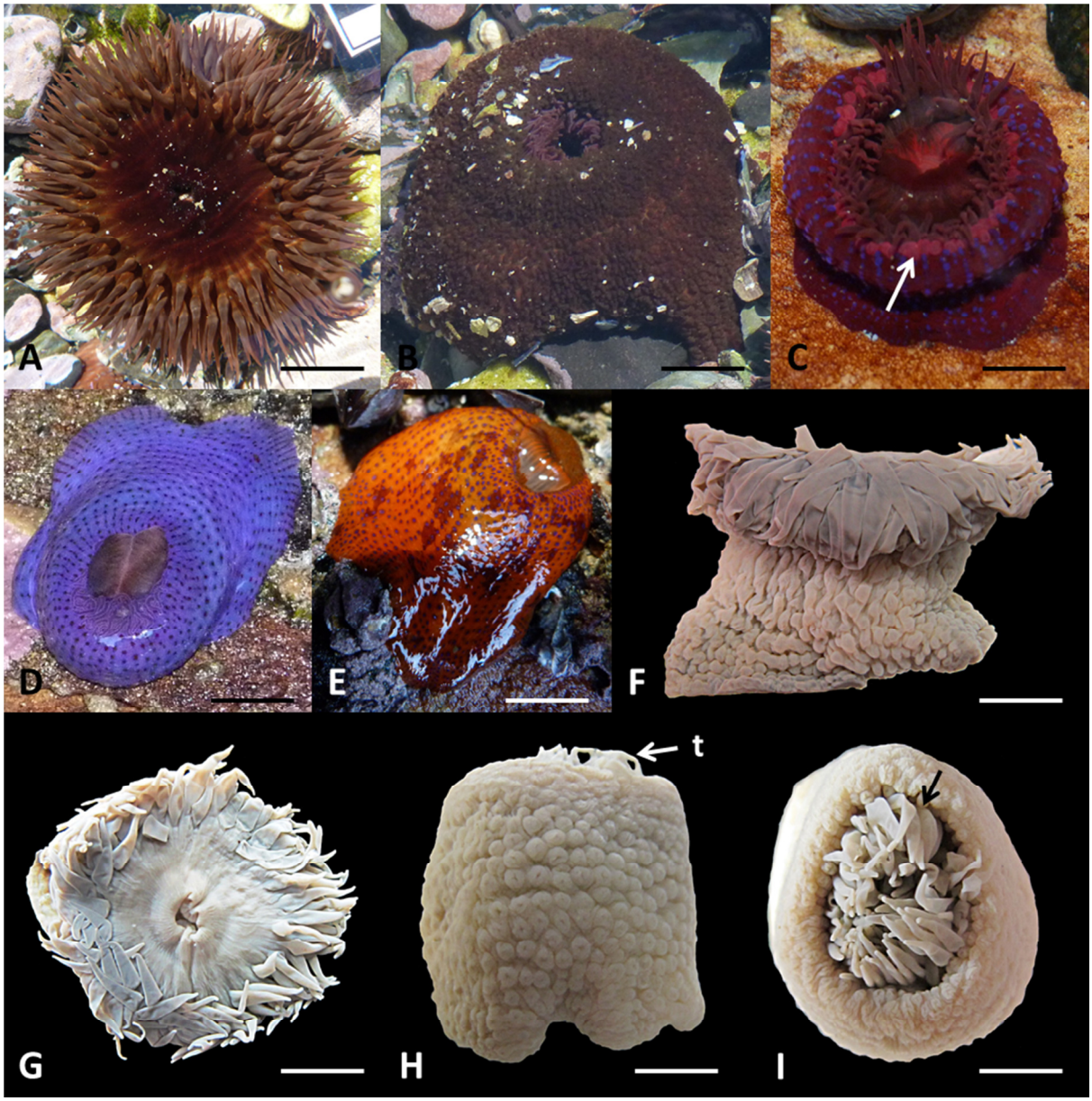

Fig. 1.12. *Bunodosoma capense* (Lesson, 1830). A) Live specimen from Kalk Bay, South Africa, oral view. Scale = 3 cm. B) Live specimen from Kalk Bay, South Africa, contracted specimen. Scale = 1.5 cm. C) Color variation in Kalk Bay, South Africa, arrow indicates acrorhagus. Scale = 1.5 cm. D) Color variation in Kalk Bay, South Africa. Scale = 0.5 cm. E) Color variation in Kalk Bay, South Africa. Scale = 0.5 cm. F) Preserved specimen, lateral view. Scale = 1 cm. G) Preserved specimen, oral view. Scale = 1cm. H) Preserved specimen, lateral view. Scale = 1 cm. I) Preserved specimen, oral view, arrow indicates acrorhagus. Scale = 1 cm. t-tentacles.

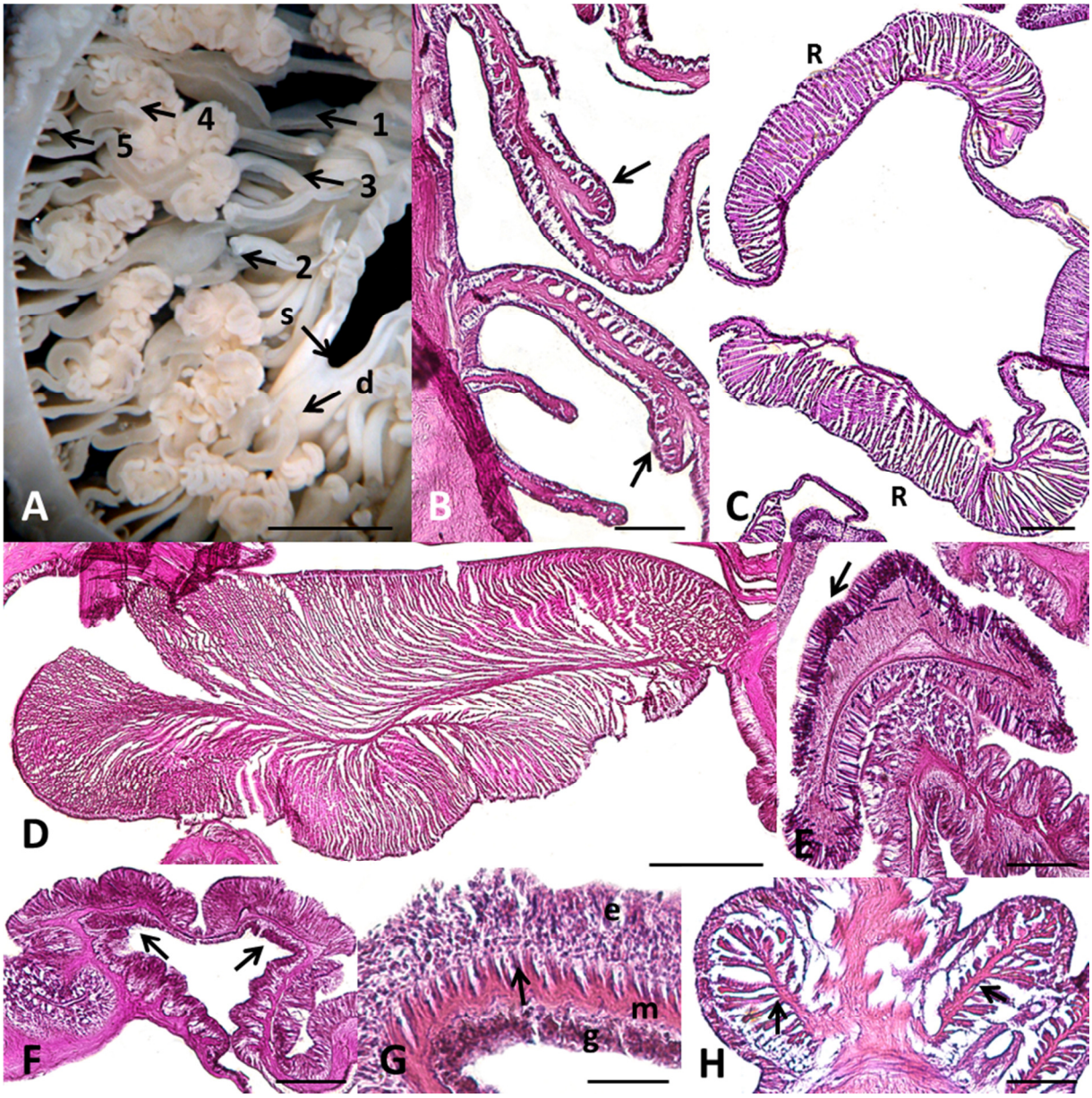

Fig. 1.13. Internal anatomy of *Bunodosoma capense* (Lesson, 1830). A) Cross section of mesenteries, at the level of the actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 7 mm. B) Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 0.05 mm. C) Cross section of mesenteries, showing retractor muscles. Scale = 0.05 mm. D) Longitudinal section through marginal sphincter muscle. Scale = 0.1 mm. E) Longitudinal section of the margin showing acrorhagus, arrow indicates ectoderm with cnidocysts. Scale = 0.3 mm. F) Cross section of columnar vesicles. Scale = 0.2 mm. G) Cross section of tentacle. Scale = 0.05 mm .H) Cross section of basilar muscle. Scale = 0.05 mm. Abbreviations: d-directive mesentery; e–epidermis; g–gastrodermis; m-mesoglea; R–retractor muscles; s-siphonoglyph.

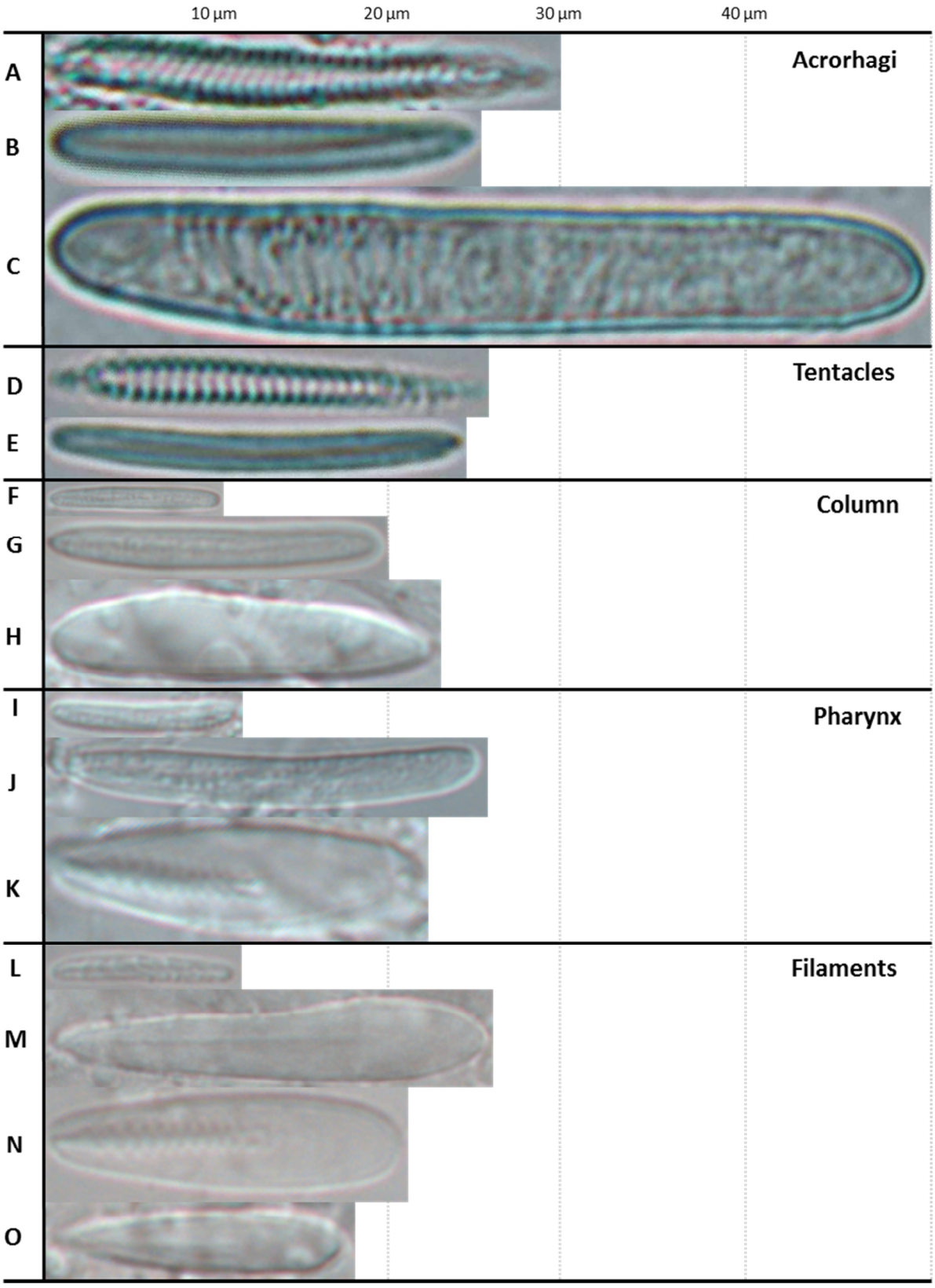

Fig 1.14. Distribution of cnidae in *Bunodosoma capense* (Lesson, 1830). A, D: Spirocyst; B, E, F, G, I, J, M: Basitrich; C, H: Holotrich; M: Microbasic *b*-mastigophore; K, N, O: Microbasic *p*-mastigophore.

Table 1.6. Size and distribution of cnidae of *Bunodosoma capense* (Lesson, 1830). Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 3); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	20.050 - 34.700 x 2.080 - 3.920	29.053 ± 3.110 x 2.969 ± 0.434	43	3/3	++
Basitrich	13.330 - 30.270 x 1.730 - 3.460	24.453 ± 3.812 x 2.368 ± 0.423	34	3/3	+
Holotrich	44.420 - 60.040 x 4.630 - 8.110	52.149 ± 2.842 x 6.441 ± 0.703	118	3/3	+++
Tentacles					
Spirocyst	15.260 - 33.420 x 1.630 - 4.530	24.903 ± 5.050 x 2.892 ± 0.555	198	3/3	+++
Basitrich	19.410 - 28.680 x 2.020 - 3.570	23.895 ± 1.538 x 2.754 ± 0.360	153	3/3	+++
Column					
Basitrich 1	8.310 - 10.990 x 1.980 - 2.820	10.037 ± 0.903 x 2.402 ± 0.325	9	1/3	+
Basitrich 2	11.870 - 23.820 x 1.870 - 3.860	19.921 ± 1.954 x 2.727 ± 0.327	79	3/3	+++
Holotrich	22.150 - 24.658 x 3.720 - 4.190	23.343 ± 1.258 x 3.933 ± 0.238	8	1/3	+
Pharynx					
Basitrich 1	9.964 - 14.160 x 2.114 - 3.077	11.327 ± 1.509 x 2.651 ± 0.421	6	3/3	+
Basitrich 2	20.510 - 31.570 x 2.440 - 3.710	26.442 ± 2.278 x 3.075 ± 0.326	79	3/3	+++
Microbasic *p*-mastigophore	20.040 - 24.820 x 4.010 - 7.180	22.637 ± 1.794 x 5.245 ± 0.894	8	3/3	+
Filaments					
Basitrich	8.300 - 12.450 x 1.990 - 2.700	11.081 ± 1.168 x 2.361 ± 0.202	14	3/3	+
Microbasic *b*-mastigophore	21.490 - 29.530 x 4.460 - 5.610	26.371 ± 3.180 x 5.110 ± 0.437	7	3/3	+
Microbasic *p*-mastigophore 1	18.310 - 25.070 x 3.610 - 7.280	20.816 ± 1.454 x 5.296 ± 0.829	40	3/3	++
Microbasic *p*-mastigophore 2	15.040 - 20.590 x 2.640 - 4.040	16.802 ± 1.206 x 3.222 ± 0.388	25	3/3	++

Taxonomical Remarks. Fautin (2013) corrected the spelling of the epithet, changing it from "*capensis*" to the neuter "*capense*", according to the Latin declension of the neuter genus name.

Discussion. According to Fautin (2013), the specimens MCZ 64179 (labeled as syntypes of *Actinia clematis*, from Chile) and MCZ 1686 (labeled as syntypes of *Actinia florida*, from Peru) are type specimens of *B. capense*. However, they are also indicated as type specimens of *Phymactis papillosa* (Häussermann, 2004; Fautin, 2013). Three aspects of the specimens in both lots lead us to the conclusion that they are not *B. capense*: the vesicles in their columns are very closely set together and larger than in *B. capense*; the number of mesenteries is larger than what we have observed and Carlgren (1928) has described for *B. capense*; both specimens have diffuse marginal sphincters as described for the genus *Phymactis*, instead of circumscribed sphincter as in *Bunodosoma*. So, we conclude that these specimens (MCZ 64179, MCZ 1686) are not *B. capense*. Therefore, our description is based only in newly collected specimens; thus there is no type material for this species. Also, *Eucladactis grandis* (in Verrill, 1899), is considered a synonym of *B. capense* (Fautin, 2013), but the information on morphology (number of tentacles and mesenteries) and geographical distribution of *E. grandis* leads to the ideia that it is probably referring to *B. grande* and not to *B. capense*.

Our description agrees mostly with the one presented by Carlgen (1928). This species is a typical *Bundosoma*, with a circumscribed sphincter and vesicles in the column. Carlgren (1928) described that this species has four cycles of complete mesenteries, but we observed only three. The fourth and fifth cycle reach the actinopharynx only at its most distal part. Also, Carlgren (1928) described some specimens with a larger number of tentacles (up to 170), which we did not observe. The specimens we observed were quite regular in their

distribution of tentacles and mesenteries, and Carlgren (1928) stated that he observed certain irregularities.

We compared the cnidae we found in the specimens to the description of the cnidom of this species provided by Carlgren (1945). We have reinterpreted the identification of some of the types of nematocysts in light of today's knowledge, but in general, the types and sizes agree with what he presented. In the acrorhagi we found spirocysts and basitrichs, which were not described by him. In the column and actinopharynx we found very small basitrichs and he didn't. We also found two spirocysts in the column of one of the specimens. Finally, we identified microbasic *b*-mastigophores and two sizes of *p*-mastigophores in the filaments.

As pointed out by Corrêa (1964) and Belém (1987), *B. caissarum* shares some features with *B. capense.* Besides having the same number of tentacles and mesenteries, *B. capense* seems to also share the color of *B. caissarum* (Carlgren, 1928, 1938 *in* Corrêa, 1964), although the author comments that the former species has fourteen color varieties. As for cnidocyst types, on one hand, both species have two size classes of basitrichs in the column, which are rare in both species. On the other hand, *B. capense* only has one size class of basitrichs in the tentacles and one of microbasic *b*-mastigophore in the filaments, instead of two size classes of each, as *B. caissarum*.

The similarities of *B. capense* to *B. goanense* are also in the number of tentacles, mesenteries and color, but also in number of transverse rows of vesicles (respectively up to 32 and 35, whereas *B. caissarum* has up to 70). A difference in their internal anatomy is that, whereas the fifth (youngest) mesentery cycle is sterile in *B. capense*, it may be fertile in *B. goanense* (according to den Hartog & Vennam, 1987; it was infertile in most specimens we observed). As for the cnidom, some differences can be pointed out: *B. capense* has two types of basitrichs in the column, and *B. goanense* has only one; the microbasic *p*-mastigophores

are slightly more common in the pharynx of *B. caissarum* than in *B. goanense*; *B. goanense* has three types of basitrichs, while *B. caissarum* has only one.

Bunodosoma cavernatum (Bosc, 1802)

(Figs. 1.15-1.17, Table 1.7)

Synonymy list

Actinia cavernata Bosc, 1802: 221-222 (original description).

Urticina cavernata – Duchassaing, 1850: 9.

Bunodes cavernata – Verrill, 1864: 17-18.

Phymactis cavernata – Andres, 1883: 448.

Bunodosoma cavernata – Verrill, 1899: 45.

Anthopleura cavernata – Cary, 1906: 51.

Bunodosma cavernata – Daly, 2003: 92.

Bunodosoma cavernatum – Fautin, 2013 (spelling corrected).

Material examined

YPM 973: 1 dissected specimen. Charleston, South Carolina, 1866, by W. Stimpson.

MCZ SCOR 1263: 4 whole specimens, 1 dissected. North America: United States: South Carolina; Charleston. 32.754788° / -79.867144°. Coll: Louis Agassiz [date unknown]. Specimens attached to mollusk shells and/or rocks. All data taken from old MCZ Catalogue; entered as *Bunodes cavernata* (etc.), 5 specimens, March, 1864. Paper label "146" is also in the jar. whole animal (70% ethanol).

USNM 22128: 3 whole specimens, 1 dissected. Hucares, Puerto Rico, Feb. 17, 1899. Steamer Fish Hawk. Id. as *Bunodosoma cavernatum* by J.W. Hedgepeth. Id. as *Bunodosoma granuliferum* by J.C. den Hartog, 1974. Acc. No. 46417

USNM 51034: 2 whole specimens, 2 dissected. St. James, Barbados, B.W.I. John B. Lewis. June 1958. Id. C.E. Cutress, 1958. Acc. No. 220661.

USNM 53332: 2 whole specimens, 2 dissected. Beaufort, North Carolina, Aug 1954, D.W. Strasburg. Id. as *Bunodosoma cavernata* by C.E.Cutress. Id. as Bunodosoma pustulata by J.C. den Hartog, 1974. Acc. No. 209154.

Diagnosis. *Bunodosoma* with up to 34 transverse rows of vesicles densely distributed in the column; 96 tentacles in five cycles and 48 mesenteries in four cycles; mesenteries of first and second cycles perfect over the pharynx's entire length, third and fourth cycles reach actinopharynx only at its most oral part. All mesenteries fertile, directives excluded. Last cycle may have some infertile mesenteries in some specimens.

Description

Pedal disc. Adherent, circular to semi-circular, 13-15 mm in diameter in preserved specimens.

Column. Cylindrical, densely covered with vesicles in 96 longitudinal rows. Large specimens have up to 34 transverse rows. The vesicles are endocoelic and exocoelic, rounded, non-adhesive, non-cup-shaped, mesoglea of similar thickness at the sides and apex, ectoderm of similar thickness and containing similar cells at sides and apex. The vesicles of the 3-4 most distal rows may be compound, 2-4 lobes each. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic with up to 48 larger acrorhagi (up to 3 mm diameter) than the second one exocoelic (up to 1.5 mm diameter). Smaller specimens may have only one row of acrorhagi. Fosse pronounced. Column more or

less of the same diameter as pedal disc, 14-17 mm in diameter and up to 28 mm height in preserved specimens.

Oral disc. 10-11 mm in diameter and without markings in the preserved specimens.

Tentacles. Short, 2-5 mm in preserved specimens, stout, tapered and pointed. Tentacles of the inner cycle may be longer than outer. Most individuals have 96 arranged in 5 cycles (6+6+12+24+48). Those of the last cycle are exocoelic.

Anatomy. Mesenteries pairs up to 48 hexamerously distributed in 4 cycles (6+6+12+24). Same number of mesenteries distally and proximally. Usually two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first and second cycles perfect over the pharynx's entire length, third and fourth cycles reach actinopharynx only at its most oral part. All mesenteries fertile, directives excluded. Last cycle may have some infertile mesenteries in some specimens. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript, strong, main mesogleal lamella thin and short. Retractors restricted and strong. Parietobasilar muscles with short and thick mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, holotrichs, basitrichs, microbasic *p*-mastigophores. microbasic *b*-mastigophores. A survey of sizes and distribution is shown in Table 1.7.

Color: According to Gonzalez-Muñoz *et al.* (2013), specimens from Mexico are as follows: column and pedal disc light-brown, orange, reddish, yellowish or olive-green; oral disc brown-yellowish, brown-reddish or pale olive-green, sometimes with white or yellowish radial stripes in endocoelic spaces of first two or three tentacle cycles; tentacles olive-green, reddish or pale-orange, with white or yellowish spots on oral side and sometimes with purple flashes. Carlgren & Hedgepeth (1952) described them as light brown with bluish vesicles, tentacles and oral disc from deep red and blue to pale olive-brown.

Distribution: Western Atlantic, from North Carolina to Barbados; Mexico (Veracruz Reef System) (Gonzalez-Muñoz *et al*., 2013), Caribbean Sea and Gulf of Mexico (Carlgren & Hedgpeth, 1952)*;* Caroline Islands, Micronesia (Bosc, 1802).

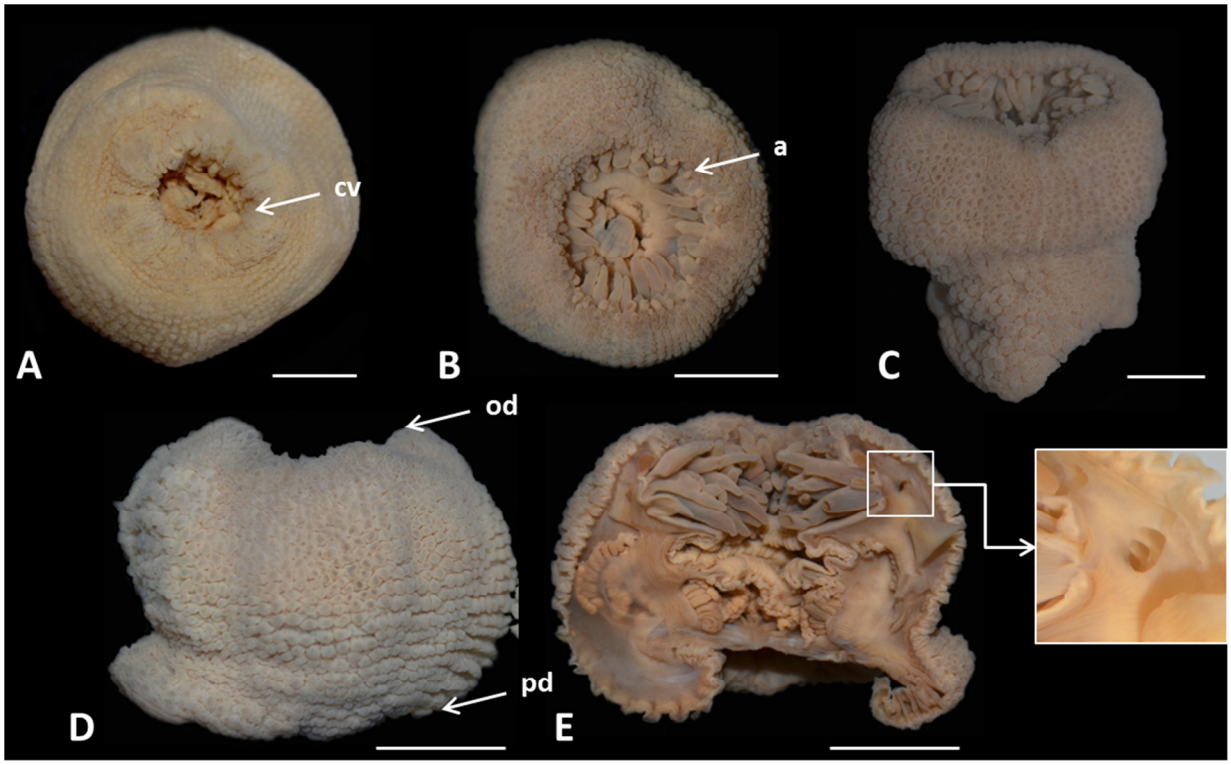

Fig. 1.15. *Bunodosoma cavernatum* (Bosc, 1802). A) YPM 973, oral view. Scale = 0.7cm. B) USNM 53332, oral view. Scale = 1cm. C) USNM 53332, lateral view. Scale = 1cm. D) USNM 53332, lateral view. Scale = 1cm. E) USNM 53332, longitudinal section of whole animal, internal view, detail showing marginal stoma. Scale = 1cm. Abbreviations: a-acrorhagus; cv-compound vesicles; od-oral disc; pd-pedal disc.

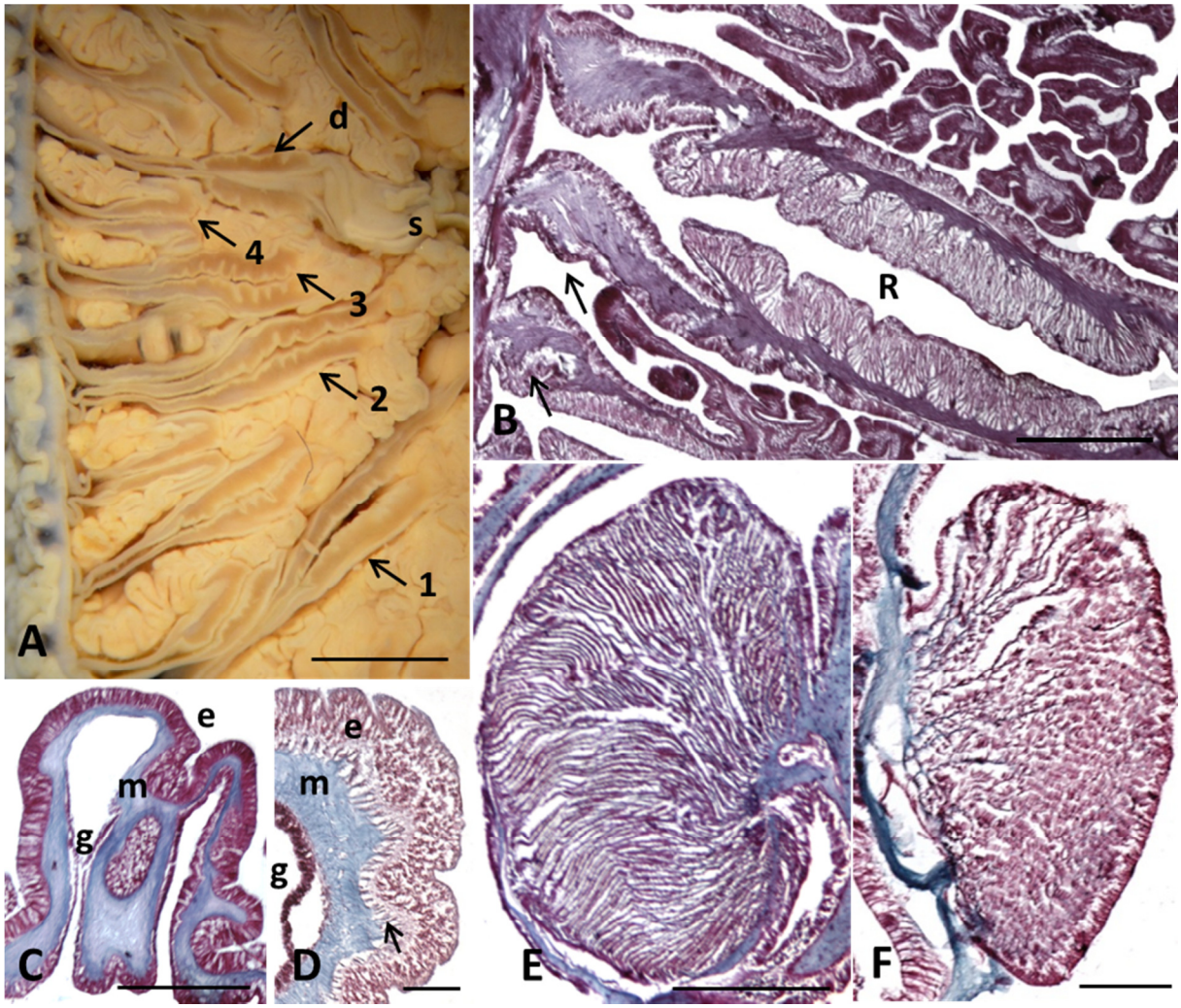

Fig. 1.16. Internal anatomy of *Bunodosoma cavernatum* (Bosc, 1802). A) Cross section of mesenteries, at the level of the actinopharynx. Numbers indicate the cycles of mesenteries. Scale = 5 mm. B) Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 3 mm. C) Cross section of columnar vesicles. Scale = 0.6 mm. D) Cross section of tentacle. Scale = 0.05 mm. E) USNM 22128, longitudinal section through marginal sphincter muscle. Scale = 0.1 mm. F) USNM 53332, longitudinal section through marginal sphincter muscle. Scale = 0.1 mm. Abbreviations: d-directive mesentery; e–epidermis; g–gastrodermis; m-mesoglea; R–retractor muscles; s-siphonoglyph.

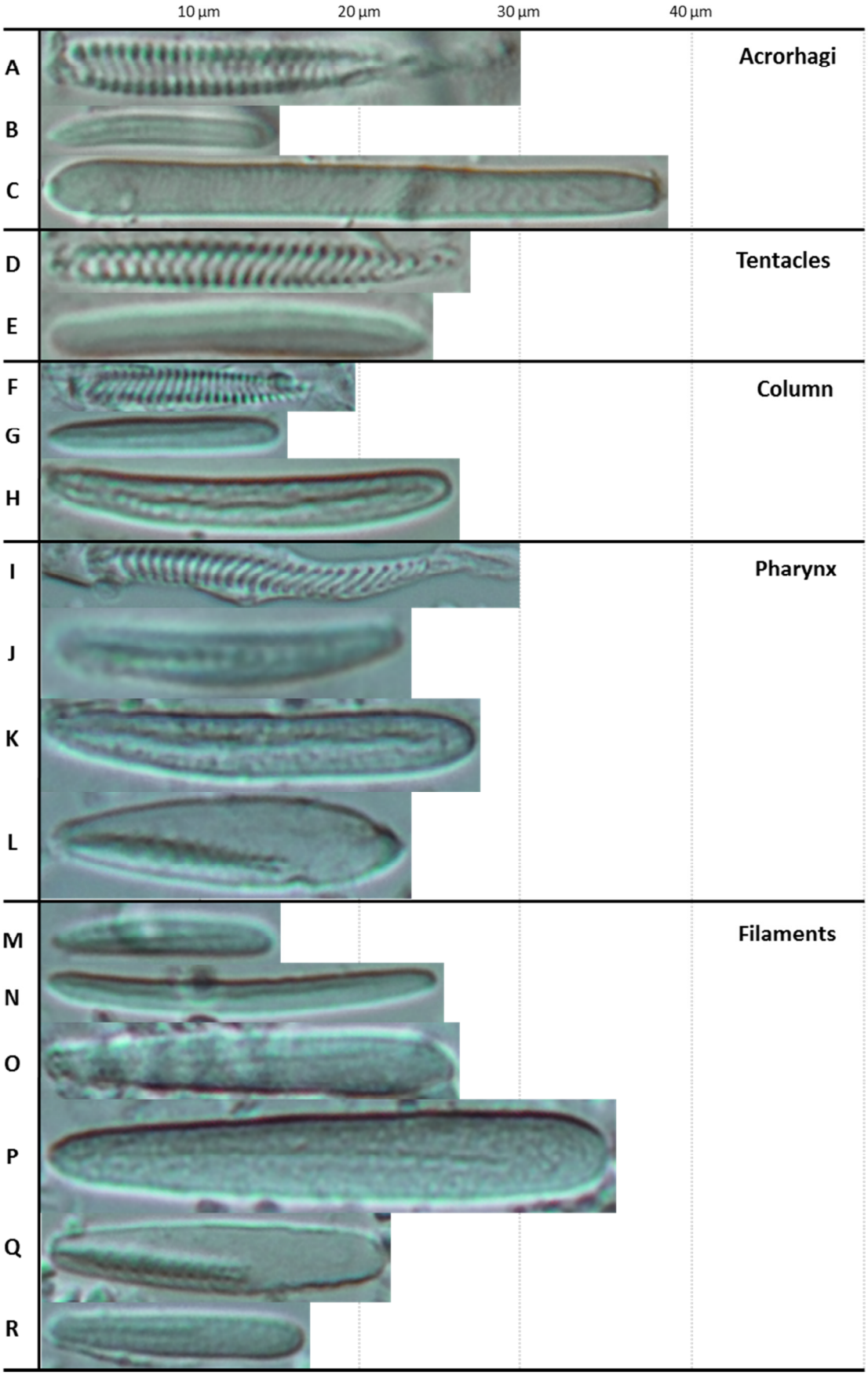

Fig. 1.17. Distribution of cnidae in *Bunodosoma cavernatum* (Bosc, 1802). A, D, F, I: Spirocyst; B, E, G, J, K, M, N: Basitrich; C: Holotrich; O, P: Microbasic *b*-mastigophore; L, Q, R: Microbasic *p*-mastigophore.

Table 1.7. Size and distribution of cnidae of *Bunodosoma cavernatum* (Bosc, 1802). Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 6); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	21.911 - 32.471 x 1.480 - 3.769	29.272 ± 2.681 x 2.932 ± 0.516	21	6/6	+
Basitrich	11.890 - 22.999 x 1.580 - 2.875	15.061 ± 2.102 x 2.188 ± 0.331	36	6/6	++
Holotrich	30.637 - 49.140 x 3.203 - 5.145	38.173 ± 4.303 x 4.195 ± 0.386	84	6/6	+++
Tentacles					
Spirocyst	15.030 - 34.559 x 1.490 - 4.165	26.252 ± 5.019 x 2.681 ± 0.517	127	6/6	+++
Basitrich	16.230 - 29.097 x 1.527 - 3.474	23.911 ± 2.094 x 2.576 ± 0.355	200	6/6	+++
Column					
Spirocyst	10.240 - 23.620 x 1.860 - 2.720	15.538 ± 5.451 x 2.368 ± 0.290	6	1/6	+
Basitrich 1	10.370 - 18.799 x 1.227 - 3.163	16.214 ± 1.703 x 2.268 ± 0.363	93	6/6	++
Basitrich 2	20.205 - 29.612 x 2.091 - 4.253	24.917 ± 2.057 x 3.188 ± 0.450	119	6/6	+++
Pharynx					
Spirocyst	27.467 - 37.320 x 2.800 - 3.681	33.531 ± 3.716 x 3.431 ± 0.372	5	2/6	+
Basitrich 1	11.730 - 28.360 x 1.680 - 2.061	22.611 ± 5.758 x 2.994 ± 0.709	36	6/6	++
Basitrich 2	23.880 - 28.359 x 2.540 - 3.040	26.219 ± 1.222 x 3.382 ± 0.417	25	6/6	++
Microbasic *p*-mastigophore	20.000 - 23.310 x 4.660 - 5.720	21.828 ± 1.358 x 5.157 ± 0.316	7	2/6	+
Filaments					
Basitrich 1	10.320 - 19.570 x 1.490 - 2.735	13.667 ± 2.820 x 2.084 ± 0.395	10	5/6	+
Basitrich 2	21.030 - 28.600 x 2.351 - 3.581	24.847 ± 2.612 x 2.953 ± 0.502	8	5/6	+
Microbasic *b*-mastigophore 1	24.910 - 27.180 x 3.680 - 4.381	26.520 ± 1.077 x 4.020 ± 0.311	4	1/6	+
Microbasic *b*-mastigophore 2	30.840 - 44.130 x 4.241 - 8.147	36.419 ± 3.063 x 5.912 ± 0.970	43	6/6	++
Microbasic *p*-mastigophore 1	18.431 - 25.330 x 4.081 - 6.385	21.986 ± 1.345 x 5.090 ± 0.485	67	5/6	++
Microbasic *p*-mastigophore 2	15.830 - 16.610 x 2.451 - 2.661	16.220 ± 0.551 x 2.555 ± 0.148	2	1/6	+

Taxonomical Remarks. Fautin (2013) corrected the spelling of the epithet, changing it from "*cavernata*" to the neuter "*cavernatum*", according to the Latin declension of the neuter genus name.

Discussion. The syntype (no number) indicated as *Actinia cavernata* by Bosc (1802) in Fautin (2013) was not found. This species is very similar to *B. granuliferum* in external and internal morphology, and the feature that separates them is the presence of a distinct chromatic pattern (= stripes or longitudinal bands, see description of *B. granuliferum*) in the column of the latter, which is absent in *B. cavernatum*. We analyzed specimens from museums identified as *B. cavernatum* and *B. granuliferum* together and posteriorly separated them based on the absence or presence of the longitudinal bands. We were not able to match this material to published descriptions, so we are not sure if the preserved specimens with no stripes were in fact like that when alive, or if the longitudinal bands faded away after preservation. Then, we compared our observations to the ones of Carlgren (1952), Carlgren & Hedgpeth (1952) and González-Muñoz *et al.* (2013), who recently collected fresh material of none-striped *Bunodosoma*.

Carlgren (1952) and González-Muñoz *et al.* (2013) observed that the marginal sphincter muscle tends to split in two parts in the specimens of *B. cavernatum* they studied (see González-Muñoz *et al.*, 2013, Fig. 5) and that this could be a morphological difference between the two very similar species. However, we did not observe that in any of our specimens of *B. cavernatum* neither *B. granuliferum*. Also, we added some types and size classes of cnidocysts in some parts of the polyp to the ones the authors described: spirocysts in the acrorhagi, actinopharynx and column; two size classes of basitrichs instead of one in the pharyx; and two size classes of microbasic *b*-mastigophores and microbasic *p*-mastigophores in the filaments.

Bunodosoma cavernatum is one of the four valid species of *Bunodosoma* that have been reported in the Gulf of Mexico and Caribbean Sea (Carlgren & Hedgpeth, 1952; González-Muñoz *et al.*, 2012, 2013; Fautin, 2013). Although this species is very similar to *B. granuliferum*, molecular evidence (allozymes) has been used to show that these species are genetically different and that should be considered as separate species (McCommas & Lester, 1980). The distinction between these species and *B. sphaerulatum* is not clear based on the information available for the latter (see description of *B. sphaerulatum*). *B. kuekenthali*, however, is very different from all of them (see description of *B. kuekenthali*).

We conclude, therefore, that the species *B. granuliferum* and *B. cavernatum* should be considered as separate species and that they must be identified based on live animals. Based on the information obtained from museum material, we could have decided to synonymize the species, but we prefer to establish that both species must be further studied so that evidences of variable morphology *in vivo* are observed and integrated to molecular information.

***Bunodosoma diadema* (Drayton *in* Dana, 1846)**

Synonymy list

Actinia diadema Drayton *in* Dana, 1846: 133, pl. 2, fig. 11 (original description).

Bunodes Diadema – Gosse, 1855: 274.

Phymactis diadema – Milne Edwards, 1857: 274.

Bunodosoma diadema – Carlgren, 1939: 794–795.

Material examined

No museum samples of this species were found; therefore our description is based exclusively on the information provided by Carlgren (1939).

Diagnosis. *Bunodosoma* with vesicles densely distributed in the column; around 90 tentacles and 48 mesenteries in four cycles; all or almost all mesenteries perfect.

Description

Column. Cylindrical, densely covered with close-set vesicles in irregular longitudinal rows. Around 48 acrorhagi. Fosse pronounced. Column 20 to 50 mm height in preserved specimens.

Oral disc. 20 mm diameter.

Tentacles. Around 90, 10 mm long in expanded preserved specimens, conical, all the same length.

Anatomy. 48 mesenteries pairs distributed in 4 cycles (6+6+12+24). Two distinct siphonoglyphs. All mesenteries, or almost all, perfect. Sterile specimen. Marginal sphincter muscle endodermal and circumscript, main mesogleal lamella ordinarily thick. Retractors well developed with fairly high folds. Parietobasilar muscles distinct. Longitudinal muscles of the tentacles ectodermal.

Cnidome. See Carlgren (1939) for sizes of the cnidocysts, he did not identify types..

Color: Vesicles purple-blue, acrorhagi pink, tentacles and oral disc scarlet (Dana *in* Carlgren, 1939)

Distribution: Cape Verde Islands, St. Vincent, Porto Grande, shore.

Discussion. The original description of this species (Drayton *in* Dana, 1846) gives only some very basic information on external morphology. The description provided by Carlgren (1939)

stated that the specimen he studied had vesicles in the column, acrorhagi (possibly with holotrichs, based on the size of the cnidocysts he described) and a marginal sphincter muscle endodermal and circumscript, all characteristics of the genus *Bunodosoma*. Although the specimen was sterile, preventing us from confirming data such as distribution of the reproductive tissue in the mesenteries, we believe that there is enough information to conclude that this is valid species and it does in fact belong to the genus *Bunodosoma*.

Bunodosoma fallax (Pax, 1922)

(Figs. 1.18-1.19, Table 1.8)

Synonymy list

Bunodactis fallax Pax, 1922: 79 (original description).

Bunodosoma fallax – Carlgen, 1928: 249 – 251[127 – 129].

Material examined

ZMB 7171: Syntype, 2 dissected specimens; one specimen sectioned longitudinally; second specimen sectioned longitudinally and transversally into three pieces; New Amsterdam, May 1903. Leg. German South Polar Expedition. Det. Pax. Amsterdam Island in the temperate southern Indian Ocean (37°55'S 77°40'E).

Diagnosis. Based on the available information, we cannot confirm if this species does in fact belong to the genus *Bunodosoma.*

Description

Pedal disc. Adherent, circular to semi-circular, 9-11 mm in diameter in preserved specimens.

Column. Cylindrical, of same diameter as pedal disk and 6 mm height in one of the specimens, possibly covered with non-adhesive vesicles (may be verrucae). Acrorhagi may be present (no cnidae found in it).

Oral disc. 4-6 mm in diameter and without markings in the preserved specimens.

Tentacles. 3-5 mm in preserved specimens, stout, tapered and pointed. 33 and 54 tentacles in the dissected specimens available. Possibly 96 tentacles in 5 cycles (6+6+12+24+48) in the whole specimen.

Internal anatomy. 26 visible mesenteries, possibly hexamerously distributed in four cycles (6+6+12+24) at the actinopharynx level. One specimen with some fertile mesenteries. No zooxanthellae.

Cnidome. We were able to observe only a few spirocysts and basitrichs in these specimens. A survey of sizes and distribution is shown in Table 1.8.

Color. Preserved specimens light pink to orange column, oral disc, tentacles and pedal disc. Pax (1922) did not provide information about the color of the living animal.

Distribution. temperate Southern Indian Ocean, Amsterdam Island (37°55'S 77°40'E)

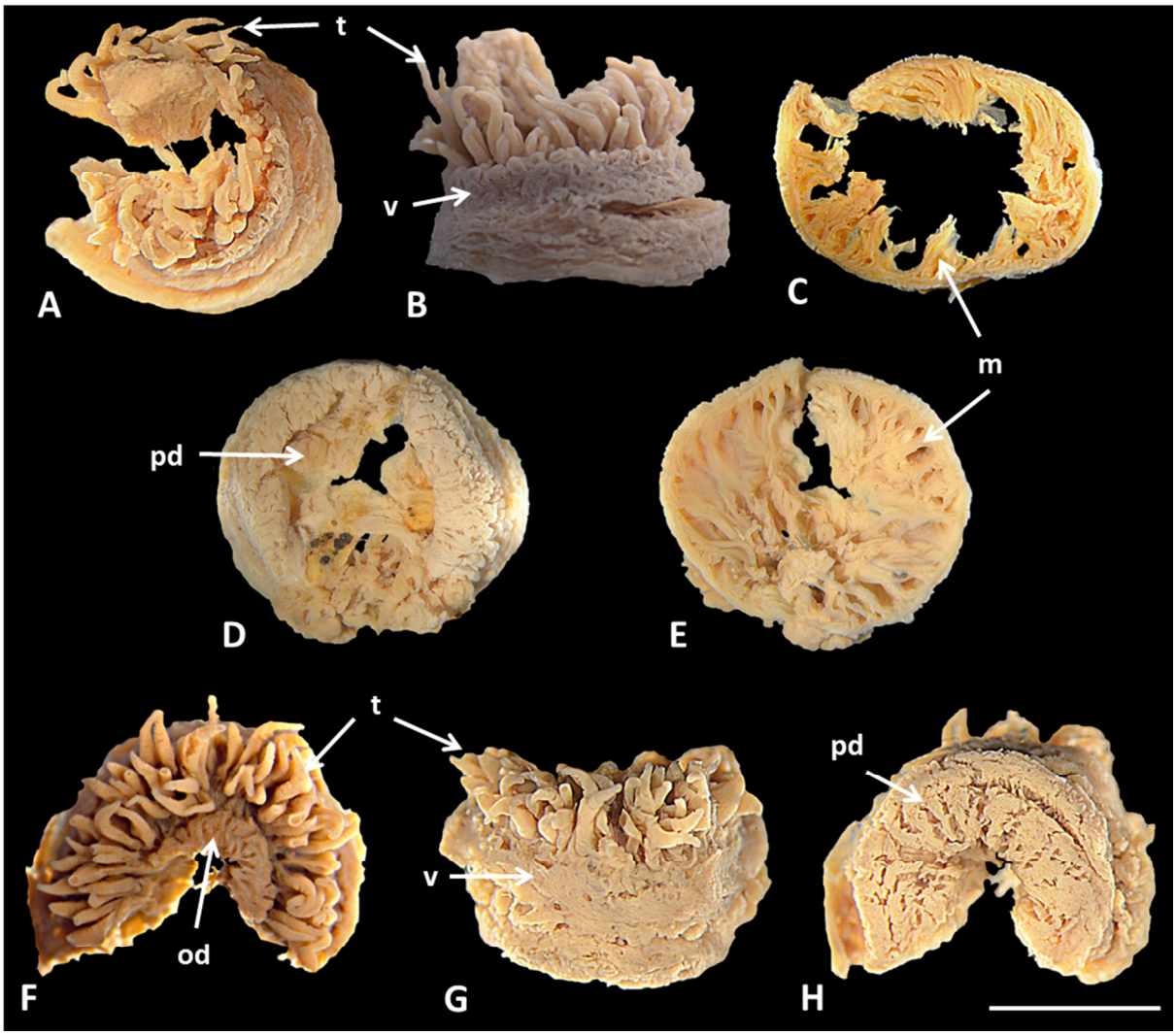

Fig. 1.18. *Bunodosoma fallax* (Pax, 1922) ZMB 7171. Specimen 1. A-E. A) Oral view. B) Lateral view. C) Cross section at middle part of column. D) Aboral view. E) Cross section of column near pedal disc. Specimen 2. F-H. F) Oral view. G) Lateral view. H). Aboral view. Abbreviations: aph-actinopharynx; m-mesenteries; od-oral disc; pd-pedal disc; t-tentacle; v-vesicles. Scale = 5 mm.

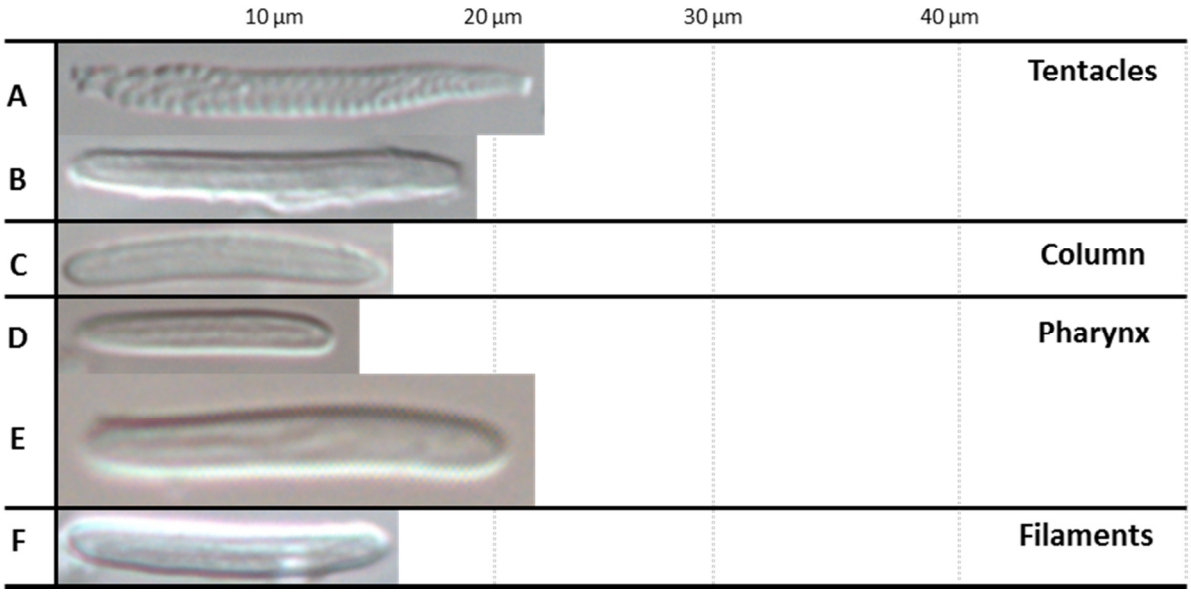

Fig. 1.19. Distribution of cnidae in *Bunodosoma fallax* (Pax, 1922). A: Spirocyst; B, C, D, E, F: Basitrich.

Table 1.8. Size and distribution of cnidae of *Bunodosoma fallax* (Pax, 1922). Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 2); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Tentacles					
Spirocyst	14.546 - 29.878 x 1.121 - 3.425	22.808 ± 5.749 x 2.524 ± 0.582	17	1/1	+++
Basitrich	17.423 - 22.769 x 1.98 - 3.000	19.824 ± 1.552 x 2.254 ± 0.329	20	1/2	+++
Column					
Basitrich	13.326 - 23.264 x 1.925 - 2.924	15.308 ± 1.839 x 2.397 ± 0.288	29	1/1	+++
Pharynx					
Basitrich 1	11.931 - 14.678 x 1.542 - 2.721	13.269 ± 0.658 x 2.160 ± 0.278	33	1/1	+++
Basitrich 2	20.659 - 22.278 x 1.723 - 2.642	21.251 ± 0.589 x 2.263 ± 0.289	7	1/1	+
Filaments					
Basitrich	16.334 1.687		1	1	+

Discussion. The description presented herein is based on the syntypes as well as on the original description of Pax (1922). However, we did not include his notes on internal morphology as we were not able to observe almost any of the features. Pax (1922) provides the following information: 24 pairs of mesenteries, including two pairs of directives; mesenteries of the first two cycles complete; retractors very strong; gonochoric; reproductive tissue on the first and second mesentery cycles; sphincter (which seems not to be mesogleal, as he comments) very circumscribed and very strongly developed. He did not provide any information on cnidae for these specimens.

According to the label on the material, there were three specimens in this lot, but we only observed what seemed to be two specimens. The type material does not allow recognition as a species of *Bunodosoma;* we cannot confirm that it has vesicles and not verrucae (in which case it would belong to *Anthopleura* or another related genus). Pax (1922) comments that the vesicles are more developed distally than proximally, however, due to the bad state of preservation, we were not able to count the number of longitudinal and transverse rows of vesicles (or verrucae). Also, both specimens had part of their oral disc missing, so it was not possible to count all tentacles. The number provided is based on a projection of the available parts of the specimens, and also on the description of Pax (1922). As for the cnidae, one specimen had no acrorhagi, while the other seemed to have a marginal projection, although we could not find any preserved cnidae. Pax (1922) comments that the acrorhagi are missing.

If this species in fact belongs to the genus *Bunodosoma*, it is one of the two species from Indian Ocean (together with *B. goanense*). However, we agree with Den Hartog & Vennam (1993) that the generic identity of this species needs to be confirmed based on freshly collected material.

Bunodosoma goanense den Hartog & Vennam, 1993

(Figs. 1.20-1.22, Table 1.9)

Synonymy list

Bunodosoma granulifera (Leseur [sic], 1817) – Parulekar, 1968: 142.

Bunodosoma granulifera (Leseur [sic]) – Haque, 1977: 36, 39.

Bunodosoma goanensis den Hartog & Vennam, 1993: 610-617 (original description).

Bunodosoma goanense – Fautin *et al.,* 2015: 45-46.

Material examined

RMNH Coel. 18434: Holotype, 1 dissected specimen; Anjuna, Goa, India.

RMNH Coel. 18435: Paratypes, 3 dissected specimens; Anjuna, Goa, India.

RMNH Coel. 18436: Paratypes, 3 dissected specimens; Anjuna, Goa, India.

RMNH Coel. 18438: Paratypes, 2 dissected specimens; Kanyakumari, Tamil Nadu, India; rocky intertidal region, 1987.

RMNH Coel. 18450; Paratypes, 2 whole specimens; Okha, Gujarate, India; reef region, intertidal, 1987.

Diagnosis. *Bunodosoma* with up to 35 transverse rows of vesicles in the column, dense proximally, sparse in the middle part and compound distally; 192 tentacles in six cycles and 96 mesenteries in five cycles; mesenteries of first and second cycles perfect over entire length of the actinopharynx, third cycle reaches the actinopharynx from the middle to its distal part, fourth and fifth cycles reach actinopharynx only at its most distal part; large specimens with all mesenteries fertile, excluding directives. Less developed specimens may have fifth cycle infertile.

Description

Pedal disc. Adherent, circular to semi-circular, 10-27 mm in diameter in preserved specimens.

Column. Cylindrical, narrower or of same diameter as pedal disc, 10-25 mm in diameter and up to 29 mm height in preserved specimens. Column densely covered with vesicles in 96 longitudinal rows and up to 35 transverse rows; in relatively relaxed specimens, vesicles visibly dense (18-23 rows) in proximal part of column, sparse in the middle (10-11 rows), and becoming compound distally (3-4 rows). Vesicles endocoelic, rounded, non-adhesive, non-cup-shaped; mesoglea and ectoderm of similar thickness at sides and apex, ectoderm with similar cells at sides and apex. Each longitudinal row of vesicles ends distally in a short marginal projection with a distinct acrorhagus on its oral side. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic, second exocoelic; inner row with larger acrorhagi (0.9-1 mm in diameter) than second one (0.6-0.7 mm in diameter). Smaller specimens may have only one row of acrorhagi. Fosse pronounced. Margin more or less undulated in relatively relaxed preserved specimens.

Oral disc. Broad, 8-18 mm in diameter in preserved specimens, wider than pedal disc diameter in some specimens; without markings in preserved specimens. Mouth with raised lips in some specimens.

Tentacles. Short, usually 2-4 mm in length in preserved specimens, tapered and pointed. 192 in 6 cycles (6+6+12+24+48+96); some specimens with fewer tentacles on outer cycle. Those of the last cycle are exocoelic.

Internal anatomy. Mesenteries pairs usually 96, hexamerously arranged in 5 cycles (6+6+12+24+48) at the actinopharynx level. Some specimens with up to 100 mesenteries pairs (sixth incomplete cycle of mesenteries present distally). Two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first and second cycles perfect over entire length of the actinopharynx, third cycle reaches the actinopharynx from the middle

to its distal part, fourth and fifth cycles reach actinopharynx only at its most distal part. Large specimens with all mesenteries fertile, excluding directives. Less developed specimens may have fifth cycle infertile. Sixth cycle when present infertile. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript, strong, main mesogleal lamella thin and short. Retractors restricted and strong. Parietobasilar muscles with short and thick mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.9.

Color. Den Hartog & Vennam (1993) described the column, tentacles and oral disc of live specimens to be entirely brownish crimson to brick red in color. Fautin *et al.* (2015) confirms these colors for the Singapore specimens, but provide additional information on color variety. Preserved specimens have column, oral disk and pedal disc of a light orange to brown or grey color, and grey tentacles. Compound vesicles may have translucid with cream extremity.

Distribution. Indo-West Pacific, from Okha (Gujarat) in India to Bangladesh (Parulekar, 1968; Haque, 1977; Den Hartog & Vennam, 1993) and Singapore (Fautin *et al.*, 2015). According to den Hartog & Vennam (1993) and Fautin *et al.* (2015) this species is intertidal and is found attached to smooth rock surfaces.

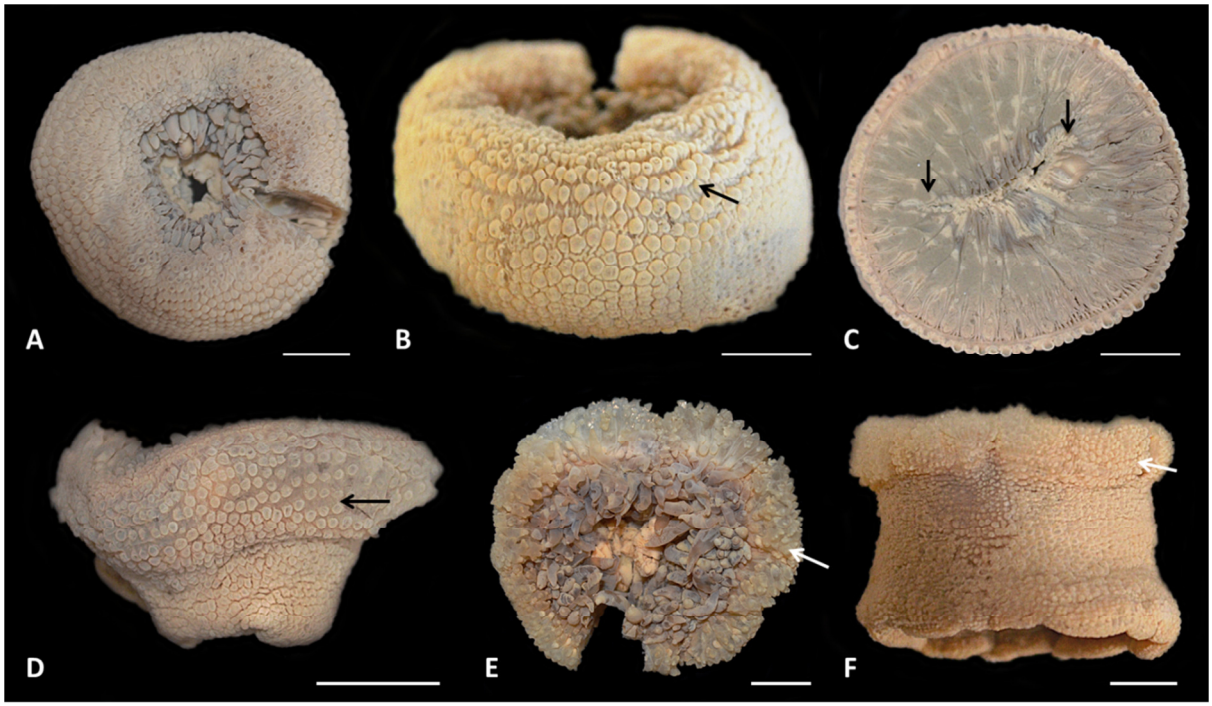

Fig. 1.20. *Bunodosoma goanense* den Hartog & Vennam, 1993. A) RMNH. Coel. 18434. Holotype, oral view. B) RMNH. Coel. 18434. Holotype, lateral view, arrow indicates start of sparse vesicles. C) RMNH. Coel. 18434. Holotype, cross section, arrows indicate siphonoglyphs. D) RMNH. Coel. 18436. Paratype, lateral view; arrow indicate start of sparse vesicles and undulated margin. E) RMNH. Coel. 18438. Paratype, oral view, arrow indicates compound vesicles and undulated margin. F) RMNH. Coel. 18450. Paratype, lateral view, arrow indicates compound vesicles and slightly undulated margin. Scale = 1 cm.

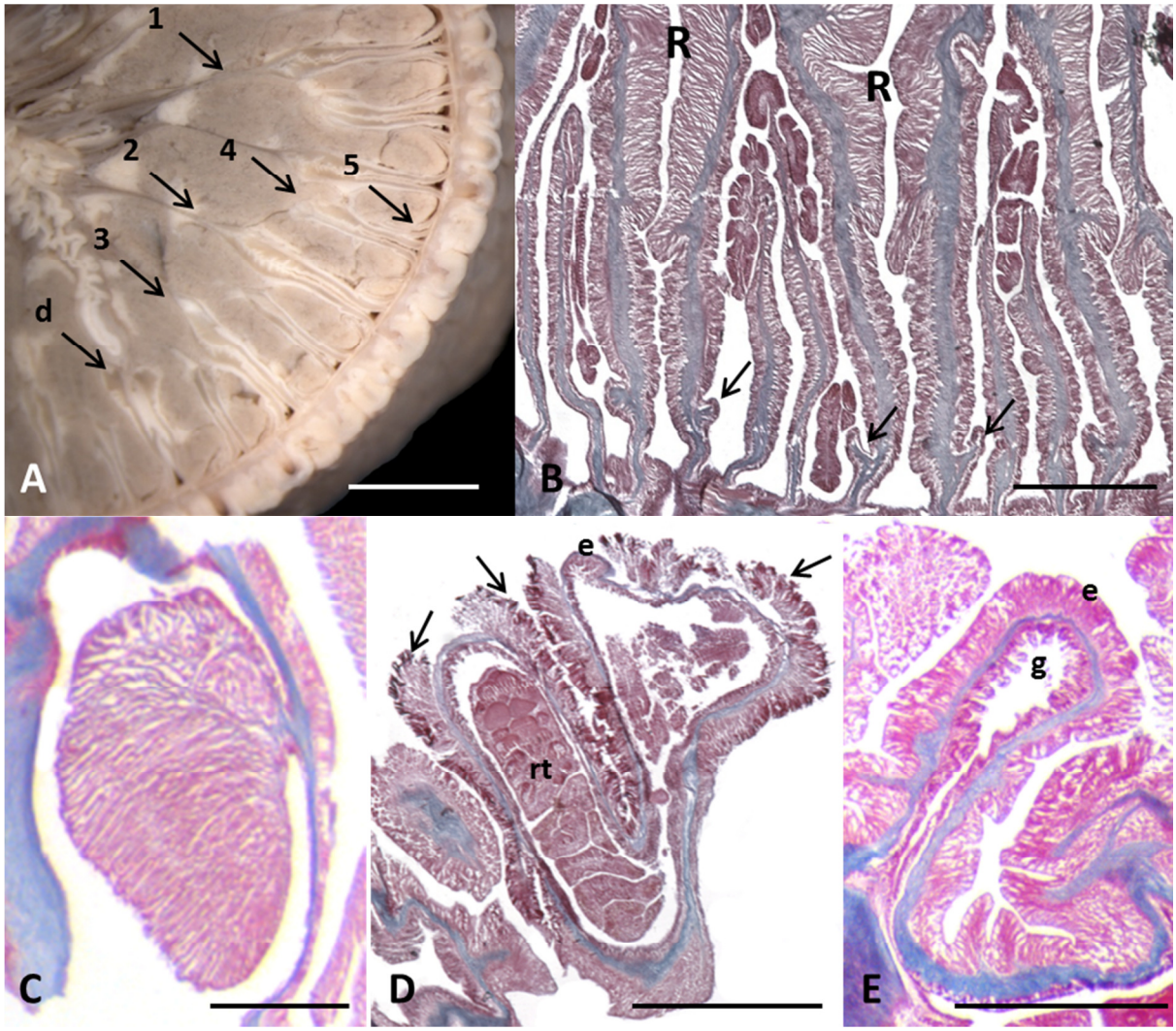

Fig. 1.21. Internal anatomy of *Bunodosoma goanense* den Hartog & Vennam, 1993. A) Cross section of the column, at the level of the actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 0.3 mm. B) Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 0.1 mm. C) Longitudinal section through marginal sphincter muscle. Scale = 0.5 mm. D) Longitudinal section of the margin showing two acrorhagi, arrows indicate ectoderm with cnidocysts. Scale = 0.8 mm. E) Cross section of columnar vesicles. Scale = 1 mm. Abbreviations: d–pair of directive mesenteries; R–retractor muscles; rt-reproductive tissue.

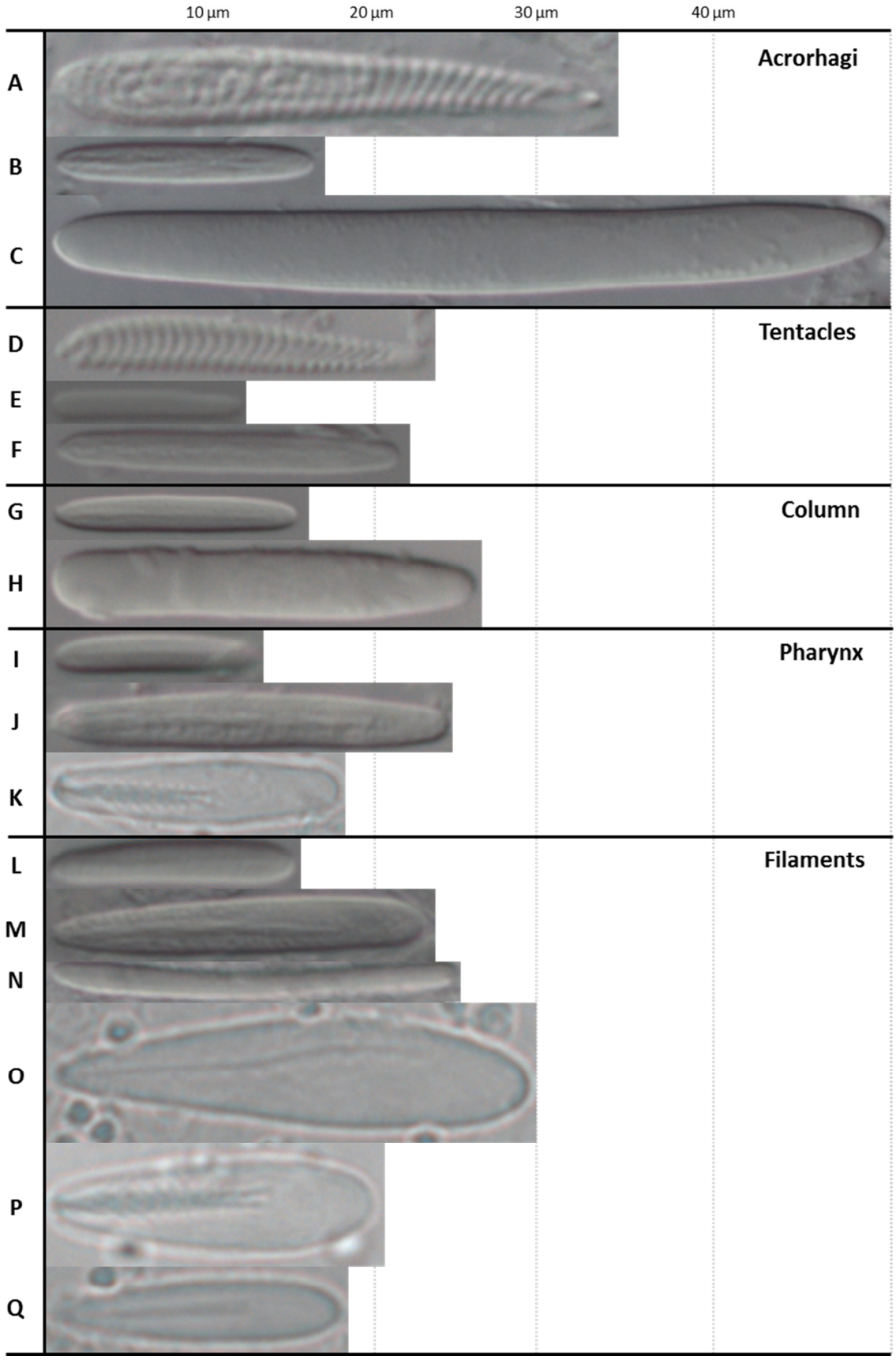

Fig 1.22. Distribution of cnidae in *Bunodosoma goanense* den Hartog & Vennam, 1993. A, D: Spirocyst; B, E, F, G, I, J, L, M, N: Basitrich; C, H: Holotrich; O: Microbasic *b*-mastigophore; K, P, Q: Microbasic *p*-mastigophore.

Table 1.9. Size and distribution of cnidae of *Bunodosoma goanense* den Hartog & Vennam, 1993. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 8); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	25.578 - 40.777 x 2.441 - 4.690	33.522 ± 4.605 x 3.341 ± 0.552	26	8/8	+
Basitrich	12.541 - 22.104 x 1.872 - 2.861	17.442 ± 2.900 x 2.388 ± 0.295	38	8/8	+
Holotrich	39.183 - 63.997 x 3.832 - 6.731	49.419 ± 4.061 x 5.349 ± 0.579	191	8/8	+++
Tentacles					
Spirocyst	16.332 - 30.581 x 1.799 - 4.224	22.230 ± 2.823 x 3.046 ± 0.542	86	8/8	+++
Basitrich 1	10.061 - 14.199 x 1.017 - 2.253	12.241 ± 1.104 x 1.739 ± 0.306	23	6/8	+
Basitrich 2	16.098 - 25.224 x 1.479 - 3.532	20.971 ± 1.721 x 2.475 ± 0.374	154	8/8	+++
Column					
Basitrich	10.997 - 22.065 x 1.476 - 16.739	17.489 ± 1.884 x 2.501 ± 1.059	200	8/8	+++
Holotrich	18.984 - 30.487 x 2.271 - 5.178	26.918 ± 2.133 x 4.406 ± 0.499	37	6/8	+
Pharynx					
Basitrich 1	11.734 - 15.748 x 1.729 - 3.163	13.682 ± 1.082 x 2.089 ± 0.310	23	8/8	++
Basitrich 2	17.213 - 25.967 x 1.568 - 3.947	22.333 ± 1.941 x 3.103 ± 0.525	125	8/8	+++
Microbasic *p*-mastigophore	17.760 - 18.630 x 4.352 - 5.362	18.195 ± 0.615 x 4.857 ± 0.714	2	1/8	+
Filaments					
Basitrich 1	11.882 - 18.067 x 1.436 - 3.163	15.396 ± 1.837 x 2.295 ± 0.438	26	8/8	++
Basitrich 2	18.905 - 27.086 x 2.159 - 3.889	22.274 ± 2.108 x 2.967 ± 0.471	45	8/8	+++
Basitrich 3	14.795 - 34.998 x 1.122 - 2.843	24.734 ± 4.364 x 1.908 ± 0.414	31	5/8	+
Microbasic *b*-mastigophore	23.781 - 35.651 x 3.471 - 8.341	29.790 ± 3.668 x 4.976 ± 1.226	16	5/8	+
Microbasic *p*-mastigophore 1	16.843 - 25.233 x 3.426 - 5.798	20.584 ± 2.050 x 4.918 ± 0.598	50	8/8	+++
Microbasic *p*-mastigophore 2	13.942 - 25.596 x 1.753 - 5.090	18.896 ± 3.801 x 3.179 ± 0.769	34	7/8	++

Discussion. This species was originally and thoroughly described by den Hartog & Vennam (1993). We have been able to verify most characteristics, although some modifications have been made to aspects of the internal anatomy and cnidae. The original description reports that in well-developed specimens the oldest cycles of mesenteries are perfect over the entire length of the actinopharynx whereas the youngest cycle reaches the actinopharynx at its oral-most part. However, we observed that mesenteries of the first and second cycles are in fact perfect over the entire length of the actinopharynx, but the third cycle reaches the actinopharynx only from the middle of this structure to its distal end. The fourth and fifth cycles reach the actinopharynx only at its distal end. In addition, according to den Hartog & Vennam (1993) all mesenteries are perfect and fertile (excluding the directives), however we observed that less developed specimens may have the fifth cycle infertile.

As for the cnidae, our observations of the types and sizes of cnidae in the acrorhagi and tentacles agree with those of den Hartog & Vennam (1993). They consider two different classes of sizes of basitrichs in the column; however, because we found some nematocysts of intermediate size to the size classes proposed in the original description we consider only one large-ranged size class of basitrichs. Also, we found only one size class of each type of microbasic *p*-mastigophore in the filaments rather than two.

The histology of the vesicles agrees with the definition of this type of columnar projection by Daly (2004), confirming that this species belongs to the genus *Bunodosoma*. This structure is the most straightforward manner to differentiate the preserved specimens of this species from the other species of the genus, as *B. goanense* is the only one with vesicles densely distributed proximally in the column and sparse and compound vesicles distally. Nonetheless, this way to distinguish this species does not seem to be true for the live and preserved specimens from Singapore described by Fautin *et al.* (2015), as the vesicles seem to be set closely together in the entire column.

As pointed out by den Hartog & Vennam (1993), *B. goanense* strongly resembles *B. caissarum,* especially as the number of tentacles and mesenteries shared by both species are unique within the genus. Nevertheless, some characteristics may be used to differentiate them: the column of *B. goanense* is shorter and has a lower number of transverse rows of vesicles than *B. caissarum* (up to 70); the youngest mesenteries tend to be fertile in *B. goanense* but sterile in *B. caissarum*. The cnidae and size ranges of the two species are very similar (as in all of the genus) but some differences can be pointed out: *B. caissarum* has two size classes of basitrichs in the column whereas *B. goanense* has only one; the microbasic *p*-mastigophores are more abundant in the actinopharynx of *B. caissarum* than *B. goanense*; *B. goanense* has three size classes of basitrichs and one size class of microbasic *b*-mastigophore in the filaments whereas *B. caissarum* has one size class of basitrich and two sizes classes of microbasic *b*-mastigophores in the filaments.

We agree with Fautin *et al*. (2015) that the records of *B. granuliferum* from Bombay and Bangladesh from Parulekar (1968) and Hague (1977) refer to *B. goanense*. So, as the status of *B. fallax* is uncertain, *B. goanense* is currently the only certain species of the genus recorded from the Indo-West Pacific. Fautin *et al.* (2015) comment that *B. goanense*, although it occurs in port areas of Singapore and it is hardy, is not invasive in that location, as it does not conform with other characteristics of invasive species such as having rapid growth, asexual reporduction, etc.

Bunodosoma grande (Verrill, 1869)

(Figs. 1.23-1.25, Table 1.10)

Synonymy list

Cladactis grandis Verrill, 1869: 472-473 (original description).

Alicia grandis – Haddon & Shackleton, 1893: 128.

Cystiactis grandis – Duerden, 1897: 4.

Eucladactis grandis – Verrill, 1899: 49-50.

Phymactis grandis – Stephenson, 1922: 285.

Bunodosoma grandis – Carlgren, 1949: 52.

Bunodosoma grande – Fautin, 2013 (spelling corrected).

Material examined

YPM 1008: Syntype, 2 whole specimens; Panama. Coll. F.H. Bradley, 1866. 70% alcohol. (According to D.G. Fautin, May 2002).

YPM 2099B: Syntype, 3 dissected specimens; Pearl Islands, Panama. Coll. F.H. Bradley, 1866. 70% alcohol. (According to D.G. Fautin, May 2002).

YPM 2100A/B: Syntype, 4 whole specimens (A), 2 whole specimens (B); Panama. Coll. F.H. Bradley, 1866. 70% alcohol. (According to D. G. Fautin, May 2002).

YPM 9117: 5 whole specimens, 1 dissected; off Chiman, Gulf of Panama; depth 3-10m; approximately 0.5km from shore; color greenish blue to blue. Coll. D.A. West, 6 June 1973.

USNM 1121706: 4 whole specimens, 2 dissected; La Perla, La Libertad, El Salvador, North Pacific Ocean. Centroid 13°30' N 89°30' W. Coll. J. Leslie, 18 September 1978. Id. Crowther, A. L. Jun 2008.

Diagnosis. *Bunodosoma* with up to 36 transverse rows of large vesicles in the column; usually more than 200 tentacles in six or more cycles and 96 mesenteries in five cycles; mesenteries of first and second cycles perfect over entire length of the actinopharynx, third cycle reaches the actinopharynx from the middle to its distal part, fourth and fifth cycles reach actinopharynx only at its most distal part. Large specimens with all mesenteries fertile, excluding directives. Less developed specimens may have fifth cycle infertile. Large basitrichs in the acrorhagi.

Description

Pedal disc. Adherent, circular to semi-circular, 0.8-56 mm in diameter in preserved specimens.

Column. Cylindrical, usually shorter than pedal disc but usually of the same diameter, 10-56 mm in diameter and up to 40 mm height in preserved specimens. Column densely covered with vesicles in 96 longitudinal rows and up to 36 transverse rows. Vesicles large, endocoelic, rounded, non-adhesive, may be slightly cup-shaped, mesoglea and ectoderm of similar thickness at the sides and apex, ectoderm with similar cells at sides and apex. In some specimens, compound vesicles in the 3-4 most distal rows with 2-4 lobes. In some specimens, each longitudinal row of vesicles ends distally in a short marginal projection, on its inner aspect provided with a distinct acrorhagus. Acrorhagi with holotrichs, large basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic, second exocoelic; inner row with up to 96 larger acrorhagi (up to 2.1 mm in diameter) than the second one (up to 1.3 mm in diameter). Smaller specimens may have only one row of acrorhagi, and badly-preserved specimens have acrorhagi with ruptured cnidocysts. Fosse pronounced.

Oral disc. 0.6-2.5 mm in diameter and without markings in the preserved specimens.

Tentacles. 2-14 mm in length in preserved specimens, tapered and pointed. 192 in 6 cycles (6+6+12+24+48+96) or up to 240 tentacles with a seventh incomplete cycle. Those of the last cycle are exocoelic.

Internal anatomy. Mesenteries pairs usually 96, hexamerously distributed in 5 cycles (6+6+12+24+48) at the actinopharynx level. Some specimens may have up to 120 mesenteries pairs with a sixth incomplete cycle. Number of mesenteries equal proximally and distally. Two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first, second and third cycles perfect over entire length of the actinopharynx, fourth and fifth cycles reach actinopharynx only at its most distal part. Large specimens with first, second, third and fourth cycles fertile, last cycle may be fertile, directives infertile. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript, main mesogleal lamella thick and short. Retractors diffuse to restricted and moderately strong. Parietobasilar muscles with long and thick mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.10.

Color. Preserved specimens may be entirely light pink, or have a light orange to dark grey column, with orange tentacles. Acrorhagi when present are usually of the same color as tentacles.

Distribution. East Pacific, from Panama to El Salvador.

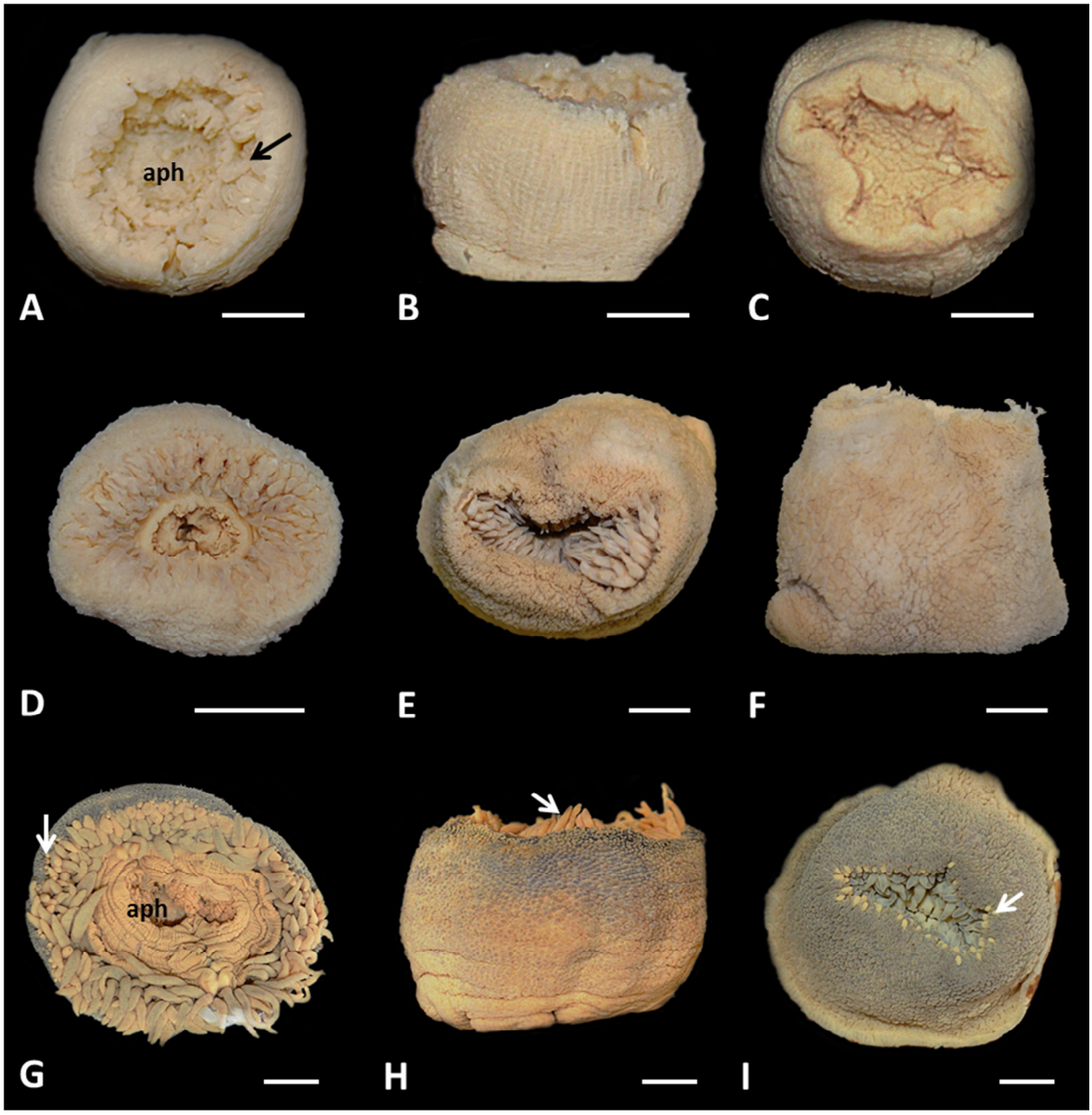

Fig. 1.23. *Bunodosoma grande* (Verrill, 1869). A) YPM 1008. Syntype, oral view, arrow indicates tentacle. B) YPM 1008. Syntype, lateral view. C) YPM 1008. Syntype, aboral view. D) YPM 2099, syntype, oral view. E) YPM 2100, syntype, oral view. F) YPM 2100, syntype, lateral view. G) YPM 9117, oral view, arrow indicates acrorhagus. H) YPM 9117, lateral view, arrow indicates tentacles. I) USNM 1121706, oral view, arrow indicates acrorhagus. Scale = 1cm. Abbreviations: aph–actinopharynx.

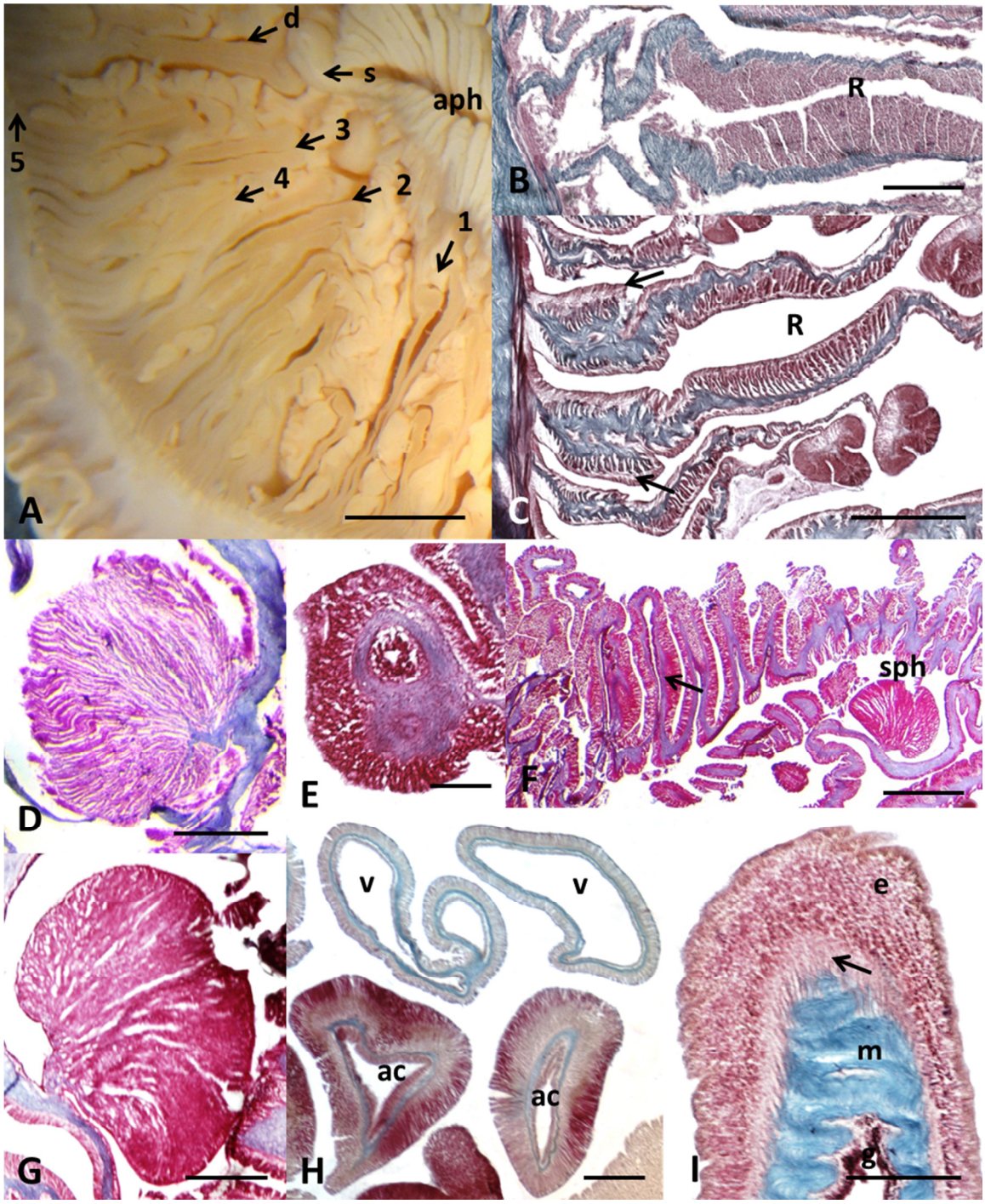

Fig. 1.24. Internal anatomy of *Bunodosoma grande* (Verrill, 1869). A) USNM 1121706. Cross section of the column, proximal to the actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 0.5 mm. B) YPM 2099. Cross section of mesenteries. Scale = 0.2 mm. C) USNM 1121715. Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 0.2 mm. D) YPM 2099. Longitudinal section through marginal sphincter muscle. Scale = 0.5 mm. E) YPM 9117. Longitudinal section of the margin showing acrorhagus. Scale = 0.5 mm. F) YPM 9117. Longitudinal section through columnar vesicles, arrow indicates large vesicle. Scale = 1 mm. G) YPM 9117. Longitudinal section through marginal sphincter muscle. Scale = 0.5 mm. H) USNM 1121706. Longitudinal section of margin showing compound vesicles and acrorhagi. Scale = 0.5 mm. I) USNM 1121706. Cross section of tentacle, arrow indicates longitudinal ectodermal muscle. Scale = 0.2 mm. Abbreviations: ac–acrorhagus; aph–actinopharynx; d–pair of directive mesenteries; e–ectodermis; g-gastrodermis; m-mesoglea; R–retractor muscles; s–siphonoglyph; v–vesicle.

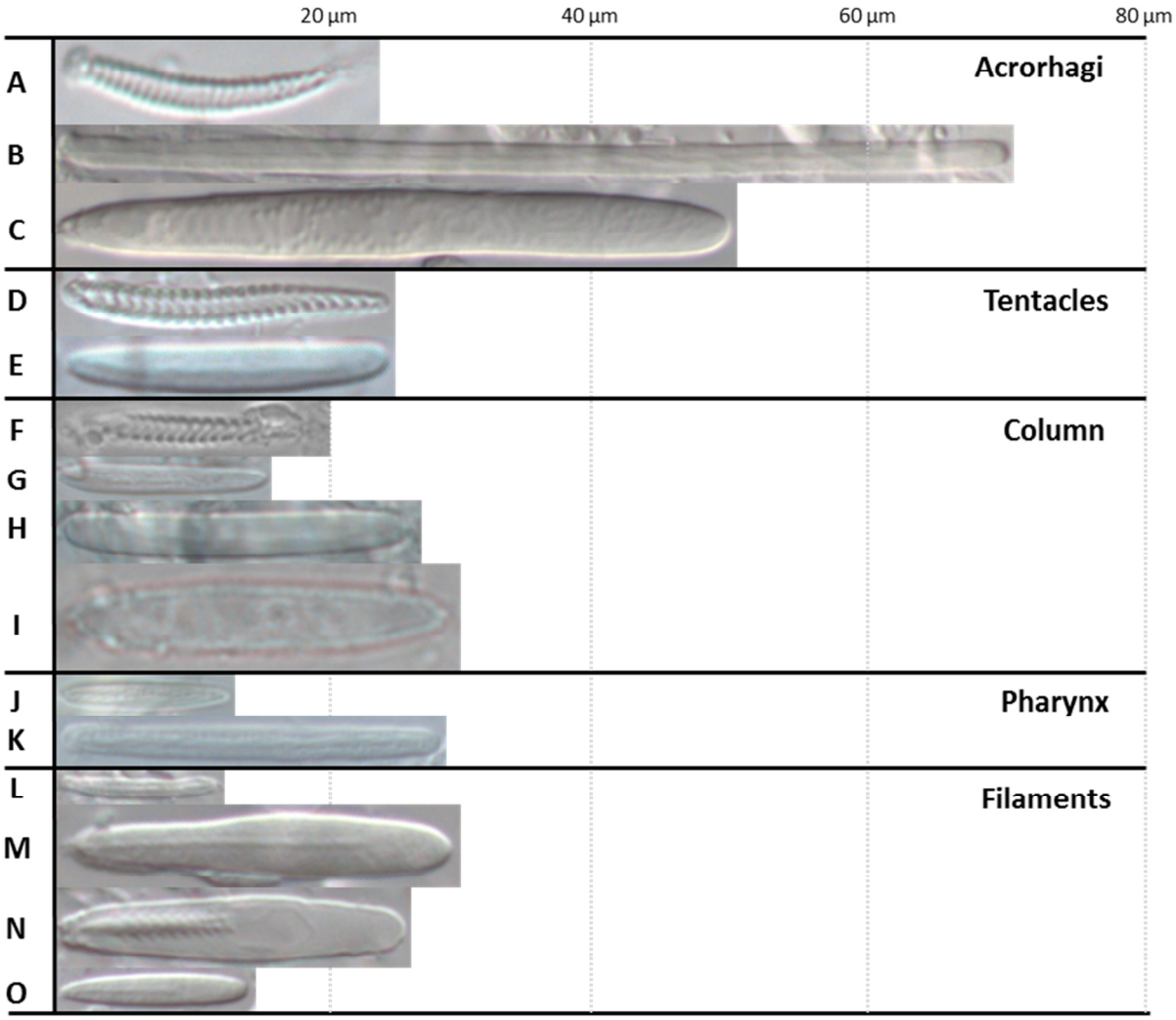

Fig. 1.25. Distribution of cnidae in *Bunodosoma grande* (Verrill, 1869). A, D, F: Spirocyst; B, E, G, H, J, K, L: Basitrich; C, I: Holotrich; M: Microbasic *b*-mastigophore; N, O: Microbasic *p*-mastigophore.

Table 1.10. Size and distribution of cnidae of *Bunodosoma grande* (Verrill, 1869). Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 3, 4, 10); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	17.273 - 31.231 x 2.060 - 3.427	22.618 ± 3.980 x 2.799 ± 0.430	19	4/4	+
Basitrich	58.286 - 80.476 x 2.421 - 3.688	68.390 ± 5.722 x 2.962 ± 0.290	45	4/4	++
Holotrich	35.972 - 69.606 x 3.521 - 7.148	49.546 ± 8.512 x 5.202 ± 1.020	125	4/4	+++
Tentacles					
Spirocyst	15.587 - 35.747 x 1.443 - 4.774	24.026 ± 4.453 x 2.897 ± 0.553	182	10/10	+++
Basitrich	17.118 - 31.372 x 1.435 - 4.144	23.772 ± 3.147 x 2.793 ± 0.473	173	10/10	+++
Column					
Spirocyst	19.260 x 3.170		1	1/10	+
Basitrich 1	12.659 - 19.338 x 1.770 - 3.030	16.163 ± 1.607 x 2.548 ± 0.228	135	9/10	+++
Basitrich 2	21.490 - 28.171 x 2.420 - 3.748	24.551 ± 1.503 x 3.134 ± 0.332	49	5/10	++
Holotrich	27.155 x 4.435		1	1/10	+
Pharynx					
Basitrich 1	12.665 - 12.989 x 2.199 - 2.441	12.827 ± 0.229 x 2.320 ± 0.171	2	2/3	+
Basitrich 2	23.612 - 31.511 x 2.169 - 3.111	27.814 ± 1.659 x 2.620 ± 0.212	42	3/3	++
Filaments					
Basitrich	12.610 - 17.600 x 1.660 - 3.260	14.771 ± 1.610 x 2.272 ± 0.607	8	2/4	+
Microbasic *b*-mastigophore	18.730 - 28.570 x 3.275 - 4.580	24.409 ± 2.434 x 4.158 ± 0.274	19	4/4	+
Microbasic *p*-mastigophore 1	18.650 - 26.270 x 3.760 - 5.470	22.849 ± 1.367 x 4.606 ± 0.411	33	4/4	+
Microbasic *p*-mastigophore 2	11.792 - 16.463 x 2.192 - 4.040	14.067 ± 1.456 x 2.867 ± 0.548	10	2/4	+

Taxomical Remarks. Fautin (2013) corrected the spelling of the epithet, changing it from "*grandis*" to the neuter "*grande*", according to the Latin declension of the neuter genus name.

Discussion. The description we present is based on the syntype material from Panama, although we include some information of a voucher lot from El Salvador, which has the same characteristics. Fautin (2013) also indicates *Actinia clematis* (MCZ 64179, from Chile), *Actinia florida* (MCZ 1686, from Peru) and *Cladactis grandis* (YPM 2101A/B, from Peru) as syntypes of *B. grande*, but due to the state of the material, we were not able to successfully make histological longitudinal sections of the column to observe the marginal sphincter and confirm if they are in fact members of the genus *Bunodosoma*.

The lots YPM 2099 A/B and YPM 2100A/B, from Panama, and YPM 2101A/B, from Peru, are syntypes of the original description of Verrill (1869) of *Cladactis grandis*. Verrill (1869), in his original description of *C. grandis*, presents only information on external morphology, so relevant musculature features have been left out. He comments that the species has up to 528 tentacles in 7-8 columns, and, in this case, it would have been an only case in the genus *Bunodosoma* of a specimen with such a high number of tentacles. Later, Verrill (1899) makes a new combination for the specimens (*Eucladactis grandis*) and comments that they have a diffuse sphincter, which, according to the current classification, would put these specimens in the genus *Phymactis*. As we have performed histological sections on the material from Panama, we can confirm that it has a circumscribed sphincter, and, therefore, belong to *Bunodosoma*. We were not able to successfully obtain histological sections of the Peruvian material due to its state of preservation, so we cannot confirm its generic assignation.

The confusion between the genus *Phymactis* and *Bunodosoma* is historical, and both genera have been synonymized by Stephenson (1922), and then *Bunodosoma* was raised again

by Carlgren (1949). The species *Phymactis papillosa*, from Chile, is very similar to *B. grande*, and both species are very easy to confuse if only external morphology is taken to account. Häusserman (2004) studied species from Chile and comments that *B. grande* only occurs in the North of the country, but we have not herein examined any specimens from this locality. Therefore, we propose that more material of sea anemones of this species should be collected at the Southern coast of the East Pacific, so that the distribution of this species can be better understood.

***Bunodosoma granuliferum* (Le Sueur, 1817)**

(Figs. 1.26-1.28, Table 1.11)

Synonymy list

Actinia granulifera Le Sueur, 1817: 173 (original description)

Urticina Lessoni [sic] – Duchassaing, 1850: 9.

Oulactis granulifera – Milne-Edwards, 1857: 293.

Urticina granulifera – Duchassaing & Michelotti, 1860: 42.

Cereus Lessoni [sic] – Duchassaing & Michelotti, 1860: 42, pl. VI, fig. 13, 14.

Anthopleura granulifera – Duchassaing & Michelotti, 1864: 32, Pl. III, fig. 8.

Anthopleura Granulifera [sic] – Duchassaing, 1870: 20.

Aulactinia granulifera – Andres, 1883: 230.

Bunodes tæniatus McMurrich, 1889: 23–27.

Bunodes taeniatus – Carlgren, 1895: 285.

Bunodes granulifera – Duerden, 1897: 454.

Bunodosoma granulifera – Verrill, 1899: 44–45.

Bunodosoma granuliferum – Pax, 1910: 162, 164, 165, 184–189.

Phymactis granulifera – Stephenson, 1922: 285.

Material examined

USNM 51068: 1 whole specimen, 1 dissected. Cabanas, Cuba (T. Barrea Exped.) Henderson Bartsch 6-8, 9-1914 Station 16 tag 592. Id. C.E.C. Acc. No. 57608.

USNM 54148: 6 whole specimens, 1 dissected. Sta. 113a-58, along shore, 1-3 ft, Gravenor Landing, Barbuda, W.I., 28 April 1958. Coll. Smith-Bredin Exped. Id. J.C. den Hartog. Acc. No. 217237.

USNM 54153: 3 whole specimens, 1 dissected; Mexico, Ouitana Roe II/4 miles north of Pt. Santa Maria. On shore, under rocks. Coll. Bousfield. 22 April 1960. Id. J.C. den Hartog. Acc. No. 229190.

MCZ 43353: 3 whole specimens, 1 dissected. US Virgin Islands; Bay on NW corner of island, St. Croix. Coll. Ken Sebens, 8/1981. Intertidal zone. Donor: K. Sebens, 27.iii.1992. Det. by: K. Sebens.

MCZ 43501: 3 whole specimens, 1 dissected. Greater Lameshur Bay, St. John, US Virgin Islands. Depth 5m. Coll. L.R. Lewand 27 July 1983. Kept in formalin until 1994, then placed in 80% ethanol in graduated steps.

Diagnosis. *Bunodosoma* with up to 35 transverse rows of vesicles densely distributed in the column; 96 tentacles in five cycles and 48 mesenteries in four cycles; mesenteries of first and second cycles perfect over the pharynx's entire length, third and fourth cycles reach actinopharynx only at its most oral part. All mesenteries fertile, directives excluded.

Description

Pedal disc. Adherent, circular to semi-circular, 10-16 mm in diameter in preserved specimens.

Column. Cylindrical, densely covered with vesicles in 96 longitudinal rows. Large specimens have up to 35 transverse rows. The vesicles are endocoelic and exocoelic, rounded, non-adhesive, non-cup-shaped, mesoglea of similar thickness at the sides and apex, ectoderm of similar thickness and containing similar cells at sides and apex. The vesicles of the 3-4 most distal rows may be compound, 2-4 lobes each. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic with up to 48 larger acrorhagi (up to 3 mm diameter) than the second one exocoelic (up to 1.3 mm diameter). Smaller specimens may have only one row of acrorhagi. Fosse pronounced. Column more or less of the same diameter as pedal disc, 9-16 mm in diameter and up to 22 mm height in preserved specimens.

Oral disc. 8-10 mm in diameter and without markings in the preserved specimens.

Tentacles. Short, 2-6 mm in preserved specimens, stout, tapered and pointed. Tentacles of the inner cycle may be longer than outer. Most individuals have 96 arranged in 5 cycles (6+6+12+24+48). Those of the last cycle are exocoelic.

Anatomy. Mesenteries pairs up to 48 hexamerously distributed in 4 cycles (6+6+12+24). Same number of mesenteries distally and proximally. Usually two pairs of directives connected with two distinct siphonoglyphs. Mesenteries of first and second cycles perfect over the pharynx's entire length, third and fourth cycles reach actinopharynx only at its most oral part. All mesenteries fertile, directives excluded. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript, strong, main mesogleal lamella thin and short. Retractors restricted and strong. Parietobasilar muscles with short and thick mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, holotrichs, basitrichs, microbasic p-mastigophores. microbasic b-mastigophores. A survey of sizes and distribution is shown in Table 1.11.

Color. According to Gonzalez-Muñoz *et al.* (2012), specimens from Mexico are as follows: column with 24 alternating dark and light bands, dark bands with about five rows of vesicles, light ones with about three; oral disc olive-green or reddish-brown; tentacles olive-green to green-greyish, often with white spots and flashes of pink or purple; pedal disc olive-green to orange. In preserved specimens, we also counted 24 alternating bands, light bands with 2-4 vesicles and the dark ones with 5-6 vesicles.

Distribution. Along the entire Caribbean Sea, including Mexico (Gonzalez-Muñoz *et al.*, 2012), Costa Rica (Acuña *et al.*, 2013), Panama (McCommas, 1991), Martinique (Le Sueur, 1817), Antilles (Duchassaing, 1850), Curaçao Islands (Corrêa, 1964), Jamaica (Duerden, 1898); Puerto Rico (McCommas & Lester, 1980).

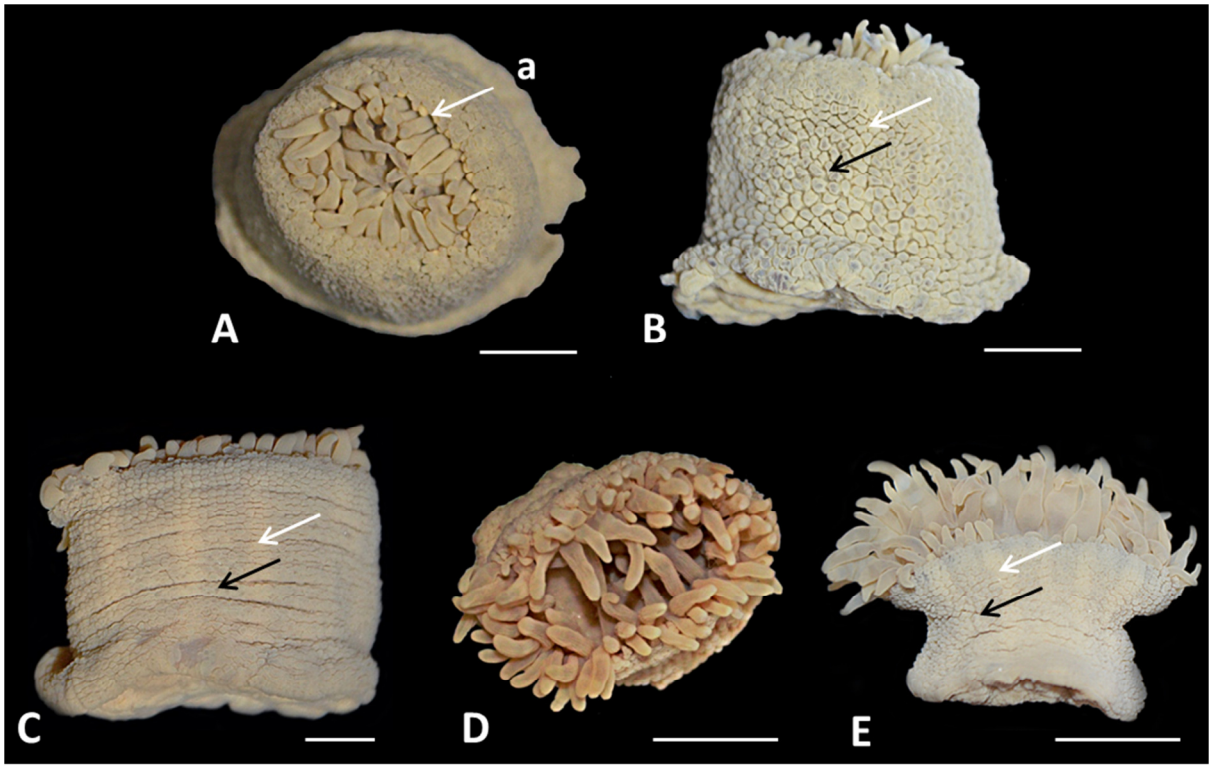

Fig. 1.26. *Bunodosoma granuliferum* (Le Sueur, 1817). A) USNM 51068, oral view. B) USNM 51068, lateral view. White arrow indicates a light band and black arrow indicates a dark band. C) USNM 54148, specimen 1, lateral view. White arrow indicates a light band and black arrow indicates a dark band. D) USNM 54148, specimen 1, oral view. E) USNM 54148, specimen 1, lateral view. White arrow indicates a light band and black arrow indicates a dark band. Scale = 1 cm. Abbreviation: a-acrorhagus.

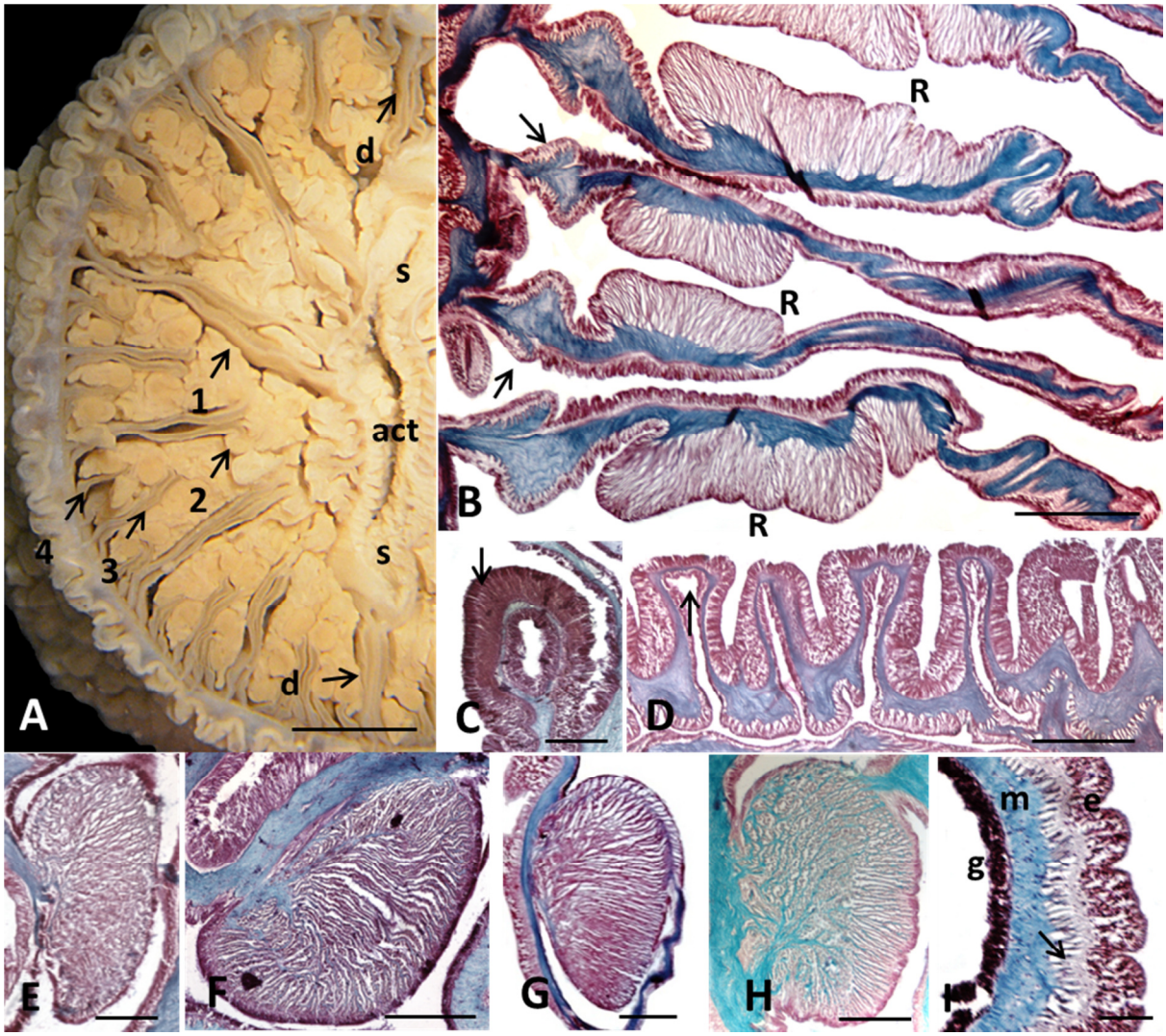

Fig. 1.27. Anatomy of *Bunodosoma granuliferum* (Le Sueur, 1817). A) Cross section through mesenteries, proximal to the actinopharynx, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 0.3 mm. B) Cross section through mesenteries, arrows show pennon of the parietobasilar muscle. Scale = 0.1 mm. C) Section through acrorhagus, arrows indicate epiderm with cnidocysts. Scale = 0.8mm. D) Section through columnar vesicles, arrow indicates a vesicle. Scale = 1 mm. E) USNM 54148. Section through marginal sphincter. Scale = 0.8 mm. F) USNM 51068. Section through marginal sphincter. Scale = 0.5mm. G) MCZ 43501. Section through marginal sphincter. Scale = 0.5 mm. H) USNM 54153. Section through marginal sphincter. Scale = 0.3 mm. I) Cross section through tentacle. Scale =0.3 mm. Abbreviations: act-actinopharynx; d–directive mesentery; e-epidermis; g-gastrodermis; m-mesoglea; R–retractor muscles; s-siphonoglyph.

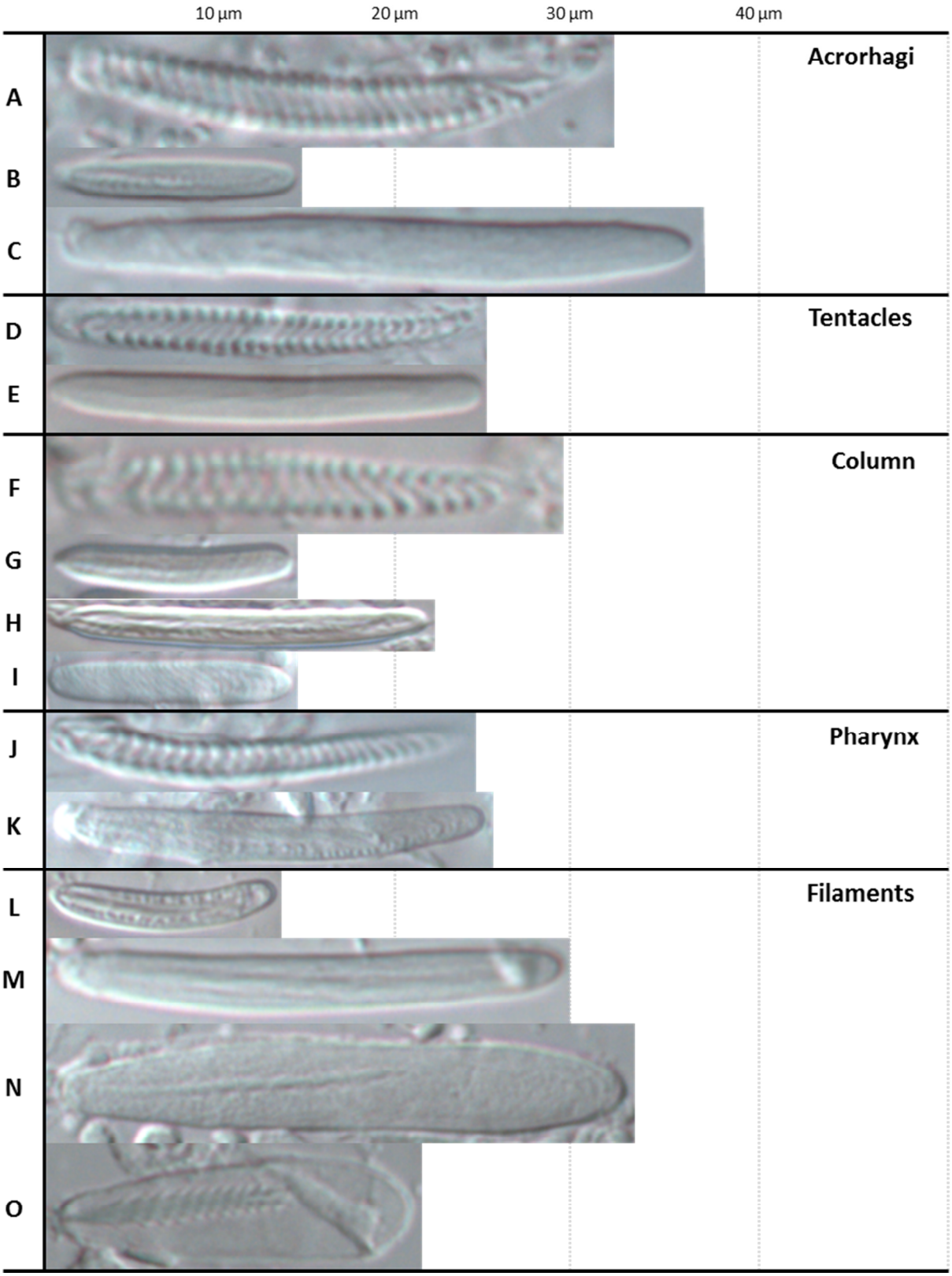

Fig. 1.28. Distribution of cnidae in *Bunodosoma granuliferum* (Le Sueur, 1817). A, D, F, J: Spirocyst; B, E, G, H, J, K, L, M: Basitrich; C, I: Holotrich; N: Microbasic *b*-mastigophore; O: Microbasic *p*-mastigophore.

Table 1.11. Size and distribution of cnidae of *Bunodosoma granuliferum* (Le Sueur, 1817). Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 6; 8; 11); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	26.697 - 37.500 x 2.369 - 3.450	31.355 ± 4.139 x 2.933 ± 0.476	17	3/6	+
Basitrich	12.720 - 24.120 x 1.950 - 3.370	15.857 ± 2.280 x 2.450 ± 0.256	67	5/6	+
Holotrich	24.558 - 43.690 x 2.903 - 5.540	35.925 ± 5.250 x 3.840 ± 0.388	210	6/6	+++
Tentacles					
Spirocyst	13.670 - 34.575 x 0.265 - 4.165	24.858 ± 4.670 x 2.686 ± 0.507	185	11/11	+++
Basitrich	18.922 - 34.720 x 0.214 - 3.480	25.049 ± 2.887 x 2.625 ± 0.409	172	11/11	+++
Column					
Spirocyst	28.170 - 29.830 x 2.390 - 3.360	29.000 ± 1.174 x 2.875 ± 0.686	2	2/8	+
Basitrich 1	11.531 - 19.654 x 1.235 - 3.247	15.210 ± 1.573 x 2.467 ± 0.334	150	8/8	+++
Basitrich 2	18.716 - 29.220 x 2.163 - 4.258	23.761 ± 2.545 x 3.109 ± 0.466	69	8/8	++
Holotrich	13.777 - 16.000 x 2.049 - 3.265	14.525 ± 0.818 x 2.829 ± 0.409	6	2/8	+
Pharynx					
Spirocyst	22.282 - 30.505 x 2.867 - 3.396	25.788 ± 3.468 x 3.181 ± 0.201	5	2/6	+
Basitrich	17.910 - 32.683 x 2.150 - 4.196	25.351 ± 3.026 x 3.451 ± 0.399	131	6/6	+++
Filaments					
Basitrich 1	12.630 - 15.802 x 1.648 - 2.466	14.068 ± 0.944 x 2.009 ± 0.277	12	4/6	+
Basitrich 2	20.421 - 34.979 x 2.248 - 3.480	30.234 ± 5.914 x 2.734 ± 0.419	7	2/2	+
Microbasic *b*-mastigophore	24.882 - 38.630 x 2.060 - 8.450	33.489 ± 3.156 x 5.644 ± 1.941	33	6/6	++
Microbasic *p*-mastigophore	17.434 - 25.050 x 4.494 - 7.409	22.207 ± 1.670 x 5.797 ± 0.753	59	6/6	++

Discussion. The original description of Le Sueur (1817) provides mainly information on external morphology of this species, so we compared most of our observations to the specimens described by Corrêa (1964) and González-Muñoz *et al.* (2012). Also, the vesicles in this species agree with the definition of these columnar projections provided by Daly (2004), so it belongs, in fact, to the genus *Bunodosoma*.

This species is very similar to *B. cavernatum* in external and internal morphology, and the feature that separates them is the presence of colored longitudinal bands in the column of *B. granuliferum* (Duerden, 1902; Pax, 1910ab; Corrêa, 1964; González-Muñoz *et al.*, 2012). We analyzed specimens from museum collections identified as *B. cavernatum* and *B. granuliferum* together, later they were separated based on the absence or presence of the longitudinal bands. As in *B. cavernatum*, we were not able to match this material to published descriptions, so we are not certain if the preserved specimens with no bands were in fact like that when alive, or if the longitudinal bands faded away after preservation. Therefore, some specimens with longitudinal bands *in vivo* may have been placed in *B. cavernatum* in this study.

In general, external and internal morphology were very similar for all the specimens we placed as *B. granuliferum*. However, we observed a variation in the morphology of the sphincter among the specimens: some had a round sphincter (Fig. 1.27.H), whereas, in others, the proximal side of the marginal sphincter had a pennon (Fig. 1.27. E, G). Some specimens had a marginal sphincter with an intermediate aspect (Fig. 1.27. F). We did not observe any specimens with a marginal sphincter muscle tending to split in two parts, which, according to Carlgren (1952) and González-Muñoz *et al.* (2013), could be a morphological difference between the two very similar species, and would characterize *B. cavernatum*.

We added some types and size classes of cnidocysts in some parts of the polyp to the ones González-Muñoz *et al.* (2012) described: spirocysts in the acrorhagi; spirocysts and

holotrichs in the column; microbasic *b*-mastigophores and another size class of basitrichs in the filaments. However, they observed microbasic *p*-mastigophore in the pharynx of *B. granuliferum*, present in most species of *Bunodosoma*, and we did not. Also, this species' cnidae are very similar to what we observed in *B. cavernatum*. The differences in *B. cavernatum* are: absence of holotrichs in the column; two size classes of basitrichs and one of microbasic *p*-mastigophore in the pharynx; two size classes of microbasic *p*-mastigophore and microbasic *b*-mastigophore in the filaments. *B. granuliferum* was the only species for which we did not find microbasic *p*-mastipgophore 2 in the filaments. This type of nematocyst is usually rare, although it was found in all other species.

Bunodosoma granuliferum is one of the four valid species of *Bunodosoma* (*B. cavernatum*, *B. sphaerulatum* and *B. kukenthali*) that have been reported in the Gulf of Mexico and Caribbean Sea (Carlgren & Hedgpeth, 1952; González-Muñoz *et al.*, 2012, 2013; Fautin, 2013). It has also been reported for the Indo-West Pacific, but we agree with Fautin *et al*. (2015) that the records of *B. granuliferum* from Bombay and Bangladesh of Parulekar (1968) and Hague (1977) refer to *B. goanense*. The distinction between *B. granuliferum* and *B. sphaerulatum* is not clear based on the information available for the latter (see description of *B. sphaerulatum*). *B. kuekenthali*, however, is very different from all of them (see description of *B. kuekenthali*). And, we reaffirm that, although the morphological feature that separates *B. granuliferum* and *B. cavernatum* may be unreliable for preserved specimens, they should be considered as separate species and must be identified based on live animals.

Bunodosoma kuekenthali Pax, 1910

(Figs. 1.29-1.30, Table 1.12)

Synonymy list

Bunodosoma kükenthali Pax, 1910: 177-178 (original description).

Phymactis kükenthali – Stephenson, 1922: 285.

Rivetia kükenthali – Carlgren, 1924: 2, 6, 12-15.

Bunodosoma kukenthali – Lewis, 1960: 430.

Bunodosoma kuekenthali – Fautin, 2013 (spelling corrected)

Material examined

ZMB 5192: Syntype, 3 whole specimens, 2 dissected. [Barbados, East Coast Bathesba, 24 February, 1907. Kükenthal and Hartmeyer].

USNM 51033: 13 whole specimens. St. James, Barbados, B.W.I. John B. Lewis. June 1958. Id. C.E. Cutress, 1958. Acc. No. 220661.

USNM 54115: 4 whole specimens. Rodney's Rock, Dominica. 18 Feb 1964. Coll. H.H.Hobbs III. Id. J.C. den Hartog 75. Acc. No. 254456.

USNM 54116: 2 whole specimens, 3 dissected. Antigua. Rock anemone; English Hbr. 1918. State University of Iowa. Coll. Barbados-Antigua Expedition. Id. JC den Hartog 175.

Diagnosis. *Bunodosoma* with up to 27 transverse rows of large vesicles in the column; up to 96 tentacles in five cycles and 48 pairs of mesenteries in four cycles; large specimens with first and second cycles perfect over entire length of the actinopharynx, third and fourth cycles reach actinopharynx only at its most distal part; last cycle and directives infertile; acrorhagi with two different sizes of holotrichs and basitrichs.

Description

Pedal disc. Adherent, circular to semi-circular, 9-24 mm in diameter in preserved specimens.

Column. Cylindrical, usually shorter than pedal disc but of the same diameter, 8-23 mm in diameter and up to 20 mm height in preserved specimens. Column densely covered with vesicles in around 96 longitudinal rows and 10-27 transverse rows. Vesicles larger than in other species (similar to *B. grande*), endocoelic and exocoelic, rounded, non-adhesive, may be slightly cup-shaped, mesoglea and ectoderm of similar thickness at the sides and apex, ectoderm with similar cells at sides and apex. Compound vesicles are rare, but may be present in the 2-3 most distal rows with 2-3 lobes. In some specimens, each longitudinal row of vesicles ends distally in a short marginal projection, on its inner aspect provided with a distinct acrorhagus. Acrorhagi may be inconspicuous, with holotrichs, two size classes of basitrichs and of spirocysts, arranged into two alternate rows, inner row endocoelic, second exocoelic; inner row with up to 48 larger acrorhagi (up to 2 mm in diameter) than the second one (up to 0.8 mm in diameter). Smaller specimens may have only one row of acrorhagi. Fosse pronounced.

Oral disc. 7-15 mm in diameter and without markings in the preserved specimens.

Tentacles. Short, 2-6 mm in preserved specimens, stout, tapered and pointed. Tentacles of the inner cycle the same length as the outer. To 96 in 5 cycles (6+6+12+24+48); some specimens with fewer tentacles on the outer cycle. Those of the last cycle are exocoelic.

Anatomy. Mesenteries pairs up to 48, usually hexamerously arranged in 4 cycles (6+6+12+24). Same number of mesenteries distally and proximally. Usually two pairs of directives connected with two distinct siphonoglyphs. Large specimens with first and second cycles perfect over entire length of the actinopharynx, third and fourth cycles reach actinopharynx only at its most distal part. Last cycle and directives infertile. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript,

main mesogleal lamella thick and long. Retractors restricted and moderately strong. Parietobasilar muscles with long and thin mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.12.

Color. Column monochrome grey-green with a faint bluish tinge, acrorhagi light yellow, tentacles with longitudinal stripes (Pax, 1910a, 1910b). Preserved specimens have brown to grey columns and tentacles, and acrorhagi light orange or brown.

Distribution: Western Atlantic, The Antilles (Curaçao, Barbados, Loango) (Pax, 1924).

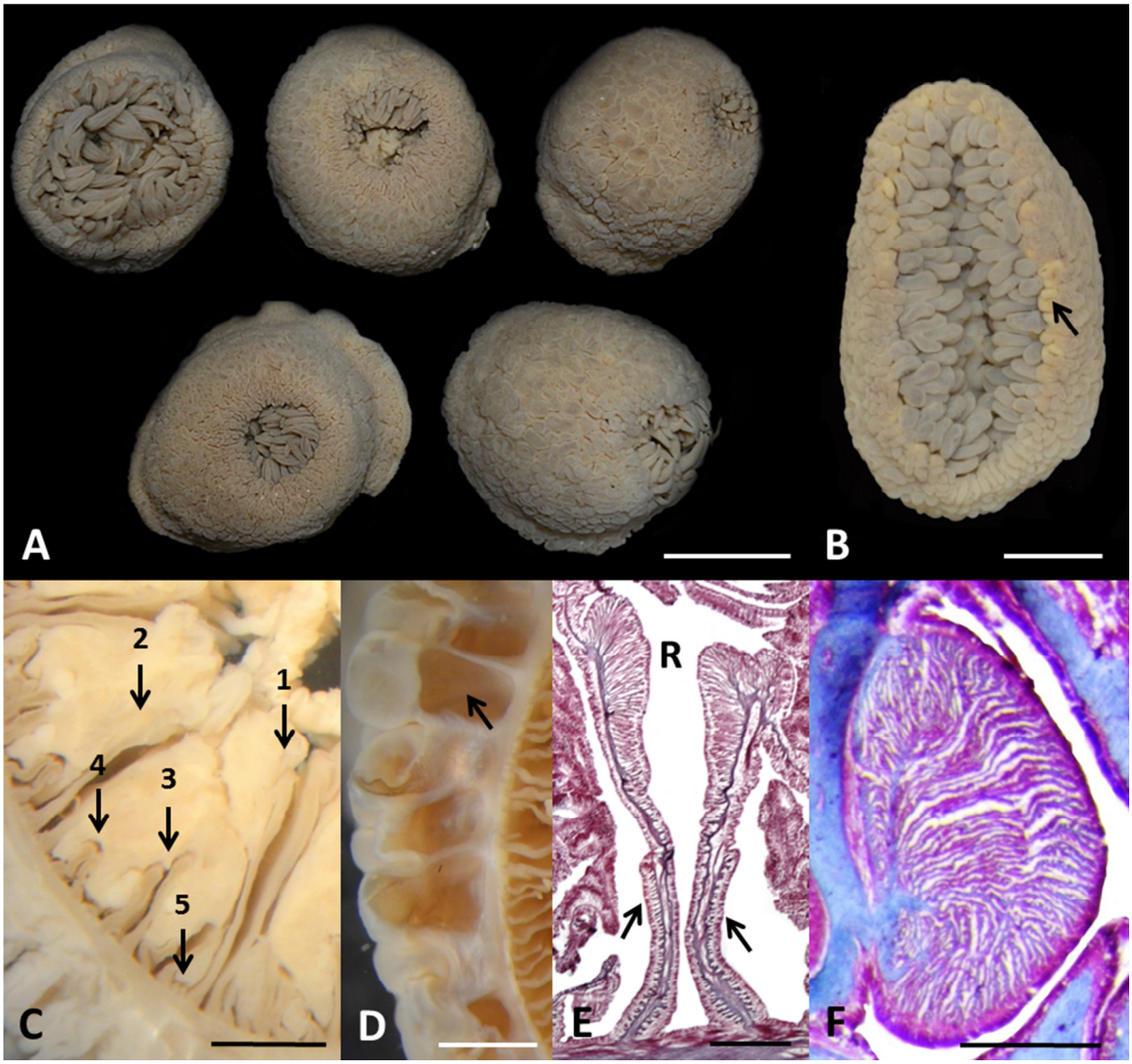

Fig. 1.29. *Bunodosoma kuekenthali* Pax, 1910. A) ZMB 5192, Syntype specimens. Scale = 1.2 mm. B) USNM 54115, oral view, arrow indicates acrorhagus. Scale = 0.9 mm. C) Cross section of the column, at actinopharynx level, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 0.4 mm. D) Cross section of column showing large vesicles. Scale = 0.3 mm. E) Cross section of mesenteries, arrows indicate parietobasilar muscles. Scale = 0.1 mm. F) Longitudinal section through marginal sphincter muscle. Scale = 0.6 mm. Abbreviation: R–retractor muscles.

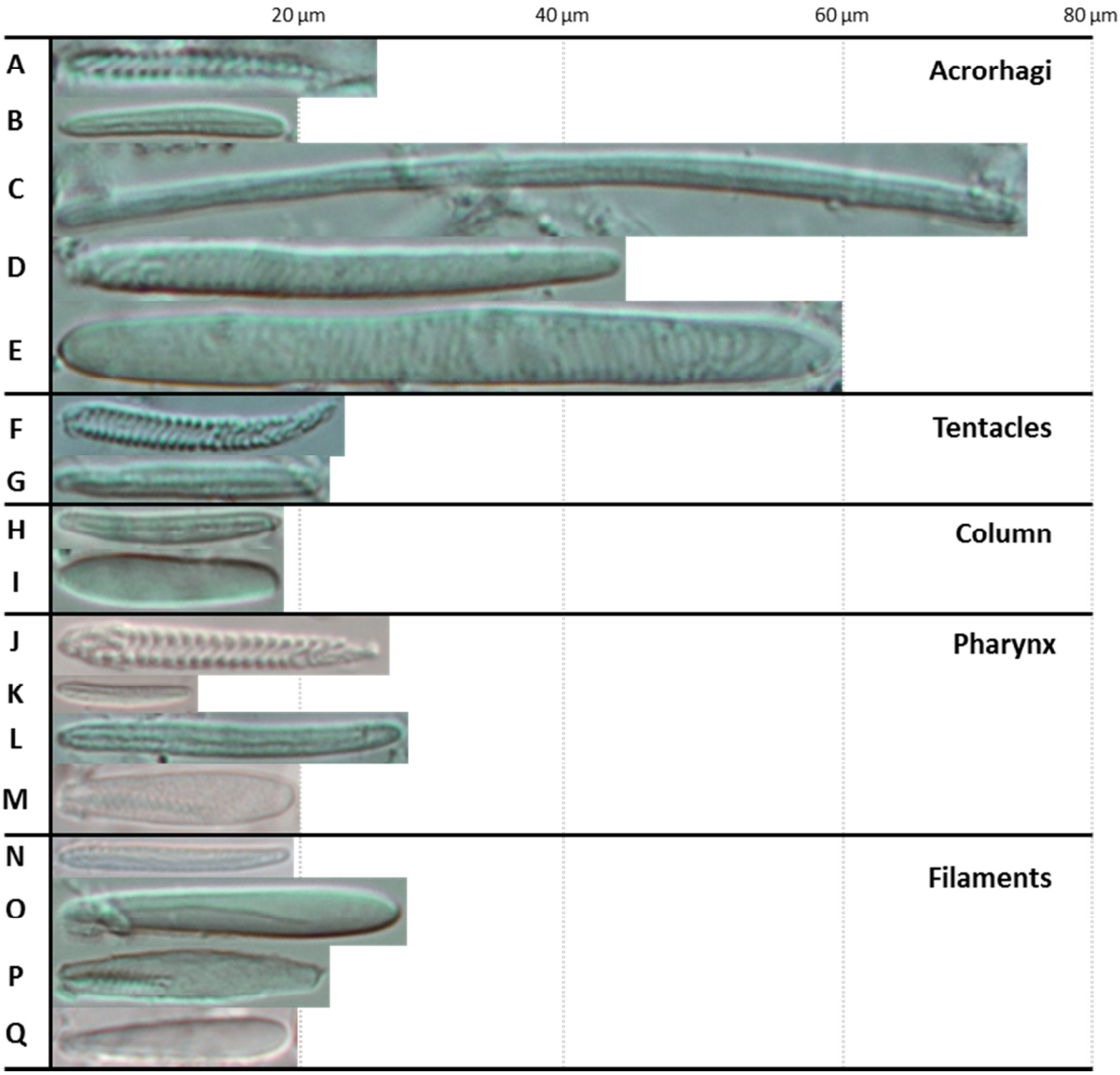

Fig. 1.30. Distribution of cnidae in *Bunodosoma kuekenthali* Pax, 1910. A, F, J: Spirocyst; B, C, G, H, K, L, N: Basitrich; D, E, I: Holotrich; O: Microbasic *b*-mastigophore; M, P, Q: Microbasic *p*-mastigophore.

Table 1.12. Size and distribution of cnidae of *Bunodosoma kuekenthali* Pax, 1910. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 6); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	22.154 - 27.839 x 2.546 - 3.200	25.032 ± 2.654 x 2.782 ± 0.264	5	4/5	+
Basitrich 1	15.390 - 22.800 x 1.810 - 3.390	17.720 ± 1.603 x 2.422 ± 0.390	24	3/5	+
Basitrich 2	62.610 - 90.226 x 1.600 - 3.063	75.063 ± 7.469 x 2.355 ± 0.325	59	5/5	+++
Holotrich 1	31.830 - 55.590 x 2.220 - 3.928	44.211 ± 6.602 x 3.460 ± 0.408	16	4/5	++
Holotrich 2	43.220 - 77.124 x 4.090 - 6.930	59.659 ± 8.105 x 5.644 ± 0.682	59	5/5	+++
Tentacles					
Spirocyst	14.840 - 29.795 x 1.660 - 4.140	23.268 ± 3.182 x 2.696 ± 0.473	103	6/6	+++
Basitrich	15.568 - 25.416 x 1.620 - 3.116	21.605 ± 1.576 x 2.297 ± 0.260	115	6/6	+++
Column					
Basitrich	15.249 - 23.777 x 1.674 - 3.768	18.569 ± 1.616 x 2.376 ± 0.305	155	6/6	+++
Holotrich	15.020 - 22.865 x 3.229 - 5.300	18.817 ± 1.571 x 4.481 ± 0.414	80	5/6	++
Pharynx					
Spirocyst	22.285 - 27.281 x 2.230 - 3.328	25.349 ± 1.744 x 2.814 ± 0.357	8	3/6	+
Basitrich 1	10.732 - 13.349 x 1.906 - 2.547	12.251 ± 1.309 x 2.199 ± 0.264	4	2/6	+
Basitrich 2	23.137 - 32.290 x 1.040 - 3.350	27.975 ± 2.278 x 2.341 ± 0.434	35	5/6	++
Microbasic *p*-mastigophore	20.990 x 5.300		1	1/6	+
Filaments					
Basitrich	10.760 - 29.576 x 2.140 - 4.167	18.669 ± 6.419 x 2.927 ± 0.701	15	4/6	+
Microbasic *b*-mastigophore	19.360 - 30.800 x 2.610 - 5.062	26.136 ± 2.651 x 4.004 ± 0.460	82	6/6	+++
Microbasic *p*-mastigophore 1	18.860 - 25.400 x 3.230 - 6.456	22.384 ± 1.425 x 4.576 ± 0.578	81	6/6	+++
Microbasic *p*-mastigophore 2	12.370 - 24.867 x 2.730 - 4.487	19.271 ± 3.121 x 3.471 ± 0.483	18	5/6	+

Discussion. Our description of this species was based mainly on the type specimens (ZMB 5192). We included some information of the different voucher lots, especially of cnidae, as they agreed with what we observed on type specimens or with the information in the original description (Pax, 1910a) and in Pax (1910b). One of the most outstanding feature of this species, which was also described in its orginal description, is the large vesicles. Pax (1910a, 1910b) comment that they are more developed in *B. kuekenthali* than in *B. granuliferum* and we found this characteristic to be very conspicuous. Nevertheless, the vesicles in this species agree with the definition of these columnar projections provided by Daly (2004). Pax (1910a, 1910b) also comments that the marginal sphincter is endordermal and has a tendency to be diffuse to circumscript. However, we disagree with this information, as all the specimens we have studied have circumscript muscles.

Pax (1910a, 1910b) did not include any information on the cnidae, which also present an interesting characteristic for this species: the large basitrichs and the two size classes of holotrichs (this feature exclusive in the genus) in the acrorhagi. In the syntype lot of *B. kuekenthali*, only one specimen has well-developed acrorhagi, in which we found the holotrichs and the large basitrichs. We also found the large basitrichs in the conspicuous acrorhagi of the specimens from lots USNM 54115 and 54116, and in the inconspicuous acrorhagi in USNM 51033.

The features we observed agree with what is described in Pax (1910a, 1910b). Due to the geographical distribution, this author compares the species to *B. granuliferum*. However, of the four species that are found in the west side of the Atlantic Ocean, *B. kuekenthali* is the most different in its external morphology. In fact, this species rather shares characteristics with *B. grande*. This species and *B. kuekenthali* have two features that are exclusive in the genus *Bunodosoma*: large vesicles and long basitrichs in the acrorhagi (over 58µm long). *B. kuekenthali* is smaller than *B. grande* in size and in number of tentacles and mesenteries.

Also, we observed some differences between both species' cnidae: *B. kuekenthali* has two size classes of basitrichs and holotrichs in the acrorhagi, whereas *B. grande* only has one; *B. grande* has spirocysts and an extra size class of basitrichs in the column; *B. kuekenthali* has spirocysts and microbasic *p*-mastigophores in the pharynx.

The fact that the distribution of *B. grande* and *B. kuekenthali* are separated by the Panama Isthmus raises an interesting question about the group's biogeography, which can be discussed if based on an appropriate phylogenetic hypothesis. Also, we find it necessary to mention that *B. kuekenthali* has a very restricted distribution if compared to *B. cavernatum* and *B. granuliferum.* It is possible that it does in fact occur in a small area, but it is also possible that it has been overlooked or errouneusly identified as one of the other species.

Bunodosoma sphaerulatum Duerden, 1902

(Figs. 1.31-1.32, Table 1.13)

Synonymy list

Bunodosoma spherulata Duerden, 1902: 329, 350-354, Pl. III, fig. 10, Pl. X, figs. 36-38; Pl. XI, figs. 39-40 (original description).

Bunodosoma sphaerulatum – Pax, 1910: 177.

Phymactis sphaerulata – Stephenson, 1922: 285.

Material examined

USNM 22129: Syntype, 1/2 dissected specimen. Puerto Rico.

Diagnosis. *Bunodosoma* with up to 10 transverse rows of large vesicles in the column and up to 24 tentacles in three cycles.

Description

Pedal disc. Adherent, circular to semi-circular, 5 mm in diameter in the preserved specimen.

Column. Cylindrical, 6 mm in diameter and 3.6 mm height in the preserved specimen. Column densely covered with large vesicles in 10 transverse rows. No compound vesicles, no acrorhagi.

Oral disc. 3.5 mm in diameter and without markings in the preserved specimen; mouth with slightly raised lips.

Tentacles. Relatively long, 0.4-1.6 mm in length in the preserved specimen, tapered and pointed. 24 tentacles in the dissected specimen in 3 cycles (probably 6+6+12 in the whole specimen).

Internal anatomy. Marginal sphincter muscle endodermal and circumscript. Retractors restricted and strong. Parietobasilar muscles mesogleal pennons.

Cnidome. We were able to observe only basitrichs and microbasic *b*-mastigophores in this specimen. A survey of sizes and distribution is shown in Table 1.13.

Color. Preserved specimens with brown column, tentacles, pedal discs and internal structures.

Distribution. Western Atlantic, Puerto Rico.

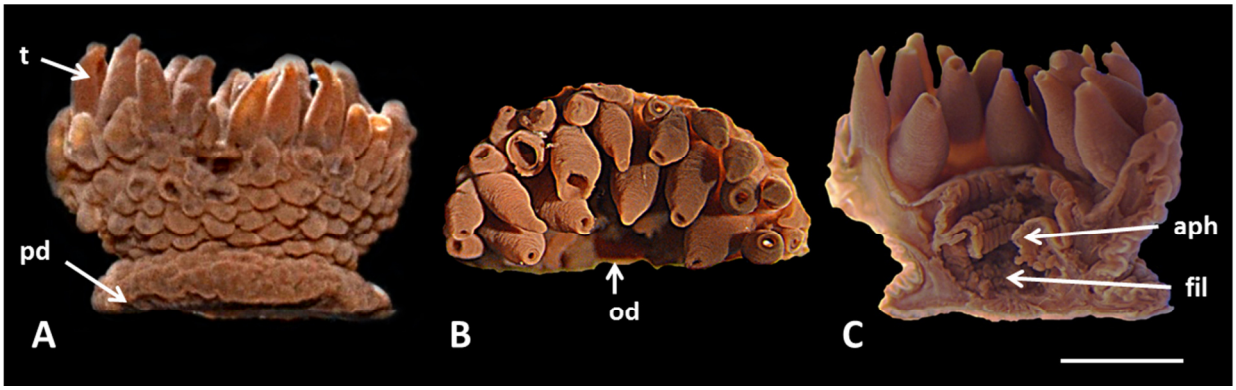

Fig. 1.31. *Bunodosoma sphaerulatum* Duerden, 1902. USNM 22129. A) Lateral view. B) Oral view. C) Internal view. Scale = 2.5 mm. Abbreviations: aph-actinopharynx; fil-filaments; od-oraldisc; pd-pedal disc; t-tentacle.

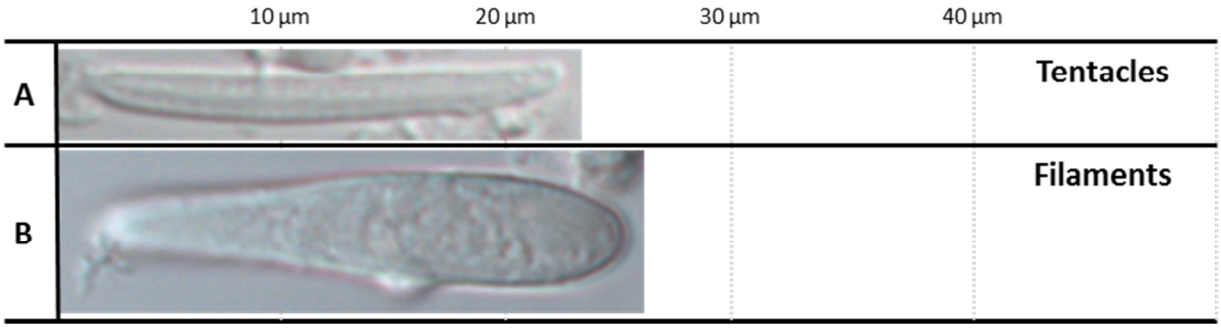

Fig 1.32. Distribution of cnidae in *Bunodosoma sphaerulatum* Duerden, 1902. A: Basitrich; B: Microbasic *b*-mastigophore.

Table 1.13. Size and distribution of cnidae of *Bunodosoma sphaerulatum* Duerden, 1902. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 1); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Tentacles					
Spirocyst	Ruptured				+++
Basitrich	18.635 - 23.332 x 1.587 - 2.911	21.142 ± 1.069 x 2.336 ± 0.328	32	1	+++
Filaments					
Bastrich	Ruptured				+
Microbasic *p*-mastigophore	Ruptured				+
Microbasic *b*-mastigophore	23.756 - 29.395 x 4.330 - 5.712	26.754 ± 1.806 x 5.250 ± 0.428	11	1	+

Taxonomical remarks. The status of this species cannot be confirmed based on the available information.

Discussion. Our description is based on the syntype of the species, which is only half of a specimen. The information on internal anatomy is based on Duerden (1902). The presence of vesicles in the column and of a circumscript and endodermal marginal sphincter indicates that this species may in fact belong to the genus *Bunodosoma*. Duerden (1902) presents a figure in which an acrorhagus is shown, but we did not find this in the specimen we studied. Based on the available information and on the fact that other three species (*B. cavernatum*, *B. granuliferum* and *B. kuekenthali*) are found in the region, we cannot conclude if *B. sphaerulatum* is a species or a juvenile belonging to one of the other species of *Bunodosoma*.

Bunodosoma zamponii Gomes, Schama & Solé-Cava, 2011

(Figs. 1.33-1.35, Table 1.14)

Synonymy list

Phymactis clematis – Zamponi, 1977; Pollero, 1983; Patronelli *et al*., 1987; Zamponi, 1989, 1993, 2000, 2005; Excoffon & Zamponi, 1991; Acuña & Zamponi, 1995, 1996, 1997; Acuña *et al.*, 1996; Zamponi & Perez, 1996, Genzano *et al.*, 1996; Acuña, 1997; Zamponi *et al.*, 1998a, b; Gomes *et al.*, 1998; Excoffon *et al.*, 1999; Patronelli *et al.*, 2005, 2008; Olivera *et al.*, 2009. not *Phymactis clematis* (Drayton *in* Dana, 1846).

Bunodosoma zamponii Gomes, Schama & Solé-Cava, 2011: 7-13 (original description).

Material examined

MACN In-35365: Holotype, 1 specimen; Punta Cantera, Mar del Plata, Argentina (38°05'S 57°32'W), Coll. P.B. Gomes, 16 September 1999, preserved in 4% formaldehyde solution.

5 specimens; Punta Cantera, Mar del Plata, Buenos Aires, Argentina (38°04'S 57°32'W); Coll. J.S. Beneti, F.H. Acuña & A. Garese, 26 February 2014; preserved in 4% formaldehyde solution.

5 specimens ; Santa Clara del Mar, Buenos Aires, Argentina (37°50'S 57°29'W) ; Coll. J.S. Beneti, F.H. Acuña & A. Garese, 3 March 2014; preserved in 4% formaldehyde solution.

Diagnosis. *Bunodosoma* with up to 38 transverse rows of vesicles densely distributed in the column; 96 tentacles in five cycles and 48 mesenteries in four cycles; mesenteries of first, second and third cycles perfect over entire length of the actinopharynx, fourth cycle reaches actinopharynx only at its most distal part; all mesenteries fertile, directives excluded.

Description

Pedal disc. Strongly adherent, circular to semi-circular, up to 42 mm in diameter in live expanded specimens, up to 37 mm in diameter in preserved specimens.

Column. Cylindrical, narrower than pedal and oral discs in live specimens, up to 42 mm in diameter and up to 60 mm height in live specimens, and up to 39 mm in diameter and up to 38 mm height in preserved specimens. Column densely covered with vesicles in 96 longitudinal rows and up to 38 transverse rows. The vesicles are endocoelic and exocoelic, rounded, non-adhesive, non-cup-shaped, mesoglea and ectoderm of similar thickness at the sides and apex, ectoderm with similar cells at sides and apex. The vesicles of the 5-6 most distal rows may be compound, usually with 2 or 3 lobes each. Acrorhagi with holotrichs, basitrichs and spirocysts, arranged into two alternate rows, inner row endocoelic, second exocoelic; inner

row with up to 48 larger acrorhagi (up to 1.3 mm in diameter) than the second one (up to 0.7 mm diameter). Smaller specimens may have only one row of acrorhagi. Fosse pronounced. Contracted specimens often dome-shaped.

Oral disc. Diameter up to 22 mm. Mouth with slightly raised lips in some specimens. In the preserved specimens, it has no special characters.

Tentacles. Long, length up to 25 mm in live specimens and up to 21 mm in preserved specimens, tapered, pointed. Most individuals have 96 arranged in 5 cycles (6+6+12+24+48). Some specimens with up to 100 tentacles with a sixth incomplete cycle. All tentacles approximately the same size. Tentacles of the last cycle are exocoelic.

Internal anatomy. Mesenteries pairs usually 48, hexamerously arranged in 4 cycles (6+6+12+24) at the actinopharynx level. Number of mesenteries usually equal proximally and distally. Some specimens with 46 (fourth cycle incomplete) to 52 mesenteries pairs (fifth incomplete cycle). Mesenteries of first, second and third cycles perfect over entire length of the actinopharynx, fourth cycle reaches actinopharynx only at its most distal part. All mesenteries fertile, directives excluded. Reproductive tissue may be poorly developed in the last cycle in smaller specimens. No indication of asexual reproduction. Gonochoric. Marginal sphincter muscle endodermal and circumscript, main mesogleal lamella thin and short. Retractors restricted and strong. Parietobasilar muscles with medium length and thin mesogleal pennons. Basilar muscles distinct. Longitudinal muscles of the tentacles ectodermal. Marginal stomata present. No zooxanthellae.

Cnidome. Spirocysts, basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores. A survey of sizes and distribution is shown in Table 1.33.

Color. In live specimens, pedal disc color cream and insertion of mesenteries are visible as pale brown lines; column dark orange to green, vesicles often darker; acrorhagi lighter than column, usually of a cream color; oral disc cream to light brown, insertion of mesenteries may

be visible as cream lines; tentacles cream to light brown. Preserved specimens with column cream proximally, becoming darker distally; oral disc and tentacles lighter then column, usually cream.

Distribution. South Atlantic, Argentina.

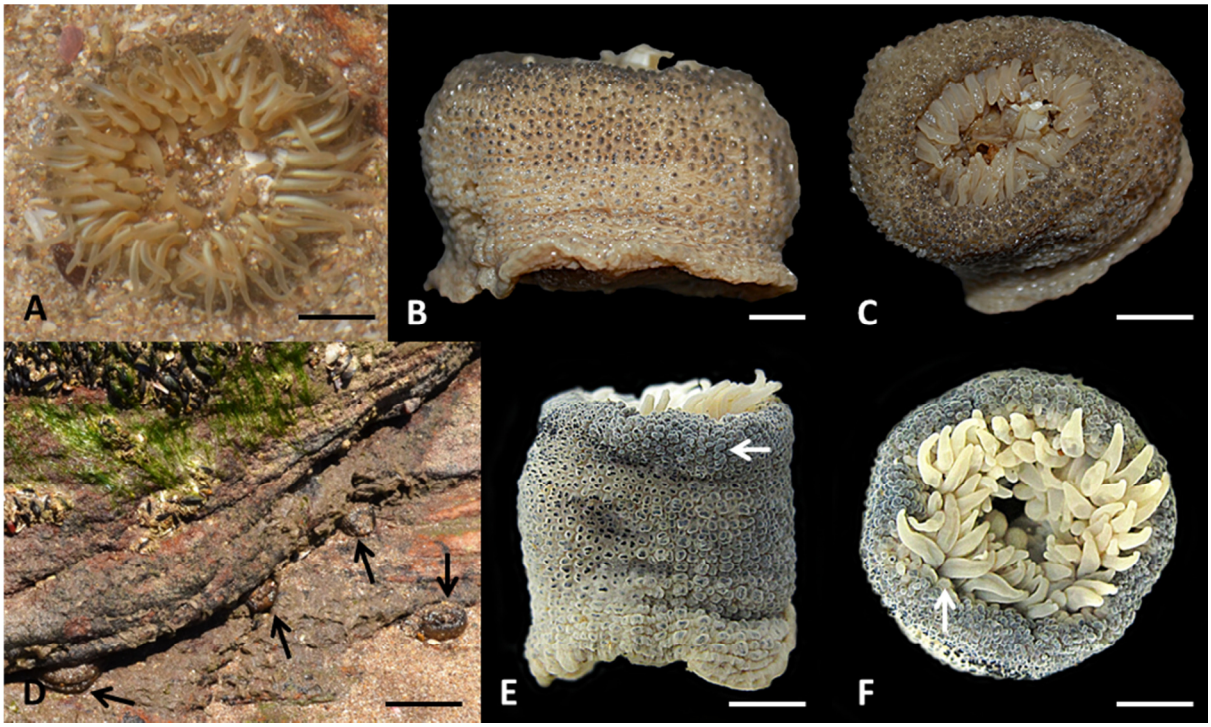

Fig. 1.33. *Bunodosoma zamponii* Gomes, Schama & Solé-Cava, 2011. A) Live specimen from Punta Cantera, Mar del Plata, Argentina. Scale = 0.9 cm. B) MACN In-35365, Holotype, lateral view. Scale = 0.7 cm. C) MACN In-35365, Holotype, oral view. Scale = 1.0 cm. D) Specimens in the field in Punta Cantera, Mar del Plata, Argentina, before collection, arrows indicate specimens. Scale = 8 cm. E) Specimen from Santa Clara del Mar, Buenos Aires, Argentina, lateral view, arrow indicates compound vesicle. Scale = 1 cm. F) Specimen from Santa Clara del Mar, Buenos Aires, Argentina, oral view, arrow indicates acrorhagus. Scale = 1 cm.

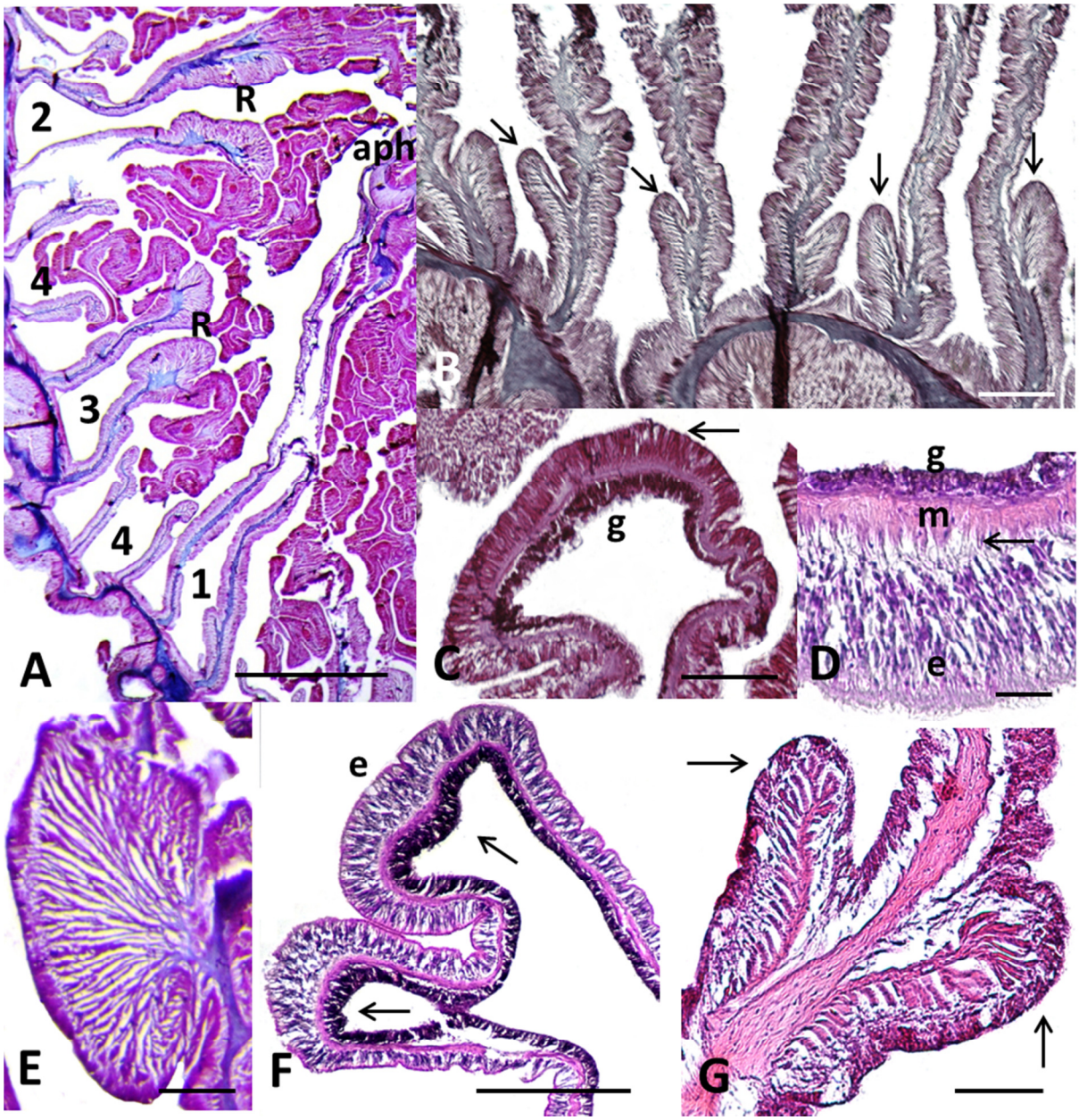

Fig. 1.34. Anatomy of *Bunodosoma zamponii* Gomes, Schama & Solé-Cava, 2011. A) Cross section of the column, at actinopharynx level, showing cycles of mesenteries. Numbers indicate the cycles of mesenteries. Scale = 6 mm. B) Cross section of mesenteries, arrows show mesogleal pennon of the parietobasilar muscles. Scale = 0.05 mm. C) Longitudinal section of the margin showing acrorhagus, arrow indicates ectoderm with cnidocysts. Scale = 0.2 mm. D) Cross section of tentacle. Scale = 0.2 mm. E) Cross section through marginal sphincter muscle. Scale = 0.2 mm. F) Cross section of bilobed compound vesicle, arrows indicate lobes. Scale = 0.2 mm. Cross section of basilar muscle. Scale = 0.03 mm. Abbreviations: aph-actinopharynx; e–epidermis; g–gastrodermis; m-mesoglea; R–retractor muscles.

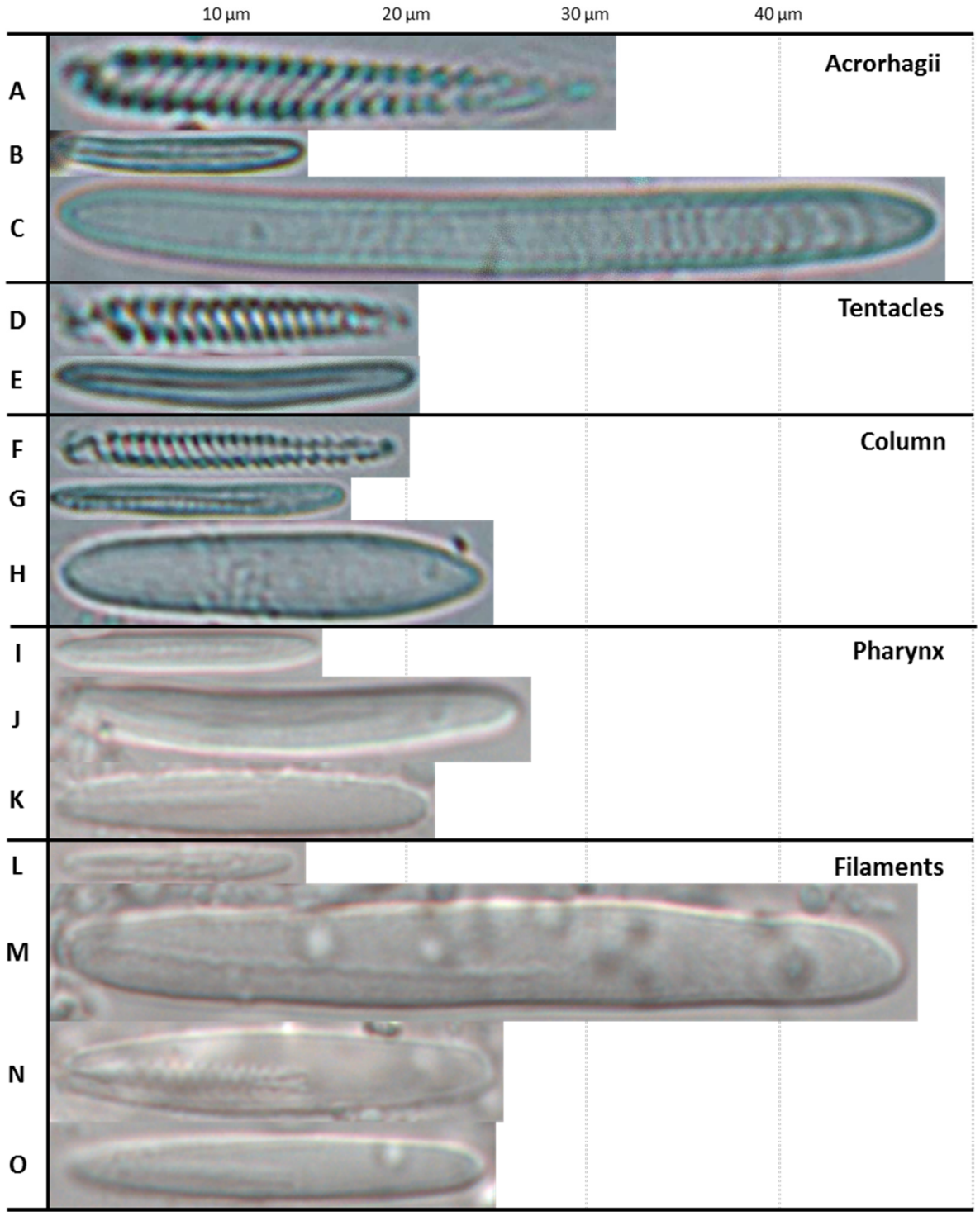

Fig. 1.35. Distribution of cnidae in *Bunodosoma zamponii* Gomes, Schama & Solé-Cava, 2011. A, F: Spirocyst; B, D, G, I, J, L: Basitrich; C, H: Holotrich; M: Microbasic *b*-mastigophore; K, N, O: Microbasic *p*-mastigophore.

Table 1.14. Size and distribution of cnidae of *Bunodosoma zamponii* Gomes, Schama & Solé-Cava, 2011. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae (total analyzed = 2; 3); F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F
Acrorhagi					
Spirocyst	23.120 - 42.016 x 1.964 - 3.949	31.980 ± 4.020 x 2.972 ± 0.480	27	3/3	++
Basitrich	11.810 - 16.720 x 1.770 - 2.750	13.979 ± 1.120 x 2.302 ± 0.219	53	3/3	++
Holotrich	21.300 - 62.128 x 2.920 - 6.710	48.495 ± 4.791 x 4.560 ± 0.549	185	3/3	+++
Tentacles					
Spirocyst	14.210 - 27.540 x 1.580 - 3.562	20.713 ± 2.705 x 2.559 ± 0.379	109	3/3	+++
Basitrich	17.610 - 26.819 x 1.702 - 3.360	21.626 ± 2.166 x 2.604 ± 0.259	153	3/3	+++
Column					
Spirocyst	17.660 - 23.630 x 2.060 - 3.130	19.870 ± 3.273 x 2.600 ± 0.535	3	1/3	+
Basitrich	11.794 - 21.762 x 0.220 - 3.313	16.163 ± 1.762 x 2.385 ± 0.333	197	3/3	+++
Holotrich	19.450 - 29.357 x 3.789 - 5.864	24.344 ± 3.015 x 4.684 ± 0.522	46	3/3	++
Pharynx					
Basitrich 1	11.515 - 19.094 x 2.000 - 2.876	15.567 ± 2.046 x 2.452 ± 0.209	26	2/2	++
Basitrich 2	24.178 - 31.929 x 2.132 - 3.769	27.710 ± 1.667 x 2.977 ± 0.288	66	2/2	+++
Microbasic *p*-mastigophore	19.780 - 22.988 x 3.338 - 3.815	21.384 ± 2.268 x 3.577 ± 0.337	2	2/2	+
Filaments					
Basitrich	10.759 - 16.666 x 1.449 - 2.381	13.910 ± 1.529 x 1.945 ± 0.215	36	2/2	+++
Microbasic *b*-mastigophore	40.193 - 58.101 x 5.001 - 6.265	47.341 ± 3.324 x 5.730 ± 0.338	31	2/2	+
Microbasic *p*-mastigophore 1	22.174 - 29.561 x 4.631 - 6.215	25.800 ± 1.748 x 5.294 ± 0.410	30	2/2	++
Microbasic *p*-mastigophore 2	20.264 - 27.298 x 2.881 - 4.096	24.065 ± 2.144 x 3.461 ± 0.361	16	2/2	++

Discussion. Before being described by Gomes *et al.* (2011), many studies had been carried out with this Argentinian species, in which it was incorrectly identified as *Phymactis clematis* (Zamponi, 1977; Pollero, 1983; Patronelli *et al.*, 1987; Zamponi, 1989, 1993, 2000, 2005; Excoffon & Zamponi, 1991; Acuña & Zamponi, 1995, 1996, 1997; Acuña *et al.*, 1996; Zamponi & Perez, 1996, Genzano *et al.*, 1996; Acuña, 1997; Zamponi *et al.*, 1998a, b; Gomes *et al.*, 1998; Excoffon *et al.*, 1999; Patronelli *et al.*, 2005, 2008; Olivera *et al.*, 2009). The genus *Phymactis* is characterized by having a diffuse marginal sphincter, which is not the case of this species. *B. zamponii* is very similar to most species of the *Bunodosoma*, as it has vesicles in the column, acrorhagi, a circumscript marginal sphincter, 96 pairs of mesenteries and also, in its cnidome. However, the species to which it is more similar is *B. cangicum.*

Although *B. zamponii* has a larger number of transverse rows of vesicles in the column (up to 38, against 35 in *B.cangicum*), it is is usually smaller than *B. cangicum*. Nevertheless, they present the same number of tentacles and pairs of mesenteries, besides a very similar distribution of mesenteries. One of the differences is that *B.cangicum* does not have a fertile last cycle of mesenteries, whereas *B. zamponii* usually does (see description of *B. cangicum*). Also, the only difference in the cnidae distribution is in the column: *B.cangicum* has only basitrichs, whereas *B. zamponii* has also spirocysts and holotrichs. This feature is revisited in Chapter 3, in which we show that spirocysts and holotrichs have been found in the column of some populations of *B. cangicum*, which were not herein considered. Therefore, we reaffirm that, despite the similarities, we chose to keep these species separate until a larger study, including various populations of both species and using appropriate data is carried out (Chapter 2).

It is important to comment that our observations on the retractor muscles are the reverse of what was observed by Gomes *et al.* (2011), who stated that the retractor muscles of *B. zamponii* is circumscript to diffuse strong, whereas in *B. cangicum* it is circumscript and

stronger. We found the retractor muscles of the former to be stronger than the latter. This may indicate that both species may have individual variations in their retractor muscle morphology, or that this feature may be different depending on preservation techniques.

Molecular data and Phylogenetic analysis

We were able to amplify CO3, 18S and partial 28S for most of the taxa, and 12S only for *B. cavernatum*. However, we were not able to amplify 16S or fragment 4 of the 28S marker due to primer contamination and to an unidentified problem, respectively. Amplified PCR products varied in length from 108 to 649 bp (in 18S-3 and 18S-2, respectively). Length variation and number of sequences amplified per species for each marker are summarized in Table 1.15.

Table 1.15. Length variation and number of sequences amplified per species and marker.

Marker	*B. caissarum*		*B. cangicum*		*B. cavernatum*		*B. granuliferum*		*B. zamponii*	
	Range (bp)	**N**	**Range (bp)**	**N**	**Range (bp)**	**N**	**Range (bp)**	**N**	**Range (bp)**	**N**
12S	-		-		540 - 588	7	-		-	
CO3	430 - 472	5	330 - 450	3	402 - 472	4	267 - 386	5	-	
18S - 1	443 - 467	5	390 - 391	2	292 - 450	7	460 - 469	6	422 - 437	2
18S - 2	434 - 582	6	466 - 613	4	474 - 554	6	548 - 649	3	445 - 507	3
18S - 3	127 - 298	4	214 - 320	2	310 - 317	4	266 - 311	3	108 - 256	3
28S - 1	471 - 478	4	355 - 478	6	471 - 494	9	458 - 477	5	456 - 478	3
28S - 3	209 - 213	3	200 - 206	5	528 - 532	4	210 - 211	5	203 - 212	3
28S - 4	277 - 586	5	580 - 591	3	-		506 - 567	5	-	
28S - 5	381 - 390	6	330 - 332	2	-		-		-	

Fragments of 18S and 28S were concatenated prior to the alignment. Each alignment had the following lengths: 12S (590bp), COIII (373bp), 18S (911bp) and 28S (1835bp). All

partitions were combined into a single data set, as combining data from different sources is more likely to accurately reflect evolutionary history (*e.g.,* Kluge 1989, 2004; Eernisse and Kluge, 1993). After 175 independent generations with 36 independent interactions of ratchet and fusing, the search yielded 15 most parsimonious trees of 805 steps. The topology of the consensus tree is presented in Figure 1.36.

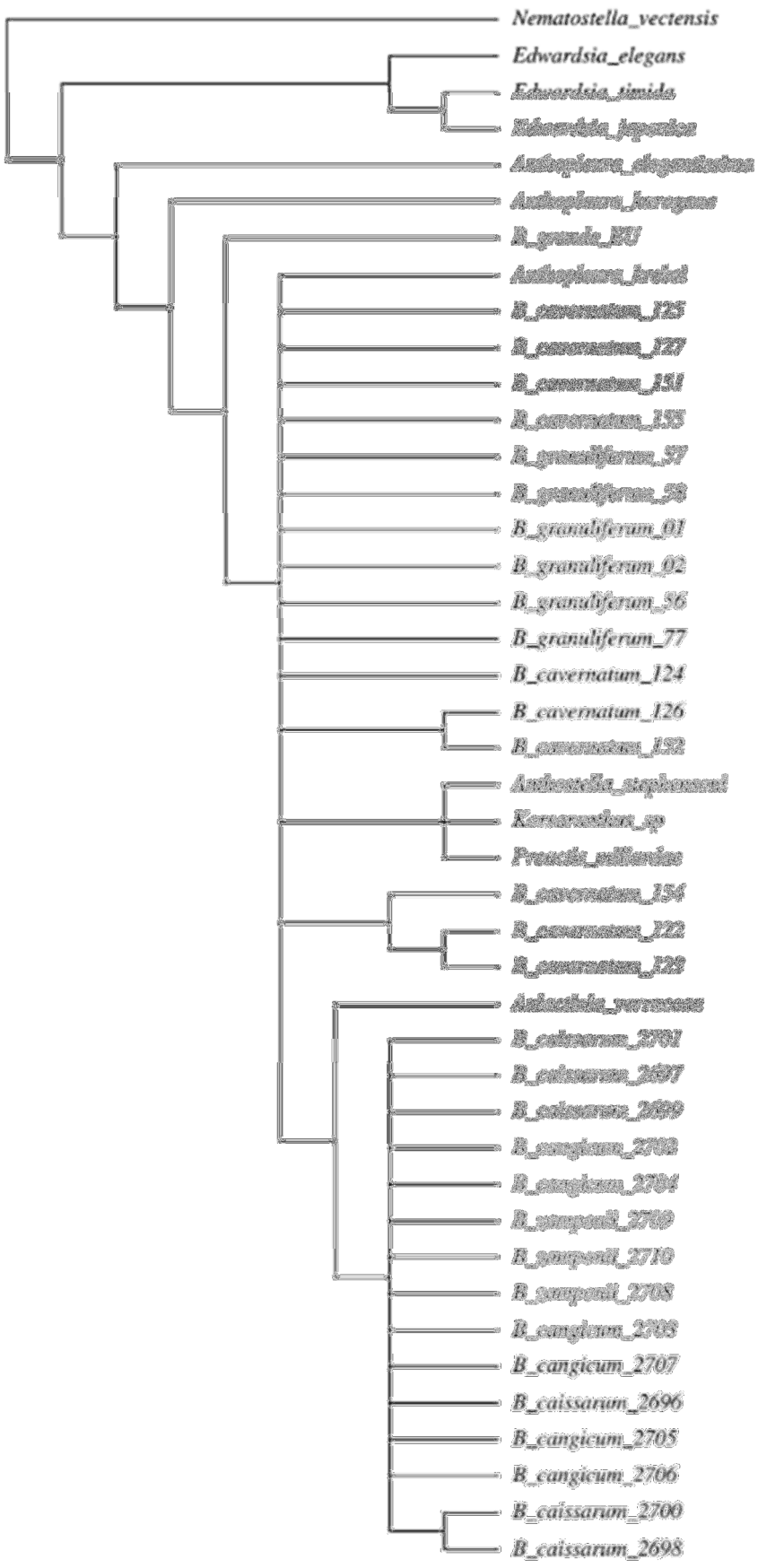

Figure 1.36. Complete cladogram of the relationships of the species based on the mitocondrial (12S and COIII) and nuclear (18S and 28S) markers.

In this tree the genus *Bunodosoma* is polyphyletic. *Bunodosoma grande* is sister to an unresolved clade containing *B. cavernatum*, *B. granuliferum*, B. *caissarum*, *B. cangicum, B. zamponii* and other included species from the family Actiniidae. Within this clade, *Aulactinia verrucosa* is sister to an unresolved clade form by species of *Bunodosoma* from South America (*B. caissarum* + *B. cangicum* + B. *zamponii*).

Discussion

Although some aspects still need to be revised, we confirm that 11 of the 14 previous species of *Bunodosoma* are valid: *B. caissarum*, *B. californicum*, *B. cangicum*, *B.capense*, *B. cavernatum*, *B. diadema*, *B. goanense*, *B. grande*, *B. granuliferum*, *B. kuekenthali* and *B. zamponii.* All of them have the diagnostic features of the genus, *i.e.* endodermal circumscript sphincter, acrorhagi, non-adherent vesicles on the column, and fertile mesenteries with well-developed muscles (Häussermann, 2004; Gomes *et al.*, 2012).

We keep *B. biscayense* in the genus because our histological observations of body sections showed that its column projections are to be considered vesicles and not verrucae, contrary to the figures and description provided by den Hartog (1987). The difference between our observations and the ones of den Hartog (1987) are possibly due to the state of contraction of the specimens we observed; there may have been cup-shaped verrucae that were deformed and therefore overlooked, so we suggest that more specimens of this species should be studied through additional histological sections.

For the species *B. fallax* and *B. sphaerulatum*, we found no evidence to remove them from the genus, although we strongly suggest that more samples of both species should be collected and their features more accurately studied. Our data are based on a few specimens which are badly preserved.

Some aspects of valid species of *Bunodosoma* must be studied further, mainly concerning the species from the Caribbean and South Atlantic west coast. Although McCommas & Lester (1980) found that *B. cavernatum* and *B. granuliferum* are genetically different and considered them as separate valid species, in our phylogenetic analysis they form one clade. It is possible that they have diverged very recently, so the molecular markers we used are not appropriate to separate them, thus we suggest that other markers should be tested in order to better understand the relationship between these two species. For the clade *B. caissarum* + *B. cangicum* + *B. zamponii* the situation is very similar. The first species has very clear morphological characteristics that separate it from the other two, but still it seems to have diverged very recently. However, the other two species, as in the Caribbean ones, are very similar in their morphology and we suggest that their molecular aspects should be better studied. We address more profoundly the molecular and cnidae aspects of the South American species in Chapters 2 and 3.

The species of the genus *Bunodosoma* are all very similar to each other and there are few characteristics that can be used to separate them. The main diagnostic features are: number of tentacles and mesenteries, number of longitudinal rows of vesicles, presence or absence of longitudinal bands and types of cnidocysts (Tables 1.16, 1.17 and 1.18). Also, we believe that geographical distribution might be of some use to help in the identification of the species, as most of them have limited distribution and do not occur in sympatry with others of the genus. The distributions of *Bunodosoma* species have not changed after the redescriptions we provided. *Bunodosoma biscayense*, *B. capense* and *B. diadema* occur on the East Atlantic coast. On the West Atlantic coast occur *B. caissarum*, *B cangicum*, *B. cavernatum*, *B. granuliferum*, *B. kukenthali*, *B. sphaerulatum* and *B. zamponii*. On the East Pacific Ocean occur *B. californicum* and *B. grande*. And, finally, *B. goanense* and *B. fallax* can be found on the Indian Ocean (Figure 1.37).

Table 1.16. Characteristics for diagnosis of the species of *Bunodosoma* Verrill, 1899. "X" indicates presence of the characteristic. "+" indicates that more tentacles, tentacle cycles, mesentery pairs or cycles have been observed in the species. "-" indicates that less tentacles, tentacle cycles, mesentery pairs or cycles have been observed in the species.

Characteristic/Species	*B. biscayense*	*B. caissarum*	*B. californicum*	*B. cangicum*	*B. capense*	*B. cavernatum*	*B. goanense*	*B. grande*	*B. granuliferum*	*B. kukenthali*	*B. zamponii*
Column											
Longitudinal bands	X								X		
Number of vesicles	45	70	25	45	32	34	35	36	35	27	38
Large vesicles								X		X	
Compound distal vesicles	X	X		X			X	X		X	X
Sparse distal vesicles							X				
Tentacles											
Number	96-	192+	48 or 96+	96+	192+	96	192-	192+	96	96-	96+
Cycles	5-	6+	4 or 5+	5+	6+	5	6	6+	5	5	5+
Mesenteries											
Number of pairs	48+	96	24-48	48+	96	48	96	96+	48	48	48+-
Cycles	4+	5	3 or 4	4+	5	4	5	5+	4	4	4+
Perfect over entire actinopharynx	1 and 2	1,2 and 3	1,2 and 3	1 and 2	1 and 2	1 and 2	1 and 2	1 and 2	1 and 2	1 and 2	1,2 and 3
Reproductive tissue present (directives excluded)	all fertile *	1,2,3,4	all fertile	1,2,3	1,2,3,4	all fertile -	all fertile-	all fertile-	all fertile	1, 2 and 3	all fertile
Indication of assexual reproduction			X								

Table 1.17. Characteristics for diagnosis of the species of *Bunodosoma* Verrill, 1899. "X" indicates presence of the characteristic. "*" indicates the type is present in specimens of Chapter 3.

Characteristic/Species	*B. biscayense*	*B. caissarum*	*B. californicum*	*B. cangicum*	*B. capense*	*B. cavernatum*	*B. goanense*	*B. grande*	*B. granuliferum*	*B. kukenthali*	*B. zamponii*
Acrorhagi											
Spirocyst	X	X	X	X	X	X	X	X	X	X	X
Basitrich 1 (< 20 µm)		X		X		X	X		X	X	
Basitrich 2 (> 20 µm)	X				X						X
Basitrich 3 (> 60 µm)								X		X	
Holotrich	X	X	X	X	X	X	X	X	X	X	X
Holotrich (2nd size class)										X	
Tentacle											
Spirocyst	X	X	X	X	X	X	X	X	X	X	X
Basitrich	X	X	X	X	X	X	X	X	X	X	X
Basitrich (2nd size class)	X	X					X				
Column											
Spirocyst				*		X		X	X		X
Basitrich	X	X	X	X	X	X	X	X	X	X	X
Basitrich (2nd size class)	X	X	X		X	X		X	X		
Holotrich	X	X		*	X		X	X	X	X	X

Table 1.18. Characteristics for diagnosis of the species of *Bunodosoma* Verrill, 1899. "X" indicates presence of the characteristic. "*" indicates the type is present in specimens of Chapter 3.

Characteristic/Species	*B. biscayense*	*B. caissarum*	*B. californicum*	*B. cangicum*	*B. capense*	*B. cavernatum*	*B. goanense*	*B. grande*	*B. granuliferum*	*B. kukenthali*	*B. zamponii*
Pharynx											
Spirocyst				*		X			X	X	
Basitrich	X	X	X	X	X	X	X	X	X	X	X
Basitrich (2nd size class)	X	X	X	X	X	X	X	X		X	X
Basitrich (3rd size class)			X								
Microbasic *p*-mastigophore	X	X	X	X	X	X	X			X	X
Filaments											
Basitrich	X	X	X	X	X	X	X	X	X	X	X
Basitrich (2nd size class)			X			X	X		X		
Basitrich (3rd size class)							X				
Microbasic *b*-mastigophore	X	X	X	X	X	X	X	X	X	X	X
Microbasic *b*-mastigophore (2nd size class)		X				X					
Microbasic *p*-mastigophore 1	X	X	X	X	X	X	X	X	X	X	X
Microbasic *p*-mastigophore 2	X	X	X	X	X	X	X	X		X	X

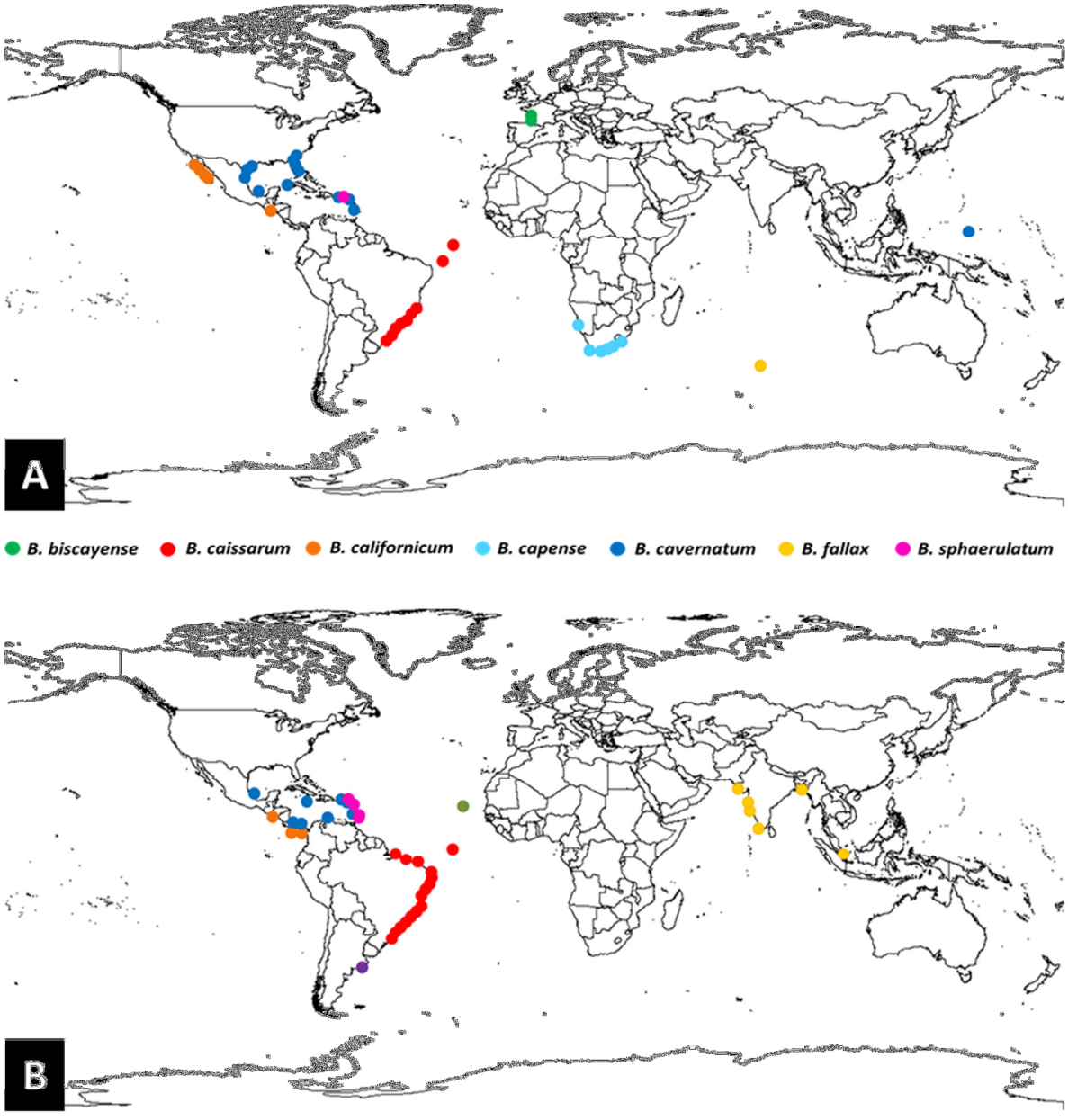

Fig. 1.37. Distribution of the genus *Bunodosoma* Verrill, 1899. A) Distributions of the species *Bunodosoma biscayense*, *B. caissarum*, *B. californicum*, *B. capense*, *B. cavernatum*, *B. fallax* and *B. sphaerulatum.* B) Distributions of *B. cangicum*, *B. diadema*, *B. goanense*, *B. grande*, *B. granuliferum*, *B. kuekenthali* and *B. zamponii.*

All specimens observed in this study do not help in the understanding of evolutionary aspects of the column projections (for the genus *Bunodosoma*: vesicles, as defined by Daly, 2004). A study with this intention and with a larger sampling of members of the family Actiniidae is being carried out by Rodríguez & Daly (in prep), and should be more

appropriate to answer these questions. Nevertheless, the vesicles may be informative inside the genus *Bunodosoma*. The number of transverse rows of vesicles is not exact for any of the species, but we believe that its maximum number is relevant for distinguishing them. This structure is probably related to the size of the polyp (personal observations in *B. cangicum*) but there may be a maximum number of vesicles that a species can have. *Bunodosoma caissarum*, *B. cangicum* and *B. grande*, for example, may reach similar sizes (up to 4cm in preserved specimens), but the former has more vesicles than the other two species. Also, Häussermann (2004) comments that the vesicles in *Bunodosoma* are never compound, but we have found that not to be true for most species, which present compound vesicles distally in the column.

Concerning the number of tentacles and mesenteries, we were able to separate the species into three groups: i) species with 6 cycles of tentacles (6+6+12+24+48+96) or more and 5 cycles of mesenteries (6+6+12+24+48), which comprise *B. caissarum*, *B. capense, B. grande* and *B. goanense*; ii) species with 5 cycles of tentacles (6+6+12+24+48) and 4 cycles of mesenteries (6+6+12+24), which comprise *B. biscayense*, *B. cangicum, B. cavernatum, B. granuliferum* and *B. zamponii*; iii) species which may have less than 5 cycles of tentacles and 4 cycles of mesenteries, such as *B. californicum* and *B. kuekenthali*. This division has solely the purpose of organizing the morphology of the group with no intention of reflecting any evolutionary pattern. Due to the lack of resolution in our proposed phylogenetic hypothesis, we cannot affirm if these features help in recognizing natural groupings or not.

The types of cnidae found in each tissue are almost the same for all species of the genus. In the column and actinopharynx, in which cnidae are much scarce, it is possible that some type was overlooked, so we recommend that species should not be distinguished exclusively based on the cnidae of such tissues. Nevertheless, some types of cnidae should be considered as being characteristic of a species. For example, the very large basitrichs in the

acrorhagi of *B. grande* and *B. kuekenthali* are exclusive, and may be an evidence of a closer relationship between these two species. Also, the two size classes of holotrichs in the latter species can be considered as exclusive.

Most of the intraspecific issues among *Bunodosoma* species highlighted by Häussermann (2004) were solved herein, although some remain to be addressed. With the review of the characteristics and redescription of the species of this genus we intend to assure that future studies provide correct identifications and contribute to the knowledge of the existing species as well as facilitating the proposal of new species for the genus. Also, we reaffirm that, to delimitate species in Actiniaria, morphological, anatomical and cnidae information must be used comparatively with molecular data, so that the knowledge in the group increases in order to aid on the comprehension of evolutionary, biological and ecological aspects of the sea anemones.

References

Acuña F.H. 1997. Ecología trófica de actiniarios (Cnidaria, Anthozoa) intermareales: selección de la talla de las presas. *Physis A* 53, 1–5.

Acuña F.H. & Zamponi M.O. 1995. Ecology of intertidal sea anemones. Density, dispersion and autoecology of *Phymactis clematis* Dana,1849 (Anthozoa: Actiniaria). *Ciencias Marinas* 21: 1–12.

Acuña F.H. & Zamponi M.O. 1996. Ecología trófica de las anémonas intermareales *Phymactis clematis* Dana, 1849, *Aulactinia marplatensis* (Zamponi, 1977) y A. *reynaudi* (Milne-Edwards, 1857) (Actiniaria: Actiniidae): relaciones entre las anémonas y sus presas. *Ciencia Marina* 22, 397–413

Acuña F.H., Zamponi, M.O. & Perez C.D. 1996. Metodología para la cuantificación de algunas estructuras taxonómicas en Actiniaria (Cnidaria, Anthozoa). *Iheringia—Série Zoologia* 80: 27–31.

Acuña F.H. & Zamponi M.O. 1997. The use of cnidocysts for ecological races identification from sea anemones populations (Anthozoa, Actiniidae). *Iheringia—Série Zoologia* 82: 9–18.

Acuña, F.H. & Griffiths, C.L. 2004 Species richness, endemicity and distribution patterns of South African sea anemones (Cnidaria: Actiniaria & Corallimorpharia). *African Zoology* 39 (2): 193-200.

Acuña F.H.; Garese, A.; Excoffon, A.C. & Cortés, J. 2013. New records of sea anemones (Cnidaria: Anthozoa) from Costa Rica. *Revista de Biología Marina y Oceanografía* 48(1): 177–184.

Amaral, F. D.; Hudson, M.M.; da Silveira, F. L.; Migotto, A.E.; Pinto, S.M. & Longo, L. 2000. Cnidarians of Saint Peter and St. Paul Archipelago, Northeast Brazil. In: *International Coral Reef Symposium, 9. Bali: International Coral Reef Society* 1: 567 –572.

Andres, A. 1883. Le attinie. *Atti dell' Accademia de Lincei Memorie* 14: 211–673.

Apakupakul, K.; Siddall; M. E., & Burreson, E. M. 1999. Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. *Molecular Phylogenetics and Evolution* 12: 350–359.

Baker, D. 2003. Quantitative parsimony and explanatory. *British Journal of Philosophy of Science* 54: 245–259.

Barnes, E.C. 2000. Ockham`s razor and the anti-superfluity principles. *Erkenntnis* 53: 353–374.

Belém, M.J. & Preslercravo, J. 1973. Contribuições ao conhecimento da fauna de cnidarios do Espírito Santo, Brasil I – Considerações sóbre Actiniaria do Município de Aracruz, E. S. *Boletim do Museu de Biologia Prof. Mello Leitao* 80: 1–14

Belém M.J.C. 1987. Anatomy and biology of *Bunodosoma caissarum* Corrêa, 1964 (Cnidaria, Anthozoa, Actiniidae). 1. Systematic position and revision of morphology and microanatomy. *Anais da Academia Brasileira de Ciencias* 60, 365–375.

Berntson, E. A.; France, S. C. & Mullineaux, L. S. 1999. Phylogenetic relationships within the class Anthozoa (phylum Cnidaria) based on nuclear 18S rDNA sequences. *Molecular Phylogenetics and Evolution* 13: 417 – 433.

Bosc, L.A.G. 1802. *Historie Naturalle des Vers. Chez Deterville*, Paris, 300 pp.

Carlgren, O. 1895. Jahresbeichte für 1889, 1890, und 1891 über die Anthozoen. *Archiv für Naturgeschichte* 61, 235–298.

Carlgren, O. 1924. On Boloceroides, Bunodeopsis and their supposed allied genera. *Arkiv für Zoologi* 1(17A): 1–20.

Carlgren, O., 1928. Actiniaria der Deutschen Tiefsee-Expedition. *Deutsche liefsee-Expedition 1898-1899* 22 (4): 123-266.

Carlgren, O. 1939. Actiniaria and Zoantharia of the Scottish National Antarctic Expedition, 1902–1904. *Transactions of Royal Society of Edinburgh* 49, 791–800.

Carlgren, O. 1945. Further contributions to the knowledge of the cnidom in the Anthozoa especially. In: Actiniaria. *Kungliga Fysiografiska Sällskapets Handlingar* 56: 1–24.

Carlgren, O. 1949. A Survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskapsakademiens Handlingar* 1(1): 1 – 121.

Carlgren, O. 1951. The actiniarian fauna of the Gulf of California. *Proceedings of the United States National Museum* 101: 415-449.

Carlgren, O. 1952. Actiniaria from North America. *Arkiv für Zoologi* 3(30): 373–390.

Carlgren, O. & Hedgpeth, J.W. 1952. Actiniaria, Zoantharia and Ceriantharia from shallow water in the northwestern Gulf of Mexico. *Publications of the Institute of Marine Science, University of Texas* 2: 143–172.

Cary, L.R. 1906. A contribution to the fauna of the coast of Louisiana. *Gulf Biologic Station Bulletin* 6: 50–59.

Chen, C. A., Wallace, C. C., & Wolstenholme, J. 2002. Analysis of the mitochondrial 12S rRNA gene supports a two-clade hypothesis of the evolutionary history of scleractinian corals. *Molecular Phylogenetics and Evolution* 23: 137–149

Corrêa DD (1964) Corallimorpharia e Actiniaria do Atlântico Oeste Tropical. Universidade de São Paulo, Ph.D Thesis, São Paulo, Brasil.

Dana, J.D., 1846. Zoophytes In: U*nited States Exploring Expedition. During the years 1838, 1839, 1840, 1841, 1842. Under the command of Charles Wilkes, U.S.N.*— Lea and Blanchard, Philadelphia. 740 pp.

Daly, M. 2003. The anatomy, terminology, and homology of acrorhagians pseudoacrorhagi in sea anemones. *Zoologishe Mededelingen* 345: 89 – 102.

Daly, M. 2004. Phylogeny and biogeography of Anthopleura in the North Atlantic Ocean. *Hydrobiologia* 530/531: 241 – 248.

Daly, M.; Fautin, D. G. & Cappola, V. A. 2003. Systematics of the Hexacorallia (Cnidaria: Anthozoa). *Zoological Journal of the Linnean Society* 139: 419 – 437.

Daly, M.; Brugler, M. R.; Cartwright, P.; Collins, A.G.; Dawson, M. N.; Fautin, D.; France, S. C.; McFadden, C.S.; Opresko, D. M.; Rodríguez, E.; Romano, S. & Stake, J. 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. In: Zhang, Z.-Q. & Shear, W.A. (Eds). Linnaeus Tercentenary: Progress in Invertebrate Taxonomy. *Zootaxa* 1668: 127 - 182.

Daly, M.; Chaudhuri, A.; Gusmão, L. & Rodríguez, E. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular Phylogentics and Evolution* 48: 292 – 301.

Daly, M.; Gusmão, L. C.; Reft, A. B.; Rodríguez, E. 2010. Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (Cnidaria, Actiniaria). *Integrative and Comparative Biology* 50 (3): 371 – 388.

Den Hartog, J.C. 1987. A redescription of the sea anemone Bunodosoma biscayensis (Fischer, 1874) (Actiniaria, Actiniidae). Zoologische Mededelingen 61: 533–559.

Dunn, D.F.; Chia, F.S & Levine, R. 1980. Nomenclature of Aulactinia (= Bunodactis), with description of Aulactinia incubans n. sp. (Coelenterata: Actiniaria), an internally brooding sea anemone from Puget Sound. *Canadian Journal of Zoology* 58: 2071-2080.

Duchassaing, P. 1850. *Animaux radiaires des Antilles*. Imprimerie Royale, Turin. 112p.

Duchassaing, P. 1870. *Revue des Zoophytes et des Spongiaires des Antilles*. Chez Victor Mas- son et Fils, Paris, 52 pp.

Duchassaing, P. & Michelotti, G. 1860. *Mémoire sur les Coralliaires des Antilles*. Imprimerie Royale, Turin, 89 pp.

Duchassaing, P. & Michelotti, G. 1864. *Supplément au mémoire sur les Coralliaires des Antilles*. Imprimerie Royale, Turin, 112 pp.

Duerden, J.E. 1897. The actiniarian family Aliciidae. *Annals and Magazine of Natural History* 20: 1–15.

Duerden, J.E. 1898. The Actiniaria around Jamaica. *Journal of the Institute of Jamaica* 2: 449-465.

Duerden, J.E. 1902. Report on the actinians of Porto Rico. *Bulletin of the United States Fish Commission* 20: 321- 374.

Eernisse, D.J. & Kluge, A.G. 1993. Taxonomic congruence versus total evidence, and the phylogeny of amniotes inferred from fossils, molecules and morphology. *Molecular Biology and Evolution* 10: 1170–1195.

Excoffon A.C., Genzano, G.N. & Zamponi M.O. 1999. Macrobenthos associated with a population of Anthothoe chilensis (Lesson, 1830) (Cnidaria, Actiniaria) in Mar del Plata Harbor, Argentina. *Ciencias Marinas* 25: 177–191.

Farris, S.J. 1983. The logical basis of phylogenetic analysis. In: Platnick, N.I. & Funk, V.A. (Eds.), *Advances in cladistics, vol. 2*. Columbia University Press, New York.

Fautin, D. G. 2013. Hexacorallians of the World. <http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm> [Access on May 10, 2016]

Fautin, D.G.; Tan, R.; Yap, N.; Liang, W.; Swee Hee, T; Crowther, A.; Goodwill,R. ; Sanpanich, K. & Chieh, T.Y. 2015. Sea anemones (Cnidaria: Actiniaria) of Singapore: shallow-water species known also from the Indian subcontinent. *Raffles Bulletin of Zoology, Supplement* 31: 44–59.

Fischer, P. 1874. Recherches sur les Actinies des côtes océaniques de France. Novelles *Archives du Muséum d`Historie Naturelle, Paris* 10: 199-244

Fischer, P., 1889. Nouvelle contribution à Tactinologie française. *Actes de la Société Linnéenne de Bordeaux* 43: 251-309.

Gabe, M. 1968. *Technique Histologique. Massou et Cie*, Paris.

Geller, J. B., & Walton, E. D. 2001. Breaking up and getting together: evolution of symbiosis and cloning by fission in sea anemones (genus Anthopleura). *Evolution* 55: 1781–1794

Genzano, G.N.; Acuña F.H.; Excoffon, A.C. & Perez, C.D. 1996. Cnidarios bentónicos de la provincia de Buenos Aires. Listado sistemático, distribución y estrategias de colonización. *Actas Jornadas Pampeanas Ciencias Naturales* 6: 113–121.

Goloboff, P. 1999. Analyzing large data set in reasonable times: solutions for composite optima. *Cladistics* 15: 415–428.

Gomes P.B.; Belém M.J. & Schlenz E. 1998. Distribution, abundance and adaptations of three species of Actiniidae (Cnidaria, Actiniaria) on an intertidal beach rock in Carneiros beach, Pernambuco, Brasil. *Miscellanea Zoologica* 21: 65–72.

Gomes, P.B.; Schama, R. & Solé-Cava. 2012. Molecular and morphological evidence that Phymactis papillosa from Argentina is, in fact, a new species of the genus Bunodosoma (Cnidaria: Actiniidae*). Journal of the Marine Biological Association of the United Kingdom* 92 (5): 895–910.

González-Muñoz, R.E.; Simões, N.; Sánchez-Rodríguez, J.; Rodríguez, E. & Segura-Puertas, L. 2012. First inventory of sea anemones (Cnidaria: Actiniaria) of the Mexican Caribbean. *Zootaxa* 3556: 1–38.

González-Muñoz, R; Simões, N; Tello-Musi, JL & Rodríguez, E. 2013. Sea anemones (Cnidaria, Anthozoa, Actiniaria) from coral reefs in the southern Gulf of Mexico. *ZooKeys* 341: 77–106.

Gosse, P.H. 1855. Description of *Peachia hastate*, a new genus and species of the class Zoophyta; with observations on the family Actiniadae. *Transactions of the Linnean Society (London)* 21: 267–276.

Grant, T.K., Kluge, A.G. 2009. Perspective: parsimony, explanatory power, and dynamic homology. *Systematic Biodiversty* 7: 357–363.

Gusmão, L. C. & Daly, M. 2010. Evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. *Molecular Phylogenetics and Evolution* 56: 868 – 877.

Haddon, A.C. & A.M. Shackleton, 1893. Description of some new species of Actiniaria from Torres Straits. *The Scientific transactions of the Royal Dublin Society* 8: 113-161.

Hartog, J.C. den & Vennam, J. 1993. Some Actiniaria (Cnidaria: Anthozoa) from the west coast of India. *Zoologist Mededelingen* 67 (42): 601-637

Haque, M.M. 1977. Some littoral coelenterates of Bangladesh and Pakistan coasts. *Bangladesh Journal of Zoology* 5: 33–40.

Häussermann, V. 2004. Re-description of Phymactis papillosa (Lesson, 1830) and Phymanthea pluvia (Drayton in Dana, 1846) (Cnidaria:Anthozoa), two common actiniid sea anemones from the south Pacific with a discussion of related genera. *Zoologische Mededelingen* 78: 345-381.

Heestand, E.N. 2009. Phylogeny and evolution of Anthopleura (Cnidaria: Anthozoa: Actiniaria). MSc. Thesis, The Ohio State University, USA. 42pp.

Hennig, W. 1966. *Phylogenetic systematics*. University of Illinois Press, Urbana.

Humason, G.L. 1967. *Animal Tissue Techniques*, 2nd Edition. WH Freeman and Company, San Francisco and London, 569 pp.

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular *Biology and Evolution* 30:772-780.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12), 1647-1649.

Kluge, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Systematic Zoology* 38: 7–25.

Kluge, A.G. 2001. Parsimony with and without scientific justification. *Cladistics*, 17:199-210.

Kluge, A.G. 2004. On total evidence: for the record. *Cladistics*, 20: 205-207.

Kluge, A.G. & Taran, T. 2006. From conviction to anti-superfluity: old and new justifications of parsimony in phylogenetic inference. *Cladistics* 22: 276–288.

Lesson, R.C. 1830. *Voyage autour du monde sur la corvette de S.M. la Coquille pendant les annés 1822–25 par L.I. Duperry* Paris 1828–1830.

Le Sueur, C.A. 1817. Observations on several species of the genus Actinia; illustrated by figures. *Journal of the Academy of Sciences of Philadelphia* 1: 149-154/169-189.

Lewis, J.B. 1960. The fauna of rocky shores of Barbados, West Indies. *Canadian Journal of Zoology* 38: 391-435.

McCommas, S.A. 1991. Relationships within the family Actiniidae (Cnidaria, Actiniaria) based on molecular characters. *Hydrobiologia* 216/217: 509-512.

McCommas, S.A. & Lester, L.J. 1980. Electrophoretic evaluation of the taxonomic status of 2 species of sea-anemone. *Biochemical Systematics & Ecology* 8: 289-292.

McMurrich, J.P. 1889. The Actiniaria of the Bahama Islands. W.I. *Journal of Morphology* 3: 1–80.

Medina, M., Collins, A. G.; Silberman, J. D. & Sogin, M. L. 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *Proceedings of the National Academy of Sciences of the United States of America* 98: 9707–9712.

Milne-Edwards, H. 1857. *Histoire Naturelle des Coralliaires ou Polypes Proprement Dits.* Paris. pp. 221–310.

Monroy-Estrada, H.I.; Segura-Puertas, L.; Galvan-Arzate, S.; Santamaría, A.; Sánchez-Rodríguez, J. 2007. The crude venom from the sea *anemone Stichodactyla helianthus* induces haemolysis and slight peroxidative damage in rat and human erythrocytes. *Toxicology in Vitro* 21: 398–402.

Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.

Oigman-Pszczol, S. S.; Figueiredo, M. A. d. O. & Creed, J. C. 2004. Distribution of Benthic Communities on the Tropical Rocky Subtidal of Armação dos Búzios, Southeastern Brazil. *Marine Ecology* 25: 173–190.

Olivera E.G., Patronelli D.L. & Zamponi M.O. 2009. Morphological and functional study of the marginal sphincter of the sea anemones Phymactis clematis and Aulactinia marplatensis from intertidal of Mar del Plata, Argentina. *Iheringia* 99, 313–316

Östman, C. 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. *Scientia Marina* 64 (Supl. 1): 31 – 46.

Padial, J.M., Grant, T. & Frost, D.R. 2014. Molecular systematics of terraranas (Anura: Brachycephaloidea) with assessment of the effects of alignment and optimality criteria. *Zootaxa* 3825: 1-132.

Patronelli D.; Olivera D. & Zamponi, M.O. (2005) Influence of intertidal environment on muscle activity in different species of sea anemones (Actiniaria). *Animal Biology* 55: 101–109.

Patronelli, D.; Olivera, E.G.; Zamponi, M.O. & Crupkin, M. 2008. Caracterización morfológica, funcional y bioquímica del esfínter marginal de la anémona de mar Phymactis clematis (Cnidaria). *Investigaciones Marinas* 29: 73–77.

Pax, F. 1910a. Diagnosen neues westindischer Actinien. *Zoologischer Anzeiger* 38 (8/9): 176-179.

Pax, F. 1910b. Studien an westindischen Actinien. *Zoologische Jahrbücher* 2: 157–330.

Pax, F. 1922. Diagnosen neuer Actiniarien aus der Ausbeute der Deutschen und franzoischen Sudepolar Expeditionen 1901–1903. *Zoologischen Anzeiger* 54: 74–92.

Pax, F. 1924. Actiniarien, Zoantharien und Ceriantharien von Curaçao. *Kungliga Zoologisch Genootschap Natura Artis Magistra, Amsterdam* 23: 93-122.

Parulekar, A. 1968. Sea anemones (Actiniaria) of Bombay. *Journal of the Bombay Natural History Society* 65: 138- 147.

Patronelli, D.; Zamponi, M.O.; Bustos, A. & Vega, F. 1987. Morphological adaptations in the marginal sphincter of anemone *Phymactis clematis* Dana, 1849 from different environment. *Biochemical Physiology* 88: 337–340.

Presnell, J.K. & Schreibman, M.P. 1997. *Humason's animal tissue techniques*, 5th ed. Johns Hopkins University Press, Baltimore, 572 pp.

Pollero, R.J. 1983. Lipid and fatty acid characterization and metabolism in the sea anemone *Phymactis clematis* (Dana). *Lipids* 18, 12–17.

Reft, A. & Daly, M. 2012. Morphology, distribution, and evolution of apical structure of nematocysts in Hexacorallia. *Journal of Morphology* 273: 121–136.

Rodríguez, E.; López-González, P. J. & Daly, M. 2009. New family of sea anemones (Actiniaria, Acontiaria) from deep polar seas. *Polar Biology* 32: 703 - 717.

Rodríguez, E.; Barbeitos, M.; Daly, M.; Gusmão, L.C.; Hausserman, V. 2012. Towards a natural classification: phylogeny of Acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). *Cladistics* 28: 375 – 392.

Rodríguez, E; Barbeitos, MS;, Brugler, MR; Crowley, LM; Grajales, A; Gusmão, L; Häussermann, V.; Reft, A. & Daly, M. 2014. Hidden among Sea Anemones: The First Comprehensive Phylogenetic Reconstruction of the Order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) Reveals a Novel Group of Hexacorals. *PLoS ONE* 9(5): e96998. doi:10.1371/journal.pone.0096998

Schmidt, H. 1972. Die nesselkapseln der Anthozoen und ihre bedeutung für die phylogenetische systematik. *Helgoländer wissenschaftliche Meeresuntersuchungen* 23: 422 – 458.

Schmidt, H. 1974. On evolution in Anthozoa. *Proceedings of the 2nd International Coral Reef Symposium* 1: 533 – 560.

Slowinski, J.B. 1998. The number of multiple alignments. *Molecular Phylogenetics and Evolution* 10: 264-266.

Stephenson, T. A. 1921. On the classification of Actiniaria. Part II. Consideration of the whole group and its relationships, with special reference to forms not treated in Part I. *Quarterly Journal of Microscopical Sciences* 65: 493 - 576.

Stephenson, T.A., 1922. On the classification of Actiniaria. Part III. - Definitions connected with the forms dealt with in Part II. *Quarterly Journal of Microscopical Sciences* 66: 247-319.

Stephenson, T.A. 1935. *The British Sea Anemones* - volume II. London: The Ray Society. 426 pp.

Varón, A., Vinh, L.S.& Wheeler, W.C. 2010. POY version 4: phylogenetic analysis using dynamic homologies. *Cladistics* 26: 72–85.

Verrill, A.E. 1864. Revision of the Polypi of the eastern coast of United States. *Memoirs of the Boston Society of Natural History* 1: 1–46.

Verrill, A.E. 1869. Review of the polyps of the west coast of America. *Transactions of the Connecticut Academy of Arts and Sciences* 1: 377-567.

Verrill, A.E. 1899. Descriptions of imperfectly known and new actinians, with critical notes on other species, II. *American Journal of Science* 7: 41- 50.

Wheeler, W.C. 1996. Optimization alignment: the end of multiples sequence alignment in phylogenetics? *Cladistics* 12: 1–9.

Wheeler, W.C. 2003. Implied alignment: a synapomorphy-based multiple-sequence alignment method and its use in cladogram search. *Cladistics* 19: 261-268.

Won, J. H.; Rho, B. J. & Song, J-I. 2001. A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. *Coral Reefs* 20: 39 – 50.

Zamponi, M.O. 1977. La anemofauna de Mar del Plata y localidades vecinas. I. Las anemonas Boloceroidaria y Endomyaria (Coelentherata: Actiniaria). *Acta Neotropica* 23: 137–153.

Zamponi, M.O. 1989. Los Cnidaria y su interaccion pelagico-bentonica. Ph.D thesis. Universidad Nacional de Mar del Plata, Mar del Plata, Argentina.

Zamponi, M.O. 1993. El ambiente intermareal subtemplado frio como un posible «pool» de tipos reproductivos. *Physis A* 51: 13–15.

Zamponi, M.O. 2000. El estuario del Rio de La Plata: una barrera geografica para los cnidarios bentonicos marinos? *Biociencias* 8: 127–136.

Zamponi, M.O. 2005. Estudio de la reproducción sexual de las anémonas de mar (Actiniaria) y la estrategia del hombre pobre (the poor man's game). *Revista Real Academia Galega de Ciencias* 24, 5–28.

Zamponi, M.O. & Perez, C.D. 1996. Comparative morphological study of different species of Actiniaria between the intertidal zone from Mar del Plata and Santa Clara del Mar

(Argentine). I. Phymactis papillosa Dana, 1849 (Anthozoa, Actiniidae). *Biociências* 4: 91–102.

Zamponi, M. O; Belém, M. J. C.; Schlenz, E & Acuña, F. H. 1998a. Distribution and some ecological aspects of Corallimorpharia and Actiniaria from shallow waters of the South American Atlantic coasts. *Physis A* 55: 31 - 45.

Zamponi, M.O.; Genzano, G.N.: Acuña, F.H. & Excoffon, A.C. 1998b. Studies of benthic cnidarian populations along a transect off Mar del Plata (Argentina). *Russian Journal of Marine Biology* 24: 7–13.

Capítulo 2

Testing nuclear markers for phylogenetic and phylogeographic studies in *Bunodosoma* Verrill, 1899 (Cnidaria: Actiniaria: Actiniidae) from the south-west Atlantic coast

Abstract

Reliable species identification and population delimitation prior to phylogenetic and phylogeographic studies are very important. In groups with few morphological characters and high morphological plasticity, as in Actiniaria, the use of genetic markers in addition to morphological characters can be extremely helpful. However, low mutation rates of mitochondrial DNA markers have been widely reported in Anthozoa, and result in insufficient divergence levels for species and population discrimination. The usage of fast evolving markers, such as rDNA internal transcribed spacer (ITS), is a potentially useful alternative. Its performance has varied in the few cases in which it has been studied. We collected *Bunodosoma cangicum, B. caissarum* and *B. zamponii* along the Brazilian, Uruguayan and Argentinian coasts and tested the variability of the nuclear markers ITS, asparagine-linked glycosylation 11 homolog (ALG11) and methionine adenosyltransferase (MAT) for these populations. Although ITS has been used to separate species and populations in Actiniaria, its levels of sequence divergence in *Bunodosoma* was not enough to separate populations, although it separated *B. caissarum* from the other two species. ALG and MAT did not present

variability between populations or among the three species. Therefore, neither of these markers is highly effective for studying populations of *Bunodosoma*. Further tests must be conducted using different nuclear markers or alternative techniques, to identify which ones could be useful for phylogeographic and phylogenetic studies in sea anemones.

Key words: ITS, ALG1 1, MAT, species delimitation, populations.

Introduction

Sea anemones (phylum Cnidaria, class Anthozoa, order Actiniaria) are polyps with a very simple morphology and few taxonomically informative features. Nevertheless, they can be very diverse ecologically and found in all marine habitats at all depths and latitudes (Daly *et al.,* 2008; Rodríguez *et al.,* 2014). They are grouped in the order Actiniaria Hertwig, 1882, for which there are approximately 1,200 described species. While different classifications for the order have been proposed (Stephenson, 1921; Carlgren, 1949; Schmidt, 1974, Rodríguez *et al.*, 2014), very few studies covering the relationships in less inclusive groups, as species and populations, have been carried out (*e.g.* Gusmão & Daly, 2010). Reliable species identification and population delimitation prior to phylogenetic and phylogeographic studies are very important, but the simplicity of the sea anemone polyp allied to the relatively high number of species worldwide makes it difficult to establish limits between species based exclusively on morphological characters. In this scenario, molecular information is a tool which has been explored with the goal to solve these problems and the genus *Bunodosoma* Verrill, 1899 (family Actiniidae Rafinesque, 1815) may be an interesting option for the study of limits between species and populations using morphological and molecular data.

The polyps of the species of *Bunodosoma* are in general relatively large (diameter higher than 2cm) and usually conspicuous in the rocky shores where they occur (Chapter 1). The genus currently has 14 valid species (Fautin, 2013; Gomes *et al.,* 2012; Chapter 1), most of them from tropical and subtropical waters. Beneti *et al.* (in prep; Chapter 1) performed a phylogenetic analysis of the genus using two mitochondrial (12S and COIII) and two nuclear markers (18S and 28S rDNA) and found that their variation rates were too low to separate the three species from the west coast of the South Atlantic Ocean: *Bunodosoma caissarum* Corrêa *in* Belém, 1987 and *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973, from Brazil; *Bunodosoma zamponii* Gomes, Schama & Solé-Cava, 2012, from Argentina. The first two species occur in sympatry, however *B. caissarum* can be easily differentiated as it has the double of the number of tentacles and mesenteries of the other two species. On the other hand, *B. cangicum* and *B. zamponii* are allopatric but have very similar morphological features, thus justifying a possible synonymization. Beneti *et al.* (in prep; Chapter 1) suggested that the separation of these three species may be very recent and that molecular markers more appropriate to study populations of these sea anemones must be tested.

Studies using molecular markers in sea anemones are relatively recent, being mostly used in phylogenetic studies including Actiniaria (*e.g.* Bernston *et al.*, 1999; Won *et al*., 2001; Daly *et al.*, 2008; Rodriguez *et al.*, 2012, 2014). *Bunodosoma* is commonly represented in phylogeny proposals that use molecular data (McCommas, 1991; Daly *et al.*, 2008; Rodríguez *et al.,* 2014), but taxonomic sampling is always very restricted. *B. cangicum, B. caissarum,* and *B. zamponii* were included in the analyses of Gomes *et al.* (2012), in which allozyme markers and the ribosomal complete internal transcribed spacer were used to place the former *Phymactis papillosa* from Argentina in the genus *Bunodosoma,* thus proposing the species *B. zamponii*. Dohna & Kochzius (2015) included the sequences of these three species generated by Gomes *et al.* (2012) in their study, but their goal was to perform an analysis of many

groups of sea anemones in search for a barcoding marker for the order. Therefore, sampling was restricted in both cases and population aspects of *Bunodosoma* remain to be comprehended.

The usage of genetic markers in addition to morphological characters has been a strategy used to overcome problems of species definition for groups in which there are few taxonomical characters and great morphological plasticity, and among the Cnidaria, this strategy has been explored, for example, in Zoantharia (*e.g.* Sinniger *et al.*, 2008) and Scyphozoa (*e.g.* Dawson, 2005). The mitochondrial cytochrome subunit I gene (COI) was established as a barcoding marker for eukaryote organisms due to its relatively high mutation rate and has been used in phylogenetic analysis of genus and family level of many groups (*e.g.* Hebert *et al.*, 2003, 2004; Ward *et al.*, 2005; Hajbabaei *et al.*, 2006). However, contrary to patterns seen in other metazoans, there is a low interspecific variability in the mtDNA of cnidarians, and the substitution rates are much slower than in the nuclear DNA (Shearer *et al.*, 2002). Therefore, the highly conserved COI segment is not an appropriate barcoding marker for Cnidaria, especially in Anthozoa (corals, sea anemones, etc) (Flot *et al.*, 2013; Dohna & Kochzius, 2015), although it has been useful in the study of certain scleractinian corals (Keshavmurthy *et al.*, 2013; contrary to Shearer & Coffroth, 2008) and Ceriantharia (Stampar *et al.*, 2014).

Mitochondrial and nuclear markers such as 12S, 16S, COIII, 18S and 28S rDNA have been successfully used in phylogenetic studies of sea anemones (*e.g.* Daly *et al.*, 2008; Gusmão & Daly, 2010; Rodriguez *et al.*, 2012, 2014; Chapter 1) but do not permit resolution between closely related species or populations. The nuclear ribosomal Internal Transcribed Spacer (ITS) is a fast evolving marker that has been incorporated into a phylogenetic framework to infer relationships from populations to families and even higher taxonomic levels in many organisms (*e.g.* Baldwin, 1992; Fritz, *et al.* 1994; Schlötterer *et al.,* 1994; Chu

et al., 2001; Chen *et al.*, 1996; Worheide *et al.*, 2006; Addis & Peterson, 2005; Drake *et al.*, 2007; Miranda *et al.* 2010). The complete ITS region includes the ITS-1, 5.8S and ITS-2 fragments and is situated between the small nuclear ribosomal unit (18S) and the large subunit (28S). It is found in tandem repeats, so polymorphism along the genome is possible, although concerted evolution is assumed to homogenize these units and reduce intragenomic diversity (Li, 1997). The rate of evolutionary change in the 5.8S gene is slow, however ITS-1 and ITS-2 have higher variation. The ITS complete sequence has already been used as a marker to detect inter-specific difference in anthozoans (*e.g.* Beauchamp and Powers, 1996; Chen & Miller, 1996; Odorico & Miller, 1997; Medina *et al.,* 1999; Lam & Morton, 2003; Reimer *et al*., 2007; Sinninger *et al.*, 2008; Acuña *et al.,* 2009; Aguilar & Reimer, 2010), but some studies have reported issues in using this marker, such as insufficient variation in the spacers (*e.g.* Lee & Song, 2000; Calderón *et al.,* 2008; Dohna & Kochzius, 2015), excessive variation preventing unambiguous alignment (Reimer *et al.*, 2007) and high intragenomic variation that can obscure phylogenetic signal (Vollmer & Palumbi, 2004; McFadden *et al*., 2010).

In sea anemones the genetic variability of ITS has been explored a few times (*e.g.* Ting & Geller, 2000; Stoletzki & Schierwater, 2005; Acuña *et al.* 2007; Gusmão, 2010; Gomes *et al.*, 2012) but each study has had different results. Stoletzki and Schierwater (2005) studied population structure in *Condylactis gigantea* and found diversity at all levels (intragenomic, intraspecific and interspecific) with 5.8S and ITS-1, being even able to use these markers to differ color morphs. Acuña *et al.* (2007), on the other hand, studied the complete ITS region and found high interspecific divergence among three species in the genus *Aulactinia* and little intraspecific variation within each species. Similarly, Gusmão (2010) found that this marker was useful to analyze closely related species of sea anemones of *Calliactis*, but not populations. And, finally, Dohna & Kochzius (2015) tested the nuclear ITS-2 as alternative to COI for barcoding in an analysis including many groups of

actiniarians, but very low variation in the marker was observed, and intraspecific variation overlapped with interspecific variation. Therefore, no generalizations can yet be made concerning the usage of ITS as a molecular marker until more studies have been carried out.

In this study we test the feasibility of using the complete internal transcribed spacer (ITS) region of ribosomal DNA as a molecular marker to separate the species *B. caissarum, B. cangicum,* and *B. zamponii*, and also to study the distribution of populations of all three species. In addition, we test the intra and interspecific variability of the nuclear molecular markers asparagine-linked glycosylation 11 homolog (ALG11) (Belinky *et al.*, 2012) and methionine adenosyltransferase (MAT) (Hill *et al.*, 2013), both developed for use in Porifera phylogeny.

Material & Methods

Sampling. Specimens of the three species of South Atlantic *Bunodosoma* were collected in Brazil, Uruguay and Argentina. (Table 2.1; Fig. 2.1). Specimens of *B. capense* were collected in South Africa (Table 2.1) and used for comparisons. All animals were collected at the intertidal zone, by gently scraping them from the substratum (rocks). Photographs were taken *in vivo* and *in situ*. Tissue from the pedal disc and 2-3 tentacles were removed and directly preserved in 100% ethanol. The rest of the polyp was anesthetized with an 8% $MgCl_2$ solution and preserved in 4% formaldehyde solution in seawater (FSW) for taxonomical confirmations and other morphological studies. A code was designated to each specimen, following the pattern "species_collection site_number of specimen". The species names were coded as Bcai (*B. caissarum*), BC (*B. cangicum*), BZ (*B. zamponii*) and Bcap (*B. capense*). Collection site codes are in Table 2.1.

Table 2.1. Number of specimens of *Bunodosoma caissarum*, *B. cangicum*, *B. zamponii* and *B. capense* used in this study from each collection site.

	Locality	Code	Coordinates (S/W)		B. caissarum	B. cangicum	B.zamponii	B. capense
Brazil	Praia do Coqueirinho, Conde (PB)	PB	7° 19' 40"	34° 47' 44"		6		
	Praia da Boa Viagem, Recife (PE)	PE1	8° 05' 33"	34° 52' 49"		6		
	Praia do Muro Alto, Ipojuca (PE)	PE2	8° 25' 35"	34° 58' 36"		5		
	Praia do Francês, Marechal Deodoro (AL)	AL1	9° 46' 13"	35° 50' 19"		2		
	Pontal de Coruripe, Coruripe (AL)	AL2/3	10° 09' 27"	36° 07' 59"		4		
	Barra Grande, Maraú (BA)	BA5	13° 52' 46"	38° 56' 54"		1		
	Praia da Ribeira, Itacaré (BA)	BA3/4	14° 17' 23"	38° 59' 05"		5		
	Praia do Backdoor, Olivença (BA)	BA1/2	14° 56' 09"	39° 00' 52"		4		
	Coqueiral, Aracruz (ES)	ES	19° 56' 42"	40° 08' 45"		6		
	Praia das Conchas, Cabo Frio (RJ)	RJ1	22° 52' 16"	41° 58' 51"	3			
	Prainha, Arraial do Cabo (RJ)	RJ2	22° 57' 42"	42° 01' 14"		6		
	Enseada do Flamengo, Ubatuba (SP)	SP1	23° 25' 02"	45° 03' 22"	3	2		
	Praia do Cabelo Gordo, São Sebastião (SP)	SP2	23° 49' 44"	45° 25' 27"		2		
	Ilha do Bom Abrigo (SP)	SP3	25° 07' 13"	47° 51' 36"	6	6		
	Praia do Farol, Ilha do Mel (PR)	PR	25° 32' 19"	48° 17' 30"	6	6		
	Pedra do Meio, Itapoá (SC)	SC1	26° 04' 11"	48° 36' 20"	4	5		
	Praia dos Ingleses, Florianópolis (SC)	SC2	27° 24' 52"	48° 24' 13"	6	5		
	Praia do Vigia, Garopaba (SC)	SC3	28° 01' 08"	48° 36' 35"	2	6		
Uruguay	Playa de los Pescadores, Punta del Diablo, Rocha	UR7	34° 02' 42"	53° 32' 12"		6		
	Puerto, La Paloma, Rocha	UR4	34° 39' 10"	54° 08' 26"		6		
	José Ignacio, Maldonado	UR2	34° 50' 53"	54° 38' 07"		6		
	Virgen de la Candelaria, Punta del Este, Maldonado	UR1	34° 57' 53"	54° 56' 25"		6		
Argentina	Santa Clara del Mar, Buenos Aires	SC	37° 50' 27"	57° 29' 9"			6	
	Playa del Aquarium, Mar del Plata, Buenos Aires	AQ	38° 05' 26"	57° 32' 31"			2	
	Punta Cantera, Mar del Plata, Buenos Aires	PC	38° 04' 50"	57° 32' 10"			6	
	Kalk Bay, Cape Town, South Africa		34° 07' 44"	18° 26' 55"				3

Fig 2.1. Collection sites of the specimens of *Bunodosoma caissarum*, *B. cangicum* and *B. zamponii* used in this study.

DNA extraction, amplification and sequencing. Total DNA was obtained from samples of tentacles and pedal discs, using the DNAdvance® kit (Agencourt®). We amplified the following molecular markers: complete ITS rDNA fragment, asparagine-linked glycosylation 11 homolog (ALG11) and methionine adenosyltransferase (MAT). Standard PCR techniques were applied to amplify the complete ITS rDNA fragment (ITS-1, 5.8S and ITS-2) using primers CAS 18S F1 (Ieji *et al.*, 2003) and CaS28sB1d (Kim & Lee, 2008) under the following conditions: 94°C for 5 min; 35 cycles at 94°C for 30s, 55°C for 40s and 72°C for 1

min; and extension at 72°C for 10 min. ALG11 (Belinky *et al.*, 2012) was amplified using the primers ALG1R1 + ALG1D2 (Tm = 55oC) and the product was reamplified with primers ALG1R2 + ALG1D2 (Tm = 58oC) as indicated by the authors. MAT was amplified according to the instructions in Hill *et al.* (2013). All PCR products were purified with AMPure® kit (Agencourt®) following manufacturer's instructions, and prepared for sequencing using the BigDye® Terminator v3.1 kit (Applied Biosystems; same primers and Tm temperature conditions as in PCR reactions). Sequencing was carried out on an ABI PRISM®3100 genetic analyzer (Hitachi). Sequences were assembled and manually inspected (checking ambiguous base calls and removing primer sequences) using Geneious™ 9.0.5 (Kearse *et al.*, 2012). Heterozygous sites were coded according to IUPAC ambiguity codes. Boundaries between ITS-1, 5.8S and ITS-2 in the complete ITS sequences were obtained by comparison to the sequence of *Bunodosoma granuliferum* (Genbank Accesion number JN118564).

Data analysis. Sequences were aligned using MAFFT v.7 (Katoh & Standley, 2013) in default parameters. A sequence of *B. granuliferum* from Genbank (accession number JN118564) was included in the analysis as outgroup. The haplotype reconstruction for the sequences with heterozygous sites was conducted in DnaSP v5.10. (Librado & Rozas, 2009) using algorithms provided by PHASE (Stephens *et al.*, 2001; Stephens & Donnelly, 2003) with a minimum posterior probability of 0.8. Base composition and genetic distance based on Kimura 2-parameter (K2P) were generated using MEGA 6.06 (Tamura *et al.*, 2013). Number of polymorphic sites and haplotype diversity were calculated in DnaSP v5.10. Median-joining haplotype networks were obtained using the software NETWORK 5.0.0.0. (Bandelt *et al.*, 1999).

A phylogenetic analysis was conducted under the parsimony optimality criteria. Parsimony was historically advocated by many researches (Hennig, 1966; Farris, 1983;

Kluge, 2001) and its epistemological justification resides on the anti-superfluity principle (Barnes, 2000; Baker, 2003; Kluge & Grant, 2006; Grant & Kluge, 2009), in which optimal hypothesis are those that require the smallest number of causal explanations. Nucleotide homology was accessed simultaneously with the tree search under the direct optimization of POY 5.1.1. (Varón *et al.*, 2010). Such approach provides an objective criterion to choose among the multiple alignments hypothesis (Wheeler, 1996; Kluge and Grant, 2009; Padial *et al.*, 2014; see also Slowinsk, 1998). Searches were performed using the command "Search", which implements a driven search building Wagner trees using random addition sequences (RAS), Tree Bisection and Reconnection (TBR) branch swapping followed by Ratchet (Nixon, 1999), and Tree Fusion (Goloboff, 1999). This command stores the shortest tree of each independent run and does final tree fusing using the pooled trees as a source of topological diversity. The resulting topologies were submitted to a final round of TBR using iterative pass optimization (Wheeler, 2003). The resulting tree was rooted in *Bunodosoma capense*.

Results

ALG11 and MAT. A test to assess the variability in the ALG11 marker was performed using 15 specimens of *B. caissarum* and *B. cangicum*. We obtained sequences with lengths ranging from 323 to 498 bp, but no difference was observed between species nor within each one. Also, we were not able to successfully amplify and obtain sequences for the molecular marker MAT. Therefore, we did not continue to explore these molecular markers.

ITS. One hundred and forty eight complete ITS sequences were obtained with lengths varying from 348 to 744bp (Table 2.2). When present, all ambiguous sites were very explicit in the chromatograms, as both F and R sequences had high peaks of two nucleotides in the same

position. All ambiguous sites were only one base pair long. Haplotype reconstruction resulted in 294 sequences. We determined the boundaries between ITS-1, 5,8S and ITS-2 comparing the obtained sequences to *B. granuliferum* JN118564, which indicated that the amplified region contained some base pairs of 18S. We chose to keep this region, as it was sufficiently conserved (all sequences identical, except for one sequence with one different nucleotide) and permitted unambiguous alignment. One sequence of *B. caissarum* (RJ1_6) was shorter than the rest (348 bp) and was removed from the analysis of haplotype frequencies, but maintained in the other analysis. After the alignment in MAFFT, the ends of the sequences were removed, thus resulting in lengths up to 523bp (18S: 27bp; ITS-1: 192 ungapped bp + 14 gaps; 5.8S: 157bp; ITS-2: 121 ungapped bp + 11 gaps) for the haplotype frequencies analysis and 607bp (ITS-2: 201 ungapped bp + 16 gaps) for the other analysis. The G+C content of the complete ITS region varies from 52.4% to 55.2%. Length variations, G+C content and sample sizes for each species are summarized in Table 2.3. The nucleotide composition was also very similar for all species (Table 2.4).

Table 2.2: Length variations, G+C content and sample sizes (N) for each species.

Species	**Complete ITS sequence length (bp)**	**G+C content (%)**	**N**
B. caissarum	348 (498) – 734	53.1 - 55.2	34
B. cangicum	553 – 733	52.4 - 54.7	97
B. zamponii	661 – 733	52.7 - 54.4	14
B. capense	703 – 718	53.5 - 54.3	3

Table 2.3: Nucleotide composition for each species and average of all samples.

	Base composition			
Species	**T**	**C**	**A**	**G**
B. caissarum	21.807	27.721	22.740	27.733
B. cangicum	22.098	27.408	22.704	27.791
B. zamponii	22.037	27.411	22.657	27.895
B. capense	23.457	25.982	21.773	28.788
All species	22.053	27.451	22.681	27.815

Haplotype diversity (H_d) was 0.7836 for the dataset (*B. caissarum* + *B. cangicum* + *B. zamponii* + *B. capense*) and 28 haplotypes were found. No intraspecific variation based on K2P distance was found within *B. caissarum* and *B. zamponii*, although 7 and 2 different haplotypes were found for each species, respectively. K2P distance between *B. zamponii* and *B. cangicum* was the lowest among all the inter and intraspecific distances (0.000218) and both haplotypes of *B. zamponii* are shared with *B. cangicum* (Fig. 2.2), so we chose to analyze them as a complex (*B. cangicum* + *B.zamponii*), as they also share morphological and ecological aspects (Chapter 1). For the complex *B. cangicum* + *B. zamponii,* 20 haplotypes were found for the complete ITS sequence. The ITS-1 segment alone had 17 haplotypes, 14 polymorphic sites and the highest K2P distance, while the 5.8S region was the least variable (Table 2.6). The two most widespread haplotypes were present in samples from all the collection sites of *B. cangicum* and *B. zamponii*.

The haplotype network of the complete ITS sequences showed that *B. capense* is separated from the three South American *Bunodosoma* by several mutational steps. *B. caissarum* and the other two species are more related, with few mutational steps separating them (Fig. 2.2).

Table 2.4: Kimura 2-parameter genetic distance within each species and between species for the complete ITS sequence.

K2P distance	Intraspecific	Interspecific				
Species		*B. caissarum*	*B. cangicum*	*B. zamponii*	*B. cangicum + B. zamponii*	*B. capense*
B. caissarum	0.000000	-				
B. cangicum	0.000434	0.003240	-			
B. zamponii	0.000000	0.003021	0.000218	-		
B. cangicum + B. zamponii	0.000380	0.003213	-	-	-	
B. capense	0.004040	0.067264	0.070844	0.070605	0.070813	-
B. granuliferum	-	0.027618	0.030989	0.030762	0.030961	0.077129

Table 2.5: Number of polymorphic sites, K2P distance, number of haplotypes and haplotype diversity (Hd) for each fragment of the ITS marker in the complex *B. cangicum + B. zamponii*.

ITS Fragment	Polymorphic sites (S)	K2P distance	Haplotypes	Hd
ITS -1	14	0.001	17	0.647
5,8 S	1	-	2	0.009
ITS -2	2	-	3	0.018

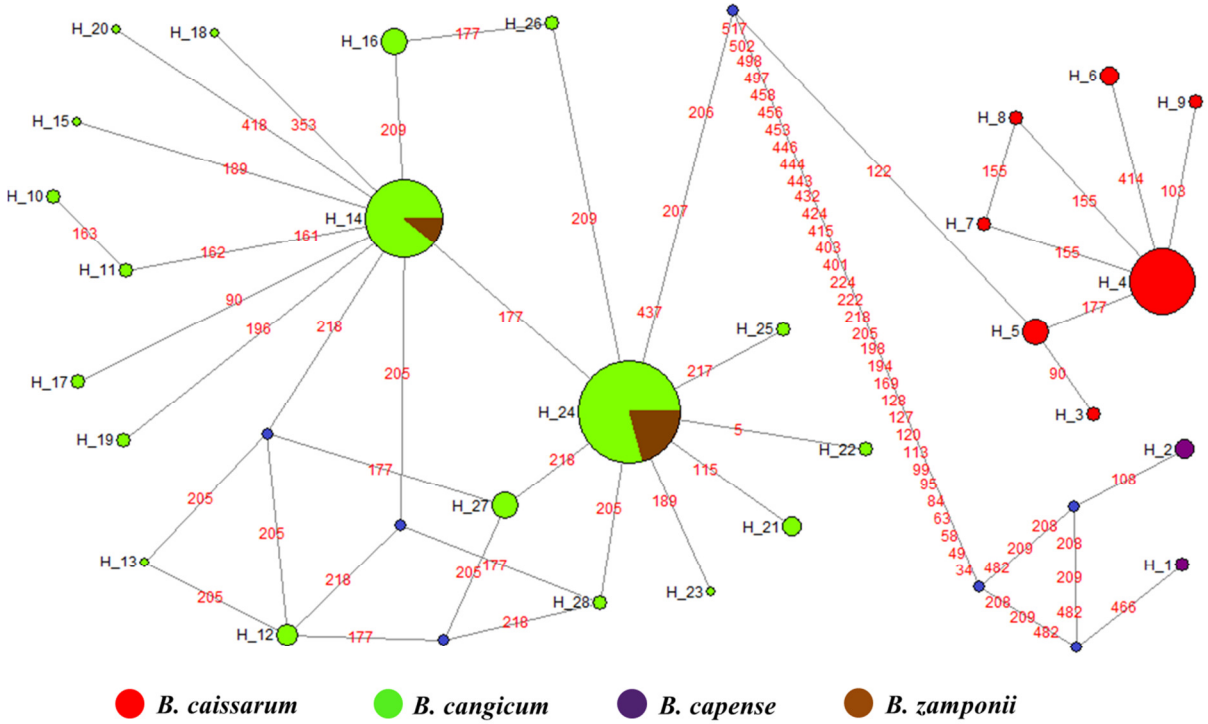

Figure 2.2. Median-Joining network analysis of the relationships among haplotypes of complete ITS sequence of nuclear DNA from 148 individuals of *Bunodosoma*. Circle area is proportional to haplotype frequency. Lines drawn between haplotypes represent mutation events identified by the numbers corresponding to the positions in the alignment where the mutations were observed. Blue circles indicate inferred haplotypes.

Of the 607 positions in the alignment of the complete ITS dataset, 85 were variable among taxa and 60 of these sites were informative for parsimony analysis. The search yielded 45 most parsimonious trees of 154 steps in 1307 generations. The shortest tree was found 58 times. The topology of the consensus tree is presented in Figures 2.3, 2.4 and 2.5.

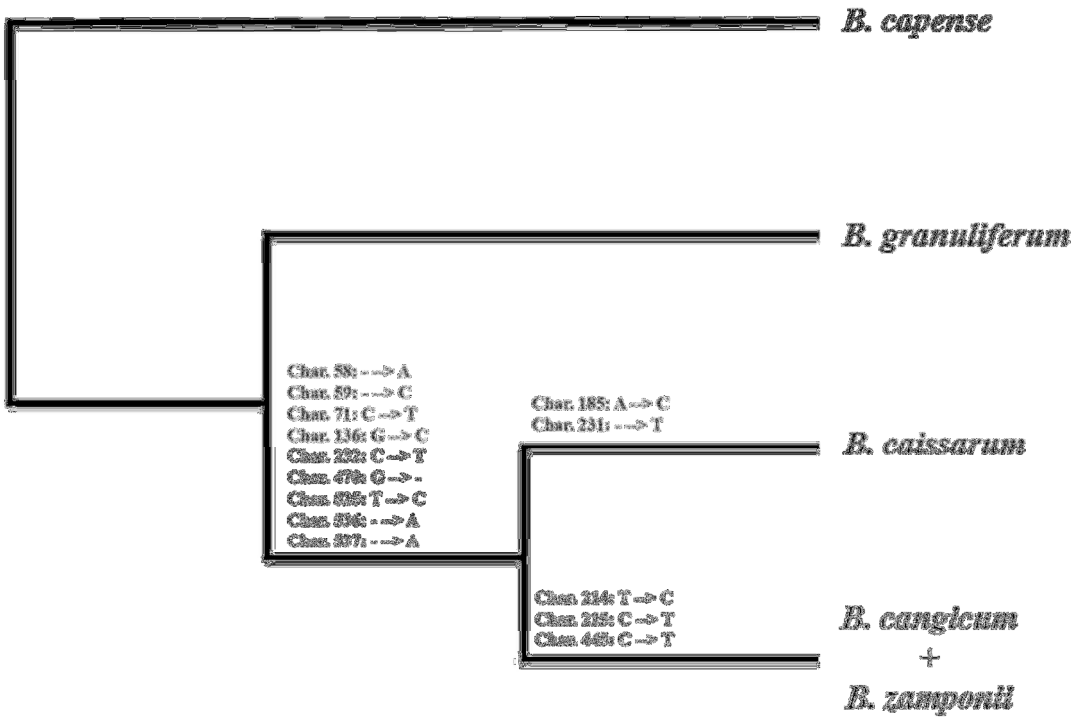

Figure 2.3: Simplified cladogram of the relationships of the species based on the complete ITS nuclear marker. Transformations (synapomorphies) supporting the clades (*B. granuliferum* (*B. caissarum* + *B. cangicum/B. zamponii*)) and (*B. caissarum* + *B. cangicum/B. zamponii*) are shown.

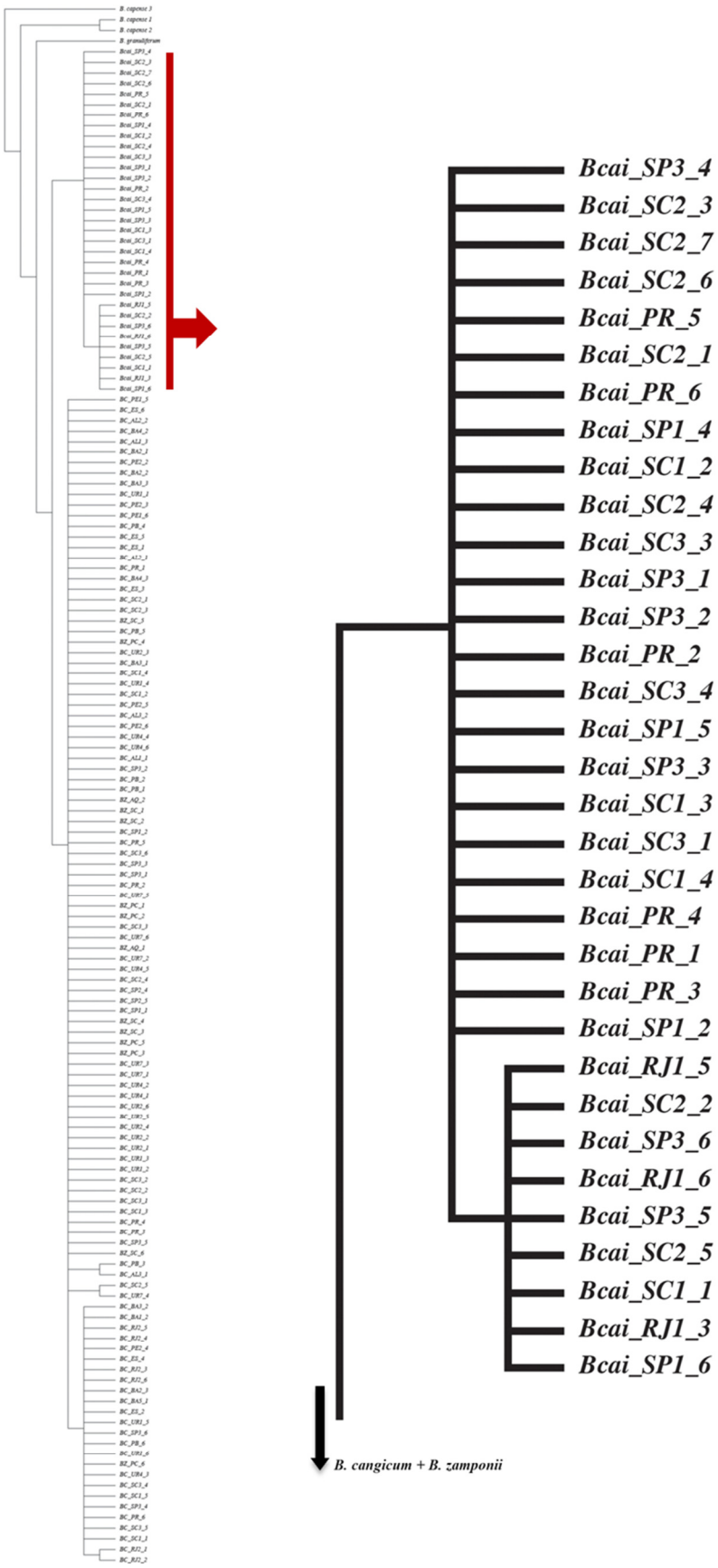

Figure 2.4. Complete cladogram of the relationships of the species based on the complete ITS nuclear marker, showing in detail the *Bunodosoma caissarum* clade. Bcai = *B. caissarum*; collection site codes follow Table 2.1.

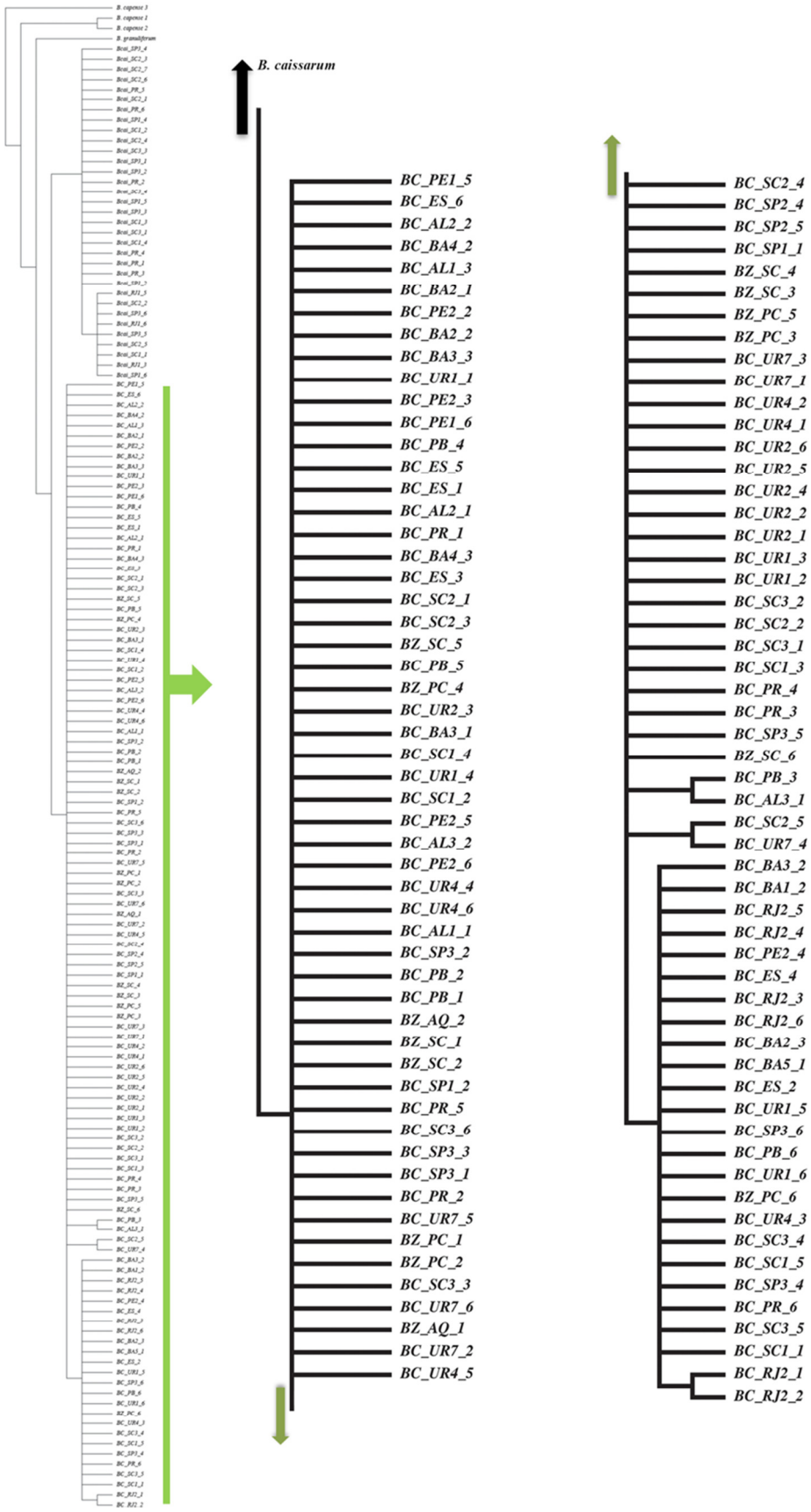

Figure 2.5. Complete cladogram of the relationships of the species based on the complete ITS nuclear marker, showing in detail the *Bunodosoma cangicum* + *B. zamponii* clade. BC = *B. cangicum;* BZ = *B. zamponii*; collection site codes follow Table 2.1.

Discussion

The protocols outlined in Hill *et al.* (2013) for the usage of the nuclear molecular marker MAT did not work for the specimens in this study. And, although we were able to obtain sequences for the marker ALG11, the lack of diversity makes them unsuitable for the study we intend to perform. Therefore, our results are all based on the analysis of the complete ITS sequence.

The difference among the sequences of complete ITS of all species was very low. The conserved 18S flanking region and 5.8S were nearly identical among the four species of *Bunodosoma*, so they did not influence the results. In this analysis, ITS-1 was the fragment with larger diversity, contrary to what was found in Gusmão (2010), who also used the complete ITS sequence to study species and population differences in sea anemones. This may be due to the fact that we were not able to successfully sequence the complete ITS-2 3'end for most samples, so it was excluded from the analysis after the alignment. ITS-2 herein considered has only 121 bp for most specimens, while Gusmão (2010) had 186-222 bp. Nevertheless, the analysis of Stoletzki & Schierwater (2005) lacked the entire ITS-2 and still found intragenomic, intraspecific and interspecific diversity for the sea anemones, meaning that ITS-1 may bear enough divergences to separate less inclusive groups.

The K2P distances, haplotype network and phylogenetic analysis showed that *B. caissarum* is in fact different from *B. cangicum* and *B. zamponii.* This difference is of 4 mutational steps in the haplotype network, while in the phylogenetic analysis *B. caissarum* is supported by two events (synapomorphies): a substitution and an insertion. The other two species, on the other hand, besides having very similar morphologies (Chapter 1), share haplotypes and form a clade supported by 3 substitution events (transitions between pyrimidines). The two most widespread haplotypes of *B. cangicum* are shared with *B.*

zamponii and are present in all the collection sites of both species; therefore it is not possible to discuss any kind of population structure based on this dataset. We did not explore intragenomic variation as did Stoletzki & Schierwater (2005) and Gusmão (2010), and this may be an interesting option to understand population aspects of these species.

Bunodosoma cangicum and *B. zamponii* have very similar morphological and ecological features, although the latter is usually smaller than the former (Gomes, 2002; Gomes *et al.*, 2012; Chapter 1). *B. cangicum* occurs in sympatry with *B. caissarum*, but they tend to occupy different parts of the rocks of the intertidal zone: *B. caissarum* is usually at the lower intertidal zone, whereas *B. cangicum* is at the upper intertidal zone. When *B. caissarum* is present, *B. cangicum* individuals are scarce. However, when the first species is not present, the latter is usually very abundant (more than 5 specimens per/m2, personal observations, Chapter 1). *B. zamponii* is also the most abundant species where it occurs (Zamponi *et al*., 1998b). Gomes (2002) and Gomes *et al.* (2012) suggest that its similarity with *B. cangicum* may be due to a very recent speciation process.

The distributional areas of occurrence of *B. cangicum* and *B. zamponii* are divided by the Rio de la Plata Estuary, which was pointed out by Gomes (2002) and Gomes *et al.* (2012) as the probable vicariant event that generated the species' divergence and consequent speciation. In this sense, the larger discharge of freshwater into the ocean in this area would be a barrier to marine species, impeding or reducing gene flux between species on each side of the barrier. The question of this estuary being a biogeographical barrier has been raised a few times and some authors think that it may be a barrier to dispersion in sea anemones (*e.g.* Zamponi *et al.*, 1998ab; Zamponi, 2000), while other studies have demonstrated it is not (*e.g.* Deserti *et al.*, 2012). The tolerance of the *Bunodosoma* larvae to different salinities or temperature flows has not been tested, so we cannot affirm if it would be able to cross a freshwater barrier. However, although not common, *B. caissarum* has been seen in the

Paranaguá Bay (personal observations) fixed on artificial substrates, which is one of the largest estuarine systems of the Southwestern Atlantic (Marone *et al.*, 2005), proving that polyps from this genus may cross low salinity systems if an appropriate substrate is encountered.

Also, *B. cangicum* has a very large distribution compared to most species of sea anemones (Chapter 1) and it is possible that the Rio de la Plata Estuary is not a barrier for the dispersion of this species, as there seems to be large gene flux between the populations from Brazil and Argentina. Although it usually occurs in rocky shores exposed to open-sea conditions, it may also be found in estuary areas (*e.g.* Salinópolis, PA, Brazil; Chapter 3). Belém (1987) studied the reproductive biology of *B. caissarum* and found that its larvae have a large capacity of dispersion (pelagic-planktotrophic larvae). The larvae of *B. zamponii* is the same as *B. caissarum*, and can live up to 5 days in the plankton (Excoffon & Zamponi, 1991; Gomes, 2002), what makes its capacity of dispersion larger. There are no studies of the larvae of *B. cangicum,* but we may assume that it is capable of considerable dispersion if we take into account the large distribution of its haplotypes. In addition, there are records of the species *B. caissarum* and *B. cangicum* in the Archipelagos of Fernando de Noronha and São Pedro e São Paulo (Amaral *et al*., 2000), which are, respectively, around 550 and 1,000 km off the Brazilian coast, but we did not have access to any material from theses localities. If a more appropriate molecular marker is found for phylogeographical studies, it would be very interesting to include specimens from these localities.

Actiniaria is a challenging group, and taxonomical, phylogenetic and phylogeographical studies would highly benefit from the finding of molecular markers capable of delimiting species and populations. Up to now, techniques such as isozymes and allozyme electrophoretic analyses (*e.g*., Solé-Cava & Thorpe, 1992; Thorpe & Solé-Cava, 1994; Monteiro *et al*., 1997; Gomes *et al,*. 2012) and Amplified Fragment Length

Polymorphisms (AFLP) (Douek *et al*. 2002; Ting & Weller 2000; Darling *et al*. 2004) have been used to carry out population and species-level studies. Reitzel *et al*. (2013) had a successful result in using restriction-site-associated DNA sequencing (RAD-seq), a next-generation sequencing technique, to study populations of a sea anemone. The molecular markers that were tested here were proven to not be completely useful, so, instead of using traditional markers, NGS methods may be the way to obtain highly-resolved species delimitation and phylogeography in *Bunodosoma*. Further tests must be conducted using different nuclear markers or alternative techniques, so that a useful marker for phylogeographic and phylogenetic studies in sea anemones can be found.

References

Acuña, F.H.; Excoffon, A.C.; McKinstry, S.R. & Martínez, D.E., 2007. Characterization of *Aulactinia* (Actiniaria: Actiniidae) species from Mar del Plata (Argentina) using morphological and molecular data. *Hydrobiologia* 592: 249–256.

Addis, J.S. & Peterson, K.J., 2005. Phylogenetic relationships of freshwater sponges (Porifera, Spongillina) inferred from analyses of 18S rDNA, COI mtDNA, and ITS2 rDNA sequences. *Zoolica Scripta* 34: 549–557.

Aguilar, C. & Reimer, J.D., 2010. Molecular phylogenetic hypotheses of *Zoanthus* species (Anthozoa: Hexacorallia) using RNA secondary structure of the internal transcribed spacer 2 (ITS2). *Marine Biodversity* 40: 195–204.

Amaral, F. D.; Hudson, M.M.; da Silveira, F. L.; Migotto, A.E.; Pinto, S.M. & Longo, L. 2000. Cnidarians of Saint Peter and St. Paul Archipelago, Northeast Brazil. In: International Coral Reef Symposium, 9. Bali: International Coral Reef Society. 1: 567 –572.

Baker, D. 2003. Quantitative parsimony and explanatory. *British Journal of Philosophy of Science* 54: 245–259.

Bandelt, H. J.; Forster, P. & Röhl, A. 1999. Median–joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48.

Barnes, E.C. 2000. Ockham`s razor and the anti–superfluity principles. *Erkenntnis* 53: 353–374.

Beauchamp, K.A. & Powers, D.A., 1996. Sequence variation of the first internal transcribed spacer (ITS–1) of ribosomal DNA in ahermatypic corals from California. *Molecular Marine Biology and Biotechnology* 5: 357–362.

Belém, MJC. 1987. Aspectos da biologia de Bunodosoma caissarum Corrêa, 1964 (Cnidaria, Anthozoa, Actiniidae) do litoral do Rio de Janeiro, com ênfase na estimativa de seu comportamento reprodutivo. PhD Thesis, Universidade de São Paulo, São Paulo, 322 pp.

Berntson, E. A.; France, S. C. & Mullineaux, L. S. 1999. Phylogenetic relationships within the class Anthozoa (phylum Cnidaria) based on nuclear 18S rDNA sequences. *Molecular Phylogenetics and Evolution* 13: 417 – 433.

Calderón, I.; Garrabou, J. & Aurelle, D., 2006. Evaluation of the utility of COI and ITS markers as tools for population genetic studies of temperate gorgonians. *Journal of Experimental Marine Biology and Ecology* 336: 184–197.

Carlgren, O. 1949. A Survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskapsakademiens Handlingar* 1(1): 1 – 121.

Chen, C.A.; Chang, C.C.; Wei, N.V.; Chen, C.H.; Lein, Y.T.; Lin, H.E.; Dai, C.F. & Wallace, C.C. 2004. Secondary structure and phylogenetic utility of the ribosomal internal transcribed spacer 2 (ITS2) in scleractinian corals. *Zoological Studies* 43: 759–771.

Chen, C.A. & Miller, D.J. 1996. Analysis of ribosomal ITS1 sequences indicates a deep divergence between *Rhodactis* (Cnidaria: Anthozoa: Corallimorpharia) species from the Caribbean an the Indo–Pacific/Red Sea. *Marine Biology* 126: 423–432.

Chen, C. A.; Willis, B. L. & Miller, D. J., 1996. Systematic relationships of the tropical corallimorpharians (Cnidaria: Anthozoa): utility of the 5.8s and internal transcribed spac– ers (ITS) of ribosmal DNA units. *Bulletin of Marine Science* 59: 269–281.

Chu, K.H.; Li, C.P. & Ho, H.Y., 2001. The first internal transcribed spacer (ITS–1) of ribosomal DNA as a molecular marker for phylogenetic and population analyses in Crustacea. *Marine Biotechnology* 3: 355–361.

Daly, M.; Chaudhuri, A.; Gusmão, L. & Rodríguez, E. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular Phylogentics and Evolution* 48: 292–301.

Daly, M.; Gusmão, L. C.; Reft, A. B. & Rodríguez, E. 2010. Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (Cnidaria, Actiniaria). *Integrative and Comparative Biology* 50 (3): 371–388.

Darling, J.A.; Reitzel, A.M. & Finnerty, J.R., 2004. Regional population structure of a widely introduced estuarine invertebrate: Nematostella vectensis Stephenson in New England. *Molecular Ecology* 13, 2969–2981.

Dawson, M. N. 2005. Renaissance taxonomy: integrative evolutionary analyses in the classification of Scyphozoa. *Journal of the Marine Biological Association of the United Kingdom* 85: 733–739.

Deserti, M.I.; Zamponi, M.O. & Riestra, G. 2012. Las anémonas de mar (Cnidaria; Anthozoa; Actiniaria) de la plataforma continental uruguaya. *Revista Real Academia Galega de Ciencias* XXXI: 115–136.

Douek, J.; Barki, Y.; Gateño, D. & Rinkevich, B. 2002. Possible cryptic speciation within the sea anemone Actinia equina complex detected byAFLP markers. *Zoological Journal of the Linnean Society London* 136: 315–320.

Dohna, T.A. & Kochzius, M. 2015. Obstacles to molecular species identification in sea anemones (Hexacorallia: Actiniaria) with COI, a COI intron, and ITS II. *Marine Biodiversity* 46(1): 291–297.

Drake, C.A., McCarthy, D.A., & von Dohlen, C.D. 2007. Molecular relationships and species divergence among *Phragmatopoma* spp. (Polychaeta: Sabellaridae) in the Americas. *Marine Biology* 150: 345–358.

Excoffon A.C. & Zamponi M.O. 1991. La biologia reproductiva de *Phymactis papillosa* Dana, 1849 (Actiniaria: Actiniidae); gametogenesis, periodos reproductivos, desarrollo embrionario y larval. *Spheniscus* 9: 25–39.

Farris, S.J. 1983. *The logical basis of phylogenetic analysis*. In: Platnick, N.I. & Funk, V.A. (Eds.), Advances in cladistics, vol. 2. Columbia University Press, New York.

Fautin, D.G. 2013. Hexacorallians of the World. Avaliable at: <http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm> [Accessed in May, 25th 2016]

Flot J–F.; Dahl M. & André C. 2013. *Lophelia pertusa* corals from the Ionian and Barents seas share identical nuclear ITS2 and near–identical mitochondrial genome sequences. *BMC Res Notes* 6:144.

Fritz, G.N.; Conn, J.; Cockburn, A. & Seawright, J., 1994. Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of Anopheles nuneztovari (Diptera: Culicidae). *Molecular Biology and Evolution* 3: 406–416.

Goloboff, P. 1999. Analyzing large data set in reasonable times: solutions for composite optima. *Cladistics* 15: 415–428.

Gomes, P.B.; Schama, R. & Solé–Cava. 2012. Molecular and morphological evidence that *Phymactis papillosa* from Argentina is, in fact, a new species of the genus *Bunodosoma* (Cnidaria: Actiniidae). *Journal of the Marine Biological Association of the United Kingdom* 92 (5): 895–910.

Grant, T.K., Kluge, A.G. 2009. Perspective: parsimony, explanatory power, and dynamic homology. *Systematic Biodiversty* 7: 357–363.

Gusmão, L. C. & Daly, M. 2010. Evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. *Molecular Phylogenetics and Evolution* 56: 868 – 877.

Hajbabaei M.; Janzen, D.H.; Burns, J.M.; Hallwachs, W. & Hebert, P.D.N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences, U S A* 103: 968–971

Hebert P.D.N.; Ratnasingham, S. & de Waard, J.R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B: Biological Sciences* 270: S96–S99

Hebert P.D.N.; Penton, E.H.; Burns, J.M.; Janzen, D.H.; & Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences, U S A* 101:14812–14817

Hennig, W. 1966. *Phylogenetic systematics*. University of Illinois Press, Urbana.

Hill, M.S.; Hill, A.L.; Lopez, J; Peterson, K.J; Pomponi, S.; Diaz, M.C; Thacker, R.W; Adamska, M.; Boury–Esnault, N.; Cárdenas, P.; Chaves–Fonnegra, A.; Danka, E.; Laine, D.; Formica, D.; Hajdu, E.; Lobo–Hajdu, G.; Klontz, S.; Morrow, C.C.; Patel, J.; Picton, B.; Pisani, D.; Pohlmann, D.; Redmond, N.E.; Reed, J; Richey, S.; Riesgo, A.; Rubin, E.; Russell, Z.; Rützler, K..; Sperling, E.A.; di Stefano, M.; Tarver, J.E. & Collins, A.G. 2013. Reconstruction of Family–Level Phylogenetic Relationships within Demospongiae (Porifera) Using Nuclear Encoded Housekeeping Genes. *PLoS ONE* 8(1): e50437. doi:10.1371/journal.pone.0050437

Ieji, Y. J.; D. X. Zhang & L Junhe. 2003. Evolutionary conservation and versatility of a new set of primers for amplifying the ribosomal internal transcribed spacer regions in insects and other invertebrates. *Molecular Ecology Notes* 3: 581–585.

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30:772–780.

Kearse, M.; Moir, R.; Wilson, A.; Stones–Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Mentjies, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647–1649.

Keshavmurthy, S.; Yang, S.; Alamaru, A.; Chuang, Y.; Pichon, M.; Obura, D.; Fontana, S.; de Palmas, S.; Stefani, F.; Benzoni, F.; MacDonald, A.; Noreen, A.M.E.; Chen, C.; Wallace, C.C.; Pillay, R.M.; Denis, V.; Amri, A.Y.; Reimer, J.D.; Mezaki, T.; Sheppard, C.; Loya, Y.; Abelson, A.; Mohammed, M.S.; Baker, A.C.; Mostafavi, P.G.; Suharsono, B.A. & Chen, C.A. 2013. DNA barcoding reveals the coral laboratory–rat, *Stylophora pistillata* encompasses multiple identities. *Scientific Reports – Nature* 3:1520.

Kim H., Lee S., 2008. Molecular systematics of the genus Megoura (Hemiptera: Aphididae) using mitochondrial and nuclear DNA sequences. *Molecules and Cells* 25: 510–522.

Kluge, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Systematic Zoology* 38: 7–25.

Kluge, A.G. 2001. Parsimony with and without scientific justification. *Cladistics* 17: 199–210.

Kluge, A.G. 2004. On total evidence: for the record. *Cladistics* 20: 205–207.

Kluge, A.G. & Taran, T. 2006. From conviction to anti–superfluity: old and new justifications of parsimony in phylogenetic inference. *Cladistics* 22: 276–288.

Lam, K. & Morton, B. 2003. Morphological and ITS1, 5.8S and partial ITS2 ribosomal DNA sequence distinctions between two species *Platygyra* (Cnidaria, Scleractinia) from Hong Kong. *Marine Biotechnology* 5: 555–567.

Lee, Y–J. & Song, J–I., 2000. Phylogenetic analysis of the genus *Dendronephthya* (Nephtheidae, Alcyonacea) based on internal transcribed ribosomal spacer sequences of nuclear rDNA. *Korean Journal Biological Sciences* 4: 319–324.

Li, W.H., 1997. *Molecular Evolution*. Sunderland, MA: Sinauer Associates. 432p.

Librado P, & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 1451–1452. doi:10.1093/bioinformatics/btp187

Marone, E; Machado, E.C.; Lopes, R.M. & Silva, E.T 2005. Land–ocean fluxes in the Paranaguá Bay estuarine system, southern Brazil. *Brazilian Journal of Oceanography* 53 (3–4): 169–181.

McCommas, S.A. 1991. Relationships within the family Actiniidae (Cnidaria, Actiniaria) based on molecular characters. *Hydrobiologia* 216/217: 509–512.

McFadden, S.C.; Sánchez, J.A. & France, S.C., 2010. Molecular phylogenetic insights into the evolution of Octocorallia: a review. *Integrative and Comparative Biology* 50(3): 389–410.

Medina, M.; Weil, E. & Szmant, A.M., 1999. Examination of the *Montastrea annularis* specie complex (Cnidaria: Scleractinia) using ITS and COI sequences. *Marine Biotechnology* 1: 89–97.

Miranda, L.S.; Collins, A.G. & Marques, A.C., 2010. Molecules clarify a cnidarian life cycle of the "Hydrozoan" *Microhydrula limopsicola* is an early life stage of the staurozoan Haliclystus antarcticus. *PLOS ONE* 5, 1–9.

Monteiro, F.A.; Solé–Cava, A.M. & Thorpe, J.P. 1997. Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands. *Marine Biology* 129: 425–433.

Odorico, D.M. & Miller, D.J. 1997. Variation in the ribosomal internal transcribed spacers and 5.8S rDNA among five species of *Acropora* (Cnidaria; Scleractinia): patterns of variation consistent with reticulate evolution. *Molecular Biology and Evolution* 14: 465–473.

Padial, J.M.; Grant, T. & Frost, D.R. 2014. Molecular sytematics of terraranas (Anura: Brachycephaloidea) with an assesment of the effects of alignment an optimality criteria. *Zootaxa* 3825(1): 1–132.

Reimer, J.D.; Takishita, K.; Ono, S. & Maruyama, T., 2007. Diversity and evolution in the zoanthid genus *Palythoa* (Cnidaria: Hexacorallia) based on nuclear ITS–rDNA. *Coral Reefs* 26: 399–410.

Reitzel, AM; Herrera, S.; Layden, M.J.; Martindale, M. Q. & Shank, T. M. 2013. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. *Molecular Ecology* 22 (11): 2953–2970.

Rodríguez, E.; Barbeitos, M.; Daly, M.; Gusmão, L.C. & Hausserman, V. 2012. Towards a natural classification: phylogeny of Acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). *Cladistics* 28: 375 – 392.

Rodríguez, E; Barbeitos, MS;, Brugler, MR; Crowley, LM; Grajales, A; Gusmão, L; Häussermann, V.; Reft, A. & Daly, M. 2014. Hidden among Sea Anemones: The First Comprehensive Phylogenetic Reconstruction of the Order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) Reveals a Novel Group of Hexacorals. *PLoS ONE* 9(5): e96998. doi:10.1371/journal.pone.0096998

Schlötterer, C.; Hauser, M.T.; von Haeseler, A. & Tautz, D., 1994. Comparative evolutionary analysis of rDNA ITS regions in *Drosophila*. *Molecular Biology and Evolution* 11, 513– 522.

Schmidt, H. 1974. On evolution in Anthozoa. *Proceedings of the 2nd International Coral Reef Symposium* 1: 533–560.

Shearer T.L.; Van Oppen, M.J.H.; Romano, S.L. & Wörheide, G. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology* 11: 2475–2487.

Shearer T.L. & Coffroth, M.A. 2008. Barcoding corals: limited by interspecific divergence, not intraspecific variation. *Molecular Ecology Resources* 8: 247–255.

Sinniger, F.; Reimer, J.D. & Pawlowski, J. 2008. Potential of DNA sequences to identify Zoanthids (Cnidaria: Zoantharia). *Zoological Science* 25: 1253–1260.

Slowinski, J.B. 1998. The number of multiple alignments. *Molecular Phylogenetics and Evolution* 10: 264–266.

Solé–Cava, A.M. & Thorpe, J.P. 1992. Genetic divergence between colour morphos in populations of the common interidal sea anemones *Actinia equina* and *A. prasina* (Anthozoa: Actiniaria) in the Isle of Man. *Marine Biology* 112: 243–252.

Stephens, M. ; Smith, N. J. & Donnelly, P. 2001. A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics* 68(4): 978–989.

Stephens, M & Donnelly, P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics* 73(5): 1162–1169.

Stephenson, T. A. 1921. On the classification of Actiniaria. Part II. Consideration of the whole group and its relationships, with special reference to forms not treated in Part I. *Quarterly Journal of Microscopical Sciences* 65: 493 – 576.

Stoletzki, N. & Schierwater, B. 2005. Genetic and color morph differentiation in the Caribbean sea anemone *Condylactis gigantea*. *Marine Biology* 147: 747 –754.

Tamura, K.; Stecher, G.; Peteron, D, Filipski, A & Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.

Thorpe, J.P. & Solé–Cava, A.M., 1994. The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta* 23: 3–18.

Ting, J.H., Geller, J.B., 2000. Clonal diversity in introduced populations of an asian sea anemone in North America. *Biological Invasions* 2: 23–32.

Varón, A., Vinh, L.S.& Wheeler, W.C. 2010. POY version 4: phylogenetic analysis using dynamic homologies. *Cladistics* 26: 72–85.

Wheeler, W.C. 1996. Optimization alignment: the end of multiples sequence alignment in phylogenetics? *Cladistics* 12: 1–9.

Ward, R.D.; Zemlak, T.S.; Innes, B.H.; Last, P.R. & Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1847–1857

Wheeler, W.C. 2003. Implied alignment: a synapomorphy–based multiple–sequence alignment method and its use in cladogram search. *Cladistics* 19: 261–268.

Won, J. H.; Rho, B. J. & Song, J–I. 2001. A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. *Coral Reefs* 20: 39–50.

Worheide, G.; Nichols, S.A. & Goldberg, J., 2006. Intragenomic variation of the rDNA internal transcribed spacers in sponges (Phylym Porifera): implications for phylogenetic studies. *Molecular Phylogenetics and Evolution* 33: 816–830.

Zamponi M.O. 2000. El estuario del Río de la Plata: una barrera geográfica para los cnidarios bentónicos marinos? *Biociências* 8: 127–136.

Zamponi, M.O.; Belém, M. J. C.; Schlenz, E & Acuña, F. H. 1998a. Distribution and some ecological aspects of Corallimorpharia and Actiniaria from shallow waters of the South American Atlantic coasts. *Physis, A* 55: 31 – 45.

Zamponi M.O.; Genzano, G.N.; Acuña, F.H. & Excoffon, A.C. 1998b. Studies of benthic cnidarian populations along a transect off Mar del Plata (Argentina). *Russian Journal of Marine Biology* 24: 7–13.

Capítulo 3

Comparing cnidocyst sizes among populations of *Bunodosoma* (Cnidaria: Actiniaria: Actiniidae) from the South Atlantic West Coast

Abstract

The simplicity of the sea anemone polyp makes it difficult to analyze differences among species and even more populations, as very few external and internal morphological characters vary enough to characterize closely related groups. The variations in the size of the different types of cnidocysts may be a way to distinguish populations of sea anemones. The aim of this study is to detect which types of cnidocysts may be useful for population studies in sea anemones. We tested this for two species of the genus *Bunodosoma* from the South Atlantic coast. Specimens of *Bunodosoma caissarum* were collected from 6 populations along the Brazilian coast and of *B. cangicum* from 24 populations along the Brazilian and Uruguayan coast. For each specimen we measured cnidocysts from the following parts of the polyp: acrorhagi, external and internal tentacles, column, actinopharynx and filaments. We compared the lengths of the cnidocysts of each tissue separately using boxplots and Generalized Linear Models. For *B. caissarum*, we found that the basitrichs of the external and internal tentacles may be used to detect differences between populations and may indicate a latitudinal gradient. And, for *B. cangicum*, we suggest that these aspects should be analyzed in

the basitrichs of the external tentacles, holotrichs of the acrorhagi and microbasic *b*–mastigophores of the filaments. A more thorough analysis of these selected cnidae should be carried out in order to confirm their usefulness for population delimitation.

Key–words: sea anemone; nematocysts; *Bunodosoma caissarum*; *Bunodosoma cangicum*; Brazil; Uruguay

Introduction

Sea anemones comprise a very plastic group, highly adaptable to different environmental conditions (Shick, 1991; Daly *et al.*, 2008). Polyps from the intertidal zone, for example, are capable of changing form and size of their bodies according to the environmental conditions, and adapting to dehydration and other adversities (Corrêa, 1964; Dube, 1974; Gomes *et al.*, 1998; Gomes, 2002). The simplicity of the sea anemone polyp and the high richness of species in the order Actiniaria — 7,500 species (Daly *et al.*, 2007) — make it difficult for taxonomists to establish diagnostic features to identify the taxa (Fautin, 1988). In this scenario, the value of cnidocysts in taxonomic studies has been much discussed. The cnidocysts (nematocysts, spirocysts and ptychocysts) are the most complex intracellular secretion known, and cnidarians have the unique ability to secrete them. The current descriptions of sea anemones include features based on internal and external morphology of the polyps as well as types and measurements of the types of cnidae present in each region of the polyp body (*e.g.* Beneti *et al.*, 2015; Gusmão, 2016). However, it is still not clear if these features' variations are larger among species or among populations of the same species.

Therefore, we carried out a population level analysis in order to understand the differences among populations of two of the *Bunodosoma* species of the South American coast.

Bunodosoma is a worldwide distributed genus of sea anemones, currently with 14 valid species (Fautin, 2013; Gomes *et al*., 2012; Chapter 1). The members of the genus *Bunodosoma* are characterized by the presence of a circumscript sphincter, acrorhagi, non–adherent vesicles and fertile mesenteries with well–developed muscles (Häussermann, 2004; Gomes *et al*., 2012; Chapter 1). Their members are relatively large and conspicuous solitary polyps found in rocky shores, and three species of this genus have records for the South Atlantic west coast: *Bunodosoma caissarum* Corrêa *in* Belém, 1987, *Bunodosoma cangicum* Corrêa *in* Belém & Preslercravo, 1973 and *Bunodosoma zamponii* Gomes, Schama & Solé–Cava, 2011. Although a systematic review of the genus has been carried out (Chapter 1), little is understood on how the cnidae of these species vary, except in *B. zamponii*, which has a more limited distribution and has been studied by Garese (2013). Gomes (2002) studied four types of cnidocysts from populations of the Brazilian coast, but her sampling was very restricted compared to the species' large distribution. *B. cangicum* is one of the most conspicuous sea anemones of the Brazilian coast, and it occurs from the north of Brazil to Uruguay. The distribution of *B. caissarum* is from the state of Rio de Janeiro to Rio Grande do Sul, and the archipelagos of Fernando de Noronha and São Pedro e São Paulo. It is almost always found in sympatry with *B. cangicum* (Chapter 1).

All species of *Bunodosoma* have the same type of cnidocysts (very few exceptions, see Chapter 1) in all parts of the polyp. The differences among the species are usually in the sizes or size classes of specific cnidocysts of each part of the polyp (Chapter 1). Gomes (2002) studied the variation of morphological aspects in three populations of *B.cangicum*, including the sizes of the nematocysts. While no difference among populations was found for most

morphological characters (*e.g.* number of tentacles and mesenteries), features such as the number of vesicles in the column were more related to the size of the animal than to populations. Also, she found that the sizes of the four types of cnidocysts on which she focused had mean values statistically the same among populations (Gomes, 2002). The latter result differs from other studies in which these structures presented a clinal variation or size differences associated to ecological features (Zamponi & Acuña, 1991; Acuña & Zamponi, 1997).

The taxonomic value of statistical analysis of cnidae measurements in sea anemones has been discussed, but it has still not been fully evaluated (Fautin, 1988; Williams, 1996, 1998, 2000; Acuña *et al.*, 2003, 2004). However, it is already common practice that no species of sea anemone can be recognized based on cnidae data alone (Fautin, 1988; Williams, 1996; Ardelean & Fautin, 2004). In the meantime, most sea anemone studies describe the cnidae in many different species, but few comparative studies involving and large quantity of data for the same species have been carried out (*e.g.* Acuña *et al*., 2004; Garese, 2013).

Besides the insufficient amount of data used in most analysis, another problem is the type of statistical analysis applied to the data. Most papers use parametric statistical tests (which presume a normal distribution) to study the distribution of capsule lengths. But it has already been demonstrated that, in some groups, cnidocysts sizes do not have normal distributions, as in acontiarian sea anemones (Acuña *et al*., 2003, 2004), some species of Actiniidae (Acuña *et al.*, 2007) and also corallimorpharians (Acuña & Garese, 2009). The most common choice in case of non–normal distribution is to use non–parametric tests, which are less powerful. Generalized Linear Models (GLM)) (developed by Nelder & Wedderburn, 1972) are mathematical extensions of linear models that do not force data into unnatural

scales via transformations, allowing non–linearity and non–constant variance structures in data (Hastie & Tibshirani, 1990; Acuña *et al.*, 2004). The data used in these analyses can be assumed from several families of probability distributions, which may be a better fit for the non–normal error structure of most biological data. This makes GLMs a better suited form of analyzing biological relationships. Allcock *et al.* (1998), Watts *et al.* (2000), Garese (2013) and Acuña *et al.* (2004) have applied GLMs to nematocysts of sea anemones. Therefore, the aim of this study is to apply GLMs to detect which types of cnidocysts may be useful for population studies of two South Atlantic species: *B. caissarum* and *B. cangicum*.

Material & Methods

Specimens of *B. caissarum* were collected in six sites along the Brazilian coast and *B. cangicum* in 24 sites along the Brazilian and Uruguayan coasts (Fig. 3.1; Table 3.1). All morphological features relevant to the group's taxonomy (*e.g.* size and form of pedal disc, presence and arrangement of the projections on the column, number and arrangement of tentacles, etc.) were examined so that we could confirm each specimen's identification to species level. For the analysis of cnidocysts, three specimens of *B. caissarum* and five of *B. cangicum* (three for column, actinopharynx and filaments) from each site were analyzed. We measured up to 30 cnidae of each type from the following parts of the polyp: acrorhagi, tentacles (from the most internal and external cycles separately), column, actinopharynx and filaments. Eventually, for very scarce types, fewer capsules were measured. Cnidae capsules were identified and measured from squash preparations made of preserved pieces of tissue from each part. The preparations were examined under an optical microscope at 1000X magnification (Nikon Eclipse 80i). We followed the classification proposed by Östman (2000), which has been used in the most recent works on Actiniaria (*e.g.* Rodríguez *et al*.,

2009; Beneti *et al.*, 2015). For each type, range, mean and standard deviation are provided. Frequencies for each type of cnidocyst were given, but are subjective impressions based on all the capsules seen on the slides.

Each type of nematocyst in each part of the polyp was considered as a separate dataset. We tested the normality of the distribution of the lengths of the capsules of all datasets using the Shapiro–Wilks test (α=0.05). Despite the fact that some types had normal distribution, we chose to evaluate all the datasets using GLMs with a gamma distribution, so that they all were analyzed using the same parameters. Datasets from populations of each species were compared pair by pair. We also compared external and internal tentacles for each species. All the analyses were performed in the software R (R Development Core Team) and the GLM model used was as follows: g (longitude) = β0 + β1 population + ε El.

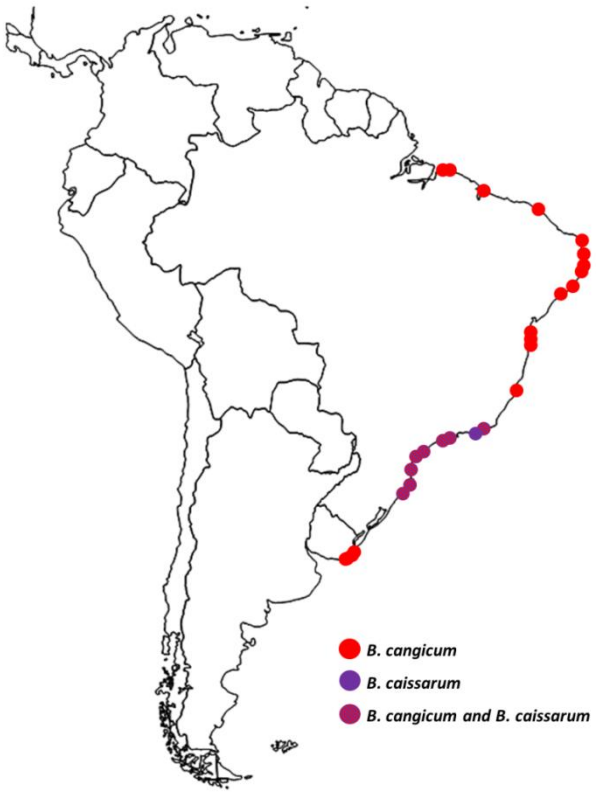

Fig 3.1. Collecting sites of each species

Table 3.1. Collecting sites of each species.

Locality	**Coordinates (S/W)**		***B. caissarum***	***B. cangicum***
Marudá, (PA)	0° 37' 25"	47° 63' 24"		X
Salinópolis (PA)	0° 36' 58"	47° 19' 59"		X
Araçagi, São Luís (MA)	2° 16' 42"	44° 07' 23"		X
Taíba (CE)	3° 30' 21"	38° 53' 36"		X
Praia da Ponta Negra, Natal (RN)	5° 52' 59"	35° 09' 52"		X
Praia do Coqueirinho, Conde (PB)	7° 19' 40"	34° 47' 44"		X
Praia da Boa Viagem, Recife (PE)	8° 05' 33"	34° 52' 49"		X
Praia do Muro Alto, Ipojuca (PE)	8° 25' 35"	34° 58' 36"		X
Praia do Francês, Marechal Deodoro (AL)	9° 46' 13"	35° 50' 19"		X
Pontal de Coruripe, Coruripe (AL)	10° 09' 27"	36° 07' 59"		X
Praia da Ribeira, Itacaré (BA)	14° 17' 23"	38° 59' 05"		X
Praia do Backdoor, Olivença (BA)	14° 56' 09"	39° 00' 52"		X
Coqueiral, Aracruz (ES)	19° 56' 42"	40° 08' 45"		X
Praia das Conchas, Cabo Frio (RJ)	22° 52' 16"	41° 58' 51"	X	
Prainha, Arraial do Cabo (RJ)	22° 57' 42"	42° 01' 14"		X
Enseada do Flamengo, Ubatuba (SP)	23° 29' 32"	45° 05' 32"	X	X
Praia do Cabelo Gordo, São Sebastião (SP)	23° 49' 44"	45° 25' 27"		X
Praia do Farol, Ilha do Mel (PR)	25° 32' 19"	48° 17' 30"	X	X
Pedra do Meio, Itapoá (SC)	26° 04' 11"	48° 36' 20"	X	X
Praia dos Ingleses, Florianópolis (SC)	27° 24' 52"	48° 24' 13"	X	X
Praia do Vigia, Garopaba (SC)	28° 01' 08"	48° 36' 35"	X	X
Playa de los Pescadores, Punta del Diablo, Rocha	34° 02' 42"	53° 32' 12"		X
Puerto, La Paloma, Rocha	34° 39' 10"	54° 08' 26"		X
José Ignacio, Maldonado	34° 50' 53"	54° 38' 07"		X
Virgen de la Candelaria, Punta del Este, Maldonado	34° 57' 53"	54° 56' 25"		X

Results

Using GLM analysis with a gamma distribution for all the types of cnidocysts we were able to evaluate the differences in the sizes of each cnidocyst among populations. As we intended to perform a preliminary identification of potential cnidocysts which depict populations or show latitudinal gradient, we chose to compare only the first population of each species (*i.e.* for *B. caissarum*, Bcai_1, and for *B. cangicum*, population 1) to all the others. If this population was similar to most of the others, that type of cnidocyst cannot be used to define a population. Based on the preliminary results obtained herein, the selected cnidocyst types shall be more thoroughly tested in a posterior study. We only performed these analyses in cnidocyst types which were present in at least one third of the populations of each species.

Bunodosoma caissarum

We measured a total of 4441 cnidae in 18 specimens from 6 different populations of *B. caissarum*, from Rio de Janeiro to Santa Catarina (Table 3.2). The types of cnidae found are the same as described in Chapter 1, except for the small microbasic *b*–mastigophore in the filaments, which were found in few populations (only Bcai_1 and Bcai_2), and therefore removed from this analysis. Normality was examined for each type of cnidae using the Shapiro–Wilks test. Size and distribution of cnidae as well as the p values of the normality tests for each type of cnidocyst are summarized in Table 3.3.

Table 3.2. Codes of the collecting sites of *Bunodosoma caissarum*.

Code	Locality
Bcai_1	Praia das Conchas, Cabo Frio (RJ)
Bcai_2	Enseada do Flamengo, Ubatuba (SP)
Bcai_5	Praia do Farol, Ilha do Mel (PR)
Bcai_6	Pedra do Meio, Itapoá (SC)
Bcai_7	Praia dos Ingleses, Florianópolis (SC)
Bcai_8	Praia do Vigia, Garopaba (SC)

Holotrichs and spirocysts were found in the acrorhagi of all specimens, whereas basitrichs were observed in only 11 of the 18 specimens. Two specimens from population Bcai_5 and the five specimens from Bcai_7 and one from Bcai_8 did not present basitrichs in the acrorhagi. The holotrichs are the most abundant cnidae in this structure and, in fact, its presence characterizes an acrorhagi (opposed to pseudoacrorhagi, which only present basitrichs; Daly, 2003). Normality was examined and both types do not have a normal distribution (Table 3.3). The GLM analysis of the basitrichs showed that population Bcai_1 is significantly different from Bcai_2 and Bcai_6, but not from Bcai_5 and Bcai_8. The analysis of the spirocysts and holotrichs showed that Bcai_1 is only different from Bcai_7 (Table 3.4).

Spirocysts and basitrichs are very abundant in the external tentacles. Basitrichs' lengths are larger in higher latitudes (Fig. 3.2), however, it lowers in population Bcai_8. Spirocysts' lengths vary very much (Fig. 3.2) and therefore do not characterize populations. Normality was examined and we found that spirocysts measurements have a normal distribution and the basitrichs do not (Table 3.3). The GLM analysis of the basitrichs showed that population Bcai_1 is significantly different from Bcai_5, Bcai_6 and Bcai_7, but not from Bcai_8. The analysis of the spirocysts showed that population Bcai_1 is significantly different from Bcai_5 and Bcai_6, and not from Bcai_2, Bcai_7 and Bcai_8 (Table 3.4).

As in the external tentacles, spirocysts and basitrichs are also very abundant in the internal tentacles. Again, the mean length of basitrichs is higher in larger latitudes, but it lowers in Bcai_8 (Fig. 3.2). Spirocysts present large variance in the internal tentacles and therefore do not characterize a population. Normality was examined for the three types of cnidocysts, and only holotrichs have a normal distribution (Table 3.3). The GLM analysis of the basitrichs showed that population Bcai_1 is significantly different from all populations. The analysis of the spirocysts showed that population Bcai_1 is not significantly different from any of the other populations (Table 3.4).

The column of *B. caissarum* is composed of basitrichs, spirocysts and holotrichs. The first cnidae is extremely abundant, but its mean values for all populations are very similar (Fig. 3.2) and, therefore do not define populations. Normality was examined and the basitrichs measurements do not have a normal distribution (Table 3.3). The GLM analysis of the basitrichs showed that population Bcai_1 is significantly different from Bcai_2, Bcai_5 and Bcai_6, but not from Bcai_7 and Bcai_8 (Table 3.4). The other two types of cnidae are quite scarce and were not found in all populations. Also, for some populations we only have measurements of one or two cnidae, so both types were removed from the GLM analysis.

In the actinopharynx the most abundant cnidocyst type is basitrichs type II, which are larger than basitrich I. Nevertheless, both types were present in all specimens, so we were able to perform GLM analysis of both. Neither have normal distributions, both have large variations within populations and no pattern was observed in their boxplots (Fig. 3.2; Table 3.3). The analysis of basitrich I showed that population Bcai_1 is significantly different from Bcai_5 and Bcai_6, and not from Bcai_2 and Bcai_7. The analysis of basitrich II showed that population Bcai_1 is significantly different from Bcai_2, Bcai_6 and Bcai_7, but not from Bcai_5 (Table 3.4). The microbasic *p*–mastigophores are very scarce and were not found in all populations, so we did not perform the GLM analysis on them.

Lastly, the filaments have four types of nematocysts and the most abundant are the microbasic *p*–mastigophore I. However, all four types were quite abundant in all populations, although no population has exclusive size ranges. Therefore, none of these nematocyst types may be used to define populations in *B. caissarum*. Normality was examined and neither of them have a normal distribution (Table 3.3). The GLM analysis of the basitrichs showed that population Bcai_1 is significantly different only from Bcai_7. For the microbasic *b*–mastigophores, Bcai_1 is significantly different from Bcai_5, Bcai_6 and Bcai_7, but not from Bcai_2. The GLM analysis of the microbasic *p*–mastigophores 1 showed that population Bcai_1 is not significantly different only from Bcai_5, whereas for microbasic *p*–mastigophores 2, Bcai_1 is not significantly different only from Bcai_6 (Table 3.4).

Table 3.3. Size and distribution of cnidae of *Bunodosoma caissarum*. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae out of the total analyzed. ; F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common. P value of the Shapiro–Wilks test, * indicates that dataset does not have normal distribution.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F	P value
Acrorhagi						
Spirocyst	21.590 – 46.150 x 1.970 – 4.560	37.065 ± 3.980 x 3.283 ± 0.476	255	18/18	++	0.017*
Basitrich	10.192 – 20.970 x 1.590 – 3.570	16.547 ± 2.510 x 2.398 ± 0.425	66	11/18	+	<0.001*
Holotrich	37.890 – 75.040 x 3.660 – 7.400	53.636 ± 5.856 x 5.391 ± 0.681	502	18/18	+++	0.064
External tentacles						
Spirocyst	14.990 – 32.600 x 1.390 – 4.520	23.345 ± 3.609 x 2.845 ± 0.527	479	18/18	+++	0.048*
Basitrich	11.330 – 28.740 x 1.760 – 4.170	22.106 ± 2.525 x 2.714 ± 0.441	432	18/18	+++	<0.001*
Internal tentacles						
Spirocyst	13.880 – 33.650 x 1.710 – 4.610	23.181 ± 3.821 x 2.901 ± 0.501	348	16/16	+++	0.280
Basitrich	11.040 – 27.320 x 1.770 – 4.490	22.031 ± 2.070 x 2.709 ± 0.412	356	16/16	+++	<0.001*
Column						
Spirocyst	22.982 – 30.690 x 2.670 – 3.643	25.340 ± 2.354 x 4.790 ± 0.846	19	2/18	+	–
Basitrich	9.998 – 25.530 x 1.553 – 3.050	18.336 ± 2.139 x 2.762 ± 0.415	504	18/18	+++	<0.001*
Holotrich	23.560 – 31.340 x 4.050 – 4.910	28.262 ± 1.935 x 4.327 ± 0.248	15	11/18	+	–
Pharynx						
Basitrich 1	10.970 – 19.950 x 1.640 – 3.510	16.504 ± 2.406 x 2.573 ± 0.450	140	15/15	++	<0.001*
Basitrich 2	18.248 – 36.390 x 1.910 – 4.390	24.208 ± 2.870 x 3.043 ± 0.427	429	15/15	+++	<0.001*
Microbasic *p*–mastigophore	18.450 – 21.619 x 4.300 – 5.226	19.559 ± 1.510 x 4.866 ± 0.345	6	4/15	+	–
Filaments						
Basitrich	9.190 – 25.220 x 1.035 – 3.020	14.320 ± 2.961 x 1.958 ± 0.382	157	15/15	++	<0.001*
Microbasic *b*–mastigophore	19.867 – 58.520 x 3.455 – 7.490	34.981 ± 7.810 x 5.239 ± 0.761	197	15/15	++	<0.001*
Microbasic *p*–mastigophore 1	16.303 – 35.070 x 2.920 – 7.890	21.342 ± 2.333 x 5.044 ± 0.643	330	15/15	+++	<0.001*
Microbasic *p*–mastigophore 2	13.240 – 22.290 x 2.040 – 4.790	17.016 ± 1.714 x 3.323 ± 0.477	206	15/15	++	<0.001*

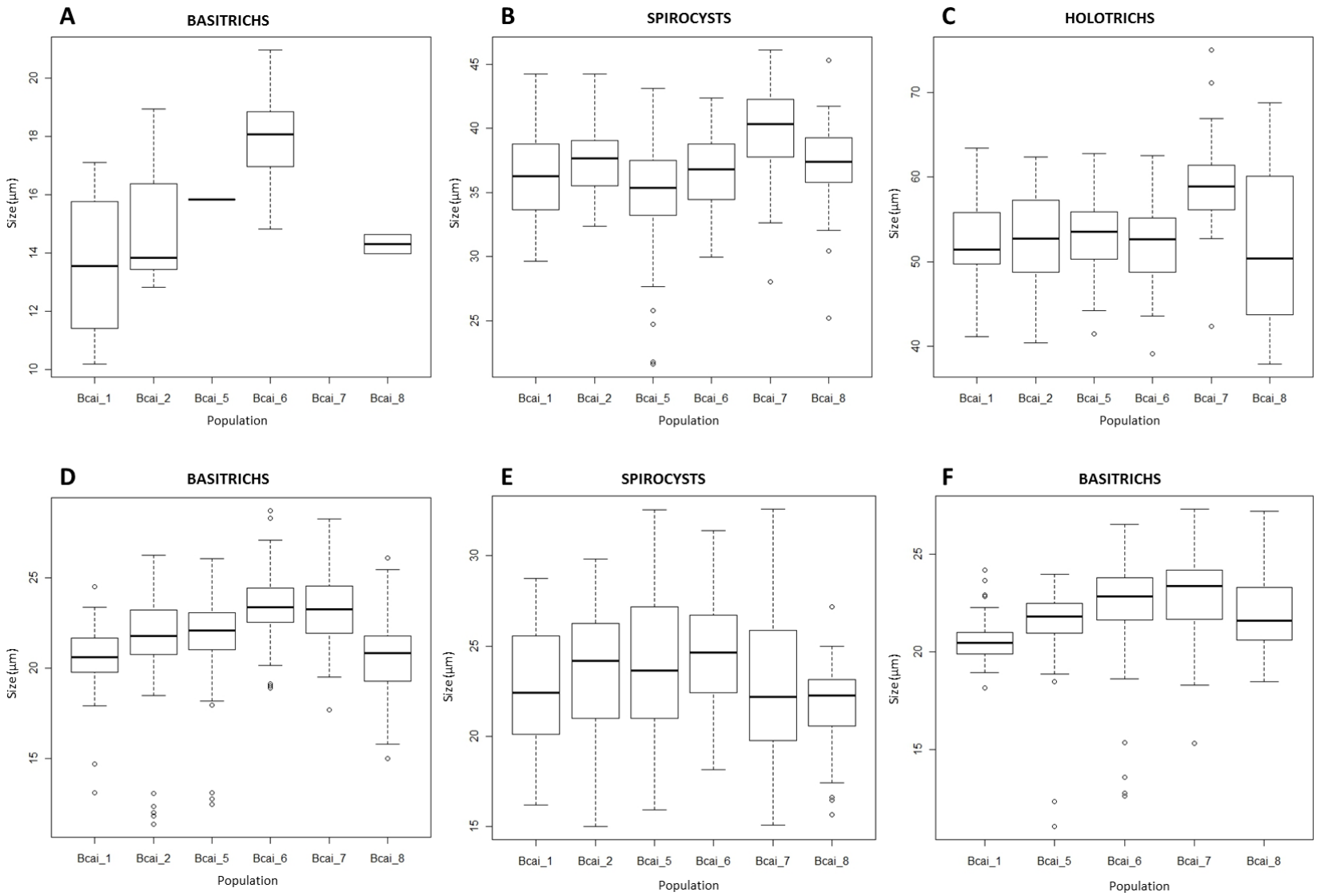

Fig. 3.2. Part 1. *Bunodosoma caissarum*. Boxplots per population of the length of the cnidocysts. Acrorhagi: A–C; External tentacles: D–E; Internal tentacles: F–G; Column: H; Actinopharynx: I–J; Filaments: K–N.

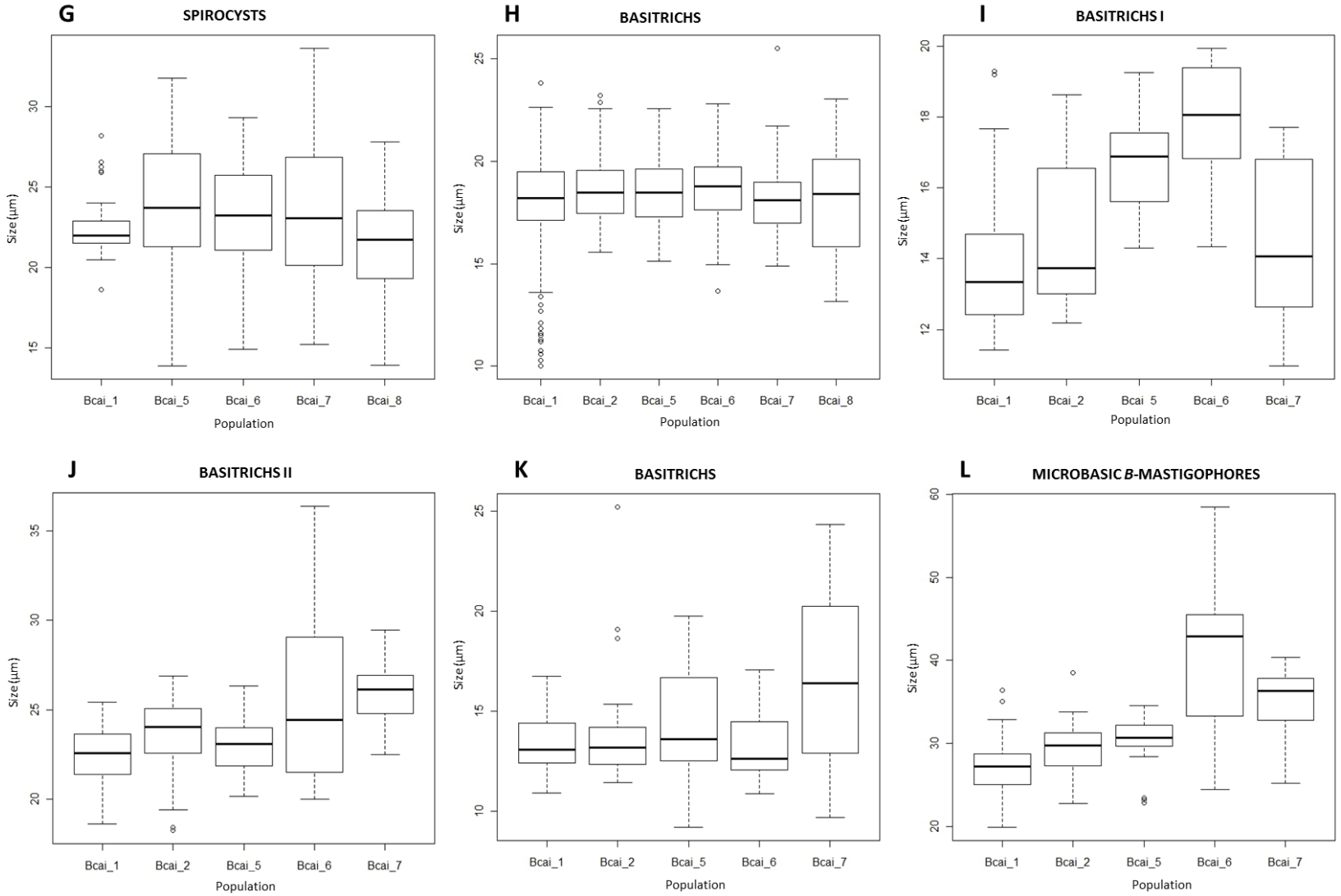

Fig. 3.2. Part 2. *Bunodosoma caissarum*. Boxplots per population of the length of the cnidocysts. Acrorhagi: A–C; External tentacles: D–E; Internal tentacles: F–G; Column: H; Actinopharynx: I–J; Filaments: K–N.

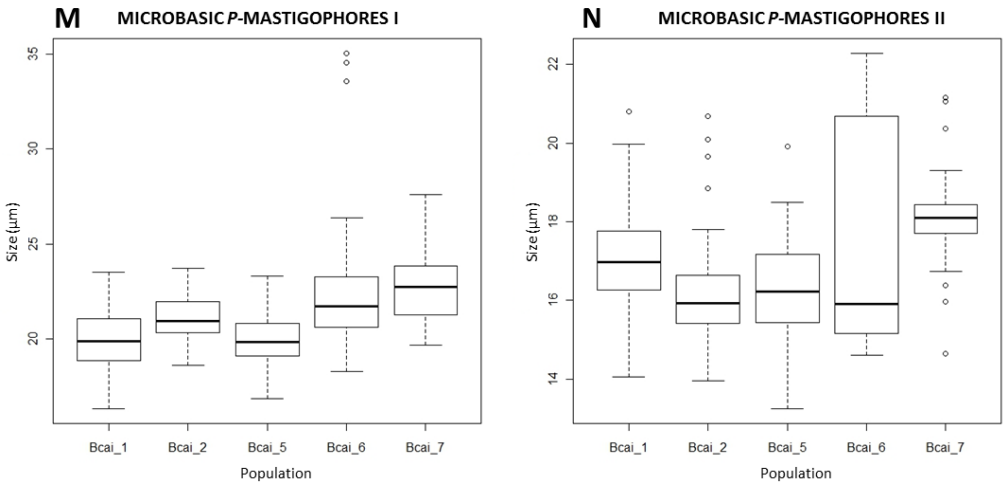

Fig. 3.2. Part 3. *Bunodosoma caissarum*. Boxplots per population of the length of the cnidocysts. Acrorhagi: A–C; External tentacles: D–E; Internal tentacles: F–G; Column: H; Actinopharynx: I–J; Filaments: K–N.

Table 3.4. Part 1. *Bunodosoma caissarum*. Comparison of each population to Bcai_1, P values of the t test for GLM coefficients (ß1), * indicates significant difference.

	Acrorhagi			External tentacles		Internal tentacles		Column	Actinopharynx	
Population	**Spirocyst**	**Basitrich**	**Holotrich**	**Spirocyst**	**Basitrich**	**Spirocyst**	**Basitrich**	**Basitrichs**	**Basitrich 1**	**Basitrich 2**
Bcai_2	0.176	0.0424*	0.472	0.225	0.003*	–	–	<0.001*	0.325	<0.001*
Bcai_5	0.097	0.187	0.353	0.036*	<0.001*	0.079	0.032*	0.002	<0.001*	0.164
Bcai_6	0.527	<0.001*	0.701	0.003*	<0.001*	0.406	<0.001*	<0.001*	<0.001*	<0.001*
Bcai_7	<0.001*	–	<0.001*	0.872	<0.001*	0.337	<0.001*	0.148	0.859	<0.001*
Bcai_8	0.497	0.559	0.707	0.080	0.856	0.720	0.005*	0.181	–	–

Table 3.4. Part 2. *Bunodosoma caissarum*. Comparison of each population to Bcai_1, P values of the t test for GLM coefficients (ß1), * indicates significant difference.

	Filaments			
Population	**Basitrichs**	**Microbasic *b*–mastigophores**	**Microbasic *p*–mastigophores1**	**Microbasic *p*–mastigophores 2**
Bcai_2	0.489	0.056	0.006*	0.01*
Bcai_5	0.152	0.009*	0.915	0.014*
Bcai_6	0.572	<0.001*	<0.001*	0.556
Bcai_7	<0.001*	<0.001*	<0.001*	0.003*
Bcai_8	–	–	–	–

Bunodosoma cangicum

We measured a total of 31152 cnidae in 113 specimens of 24 different populations of *B. cangicum*, from Pará (Brazil) to Punta del Este (Uruguay) (Table 3.5). Apart from the types of cnidae described in Chapter 1, we also found spirocysts and holotrichs in the column as well as spirocysts in the actinopharynx. Normality was examined for each type of cnidae using the Shapiro–Wilks test. Size and distribution of cnidae as well as the p values of the normality tests for each type of cnidocyst are summarized in Table 3.6.

Only holotrichs were found in the acrorhagi of all specimens, whereas spirocysts and basitrichs were observed in, respectively, 110 and 86 of the 113 analyzed specimens. Specimens from populations 4 (2 specimens), 22 (1) did not present spirocysts in the acrorhagi, and specimens from populations 6(1), 7(2), 10 (3), 12(2), 16(1), 17(2), 19(1), 21 (1), 23 (2), 24 (1) and 26 (1) did not present basitrichs. The holotrichs are the most abundant cnidocyst in this structure and its presence characterizes it as an actual acrorhagi (Daly, 2003). Normality was examined for the three types of cnidocysts, and none of them have a normal distribution (Table 3.6). While no pattern can be observed in the boxplots of the basitrichs and spirocysts, for the holotrichs there seems to be higher values of lengths in populations found in higher latitudes (Fig. 3.3). The GLM analysis of the spirocysts showed that population 1 is not significantly different from population 16. Based on the analysis of the basitrichs, population 1 is not significantly different from populations 21, and the holotrichs showed that population 1 is not significantly different from populations 2, 5 and 6 (Table 3.7).

Table 3.5. Codes of the collecting sites of *Bunodosoma cangicum*.

Code	Locality
1	Marudá, (PA)
2	Salinópolis (PA)
3	MA
4	Taíba, CE
5	Praia da Ponta Negra, Natal (RN)
6	Praia do Coqueirinho, Conde (PB)
7	Praia da Boa Viagem, Recife (PE)
8	Praia do Muro Alto, Ipojuca (PE)
9	Praia do Francês, Marechal Deodoro (AL)
10	Pontal de Coruripe, Coruripe (AL)
12	Praia da Ribeira, Itacaré (BA)
13	Praia do Backdoor, Olivença (BA)
14	Coqueiral, Aracruz (ES)
15	Prainha, Arraial do Cabo (RJ)
16	Enseada do Flamengo, Ubatuba (SP)
17	Praia do Cabelo Gordo, São Sebastião (SP)
19	Praia do Farol, Ilha do Mel (PR)
20	Pedra do Meio, Itapoá (SC)
21	Praia dos Ingleses, Florianópolis (SC)
22	Praia do Vigia, Garopaba (SC)
23	Playa de los Pescadores, Punta del Diablo, Rocha (URUGUAY)
24	Puerto, La Paloma, Rocha (URUGUAY)
25	José Ignacio, Maldonado (URUGUAY)
26	Virgen de la Candelaria, Punta del Este, Maldonado (URUGUAY)

Spirocysts and basitrichs are very abundant in the external tentacles, are present in all populations and neither have normal distribution (Table 3.6). The boxplots of the spirocysts show that all populations have large variations in the size of this cnidocyst. However, for the

basitrichs there seems to be a tendency of higher values of lengths in populations from higher latitudes (Fig. 3.3). The GLM analysis of the basitrichs showed that population 1 is not significantly different only from population 15, whereas for the spirocysts no significant difference was found between population 1 and populations 4, 6, 8, 10, 14, 15, 23, 25 and 26 (Table 3.7).

As in the external tentacles, spirocysts and basitrichs are also abundant in the internal tentacles, are present in all populations and neither have normal distribution (Table 3.6). The boxplots of the spirocysts also show that all populations have large variations in the size of this cnidocyst. However, for the basitrichs the tendency of higher values of lengths in populations from higher latitudes cannot be seen as in the external tentacles (Fig. 3.3). The GLM analysis of the basitrichs showed that population 1 is not significantly different only from populations 15, 19, 20 and 25, whereas for the spirocysts no significant difference was found between population 1 and populations 6, 7, 8, 12, 13, 14, 16 and 22 (Table 3.7).

In Chapter 1, we found only basitrichs in the column of *B. cangicum*, however, after exploring more populations, spirocysts and holotrichs were also found. As in *B. caissarum*, basitrichs are extremely abundant and were found in all specimens, but contrary to that species, its values for all populations have large variations (Fig. 3.3). The other two types of cnidae were found in less than a third of the analyzed populations, so they were removed from the GLM analysis. The basitrichs lengths do not have a normal distribution, and the GLM analysis showed that population 1 is not significantly different from populations 2, 3, 4 and 24 (Tables 3.6 and 3.7).

Also as in *B. caissarum*, in the actinopharynx the most abundant cnidocyst type is basitrich type II, which are larger than basitrich I. The first was present in all analyzed specimens, whereas the latter was not present in 4 specimens: one from population 4, two from 1 and 1 from 17. Neither have normal distributions, both have large variations within

populations and no pattern was observed in their boxplots (Fig. 3.3). The analysis of basitrich I showed that population 1 is not significantly different from 3, 6, 8, 10, 13, 19, 22, 23 and 26. The analysis of basitrich II showed that population 1 is not significantly different from 2, 4, 5, 6, 10, 19 and 23 (Table 3.7). The microbasic *p*–mastigophores were found in less than a third of the populations, so we did not perform the GLM analysis on them.

And finally, the filaments have four types of nematocysts, as described in Chapter 1, but the most abundant are the microbasic *b*–mastigophore (different from *B. caissarum*), which were also the only type found in all specimens and populations. Microbasic *p*–mastigophore I was also very abundant in most specimens, although it was not found in 2 specimens from population 5. Basitrichs were not found in 1 specimen from population 5 and microbasic *p*–mastigophore II in population 1 (2 specimens), 4 (1), 10 (2), 8(1) and 9 (2). The four types do not have a normal distribution (Table 3.6). While no pattern can be observed in the boxplots of the basitrichs and both types of microbasic *p*–mastigophores, for the microbasic *b*–mastigophores there seems to be higher values of lengths in populations found in higher latitudes (Fig. 3.3). The GLM analysis of the basitrichs showed that population 1 is not significantly different from populations 5, 6, 9, 13, and 19. For the microbasic *b*–mastigophores, population 1 is not significantly different only from population 2. The GLM analysis of the microbasic *p*–mastigophores I showed that population 1 is not significantly different only from populations 4, 6, 7, 8, 9, 13 and 19, whereas for microbasic *p*–mastigophores II, population 1 is significantly different from populations 14 to 17, and from 20 to 26 (Table 3.7).

Table 3.6. Size and distribution of cnidae of *Bunodosoma cangicum*. Measurements in micrometers (µm). N = number of capsules measured; P = number of specimens that showed the particular kind of cnidae out of the total analyzed. ; F = frequency of each type of cnidae: +++ = very common, ++ = common, + = not common. * indicates that data does not have normal distribution.

Tissue / Type of cnida	Range of length x width	Mean ± SD	N	P	F	P value
Acrorhagi						
Spirocyst	11.350 – 51.360 x 1.280 – 4.350	29.496 ± 5.180 x 2.831 ± 0.440	1600	110/113	++	<0.001*
Basitrich	7.880 – 27.660 x 1.120 – 3.960	16.048 ± 2.243 x 2.471 ± 0.401	1450	86/113	+	<0.001*
Holotrich	20.370 – 66.340 x 2.610 – 7.640	44.322 ± 5.300 x 5.188 ± 0.608	3213	110/113	+++	<0.001*
External tentacles						
Spirocyst	9.360 – 31.240 x 1.190 – 5.030	20.826 ± 3.474 x 2.634 ± 0.461	3290	113/113	+++	<0.001*
Basitrich	9.980 – 32.070 x 1.050 – 4.500	21.222 ± 2.687 x 2.678 ± 0.387	3307	113/113	+++	<0.001*
Internal tentacles						
Spirocyst	9.880 – 32.420 x 1.020 – 4.850	21.308 ± 3.326 x 2.656 ± 0.468	3308	113/113	+++	<0.001*
Basitrich	6.720 – 29.110 x 1.350 – 5.640	20.706 ± 2.138 x 2.643 ± 0.394	3289	113/113	+++	<0.001*
Column						
Spirocyst	9.680 – 32,42 x 1.250 4.660	21.750 ± 3.314 x 2.650 ± 0.477	1380	20/72	+	–
Basitrich	9.490 – 29.110 x 1.290 – 4.350	18.670 ± 3.349 x 2.622 ± 0.402	3304	72/72	+++	<0.001*
Holotrich	15.050 – 54.720 x 3.380 – 6.220	24.976 ± 8.273 x 4.827 ± 0.606	68	13/72	+	–
Pharynx						
Spirocyst	10.290 – 27.940 x 1.450 – 3.400	20.224 ± 3.554 x 2.520 ± 0.489	49	17/66	+	–
Basitrich 1	7.090 – 20.240 x 1.250 – 3.380	15.051 ± 2.179 x 2.399 ± 0.457	734	62/66	++	0.046*
Basitrich 2	19.930 – 35.720 x 1.730 – 5.560	26.412 ± 2.507 x 3.059 ± 0.449	1755	66/66	+++	<0.001*
Microbasic *p*–mastigophore	15.830 – 26.590 x 2.730 – 7.160	20.623 ± 2.126 x 5.270 ± 0.906	71	21/66	+	–
Filaments						
Basitrich	6.780 – 22.820 x 0.630 – 3.670	13.209 ± 2.062 x 1.995 ± 0.393	932	59/60	++	<0.001*
Microbasic *b*–mastigophore	20.260 – 58.480 x 2.180 – 8.210	37.044 ± 6.117 x 5.442 ± 0.784	1553	60/60	+++	<0.001*
Microbasic *p*–mastigophore 1	15.300 – 28.440 x 3.000 – 7.850	21.675 ± 2.037 x 5.369 ± 0.678	1386	58/60	+++	0.039*
Microbasic *p*–mastigophore 2	10.650 – 28.060 x 2.980 – 6.470	18.968 ± 3.596 x 3.321 ± 0.672	463	52/60	+	<0.001*

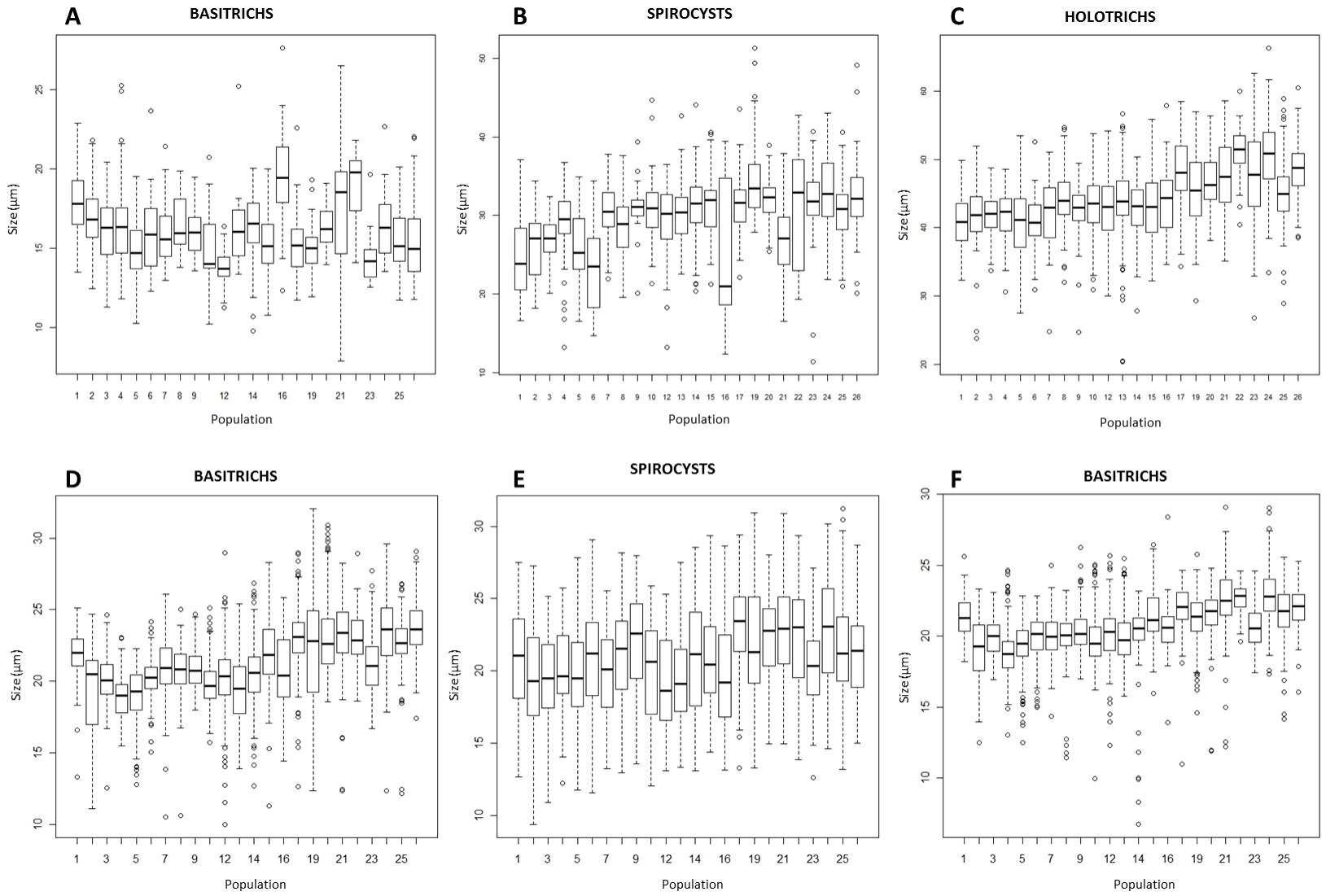

Fig. 3.3. Part 1. *Bunodosoma cangicum*. Boxplots per population of the length of the cnidocysts. Acrorhagi: A–C; External tentacles: D–E; Internal tentacles: F–G; Column: H; Actinopharynx: I–J; Filaments: K–N.

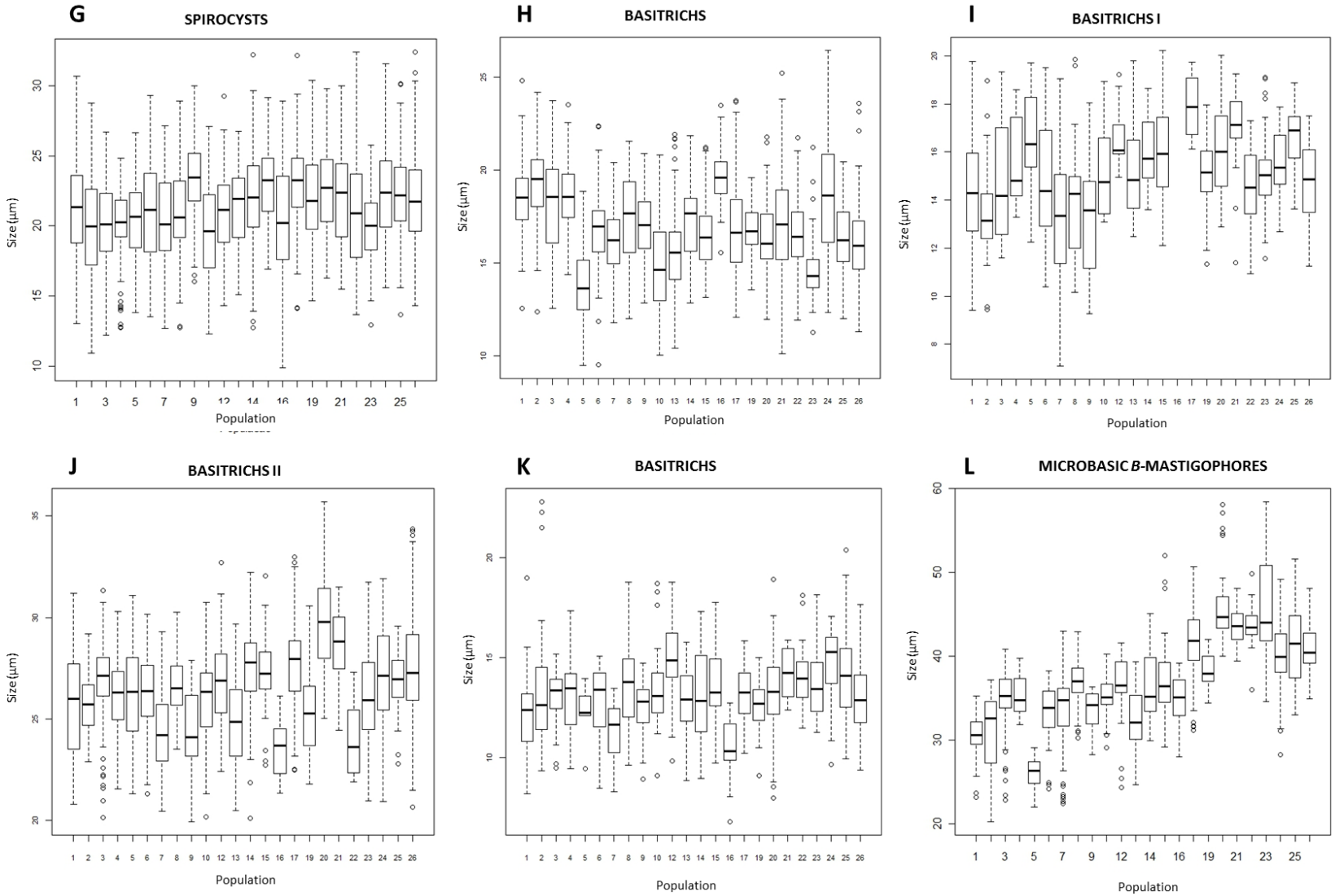

Fig. 3.3. Part 2. *Bunodosoma cangicum*. Boxplots per population of the length of the cnidocysts. Acrorhagi: A–C; External tentacles: D–E; Internal tentacles: F–G; Column: H; Actinopharynx: I–J; Filaments: K–N.

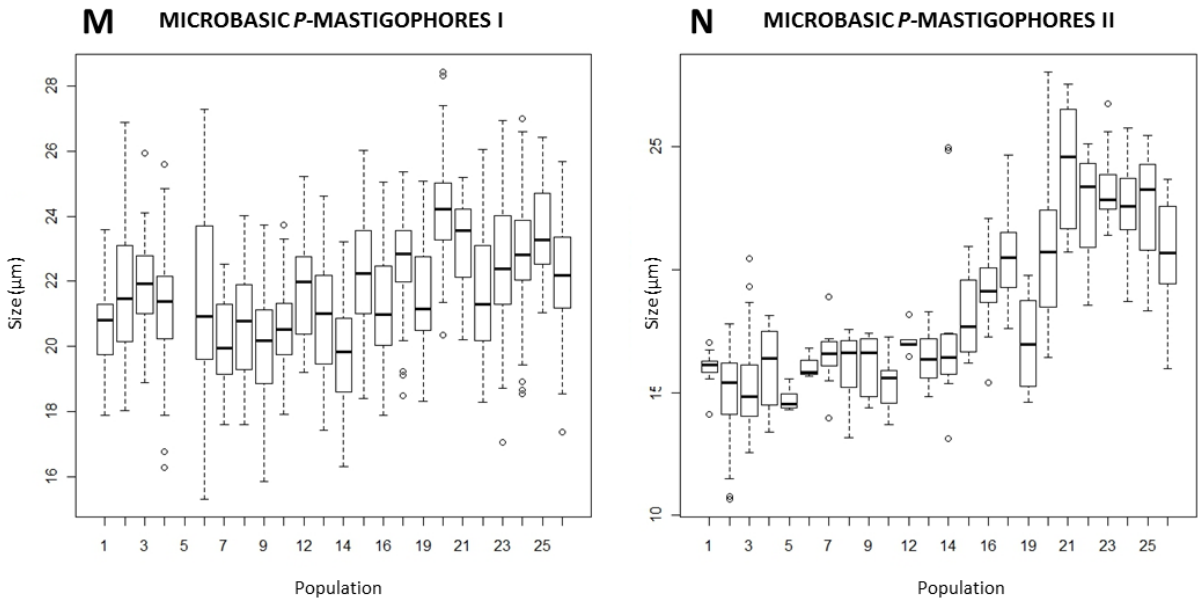

Fig. 3.3. Part 2. *Bunodosoma cangicum*. Boxplots per population of the length of the cnidocysts. Acrorhagi: A–C; External tentacles: D–E; Internal tentacles: F–G; Column: H; Actinopharynx: I–J; Filaments: K–N.

Table 3.7. Part 1. *Bunodosoma cangicum.* Comparison of each population to population1, P values of the t test for GLM coefficients (ß1), * indicates significant difference.

	Acrorhagi			External tentacles		Internal tentacles		Column	Actinopharynx	
Population	Spirocyst	Basitrich	Holotrich	Spirocyst	Basitrich	Spirocyst	Basitrich	Basitrichs	Basitrich 1	Basitrich 2
2	0.007*	0.001*	0.054	<0.001*	<0.001*	<0.001*	<0.001*	0.047	0.041*	0.691
3	<0.001*	<0.001*	0.040*	<0.001*	<0.001*	0.006*	<0.001*	0.218	0.725	0.004*
4	<0.001*	<0.001*	0.018*	0.061	<0.001*	0.005*	<0.001*	0.732	0.023*	0.308
5	0.006*	<0.001*	0.964	0.006*	<0.001*	0.044*	<0.001*	<0.001*	<0.001*	0.312
6	0.009*	<0.001*	0.956	0.983	<0.001*	0.799	<0.001*	<0.001*	0.444	0.136
7	<0.001*	<0.001*	<0.001*	0.015*	<0.001*	0.060	<0.001*	<0.001*	0.002*	<0.001*
8	<0.001*	0.002*	<0.001*	0.556	<0.001*	0.300	<0.001*	<0.001*	0.428	0.019*
9	<0.001*	<0.001*	<0.001*	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.001*	<0.001*
10	<0.001*	<0.001*	<0.001*	0.054	<0.001*	<0.001*	<0.001*	<0.001*	0.322	0.890
12	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.792	<0.001*	–	<0.001*	0.001*
13	<0.001*	0.006*	<0.001*	<0.001*	<0.001*	0.678	<0.001*	<0.001*	0.064	0.003*
14	<0.001*	<0.001*	<0.001*	0.740	<0.001*	0.058	<0.001*	<0.001*	<0.001*	<0.001*
15	<0.001*	<0.001*	<0.001*	0.653	0.581	<0.001*	0.495	<0.001*	<0.001*	<0.001*
16	0.215	0.026*	<0.001*	0.038*	0.001*	0.395	0.010*	0.009*	–	<0.001*
17	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.009*	<0.001*	<0.001*	<0.001*
19	<0.001*	<0.001*	<0.001*	0.005*	0.038*	0.010*	0.409	<0.001*	0.196	0.187
20	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.367	<0.001*	0.002*	<0.001*
21	<0.001*	0.188	<0.001*	<0.001*	<0.001*	0.002*	<0.001*	<0.001*	<0.001*	<0.001*
22	<0.001*	<0.001*	<0.001*	0.006*	<0.001*	0.933	<0.001*	<0.001*	0.786	0.003*
23	<0.001*	<0.001*	<0.001*	0.143	0.008*	0.001*	0.007*	<0.001*	0.092	0.351
24	<0.001*	0.011*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.941	0.011*	0.003*
25	<0.001*	<0.001*	<0.001*	0.058	0.001*	0.002*	0.208	<0.001*	<0.001*	0.024*
26	<0.001*	<0.001*	<0.001*	0.162	<0.001*	0.026*	0.002*	<0.001*	0.397	<0.001*

Table 3.7. Part 2. *Bunodosoma cangicum.* Comparison of each population to population1, P values of the t test for GLM coefficients (ß1), * indicates significant difference.

	Filaments			
Population	**Basitrichs**	**Microbasic *b*–mastigophores**	**Microbasic *p*–mastigophores1**	**Microbasic *p*–mastigophores 2**
2	0.004*	0.796	<0.001*	0.055
3	0.005*	<0.001*	<0.001*	0.183
4	0.039*	<0.001*	0.104	0.955
5	0.917	<0.001*	0.009*	0.112
6	0.181	<0.001*	0.095	0.917
7	0.049*	<0.001*	0.849	0.456
8	<0.001*	<0.001*	0.078	0.894
9	0.571	<0.001*	0.520	0.774
10	<0.001*	<0.001*	–	0.402
12	<0.001*	<0.001*	0.001*	0.208
13	0.063	<0.001*	0.317	0.565
14	0.013*	<0.001*	0.003*	0.043*
15	<0.001*	<0.001*	<0.001*	0.002*
16	<0.001*	<0.001*	0.030*	<0.001*
17	0.007*	<0.001*	<0.001*	<0.001*
19	0.472	<0.001*	0.053	0.190
20	<0.001*	<0.001*	<0.001*	<0.001*
21	<0.001*	<0.001*	<0.001*	<0.001*
22	<0.001*	<0.001*	0.012*	<0.001*
23	<0.001*	<0.001*	<0.001*	<0.001*
24	<0.001*	<0.001*	<0.001*	<0.001*
25	<0.001*	<0.001*	<0.001*	<0.001*
26	0.014*	<0.001*	<0.001*	<0.001*

External versus *Internal tentacles*

The comparison between the whole datasets of basitrichs and spirocysts of external *versus* internal tentacles in *B. caissarum* revealed that there is no significant difference between the cnidocysts in these cycles of tentacles (bastrichs, p value of the t test for the GLM coefficients (ß1) = 0.648; spirocysts, p value = 0.535). However, for *B. cangicum* significant differences were found for both cnidocysts: the basitrichs are larger in the external tentacles of this species (p <0.001), whereas spirocysts are larger in the internal cycles (p <0.001) (Fig 3.4).

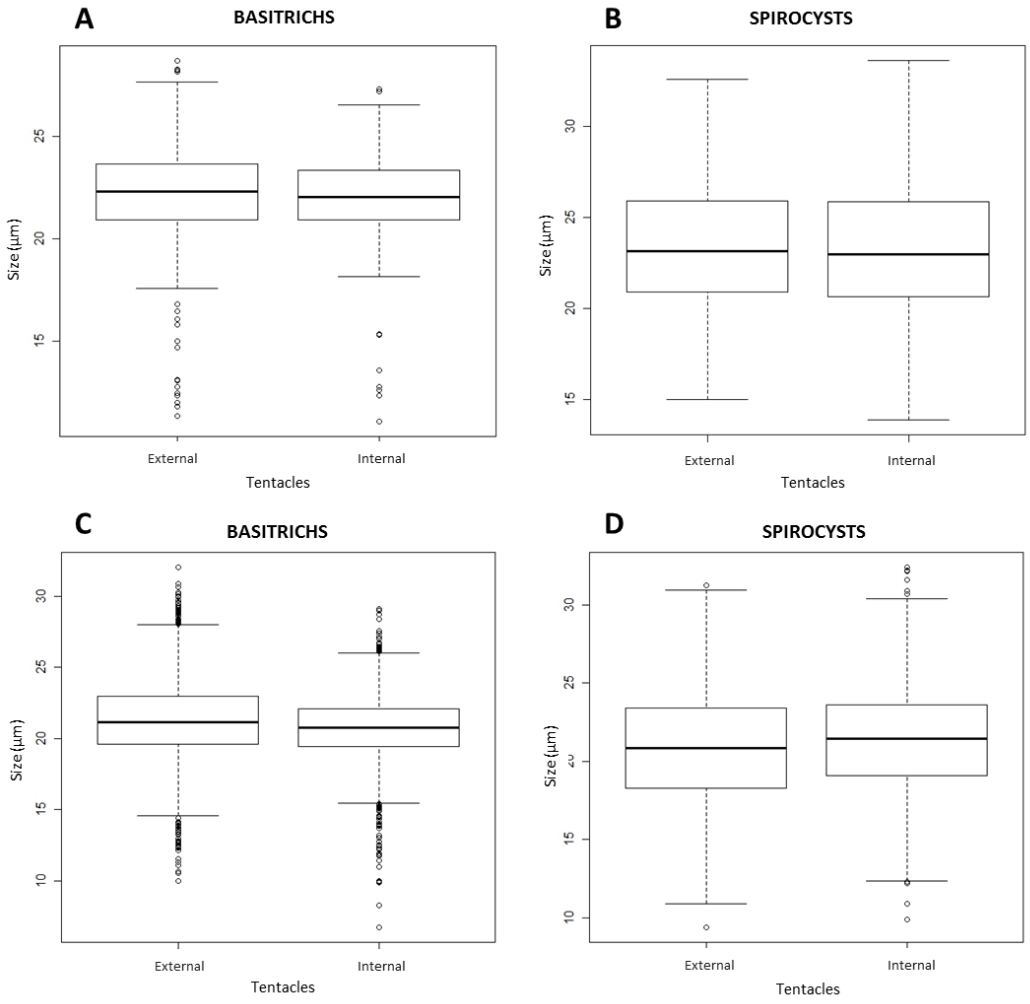

Fig. 3.4. Boxplots of the length of the cnidocyst of the tentacles of external and internal cycles. *Bunodosoma caissarum:* A–B. *Bunodosoma cangicum*: C–D.

Discussion

The fact that most cnidocyst types did not present normal distribution contributes to the idea that using GLM with gamma distribution is more appropriate for dealing with this type of data. Cnidocyst types for which most populations did not present any difference compared to Population 1 were considered as not being suited for characterizing populations or showing latitudinal gradient. Therefore we will comment only on the ones that may be useful for these purposes and for which more thorough analysis should be carried out.

The types of cnidocysts found for *B. caissarum* and *B. cangicum* in the analysis herein were almost the same as in Chapter 1. For the first species, the small microbasic *b*–mastigophore in the filaments, which were found only in the populations of Rio de Janeiro and São Paulo, were removed from this analysis. For the latter, aside from the types of cnidae described in Chapter 1, we also found spirocysts and holotrichs in the column as well as spirocysts in the actinopharynx. These cnidae were also removed from our GLM analyses as they were present in less than one third of the populations examined, and therefore, do not contribute to the purpose of separating populations.

In *B. caissarum*, it is possible to observe a tendency of basitrichs from the external and internal tentacles being larger in higher latitudes. The exceptions to this possible pattern are the specimens collected in Garopaba (SC; Bcai_8). These specimens are smaller (pedal disc in preserved specimens with diameter up to 3cm) than the sea anemones from other populations (pedal disc diameter up to 8cm), and that may be an explanation for the difference observed. For *B. cangicum* more than one type of nematocyst seems to present latitudinal gradient: the holotrichs in the acrorhagi, the basitrichs in the external tentacles and the microbasic *b*–mastigophores in the filaments. Our results show a different tendency than demonstrated by Gomes (2002), as she found no difference in the lengths of the holotrichs of the acrorhagi

from three different populations of *B. cangicum*, but agree with her findings for the other types.

Both species have, in general, the same types of cnidocysts in each tissue, as in most of the species of the genus *Bunodosoma* (Chapter 1). However, some aspects should be noticed. In both species, basitrichs were abundant in the columns of all specimens, while spirocysts and holotrichs were very scarce and were not found in all populations. However, the species differ in their distribution of the sizes of the basitrichs among the populations, as in *B. caissarum* variation in the mean values among the populations and in all values of each population are much smaller than in *B. cangicum*. For cnidae in the actinopharynx, both species present the same result: basitrichs have large variations in length within and among populations, and microbasic *p*–mastigophores are scarce. Another aspect that distinguishes species is in the filaments. The microbasic *p*–mastigophore II were found in most specimens of *B. cangicum*, however it was never abundant. In *B. caissarum*, this type of nematocyst was very easily found and in some specimens it was the most abundant type in the filaments. Also, it is interesting to notice that in *B. caissarum* the most abundant nematocysts are the microbasic *p*–mastigophore I, whereas in *B. cangicum* the most abundant are the microbasic *b*–mastigophores.

The analysis of the external and internal tentacles also had some interesting results. Although no difference was found between the cnidocysts of the external and internal tentacles of *B. caissarum*, the findings in *B. cangicum* show that sampling of the tentacles is very important when describing a species or populations of sea anemones, as the sizes of both types of cnidocysts are different. This aspect should be taken into account when describing other species of sea anemones.

Cnidocysts are used for a variety of functions, such as prey capture, defense and locomotion (Anderson & Bouchard, 2009). The results for each type of cnida may be

explained by their functions or by local ecological factors. Spirocysts, for example, are adhesive and their function is to capture prey as well as acting in the fixation of the animal to the subtrate (Mariscal *et. al*, 1977). Zamponii & Acuña (1991) and Williams (2000) confirmed that spirocysts show higher than usual coefficients of variation, validating the general impression that variability is higher for spirocysts than for other cnidae (Francis, 2004; Acuña *et al.*, 2004), which was also observed here. On the other hand, holotrichs from the acrorhagi are used for defense and aggression (Shick, 1991), and in some species of sea anemones they are known to appear as a reaction to intra or interspecific aggression (Watson & Mariscal, 1983).

This is the first time that cnidae of the species *B. caissarum* are studied throughout many populations and also that so many populations of a widespread sea anemone is analyzed, as we did for *B. cangicum*. It is interesting to observe that the most promising types of cnidae are not the same for *B. caissarum* and *B. cangicum* even though both species are closely related (Chapters 1 and 2). To confirm if the selected cnidocyst types herein selected can be in fact used for population differentiation, this data should be better explored. Variations within each population should be better analyzed as well as the differences between adjacent populations. Our results can only be confirmed if a more thorough analysis of this data is carried out using appropriate GLM comparisons.

References

Acuña, F. H. & Zamponi, M. O. 1997. The use of cnidocysts for ecological races identification from sea anemones populations (Anthozoa, Actiniidae). *Iheringia* 82: 9–18.

Acuña, F. H., Excoffon, A. C., Zamponi, M. O. & Ricci, L. 2003 Importance of nematocysts in taxonomy of acontiarian sea anemones (Cnidaria, Actiniaria): A statistical comparative study. *Zoologischer Anzeiger* 242: 75–81.

Acuña, F. H., Ricci, L., Excoffon, A. C. & Zamponi, M. O. 2004. A novel statistical analysis of cnidocysts in acontiarian sea anemones (Cnidaria, Actiniaria) using generalized linear models with gamma errors. *Zoologischer Anzeiger* 243: 47–52.

Acuña, F. H., Excoffon, A. C. & Ricci, L. 2007. Composition, biometry and statistical relationships between the cnidom and body size in the sea anemone *Oulactis muscosa* (Cnidaria: Actiniaria). *Journal of the Marine Biological Association of the United Kingdom* 87: 415–419.

Acuña, F. H. & Garese, A. 2009. The cnidae of the acrospheres of the sea anemone *Corynactis carnea* Studer, 1878 (Cnidaria, Corallimorpharia, Corallimorphidae): composition, abundance and biometry. *Belgian Journal of Zoology* 139(1): 50–57.

Allcock, A. L., Watts, P. C. & Thorpe, J. P. 1998. Divergence of nematocysts in two colour morphs of the intertidal beadlet anemone *Actinia equina*. *Journal of the Marine Biological Association of the United Kingdom* 78: 821–828.

Anderson, P. A. V. & Bouchard, C. 2009. Regulation of cnidocyte discharge. *Toxicon* 54: 1046–1053.

Ardelean, A; Fautin, D. G. 2004. Variability in nematocysts from a single individual of the sea anemone *Actinodendron arboretum* (Cnidaria: Anthozoa: Actiniaria). *Hydrobiologia* 530/531: 189–197.

Beneti, J. S.; Stampar, S.N.; Maronna, M.M.; Morandino, A. C. & da Silveira, F. L. 2015. A new species of *Diadumene* (Actiniaria: Diadumenidae) from the subtropical coast of Brazil. *Zootaxa* 4021 (1): 156–168.

Belém, M.J. & Preslercravo, J. 1973. Contribuições ao conhecimento da fauna de cnidários do Espírito Santo, Brasil I – Considerações sobre Actiniaria do Município de Aracruz, E. S. *Boletim do Museu de Biologia Prof. Mello Leitao* 80: 1–14

Belém, M.J.C. 1987. Anatomy and biology of *Bunodosoma caissarum* Corrêa, 1964 (Cnidaria, Anthozoa, Actiniidae). 1. Systematic position and revision of morphology and microanatomy. *Anais da Academia Brasileira de Ciencias* 60: 365–375.

Corrêa, D.D. 1964. *Corallimorpharia e Actiniaria do Atlântico Oeste Tropical*. Universidade de São Paulo, Ph.D Thesis, São Paulo, Brazil, 139 p.

Daly, M. 2003. On the anatomy, terminology, and homology of acrorhagi and pseudoacrorhagi. *Zoologishe Mededelingen* 345: 89–102.

Daly, M.; Brugler, M. R.; Cartwright, P.; Collins, A.G.; Dawson, M. N.; Fautin, D.; France, S. C.; McFadden, C.S.; Opresko, D. M.; Rodriguez, E.; Romano, S. & Stake, J. 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. *In*: Zhang, Z.–Q. & Shear, W.A. (Eds). Linnaeus Tercentenary: Progress in Invertebrate Taxonomy. *Zootaxa* 1668: 127–182.

Daly, M.; Chaudhuri, A.; Gusmão, L. & Rodríguez, E. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular Phylogenetics and Evolution* 48: 292–301.

Dube, V. M. C. 1974. Anêmonas do Mar (Ordem Actiniaria) do Estado da Bahia. MSc. Dissertation. Instituto de Biociências, Universidade de São Paulo, São Paulo, 81pp.

Fautin, D. G. 1988. Importance of nematocysts to actinian taxonomy. In: Hessinger, D. A. & Lenhoff, H. M (Ed.). *The Biology of Nematocysts*. San Diego and other cities: Academic Press. p. 487 – 500.

Fautin, D. G. 2013. Hexacorallians of the World. Available in <http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm> [Access em May 10, 2016]

Francis, L. 2004. Microscaling: Why larger anemones have longer cnidae. *Biological Bulletin* 207: 116–129.

Garese, A. 2013. Estudio del cnidoma de anémonas de mar (Cnidaria: Actiniaria y Corallimorpharia): composición, abundancia y biometría. PHD thesis, Departamento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales Universidad Nacional de Mar del Plata. 137p.

Gomes, P.B., Belém, M.J.C. & Schlenz, E. 1998. Distribution, abundance and adaptation of some Actiniidae (Cnidaria, Actiniaria) on an intertidal sand reef in Carneiros Beach, Pernambuco, Brasil. *Miscelania Zoologica* 21(2): 65–72.

Gomes, P.B. 2002. Estudio de la vicariancia en algunas especies de anémonas de mar (Cnidaria, Actiniaria) del intermareal de Brasil y Argentina con el uso de datos morfológicos y genéticos. Ph.D Thesis – Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina, 101p.

Gomes, P.B.; Schama, R. & Solé–Cava. 2012. Molecular and morphological evidence that *Phymactis papillosa* from Argentina is, in fact, a new species of the genus *Bunodosoma* (Cnidaria: Actiniidae). *Journal of the Marine Biological Association of the United Kingdom* 92 (5): 895–910.

Gusmão, L. C. 2016. *Metapeachia schlenzae* sp. nov. (Cnidaria: Actiniaria: Haloclavidae) a new burrowing sea anemone from Brazil, with a discussion of the genus *Metapeachia*. *Zootaxa* 4072 (3): 373–383.

Hastie, T. J. & Tibshirani, R. J. 1990. *Generalized additive models*. Washington: Chapman & Hall. 352 p.

Haussermann, V. 2004. Re–description of *Phymactis papillosa* (Lesson, 1830) and *Phymanthea pluvia* (Drayton in Dana, 1846) (Cnidaria:Anthozoa), two common actiniid sea anemones from the south Pacific with a discussion of related genera. *Zoologische Mededelingen* 78: 345–381.

Mariscal, R. N., McLean, R. B. & Hand, C. 1977. The form and function of cnidarian spirocysts. 3. Ultraestructure of the thread and the function of spirocysts. *Cell and Tissue Research* 178, 427–433.

Nelder, J. A. & Wedderburn, R. W. M. 1972 Generalized linear models. *Journal of the Royal Statistical Society: Series A* 135: 370–384.

Östman, C. 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. *Scientia Marina* 64 (Supl. 1): 31–46.

R Development Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available in [http://www.R–project.org/].

Rodríguez, E.; López–González, P. J. & Daly, M. 2009. New family of sea anemones (Actiniaria, Acontiaria) from deep polar seas. *Polar Biology* 32: 703–717.

Shick J. M. 1991. *A Functional Biology of Sea Anemones*. London: Chapmann and Hall. 395p.

Watson, G. M. & Mariscal, R. N. 1983. The development of a sea anemone tentacle specialized for aggression: morphogenesis and regression of the catch–tentacle of *Haliplanella luciae* (Cnidaria, Anthozoa). *Biological Bulletin* 164 (3): 506–517.

Watts, P. C., Allcock, A. L., Lynch, S. M. & Thorpe, J. P. 2000. An analysis of the nematocysts of the beadlet anemone *Actinia equina* and the green sea anemone *Actinia prasina*. *Journal of the Marine Biological Association of the United Kingdom* 80: 719–724.

Williams, R. B. 1996. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria): statistical parameters and taxonomic relevance. *Scientia Marina* 60: 339–351.

Williams, R. B. 1998. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), II: further studies of differences amongst sample means and their taxonomic relevance. *Scientia Marina* 62: 361–372.

Williams, R. B. 2000. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), III: ranges and other measures of statistical dispersion, their interrelations and taxonomic relevance. *Scientia Marina* 64: 49–68.

Won, J. H.; Rho, B. J. & Song, J–I. 2001. A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. *Coral Reefs* 20: 39–50.

Zamponi, M. O. & Acuña, F. H. 1991. La variabilidad de los cnidocistos y su importancia en la determinación de clines. *Physis* 49: 7–18.

Conclusão geral

A partir das evidências disponíveis, pudemos concluir no Capítulo 1 que o gênero *Bunodosoma* engloba 14 espécies válidas e é parafilético em relação a outras espécies da Família Actiniidae. Além disso, os dados evidenciam que três espécies reconhecidas (*B. biscayense*, *B. fallax* e *B. sphaerulatum*) seguem apresentando problemas taxonômicos, e uma revisão mais detalhada focada nas mesmas é necessária. Em *B. biscayense* é necessário compreender melhor a natureza das projeções da coluna. Já para *B. fallax* e *B. sphaerulatum* é necessário que mais material seja coletado em suas localidades tipo para que possa se realizar estudos mais completos de seus aspectos de anatomia interna e cnidoma. Não há evidências até o momento que suportem o posicionamento de *B. fallax* como pertencente ou não a *Bunodosoma*. *Bunodosoma sphaerulatum* por outro lado apresenta caracteres que permitem sua alocação no gênero *Bunodosoma*, no entanto, existe a possibilidade de que os espécimes associados e está espécie sejam na realidade pólipos juvenis de alguma das outras espécies que ocorrem no mar do Caribe (*B. cavernatum*, *B. granuliferum* e *B. kuekenthali*).

Em nossa hipótese filogenética apresentada no Capítulo 1, as espécies *B. cavernatum* e *B. granuliferum* formaram um clado, porém, com nosso conjunto de evidências, não podemos afirmar nada acerca de seus relacionamentos internos, e o consenso estrito resulta em uma politomia. Associado a isso, ambas espécies são fenotipicamente muito similares, compartilhando características de morfologia externa, interna e, em grande parte, de cnidoma. Estas espécies são diferenciadas apenas por bandas/listras longitudinais presentes na segunda espécie e ausentes na primeira. Dessa forma, apenas com o uso de uma quantidade maior de evidências moleculares se pode testar de forma robusta a validade taxonômica dessas espécies.

As espécies do Atlântico Sul, *B. caissarum*, *B. cangicum* e *B. zamponii*, também formaram um clado em nossa hipótese filogenética do Capítulo 1. *Bunodosoma caissarum* pode ser fenotipicamente e geneticamente (com o uso do marcador molecular ITS) diferenciada das outras espécies do clado. Já as espécies *B. cangicum* e *B. zamponii* são fenotipicamente semelhantes e mesmo com o uso do marcador molecular ITS (Capítulo 2) não podemos afirmar nada conclusivo acerca de suas relações, que devem ser mais bem exploradas em estudos futuros.

No capítulo 2 concluímos que os marcadores moleculares nucleares ITS (espaçador transcrito interno), ALG11(em inglês, *asparagine-linked glycosylation 11 homolog*) e MAT (em inglês, *methionine adenosyltransferase*) não são apropriados para a realização de estudos populacionais no gênero *Bunodosoma*. Enquanto o primeiro marcador apresentou variação suficiente para separar *B. caissarum* de *B. cangicum* e *B. zamponii*, os outros dois marcadores não apresentaram variabilidade entre as espécies analisadas. Mais testes com outros marcadores nucleares ou técnicas alternativas, como as de sequenciamento de nova geração (NGS, sigla em inglês) necessitam ser realizados para que se possa encontrar um marcador que permita a realização de estudos filogeográficos em anêmonas do mar.

Por fim, no capítulo 3 conseguimos detectar alguns tipos de nematocistos que podem auxiliar no estudo de populações de *B. caissarum* e *B. cangicum*. Para a primeira espécie, o único tipo de cnidocisto que parece ter potencial para esses estudos são os basítricos dos tentáculos. Já para *B. cangicum*, os resultados dos seguintes nematocistos se destacaram: holótricos dos acrorrágios, basítricos dos tentáculos externos e microbásico *b*-mastigóforo dos filamentos. Sugerimos que os dados levantados para esses tipos detectados sejam analisados com maior detalhe, para que se possa compreender se realmente contribuem para estudos de população. Além disso, verificamos que na espécie *B. cangicum*, há diferenças significativas

nos tamanhos dos basítricos e dos espirocistos dos tentáculos do ciclo mais externo e mais interno. Este resultado deve ser levando em conta em futuras descrições de anêmonas do mar.

De forma geral, podemos concluir que o conjunto de resultados do presente estudo contribui para um melhor conhecimento da sistemática e biologia das anêmonas do mar. É de grande importância que estudos integrativos sejam realizados com outros grupos desta ordem, para gerar subsídios para a compreensão de aspectos evolutivos da ordem Actiniaria.